THE MECHANISM OF MONOSYNAPTIC REFLEX DEPRESSION BY SUCCINYLCHOLINE

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A THESIS
Presented to the Department of Physiology
and the Graduate Division of the University of Oregon Medical School
in partial fulfillment of
the requirements for the degree of
Master of Science

June 1963

APPROVED:

[redacted]

(Professor in Charge of Thesis)

[redacted]

(Chairman, Graduate Council)

ACKNOWLEDGEMENT

The author is indebted to Professor John M. Brookhart for his advice, his encouragement and his friendship. The efforts of Mr. Duncan Neilson were essential for the completion of this investigation. The suggestions of Drs. P. Crepax and K. Kubota with regard to various aspects of this study were greatly appreciated.

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INTRODUCTION

A. STATEMENT OF THE PHENOMENON

For more than ten years, succinylcholine (SCh) and, to a lesser degree, decamethonium (C10) have been employed in medicine as neuromuscular blocking agents. The paralyzing actions of these drugs have been and are currently used to produce muscular relaxation for various surgical and electroshock procedures. This usage has extended to neurophysiological investigations, wherein it has become common practice to immobilize the experimental preparation with one of these agents. In some of these cases, the unwarrented assumption has been made that SCh or C10 exert only neuromuscular blockade and are without influence on the central nervous system.

In 1953, Granit and co-workers (60) reported that the intravascular injection of SCh or C10 in the cat was followed by an increased rate of afferent discharge from muscle spindles. It was then observed by other investigators, that the actions of SCh and C10 were not limited to neuromuscular blockade or to augmenting muscle spindle discharge, but included a transient depression of the monosynaptic reflex response. This reflex depression was first reported in 1957 by Eldred and others (43), and later, was observed independently in Germany (68) and in this laboratory.

The experiments upon which this dissertation is based were concerned with an elucidation of the mechanisms by which SCh depresses the monosynaptic reflex response in the cat. In a few experiments, the effects of C10 administration were compared with those of SCh. A review of the pertinent spinal cord physiology and of the known properties of SCh and C10 are in order.

B. MONOSYNAPTIC REFLEX RESPONSES

A monosynaptic reflex has but a single synapse between its afferent and efferent nerve components. Monosynaptic reflexes in the spinal cord may be activated by electrical stimulation of a dorsal root (38, 117, 91) or a muscle nerve (92, 93), or by phasic stretch of a muscle (93). The resulting response, oscillographically recorded from a cut ventral root, consists of a well defined, negative potential of brief duration (117, 93). The amplitude of the monosynaptic reflex response (MSRR), recorded in this fashion, has been employed widely in neurophysiologic investigations as an indirect measure of motoneuronal excitability (see 117, 119, 58, 76, 100) and was utilized here for the same purpose. The concepts underlying this use and more importantly, the limitations of its use are pertinent.

Anatomical Evidence

The anatomical basis of the monosynaptic reflex was provided by the studies of Cajal (16; Fig. 113), who observed collaterals from the larger dorsal root fibers extending through the spinal grey to make direct synaptic contact with motoneurons in the ventral horn. Cajal's demonstration of monosynaptic reflex collaterals was later confirmed by the Wallerian technique (132, 131), which showed a degeneration of synaptic terminals on motoneurons five to eight days subsequent to dorsal rhizotomy in cats. Reflex collaterals which establish monosynaptic connections with motoneurons are found throughout the spinal cord, and the greatest density of collaterals occurs in the cervical and lumbar enlargements. The collaterals arising from the fibers of a given dorsal root are mainly distributed to the motoneurons of

that segment; however, some reflex collaterals extend beyond the zone of entry by as much as two segments (132, 131). In general the segmental distribution of afferent fibers in a muscle nerve matches closely that of the efferent fibers in the same nerve (79). Anatomical studies have not indicated whether the functions of these reflex collaterals are inhibitory or excitatory.

Physiologic Evidence

The anatomic demonstration of monosynaptic reflex pathways provided a rich substrate for speculation and experimentation with regard to the function of the MSR in the intact organism. Cajal (16; Fig. 209) postulated a monosynaptic circumscribed reflex mechanism, Jolly (82) and Hoffman (70) believed the stretch reflex to be mediated by a two-neuron arc, and Eccles and Pritchard (38) indicated that the first portion of the segmental reflex (the ventral root response evoked by dorsal root stimulation) was probably monosynaptic in nature. However, physiologic demonstration of spinal monosynaptic pathways awaited the determination of synaptic delay, that is, the time required by central afferent terminals to activate their motoneurons. This information was provided by Renshaw (117) in 1940, who applied the technique of Lorente de Nó (104, 105) to the spinal cord. The use of intramedullary electrodes enabled Renshaw to stimulate motoneurons directly, and at the same time, to activate motoneurons trans-synaptically by stimulating their afferent terminals. The latency difference between ventral root response evoked by direct stimulation and that evoked trans-synaptically was found to vary between 0.5 and 1.0 msec. Since the value of 0.5 msec could not be reduced by prior conditioning it was concluded to represent the minimal value for synaptic delay. A

similar value for the synaptic delay of oculomotor motoneurons had been established previously (104, 105).

Having resolved the duration of synaptic delay, Renshaw (117) then proved with latency measurements the monosynaptic nature of Eccles and Pritchard's segmental reflex. The latency of the segmental reflex minus the time required by the afferent volley to conduct to the spinal cord and the reflex efferent volley to conduct to the recording electrodes equaled synaptic delay plus a few tenths of a millisecond for intramedullary conduction. Thus, the earliest deflection of the segmental reflex was derived from motoneurons which were discharged monosynaptically since there was insufficient time for the interposition of an interneuron in the reflex pathway.

Indirect Measure of Motoneuronal Excitability

Basis of Test System

Renshaw (117) further demonstrated that the monosynaptic component of the segmental reflex was generated by those afferent volleys with the greatest conduction velocity. These observations were the basis for his suggestion (117) and utilization (117, 119) of the monosynaptic component of the segmental reflex as an indirect measure of motoneuronal excitability. Electrical stimulation of a dorsal root activates numerous fibers with diverse central actions; however, the afferent volley in the largest fibers would reach and discharge motoneurons before the volleys in slower conducting fibers could alter motoneuronal excitability either directly or via internuncial pathways. The number of motoneurons discharged monosynaptically

by the test volley and hence the amplitude of the recorded monosynaptic potential would depend on the excitability of the motoneurons at that instant.

Lloyd (91) confirmed and extended these observations by demonstrating an almost linear relationship between the amplitude of the MSRR and the amplitude of the dorsal root volley producing it. The amplitude of the MSRR was approximately 90 percent of maximum before the presynaptic volley reached 50 percent of maximum. Further increases in the size of the presynaptic volley produced polysynaptic discharge. In view of the relationships between spike height, fiber threshold, fiber conduction velocity and fiber diameter (see 54, 78), Lloyd concluded that the low threshold, fast conducting afferent fibers evoked monosynaptic reflex discharge, in contrast to the high threshold, slow conducting afferent fibers which were responsible for generating polysynaptic reflex discharge. When the MSRR was evoked by an electric shock to a muscle nerve or by phasic stretch of a muscle, the responsible afferent volley was found to conduct at an average maximum velocity of 116 M/sec (93). This observation again confirms the low threshold, fast conducting properties of the afferent fibers which generate the MSRR.

The MSRR as a test of motoneuronal excitability was further refined by Lloyd's (92) demonstration that stimulation of a muscle nerve discharges monosynaptically only those motoneurons destined for the muscle whose afferent fibers had been stimulated. As Lloyd (98) points out, this observation was not new; weak stimulation of afferent nerves from the sternomastoid muscle was noted to evoke contraction in that muscle alone more than seventy years ago (19). The similarity between the properties of the monosynaptic reflex and those of the myotatic reflex (89) led Lloyd to conclude (92, 93, 98, 100) that monosynaptic pathways mediated the

myotatic reflex.

Limitations of Test System

The use of the amplitude of the MSRR evoked by stimulation of a muscle nerve as a measurement of motoneuronal excitability is based on three assumptions: 1) that changes in motoneuronal excitability are linearly related to the number of motoneurons discharged by the afferent test volley; 2) that the number of discharged motoneurons is linearly related to the amplitude of the recorded ventral root potential; and 3) that the discharged motoneurons only innervate the muscle whose afferent fibers had been stimulated. The validity of these assumptions will be discussed now, as will a number of other factors which limit the use of the MSRR test system.

The proposition that the excitability of the tested motoneurons linearly relates to the number of discharged motoneurons is true in some cases but not in others. This must be so for the MSRR test system because this technique does not distinguish between presynaptic and postsynaptic events. For example, following tetanic stimulation of a muscle nerve, the excitability of the activated motoneurons is actually depressed (97), but MSRR testing reveals considerable potentiation. The factor responsible for the MSRR facilitation following tetanic stimulation is confined to the afferent terminals (97, 36) and not the tested motoneurons. A similar example is provided by the recent demonstration of presynaptic inhibition (32, 37). In this case the excitability of the motoneurons is not altered, but a persistent depolarization of the afferent terminals renders the testing afferent volley less effective in generating a postsynaptic potential. In contrast, the amplitude of the MSRR does reflect with a considerable degree of accuracy those changes in excitability con-

fined to the tested motoneurons (103).

A truly linear relationship probably does not exist between the amplitude of the recorded MSRR and the number of active ventral root axons contributing to that response. The apparent magnitude of the action potential of a single axon is dependent on three factors: 1) the diameter of the fiber (54); 2) the distance separating the active fiber from the recording electrode (106); and 3) the amount of tissue fluid and inactive tissue surrounding the active fiber (80). It is a logical conclusion of such observations that some active fibers will contribute more than others to the composite potential that is recorded from a ventral root. Thus a 50 percent reduction in the amplitude of the MSRR does not imply that one-half of the motoneurons failed to respond to the testing afferent volley.

Lloyd (92) provided evidence that monosynaptic discharge was confined to those motoneurons innervating the muscle whose afferent fibers had been stimulated. In other words, it was possible to test the excitability of a given motoneuronal pool by the simple expedient of stimulating the large afferent fibers in that particular muscle nerve. This is in contrast to the MSRR evoked by dorsal root stimulation which represents the excitability of motoneurons to many muscles. However, Lloyd's experiments were conducted on decapitate animals in which both the dorsal and ventral roots were left intact. In such a preparation and following stimulation of a muscle nerve, the orthodromic volley conducted in the afferent fibers would clash somewhere in the reflex arc with the antidromic volley evoked in the afferent fibers. To avoid a significant blockade of the MSRR recorded from the stimulated muscle nerve, a very critical adjustment of the stimulus intensity was necessary (92). It follows that such stimulation may or may not mimic the shocks applied to muscle

nerves when MSRR's are recorded from cut ventral roots. It would seem a distinct possibility that monosynaptic activation and discharge of motoneurons other than those supplying the muscle of origin would occur when the latter technique is employed. Intracellular recordings in single motoneurons have also indicated that monosynaptic activation is not confined to the muscle of origin (41).

Other factors which limit the usefulness of the MSRR test system or which must be considered when employing this measurement of motoneuronal excitability include: the size of the sample versus the size of the population; the inability to compare quantitatively the changes in MSRR amplitude obtained from one experiment with those obtained from another; the "spontaneous" temporal variability in the amplitude of the MSRR; and the interpretation of a change in response amplitude.

With the reservation that the MSRR may represent motoneurons other than those innervating the muscle whose afferent fibers were stimulated, it is of interest to compare the size of the discharged population with that of the motoneuronal pool. In response to a synchronous afferent volley only a fraction of the motoneuronal pool is discharged (91, 94). Jefferson and Benson (80) quantitated the size of the monosynaptic discharge zone and found that single shock stimulation of the gastrocnemius-soleus nerve discharged only 12 to 26 percent of that motoneuronal pool in decapitate preparations. These figures probably err on the low side (7) but even so, do emphasize the small size of the discharge zone. Thus, the behavior of motoneurons in the subliminal fringe goes undetected with regard to depressive influences, whereas the influence of excitatory factors is lost with regard to those motoneurons which discharge in response to every test volley. This must be so, because the MSRR only indicates discharge or lack of discharge. The MSRR test system can

detect only those excitability changes which exceed or which fall below the threshold of the involved motoneurons; the more subtle changes in excitability which do not cross the threshold are not detected.

The sample size, which is small in many instances to begin with, is further reduced by the recording technique. The ratio of inactive tissue to active axons in a ventral root is large and the shunting effect of inactive tissue has been clearly demonstrated (80). The actual magnitude of this shunting effect was estimated by Jefferson (79) who showed that 16 synchronously active axons in a ventral root containing 2,500 fibers with diameters of 7 microns or greater could be detected by gross root recording. Therefore, a number of discharged axons less than 16 will not be available for recording. In a given experiment, the actual magnitude of this loss would vary with the size of the ventral root and with the total number of motoneurons discharged by the test volley.

Whatever the relationship between the amplitude of the MSRR and the number of discharged fibers may be, the relationship is only valid for that particular recording situation. The proximity of active fibers to the recording electrode; the amount of intervening inactive tissue; and the size of the ventral root vary from one experiment to the next. In addition, differences in interelectrode distance affect the size of the recorded potential; the potential linearly increases as the log of the interelectrode distance increases (80). These factors mean that a comparison of changes in amplitude in terms of voltage between MSRR's recorded from different ventral roots in the same animal or from roots of different animals is without meaning. This problem can be circumvented by evoking relatively similar responses in different experiments, for example, just maximal MSRR's. By converting the amplitudes

of all responses to percent and relative to baseline amplitude, comparisons can be made in terms of percent changes (117, 119). However, this technique is not entirely satisfactory since the number of discharged units for a particular recording situation is not a linear function of the amplitude of the recorded potential (see above).

When evoked in successive trials by a constant test volley, the MSRR demonstrates characteristic "spontaneous" fluctuations in amplitude (74). As the size of the testing afferent volley is increased from zero, the amplitude of the MSRR increases as does its range of variation in amplitude. The variation increases until the amplitude of the MSRR is sufficiently large to bring the full range of temporal variation into view; thereafter, the range of the variation in response amplitude remains constant until the testing afferent volley is large enough to discharge the majority of the motoneuronal pool at which time the variation decreases and with activation of the entire pool the amplitude of the MSRR remains constant (74).

The temporal variability of the MSRR has been investigated on a unit basis by recording reflex responses from single ventral root axons (75, 103). In these cases the excitability of the tested motoneuron is expressed by the number of monosynaptic discharges which resulted from a fixed number of testing volleys. This technique was first employed by Granit and Strom (61) and the expression of motoneuronal excitability was subsequently referred to as a firing index (75, 103). With reference to a constant monosynaptic input, it has been shown (103) that some motoneurons respond on every trial, some on certain trials and others on no trials. Regardless of the level of synaptic excitation the number of motoneurons with intermediate firing indices remained constant, i.e., those units responding on certain trials but not on others (103). The motoneurons with intermediate firing indices account for the

variability of the population MSRR's.

With reference to graded afferent input, unit studies (75) have shown that a given motoneuron demonstrates low, intermediate and maximal firing indices in that order as the afferent volley is increased in size from zero. Since the MSRR only detects discharge or lack of discharge, these motoneurons with intermediate firing indices are the most labile fraction of the population with respect to factors which produce a generalized increase or decrease in motoneuronal excitability.

The large temporal variability in the amplitude of the population MSRR means that a number of responses must be evoked for a baseline and that small induced changes in amplitude will probably not be statistically significant.

The interpretation of a given change in the amplitude of the MSRR may be considered. As a number of synchronously discharged units contribute to the composite potential, the MSRR is only an average index of motoneuronal excitability. As such, the actual change in excitability of a given motoneuron can only be inferred from the response of the gross population. It is entirely possible for facilitation to co-exist with depression and yet the composite response would only demonstrate depression.

In addition to being an average index of motoneuronal excitability, the MSRR is an indirect measurement and as such does not distinguish between presynaptic and postsynaptic events. Thus, MSRR depression may not imply decreased motoneuronal excitability.

In spite of the objectionable aspects associated with the MSRR test system, its use in the past has provided a wealth of information. The MSRR will continue to be a useful adjunct to the future student of neurophysiology if the factors enumerated

in this discussion are understood.

Inhibition of Monosynaptic Reflex Responses

In anticipation of the forthcoming experiments which deal with the mechanism of SCh-induced MSRR depression, it is appropriate to consider three different inhibitory mechanisms: direct, recurrent and presynaptic inhibition.

Direct Inhibition

With reference to a MSRR evoked by stimulation of an extensor muscle nerve, Lloyd (95) demonstrated that the response was depressed by a preceding conditioning volley set up in large afferent fibers which originated in an antagonistic flexor muscle. The reverse situation in which a flexor MSRR was depressed by an extensor afferent volley was also shown to exist. The time course of this depressive influence was found to be quite brief. The onset of MSRR depression and maximal MSRR depression occurred with a conditioning interval of less than 1 msec. Thereafter, the degree of depression decreased exponentially as the time the conditioning volley preceded the MSRR was increased. When the conditioning afferent volley preceded the MSRR by more than 15 msec reflex depression was no longer apparent. This phenomenon, termed direct or postsynaptic inhibition, results from a hyperpolarization of the motoneuronal membranes which decreases their excitability to either direct stimulation or an excitatory test volley (29). Since the change in excitability is confined to the postsynaptic elements, the number of units discharged by the monosynaptic test volley would vary in linear relation to the excitability of the involved motoneurons.

Our understanding of direct inhibition is based on the characteristics of afferent

fibers which originate in muscles and on the central actions of these fibers. The diameter spectrum of afferent fibers in muscle nerves has been studied (121, 101) and, based on the modes of diameter distribution, muscle afferent fibers have been classified into three groups (73): Group I (12-20 μ), Group II (4-12 μ) and Group III (1-4 μ). Group I is further divided into Group IA consisting of those fibers arising from muscle spindles, and Group IB fibers which arise from Golgi tendon organs. Group II fibers also originate from muscle spindles and evidence has been presented that these fibers are activated by secondary endings (flowerspray) on the spindle, whereas the primary or annulospiral endings are related to Group IA fibers (73). In the gastrocnemius-soleus nerve, afferent fibers in Groups I and II are accounted for by fibers arising from muscle spindles or tendon organs (73). However, in other muscle nerves, it is stated that receptors from joints or associated structures contribute afferent fibers of the Group I and II range (127).

The central actions of these muscle afferent fibers may be summarized as follows. The Group IA fibers from a given muscle monosynaptically excite the motoneurons to that muscle and its synergists, and they also inhibit motoneurons of antagonistic muscles (95, 96, 87). The afferent fibers from tendon organs (Group IB) inhibit the motoneurons to the muscle from which they originated and facilitate motoneurons innervating muscles with antagonistic function (109, 57, 87). The general action of Group II afferents is a generalized excitation of motoneurons supplying flexor muscles and an inhibition of extensor motoneurons (96, 87). A similar pattern of central action is thought to hold true for Group III fibers (3, 30).

The study of the central actions of afferent fibers was greatly facilitated by the intracellular recording technique. This technique has permitted the essential con-

firmation of the above observations and, in addition has revealed many variations not detectable by MSRR testing (29, 41, 30). For the most part, these variations are minor and for the purposes of this study need not be reiterated.

The foregoing conclusions regarding the central actions of afferent fibers were derived mainly from experiments in which the afferent conditioning volleys were evoked by non-physiological electrical stimulation of muscle nerves. The effects of a pure Group I afferent volley on the tested motoneurons may be observed with appropriate stimulus adjustment. Further increases in stimulus strength add Group II afferents to the conditioning volley, and the central effects of Group I plus Group II volleys may then be detected. How the response of the tested motoneurons to the Group II afferents is modified by the faster conducting Group I fibers activated as well by the conditioning shock is not known. This reservation applies regardless of the means employed for measurement of motoneuronal excitability, i.e., MSRR testing or intracellular recording.

Another factor which modifies the apparent central action of afferent fibers is the type of experimental preparation employed. For example, the excitatory and inhibitory actions of Group II afferents are more clearly demonstrable in decerebrate animals which have been subjected to spinal cord transection than in animals which have been only decerebrated (42).

Recurrent Inhibition

Renshaw (118) showed that antidromic volleys set up in motor nerves of various spinal nuclei caused the MSRR of other nuclei to be inhibited or, to a lesser degree, facilitated. The inhibition and facilitation both appear to result from the activation

of recurrent collaterals of large motoneurons (33, 13, 140). Specifically, the impulses in the recurrent collaterals result in the release of a cholinergic mediator (33, 29) which activates a special group of interneurons which were first described physiologically by Renshaw in 1946 (120). The interneurons in turn exert the inhibitory or facilitatory influence on the involved motoneurons. As with direct inhibition, recurrent inhibition is manifest as a hyperpolarization of the inhibited motoneurons (33), but differs from direct inhibition in its time course. The onset of recurrent inhibition as determined by intracellular recording is approximately 1.5 msec. The maximum inhibition occurs about 10 msec later and then gradually decays over 50-100 msec. The time course of recurrent inhibition as determined by MSRR testing is quite similar to that obtained by intracellular recording (118, 13).

It has been demonstrated that a relatively large proportion of spinal motoneurons give rise to recurrent collaterals (16). There can be no doubt about the significance of this phenomenon of recurrent inhibition from the point of view of its intensity. Nevertheless, the role played by recurrent inhibition in the integrated organization of spinal cord function has not yet been elucidated.

Presynaptic Inhibition

Frank and Fuortes (50) and Frank (48) observed that single afferent volleys in the hamstring nerve were capable of depressing the monosynaptically evoked gastrocnemius excitatory postsynaptic potential (EPSP). In contrast to direct and recurrent inhibition, this EPSP depression occurred without a change in the resting membrane potential of the gastrocnemius motoneurons. Furthermore, the afferent volley in the hamstring nerve did not alter the excitability of the gastrocnemius

motoneurons or the ability of an antidromic volley to invade these motoneurons. The demonstration of EPSP depression without a change in the electrical characteristics of the motoneuron's soma led these investigators to postulate that the inhibition occurred before the motoneuron was reached (presynaptic inhibition) or that the inhibition was exerted far out on the dendrites of the motoneuron (remote inhibition).

These observations have been confirmed in part and extended by Eccles and coworkers (33, 37, 34, 35, 39). The EPSP depression evoked by a single or a brief train of afferent volleys in the posterior biceps-semitendinous (PBST) nerve varies from 100 to 200 msec in duration. The latency for the EPSP depression was found to be 3 to 5 msec and with the maximal depression occurring 10 to 20 msec later (32, 37). The observation that the reduction in the size of the EPSP occurred without an alteration in its time course, and the earlier observations of Frank and Fuortes (50) that the EPSP reduction took place without a change in the excitability of the tested motoneuron, led Eccles' group (32) to conclude that the inhibition was exerted at the presynaptic level; thus, the name, presynaptic inhibition was adopted.

The actual event occurring at the presynaptic level was demonstrated to be a depolarization of the intraspinal portions of large afferent fibers arising from muscle and this phenomenon was termed primary afferent depolarization. The depolarization of the membrane of the large afferent fibers was demonstrated by both an enhanced excitability of the involved afferent terminals and by intra-axonal recordings (37). Presumably, the afferent terminals are depolarized by an interneuron activated by the conditioning volley (34). The time courses of both the EPSP depression and the primary afferent depolarization were found to be essentially identical and both phenomena were noted to come and go together in response to various

maneuvers. Thus, the EPSP depression was concluded to result from the afferent fiber depolarization (37). The magnitude of the presynaptic spike is thought to be reduced by the depolarization, and as such, the spike liberates less transmitter substance which accounts for the reduced EPSP. Such a situation is known to exist in the squid (64) and an analogous situation exists in the mammalian nervous system in the form of post-tetanic potentiation (97, 36).

The original scheme describing the anatomical distribution of presynaptic inhibition was relatively simple (32, 37). All Group I afferents were equally capable of being depolarized but afferent fibers arising from flexor muscles exerted a greater depolarizing action than did afferents originating from extensor muscles. Of the particular afferents, Group IA plus Group IB volleys were more effective depolarizers than Group IA volleys alone and Groups II and III were without apparent effect. However, the concept has now evoluted to a more complex stage. In addition to the above, the system now stands: 1) Group I muscle afferents, cutaneous afferents and high threshold muscle afferents inhibit presynaptically all central actions of those afferents which mediate flexion reflexes (35); 2) Group IA afferents are depolarized largely by Group I afferents from flexor muscles (32, 37); and 3) Group IB fibers are depolarized mainly by Ib volleys from both flexor and extensor muscles (39).

It is understood that the subdivision of Group I volleys into IA and IB components in these experiments rests on the assumption that it is possible to separate the afferent fibers which arise from muscle spindles and tendon organs by virtue of their differential thresholds to electrical stimulation and the different conduction velocities of the two groups. In this writer's mind, there is not sufficient experimental

evidence to justify this assumption. The arguments for and against this assumption have been summarized elsewhere (76, 30). Thus, there is room for doubt about the reality of the pattern of distribution which has been described for presynaptic inhibition. At the present time it appears impossible to determine whether or not a definite anatomical pattern does in fact exist at all.

C. PHARMACOLOGY OF SUCCINYLCHOLINE

The dicholine ester of succinic acid, which is referred to as succinylcholine, succinyldicholine, suxamethonium or simply SCh, was first synthesized in 1906 by Hunt and Taveau (77). The compound was again synthesized in 1941 by Glick (56) who demonstrated that SCh was readily hydrolyzed in vitro by the cholinesterase of horse serum. However, systematic research in the field of synthetic neuromuscular blocking agents did not start until 1948 when Paton and Zaimis (113) and, independently of them, Barlow and Ing (4) reported the neuromuscular blocking action of the "methonium salts". Of the investigated series, decamethonium (C10) was the most potent with 2-3 times the potency of d-tubocurarine on a weight for weight basis.

The following year (1949), the first observation of the potent, short term paralyzing action of SCh was made by Bovet and others (8). Since that time a large series of similar compounds have been synthesized in an effort to develop blocking agents as potent as SCh and C10, but with fewer side effects (62, 24). Before considering the effect of SCh on muscle spindle afferents and the drug-induced MSRR depression, it may be of value to review the pharmacology of SCh with respect to the neuromuscular junction and the cardiovascular system.

Neuromuscular Junction

Neuromuscular blocking agents can be divided into those which prevent depolarization of the endplate region, and those which depolarize this region themselves (133). The accepted action of the first group, of which d-tubocurarine is a member is to inhibit, competively, the transmitter substance but not to alter the electrical characteristics of the endplate membrane.

In contrast to the flaccid paralysis which follows the administration of d-tubocurarine, the second group or the depolarizing neuromuscular blocking agents first potentiate the indirect muscle twitch and evoke muscle fasciculations before producing neuromuscular paralysis (113, 133, 130). The specific depolarizing action of this class of drugs was defined by Burns and Paton (15) who studied the effects of intravascular injections of decamethonium (C10) on the cat's gracilis muscle. With surface electrodes they showed that C10 depolarized the muscle in the region of the endplate and that the degree of depolarization was well correlated with the degree of neuromuscular block. On the basis of their findings, Burns and Paton (15) suggested that C10 produced neuromuscular blockade by persistently depolarizing the endplate region. The initial depolarization accounted for the potentiation of the muscle twitch and muscle fasciculations produced by this group of drugs, whereas the persistent depolarization prevented repolarization of the endplate and accounted for the neuromuscular block. This hypothesis was well accepted and was adopted to explain the neuromuscular block produced by SCh (133).

Thesleff reinvestigated the phenomenon of depolarizing neuromuscular drugs in the frog (134) and the rat (135) with intracellular recording techniques. Both SCh

and C10 were found to produce an initial depolarization of the muscle membrane; however, the membrane potential returned to its resting value even though the drug remained in contact with the muscle and in the face of persistent neuromuscular block. In view of the difference in time courses of the depolarization and neuromuscular block, Theleff (135) concluded that the neuromuscular blockade was initially that of depolarization but then converted to a type of block which resembled the paralysis produced by d-tubocurarine, or in other words, a mixed block.

The concept of a third group of neuromuscular blocking agents, i.e., mixed, is now well accepted and a large body of indirect evidence has accumulated to support this concept (143, 111, 62, 24). The mixed action has been utilized to explain such observations as the following: the amount of depolarization and of muscle fasciculations produced by successive administrations of SCh becomes progressively less without altering the degree of neuromuscular block; and neostigmine may first potentiate the neuromuscular block but later serves as an antagonist to the block.

The duration of action of SCh is quite transient, usually less than 10 minutes (133), whereas C10 produces a block lasting for as long as one hour (4, 113). Presumably, the rapid hydrolysis of SCh by pseudocholinesterase (see 139) accounts for its short action, in contrast to C10 which is excreted unchanged in the urine (142). Factors which augment and prolong the neuromuscular blocking action of SCh include: decreased peripheral blood flow (20); decreased temperature (139); decreased amounts of serum cholinesterase (45); the presence of procaine (122); and hyperventilation (114).

Cardiovascular System

The earliest observations regarding the effect of SCh on the cardiovascular system were those of Hunt and Taveau (77). Since that time numerous investigations have been conducted, and of these, Thesleff's study (133) is quite informative. SCh in doses of 2 to 5 mg/kg produces a moderate pressor response (10-30 mm Hg) which persists for several minutes in the cat. Pretreatment with a ganglionic blocking agent (hexamethonium) was shown to prevent, in varying degrees, the SCh-induced pressor response. Thesleff inferred from this observation that the pressor response resulted from an excitatory effect exerted by SCh on the autonomic ganglia with the subsequent release of epinephrine.

The findings of Thesleff are in general agreement with the observations of later studies (130, 21), the general consensus being that SCh in doses of less than 0.5 mg/kg is without effect on the blood pressure. However, it was interesting to note that one investigator (130, see Fig. 1) illustrated a 20 mm Hg pressor response following a 70 µg/kg injection of SCh but failed to comment on his observation.

Histamine release and resultant hypotension, is said to be a potential side effect following the administration of all neuromuscular blocking agents (112). In series from most active to least active with respect to histamine release are d-tubocurarine, C10, SCh and gallamine; however, histamine release usually occurs only with the first mentioned.

Other vascular phenomena which are induced by SCh include conjunctival injection, increased peripheral blood flow and increased cerebrospinal fluid pressure (137). The pressure of the cerebrospinal fluid has been observed to increase

as much as 320 mm of water in man and was attributed to an increased cerebral blood flow (137).

Activation of Muscle Spindle Afferents

Chemical activation of muscle spindle afferents was first reported by Hunt (72), who demonstrated spindle discharge following acetylcholine administration. In 1953, Granit and others (60) reported that the intraarterial injection of subparalytic doses of SCh produced a transient discharge in afferent fibers identified as arising from muscle spindles. The initiation or augmentation of spindle discharge lasted from 3 to 10 minutes depending on the dosage and preparation employed and was demonstrated to be independent of any changes in length of the extrafusal muscle fibers since the discharge could be evoked from completely paralyzed muscles by increasing the dosage employed. In a few experiments these investigators employed C10 which was observed to produce an irregular augmentation of spindle discharge which persisted for approximately one hour.

The chemical excitation of spindle discharge was reinvestigated by Brinling and Smith (51) who demonstrated that 12.5 to 800 μg/kg doses of SCh increased the rate of Group IA discharge 4 to 8 times, with the peak frequency of discharge being reached within 30 seconds, followed by a gradual decay over 10 minutes. The onset of the discharge and the time of occurrence of the peak response was less with increased rates of infusion. In a few instances the frequency was noted to decrease before the augmented discharge occurred; this transient decrease in frequency was attributed to muscle shortening.

The increased rate of muscle spindle discharge in mammals following the admin-

istration of SCh has been noted by many investigators (68, 52, 128, 51, 85, 66, 115) and was also reported by Henatsch (67) to occur in the frog. In addition to activating Group IA afferents, SCh is said to activate those Group II afferent fibers which arise from muscle spindles (52, 85).

With regard to the mechanism of initiating spindle discharge, Granit and others (60) suggested that SCh affected the sensory afferents directly in addition to an indirect stimulatory effect produced by evoking contractions in the intrafusal muscle fibers. On the other hand, Smith and Eldred (129) concluded that the augmented rate of discharge was the direct result of intrafusal fiber contraction, since the discharge evoked by SCh was reduced or abolished by pretreatment with ryanodine which presumably prevented intrafusal fiber contraction.

Apparently the initiation of spindle discharge by SCh is limited to those afferent fibers arising from muscle spindles. The discharge rate in afferent fibers identified as arising from tendon organs is not altered by SCh (60, 52) or C10 (52, 85). Furthermore these blocking agents are without effect on cutaneous receptors or nerve fibers (27, 52). However, the effect of SCh on other receptors such as those located in joints or on the smaller afferent fibers which arise from muscle spindles is not known to date.

Depression of the Monosynaptic Reflex Response

The first observation that the intravascular administration of a depolarizing neuromuscular blocking agent resulted in depression of the MSRR was reported by Eldred and others (43) who observed the effects of C10 on the MSRR of the cat in

1957. This report was soon confirmed (52) and was extended by the demonstration that SCh exerts a similar action (68, 52, 51, 66, 22). Systemically administered SCh evokes MSRR depression lasting 5 to 10 minutes, whereas the depression produced by C10 lasts for approximately one hour. The reflex depression was apparently dependent on the altered afferent input since MSRR depression was not observed following dorsal rhizotomy or when the drug was prevented from reaching the hind limb from which the MSRR was evoked (52).

The apparent relationship between the reflex depression and the drug-induced spindle discharge is enhanced since a direct action by SCh or C10 on the central nervous system has not been conclusively demonstrated. The close intraarterial injection of SCh to the spinal cord did not influence the MSRR evoked by stimulation of distally severed dorsal roots (23). A desynchronization of the cortical activity of the rabbit was observed following large doses of SCh and C10 (9), and action potentials in the phrenic nerve of the cat and dog are stated to be abolished by SCh and C10 (44). However, in neither of these investigations was a direct action of SCh or C10 demonstrated. In fact, some investigators believe that the possibility of a direct central action is remote because of the chemical and ionic nature of SCh which would not favor penetration of the blood brain barrier (21).

A most unique contribution regarding the central actions of these agents was made by Davis and others (25). These investigators first immobilized their animals with one of the blocking agents and then gave additional amounts of the same agent to determine its effect on evoked thalamic and midbrain responses. It is not astonishing to note that the evoked responses were not altered by the second administration of the blocking agent.

D. EXPERIMENTAL DESIGN

The problem at hand, the mechanism by which SCh produces MSRR depression, was simplified by the apparent relationship between the altered afferent input to the spinal cord and the reflex depression. Therefore, the plan of attack was to first confirm this observation and then to proceed to test the role of mechanisms which might be evoked by the increased rate of spindle discharge. Mechanisms to be considered or which have been considered by other investigators include: supraspinal effects (52); direct spinal cord drug actions; direct inhibition (14, 51); recurrent inhibition (68, 51, 66); occlusion or excessive seizure (66); post spike hyperpolarization (51); accomodation; and presynaptic inhibition. To avoid repetition, the hypotheses concerning each of these mechanisms and the actual experimental methods employed to evaluate their importance will be discussed in the appropriate section of the RESULTS.

METHODS AND MATERIALS

Experiments were performed on more than 100 acutely prepared adult cats, the mean weight of which equaled 3.4 kg (1 standard deviation = 1.1). Sixteen of the animals had been employed previously in a study which involved the use of chronically implanted, cortical electrodes.[1]

A. BASAL PREPARATION

The basic procedure common to most experiments consisted of recording monosynaptic reflex responses (MSRR's) before and after the administration of succinylcholine or decamethonium in an animal subjected to decerebration, decapitation or barbiturate anesthesia. To avoid unnecessary repetition the surgical preparation and procedures common to all experiments are presented as a unit before describing the various special techniques that were employed in different experiments.

Surgical Preparation

Anesthetics

Sodium-pentobarbital (Nembutal; Abbott Laboratories) was used as the general anesthetic in intraperitoneal doses of 20 to 30 mg/kg. The initial dose was usually sufficient for all surgical manipulations and maintained the animal in a quiet state from three to eight hours. Additional amounts, usually in 15 or 30 mg increments,

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1. Drs. J.G. Roth and V. Milstein kindly supplied these animals for the present work.

were given intravenously as needed. During the periods of experimentation the level of anesthesia was such as to prevent movement but not deep enough to produce undue MSRR depression.

Decerebration and decapitation were performed under ether anesthesia, which was administered by the open-drop method. Immediately following the decerebration or decapitation procedure, the ether was discontinued and at least three hours elapsed before experimentation was begun.

Tracheotomy and Blood Pressure Recording

In all animals, once anesthetized, the trachea was exposed with a midline incision and cannulated about 2 cm below the cricoid cartilage. The left common carotid artery was isolated by blunt dissection and cannulated with polyethylene tubing (# 190). The cannula was fitted to a three way stop-cock which in turn was attached to a pressure transducer (Statham) for the continuous monitoring of systemic blood pressure during the remainder of the preparation and experimental period. The contralateral common carotid artery was also isolated and ligated at this time in those animals to be decerebrated. The incision was closed with Michel metal wound clips to prevent the loss of fluid and resultant desiccation of the exposed tissues. In a few instances the femoral artery supplying the limb contralateral to the one under study was utilized to monitor systemic blood pressure. The cannula and head of the transducer were filled with a solution of 0.9 percent sodium chloride which contained heparin (50 USP units/cc saline). A syringe containing the same solution and attached to the stop-cock was utilized to flush the system as needed. The signal from the transducer was led directly to a polygraph (Grass-Model 5) or to an

electroencephalograph (Grass - Model III D) via a converter - demodulator (Grass - Model CD3). With appropriate adjustment of the high frequency response it was possible to record either the mean arterial blood pressure or the systolic and diastolic fluctuations. The system was calibrated with a mercury manometer before each experiment.

Decapitation

For decapitating purposes, the spinous process of the second cervical vertebra (axis) was exposed by a midline incision through the skin and dorsal paravertebral muscles. Hemorrhage was minimized by adhering strictly to the midline, the structures so encountered being fascial. The spinous process of the second cervical vertebra and a portion of its dorsal arch were rongeured away with modeling clay or bone wax being used to control bone hemorrhage. The dura was incised and the exposed spinal segment was transected with a blunt resector. At this time the ether anesthesia was discontinued and artificial respiration initiated (Bird respirator at pressures of 8 - 12 cm H_2O and frequencies of 20 - 30/min). Hemostasis was achieved by placing a small piece of hemostatic gelatin (Gelfoam; UpJohn Laboratories) between the severed ends of the cord. The effectiveness of the gelatin sponge was enhanced by first saturating it with a solution of bovine thrombin (100 NIH units/cc saline). The exposed muscle and skin edges were infiltrated with 2 to 3 cc of procaine hydrochloride (2 % solution) and the wound closed with clips. The necessity of infiltrating the tracheotomy wound as well was realized after several experiments. The following procedures were found to aid in the optimal maintenance of the decapitate animal: 1) a light level of ether anesthesia at the time of transection; 2) brief period of

hyperventilation following the transection to hasten the dissipation of the ether; and 3) section of the second cervical segment in preference to section at the level of the dorsal alanto-occipital membrane (107). A complete cervical transection was indicated by: a prompt cessation of respiration, a mean arterial blood pressure of 60 to 80 mm Hg, and a bilateral, equal relaxation of the nictating membranes and pupil dilator muscles.

Decerebration

In the hands of this experimenter the following method of decerebration, a modified form of Sherrington's procedure, was found to be the most valuable with respect to conservation of time, minimal trauma, and the greatest percentage of successful preparations. Succeeding the bilateral ligation of the common carotid arteries, the animal's head was fixed with either ear bars or a head holder approximately 15 cm above the heart. This manuver significantly reduced the venous hemorrhage which accompanied decerebration. The scalp was incised along the midline from the glabella to the lambda. The fascial attachment of the left temporal muscle to the superior temporal line was severed and the muscle pushed down and away from the calvarium. A large bore trephine (2.5 cm) was used to expose the left occipital cortex. The careful application of bone wax to the exposed diploic spaces ensured hemostasis, but more importantly, prevented aspiration of air with fluctuations in venous pressure. Two animals which expired prematurely were shown to have large air emboli in the superior vena cava at autopsy. The dura was incised and the left occipital pole was removed by suction. By utilizing the tentorium cerebelli for

orientation, sufficient cortex was removed to expose the dorsal aspect of the midbrain. The brain stem was transected at the desired level by a blunt resector or suction. The level of transection varied from precollicular to intercollicular in different preparations. Gelatin sponges, saturated with thrombin, were used as necessary to control hemorrhage. Once hemostasis was achieved, the head was lowered and fixed at or just above the level of the heart. The wound was closed with clips and the ether anesthetic discontinued.

At the completion of some experiments, the head was perfused with ten percent formalin; the brain was removed and fixed in the same solution for several days and 90 micron, frozen sections of the transected midbrain were obtained and photographed (63, 47). As should be expected, the completeness of the transection as determined at surgery and its completeness as demonstrated by frozen sections was widely discrepant in some cases. This is without bearing on the present work but serves to emphasize the necessity of histologic controls for any transection procedure which is an experimental variable.

Spinal Transection

In most anesthetized preparations and in an occasional decerebrate preparation the spinal cord was transected at a low thoracic (T10-T12) or high lumbar level (L1-L2). The desired spinal segment was exposed by laminectomy and the spinal cord was transected through a dural incision. The surgical transection was usually preceded by the intramedullary injection of 0.25 cc procaine hydrochloride (2 % solution). The use of procaine abolished the violent muscular response to surgical transection. Hemorrhage was controlled with gelatin sponges under pressure and the

wound closed with clips.

Laminectomy

The lumbosacral spinal cord was exposed by laminectomy in all animals. The following facts afforded initial topographical orientation: a line connecting the anterior margins of the iliac crests passes opposite the spinous process of the seventh lumbar vertebra (L7); and the spinous process of L7 is considerably larger than that of the first sacral (S1) (84). The lumbodorsal fascia was exposed by a midline skin incision extending from the spinous process of L4 to that of S3. Two incisions were then made, on either side of the spinous processes and extending the length of the skin incision, through the lumbodorsal fascia and the underlying paravertebral muscles. The tissue and spinous processes between the incisions were removed by rongeurs. The remaining muscle and ligaments were dissected from the dorsal aspect of the laminae. Muscular hemorrhage was significantly reduced by limiting the ventral extent of the spinal column exposure to the dorsal lip of the articular processes. The laminectomy was initiated at the lumbosacral articulation. This minimized the danger of damaging the underlying spinal structures since the canal to spinal cord diameter ratio is relatively large at this point and the cord segments of interest (L6 - S1) underlie the laminae of L5. The laminectomy was continued until spinal roots L5 - S3 were exposed. The opportunity of encroaching on the prominent extradural venous plexi was reduced by removing only the more dorsal portions of the laminae. When such a plexus was torn elevation of the caudal portion of the animal was found to reduce venous pressure sufficiently for hemostasis to occur. Upon completion of the laminectomy a clamp was applied to the body of the fourth lumbar vertebra and

attached to supporting rods fixed to the table top. The iliac crests were stabilized by pins and fixed to the table top in a similar fashion. The hind legs were extended and taped to supporting poles so that the final position of the animal was prone with hind limb extension. Sutures were passed through the skin flaps and tied so that a pool was formed around the exposed tissue and spinal cord. The paravertebral muscles were sutured to the skin, thus pulling the muscles away from the cord and preventing the escape of mineral oil between fascial planes. The exposed tissues were thoroughly moistened with saline and the wound covered with saline-soaked sponges or mineral oil. The dura was left intact until the remainder of the preparation was completed.

Subsequent to the peripheral nerve isolation the spinal dura was incised and reflected laterally. Ventral roots L5 to S3 were isolated on the left side and severed at their dural exit; the contralateral ventral roots were left intact. The severed, central ends of VRL7 and S1 were killed by crushing and laid upon platinum recording electrodes. The pool formed by skin flaps was filled with mineral oil warmed to 37.5°C.

The handling of spinal roots was facilitated by the prior incision of the dentate ligaments. Another procedure which further reduced trauma was the utilization of glass hooks and rods for all manipulations of spinal roots. The use of glass permitted the manufacture of any shape or size of instrument and prevented the stimulation of nerve tissue which did occur with the use of dissimilar metal instruments (88-Ch.2). In retrospect colored glass instruments would have been more useful in view of the similar refractive indices of clear glass and mineral oil.

In later experiments the rhizotomy was restricted to ventral roots L6 to S2 to re-

duce the trauma of exposure and the left side of the spinal cord was not always used, the side of choice being dependent on the state of the deep tendon and withdrawal reflexes. The limitation of the rhizotomy to VRL6-S2 was adequate to prevent antidromic volleys, evoked by peripheral stimulation of the gastrocnemius-soleus, posterior biceps-semitendinosus or ankle flexor nerves, from invading the motoneurons under study (125, 124, 79). Disadvantages of ventral rhizotomy included an interruption of the tonic spinal efferent nerve activity with a consequent alteration of tonic afferent input to the spinal cord and the severance of the ventral radicular arteries which altered the blood supply to one side of the spinal cord. However, the procedure enabled cord reflexes to be recorded monophasically, prevented reflex contraction of muscle, prevented the complications of recurrent inhibition and facilitation (118, 120, 13) secondary to peripheral muscle nerve stimulation and left intrafusal and extrafusal fibers in a constant, unstimulated state.

Peripheral Nerve Isolation

The peripheral nerves from which MSRR's were evoked included: the gastrocnemius-soleus (GS), the medial gastrocnemius (MG), the lateral gastrocnemius and soleus (LGS), the posterior biceps-semitendinosus (PBST), the medial (tibial) and lateral (common peroneal) divisions of the sciatic nerve and muscular branches of the common peroneal nerve. These nerves were identified with the aid of Jefferson's excellent paper (79). The GS nerve was isolated via a longitudinal incision that extended the length of the popliteal fossa. The PBST nerve was isolated by extending the popliteal skin incision to the ischial tuberosity, and the desired branches of the common peroneal nerve were approached from the lateral aspect of the limb. In each case a

sufficient amount of adipose and connective tissue was removed to provide adequate exposure; however, the blood supply to each nerve was judicously preserved. The GS and PBST nerves were placed on platinum hook stimulating electrodes and were protected from desiccation by suturing the skin edges of the incision to supporting poles and filling the pool so formed with mineral oil warmed to 37.5°C. Construction of a mineral oil pool was not possible when stimulating branches of the common peroneal nerve due to the position of the animal. To remedy this the stimulating electrodes were enclosed within a polyethylene tube into which the nerve was threaded. The tube was filled with isotonic saline to ensure contact and the incision was filled with white petroleum jelly and closed with wound clips.

Maintenance of Preparation

A great deal of effort was expended to maintain each animal in a stable and physiologic state throughout the preparation and experiment. This stemmed from the observation that in most instances the quality of the data was directly related to the quality of the preparation. Those preparations which did not meet the criteria to be outlined were discarded. It is understood that the evaluation of a given preparation was rather subjective and that the evaluation of the initial preparations undoubtably differed from the evaluation of later preparations when a backlog of experience had been gained. It also follows that the quality of reflex activity of the spinal cord was the major criterion for the inclusion or exclusion of any given animal.

The various maneuvers performed to maintain each preparation include those to be discussed now and those alluded to in the previous section, for example, adequate hemostasis, atraumatic handling of tissues and prevention of tissue desiccation.

Pre-experimental Care

Animals were not chosen randomly for experimentation. The only animals utilized were ones whose general condition suggested they would survive the surgical ordeal and present for experimentation in a stable state. A number of these animals were received in a semistarved and dehydrated state. In an attempt to improve their general condition, they were isolated, fed and watered from several days to a week prior to experimentation.

Blood Pressure

The method of continuous blood pressure monitoring was described in the preceding section. The information gained from this routine procedure more than off set the time and energy required to obtain it. Even though the blood supply to a given organ may or may not be reflected by the systemic blood pressure (136), a direct relationship between the mean arterial pressure and the excellence of the preparation was found. This relationship was noted in a sufficient percentage of experiments that any preparation with a mean arterial pressure of 50 mm Hg or less was discarded because the spinal cord reflexes were so unstable and highly variable that interpretation of the records was impossible. Blood pressures of this low value were most frequently encountered in the decapitate preparations. Mean arterial blood pressures of 60 to 80, 80 to 110, and 90 to 120 mm Hg characterized respectively those decapitate, anesthetized and decerebrate preparations which demonstrated active and stable cord reflexes.

Respiration

Every preparation, except 6 anesthetized and spinal animals, was respirated with either an Ensco or Bird respirator during each experimental run. The decapitate preparations were respirated continuously out of necessity; the remainder were respirated to avoid any complications of hypoventilation secondary to the blocking action of SCh or C10. The animals were placed on the respirator at least one-half hour prior to the recording period to permit equilibration. In each case the rate and depth of respiration was adjusted so that the animal took only an occasional spontaneous breath or none at all. Presumably this level of respiratory drive reflected a decreased arterial PCO_2. The dead space was reduced to a minimum and tracheal toilet was observed to prevent asphyxiation secondary to retained secretions.

The Ensco respirator which delivered room air was discarded in later experiments in favor of the Bird respirator which enabled a greater degree of respiratory control. The instrument was set to deliver 40 percent oxygen and room air or 100 percent oxygen at pressures of 8 to 12 cm of water and at rates of 20 to 30 per minute. A further advantage of the Bird respirator was its nebulizer which was kept filled with saline in order to prevent drying of the respiratory mucosa.

As a control 6 anesthetized preparations were allowed to breathe spontaneously while being exposed to subparalytic doses (50 μg/kg) of SCh. The behavior of the cord reflexes to SCh in these preparations was identical to the behavior observed in the artificially respirated animals.

Temperature

The decerebrate, decapitate and anesthetized preparations all required an external heat source to maintain body temperature. Alterations of temperature in either direction from normal, which is approximately 38.3 degrees Centigrade as measured rectally (116), influence spinal cord reflexes as well as influencing general metabolism as a whole. The magnitude of MSR, PSR, and dorsal root reflex responses were first increased and then decreased by cooling whereas overheating produced a progressive diminution in the magnitude of all of these responses (12).

In these experiments body temperature was estimated rectally and unless stated otherwise, was maintained between 35 and 39°C with a shielded heating pad. These values refer to different experiments, the drift of temperature within a given experiment was usually less than 0.5°C.

The mineral oil surrounding the spinal cord was kept at approximately 37.5°C by an incandescent heat source. The temporal drift of the mineral oil's temperature was usually less than 0.2°C/hour.

The mineral oil was added to the peripheral nerve pools at a temperature of 37.5°C and its temperature was then allowed to equilibrate with that of the room which varied between 23 to 26°C.

Fluids

Lewis suggested that tolerance to hypolvolemic stress was increased by loading the stomach with water. In 23 animals, once anesthetized but prior to surgery, warm water in doses of 10 or 15 cc/kg was introduced into the stomach with a gastric

tube. The results of this procedure were equivocal, in that some preparations were apparently benefited whereas others were not. In view of the large number of variables and the lack of a suitable index to judge its value, the procedure was discontinued.

Dextran[2] which is a high molecular weight glucose polymer was utilized in a few experiments as a plasma volume expander. Hypotension, tachycardia, and an established loss of blood constituted the indications for its use. The expander was given by intravenous drip at a rate of approximately 1 cc/minute until the mean arterial blood pressure stabilized between 80 and 100 mm Hg. In general the use of Dextran was disappointing in that upon its discontinuance the blood pressure fell. Most preparations which required Dextran to remain normotensive demonstrated small, extremely variable spinal cord reflexes and were discarded without experimentation.

Glucose (5 percent in water) was administered to a few preparations in which the experiment was prolonged greater than 12 hours to replace fluid losses. The dosage in these preparations was 50 to 80 cc administered by intravenous drip over a period of two hours.

Vesical Catheterization

It has been shown that a full bladder depresses spinal cord reflexes (28, 46). To avoid this source of depression the bladder was cannulated with polyethylene tubing and emptied in some experiments. The procedure was not difficult in the anesthetized

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2. Dextran (Cutter Laboratories) 6 percent solution in normal saline.

animal and, in addition to preventing reflex depression, provided an excellent index to the function of the cardiovascular system as was indicated by urine excretion.

Stimulation and Recording

Platinum wires (diameter of 0.5 mm) in the form of hooks were used for stimulating and recording electrodes. Platinum was chosen because it is easy to manipulate, it is relatively non-toxic, to living tissue, and it does not polarize significantly as long as small, brief currents are used (26-Ch. 36). Accumulated debris was removed from the electrodes by flaming prior to each experiment. Interelectrode distance varied between 5 and 10 mm in different experiments.

Monosynaptic and Polysynaptic Reflex Responses

The parameters and combinations of stimuli were controlled by various arrangements of Tektronix waveform (Type 162) and pulse (Type 161) generators and were designed to evoke MSRR's not polysynaptic discharge. If the stimulation provided PSRR's this was considered a bonus; however, the intensity and duration of the stimulus was not increased beyond those values required to evoke a maximal MSRR if there was no polysynaptic discharge. Stimuli consisted of rectangular pulses, 0.01 to 0.1 msec in duration, led to the muscle nerve through an isolation transformer (General Radio Co.-Type 578) at intervals of eight seconds. This stimulus frequency represents a compromise between the ideal and the practical. The eight second interval was sufficiently great so that the first MSRR did not greatly inhibit subsequent responses (81, 99) and yet provided a sufficient number of responses to deal statistically with

the inherent variability of the MSRR (74) and with the time course of the SCh induced MSRR depression (69, 52). The stimulus strength was adjusted to evoke just maximal MSRR's in every experiment. The maximal MSRR offered the following advantages: 1) the relative variation in response amplitude was reduced (74); 2) the full range of variation in response amplitude was exposed (74); 3) the response amplitude was not influenced by small changes in the stimulating current at this degree of synaptic drive (91); and 4) the behavior of MSRR's from different preparations was comparable in the sense that each motoneuronal pool was maximally activated. A theoretical disadvantage to the use of the maximal response is the inability to detect facilitation; however, Lloyd (91, 103) has demonstrated that a large subliminal fringe exists when employing single, supramaximal Group I shocks.

Reflex responses were recorded from ventral roots L7 or S1 with the proximal (active) electrode placed at least 2 cm from the spinal cord to avoid complicating electrotonic potentials. By crushing of the end of the ventral root before placing it on the reference electrode, all recordings were rendered monophasic. The responses were led via shielded leads to preamplifiers (Tektronix - Type 122) with the leads so arranged that negative potentials at the active electrode were indicated by an upward deflection on the cathode-ray oscilloscope. The band width of the preamplifiers was adjusted to give an essentially uniform response between the frequencies of 0.2 and 40,000 cps. The low frequency response corresponded to a time constant of 1.0 second. Responses were displayed on monitoring cathode-ray oscilloscopes (Tektronix - Type 360) and two additional slave cathode-ray oscilloscopes which faced the recording camera (Grass - Model C4). In most experiments the camera

was operated continuously at a film speed of 0.25 mm/sec with the camera adjusted so that the film moved at right angles to the sweep being photographed. The film speed was not sufficient to distort MSRR's recorded on fast sweeps (10 to 40 msec). At the conclusion of each experiment a time and voltage calibration, generated by an audio oscillator (Hewett Packard-Model 200 CD), was photographed.

Double shocks were used to evoke MSRR's in a number of experiments. This technique (42) served to increase the absolute amplitude of the MSRR and to reduce its relative variation in response amplitude. The time interval between the two shocks was adjusted to yield the greatest facilitation; this was usually between 0.9 and 2.0 msec. Independent intensity control of the conditioning (first) and testing (second) shocks was not possible in the earlier experiments due to an electrical interlocking of the pulse generators. The use of two sets of stimulating electrodes on the muscle nerve or the use of a mixing circuit provided independent intensity control of the conditioning and test shocks in later experiments. In these cases the conditioning shock was usually adjusted to a just subliminal value and the testing shock to a just maximal value with respect to the amplitude of the evoked MSRR.

Before leaving the subject of evoking and recording MSRR's a serious source of error must be considered. With reference to a specific motoneuronal pool, the MSRR is a valid index of motoneuronal excitability only if the effective stimulating current is confined to the stimulated nerve and does not activate adjacent nerves. For example, the effect of succinylcholine on extensor and flexor MSRR's was informative only if pure populations of extensor and flexor motoneurons were tested. Early in the course of this investigation examples of stimulus spread to nerves other than the stimulated nerve were observed. This point may be illustrated with several examples.

Stimulation of an intact muscle nerve was noted to evoke a contraction in a muscle not innervated by the stimulated nerve. Since the ventral roots had been transected the contraction could not have been reflex in origin. The contraction was not altered by crushing the stimulated nerve distal to the electrodes but was abolished by transecting the nerve at this location. Thus, the intact nerve provided an alternate path for current flow with resultant excitation of adjacent nerve fibers. A more subtle example was detected when stimulating the distally severed GS nerve with a rather wide pulse (0.5 msec). Recordings from the medial sciatic division distal to its junction with the GS nerve revealed propagated nerve action potentials. The stimulus spread was removed in this case by moving the stimulating electrodes further from the junction of the GS nerve and by reducing the pulse width. The MSRR was reduced by these manuvers which indicated that afferent fibers other than gastrocnemius-soleus contributed to its generation. In summary the following factors were found to contribute to stimulus spread: 1) stimulation of intact muscle nerves; 2) wide stimulating pulses; 3) damage to nerve fibers at the junctions of nerve trunks with resultant formation of artificial synapses (59); and 4) placement of the stimulating electrodes within 1 cm of the junction of the nerve with its parent trunk. In each experiment where localization of the effective stimulating current to one nerve was mandatory these factors were considered in the preparation of the animal and the presence of stimulus spread ruled out by examining adjacent nerve trunks for the presence of propagated action potentials. When the medial or lateral divisions of the sciatic nerve were stimulated it was not feasible to transect the stimulated nerves because this would have removed a large source of necessary afferent input. Spread in these instances was avoided by placing the flexor electrodes on the lateral aspect

of the limb far from the extensor division and encasing the electrodes within polyethylene tubing. The tubing was filled with saline which shunted a sufficient amount of the current to the surrounding tissue so that the nerve fibers in the medial division were not activated.

Cord Dorsum Response

The cord dorsum response was utilized in some experiments as an estimate of the arrival time of the evoked afferent volley at the spinal cord. This was accomplished by placing a platinum electrode on the dorso-lateral sulcus of the desired spinal segment (L7 or S1). The active electrode consisted of platinum wire with the end fashioned into a 1 mm ball. The reference electrode was fixed to a metal clamp attached to the spinal column and the responses were recorded by the method described for the MSRR.

Indirect Muscle Twitch

The time course of the drug-induced MSRR depression was compared with the time course and extent of muscle twitch depression in some experiments. Submaximal twitches were evoked by single shock stimulation of the GS nerve and were recorded isometrically from the GS tendon. In addition to the usual fixation, these preparations were further immobilized by pinning the distal ends of the femur and tibia of the ipsilateral hind limb and fixing the pins to supporting rods. The tendon of the GS muscle was isolated and attached with piano wire to a strain gauge transducer (Grass) with the muscle placed under a resting tension of 100 to 200 gms. In initial experi-

ments the twitch and MSRR were evoked simultaneously using a single pair of stimulating electrodes placed on the intact GS nerve (this method is schematically illustrated in Figure 1-A). With this arrangement the magnitude of the twitch was dependent on the stimulus strength required to evoke a maximal MSRR. However, once the problems associated with stimulus spread became manifest this technique was discarded. The GS nerve was transected and electrodes fitted to each end; one pair to evoke the twitch and the other to evoke the MSRR. At the conclusion of each experiment the system was calibrated with metal weights.

Experimental Drugs

Solutions of succinylcholine chloride (SCh)[3] or decamethonium bromide (C10)[4] were diluted before each experiment with 0.9 percent sodium chloride solution to a final concentration of 200 μg/cc for SCh and 100 μg/cc for C10. In most experiments doses of 50 to 200 μg/kg were used for both drugs and unless stated otherwise, the dosage was 50 μg/kg.

In all but two experiments the desired blocking agent was administered into the left cephalic (superficial forelimb) vein through an indwelling polyethylene cannula. In the two exceptions SCh was administered intrarterially as described elsewhere.

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3. SCh (Sucostrin Chloride-Squibb) was obtained in multiple dose vials (20 mg/cc) with NaCl added for isotonicity and containing 0.1 % methylparaben and 0.01 % propylparaben as preservatives. The drug was refrigerated until the day of use.

4. C10 (Syncurine-Burroughs Wellcome) was obtained as an isotonic solution in multiple dose vials (1 mg/cc) and stored at room temperature.

The drug injection in the majority of experiments was preceded by an equal volume injection of saline. The blocking agent was injected over a time course of approximately 10 seconds and was followed by a 1 cc flushing dose of saline. A four minute recording of the desired response was obtained in each experiment before the saline was injected and then another four minute recording was obtained before the blocking agent was injected. The recording was continued until the control value of the response was reestablished following drug administration. This ideal was accomplished in the majority of instances. The four minute recording period before and between the two injections was based on preliminary experiments which indicated that this time interval was sufficient to detect any trends in the control responses and to provide a sufficient number of scores to deal with the responses variation. In addition the responses were evoked and observed for 10 minutes or longer prior to the recording period to ensure steady state conditions.

B. OTHER PROCEDURES AND TECHNIQUES

Unit and Dorsal and Ventral Root Nerve Activity

The method of Adrian and Bronk (1), with several modifications, was employed here to study the unit activity of selected dorsal and ventral root nerve fibers. The dissections were carried out under mineral oil with the aid of a low-power binocular microscope. The electrical activity of the nerve fibers was monitored aurally as well as visually during the dissection and experiment by leading the preamplifier output to an audio amplifier and speaker system and to a cathode-ray oscilloscope. The central end of a ventral root was divided into its naturally occurring filaments

by grasping the severed end with two pairs of jeweler's forceps and retracting laterally. Having obtained several filaments, each was tested for the presence of a monosynaptically evoked response by laying the filament on recording electrodes and stimulating the desired muscle nerve. Recordings were rendered monophasic by placing the crushed end of the filament on the reference electrode. An active filament was further subdivided until it contained only a single active fiber. If the latency of the spike was comparable to that of the population MSRR being recorded simultaneously, the fiber was accepted as being monosynaptically activated. A spike was considered to represent the action potential of a single fiber if it was all or none relative to stimulus intensity and was constant temporally with respect to amplitude, waveform and duration.

The same dissection technique was used to isolate single dorsal root fibers. Only tonically active fibers were studied. A dorsal root filament was subdivided until the repetitive discharge of a single fiber could be distinguished. Constant amplitude and regularity of discharge interval ensured that a single unit was being dealt with. The method employed did not determine the fiber's receptor type; however, by manipulation of the limb it was sometimes possible to localize the receptor to a given muscle. The conduction velocity could then be calculated from the latency of the spike and the distance between the stimulating and recording electrodes. The diameter of the single fiber was obtained by dividing the conduction velocity by six (76).

The dissection technique employed did not isolate anatomically a single fiber. In most instances the filament could be seen with the unaided eye, implying the presence of several nerve fibers, of which only one responded to the stimulus. It

follows that isolation of monosynaptically evoked spikes was accomplished more readily in those preparations with little spontaneous ventral root discharge, e.g., anesthetized and spinal.

The major difficulty encountered with this unit recording technique was the small signal to noise ratio. With each anatomical subdivision the amount of inactive tissue and fluid surrounding the active fibers was reduced and the interelectrode resistance correspondingly increased. A number of manuvers served to improve the signal to noise ratio. These included a reduction of inter-electrode distance to 3 to 4 mm; an improvement of the contact between the active fiber and the electrode with a drop of saline; the use of grounded aluminum shielding around the spinal cord and electrodes; and a reduction in the high frequency response of the preamplifier to the value that provided the best signal to noise ratio.

The evoked unit potentials (e.g., MSRR) were recorded as was the population MSRR. Three methods were employed to obtain permanent records of spontaneous unit nerve activity. 1) The spikes were photographed on slow sweeps (100-250 msec) every two seconds so that only a fraction of the total activity was recorded. 2) The input to the horizontal deflection plates of the recording cathode-ray oscilloscope was removed, and the camera was run at continuous, high speeds to provide the time base. This method recorded all unit activity in contrast to the above. 3) The input to the monitoring cathode-ray oscilloscope was led to a two channel tape recorder (*Magnecord*, Type 728-4). The recordings were then played back at leisure following the experiment and the desired portions photographed on moving film for quantitative analysis.

Detection of Excitability Changes In the Intramedullary Portion of Large Afferent Axons

Wall (138) has perfected a simple, ingenious method of detecting excitability changes in the intramedullary portion of afferent axons. Intramedullary stimulation of large afferent fibers evokes an antidromic response in dorsal root and afferent peripheral nerve fibers and an orthodromic response in fibers comprising the dorsal columns. Excitability changes are inferred from changes in the area of the recorded antidromic response.

In this study, the antidromic response was recorded with platinum wire hooks from the medial or lateral division of the GS nerve, or the central end of a dorsal root filament. The stimulating microelectrode (cathode) consisted of 10 micron platinum wire insulated with glass. The anode was formed by the multiple metal table appliances in contact with the animal. This circuit is schematically illustrated in Figure 14-A.

Microelectrode Construction

The microelectrodes were constructed from silver-coated, 10 micron platinum wire. Boiling, concentrated nitric acid was used to remove the silver from the central one-third of 12 inch lengths of Wollaston wire. The wire was threaded into a 6 inch glass capillary tube (outside diameter of 0.8 mm) and arranged so that the bared platinum was centrally located and provided with sufficient slack to prevent its breakage during the pulling process. With the wire in place, the glass tube was pulled into two microelectrodes with a standard micro-pipette puller. The wire and

glass were permanently united by sealing wax applied to the large end of the glass tube. The extension of the glass tip beyond the wire was removed by touching it to the finger. Such construction consistently yielded electrodes with outside tip diameters of 15 microns and shaft diameters of 40 to 90 microns, 2 mm above the electrode tip. Each electrode was checked by placing the tip in a saline bath, applying a 3 volt potential across the electrode, and microscopically observing bubble formation. The presence of bubbles at the tip indicated the ability of the electrode to conduct a current, whereas bubble formation along the shaft signified faulty insulation. The resistance of the electrodes, measured in saline with a dc current, was approximately 100,000 ohms. Values of approximately 100 ohms were obtained when the resistance was measured in mercury. These electrodes were convenient to manufacture and a yield of greater than 50 percent was obtained with little practice. Pial dissection was not a prerequisite to intramedullary placement of these electrodes.

Spinal Column Immobilization

The use of intramedullary microelectrodes necessitated complete immobilization of the lumbo-sacral spinal column to prevent movement artifact. This was achieved by: 1) bilateral pinning of the iliac crests and the body of the 3rd or 4th lumbar vertebra; 2) rigid clamping of the 3rd or 4th lumbar spinous process; and 3) pinning the body of the 5th lumbar vertebra from below on one side and above on the other to prevent movement in the vertical plane. A bilateral thoracotomy, elevation of the spinal cord with dural slings, and fixation of the animal so the thorax rested lightly on the table minimized movements associated with respiration. The prepar-

ation was maintained on a Bird respirator set to deliver low tidal volumes at a rapid rate (100 % O_2 at pressures of 5-8 cm of H_2O and frequencies of 35 to 40/min). Paravertebral muscle fasciculation was found to induce movement of the mineral oil surrounding the spinal cord. This source of movement artifact was diminished by the partial removal of these muscles. The remaining muscles were pulled laterally and firmly sutured to supporting posts. During the course of each drug experiment the spinal cord and microelectrode were observed with a dissecting microscope.

Intramedullary Penetration and Stimulus Characteristics

The microelectrode was introduced into the spinal substance parallel to those afferent fibers proceeding to the ventral horn (see Fig. 14-A). Penetration was initiated 1 mm lateral to the midline of the desired spinal segment, usually L7, and proceeded ventro-laterally approximately 15° from the vertical axis. At 0.5 mm steps throughout the downward penetration an antidromic response was evoked and subjected to a standard posterior biceps-semitendinous conditioning volley (32). The antidromic response which exhibited the greatest facilitation to this conditioning volley was utilized for the experiment. It was possible to make several penetrations in the same preparation without observing untoward MSRR depression. The route of this penetration undoubtably damaged a percentage of the fibers under study. However, a higher yield of sensitive antidromic responses were found when the microelectrode paralleled the large afferent axons instead of approaching the fibers at right angles as from the contralateral side of the cord or from the ipsilateral side with the penetration initiated just above the dentate ligament.

Rectangular pulses, 0.1 to 0.2 msec in duration, were led to the preparation through an isolation transformer at 8 second intervals. These relatively wide pulse durations were required to overcome the increased threshold consequent to the diminished diameter of the intramedullary fibers. The stimulating current was adjusted to values of 1.5 to 4 times that required to evoke a threshold response. A second pulse of equal amplitude but opposite polarity was delivered to the preparation approximately 10 msec following the stimulating pulse. In theory, the second pulse would counteract the electrode polarization induced by the stimulating pulse. The stimulating current was monitored in five experiments by measuring the IR drop across a fixed resistance (set between 30 and 5000 ohms) with a differential amplifier and cathode-ray oscilloscope.

Anatomical Controls

The position of the microelectrode tip was marked by coagulation of adjacent tissue in eight experiments. Coagulation was accomplished by connecting a 1.5 volt dry cell to the preparation for 20 to 40 seconds (cathode to microelectrode and anode to spinal column clamp). At the completion of the experiment the thoracic aorta was cannulated and 50 to 100 cc of 10 percent formalin infused by hand pressure. The spinal segment was removed from the preparation and allowed to fix in the same formalin solution for several days before freezing and sectioning. The 60 or 90 micron frozen sections were mounted in glycerin between glass slides (63, 47) and photographed at 10 to 20 times magnification directly on photographic paper (Kodabromide, F-4).

C. ANALYSIS OF DATA

Monosynaptic Reflex Responses

All measured responses were dealt with as will be described for the MSRR and for this reason only the MSRR will be considered in detail. The photographed responses were magnified approximately seven times with a projector and were measured to the nearest millimeter. The latency of the MSRR was measured from the beginning of the stimulus artifact to the beginning of the first upward deflection; the duration from the first upward deflection to where the best fitting line applied to the descending deflection crossed the baseline; and the amplitude from the baseline to the point of maximum negativity. With regard to the amplitude measurements, the accuracy and precision of measurement for three different persons was greater than 98 percent. The precision of measurement dropped to 93 percent with small or depressed MSRR's. In view of the percent change in amplitude which followed drug administration this degree of error was negligible.

The raw amplitude measurements were converted to percent values relative to the mean amplitude of all scores which preceded the saline injection which was designated as 100 percent. The percent amplitudes of the MSRR's were plotted graphically as a function of time. In most graphic descriptions only the mean percent amplitude of five consecutive responses was indicated, instead of plotting the amplitude of each score. As demonstrated by Figures 3 and 4 this method of presentation does not mask the variability of MSRR amplitude.

In some experiments, the statistical significance of the MSRR depression was

proven with Student's t test. The amplitudes of those MSRR's evoked between 40 and 160 seconds subsequent to the SCh injection were compared to the similar group of MSRR's which followed the saline injection ($n = 15$; $df = 14$).

Polysynaptic Reflex Responses

The PSRR does not lend itself to amplitude measurements, contrary to the belief of some (6), due to the asynchronous variable nature of the responding units. Area measurements of the PSRR were not undertaken in this study but rather each record was inspected and an overall impression formed. This impression was then confirmed by the quantitative data obtained from unit PSRR studies.

Unit Nerve Activity

Evoked unitary reflex responses (monosynaptic or polysynaptic) are a manifestation of the tested motoneuron's excitability at that moment (61, 103). This statement assumes a constant presynaptic drive and will be modified by the forthcoming results.

The excitability of the tested motoneuron was expressed by a firing index (103) which is the probability of a discharge occurring in response to a constant afferent nerve volley. The firing index utilized here equaled the number of times the motoneuron responded to five afferent nerve volleys delivered at eight second intervals ($FI = \frac{\text{No. of responses}}{5}$). A maximal firing index ($FI = 1.0$) indicated the motoneuron was discharged by every afferent volley and implied a high level of motoneuronal excitability. Intermediate firing indices (FI less than 1.0 but 0.2 or greater) were

indicative of motoneurons with relatively greater thresholds. It follows that the observance of facilitation was dependent on the use of motoneurons with intermediate firing indices.

The spontaneous unit activity (dorsal or ventral root) was dealt with by calculating the number of discharges per second and by plotting the mean value for 40 second intervals as a function of time.

RESULTS

These data were obtained from 75 preparations, either decerebrate, decapitate or anesthetized. Those preparations which did not meet the criteria outlined in the METHODS were excluded. The three types of preparations yielded results which were so similar as to obviate the need for further differentiation except under special circumstances which will be noted below.

The study to be described was based upon the changes induced in the monosynaptic reflex response (MSRR) recorded electrically from cut ventral roots. The design of the study involved the elicitation of the MSRR using artificial, non-physiological forms of excitation. It therefore was appropriate to examine this response in considerable detail in order to define the limits of its utility and of its suitability for the purposes intended. Recognition and identification of the monosynaptic reflex elements of an evoked ventral root discharge were based on the criteria of configuration, threshold, latency and duration.

A. MONOSYNAPTIC REFLEX RESPONSES

Characteristics of Electrical Records

Configuration

Examples of MSRR's, generated by single and double shock stimulation, are illustrated in Figure 1. Record A shows a maximal reflex response that was evoked by single shock stimulation of the medial division (extensor nerve) of the sciatic nerve. The response was an abrupt, negative-going deflection which began 3.0

FIGURE 1

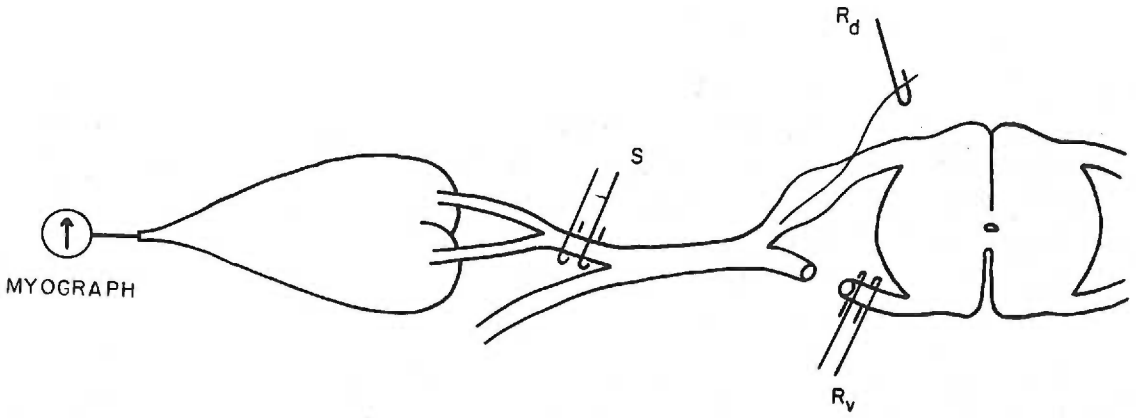
Upper: A schematic illustration of the basic experimental arrangement. Stimulating electrodes (S) were fitted to the gastrocnemius-soleus nerve and the evoked reflex discharges were recorded from the appropriate, distally severed ventral root (Rv). In most experiments the stimulated muscle nerve was severed in contrast to what is illustrated. The evoked afferent volley or spontaneous unit afferent activity was monitored from small dorsal root filaments (Rd). Variations of this basic design will be pointed out as the occasion arises.

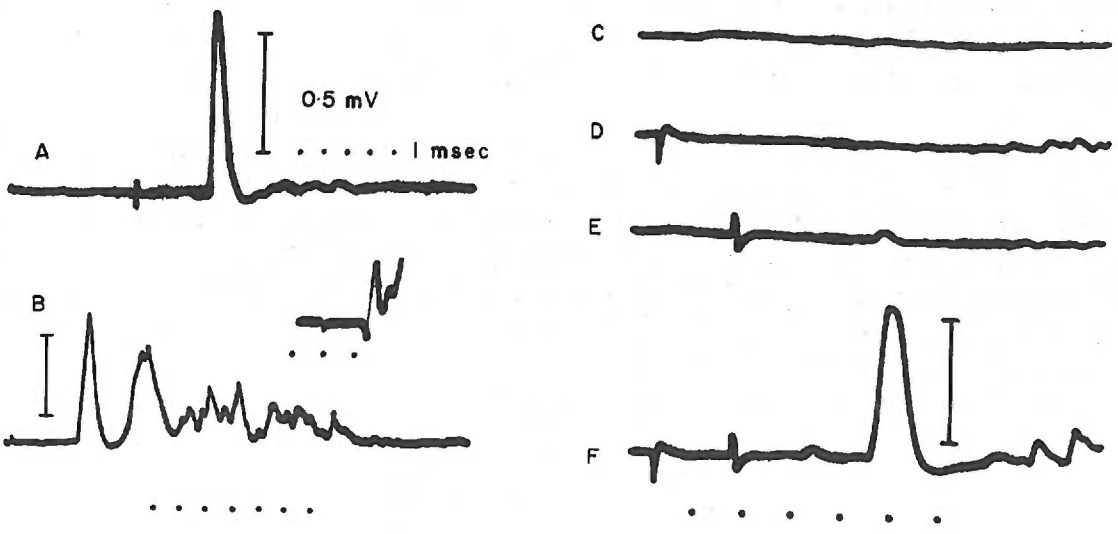
Lower: Examples of monosynaptic reflex responses (MSRR's) elicited by single and double shock stimulation. In this, and all subsequent figures, an upward deflection denotes negativity at the active electrode with respect to the reference electrode. Calibrations for this figure: 1.0 msec; 0.5 mV.

A. MSRR which was recorded from VRS1 and was evoked by single shock stimulation of the medial division of the sciatic nerve in a decerebrate, spinal preparation.

B. A reflex discharge that was recorded from VRS1 and was evoked by single shock stimulation of the lateral sciatic division in another decerebrate, spinal preparation. The initial portion of the cord dorsum response (inset) was recorded simultaneously with a unipolar electrode placed on the dorso-lateral sulcus at the entrance of DR-L7. The reference electrode consisted of a metal clamp that was fixed to the spinal column. This record was retouched to prevent losses in reproduction.

C-F. In this anesthetized, spinal preparation the reflex discharges were evoked by stimulation of the severed gastrocnemius-soleus nerve and were recorded from VRS1. Record C is the baseline that the subsequent responses were imposed upon. The conditioning shock (record D) and the testing shock (record E) failed to evoke a usable MSRR when delivered separately, but produced the MSRR in record F when delivered at a stimulus interval of 1.6 msec. The small negative deflection just preceding the large MSRR in record F is the MSRR generated by the conditioning volley. A similar response to the test volley is apparent in record E.


R_d
S
MYOGRAPH
R_v


0·5 mV
A
1 msec
B
C
D
E
F

msec after the stimulus artifact and persisted for 1.2 msec. The brief latency and synchronous appearance of this response characterized it as having been produced by activation of a reflex pathway containing only one synapse (93). The monosynaptic discharge was followed by small baseline irregularities which were superimposed on a persisting, low amplitude, negative potential. The baseline irregularities are indicative of minimal, polysynaptic reflex discharge. The persistent, low amplitude negativity seen in this record and in Figure 15 (record B) was only an occasional finding. Since it appeared to be related to the distance between the spinal cord and the proximal recording electrode, it has been ascribed to the electrotonically conducted post-synaptic potential (5).

In contrast to the ventral root discharge shown in record A, the flexor MSRR illustrated in record B was followed by an intense polysynaptically conducted reflex discharge. Polysynaptic reflex responses (PSRR's) of this magnitude were usually observed with stimulation of mixed flexor nerves and presumably resulted from the wider stimulating pulses that were required to evoke flexor MSRR's.

Threshold

Lloyd (93) demonstrated that the MSRR was evoked by stimulation of the low threshold, fast conducting afferent fibers of muscle nerves. On this basis, one would expect MSRR's to exhibit lower thresholds than other varieties of reflex activity. This was found to be true when stimulating extensor or flexor muscle nerves in that maximal MSRR's could sometimes be obtained with little or no polysynaptic reflex discharge (Figs. 1-F, 2). However, the difference in threshold of monosynaptic and polysynaptic reflex responses was not always so marked when the responses were

evoked by stimulation of mixed flexor nerves. For example, the thresholds of the MSRR and PSRR shown in record B (Fig. 1) were essentially identical and both responses increased in amplitude together as the stimulus intensity was increased from zero.

The lowest threshold extensor MSRR's were consistently found in the decerebrate preparation as would be predicted from the augmented excitability of extensor motoneurons which characterizes this preparation (126). In contrast flexor MSRR's evoked from either muscle or mixed nerves, were most easily elicited following spinalization.

Latency

The events which contribute to the latency of the MSRR are defined in Figure 1 (record B). The 2.5 msec latency of this MSRR represents the time that was required for stimulus utilization, afferent and efferent conduction and central delay. The utilization and efferent conduction times can be estimated from the latency of the simultaneously recorded cord dorsum response of which the initial portion, the triphasic spike (inset), was a manifestation of activity in Group I fibers in the dorsal column (53, 71, 6, 102, 55). Specifically, the initial positivity of the spike signaled the approaching afferent volley; the negativity indicated the passage of the volley under the recording electrode; and the ensuing positivity signified that the volley has passed the electrode and was moving away. The reversal from initial positivity to negativity was taken to indicate the arrival of the afferent volley at the spinal cord. At best, this endpoint was only an approximation since the potential changes do not reflect the cessation of one process and the beginning

of another with absolute accuracy (106). Be that as it may, the latency of the triphasic spike measures 1.4 msec of which 1.3 msec was required for afferent conduction to the spinal cord with a 0.1 msec allowance for stimulus utilization (86). Efferent conduction from the spinal cord to the proximal recording electrode required an additional 0.2 to 0.3 msec on the basis of the conduction distance and diameters of ventral root axons (40). The 2.5 msec latency of the MSRR minus the above times provides a central delay -- synaptic delay plus intramedullary conduction -- of 0.8 to 0.9 msec. Central delays of this magnitude are accepted as indicative of a monosynaptic pathway (117).

The mean latency of 29 MSRR's evoked by stimulation of the gastrocnemius-soleus nerve and recorded from a distally severed ventral root (VRL7 or S1), was 2.9 msec with a range of 2.2 to 3.7 msec. This variation in latency from animal to animal was accounted for in part by different conduction distances; however, on occasion, central delays with values as high as 1.4 msec were observed. Prolonged central delays of this order did not eliminate the possibility of monosynaptic responses because the upper limit for monosynaptic delay has not been established (117) and such responses fulfilled other characteristics of extensor MSRR's: post tetanic potentiation (97); response depression by Group I conditioning volleys in flexor nerves (95); and in retrospect, response depression by the intravenous administration of SCh. It was concluded that ventral root responses evoked by stimulation of the gastrocnemius-soleus nerve can demonstrate latencies of nearly 4 msec and yet be monosynaptic in nature.

Duration

The durations of the MSRR's in Figure 1 (records A, B and F) measure 1.2, 1.1 and 1.0 msec. The mean duration of 10 other MSRR's evoked from the GS nerve equaled 1.1 msec with a range of 0.63 to 1.4 msec. Inview of the 0.5 to 0.6 msec duration of a single Group A spike (54, 49) it follows that these reflex discharges were dispersed temporally. The temporal dispersion of the MSRR is adequately accounted for by temporal dispersion of the afferent volley (92), by variations in synaptic delay due to differing degrees of spatial summation (29), and to a lesser degree, by temporal dispersion of the efferent volley within the ventral root itself. In those experiments which involved the use of SCh or C10 the actual duration of the MSRR was of no concern as long as it remained constant throughout the experiment. However, alterations in the duration were of importance. For example, an increased duration would manifest itself as depression of response amplitude even though the total number of units contributing to the response remained constant.

Double Shock Test System

A MSRR evoked by the double shock technique (42) is illustrated in record F (Fig. 1). In this anesthetized preparation, the application of either the conditioning (record D) or the testing shock (record E) in isolation failed to evoke a usable MSRR. However, when the two shocks were delivered in combination at the proper stimulus interval, (1.6 msec for this preparation) a marked facilitation of the MSRR resulted (record F). This is but another example of temporal facilitation. The value of double shock test system was threefold: 1) the percentage of usable pre-

parations was increased as is exemplified in Figure 1 (record F); 2) the relative variation and in some cases the absolute variation of response amplitude was reduced (80, 74); and 3) the absolute amplitude of the response was increased, thereby increasing the size of the discharge zone at the expense of the subliminal fringe (9, 94, 74, 103). An enlarged discharge zone more nearly approximates the total population of motoneurons in a motoneuronal pool and thus, a given degree of depression of the MSRR assumes more significance.

Size of the Monosynaptic Discharge Zone

The MSRR was employed in this study as an indirect measure of motoneuronal excitability. As such, it would be worthwhile to know what percentage of the motoneuronal pool was discharged monosynaptically. For example, a complete depression of the MSRR which comprised only 30 percent of the total pool would have less meaning than a complete depression of the MSRR which represented the entire population of motoneurons. In the former instance, the depressive agent's effect on the excitability of 70 percent of the motoneurons would have gone undetected.

Post-tetanic potentiation (97) has been suggested (80, 69) as a means of estimating the size of the monosynaptic discharge zone relative to the size of the motoneuronal pool; the rationale of this test being that the maximally potentiated MSRR represents discharge from the entire motoneuronal pool (80, 69). A number of MSRR's were subjected to tetanic stimulation in this study and the two extremes of observed behavior are shown in Figure 2. Records A and B describe a very large, maximal MSRR which was evoked by single shock stimulation and which potentiated only 100 percent following tetanic stimulation. In view of other experiments, this degree of

FIGURE 2

A characterization of MSRR's by tetanic stimulation and an estimate of the size of the monosynaptic discharge zone. The data were obtained from decerebrate preparations, and in each case, the maximal MSRR's were elicited at 8 second intervals from the distally severed gastrocnemius-soleus nerve. Following approximately 20 control MSRR's, the gastrocnemius nerve was stimulated tetanically (500/sec) for 10 seconds by stimuli which were greatly supramaximal for Group I fibers.

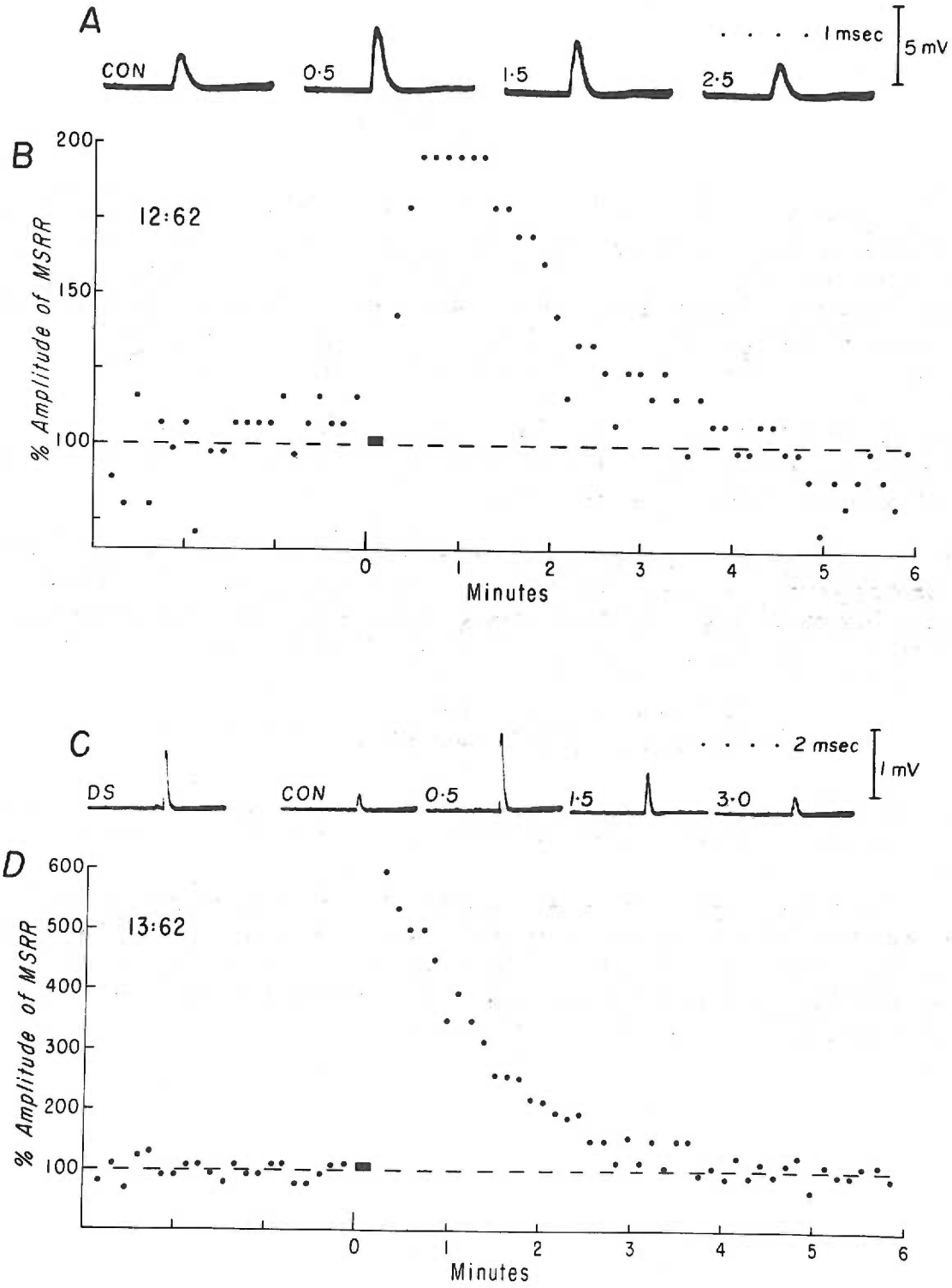
A. Maximal MSRR's before and after tetanic stimulation. The time that each response followed the onset of tetanic stimulation is indicated in minutes. (Con: control MSRR preceding tetanic stimulation)

B. Plot of response amplitude describing the time course of post-tetanic potentiation for the series of responses partly illustrated in record A. The points in the graph express the amplitude of each MSRR in percent and relative to the mean amplitude of all MSRR's that preceded the tetanic stimulation, i.e.,

$$\frac{\text{amplitude of individual MSRR} \times 100}{\text{mean amplitude of control MSRR's}} = \% \text{ amplitude}$$

C. Maximal MSRR's from another decerebrate preparation. Identical to record A with the exception of the MSRR evoked by double shock (DS) stimulation.

D. The time course of potentiation is graphed for the responses partially illustrated in record C. The temporal variation in the amplitude of the control MSRR's should be noted in each graph. The temporal variation of motoneuronal excitability and thus MSRR amplitude must be considered when assaying the significance of a given percentage change in MSRR amplitude.


A
CON
0·5
1·5
2·5
1 msec
5 mV
B
12:62
% Amplitude of MSRR
200
150
100
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2
3
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6
Minutes
C
DS
CON
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3·0
2 msec
1 mV
D
13:62
% Amplitude of MSRR
600
500
400
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200
100
0
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5
6
Minutes

potentiation was quite minimal and in terms of discharge zone size indicates that 50 percent of the motoneuronal pool was discharged by single shock stimulation. This preparation was quite unusual in that double shock stimulation did not augment the amplitude of the single shock MSRR. Record C demonstrates a small but maximal MSRR of another preparation which was quite susceptible to double shock and to tetanic stimulation. The single shock MSRR potentiated approximately 6 times (record D), which indicates that single shock stimulation discharged less than 20 percent of the available motoneurons. In contrast, double shock stimulation evoked a MSRR (record C - DS) which represented approximately 70 percent of the available motoneurons and thus, was of considerable value in this preparation.

The basic assumption underlying the post-tetanic potentiation test of discharge zone size is that all discharged motoneurons are destined for the muscle whose afferent fibers were stimulated. However, it has been demonstrated (7) that tetanic stimulation discharges other motoneurons as well. Thus, the above estimates of discharge zone size are in error. The actual percentage of the motoneuronal pool discharged monosynaptically would be greater than the above figures indicate.

Eccles and Lundberg (42) have observed that MSRR's evoked by double shock stimulation often failed to potentiate with tetanic stimulation. In this study the maximal MSRR that was elicited by double shocks in eight consecutive preparations always potentiated when subjected to tetanic stimulation at rates of 500/sec for 10 seconds. The reason for this descrepancy in results was not apparent.

On the basis of the considerations outlined above, it was concluded that it was possible to recognize and utilize the electrical signs of activity in monosynaptic

reflex area for further study of the effects of certain pharmacologic agents on this activity.

B. ACTIONS OF INTRAVENOUS SUCCINYLCHOLINE AND DECAMETHONIUM

Depression of Extensor Monosynaptic Reflex Responses

Succinylcholine

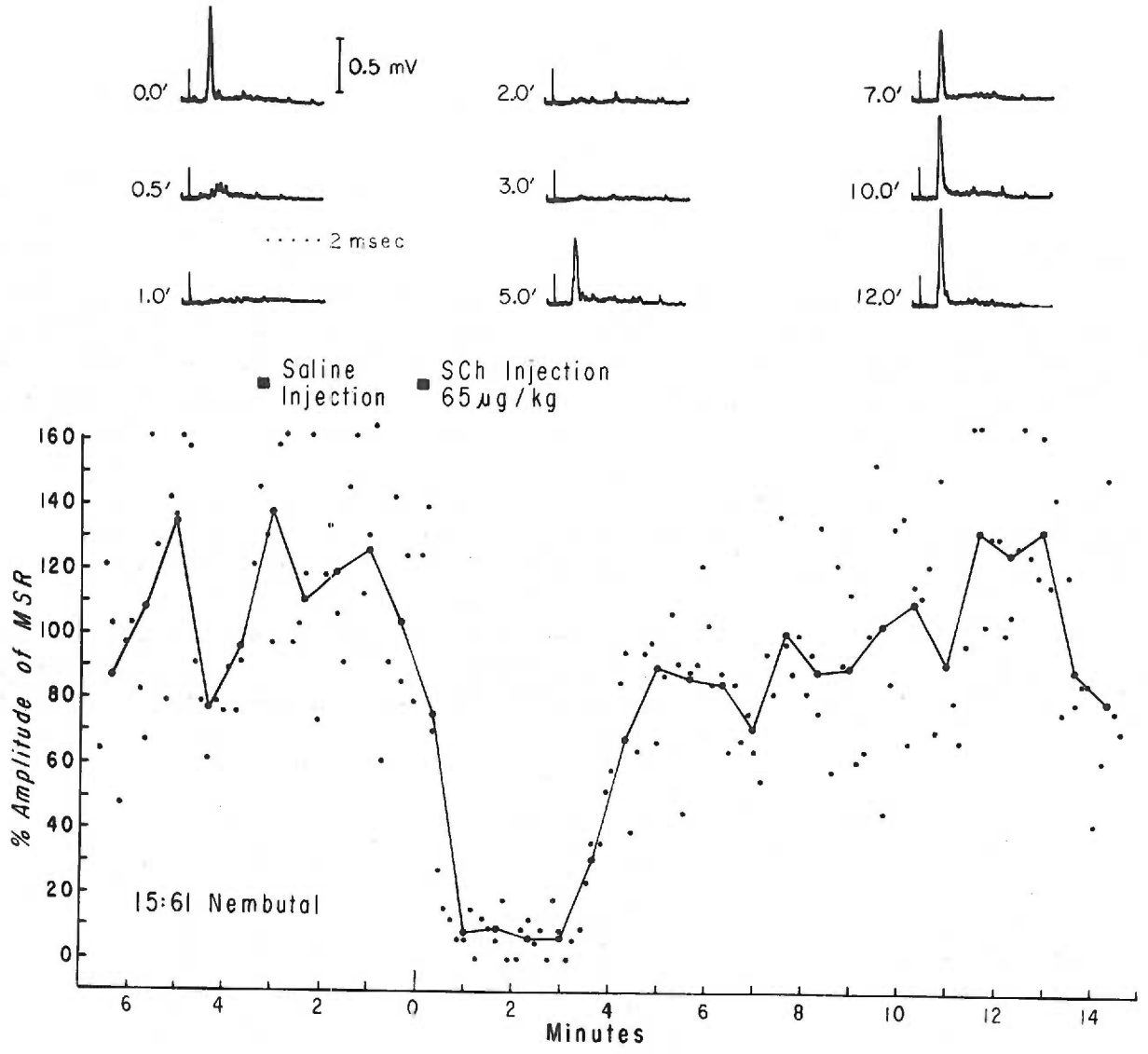
The intravenous administration of SCh is known to produce a transient depression of the cat's extensor MSRR (68, 52). The time course of this reflex depression is illustrated by data from a preparation (Fig. 3) in which the control MSRR's exhibited an unusually large variation in amplitude. In this preparation the intravenous administration of SCh (65 μg/kg) evoked a significant MSRR depression which was apparent within 30 seconds. The maximal reflex depression occurred slightly before 1 minute and persisted for another 2 minutes before the recovery process began. The amplitude of the response did not regain its former magnitude until approximately 12 minutes following the drug injection. The mean amplitude of the MSRR's before and after SCh equaled 107 and 8 percent respectively. The difference between the mean amplitudes is highly significant ($P<0.001$; $N=15$) in spite of the large amplitude variation exhibited by this preparation. The data in Figure 3 also illustrates that the equal volume injection of saline had no comparable effect on the amplitude of the extensor MSRR, and that a small, but definite, augmentation of the polysynaptic reflex response followed the administration of SCh (record 0.5). The time course of reflex depression exhibited by this preparation was somewhat atypical with regard to the recovery curve. More frequently, the recovery process was

FIGURE 3

Extensor MSRR's: range of variation in response amplitude and the response to succinylcholine (SCh). Single shocks were delivered to the intact gastrocnemius-soleus nerve at 8 second intervals and the maximal MSRR's were recorded from VRS1 of an anesthetized preparation.

Upper: Records of the ventral root discharges. To the left of each sweep is the time, in minutes, that the response followed the onset of the SCh injection. The transient and nearly complete depression of the MSRR is apparent.

Lower: Percent amplitude of the MSRR expressed as a function of time. The data were obtained from responses partially illustrated in the above record. The amplitude of each MSRR was expressed in percent relative to the mean of its control responses as were the responses in Figure 2. A mean curve was superimposed upon the individual scores; each point of the curve represents the average amplitude of 5 consecutive responses. The onset and duration of the saline and drug injections are indicated by their respective blocks.


0.5 mV
0.0′
0.5′
1.0′
2.0′
3.0′
5.0′
7.0′
10.0′
12.0′
2 msec
Saline Injection
SCh Injection 65 µg/kg
% Amplitude of MSR
15:61 Nembutal
Minutes

observed to be complete 6 to 9 minutes following 50 to 200 μg/kg doses of SCh (Figs. 4 and 5).

A MSRR which demonstrated a minimal variation in response amplitude and a complete reflex depression following a small dose of SCh is illustrated in Figure 4. The difference between the temporal variation in motoneuronal excitability exhibited by this preparation and that shown in Figure 3 can be accounted for by differences in the experimental preparations. Both preparations were anesthetized with comparable doses of sodium pentobarbital; however, the preparation which is shown in Figure 4 was subjected to spinal transection and the stimulated muscle nerve was severed. Both transection procedures decreased the amount of spontaneous nerve activity acting on the motoneurons under question and hence the absolute fluctuations in motoneuronal excitability were reduced. In addition the MSRR which demonstrated the lesser variation was evoked by double shocks. The enlarged discharge zone which resulted would, in view of Hunt's observation (74), decrease the relative variation in response amplitude.

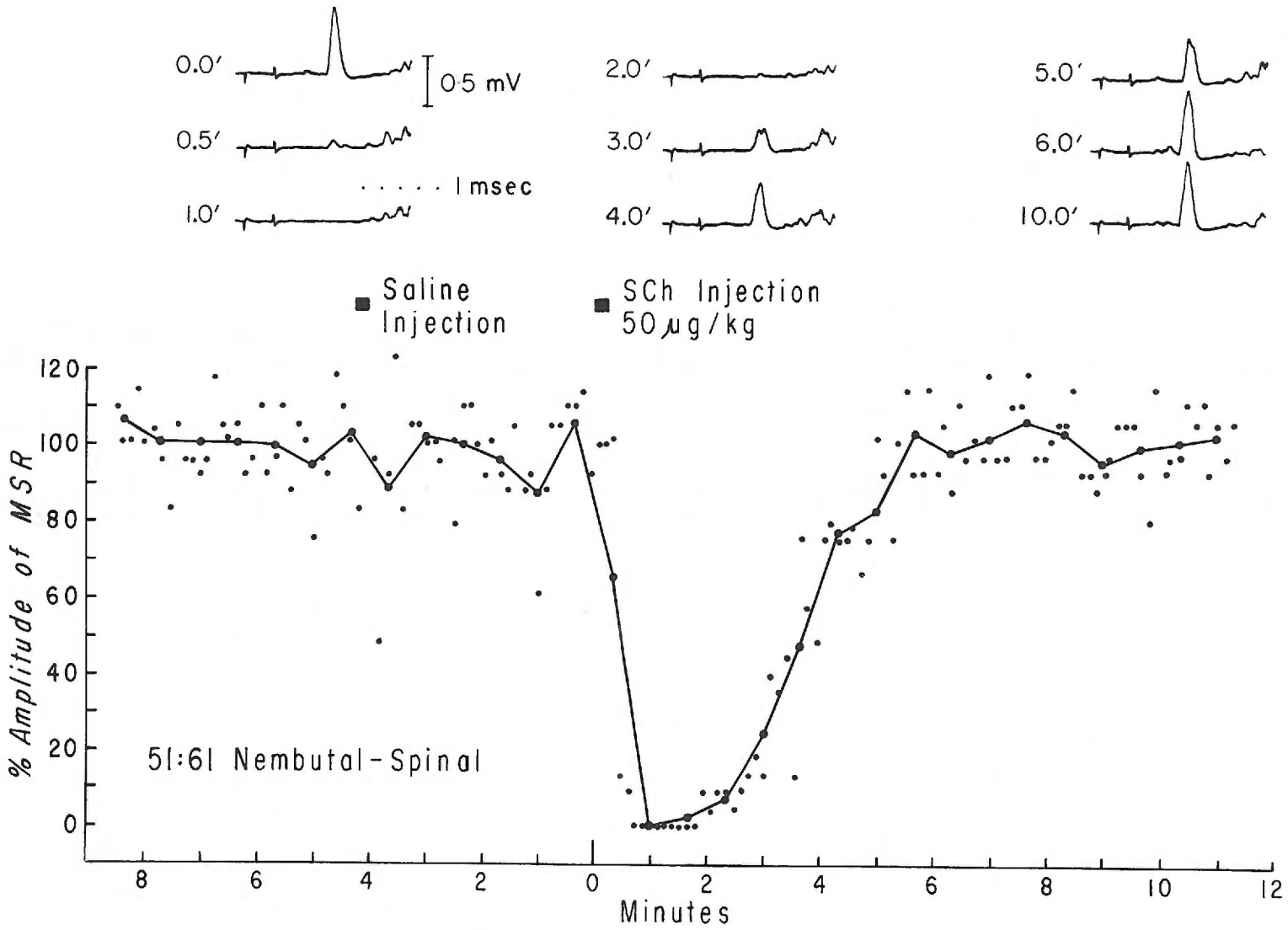
The time course of the SCh-induced MSRR depression is better appreciated from Figure 5 in which the data from 24 experiments are summarized graphically. Following the drug administration, the maximal reflex depression was manifest within 1 to 2 minutes. The recovery process began within 2 to 4 minutes after SCh injection and was essentially complete 6 to 8 minutes later. This time course of reflex depression is in general agreement with the illustrated observations of others (52, 51, 66). The data also illustrates that the reflex depression occurred regardless of the preparation type (decerebrate, decapitate or anesthetized) and regardless of the relative threshold of the MSRR; i.e., the low threshold of the MSRR in the decere-

FIGURE 4

Extensor MSRR's: range of variation in response amplitude and the response to SCh. Double shocks were delivered to the distally severed gastrocnemius-soleus nerve and the maximal MSRR's were recorded from VRS1.

Upper: Ventral root discharges.

Lower: Percent amplitude of the MSRR expressed as a function of time. Construction of the graph was identical to that presented in Figure 3.


0.0′
0.5 mV
0.5′
1 msec
1.0′
2.0′
3.0′
4.0′
5.0′
6.0′
10.0′
Saline Injection
SCh Injection 50 µg/kg
% Amplitude of MSR
120
100
80
60
40
20
0
51:61 Nembutal-Spinal
8
6
4
2
0
2
4
6
8
10
12
Minutes

brate preparations versus the high threshold in the decapitate or anesthetized and spinal animals. The slight differences between the mean curves of the different preparation types in Figure 5 are not statistically significant in view of the inherent variation in amplitude of the MSRR (Figs. 2, 3, 4, 10). During the period since this graph was constructed, the MSRR's of an additional 30 preparations, mainly decerebrate or anesthetized with spinal transection, were subjected to SCh. Similar reflex depressions were obtained in every case. A facilitation of the extensor MSRR was never observed with the experimental conditions employed here. This is in contrast to the observations of Henatsch (66) who occasionally recorded facilitation with 0.5 mg/kg doses of SCh.

Decamethonium

The influence of decamethonium (C10) on the MSRR was identical to that of SCh except for the extended duration of the depressive action. In the preparation from which the data for Figure 5 were derived, an extensor MSRR was influenced by a small, intravenous dose of C10 which evoked a prompt depression of the MSRR quite similar to that produced by SCh; however, the response had only partially recovered by the end of the thirty minute recording period. The time course of the C10 induced reflex depression was not accurately determined due to the difficulty of maintaining acute preparations of this type constant for extended periods of time. In 4 experiments the reflex depression was observed to persist for as long as 60 minutes.

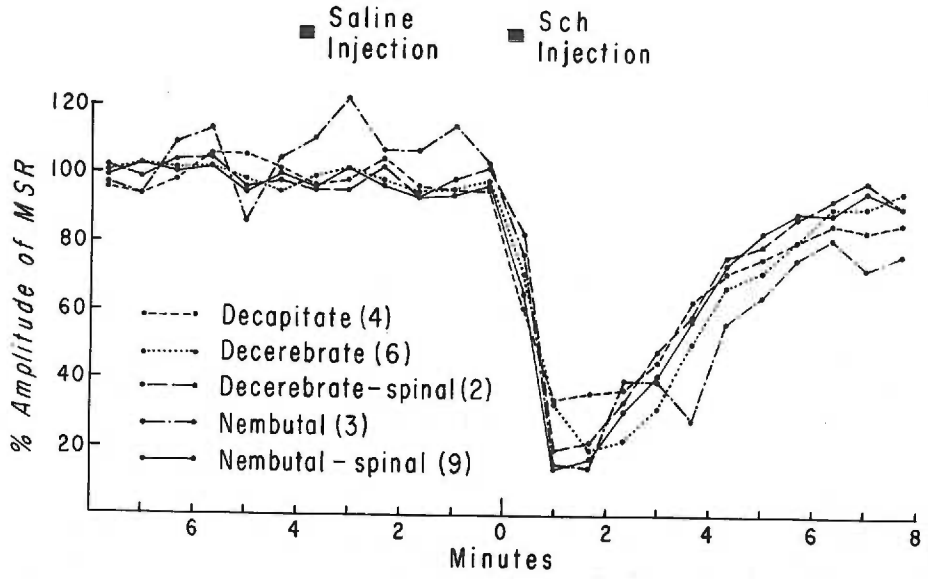
Increased Rate of Muscle Spindle Discharge

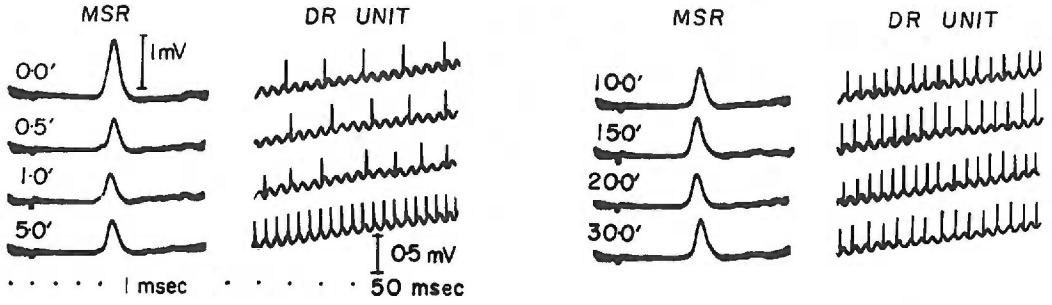
During the course of this investigation spontaneous afferent activity was recorded

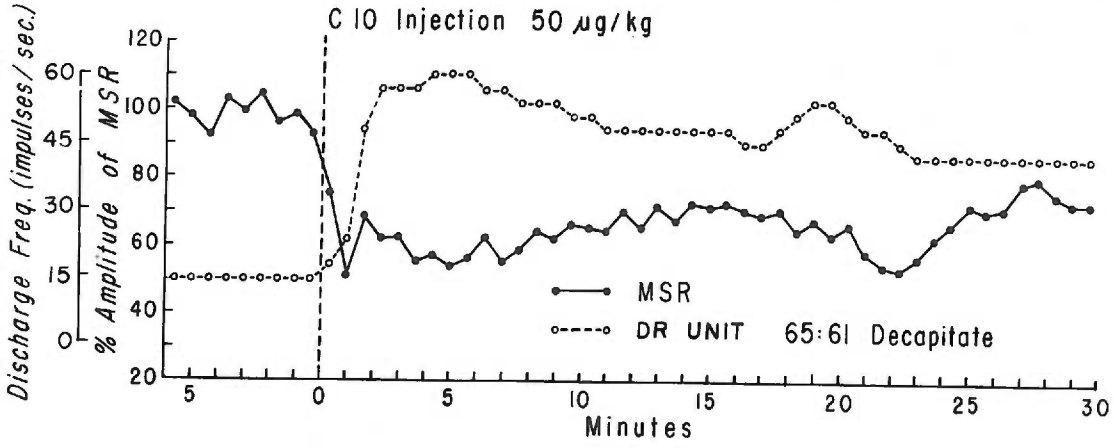
FIGURE 5

Upper: The percentage amplitudes of 24 extensor MSRR's are plotted as a function of time. The number of experiments contributing to each curve is indicated; otherwise the construction is identical to the mean curves of Figures 3 and 4. For example, 20 scores were used to calculate the mean for each point of the curve describing the decapitate preparations. The inherent variance of the MSRR's amplitude should be considered when interpreting this data.

Middle and Lower: The influence of decamethonium (C10) on the extensor MSRR and a single, stretch sensitive dorsal root unit (DR) which originated in the gastrocnemius-soleus muscle. Single shocks were applied to the intact gastrocnemius-soleus nerve and the maximal MSRR was recorded from VRS1. The tonically active dorsal root unit was isolated from DRS1 (see Fig. 1) and was recorded on a baseline of 60 cps. Each point is the mean of 5 consecutive responses.


Saline Injection
Sch Injection
% Amplitude of MSR
120
100
80
60
40
20
Decapitate (4)
Decerebrate (6)
Decerebrate-spinal (2)
Nembutal (3)
Nembutal - spinal (9)
6
4
2
0
2
4
6
8
Minutes


MSR
DR UNIT
1mV
0·0′
0·5′
1·0′
5·0′
0·5 mV
1 msec
50 msec
MSR
DR UNIT
10·0′
15·0′
20·0′
30·0′


C 10 Injection 50 µg/kg
Discharge Freq. (impulses/sec.)
% Amplitude of MSR
120
100
80
60
40
20
60
45
30
15
0
MSR
DR UNIT 65:61 Decapitate
5
0
5
10
15
20
25
30
Minutes

from a large number of dorsal root filaments in order to determine the effects of intravenous SCh or C10. The influence of C10 on the discharge rate of a single afferent fiber is shown in Figure 5. This fiber originated from the gastrocnemius-soleus muscle and was identified as a Group I fiber by its conduction velocity. The marked increase in rate of discharge which followed C10 injection indicated that the fiber originated from a muscle spindle and not a tendon organ (60, 52). The time course of the increased rate of spindle discharge was quite similar to that of the simultaneously recorded MSRR depression despite the fact that the comparison involved a single unit on one hand and numerous units on the other.

The increased rate of afferent discharge which followed SCh injection is illustrated in Figure 6. In both of these preparations, the time course of the altered discharge rate was essentially identical to the time course of the MSRR depression.

The majority of the afferent units that were examined increased their rate of discharge following SCh injection and some units which were silent prior to drug injection began to fire. A silencing of spontaneous afferent discharge by SCh was not observed. The lack of response by some units to SCh is not surprising because the unit isolation technique did not identify the type of receptor from which the fiber originated and a certain percentage of the isolated fibers undoubtably arose from tendon organs.

In two experiments the action of SCh (intravenous doses up to 1 mg) was tested on nerve filaments dissected from the sural nerve. No effect, in terms of evoking afferent discharge was noted and, in agreement with others (52), it would seem likely that the increased discharge rate is limited to those fibers which arise from muscle spindles.

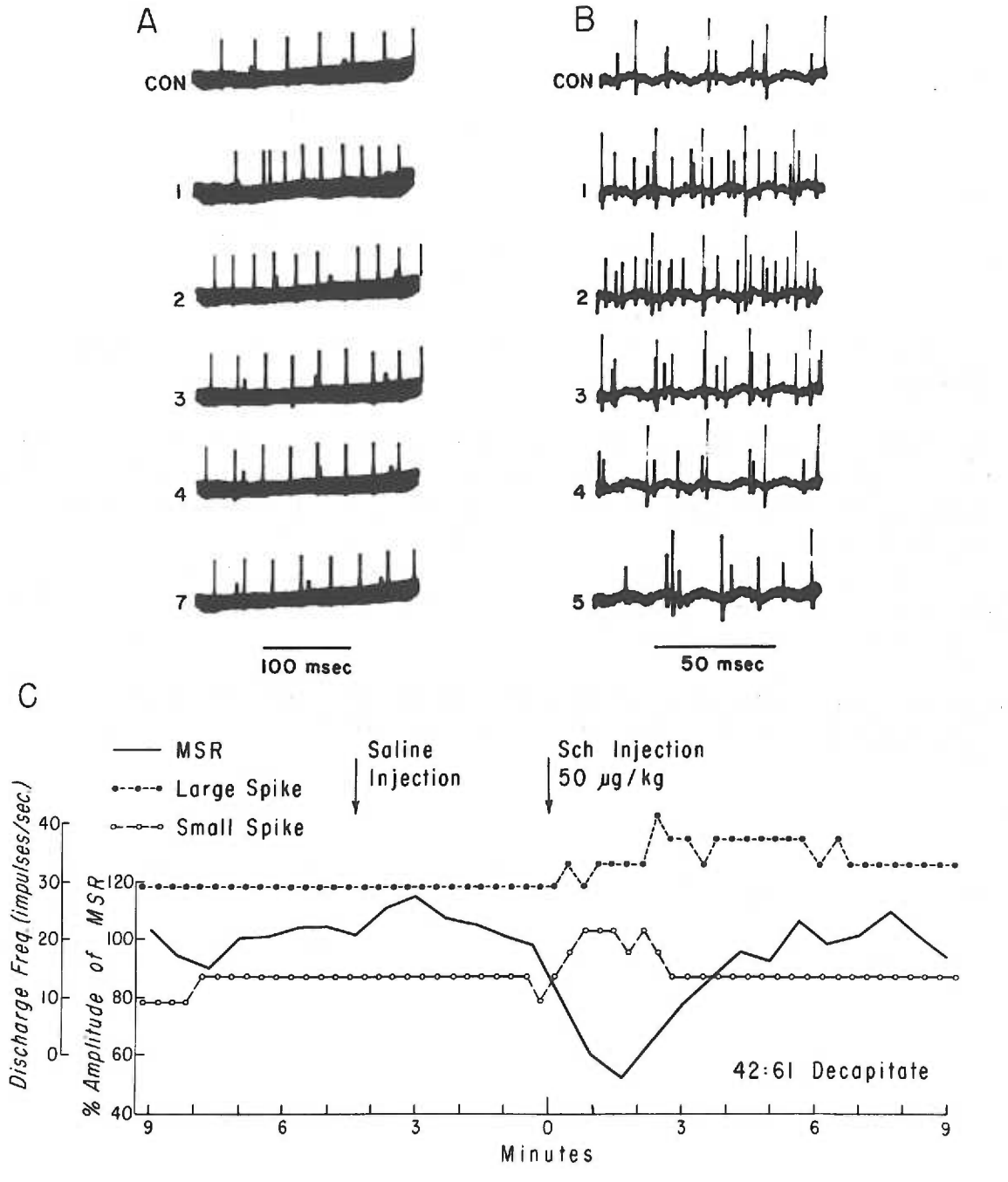
FIGURE 6

The effect of SCh on spontaneous unit afferent discharge originating from stretch sensitive receptors.

A. Records of spontaneous afferent discharge which were recorded monophasically from the peripheral end of a small dorsal root filament (DRL7). The time, in minutes, that the sample record was taken after SCh administration is indicated to the left of each sweep.

B. Similar records that were obtained from a filament of DRL7 of another decapitate preparation.

C. The graphic description of the increased rate of afferent discharge and the reflex depression was constructed from records partially illustrated in record A.


A
CON
1
2
3
4
7
100 msec
B
CON
1
2
3
4
5
50 msec
C
MSR
Large Spike
Small Spike
Saline Injection
Sch Injection 50 µg/kg
Discharge Freq. (impulses/sec.)
% Amplitude of MSR
42:61 Decapitate
Minutes

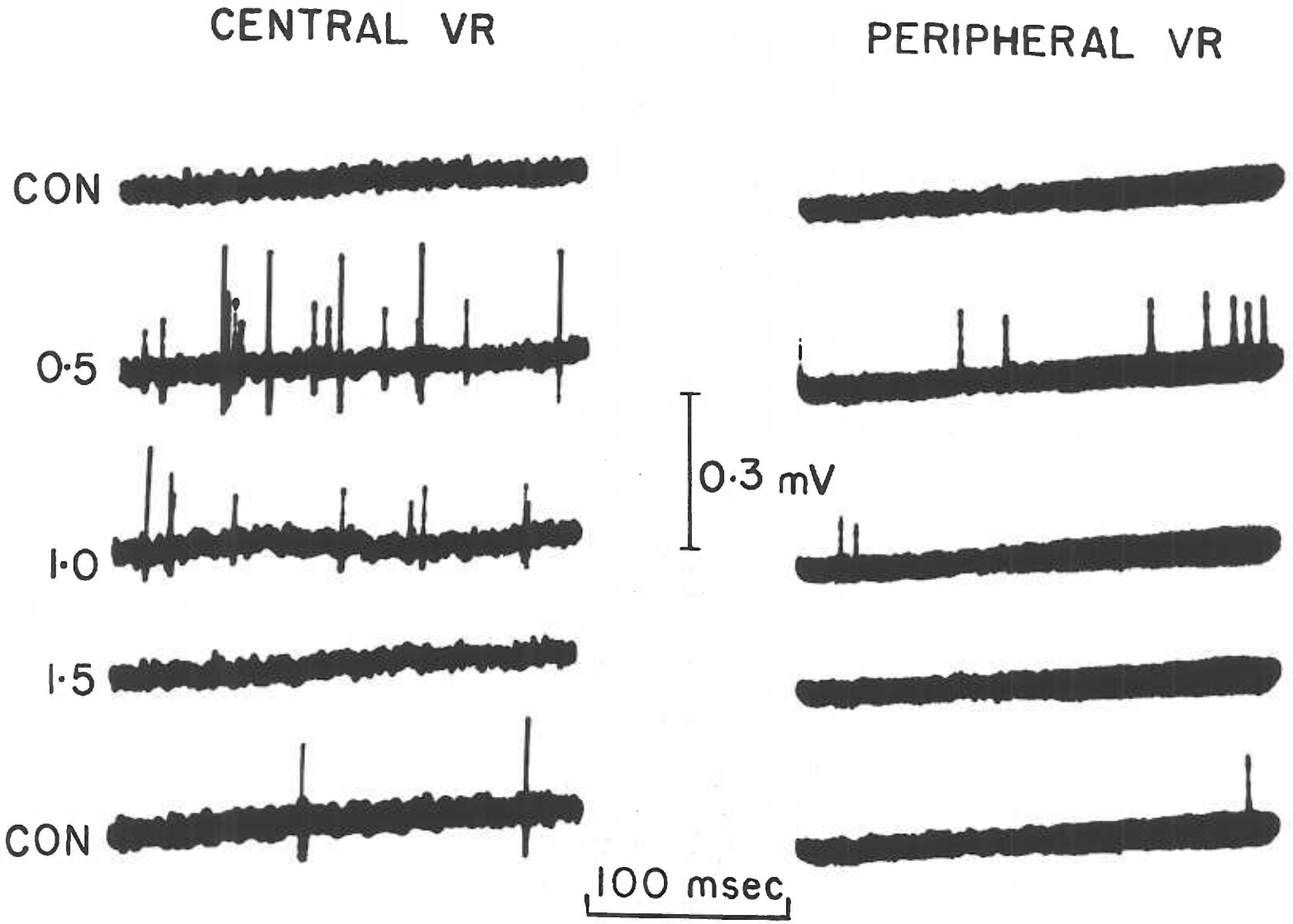
Increased Random Firing of Motoneurons

The barrage of afferent impulses which follows the injection of SCh might be expected to exert a central facilitatory action which would result in reflex motoneuronal discharge. Such discharges were not observed from whole ventral roots as was demonstrated by Figures 3, 4 and 5; however, unsynchronized spikes may not reveal themselves in gross root recordings. The attenuation of single action potentials by tissue fluid and inactive tissue is sufficient to mask their presence. To circumvent this technical difficulty the spontaneous behavior of motoneurons was observed by recording from fine ventral root filaments before and after the administration of SCh. That SCh does induce a random firing of previously quiescent motoneurones is shown by Figure 7. The motoneuronal discharge began approximately 15 seconds after the SCh injection and persisted for about 70 seconds in this decapitate preparation. The time course of this random firing of motoneurons is noted to be considerably less than the duration of the drug-induced MSRR depression established previously. It was further noted that spike discharges were not present following dorsal rhizotomy which demonstrated the motoneuronal discharge to be both reflex in nature and dependent on the afferent barrage which follows SCh injection.

In addition to the predicted motoneuronal reflex discharge following SCh there was the transient appearance of a few spikes which were antidromically conducted from the periphery in ventral root axons (Fig. 7). The antidromic discharges were noted in the two experiments designed to detect their presence and presumably resulted from a depolarization of motor nerve terminals, either by the direct action of SCh, such as occurs with neostigmine (106, 123), or by action potentials in adja-

FIGURE 7

Increased random firing of motoneurons secondary to SCh administration. Recording electrodes were fitted to the central (central VR) and peripheral (peripheral VR) ends of a transected ventral root filament (S1) in a decapitate preparation. In the resting state (upper control records) there was no discharge from either the central or peripheral end of the filament. When mechanical pressure was applied to the ipsilateral foot (lower control records) discharges appeared in both ends of the filament. The spikes recorded from the central end were evoked reflexly in contrast to the spike which was conducted antidromically from the muscle in a motor nerve axon to the peripheral recording electrodes. The records interposed between the control responses were obtained at the indicated time (minutes) following the intravenous injection of SCh (100 μg/kg).


CENTRAL VR
PERIPHERAL VR
CON
0·5
1·0
1·5
CON
0·3 mV
100 msec

cent active muscle and nerve fibers (90). Regardless of their origin, the antidromic spike discharges were of no concern with respect to the MSRR depression because the ipsilateral ventral rhizotomy prevented such impulses from reaching the motoneurons under study. For this reason, the phenomenon was not further investigated. In contrast, the reflex firing of motoneurons was of immediate interest in that it provided the basis for at least two possible explanations to account for the MSRR depression. This random firing of motoneurons was noted independently by others (68, 51) and was investigated in detail in this study. To avoid repetition further discussion of these results will be deferred until the mechanism of MSRR depression is considered.

Depression of the Indirect Muscle Twitch

The intravenous or intraarterial injection of SCh is known to produce a transient paralysis of skeletal musculature (115, 133, 130). The magnitude and duration of the paralysis are illustrated in Figure 8 (records A-C) which describes the response of two gastrocnemius-soleus twitches to SCh. In every experiment (N=7) in which both the MSRR and the twitch were evoked with the same pair of stimulating electrodes the twitch depression persisted two or more minutes longer than did the simultaneously recorded MSRR depression (record B). In contrast, in those experiments (N=4) in which separate pairs of electrodes were utilized to evoke the two responses the time courses of depression were identical (record C). The difference in time courses was most likely a result of one of the factors known to influence the duration of neuromuscular blockade (see INTRODUCTION), rather than a reflection of the technical differences. For example, both decreased peripheral temperature (139)

FIGURE 8

The depression of the indirect muscle twitch, the time course of muscle fasciculation and the alterations in carotid blood pressure which follow the intravenous injection of SCh.

A. Submaximal gastrocnemius-soleus muscle twitch evoked by single shock stimulation of the GS nerve in a decerebrate preparation. The experimental method is illustrated in Figure 1-A. Resting tension was 100 gms. The increased resting tension and muscle fasciculations which followed SCh injection and the omitted 5 minute portion of the record are noted.

B. The relative amplitudes of the indirect twitch and of the simultaneously recorded MSRR are plotted as a function of time. This data was obtained from the series of responses partially illustrated in record A.

C. Just submaximal GS twitch of an anesthetized and spinal preparation. In this experiment the GS nerve was severed between the two pairs of electrodes that evoked the twitch and MSRR in contrast to the experimental method employed in record A and illustrated in Figure 1-A. The resting tension was 150 gms.

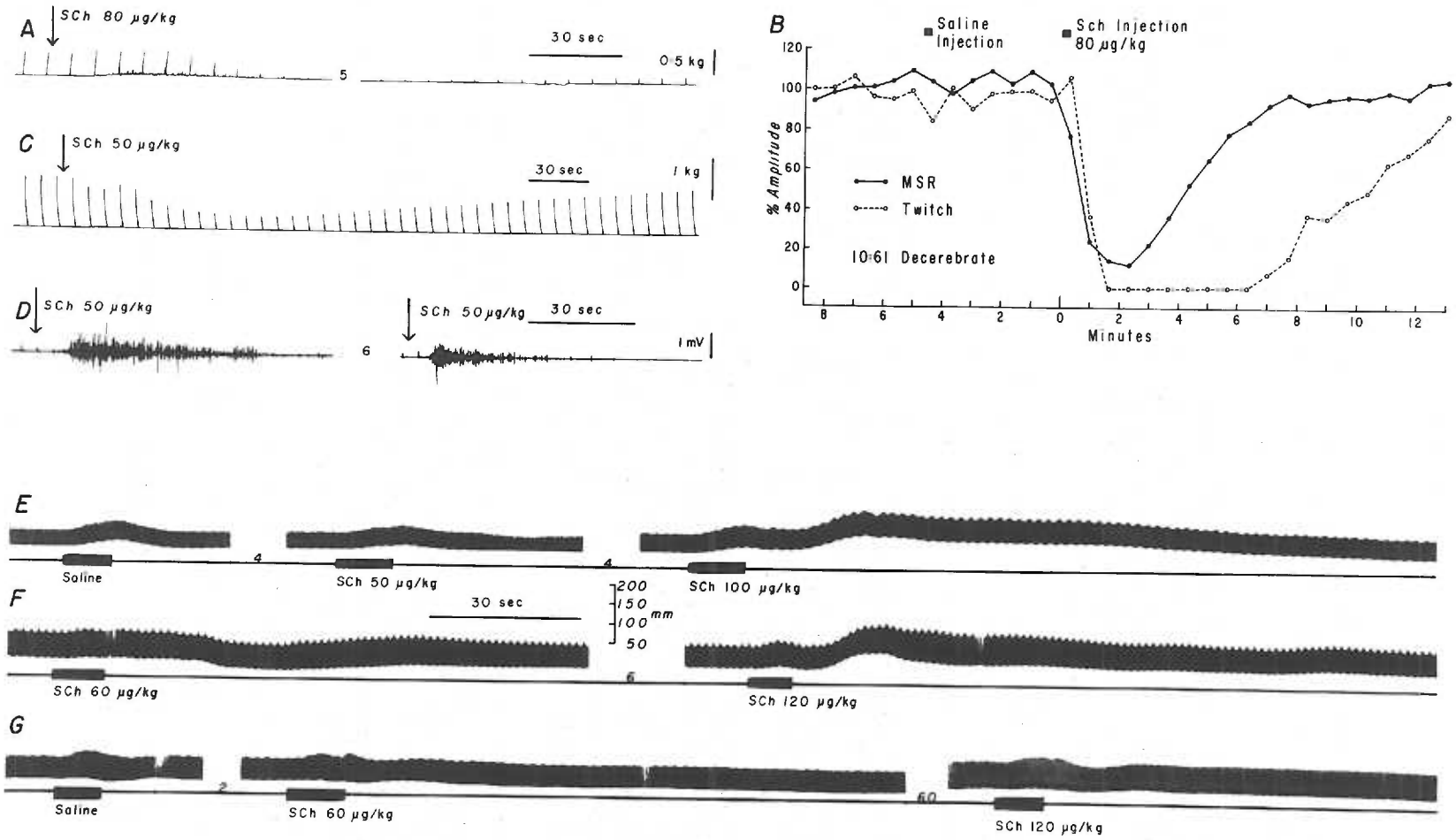
D. The time course of SCh induced muscle fasciculation as determined by electromyography. A 26 gauge steel needle insulated to the tip was plunged into the belly of the GS muscle. The reference electrode was attached to a metal clamp on the spinal column. The same dosage of SCh was readministered 6 minutes following the cessation of the fasciculations. The diminished magnitude and duration of the fasciculations which followed the second injection are noted.

E-G. The line under each common carotid blood pressure tracing corresponds to 25 mm Hg. The onset and duration of each injection is indicated by the signal and the duration of the omitted portion of each record is indicated in minutes. The rhythmic variations in the tracings were secondary to the positive phases of the artificial respirations. The calibration scales between E and F indicate mm of Hg and seconds for all arterial pressure records.

E. Anesthetized and spinal preparation.

F. Anesthetized and spinal preparation. See Figure 19-A.

G. Anesthetized and spinal preparation. See Figure 18-A, B.


A
SCh 80 µg/kg
30 sec
0·5 kg
5
C
SCh 50 µg/kg
30 sec
1 kg
D
SCh 50 µg/kg
SCh 50µg/kg
30 sec
1 mV
6
B
Saline Injection
Sch Injection 80 µg/kg
% Amplitude
MSR
Twitch
10·6l Decerebrate
Minutes
E
Saline
SCh 50 µg/kg
SCh 100 µg/kg
4
4
F
30 sec
200
150
100
50
mm
SCh 60 µg/kg
SCh 120 µg/kg
6
G
Saline
SCh 60 µg/kg
SCh 120 µg/kg
2
60

and decreased blood flow (20) prolong the duration of the neuromuscular blockade produced by SCh. There was little doubt that both of these factors were operating to a greater degree in the earlier experiments in which the stimulating electrode was fitted to the intact muscle nerve.

The intravenous administration of 50 μg/kg of SCh was not sufficient to produce complete neuromuscular blockade. This is illustrated by the incomplete depression of the nearly maximal GS twitch shown in record C and by the fact that the 6 preparations which were not ventilated artificially did not cease breathing when subjected to this dose of SCh.

Record A (Fig. 8) demonstrates two additional features of the neuromuscular blocking actions of SCh. Approximately 15 seconds after the drug injection the resting tension of the GS muscle increased and muscle fasciculations became apparent. Both the increased tension and the muscle fasciculations were shorter in time course than was the twitch depression and usually did not persist for more than one minute. The time course and extent of the muscle fasciculations were more apparent from electromyographic recordings as record D demonstrates. Other features well demonstrated by electromyography included the tachyphylaxis of muscle fasciculations observed with repeated injections of the same dosage of SCh (record D). This observation is in accord with the results of previous investigations (21).

The depolarizing action of SCh (135) would seem adequate to account for both the increased muscle tension and the muscle fasciculations. However, another contributing factor might be the increased random discharge of motoneurons which follows SCh injection since these impulses would be propagated to the periphery and would discharge the muscle fibers supplied by the activated axons. If the random ventral

root discharges were a significant cause of muscle fasciculation they should be more apparent in the hind limb not subjected to ventral rhizotomy. This hypothesis was tested by recording electromyographically from both GS muscles before and after SCh administration in four experiments. In two of these animals the muscle fasciculations recorded from the de-efferented hind limb were definately less than the fasciculations recorded from the contralateral innervated muscle. In the other two experiments the magnitude and duration of the fasciculations recorded from the two limbs were essentially identical. As such, the results were equivocal and further experimentation along these lines was not conducted.

Variable Influence On Carotid Blood Pressure

The effect of the intravenous administration of SCh on the common carotid blood pressure was totally unpredictable from one preparation to the next. Examples of the various pressure alterations that were observed are shown in Figure 8 which illustrates the pressure responses of three anesthetized and spinal preparations. The preparation described by record E responded to 50 μg/kg of SCh with a brief, mild increase in blood pressure quite similar to the pressor response which followed the prior, equal volume injection of saline. The same animal responded to a 100 μg/kg dosage with a large, long lasting increase in carotid blood pressure. In contrast, record F shows a preparation which responded to 60 μg/kg of SCh with a drop in pressure and responded to 120 μg/kg with a marked increase. The preparation shown in record G was not particularly responsive to either 60 or 120 μg/kg dosages and demonstrated only a very mild decrease in carotid blood pressure. The pressure responses of ten animals of each preparation type to SCh (50 μg/kg) were analyzed in detail. Only

blood pressure alterations of 10 mm Hg or greater and which persisted for longer than 30 seconds were considered significant. With this standard the following results were obtained: 1) decerebrate - 7 increased, 1 decreased and 2 remained stable; 2) decapitate - 5 increased and 5 remained stable; and 3) anesthetized and spinal - 3 increased, 1 decreased and 6 remained stable. As demonstrated by Figure 8 the percentage of preparations responding with pressor responses and the magnitude of the response were increased by increasing the dosage of SCh.

Pressor responses following the small doses of SCh employed here have not been described previously (133, 130, 21). The mechanism for the pressor responses observed here was not established; however, a descending spinal sympathetic discharge does not seem to be a likely factor since the response was observed in the decapitate preparations. The nicotinic action of SCh, to which the pressor responses following large doses has been attributed (133, 130, 21), would appear to be a likely cause.

The carotid blood pressure alterations were not found to correlate with any of the changes in nerve activity which followed SCh administration. It will be demonstrated that the SCh-induced MSRR depression was entirely independent of any changes which might have occurred in blood flow to the central or peripheral nervous systems.

C. THE MECHANISM OF MONOSYNAPTIC REFLEX RESPONSE DEPRESSION

A variety of mechanisms have been proposed (see INTRODUCTION) to account for the depression of extensor MSRR's which follows the intravascular administration of SCh or C10. This section presents the results from experiments that were designed specifically to test for the presence of these mechanisms. Evidence will be presented

to show that the majority of these factors have little if anything to do with the drug-induced reflex depression. The presence of a previously untested factor which adequately explains the reflex depression and associated phenomena will be demonstrated.

Validity of the Reflex Depression

The extensor MSRR's of more than 60 preparations were exposed to intravenous injections of SCh in doses of 50 μg/kg or greater. In these preparations the effects of approximately 150 injections were observed, and, in all but 3 experiments depression of the extensor MSRR's resulted. The MSRR's of the 3 experimental exceptions were not altered by SCh. These negative observations were the direct result of technical difficulties, and with correction of the offending factor MSRR depression then followed SCh administration. A facilitation of the extensor MSRR was never observed, even in the experiments in which doses of 10 and 25 μg/kg of SCh were injected. Small doses of this order evoked either no effect or quite minimal reflex depression.

The ventral root records in Figures 3, 4, and 5 illustrated that the duration of the MSRR's did not increase during the drug-induced reflex depression. Therefore, the depression cannot be attributed to a desynchronization of the reflex discharge with a consequent reduction in response amplitude. The reduction of reflex amplitude, in view of the constant or even decreased duration (Fig. 5), can only mean that the average number of motoneurons responding monosynaptically to the test volley was reduced in each case by SCh or C10. Further evidence that the reduction in response amplitude was not artifactual was provided by the investigation on unit

reflexes to be discussed in a later section.

Supraspinal Effects

The SCh-induced MSRR depression could have resulted from either a suppression of descending spinal facilitation or an enhancement of descending spinal inhibition or from a combination of these two mechanisms. The loss of facilitation or the enhancement of inhibition could have followed a direct supraspinal action which these drugs may exert (44). Alternately, a supraspinal action may have been secondary to the altered afferent input (9). However, data which has already been presented demonstrates that the MSRR depression was entirely independent of any actions which may have occurred at a supraspinal level. Figures 3 and 4 illustrated two MSRR's which were depressed by SCh and yet in one case the spinal cord had been transected (Fig. 4) and thus, descending spinal activity could not have altered the MSRR under investigation. Furthermore the depression occurred regardless of the level at which the neural axis was interrupted (cervical, thoracic or lumbar spinal cord) as the composite graph in Figure 5 demonstrates. The independence of the reflex depression on supraspinal events found in these experiments is in agreement with the findings of Fugimori and others (31).

Dependence of Reflex Depression on the Peripheral Action of SCh

The similarity between the time course of the reflex depression and that of the increased rate of spindle discharge (Figs. 5 and 6) suggested the existence of a cause and effect relationship. That such a relationship did exist was demonstrated

by nerve transection and drug localization experiments. MSRR's evoked by dorsal root stimulation were not depressed by the intravenous administration of SCh if the increased spindle discharge was prevented from reaching the spinal cord by unilateral dorsal rhizotomy. However, the MSRR evoked simultaneously from the opposite side of the spinal cord was depressed in a characteristic fashion. The comparable maneuver in which the increased spindle discharge was prevented from reaching the spinal cord by deafferentation of the hind limb also prevented MSRR depression (Fig. 20-A). It would appear from such experiments that the drug-induced spindle discharge must reach the side of the spinal cord from which the reflex is recorded in order for the MSRR depression to be manifest. However, there is a theoretical objection to the use of dorsal root or peripheral nerve transection procedures; the transection not only prevents the drug-induced afferent input from reaching the spinal cord but, in addition, deprives the spinal cord of its spontaneous, tonic afferent input. How the response of tested motoneurons would be altered by the lack of tonic afferent input is not known and is impossible to predict.

The objections associated with transection experiments were circumvented by close intraarterial injections of SCh (Fig. 20-B). When a total dose of 50 μg of SCh was injected into one femoral artery the MSRR evoked from the ipsilateral GS nerve was significantly depressed. However, the amplitude of the MSRR evoked simultaneously from the contralateral GS nerve was not altered. In contrast, the intravenous injection of SCh depressed both MSRR's. From these experiments it follows that the MSRR depression was the direct result of a peripheral action of SCh and was not secondary to a direct action by SCh on the spinal cord. The only known peripheral action of SCh that could account for these observations is the

initiation or augmentation of muscle spindle discharge. These experiments complement the nerve transection procedures and provide conclusive evidence that the reflex depression was secondary to the increased rate of discharge from muscle spindles. Furthermore, the possibility that the reflex depression resulted from any alterations in blood flow to the spinal cord was eliminated by the drug localization experiments.

Increased Refractoriness of Stimulated Muscle Nerves

The administration of SCh increases the rate of discharge in those Group IA afferent fibers which arise from muscle spindles (60, 11). The afferent volley which evokes the MSRR is also conducted by Group I nerve fibers (93). It would appear likely that if the increased rate of discharge in Group I fibers which follows SCh were sufficiently great, the discharging afferent fibers would be left in a partially refractory state and that the stimulating current which was maximal for the MSRR prior to drug injection would have become less effective. Again, previously illustrated data eliminates this possibility as a cause of MSRR depression. The MSRR shown in Figure 4 was evoked from a muscle nerve which had been transected distally to the stimulating electrodes and yet reflex depression occurred in typical fashion. In most experiments conducted during this investigation, the muscle nerve from which the reflex was evoked had been transected, and in all cases the reflex depression which resulted from SCh administration was indistinguishable from the reflex depression observed in those preparations with intact stimulated muscle nerves.

Direct Inhibition

The data that have been presented leads to the conclusion that the depression of

extensor MSRR's results directly from the increased rate of muscle spindle discharge which follows SCh administration. With regard to the central actions of these spindle afferents, there is general agreement (see INTRODUCTION) that Group IA fibers facilitate the motoneurons innervating the muscle from which the spindle afferents arose. These afferents also facilitate the motoneurons supplying muscles of synergistic function but inhibit motoneurons innervating muscles of antagonistic function. In contrast, Group 2 afferent fibers exert a central action which results in a generalized facilitation of flexor motoneurons and an inhibition of extensor motoneurons. Those afferent fibers which arise from tendon organs are not activated by SCh (60, 52) and therefore, their central actions do not appear pertinent to the problem at hand.

It must therefore be admitted hypothetically that the depression of extensor MSRR's might be due to the central inhibitory actions of the activated Group 2 afferent fibers which arise from muscle spindles. This hypothesis assumes that the concepts regarding the central actions of muscle spindle afferents are correct and that the SCh-induced afferent barrage is limited to those Group IA and Group 2 afferent fibers which arise from muscle spindles. If such a mechanism of direct inhibition is responsible for this phenomenon, the depression of extensor MSRR's should be coupled with a generalized facilitation of flexor motoneurons. This hypothesis was tested by exposing simultaneously extensor and flexor MSRR's to the administration of SCh or C10.

The use of stimuli delivered simultaneously to flexor and extensor muscle nerves was precluded by the possibility of presynaptic or postsynaptic interaction of the two volleys. To maintain the previously adopted stimulus interval of 8 seconds and to

avoid the foremantioned complicating factors, the test shocks were delivered alternately at 4 second intervals. However, the interpretation of the forthcoming data would be complicated by any interaction between the alternately evoked volleys. In otherwards, the extensor MSRR might have influenced the response of the flexor MSRR to SCh or vice versa. In view of the short time courses of direct (95) and recurrent inhibition (120), this possibility did not seem likely; however, as a control a few experiments were conducted in which only the flexor MSRR was subjected to SCh. The results of such experiments were identical to those in which both reflexes were alternately evoked and recorded. In each of the experiments described here, the occurance of stimulus spread was prevented or eliminated by the techniques described previously. The experimental arrangement is illustrated schematically in Figure 9.

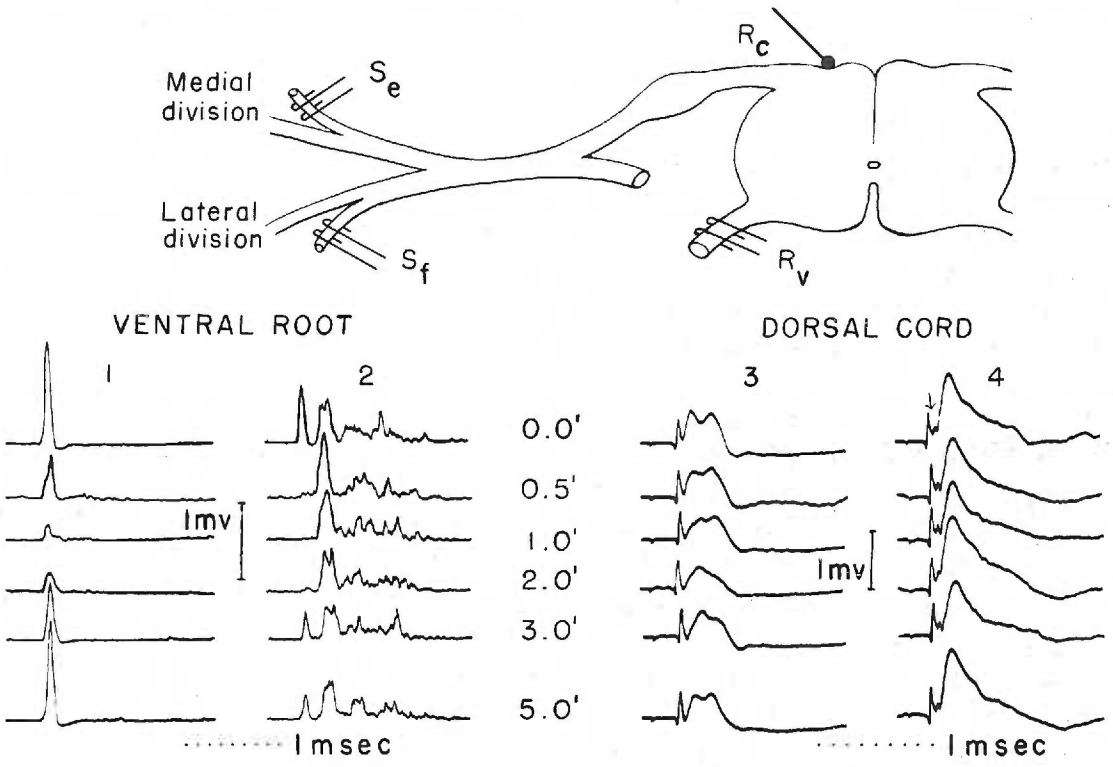
The response of alternately evoked flexor and extensor MSRR's to the intravenous administration of SCh is illustrated in Figure 9. However, before this is considered several points of interest are to be noted from inspection of the control responses (row 0.0) which include the magnitude of the intermediary cord potentials (columns 3 and 4) and the small potential indicated by the arrow in column 4. The large intermediary cord potential suggests that either Group I afferent fibers mediate activity via multisynaptic pathways or that the stimulus required to evoke maximal MSRR's was supramaximal for Group I afferents. The small potential following the triphasic spike (column 4) has been associated with activity arising from afferent presynaptic terminals (3).

The predicted results of the direct inhibition hypothesis were not obtained when this preparation was exposed to a 50 μg/kg dose of SCh. Instead, both the flexor

FIGURE 9

Upper: Diagram of the experimental arrangement used to alternately test the excitability of extensor and flexor motoneuronal pools. S_e and S_f - stimulating electrodes applied to extensor and flexor muscle nerves respectively. In some experiments the electrodes were fitted to the intact lateral and medial divisions of the sciatic nerve; in others, severed muscle nerves were used as illustrated. R_v - ventral root pickup. R_c - monopolar electrode for recording dorsal cord potentials. The reference electrode consisted of a metal clamp on the spinal column.

Lower: Records from a decerebrate - spinalized animal which illustrate the simultaneous depression of extensor and flexor MSRR's. Single shocks were applied alternately to the intact medial and lateral divisions of the sciatic nerve and the extensor and flexor MSRR's were recorded from VRS1. The dorsal cord electrode was placed on the dorsolateral sulcus at the entry of DRL7. Columns 1 and 3 - records of ventral root and dorsal cord potentials generated by stimulation of the medial division (extensor). Columns 2 and 4 - responses evoked by stimulation of the lateral division (flexor). For a given horizontal row the records in columns 2 and 4 were obtained 4 sec following the records in columns 1 and 3. The numbers at the center of the figure indicate the time (minutes) that the corresponding row followed the injection of SCh (50 μg/kg). The records were retouched to prevent losses in reproduction.


Medial division
Lateral division
S_e
S_f
R_c
R_v
VENTRAL ROOT
DORSAL CORD
1
2
3
4
0.0'
0.5'
1.0'
2.0'
3.0'
5.0'
1mv
1mv
1msec
1msec

and extensor MSRR's underwent virtually complete depression and as the plot of their amplitudes indicates (Fig. 10-A), there was little difference between the two MSRR's and their response to SCh. In contrast to the MSRR depression, the PSRR evoked by stimulation of the flexor nerve was facilitated (column 2; rows 0.5 and 1.0) by SCh. The appearance of minimal polysynaptic discharge was also noted following the extensor MSRR (column 1; rows 0.5 and 1.0). The alterations in the intermediary cord potentials which followed the SCh injection are not meaningful. These experiments were performed before adequate immobilization of the spinal column was instituted and thus, the observed changes may be nothing but movement artifact.

In the preparation from which the data described by Figure 10 was derived (record B), the flexor MSRR was evoked by single shock stimulation, whereas the extensor MSRR was elicited by the double shock technique. Again the response to SCh administration was universal MSRR depression.

An interesting experiment is illustrated by the records shown in Figure 11 (record A). In this preparation the injection of saline was followed by a significant facilitation of the flexor MSRR in contrast to the 50 μg/kg dosage of SCh which produced a minimal depression of both varieties of MSRR's. A second, larger dose of SCh was required to bring about the usual degree of reflex depression. Even though this particular flexor MSRR was extremely labile as indicated by its response to saline, depression of the reflex resulted from both injections of SCh.

The data of 9 observations obtained from 6 preparations are summarized by the composite graph illustrated in Figure 11 (record B). The mean amplitudes of the flexor and extensor MSRR's when considered with their respective variations demon-

FIGURE 10

Graphic description of the effect of SCh on alternately evoked extensor and flexor MSRR's. Each point is the mean of 5 consecutive scores expressed in percent and relative to the mean amplitude of the control responses.

A. Graphic plot for the series partially illustrated in Figure 9.

B. MSRR's were evoked by applying single test shocks to the anterior tibial nerve (flexor) and double shocks to the gastrocnemius-soleus nerve. Both muscle nerves were severed distally to the stimulating electrodes.

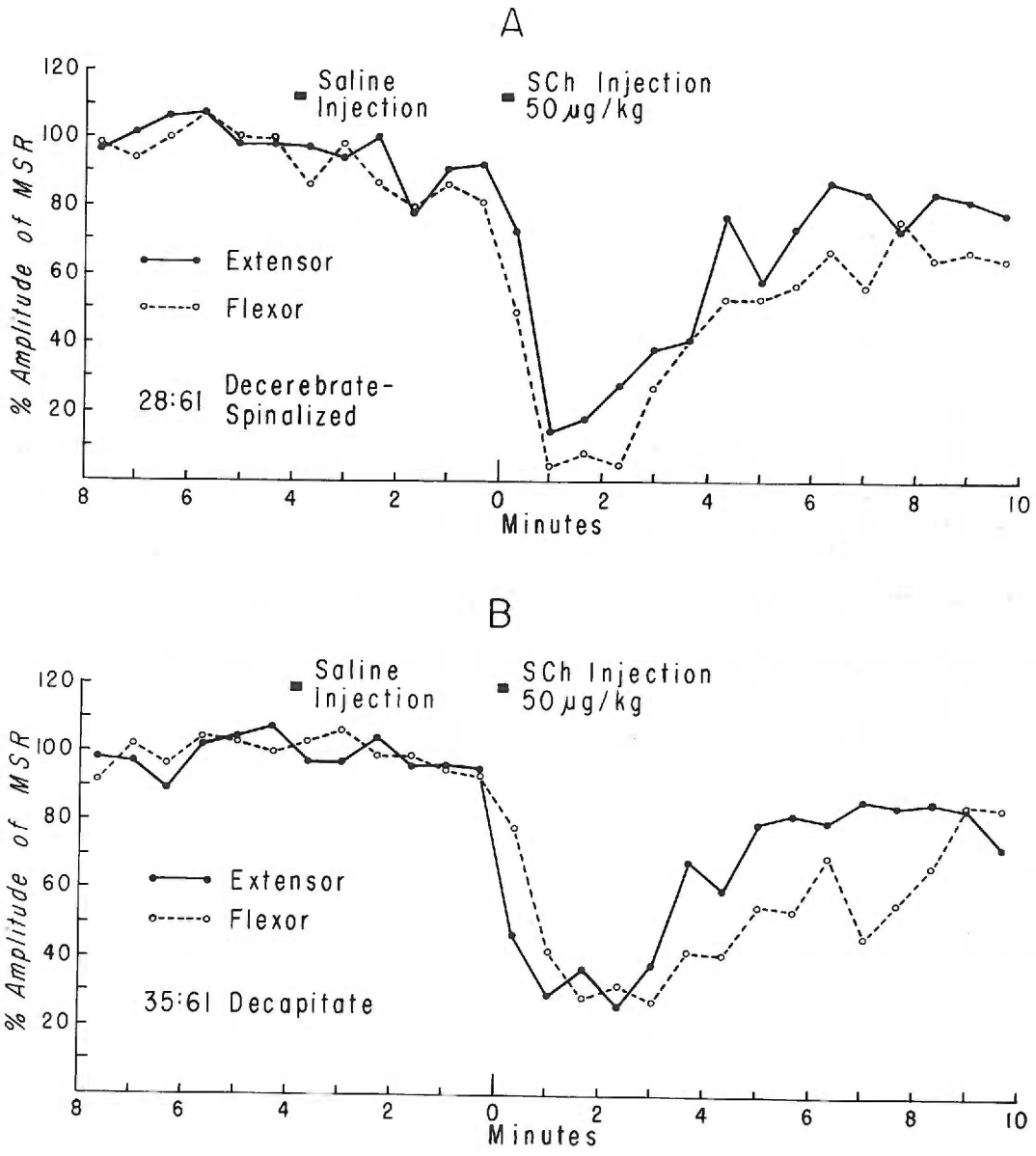
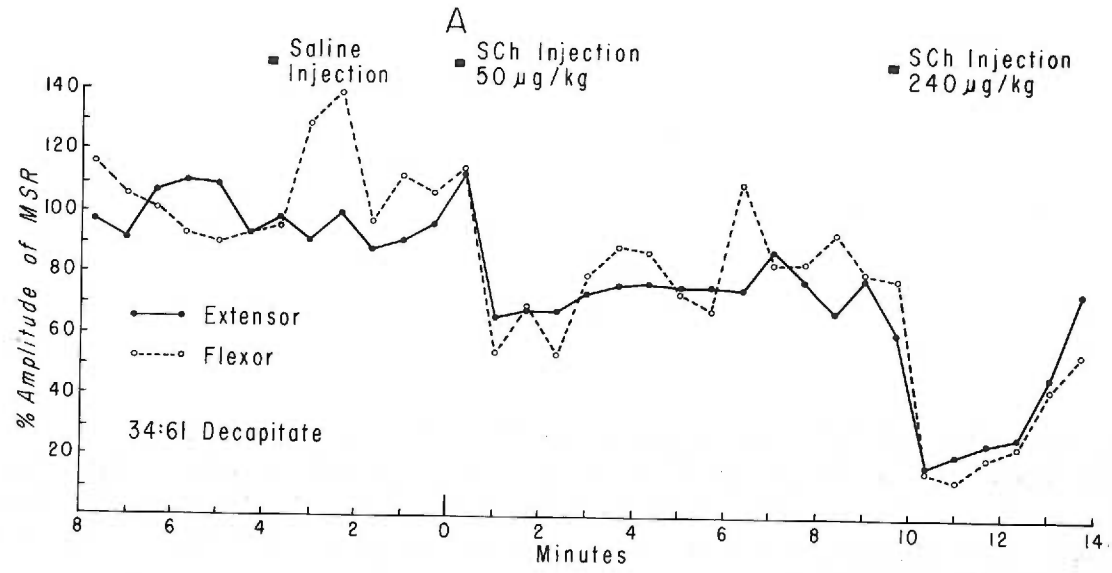
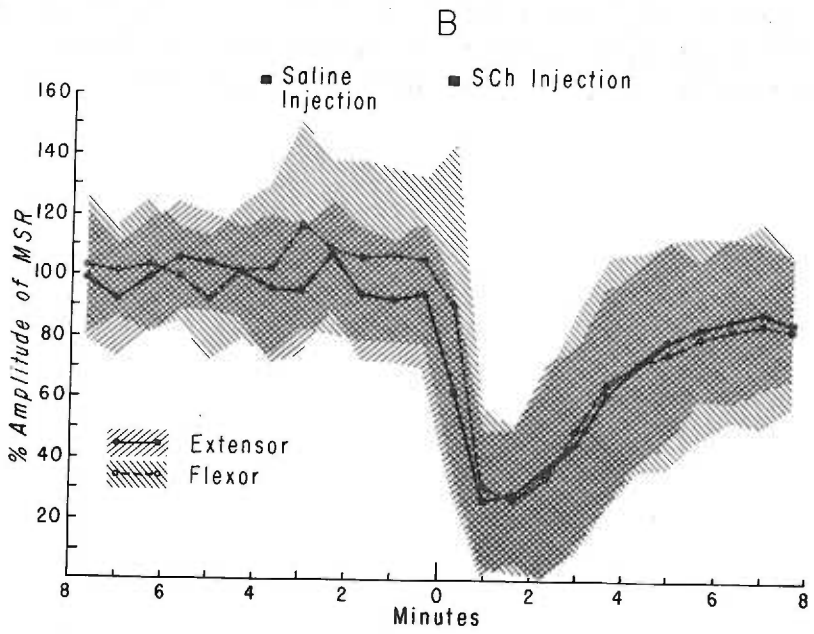

A
Saline Injection
SCh Injection 50 µg/kg
% Amplitude of MSR
120
100
80
60
40
20
Extensor
Flexor
28:61 Decerebrate-Spinalized
8
6
4
2
0
2
4
6
8
10
Minutes
B
Saline Injection
SCh Injection 50 µg/kg
% Amplitude of MSR
120
100
80
60
40
20
Extensor
Flexor
35:61 Decapitate
8
6
4
2
0
2
4
6
8
10
Minutes

FIGURE 11

A. Data obtained from a decapitate animal by delivering double shocks to the intact lateral division of the sciatic nerve and to the severed medial gastrocnemius nerve. The extensor MSRR's were recorded from VRL7; the flexor MSRR's from VRL6. Note the second injection of SCh which was given approximately 9 minutes after the first injection.

B. A composite graph which summarizes the data from 9 experiments performed on 6 animals. The construction of the graph is similar to those previously presented with the exception that each point is the mean of 45 scores; 5 consecutive scores from each experiment. The slanted lines indicate the magnitude of 1 standard deviation from the respective mean. A S.D. was calculated for each point (N=45).


A
Saline Injection
SCh Injection 50 µg/kg
SCh Injection 240 µg/kg
% Amplitude of MSR
Extensor
Flexor
34:61 Decapitate
Minutes


B
Saline Injection
SCh Injection
% Amplitude of MSR
Extensor
Flexor
Minutes

strates conclusively that a generalized monosynaptic reflex depression is the result of SCh administration and not a selective facilitation of flexor MSRR's coupled with depression of extensor MSRR's. The slight differences between the onset, the magnitude and the duration of depression in extensor and flexor MSRR's (Figs. 9, 10, 11) are not significant in view of the variation of response amplitude. The large variation demonstrated by the MSRR's during the depressed phase in this graph (Fig. 11-B) was secondary to the differing degrees of reflex depression in different experiments. The variation in MSRR amplitude during the SCh-induced depression was actually less as Figures 3 and 4 illustrated.

In addition to the data presented, 6 MSRR's evoked by stimulation of the PBST nerve were subjected to the influence of SCh. Of these, 5 demonstrated a significant depression whereas the remaining MSRR responded to SCh with a marked facilitation. Perhaps the facilitation was an example similar to the rare MSRR facilitation observed by Henatsch (66) or perhaps nothing more than a response similar to that shown in Figure 11 (record A) in which the flexor MSRR was augmented by the saline injection. In either case, the phenomenon was not duplicated by the 5 succeeding experiments.

The flexor and extensor MSRR's of one decapitate preparation were exposed to 50 μg/kg of C10. A depression of both reflex responses similar to that evoked by SCh but of extended duration resulted.

On the basis of the observations presented here it was concluded that reflex responses evoked by stimulation of flexor and extensor nerves responded in an identical fashion to the administration of SCh. The MSRR's underwent depression in contrast to the PSRR's which demonstrated varying degrees of facilitation. These

findings are in agreement with the observations of others (32). The MSRR depression occurred regardless of the preparation type (decerebrate and spinal, decapitate or anesthetized and spinal) and regardless of whether the reflexes were evoked by single or double shocks. These experiments do not support the hypothesis that direct inhibition is a major factor in the SCh-induced MSRR depression.

Evoked Ventral Root Unit Activity

Depression of Unit Monosynaptic Reflex Responses

Reflex discharges that are recorded from whole ventral roots represent a relatively synchronized discharge of many motoneurons. An alteration in the amplitude of the MSRR so recorded is assumed to indicate a change in motoneuronal excitability; however, such an amplitude alteration is only an average index of excitability and does not indicate the direction of change for a given motoneuron except by inference. Thus, the incomplete depression of those MSRR's shown in Figures 5, 10 and 11 could be explained by facilitation of one segment of the population of stimulated motoneurons coupled with depression of the remainder. The presence of masked facilitation appears probable in view of the increased random firing of motoneurons which follows SCh administration (Fig. 7). This random discharge may reflect an increased excitability of some motoneurons, as yet unidentified, and not a decreased excitability as the widespread depression of flexor and extensor MSRR's indicates. The averaging feature of population MSRR was circumvented and the presence of coexistent MSRR facilitation was looked for by recording reflex discharges from single ventral root fibers before and after the administration of SCh.

An example of an extensor, unit MSRR and the response of this unit to SCh is illustrated in Figure 12 (records A, B). The control records indicate that the reflex discharge was all or none with respect to the size of the evoked volley and therefore this discharge represented the action potential of a single ventral root axon. The latency of the unit discharge measured 2.9 msec, whereas the latency of the population MSRR (which was recorded simultaneously and is illustrated in Figure 4) measured 2.7 msec. Considering the temporal dispersion of the population MSRR and the error of measurement, the latency of the unit discharge indicates that this unit reflex response was evoked monosynaptically. The afferent test volleys evoked a unit MSRR on nearly every trial preceding and following the injection of saline (record B), in contrast to its failure to evoke MSRR's subsequent to the administration of SCh. The time courses of depression for the unit MSRR and for the population MSRR were essentially identical. In record C the firing indices of five extensor motoneurons are plotted as a function of time before and after SCh administration. The time course of the drug-induced depression of the unit MSRR's is identical to that previously established for the population MSRR (Figs. 5, 11). Nine additional extensor unit MSRR's were subjected to SCh and in every case the firing index of the motoneuron was depressed (Table I). The depression occurred regardless of the tested motoneuron's excitability, that is regardless of whether the control firing index was high (FI = 1.0) or low (FI = 0.2 or greater). This data confirms the information provided by the study of population MSRR's but does not support the contention that incomplete depression of the MSRR results from a facilitation of some motoneurons coupled with a depression of other motoneurons in the same pool. If facilitation were present to any degree in these experiments it should have been detected by

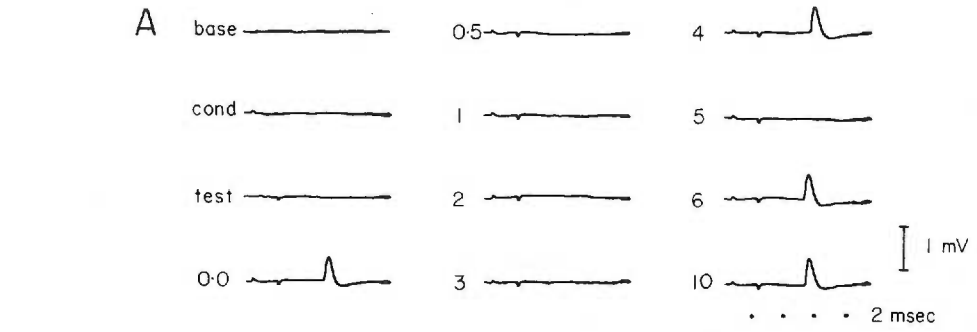
FIGURE 12

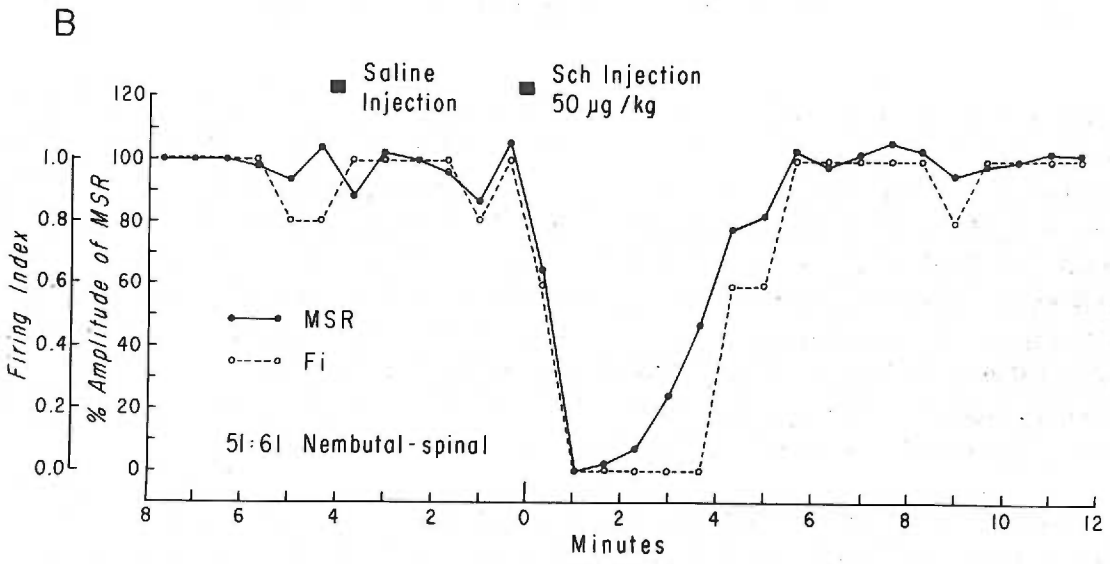
The unit MSRR and its response to intravenous SCh

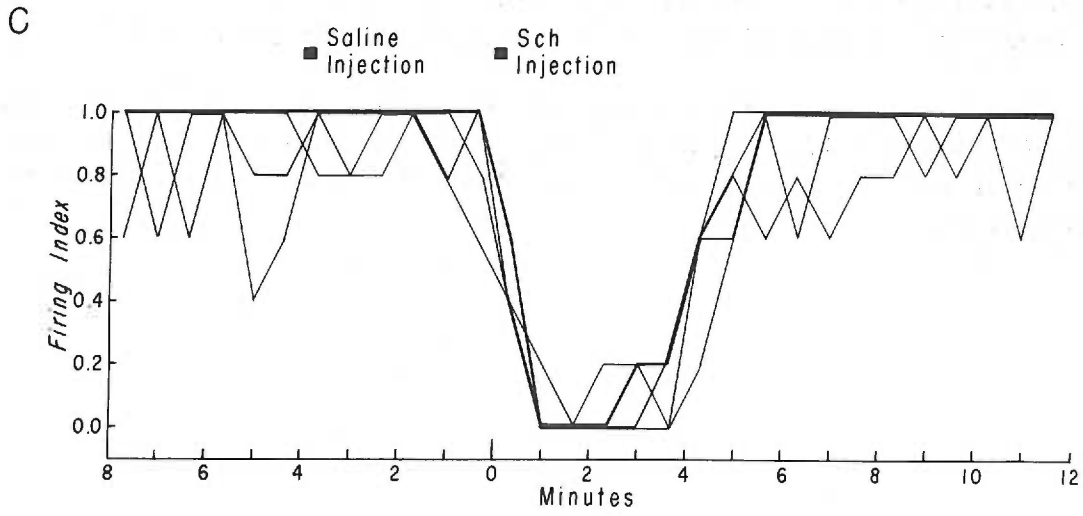
A. The unit MSRR was evoked by double shock stimulation of the GS nerve and was recorded monophasically from a small filament of VRS1 in an anesthetized, spinal preparation. The records in the column at the extreme left of the figure show from above to below: the baseline (base) upon which the subsequent potentials were written; the inability of the conditioning (cond) or test shocks to evoke a reflex response when applied separately; and the unit response (0.0) evoked by the combined application of the two shocks. The remainder of the records were obtained at various times subsequent to SCh administration (50 μg/kg). The time following the drug injection is indicated to the left of each sweep in minutes. The prolonged latency of the unit response recorded at 4 minutes is noted.

B. A graphic description of the simultaneously recorded unit and population MSRR's before and after SCh administration. The firing index (number of responses evoked by 5 afferent volleys by 5) of the unit MSRR was calculated from the series partially illustrated in record A. The mean amplitude of the population MSRR was computed in standard fashion from the series of responses partially shown in Figure 4.

C. The response of five extensor motoneurons to SCh. Each line in the graph represents the firing index of one motoneuron. The 50 μg/kg doses of SCh are noted to have depressed those motoneurons with intermediate as well as those with maximal firing indices.


A
base
cond
test
0·0
0·5
1
2
3
4
5
6
10
1 mV
2 msec


B
Saline Injection
Sch Injection 50 µg/kg
Firing Index
% Amplitude of MSR
MSR
Fi
51:61 Nembutal-spinal
Minutes


C
Saline Injection
Sch Injection
Firing Index
Minutes

those unit reflexes which demonstrated intermediate firing indices.

The latencies of a number of population MSRR's were measured before and after SCh administration, and in some experiments (Figs. 5, 9), the latency was noted to increase slightly (0.2 to 0.3 msec) during the period of MSRR depression. This was not a consistent finding for all experiments in that only one-third of the population MSRR's demonstrated a measurable increase in latency. With the unit reflex studies, the accuracy of latency measurements was increased as record A (Fig. 12) illustrates. The latency of this unit response increased from 2.9 msec during the control period to 3.3 msec four minutes subsequent to the SCh injection. Within the limits of accuracy imposed by the absence of unit responses during the first four minutes following SCh, the time course of the increased latency paralleled the time course of the reflex depression. About one-half of the unit MSRR's demonstrated an increased latency following SCh injection, with 0.5 msec being the greatest latency increase observed. The increased latency would not appear to be related to any events occurring at the peripheral nerve level since the latency of the triphasic dorsal root spike was not altered by SCh (Fig. 9). One of the following factors may have accounted for the observed increased latencies: 1) diminished intraspinal conduction velocity; 2) diminished output of transmitter substance secondary to primary afferent fiber depolarization; 3) hyperpolarization of the membrane of the motoneuron; or 4) accomodation at the postsynaptic level. There is not sufficient evidence on hand to choose between these mechanisms.

Facilitation of Unit Polysynaptic Reflex Responses

The PSRR was augmented by SCh administration to varying degrees in some ex-

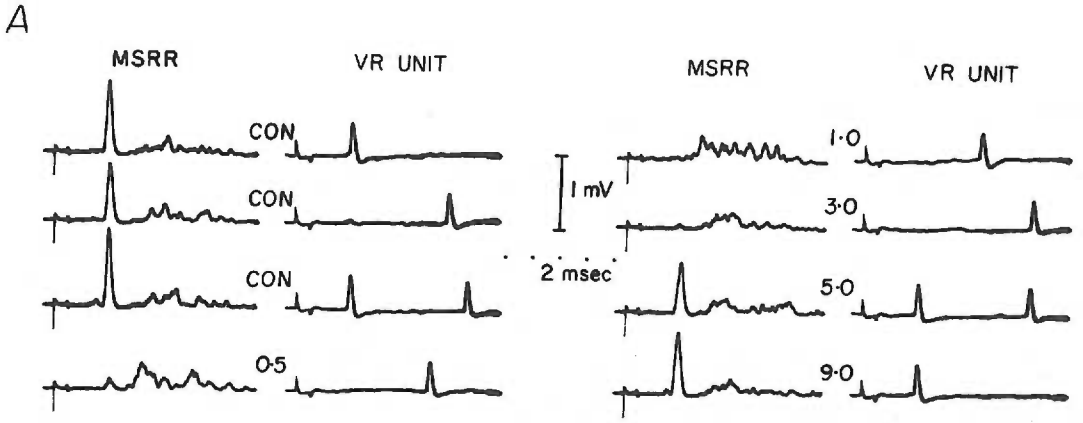
periments (Figs. 3, 9). However, the gross ventral root recordings did not indicate if the facilitation represented an increased number of motoneurons contributing to the multisynaptic discharge or merely a synchronization of the preexisting motoneuronal discharge. In some experiments dealing with unit activity the dissection fortuitously yielded two different units which responded to a single afferent test volley. Figure 13 (record A) shows records from two such units, one of which responded monosynaptically and the other polysynaptically to the afferent volley. Both spikes responded to the test volley in an all or none fashion, which established their unitary nature, and the difference in amplitude between the two spikes established that they were generated by different ventral root axons. During the control period both units displayed intermediate firing indices which were prerequisite for the detection of facilitation. Following the injection of SCh, the population reflex discharges responded in a typical fashion; the MSRR was depressed whereas the PSRR was facilitated. The behavior of the unit responses was identical to that of the simultaneously recorded population responses. The firing index of the unit PSRR increased following SCh administration and the time course of this change was a mirror image of the time course of depression shown by the unit MSRR. Three additional unit PSRR's with intermediate firing indices were subjected to the action of SCh and in each case the firing index was increased (Table I). In one of these experiments both the unit MSRR and the unit PSRR apparently originated from the same ventral root axon in that their amplitudes were indistinguishable. The response of these two discharges to SCh, which presumably originated from the same motoneuron, was identical to those previously described. The firing index of the spike which responded monosynaptically was depressed in contrast to the firing index of

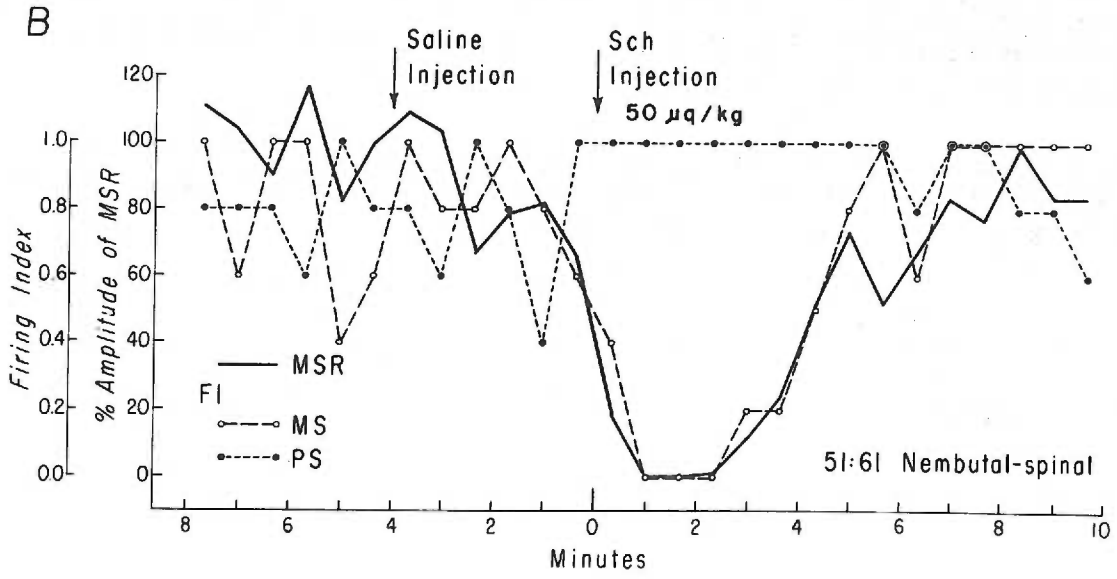
FIGURE 13

The effect of SCh on simultaneously recorded monosynaptic and polysynaptic unit and population reflexes in an anesthetized and spinal preparation.

A. The first and third columns are the population reflex discharges that were recorded from VRL7 whereas the second and fourth columns are the unit discharges that were recorded simultaneously from a small filament of VRL7. The three control (con) records demonstrate that both units responded to the evoked afferent volleys with intermediate firing indices. The remainder of the traces were recorded at the indicated time (minutes) after the administration of SCh (50 μg/kg).

B. Graphic description of the series of responses partially illustrated in record A. The construction of the graph is identical to that of Figure 12 excepting the addition of the firing index of the polysynaptic unit.


A
MSRR
VR UNIT
MSRR
VR UNIT
CON
CON
CON
0·5
1·0
3·0
5·0
9·0
1 mV
2 msec


B
Saline Injection
Sch Injection
50 µq/kg
Firing Index
% Amplitude of MSR
MSR
FI
MS
PS
51:61 Nembutal-spinal
Minutes

the PSR which was increased to unity. The elevation of the firing indices of these unit PSRR's eliminates the possibility that synchronization of the polysynaptic discharge accounted for the observed increase in the amplitude of the population PSRR's.

It is understood that the alterations in the PSRR (unit or population) following SCh administration do not have the same significance as do changes in the amplitude of the MSRR. The presence of one or more interneurons in the polysynaptic reflex pathway provide for an unknown number of uncontrolled variables. Thus we do not know if the augmented PSRR implies a lowered threshold of its motoneurons or if the total effective presynaptic drive to its motoneurons was increased by the drug-induced afferent barrage.

Increased Random Discharge of Motoneurons

The increased random discharge of motoneurons that follows the intravascular administration of SCh and which was illustrated in Figure 7 will be considered in some detail. Possible mechanisms suggested by this phenomenon which could explain the drug-induced reflex depression include: recurrent inhibition, after hyperpolarization and occlusion or excessive seizure of the involved motoneurons. It is understood from the onset that the discharge of previously quiescent motoneurons does not prove that the excitability of these motoneurons was actually increased. We have no information relating to the effect of the total, spontaneous, presynaptic drive acting on these motoneurons subsequent to the administration of SCh.

Quiescent motoneurons began to discharge 10 to 20 seconds after the injection of SCh with the maximum rate of discharge being reached in another 10 to 20 seconds (Fig. 7). The duration of this random discharge was usually quite short relative to

that of the MSRR depression, and in these experiments persisted no longer than 2 minutes. Table I summarizes the duration of the drug-induced motoneuronal discharge for a total of 14 preparations: decerebrate, decapitate and anesthetized. It is noted that the discharge in the anesthetized and spinal preparations was quite brief if present at all. In contrast to the anesthetized preparations, random discharges were evoked regularly in the majority of decerebrate or decapitate preparations with small doses of SCh (50 μg/kg).

In agreement with Henatsch and Schulte (68) it was observed that the random discharge of motoneurons was apparently dependent on the drug-induced afferent barrage since the discharge was abolished by dorsal rhizotomy. However, at best this conclusion is only tentative due to the limitations of transection procedures that were discussed previously. Drug localization experiments which might have provided a conclusive answer to this question were not attempted.

It was of interest to learn which motoneurons contributed to the random discharge after SCh injection. Specifically, the question arose whether or not those motoneurons which responded monosynaptically to the evoked afferent volley and whose firing indices were depressed following SCh injection also underwent a random discharge. If so this would provide evidence to support the hypothesis that the MSRR depression was due to after hyperpolarization or occlusion of those motoneurons contributing to the MSRR. If the monosynaptically discharged motoneurons did not undergo a random discharge following SCh administration this would favor an inhibitory mechanism. This problem was approached experimentally by recording the unit's behavior continuously in the 8 second intervals between the evoked monosynaptic discharges. The results of 14 such experiments are summarized in Table I.

TABLE I

Changes in Evoked and Spontaneous Unit Behavior Following SCh

PREPARATION	MSRR		PSRR		DURATION*
	FI	Spont	FI	Spont	
Decerebrate	Decr	Decr			90
Decerebrate	Decr				-
Decerebrate	Decr	Decr			120
Decapitate	Decr		Incr	Incr	120
Decapitate	Decr	Incr	Incr	-	50
Decapitate	Decr	-			90
Decapitate	Decr	-			70
Decapitate	Decr	-			120
Anesthetized	Decr	-	Incr	-	-
Anesthetized	Decr	-			30
Anesthetized	Decr	-	Incr	-	-
Anesthetized	Decr	-			-
Anesthetized	Decr	-			60
Anesthetized	Decr	-			-

* Duration in seconds of spontaneous discharge in ventral root fibers other than those identified as participants in evoked reflex activity.

The 50 μg/kg dosages of SCh depressed the monosynaptic firing indices of all 14 units but only one of these units underwent a random discharge following the drug injection. In contrast 1 of the 4 isolated unit PSRR's demonstrated random firing. The small percentage of unit MSRR's which demonstrated a random discharge following the administration of SCh does not lend support to the forementioned hypotheses of MSRR depression. However, the data summarized in Table I was biased by a sampling problem. In some of the earlier experiments the dissected ventral root filaments were too large and what appeared to be a filament containing only one or two easily distinguishable units prior to the drug injection revealed the presence of 5 or 6 such

units following SCh administration. In these cases it was not possible to tell with accuracy whether or not the monosynaptically evoked unit underwent a random discharge. Therefore, these data were discarded and only results from those decerebrate or decapitate preparations about which there was no doubt were included in Table I. Further experimentation was confined to the anesthetized and spinal preparations in which the level of motoneuronal excitability was low so that random discharges were only occasionally observed. In these preparations none of the unit MSRR's underwent a random discharge following SCh. When the technique of dissection was perfected, further experiments on decerebrate and decapitate animals were not conducted since the results of other investigators (68, 51, 66) had become available. In view of the sampling problem there is little doubt that the percentage of decapitate and decerebrate preparations which actually undergo a random discharge of motoneurons is greater than the above data would indicate.

Two of the units which responded monosynaptically to the evoked afferent volley were also discharging spontaneously prior to the drug injections at rates of 20 to 40 impulses per second. The observed effect of intravenous SCh was not an enhancement of the existing discharge rate as reported by Fugimori and Eldred (51) but rather a depression. Such behavior implies inhibition but the data does not indicate where the inhibition occurred, nor does it indicate the nature of the inhibition.

Before leaving the subject of random discharges, a serious source of error must be considered. The role of recurrent inhibition in the SCh-induced MSRR depression is considered to be prominent by some investigators (68, 51, 66). This conclusion was based on the observation that pretreatment of the animal with dihydro-beta-erythradine (DHE) lessens the degree of MSRR depression produced by SCh. How-

ever, in addition to the known central action of DHE on cholinergic synapses (33), it might be anticipated that DHE would exert a peripheral action. To examine the possibility that DHE might have interfered with the peripheral action of SCh, a dorsal root filament was isolated and exposed to SCh injection in standard fashion. The spindle responded to the 50 μg/kg dosage with a large increase in discharge rate; however, when the same spindle was subjected to the same dose of SCh one-half hour later, but after pretreatment with DHE (0.5 mg/kg), only minimal discharge occurred. Thus, it would appear that DHE does prevent SCh from exerting its full peripheral action on muscle spindles and this observation should be considered when interpreting the results of experiments in which DHE was utilized.

Presynaptic Inhibition and Excitability Changes of the Intraspinal Portions of Large Afferent Axons

As was stated in the INTRODUCTION, it has recently been demonstrated that a synchronized afferent nerve volley exerts a central action which depolarizes the intraspinal portions of other large afferent fibers. A subsequent afferent nerve volley conducted by depolarized primary afferent fibers generates a reduced excitatory postsynaptic potential (EPSP), which presumably is the result of a diminished transmitter output. The phenomenon of EPSP reduction secondary to the depolarization of primary afferent fibers has been termed presynaptic inhibition (32).

In view of the afferent barrage which follows the administration of SCh, presynaptic inhibition is another mechanism which could account for the drug-induced reflex depression; any factor which produced sufficient EPSP depression would be expected to manifest itself as MSRR depression. However, only synchronized

afferent nerve volleys have been demonstrated to evoke intra-spinal afferent fiber depolarization and it is not yet known if an asynchronous afferent input, such as that occurring after SCh administration, is capable of inducing a similar change.

The hypothesis that MSRR depression following SCh administration is due at least in part to augmented presynaptic inhibition was tested by measuring the excitability of the intraspinal portions of large afferent fibers before and after the administration of SCh. The results to be described were performed on animals subjected to decerebration or sodium pentobarbital anesthesia with spinal transection. In every experiment the ipsilateral GS and PBST nerves were transected. A schematic diagram of the experimental method is shown in Figure 14-A. A number of preliminary observations which define this technique and its limitations will be presented before the results of the experiments which involved the use of SCh are described. A preliminary account of this investigation was reported elsewhere (22).

Characteristics of Antidromic Responses Evoked by Intraspinal Stimulation

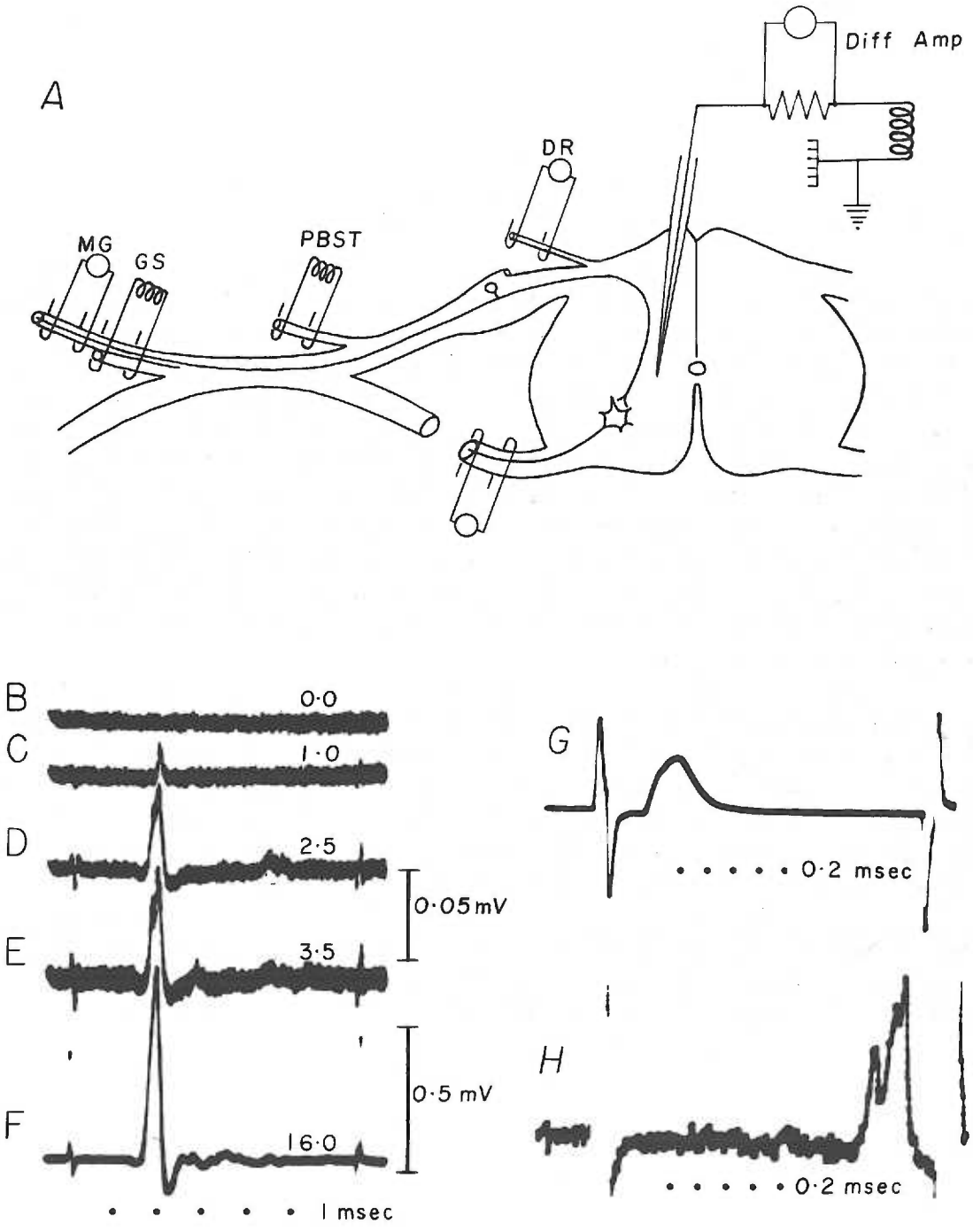
Intraspinal stimulation evokes an antidromic volley which can be recorded from large afferent fibers in the appropriate peripheral nerve (138, 37). One such response evoked by stimulation within the seventh lumbar segment and recorded from the MG nerve is illustrated in Figure 14, (records B-F). The amount of current entering the tip of the microelectrode was increased from that value which evoked a just threshold response (record C) to that value which evoked a maximal response (record F). Since the ipsilateral ventral roots (L6-S2) had been transected the responses could have only been antidromically conducted in afferent fibers which originated from the medial head of the gastrocnemius muscle. It is noted that the

FIGURE 14

A. Schematic illustration describing the method that was utilized to test intramedullary, afferent fiber excitability. A stimulating microelectrode (cathode) was inserted parallel to the intraspinal pathway of the medial division of the dorsal root. The indifferent stimulating electrode consisted of multiple contacts, established whereever metal table appliances touched the animal. A differential cathode-ray oscilloscope served to monitor the stimulating current in five experiments. The evoked antidromic volley was recorded from the central end of a dorsal root filament (DR) or one branch of the nerve to the gastrocnemius-soleus muscle. Stimulating electrodes were fitted on the nerves supplying the gastrocnemius-soleus (GS) and posterior biceps-semitendinosus (PBST) muscles; The former to elicit a MSRR and the latter to evoke an afferent conditioning volley. MG: nerve to the medial head of the gastrocnemius muscle.

B-F. Records of antidromic responses monophasically recorded from the MG nerve and evoked by stimulation within the seventh lumbar segment. Each record is composed of 10 superimposed sweeps. The stimulating current was increased from zero (record B) to that yielding the maximal antidromic response (record F). The stimulus intensity, relative to threshold stimulating current, is indicated above each record. The artifact from the second stimulating pulse is apparent in records C-F.

G-H. Single traces of an antidromic volley simultaneously recorded from the central end of a dorsal root filament (record G) and the MG nerve (record H). The intervening conduction distance was 16.0 cm.


A
Diff Amp
DR
MG
GS
PBST
B
0·0
C
1·0
D
2·5
0·05mV
E
3·5
F
16·0
0·5 mV
1 msec
G
0·2 msec
H
0·2 msec

small, late antidromic discharges appeared only with the greater stimulus intensities. The short latency (1.7 msec) and low threshold of the early, large antidromic response suggested that the stimulating current was activating low threshold, fast conducting afferent fibers. This contention was supported by conduction velocity studies. The antidromic responses in records G and H were evoked by stimulation within the seventh lumbar segment and recorded simultaneously from the central end of a dorsal root filament (record G) and the MG nerve (record H). The difference in latency of the two responses measures 1.6 msec and the distance separating the two pairs of recording electrodes was 16.0 cm. Therefore, the first component of this antidromic response was conducting at a rate of 100 m/sec. The antidromic responses from two other experiments were tested in a similar manner and were found to conduct at velocities of 92 and 120 m/sec. Conduction velocities of 92, 100 and 120 m/sec are equivalent to 15, 17 and 20 micron nerve fibers, respectively (78). Afferent fibers of these diameters belong to Group I (73) as do those fibers which form the afferent arc of the MSRR (93). In the remainder of experiments the conduction velocity was not determined; the short latency and low threshold of the antidromic response ensured that Group I afferent fibers were activated.

It is understood that the technique of intraspinal stimulation employed here did not distinguish between the IA and IB components of Group I afferent fibers. Hunt (73) has provided conclusive evidence that the diameter overlap of IA and IB fibers in the gastrocnemius-soleus nerve is so great that differentiation of these fibers by conduction velocity is not possible. Nor does it seem likely, as advocated by some (37), that placement of the microelectrode tip in the ventral horn permits selective stimulation of the Group IA central terminals to the exclusion of the more dorsally

terminating Group IB afferent fibers. In view of the unknown current field which accompanies monopolar stimulation this viewpoint cannot be accepted without experimental proof. Therefore, in this study the antidromic responses are considered to represent active Group I afferent fibers and no distinction is made between the IA and IB components.

Excitability Changes of Large Afferent Fibers

In agreement with others (138, 37), it was found that the amplitude of the antidromic response could be augmented by afferent conditioning volleys in nerves other than the nerve conducting the antidromic volley. Record A (Fig. 15) describes graphically the influence of afferent PBST volleys on submaximal antidromic responses recorded from the nerve to the MG muscle. Not until the PBST volley preceded the antidromic response by 4 to 5 msec did facilitation of the antidromic volley appear. As the conditioning interval was increased further, the degree of facilitation rapidly increased and maximum facilitation was observed at intervals of 15 to 25 msec. The facilitatory effect gradually diminished thereafter, and no apparent effect remained at conditioning intervals of 100 msec or more. Since the duration of the antidromic response remained constant (see Fig. 17-A, B), the augmented amplitude of the conditioned volley implies that additional fibers were discharged by the constant test stimulus secondary to an enhanced excitability of these fibers. The enhanced excitability or lowered threshold of the intraspinal portions of these large afferent fibers is known to result from a depolarization of their central terminals (138, 37).

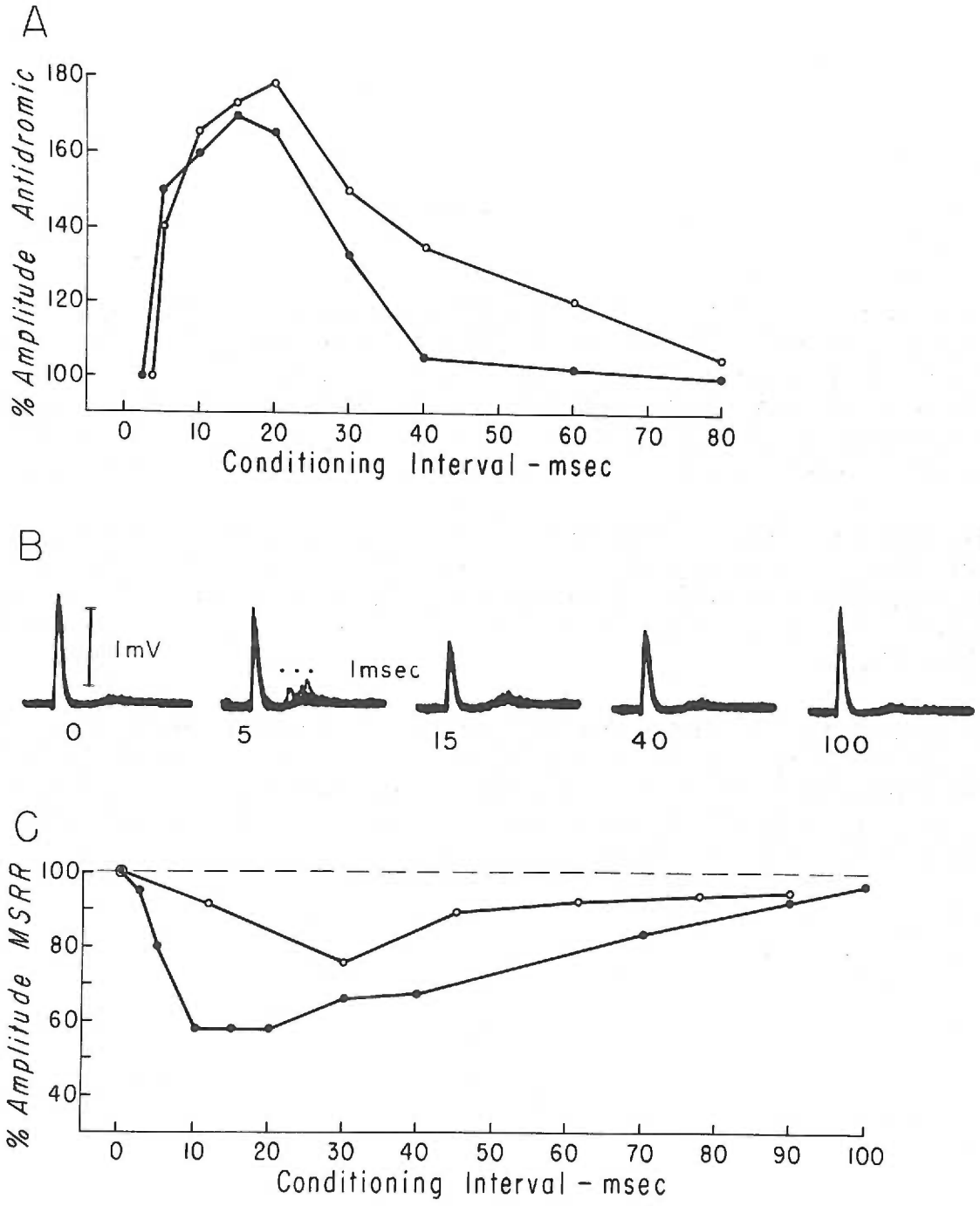
The time course of the excitability change shown in record A is less than the

FIGURE 15

A. The percent amplitudes of two antidromic responses are plotted as a function of the time that a single PBST volley preceded each antidromic response. Each point is the mean amplitude of 10 antidromic responses at the same conditioning interval. The two curves were obtained from different preparations, but otherwise are identical. In each case the antidromic stimulus was 1.5 times threshold value. Records similar to those illustrated in Figure 17 (records A, B) were used to construct these curves.

B. Records of maximal gastrocnemius MSRR's which were recorded from VRS1 in a decerebrate preparation. Each MSRR was preceded by single shock stimulation of the ipsilateral PBST nerve. The conditioning interval is indicated beneath each record in msec. Each record consists of 10 superimposed sweeps. Rectal temperature - 37.0 °C.

C. The influence of single afferent PBST volleys on maximal gastrocnemius MSRR's is graphically described by the data obtained from two decerebrate preparations. The construction of the curves is identical to those illustrated in A. The curve exhibiting the greatest depression (filled circles) was constructed from responses partially illustrated in record B.


A
% Amplitude Antidromic
180
160
140
120
100
0
10
20
30
40
50
60
70
80
Conditioning Interval - msec
B
1mV
1msec
0
5
15
40
100
C
% Amplitude MSRR
100
80
60
40
0
10
20
30
40
50
60
70
80
90
100
Conditioning Interval - msec

200 msec duration which is described as characteristic for this phenomenon (37). In view of the observations of Eccles' group (37 - cf. Figs. 10 and 12), the brief time course observed here most likely resulted from stimulus shocks which did not activate all Group I fibers in the PBST nerve.

Primary Afferent Depolarization and Reflex Depression

The magnitude of the monosynaptic EPSP is reduced when the intraspinal portions of the afferent fibers evoking the EPSP are depolarized (37). If the EPSP depression was of sufficient magnitude, primary afferent depolarization (Fig. 15 - A) would be expected to manifest itself as MSRR depression. This hypothesis was tested by evoking MSRR's by stimulation of the GS nerve, and by preceding the evoked reflex responses at increasing time intervals with afferent PBST conditioning volleys. The results of two such experiments are described by Figure 15 (records B, C) and as predicted, the MSRR was depressed by the afferent PBST volleys. The time course of the reflex depression was essentially identical to the time course of the afferent fiber depolarization described by record A. The presence of augmented multisynaptic discharge is also to be noted in record B. Thus, afferent PBST volleys mimic the action of SCh; MSRR depression coupled with PSRR facilitation.

Before concluding that the reflex depression illustrated in records B and C resulted from presynaptic inhibition, the possible roles of direct and recurrent inhibition should be considered. Direct inhibition can be dismissed as a contributing factor in view of the difference in time courses of direct (95) and presynaptic (32, 37) inhibition. However, recurrent inhibition is not so easily dealt with in these experiments, since the conditioning volley (PBST) did generate a minimal monosynaptic ventral

root discharge. This raises the question of whether or not recurrent inhibition contributed to any of the observed MSRR depression. The time course of recurrent inhibition as determined by both reflex testing (118) and intracellular recording (33) does differ from the time course of the reflex depression illustrated in records B and C. However, the difference is not sufficient to exclude entirely recurrent inhibition.

The Sensitivity of the Antidromic Response

Before negative results can be considered significant, it must be known whether or not the sensitivity of the utilized test system was sufficient to detect the sought after change. This concept relates directly to the present study. A number of factors were observed to alter the sensitivity of the antidromic response to the known depolarizing influence of afferent PBST conditioning volleys. In addition to the conditioning interval (Fig. 15-A); the placement of the microelectrode, the strength of the antidromic test stimulus, and the temporal stability of the antidromic volley were found to relate to a given antidromic volley's response to a constant PBST volley.

The positioning of the microelectrode tip was a crucial factor in the elicitation of sensitive antidromic responses. The following procedure was employed to determine what intraspinal portion of the large afferent fibers demonstrated the greatest excitability change following a standard PBST volley. The electrode was introduced into the spinal cord in a dorsoventral direction and approximately parallel to the intraspinal course of primary afferent fibers (Fig. 14-A). At 0.5 mm intervals along the course of this downward penetration an antidromic volley was evoked, and its response to a constant PBST conditioning volley ascertained. The current required to elicit antidromic responses was not constant at each tested depth, but

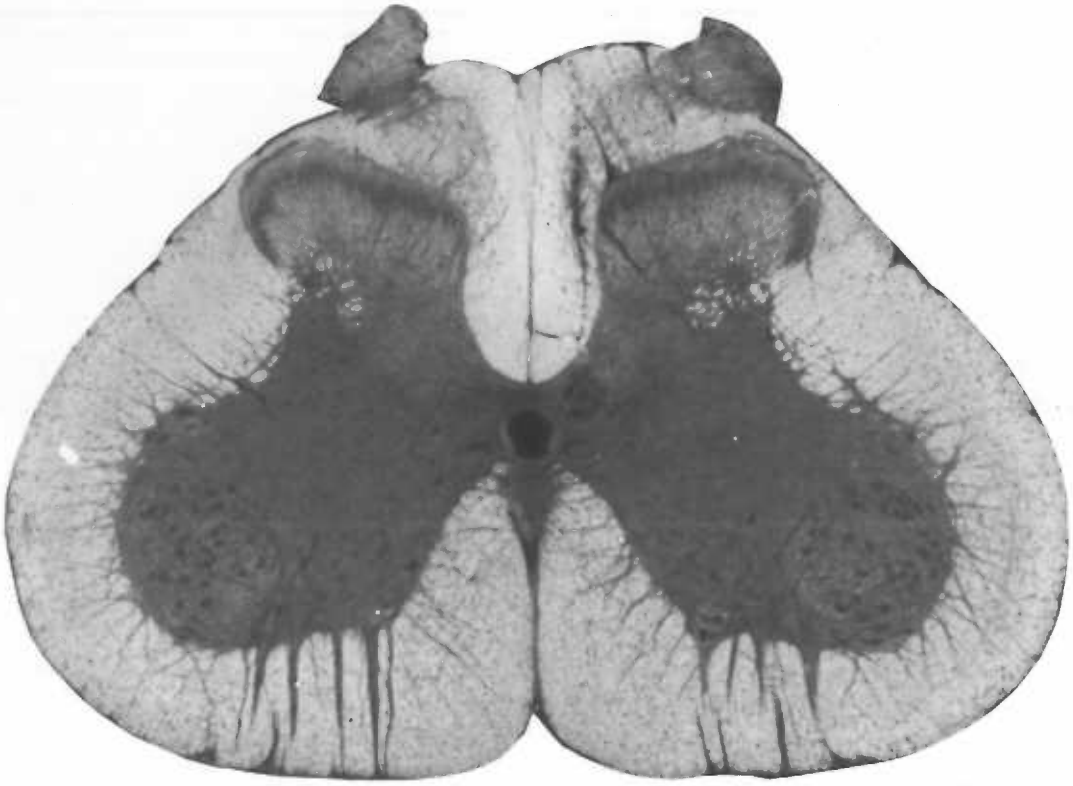
rather, increased as the tip of the microelectrode was advanced ventrally. To enable comparison of the effect of the PBST volley on the antidromic response in face of the changing current requirements, each intraspinal shock was adjusted to the same relative value. For example, the amount of current employed at each tested depth was three times that required to evoke a threshold response at that depth. Thus along the intraspinal course of the primary afferent fibers, antidromic responses composed of approximately the same number of units were evoked. The relative percent change in amplitude of the antidromic volley by the PBST volley could then be compared.

With this procedure, the results obtained here confirmed the observations of others (37). With reference to a standard PBST conditioning volley, a minimal increase in the excitability of the intraspinal afferents occurred with the tip of the microelectrode placed in the dorsal column. With further ventral advancement of the tip, the enhancement of excitability increased and maximal excitability increases were observed in the lower portions of the central grey or the upper portions of the ventral horn. The histological controls shown in Figures 16, 17, 18 and 19 localize the position from which the greatest change in excitability was observed for that penetration.

The apparent loss of excitability changes in the ventral horn is thought to be artifactual. As primary afferent fibers descend to the ventral horn and diacotomize, the density of the fibers decreases and the diameter of each fiber becomes progressively smaller (16). An increased threshold would be the expected manifestation of decreased fiber diameter, and this would account adequately for the relatively large stimulating currents required to evoke antidromic responses from the ventral horn.

FIGURE 16

Photomicrograph of an unstained, 60 micron frozen section of a cat's seventh lumbar spinal segment. The electrode tract is apparent extending down through the dorsal column and the lesion, which locates the electrode tip, is just dorsolateral to the central canal. Approximately 18x.



The large currents would undoubtably discharge antidromically some of the finer terminals in the ventral horn, but many more fibers would be expected to be discharged from more dorsal locations, where the density of primary afferent fibers is greater and their threshold is less. The discharge of afferent fibers from their more dorsal portions and where the degree of excitability change is small, would tend to mask any changes that occurred in the finer terminals.

An alternate explanation for the absence of demonstrable excitability changes in fibers coursing through the ventral horn is that the membrane depolarization, which underlies the excitability change, does not occur in these portions of the afferent fibers. This viewpoint, according to Eccles' group (37), does not seem likely.

Another factor which influenced the apparent sensitivity of the antidromic response was the number of afferent fibers discharged by the testing stimulus. Again, with reference to a standard conditioning volley, the largest amplitude increases were observed when the antidromic response was evoked by currents 1.5 to 4 times the threshold value. It is appreciated from Figure 14 (records B-F) that responses of this magnitude were greatly submaximal. With increases in response size above these values, the amount of facilitation progressively decreased and antidromic volleys evoked by currents 6 to 7 times the threshold value failed to demonstrate any change. It would seem that the failure of the larger antidromic volleys to demonstrate facilitation was related to the same considerations discussed under microelectrode placement.

In some experiments, the amplitude of the antidromic response was noted to progressively decline over a short period of time. The loss of amplitude in most

instances was associated with a loss of response amplitude variation as well. The factors responsible for the loss of amplitude and the variation in response amplitude were not well defined but may have included: injury or death of the stimulated afferent fibers; a gradual movement of the stimulating microelectrode; polarization at the electrode tip or an increased intraspinal resistance. Regardless of the cause, such responses were observed to have lost their ability to respond with facilitation to PBST conditioning volleys. As such, responses with diminished amplitude and diminished variation in amplitude were of no value as sensitive indicators of afferent fiber depolarization.

The Effect of SCh on the Antidromic Response

A number of the preceding techniques and principles were utilized to test the hypothesis that the SCh-induced MSRR depression is due to augmented presynaptic inhibition. These are summarized as follows:

1) Stimulation within the appropriate spinal segment evokes an antidromic volley in afferent fibers originating from the GS muscle. Both the orthodromic volley which generates the gastrocnemius-soleus MSRR and the antidromic volley are conducted by Group I afferent nerve fibers.

2) The area of the antidromic response is a direct measure of the excitability of the stimulated intraspinal afferent fibers. In place of area measurements, the amplitude of the antidromic response may be utilized as an index to the excitability of the stimulated fibers if the duration of the response remains constant. An increase

in the amplitude of the response implies an enhanced excitability of the stimulated intraspinal fibers and vice versa.

3) Afferent conditioning volleys in the PBST nerve are a convenient and useful means of testing the sensitivity of a given antidromic volley to presynaptic depolarizing influences. The sensitivity of the antidromic volley must be ascertained before the response can be used to measure the amount of intraspinal afferent fiber depolarization produced by other factors.

4) In this study the greatest facilitation of the antidromic response was produced by constant PBST conditioning volleys when the following conditions were met: the antidromic response was evoked by stimulation within the lower portion of the central grey or the upper portion of the ventral horn; the response was evoked by a stimulus 1.5 to 4 times the threshold value of that stimulus; and the amplitude and variation in amplitude of the antidromic volley remained stable and did not alter with the passage of time.

The application of the foregoing techniques and principles to the problem of presynaptic inhibition and the drug-induced reflex depression is illustrated by the data obtained from a single experiment (Fig. 17). In this and subsequent experiments the antidromic response and the MSRR were evoked alternately at 4 second intervals to maintain the standard stimulus interval of 8 seconds. Record A shows 10 superimposed antidromic responses evoked by stimuli 3 times the threshold value. The same stimuli evoked the antidromic responses shown in record B but in this case each antidromic response was preceded at a fixed conditioning interval by a con-

FIGURE 17

The effect of intravenous SCh on alternately evoked antidromic and MSR responses in a decerebrate preparation (rectal temperature 37.4° C).

A. Ten superimposed antidromic responses recorded from the MG nerve and evoked by stimulating currents three times the threshold value (the inset in record E locates the tip of the microelectrode in the seventh lumbar segment).

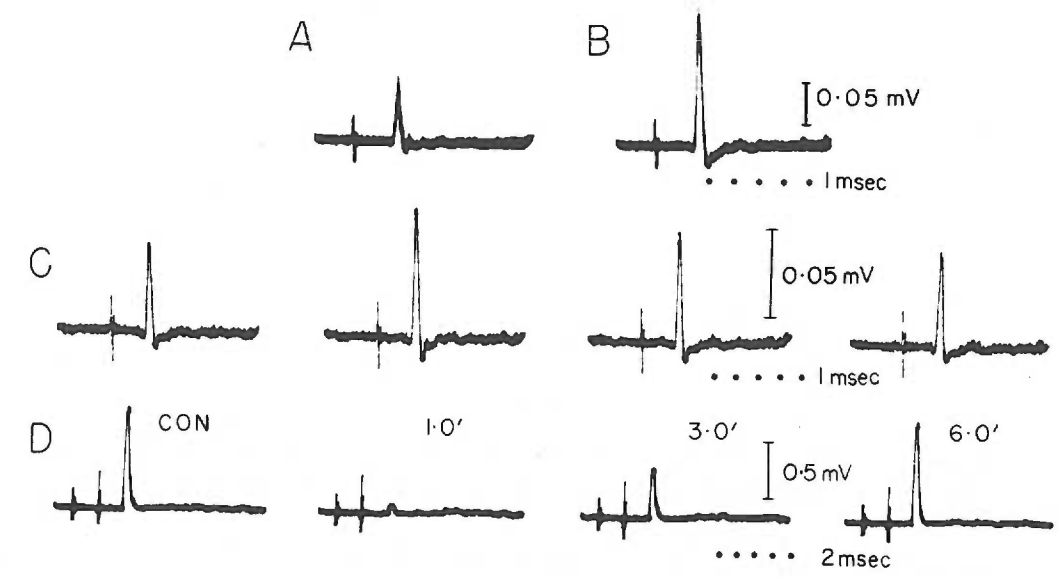
B. Identical to A, but each of the ten antidromic responses was preceded by a single PBST afferent volley. The conditioning interval was fixed at 20 msec.

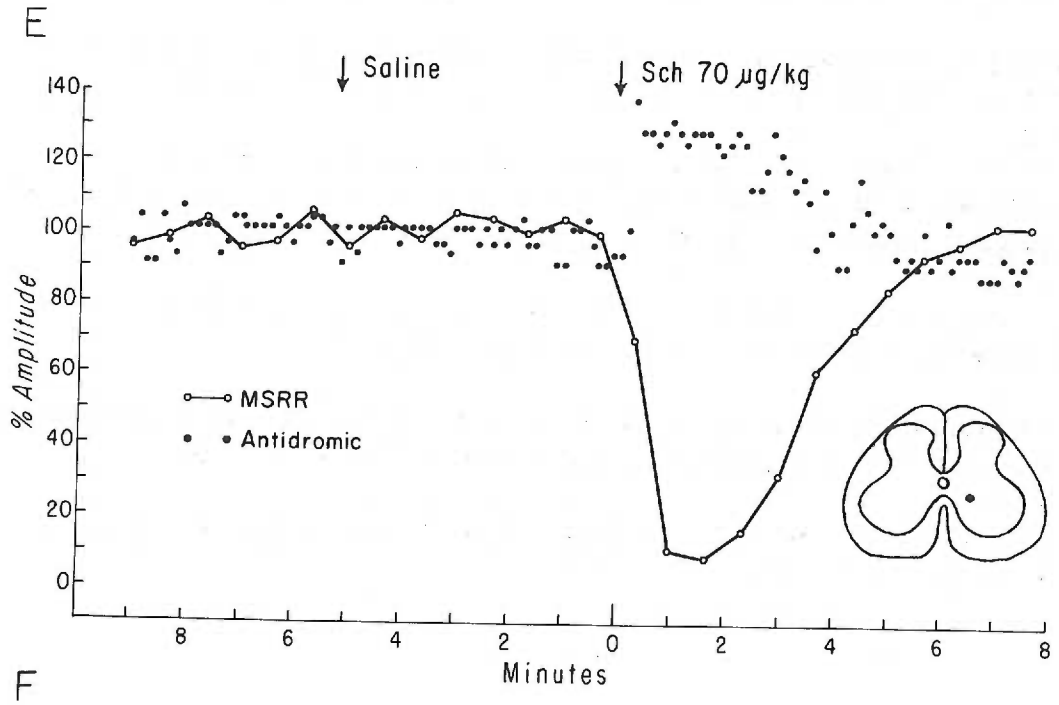
C. Single traces of the same antidromic response at higher gain before and after SCh administration. The time that the response followed the injection of SCh is indicated in minutes beneath each record.

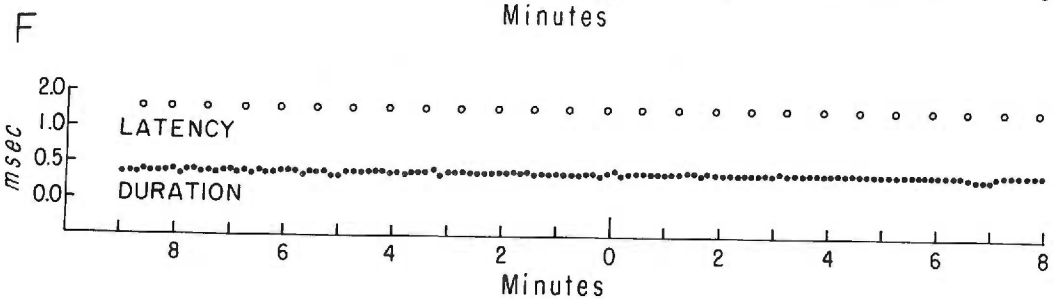
D. Records of the alternately evoked maximal MSRR, generated by double shock stimulation of the GS nerve and recorded from VRSI.

E. The percent amplitude of each antidromic response and the mean percentage amplitude of 5 consecutive MSRR's is plotted as a function of time.

F. The latency of every fifth antidromic response and the duration of each is plotted as a function of time.


A
B
0·05 mV
1 msec
C
0·05 mV
1 msec
D
CON
1·0′
3·0′
6·0′
0·5 mV
2 msec


E
Saline
Sch 70 µg/kg
% Amplitude
MSRR
Antidromic
Minutes


F
msec
LATENCY
DURATION
Minutes

stant PBST afferent nerve volley. The approximate 100 percent facilitation of the antidromic response which resulted demonstrated this antidromic response to be a sensitive indicator of primary afferent depolarization. The behavior of this antidromic response and that of the alternately recorded MSRR to the intravenous administration of SCh are shown in records C and D. Following the drug injection the MSRR underwent a typical depression whereas the antidromic volley was facilitated. The identical time courses of the reflex depression and of the facilitation of the antidromic response are best appreciated from the graphic plot of response amplitudes in record E. The augmented amplitude of the antidromic response is not attributable to a synchronization of the responding units since the duration of the antidromic response remained constant throughout the experiment (record F). Thus, the augmented antidromic response reflects an enhanced excitability of the stimulated gastrocnemius afferent fibers which provisionally will be attributed to a depolarization of these fibers.

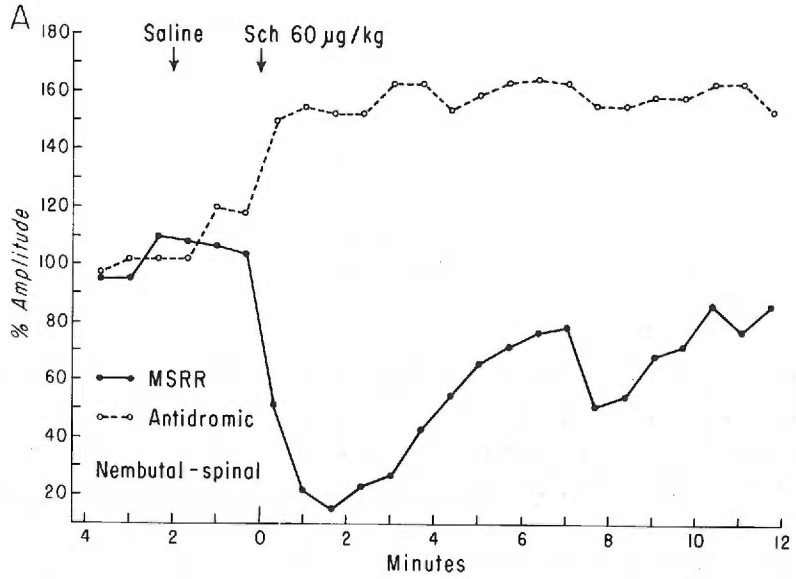
Further information regarding the antidromic volley and its response to SCh is provided by Figures 18 and 19. The data of any one of these experiments were not considered typical by themselves; however, taken as a group, experiments of the type exemplified by these figures were the basis for the conclusions drawn from this study.

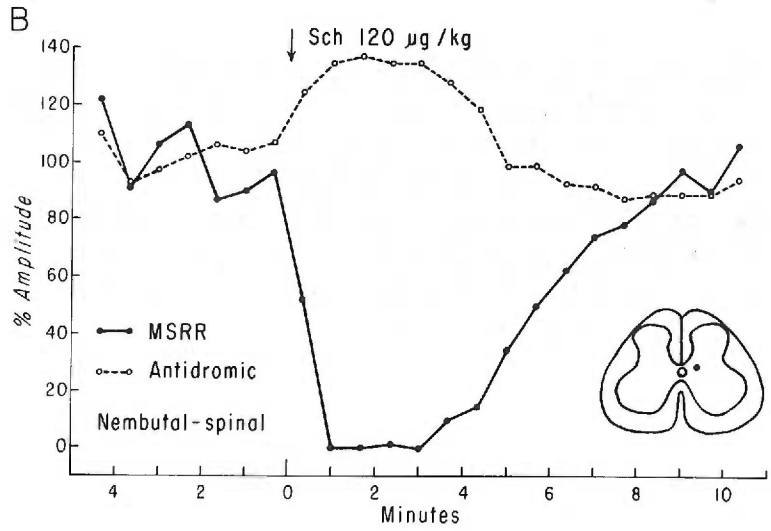
Records A and B of Figure 18 graphically illustrate the amplitude changes of an antidromic volley which responded to a small dose of SCh with a large amplitude increase but which failed to return to its preinjection value. The reason for such behavior was not apparent but the failure of the amplitude of the MSRR to regain

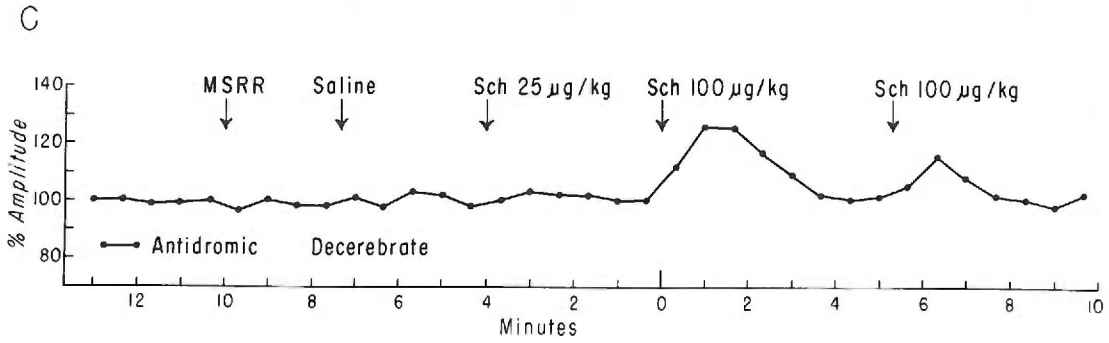
FIGURE 18

A-B. The percentage amplitudes of antidromic and MSR responses are plotted as a function of time. Each point is a mean value for 5 consecutive response amplitudes. The antidromic response was elicited by stimulation within the seventh lumbar segment (see inset) and was recorded from the MG nerve. The MSRR was evoked by double shock stimulation of the GS nerve and recorded from VRL7. The population of intraspinal fibers described by record A was retested one hour later with a larger dose of SCh (record B).

C. Data from another preparation in which the microelectrode was inserted into the caudal portion of the seventh lumbar segment and the antidromic volley was recorded from the GS nerve. The MSRR was not photographed during this experiment, but instead was displayed on a monitor scope and observed. A series of control antidromic responses was obtained before the MSRR was evoked alternately. The amplitude of the antidromic response was not altered significantly by the addition of the MSRR. Thus, the excitability changes produced by the afferent MSRR volley were not detectable four seconds later.


A
Saline
Sch 60 µg/kg
% Amplitude
MSRR
Antidromic
Nembutal-spinal
Minutes


B
Sch 120 µg/kg
% Amplitude
MSRR
Antidromic
Nembutal-spinal
Minutes


C
MSRR
Saline
Sch 25 µg/kg
Sch 100 µg/kg
Sch 100 µg/kg
% Amplitude
Antidromic
Decerebrate
Minutes

completely its control value was noted as well. The same MSR and antidromic responses were retested one hour later with a larger dose of SCh and in this case the amplitudes of both responses returned to their control values. The behavior of the recorded responses was not correlated to the observed changes in systemic blood pressure as can be appreciated by comparing these records with the pressure tracings illustrated in Figure 8 (record G).

Movement of the electrode during the experiment is a serious, potential source of error always present with microelectrode studies. This problem was compounded in this investigation by the SCh-induced muscle fasciculations. In addition to the rigid fixation of the spinal column and the removal of the paravertebral muscles to reduce movement of the spinal cord, experiments were designed to eliminate this source of artifact as being responsible for the facilitation of the antidromic response following SCh administration. One such experiment is described by the data shown in record C (Fig. 18). In this decerebrate preparation the injection of 25 μg/kg of SCh was followed by violent muscle fasciculations but the amplitude of the antidromic response was not altered. The first of the two 100 μg/kg injections of SCh was followed by a lesser degree of fasciculations but marked facilitation of the antidromic response resulted. In contrast the second 100 μg/kg injection was not followed by fasciculations but the antidromic valley responded in typical fashion. Additional evidence that the augmented amplitude was not secondary to movement was provided by the occasional animal which did not fasciculate in response to SCh and by the drug localization experiments to be described in a later section.

It would seem that the time course of afferent fiber depolarization and the time

course of the MSRR depression should be similar before presynaptic inhibition can be considered as a cause of the drug-induced reflex depression. Similar time courses were observed in the experiments illustrated by Figures 17 and 18 but this relationship was not found in all experiments. For example, in the preparation whose data is plotted in Figure 19 (record A) the 60 ug/kg injection of SCh resulted in a marked facilitation of the antidromic response. But when a larger dose of SCh was injected soon thereafter only minimal facilitation resulted. In fact the facilitation of the antidromic response was over before the depressed MSRR had started to recover. Such discrepancies in time course do not support a cause and effect relationship for presynaptic inhibition and the reflex depression. However, it is thought that evidence relating to the reason for such behavior is contained within the record. The amplitude of the antidromic response was declining rapidly after the first drug injection and stabilized at approximately 50 percent of its original amplitude. In addition this response had lost its characteristic temporal variation in amplitude as indicated by the almost straight line which describes this response prior to and following the second drug injection. These two factors, loss of response amplitude and loss of variation in response amplitude, were previously described as indicating a loss of sensitivity to a known and standard depolarizing influence. On this basis it seemed reasonable to attribute the failure of the antidromic volley to respond to the second drug injection to a loss of sensitivity.

Record B (Fig. 19) describes graphically another experiment in which the amplitude of the antidromic response was declining prior to drug administration. Again, the time course of enhanced excitability was considerably less than that of the reflex depression. To correct for the suspected loss of sensitivity, the stimulus

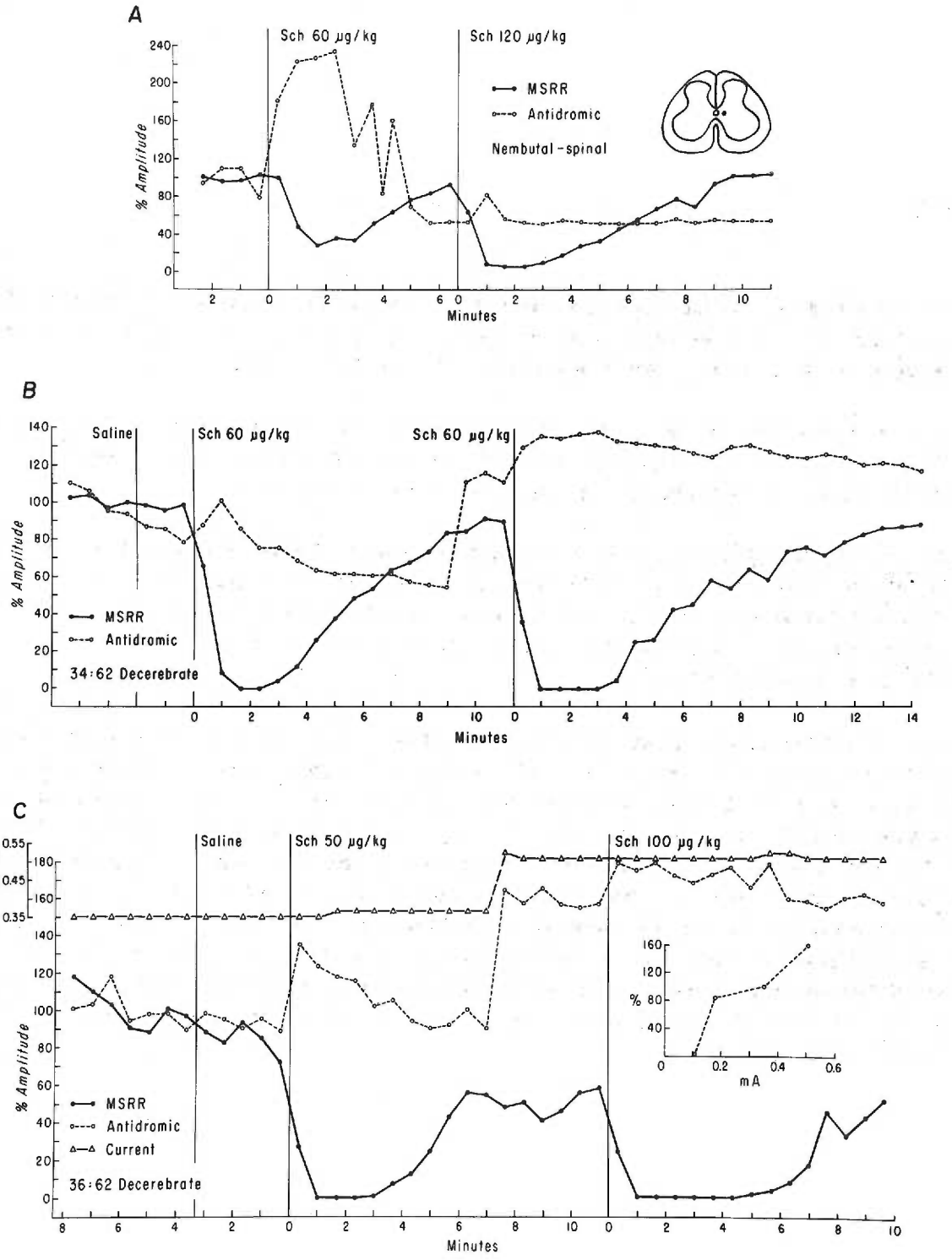
FIGURE 19

Time course comparisons between the increased excitability of afferent fibers and the reflex depression. In each record the percentage amplitudes of the antidromic volley and the MSRR are plotted as a function of time.

A. Anesthetized, spinal preparation in which the antidromic volley was evoked from L7 and was recorded from the MG nerve. The failure of a sustained excitability change is noted following the second injection of SCh.

B. In this decerebrate preparation the amplitude of the antidromic volley was rapidly declining prior to the first injection of SCh. Following the first injection of SCh, the stimulating current was increased (signaled by the abrupt change in amplitude) so as to evoke a stable antidromic response which was then retested with the same dosage of SCh.

C. The stimulating current for another decerebrate preparation's antidromic volley is plotted in milliampheres (mA) and the stimulus-response curve for the same antidromic volley is plotted in the insert. Following the first drug injection the stimulating current was increased and the drug readministered as was done in record B. The small increase in current which immediately followed the first injection of SCh was not responsible for the enhanced excitability because: 1) the excitability change occured before the increase in stimulating current; 2) the observed increase in stimulating current was within the range of error of the technique and; 3) the slight increase in current could not have produced the degree of amplitude change as is apparent from the stimulus-response curve established for this population of fibers (inset).


A
Sch 60 µg/kg
Sch 120 µg/kg
MSRR
Antidromic
Nembutal-spinal
% Amplitude
Minutes
B
Saline
Sch 60 µg/kg
Sch 60 µg/kg
MSRR
Antidromic
34:62 Decerebrate
% Amplitude
Minutes
C
Saline
Sch 50 µg/kg
Sch 100 µg/kg
mA
% Amplitude
MSRR
Antidromic
Current
36:62 Decerebrate
Minutes

intensity was increased until a new stable antidromic response was obtained. The changes in amplitude which then followed the second injection of SCh were identical with respect to time course. Record C describes a similar experiment in which the current that evoked the antidromic response was monitored as well. It was concluded from observations such as these that the time courses of the excitability increase and reflex depression were identical.

The preparation whose data was illustrated in record A (Fig. 19) was of further interest in regard to the blood pressure responses which followed the two injections of SCh (see Fig. 8-F). The 60 μg/kg injection was followed by a reduction in carotid blood pressure whereas the 120 μg/kg dosage resulted in a marked pressor response. These observations were interpreted as further evidence that the changes in systemic blood pressure were in no way related to either the MSRR depression or the antidromic response facilitation.

The data which were obtained from 19 observations in 10 consecutive preparations and which summarize the behavior of the antidromic response to the administration of SCh are presented in Table II. The amplitude of every antidromic response subjected to doses of 50 μg/kg or more demonstrated a statistically significant increase. The only amplitude increase which was not significant was that following a SCh dose of 25 μg/kg. This dosage was not sufficient to depress the alternately evoked MSRR. It is to be noted that the amplitude increases occurred regardless of the preparation type, regardless of the rectal temperature of the preparation, and regardless of whether the antidromic response was recorded from a dorsal root filament, from the GS nerve, or from one of its branches.

TABLE II

The effect of SCh on the excitability of the intraspinal portions of large afferent fibers. The columns from left to right read: the preparation (anesthetized and spinalized or decerebrate); the spinal segment utilized for intramedullary stimulation; the nerve the antidromic volley was recorded from; the rectal temperature of the preparation in degrees Centigrade; the dose of SCh in μg/kg; the mean percentage increase in the amplitude of the antidromic response following SCh administration; and the probability (P) that the increase in amplitude was due to chance alone. For each experiment the mean amplitude of the twelve antidromic responses beginning 24 seconds after the SCh injection was compared with the mean amplitude of the twelve responses that just preceded the drug injection with Student's t test. The probabilities of the observed differences being due to chance are much less than the stated value with the one indicated exception. GS – nerve to the gastrocnemius-soleus muscle; MG – nerve to the medial head of the gastrocnemius muscle; LGS – nerve to the lateral head of the gastrocnemius and soleus muscles; DR – dorsal root filament.

THE EFFECT OF SCh ON THE EXCITABILITY OF INTRASPINAL PORTIONS OF LARGE AFFERENT FIBERS

PREPARATION	SEGMENT	NERVE	TEMP	DOSE	MEAN AMPLITUDE INCREASE	P
Anesth	Mid S1	MG	37.3	60	27	0.001
Anesth	Mid L7	MG	35.7	60	125	0.001
Anesth	Mid L7	MG	37.4	60	41	0.001
Anesth	Hi L7	MG	38.8	60	43	0.001
Anesth	Hi L7	MG	37.2	120	30	0.001
Anesth	Mid L7	MG	36.8	120	37	0.001
Anesth	Low L7	MG	37.4	70	21	0.01
		DR			7	0.001
Decere	Mid L7	MG	37.4	70	31	0.001
Decere	Mid L7	MG	36.0	60	12	0.01
				60	20	0.001
Decere	Mid L7	MG	36.3	50	37	0.001
				100	19	0.001
Decere	Low L7	GS	34.0	25	1	>0.1
				100	24	0.001
				100	11	0.01
Decere	Mid L7	LGS	36.3	75	12	0.001
		DR			21	0.001
	Low L7	DR	37.0	75	19	0.001

The results from four preliminary experiments and from the experiments illustrated in Figure 20 were not included in Table II. The antidromic volleys in three of the four earlier experiments did not respond to the administration of SCh even though their alternately recorded MSRR's underwent a typical depression. However, the same antidromic responses also failed to respond with facilitation to PBST conditioning volleys. The failure of these volleys to respond to a known depolarizing influence and the failure to duplicate such negative results when the intricacies of the technique had been learned were considered sufficient reasons to discard these negative results as meaningless. The amplitude of the remaining antidromic volley responded to SCh administration with marked depression. This particular experiment was conducted before the necessity of rigid immobilization of the spinal cord was realized, and since that time the injection of SCh never resulted in antidromic response depression. It seemed justified to discard this result on the basis of movement artifact.

From inspection of Table II it would appear that the antidromic responses of the anesthetized and spinal preparations demonstrated a greater amplitude increase to similar doses of SCh than did those of the decerebrate preparations. This impression, although attractive in view of the recent demonstration of descending spinal fibers capable of inducing primary afferent depolarization (17, 18), is not justified. The percent changes in amplitude only have meaning for the given experiment, as an interexperimental comparison between the sensitivities of the antidromic volleys was not conducted.

It has been demonstrated that the excitability of the intraspinal portions of large afferent fibers arising from the GS muscle was increased by the administration of

SCh in doses of 50 µg/kg or greater. It was also shown that the time course of the MSRR depression was identical to the time course of the excitability change providing that the sensitivity of the antidromic volley was maintained. However, these observations by themselves are not sufficient evidence to conclude that the drug-induced MSRR depression is the result of augmented presynaptic inhibition. It is entirely possible that the depolarization of intraspinal afferent fibers and hence the observed excitability change resulted from a direct effect on the spinal cord by SCh. If such were the case the phenomenon would be of no consequence since the MSRR depression does not occur unless the drug-induced afferent barrage reaches the spinal cord. In other words any direct depolarizing action that SCh may exert on the spinal cord is entirely irrelevent with regard to the MSRR depression.

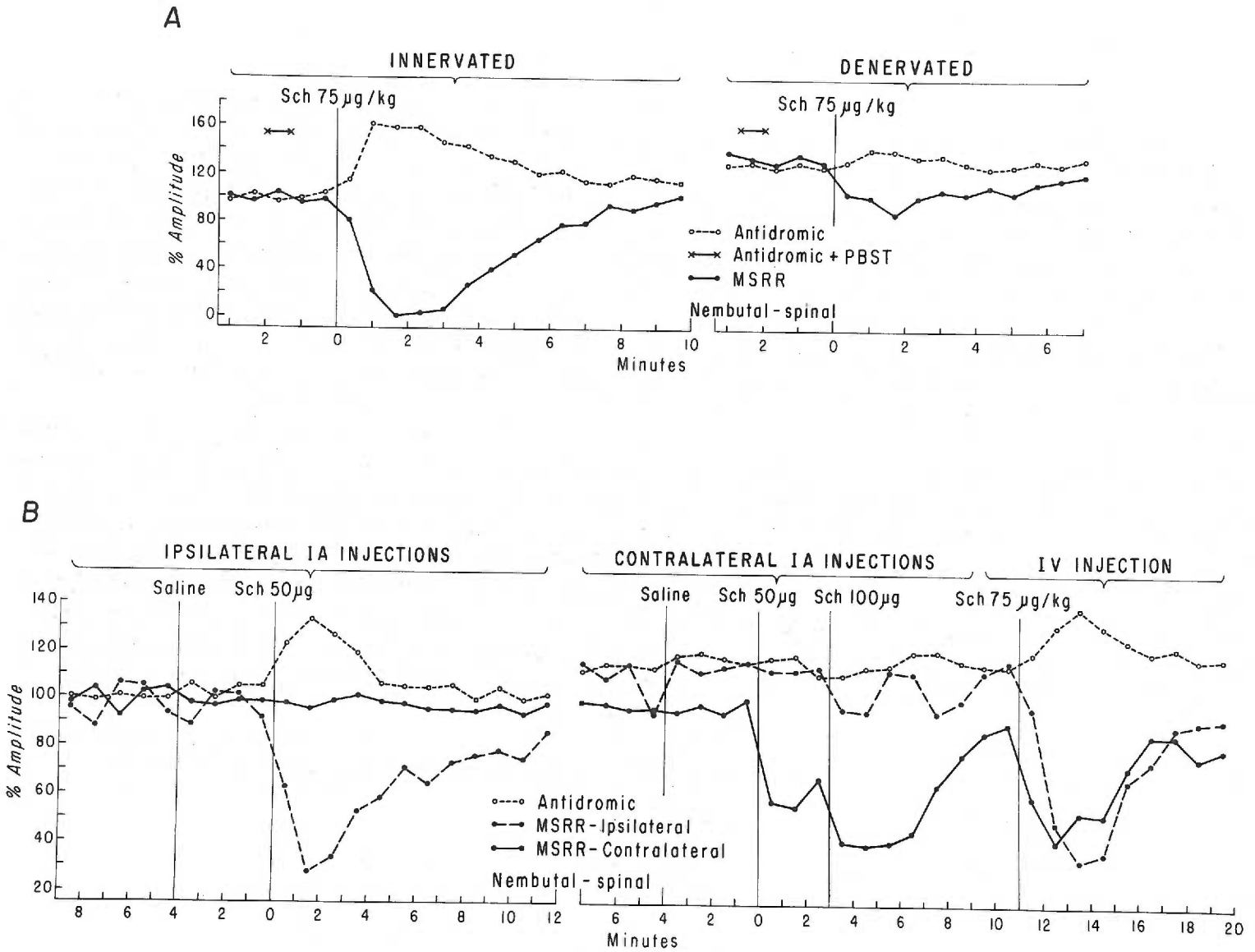
The possibility that SCh exerted a direct central action which would account for the observed excitability changes was eliminated by two different experimental techniques. In one preparation whose data are shown in Figure 20 (record A), the MSR and antidromic responses were evoked and recorded in standard fashion. Subsequent to the first injection of SCh typical changes were observed; the MSRR underwent depression whereas the antidromic response was facilitated. Once the amplitudes of the recorded responses returned to their control values the hind limb ipsilateral to the antidromic response was subjected to extensive peripheral deafferentation. One-half hour later the same dosage of SCh was readministered and in contrast to the results which followed the first injection, the alterations in the amplitudes of the recorded responses were quite minimal. This observation was suggestive that the response of the antidromic volley as well as the MSRR to SCh was secondary to the drug-induced afferent barrage. As was noted before, the results of transection

FIGURE 20

The peripheral action of SCh and the secondary central effects which are induced by the increased rate of muscle spindle discharge.

A. The percent amplitudes of the MSR and antidromic responses are plotted in standard fashion. The antidromic and MSR responses were alternately evoked and recorded (L7 and MG; GS and VRS1) from an anesthetized, spinal preparation in which the ipsilateral femoral and obturator nerves had been sectioned. The 75 μg/kg dosage of SCh produced significant changes in both response's amplitudes (labeled innervated) even though the majority of the remaining ipsilateral afferent input was restricted to the sciatic nerve (minus the PBST and GS nerves which were routinely transected). Immediately following this drug injection the lateral and medial (distal to the GS nerve's junction) divisions of the sciatic nerve were transected, thus removing the majority of the limb's remaining afferent input. One half hour later the MSR and antidromic responses were retested with the same dosage of SCh (labeled denervated).

B. Data from an anesthetized, spinal preparation which differed from other experiments in this section as follows: 1) the ventral rhizotomy was bilateral (L6-S2); 2) gastrocnemius-soleus MSRR's were evoked and recorded (VRS1) bilaterally; and 3) each hind limb was prepared for the close intrarterial injection of SCh by cannulating a muscular branch of each femoral artery with polyethylene tubing high within the femoral canal. Each cannula was inserted in retrograde fashion until its end just projected into the femoral blood stream. The antidromic response was evoked from L7 and was recorded from the MG nerve of the hind limb which is designated as ipsilateral. The stimuli were applied in the following order: antidromic, ipsilateral MSR, and contralateral MSR with a 3.3 second interval between each, or in other words, 10 seconds between consecutive responses of the same species. Each point in the graph is the mean percent amplitude of 6 consecutive responses in contrast to other graphic material in this thesis.


A
INNERVATED
DENERVATED
Sch 75 µg/kg
Sch 75 µg/kg
% Amplitude
160
120
80
40
0
Antidromic
Antidromic + PBST
MSRR
Nembutal - spinal
2 0 2 4 6 8 10
2 0 2 4 6
Minutes
B
IPSILATERAL IA INJECTIONS
CONTRALATERAL IA INJECTIONS
IV INJECTION
Saline
Sch 50µg
Saline
Sch 50µg
Sch 100µg
Sch 75 µg/kg
% Amplitude
140
120
100
80
60
40
20
Antidromic
MSRR - Ipsilateral
MSRR - Contralateral
Nembutal - spinal
8 6 4 2 0 2 4 6 8 10 12
6 4 2 0 2 4 6 8 10 12 14 16 18 20
Minutes

experiments must be accepted with reservation.

The inherent limitations of transection procedures were bypassed by drug localization experiments such as that illustrated by the data shown in record B (Fig. 20). This animal was prepared in such a fashion that SCh could be administered directly to either femoral artery in contrast to the intravenous injections employed in all prior experiments. When 50 μg of SCh was injected intraarterially into the hind limb from which the antidromic response was recorded (ipsilateral limb) the antidromic response was facilitated. The MSRR which was evoked from the injected hind limb underwent depression in contrast to the MSRR that was alternately evoked from the contralateral limb and which was not affected. The fact that the MSRR evoked from the limb opposite to the injected limb was not altered indicates that a negligible concentration of SCh reached the systemic circulation. Conversly, when SCh was administered to the contralateral hind limb (with reference to the side from which the antidromic response was recorded) only the MSRR evoked from that limb was depressed. The antidromic response and its ipsilateral MSRR were not affected significantly, even when the dosage of SCh was increased to 100 μg. Soon thereafter, an intravenous dosage of SCh was administered and both MSRR's underwent depression and the antidromic response was facilitated. Thus the failure of the antidromic volley to respond to the contralateral intraarterial injection of SCh cannot be attributed to a loss of sensitivity.

These observations provide conclusive evidence that the enhanced excitability of the intraspinal portions of large afferent fibers originating from the GS muscle was not the result of a direct depolarizing action by SCh on the spinal cord. Rather, the excitability changes resulted from the peripheral action of SCh which is known

to be the elicitation of muscle spindle discharge. These results also eliminate movement of the microelectrode, either secondary to muscle fasciculations or to changes in blood flow to the spinal cord, as possible causes of the observed excitability changes.

DISCUSSION

In more than 150 experiments, the administration of SCh, in intravenous doses of 50 μg/kg or greater, resulted in a significant depression of the MSRR. Only one exception to this statement was observed, and in this case, it is now considered that the observed reflex facilitation was most likely artifactual in nature. The time course of the reflex depression (Figs. 5, 11) and the time course of the drug-induced afferent barrage (Figs. 5, 6) were found to be identical. Furthermore, the reflex depression was not dependent on any direct CNS action that SCh may exert, but only occurred when the increased afferent input was allowed to reach the spinal cord (Fig. 20). This data confirms the previous findings of other investigators (52), and it would appear reasonable to conclude that the MSRR depression which follows SCh administration is the direct result of the central actions of the activated afferent fibers.

The remainder of this discussion will be concerned with the mechanisms responsible for the reflex depression. Reports of other studies (60, 52, 55) make it reasonable to assume that SCh activates only those receptors in muscle spindles which are related to Group IA and Group II afferent fibers. If subsequent experimentation demonstrates that other varieties of receptors, for example, joint receptors, are activated by SCh, the statements made here will require appropriate modification.

The data obtained here offer strong support of the conclusion that the MSRR depression results from augmented presynaptic inhibition. It was shown that the excitability of Group I afferent fibers arising from the GS muscle was increased following SCh administration (Table II). The time course of this excitability change

was found to be identical with the time course of the reflex depression (Figs. 17, 18, 19, 20). As was the case for the MSRR depression, the excitability increase resulted from the central actions of the activated muscle spindle afferents and not from a direct action by SCh on the spinal cord (Fig. 20). It would appear that this situation is essentially identical to that in which synchronized afferent nerve volleys depolarize the intraspinal portions of other afferent nerve fibers (37). Therefore, the excitability change in the afferent terminals which follows SCh administration can be attributed without reservation to a depolarization of the stimulated fibers (138, 37). It has been clearly demonstrated by others that the EPSP's evoked by activation of depolarized afferent terminals are reduced in amplitude below normal values (32, 37). Thus, the essential requirements for augmented presynaptic inhibition appear following SCh administration and follow the same time course as the MSRR depression.

The causal relationship between the drug-induced primary afferent depolarization and the reflex depression gains further support from the anatomical distribution of both phenomena. All tested MSRR's (flexor and extensor) responded to SCh with depressions; it has been shown by Eccles and his co-workers (32, 37) that all Group I fibers in the hind limb are equally capable of being depolarized. The observation that MSRR depression was independent of preparation type is also in accord with the conclusion that inhibition is exerted at a presynaptic level.

In addition, presynaptic inhibition would seem to provide an adequate explanation for the increased latency of the MSRR (Fig. 12), the augmented PSRR (Fig. 13), and the random firing of motoneurons (Fig. 7) which follow SCh administration. Diminished transmitter output which presumably accounts for presynaptic inhibition

(37) would be expected to increase the time required for the EPSP's to reach the firing thresholds of the motoneurons. Other factors which might relate to the increased latency were enumerated in the RESULTS (p. 93). Primary afferent depolarization may extend to involve those central terminals of Group I fibers which activate inhibitory interneurons. Thus, for the duration of the drug-induced depolarization the "spontaneous" input to the motoneurons may be so altered that release occurs, allowing random discharges and PSRR augmentation. Such a mechanism is suggested by the observation (Fig. 15-B) that Group I PBST volleys depressed the MSRR evoked by stimulation of the GS nerve but augmented multisynaptic reflex transmission.

Alternatively, it is possible that the increased rate of discharge in Group II afferents underlies the PSRR augmentation. However, increased Group II input would not account in total for the random discharges since random firing of extensor motoneurons is known to occur (51).

If the excitatory actions of Group II afferents are considered as possible causes of PSRR augmentation, then the failure of the MSRR test system to demonstrate facilitation of flexor motoneurons must be reckoned with. Presynaptic inhibition of the testing afferent volley is one obvious answer.

The recent demonstration of presynaptic inhibition of flexor reflex afferents (35) evokes the question of whether or not the central terminals of Group II afferents were depolarized by the drug-induced afferent barrage. The answer is not known since the excitability of Group II afferents was not measured by antidromic testing.

This investigation did not yield any data about the actual excitability changes of the tested motoneurons following SCh administration. The MSRR test was

invalidated as an index of motoneuronal excitability by the demonstration of primary afferent depolarization. And, we do not know if the augmented PSRR and random discharge of motoneurons implies an increased motoneuronal excitability or merely increased presynaptic drive to those motoneurons.

The random discharge of motoneurons which follows SCh administration (Fig. 7) has been suggested (68, 51, 66) as a cause of the drug-induced MSRR depression. The axonal impulses set up by the firing of motoneurons would presumably invade the recurrent collateral system and activate Renshaw inhibitory and facilitatory interneurons. Since the inhibitory component of these interneurons predominates (118), MSRR depression would be the expected result. This concept is supported by the observation (51, 66) that pre-treatment of the preparation with dihydro-beta-erythrodine (DHE) or strychnine lessens the degree of MSRR depression following SCh administration.

However, on the other hand, there is evidence suggesting that recurrent inhibition does not contribute significantly to the reflex depression. The fact that strychnine lessens the degree of reflex depression which follows SCh administration does not imply the type of inhibition involved. Strychnine increases the threshold of primary afferent fibers (110) as well as depressing other central inhibitory mechanisms (29). As such strychnine would prevent the manifestations of presynaptic inhibition in addition to those of postsynaptic or recurrent inhibition.

The observation that MSRR depression is reduced by pre-treatment with DHE (51, 66) has been offered as an argument that recurrent inhibition is one mechanism responsible for the reflex depression. However, DHE reduces or prevents the action of SCh on spindle receptors and thus reduces the afferent barrage which is necessary

for the depression (see p. 100). On the other hand, Henatsch's unpublished results (65) involving cross-circulation experiments force the adoption of a cautious attitude about the actions of DHE.

Another objection to the recurrent inhibition hypothesis is the short duration of the random discharge. In this study, the discharge was not found to persist beyond two minutes (Table I). The illustrated observations of other investigators (68, 66) do not reveal random discharges persisting beyond two and one-half minutes. It would seem obvious that the causative factor must equal in time course the reflex depression as does the increased rate of muscle spindle discharge. The hypothesis of recurrent inhibition initiated by random firing does not meet these requirements.

The observation that MSRR depression occurred in typical fashion in the anesthetized preparations is the most serious objection to the recurrent inhibition hypothesis, since these preparations demonstrated little if any random firing in contrast to the decerebrate or decapitate preparations (Table I). The same observation prevents acceptance of the concept that after-hyperpolarization or occlusion are significant causes of reflex depression.

The data obtained in this investigation and the conclusions derived therefrom suggest that presynaptic inhibition is the dominant factor responsible for the MSRR depression evoked by SCh. Complete resolution of the problem will require intracellular recording techniques to define the role of postsynaptic inhibition and the actual excitability changes of the tested motoneurons.

SUMMARY

The mechanism of monosynaptic reflex response (MSRR) depression produced by the intravenous injection of succinylcholine (SCh) was studied in 75 cats subjected to decerebration, decapitation, or general anesthesia with spinal cord transection. The results and conclusions of this study were as follows:

1) Intravenous SCh in doses of 50 μg/kg or greater produces a characteristic depression of both flexor and extensor MSRR's, an augmentation of polysynaptic reflex responses, and sometimes a random firing of previously quiescent motoneurons. The MSRR depression was found to result from the central actions of those muscle spindle afferents activated by SCh.

2) The roles of direct inhibition, recurrent inhibition and after-hyperpolarization were investigated as possible causes for the reflex depression. Evidence was presented that these factors were *not* significantly involved in the drug-induced MSRR depression.

3) The excitability of the intraspinal portions of Group I afferent fibers was found to increase after SCh administration; the time course of the excitability change was identical to the time course of the MSRR depression. The excitability increase was shown to result from the central actions of the activated afferent fibers and was attributed to a depolarization of the tested fibers.

4) On the basis of the known relationship between afferent fiber depolarization and presynaptic inhibition, it was concluded that the MSRR depression which follows SCh administration results for the most part from augmented presynaptic inhibition.

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