STIMULANT EFFECTS OF BARBITURATES ON SMOOTH MUSCLE

by

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DEDICATION

This thesis is dedicated to my mother and father, Marianne Allen Edney and Victor M. Edney. It is my hope that this thesis will give them some pleasure in return for the life they have given me.

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INTRODUCTION

Central Nervous System Activity of Barbiturates. The use of barbiturates in clinical medicine began with the introduction of diethyl barbituric acid (barbital) as a sedative and hypnotic by Fisher and von Mering in 1903 (Carter, 1950), followed by phenobarbital in 1912 (Dundee, 1956; Sharpless, 1970). The success of these barbituric acid derivatives prompted a search for and clinical use of other derivatives with the same depressant action on the central nervous system (CNS) but with slightly different pharmacological parameters, such as greater potency, shorter duration of action, and a more rapid induction of anesthesia. Eventually over 2,500 different barbiturate molecules were synthesized and many were evaluated for pharmacologic activity (Doran, 1959; Sharpless, 1970).

Both clinical experience and laboratory investigation established that while the predominant CNS effect of the majority of barbiturate analogs was depression, many barbiturates had the potential to produce excitation as well as depression and a few had prominent convulsant activity. For convenience, barbiturates can be grouped into three classes on the basis of the predominant CNS activity. Structures of representative examples of each class are shown in Figure 1.

1. Depressants. Barbiturates of this group have been used as sedatives, hypnotics, or anesthetics. Some depressant barbiturates may manifest excitatory effects on the CNS, but these are usually

minor, transient, and confined to Stage II of anesthesia. Readily apparent excitatory effects include tremors, hyperactive stretch reflexes, clonus, and rigidity (Etsten and Himwich, 1946; Wynne, 1948; Dundee, 1956). Typical examples of depressant barbiturates are:

5-ethyl-5-(1-methylbutyl) barbituric acid or pentobarbital;

5-ethyl-5-(1-methylbutyl) thiobarbituric acid or thiopental;

5-ethyl-5-phenyl barbituric acid or phenobarbital;

5-ethyl-5-cyclohexylethyl barbituric acid (Blicke and Zienty, 1941);

α-dl-1-methyl-5-allyl-5-(1-methyl-2-pentynyl) barbituric acid or methohexital.

2. Convulsants. The barbiturates of this group produce maximal CNS excitation resulting in a lethal, tonic extensor seizure, similar to the seizure provoked by electroshock or pentylenetetrazol. Death occurs from tonic respiratory arrest. The term "convulsant barbiturate" has been applied to any barbiturate which was unsuitable for clinical use due to excessive CNS excitation. However, in the present context, the term "convulsant" will be applied to only those barbiturates that produce lethal, tonic extensor seizures.

The seizure activity of the convulsant barbiturates is manifest at doses lower than those required for CNS depression, and animals die of convulsions before depressant concentrations can be established (Cain and Kleis, 1959; Downes, Perry, Ostlund and Karler, 1970). Thus under normal conditions, convulsants cause CNS stimulation with little or no sign of depression. Typical convulsant barbiturates are:

(+)-5-ethyl-5-(1,3-dimethylbutyl) barbituric acid or (+)-DMBB (Downes et al., 1970);

5-ethyl-5-(1,3-dimethyl-2-butenyl) barbituric acid or πDMBB (Cain and Kleis, 1959);

5-ethyl-5-(2-cyclohexylideneethyl) barbituric acid, or CHEB (Allais and Mathieu, 1951; Downes et al., 1970).

3. Semiconvulsants. Barbiturates with CNS effects intermediate between those described in the first two categories are contained in this group. Semiconvulsant barbiturates are arbitrarily distinguished from depressants in that they produce preanesthetic excitation sufficiently intense to be intolerable in the clinical situation, and they are distinguished from the convulsants in that they cannot produce the lethal, tonic extensor seizure. The semiconvulsants cause CNS excitation accompanied by depression, but as the dose is increased, excitation is replaced by anesthesia. Death occurs by respiratory arrest in deep anesthesia. Typical semiconvulsants are:

5-ethyl-5-cyclohexyl-O-methyl barbituric acid or CHOMB (Downes et al., 1970);

5-ethyl-5-benzyl barbituric acid or benzylbarbital (Dox and Yoder, 1922);

(—)-5-ethyl-5-(1,3-dimethylbutyl) barbituric acid or (—)-DMBB (Downes et al., 1970).

Although CNS activity has not been demonstrated to depend on any identifiable chemical characteristic, small differences in chemical structure are quite important in determining the predominant action of the barbiturate; for example, DMBB, a lethal convulsant, differs from pentobarbital by only one additional methyl group (Figure 1). Benzylbarbital, a semiconvulsant, and phenobarbital differ by a single methylene group in the alkane linkage between the barbiturate and aromatic rings; similarly, CHEB, a convulsant, and 5-ethyl-5-cyclohexylethyl barbituric acid, a depressant, differ simply in the presence of an alkane or alkene linkage between the barbiturate and the cyclohexyl rings. Studies of the separated optical isomers of two depressants, pentobarbital (Kleiderer and Shonle, 1934) and methohexital (Gibson, Doran, Wood and Swanson, 1959), and the convulsant DMBB (Downes et al., 1970), have demonstrated that the relative degree of CNS excitatory and depressant effects of a barbiturate molecule are also dependent on enantiomorphic configuration.

Theories of Barbiturate Excitation. Traditionally, barbiturate excitation has been explained on the basis of selective depression of CNS inhibitory centers. The release from inhibition hypothesis of barbiturate excitation assumes that all barbiturates have only one effect, depression, and CNS stimulation produced by barbiturates is due to a selective depression of centers which control by active inhibition the activity of other functional units (Guedel, 1937; Etsten and

Himwich, 1946). Alternatively, barbiturates might have both a direct stimulant and a direct depressant action on the CNS (Downes et al., 1970; Downes and Franz, 1971).

Although the selective inhibition hypothesis is consistent with the results of many studies and may account for much of the CNS excitation seen with the use of barbiturates (Domino, Fox and Brody, 1955; Frank and Sanders, 1963), there has been no demonstration of selective depression by either depressant or convulsant barbiturates of pre-or postsynaptic inhibition (Esplin, 1963; Eccles, Schmidt and Willis, 1963; Miyahara, Esplin and Zablocka, 1966; Weakly, Esplin and Zablocka, 1968). On the other hand, direct excitatory effects of the convulsant barbiturates have been demonstrated in dorsal root ganglion cells (Downes and Franz, 1971).

In Vitro Activity of Barbiturates. Much of the previous work, monitoring either metabolic or contractile activity of isolated tissue, failed to detect qualitative differences between depressant and convulsant barbiturates. Fuhrman, Martin and Dille (1941) reported that both DMBB and pentobarbital depressed oxygen metabolism in isolated rat brain slices. Brody and Bain (1956) found that all of the barbiturates in a large series including pentobarbital and DMBB had the same qualitative depressant action on oxidative phosphorylation of isolated liver and brain mitochondria. Also Maxwell and Nickel (1954) found that the depressant barbiturates pentobarbital, phenobarbital and barbital, as well as the convulsant barbiturate DMBB, stimulated ATPase

activity of rat brain and liver homogenates. Clowes, Keltch and Krahl (1940) reported that DMBB, as well as pentobarbital, depressed the rate of division of sea urchin eggs, and the contractile activity of isolated frog heart and intestine was also depressed by both pentobarbital and DMBB (Powell, Lee and Swanson, 1943). A problem in interpretation of these studies is that a racemic mixture of DMBB was used, and DMBB was later demonstrated to have both excitatory and depressant properties separable on the basis of enantiomorphic structure (Downes et al., 1970). If these systems had the ability to detect qualitative differences between depressant and excitatory barbiturates, they might have failed to do so because of a predominance of depressant activity in the racemic mixture.

Not all in vitro systems have failed to detect qualitative differences between convulsant and depressant barbiturates. Powell et al. (1943) reported that racemic DMBB stimulated contraction of isolated rabbit intestinal strips, while pentobarbital had only a depressant effect. Schaer (1966), working with isolated guinea pig atrial strips, recorded transient increases in the rate and force of contraction when racemic DMBB was used at low concentrations, while at higher concentrations, depression was observed. In comparison, pentobarbital had only a depressant action on this preparation. Hupka, Williams and Karler (1969) demonstrated that the convulsant barbiturates, (+)-DMBB and CHEB, stimulated isolated aortic smooth muscle, while (−)-DMBB and pentobarbital had no stimulant action. Analogous

to the activity of these isomers in the CNS, (—)-DMBB blocked the stimulant action of (+)-DMBB in the aortic strip. Hupka et al. suggested that "the in vitro muscle preparation may serve as a working model for study of the mechanism of action of the convulsant barbiturates on the CNS". While two convulsant barbiturates have produced contraction of smooth muscle, the depressant barbiturate, thiopental, also stimulated contraction of isolated vascular smooth muscle including the aortic strip preparation (Burn, 1959; Price and Price, 1962). Thus the effect of thiopental contradicts the analogy between CNS activity of barbiturates and barbiturate activity in isolated smooth muscle.

Site of Stimulant Action of Barbiturates in Isolated Smooth Muscle. In terms of gross morphology, there are two potential sites of drug action in isolated smooth muscle preparations, muscle and nerve. Drugs may act selectively as a function of dose, time, or chemical structure on either site. The characteristic loss of responsiveness of aortic strips with repeated stimulation by CHEB, which Hupka et al. (1969) termed "tachyphylaxis", suggests that the activity of convulsant barbiturates on smooth muscle might be due to release and depletion of some vasoactive substance. In isolated aorta, known agonists which might be released by indirect action on nerve endings are norepinephrine (NE) and acetylcholine (Ach).

The lack of cross-tachyphylaxis between CHEB and tyramine suggests that CHEB is not acting indirectly by release of NE (Hupka

et al., 1969). Similarly, phenoxybenzamine, which blocks the constrictor effect of NE, does not block smooth muscle stimulation by CHEB (Hupka et al., 1969). However, these studies do not eliminate the possibility that the convulsants might cause release of some unidentified agonist.

Burn (1959) believed that contraction of vascular smooth muscle by thiopental is an indirect effect mediated by release of catecholamines from nerve endings. However, Price and Price (1962) suggested that thiopental might be acting directly to increase the sensitivity of smooth muscle to endogenous catecholamines rather than releasing catecholamines. Thus, studies with thiopental illustrate the uncertainty which prevails with regard to site of action for barbiturate stimulation of smooth muscle.

PURPOSE OF THE INVESTIGATION

The purposes of this investigation were first, to test the extent of correlation between stimulant activity of barbiturates in CNS and in smooth muscle and second, to compare thiopental to the convulsant barbiturates with respect to possible sites and mechanisms of stimulant activity in smooth muscle. In order to determine extent of correlation between CNS and smooth muscle stimulant activities, a series of barbiturates, with CNS effects ranging from mild preanesthetic excitation to maximal (tonic extensor) seizures, was tested for stimulant and depressant effects in different types of isolated smooth muscle preparations. The series included convulsants (CHEB and π DMBB), semiconvulsants (CHOMB and benzylbarbital) and clinically useful depressants (pentobarbital, thiopental, and methohexital). All seven barbiturates were tested on rabbit aortic strips, tracheal chains and jejunal segments.

Possible sites of stimulant activity of thiopental and CHEB were investigated using adrenergic and cholingergic antagonists; however, the question of whether these drugs act selectively on nerve or muscle was also examined by means of the localized sweat response to intradermal drug injection. Intradermal injection of drugs which stimulate autonomic nerve terminals produce sweating through an axon reflex in a large area surrounding the site of injection (Coon and Rothman, 1941).

An element common to many studies of the mechanism of action

of smooth muscle agonists (Somlyo and Somlyo, 1968, 1970) has been the importance of the calcium ion (Ca). Hudgins and Weiss (1968) and Garrett and Carrier (1971) have demonstrated the different sensitivities of contractions induced by NE and potassium (K) to changes in extracellular Ca. Also, the dependence of agonist effect on intracellular and extracellular Ca can be distinguished by the use of lanthanum. This trivalent cation "stabilizes" the cell membrane to prevent the influx of extracellular Ca. Because of the importance of Ca to the action of other smooth muscle agonists, the relationship of extracellular Ca and the influence of lanthanum on the constrictor effects of CHEB and thiopental were studied in this thesis investigation.

MATERIALS AND METHODS

Animals. Smooth muscle preparations were isolated from male New Zealand white rabbits (2.5 to 3.0 kg) that had been sacrificed by injection of a bolus of air into the ear vein. The rabbits were purchased from V-R Research, Newberg, Oregon and maintained on standard laboratory diet until sacrificed.

Organ Baths and Recording Equipment. An isolated organ was placed in a 50 ml cylindrical glass chamber filled with physiologic salt solution (see page 13) and oxygenated by a constant flow of gas through a sintered glass disc at the bottom of the chamber. Each chamber was surrounded by an outer jacket perfused with water at 37.5°C by a Lauda K-2/R circulator and thermoregulator. In order to change bath solutions, a chamber was drained from the bottom and refilled from the top with new solution previously brought to 37.5°C in a separate thermoregulated bath.

One end of the smooth muscle preparation was tied to a hooked glass rod at the bottom of the chamber, and the other end of the preparation was tied to the lever arm of a Harvard 356 isotonic muscle transducer. Contractions were recorded on paper with a Harvard 350 recorder and 486 chartmover.

Smooth Muscle Preparations. Rabbit aortic strips were prepared by the technique described by Burchgott and Bhadrakom (1953). The descending thoracid aorta was excised, cleaned of external fatty tissue, and spirally cut from right to left into a strip 5 to 10 cm long and 4 to

6 mm wide. This was then cut lengthwise into two narrower strips and these were divided transversely into shorter strips, about 3 cm long. Aortic strips were mounted under a tension of 2 g.

Jejunal segments were prepared by the procedure described by the Staff of the Department of Pharmacology, University of Edinburgh (1968). Segments approximately 2.5 cm long were cut from the small intestine at a point between 5 to 10 cm distal to the stomach. To mount the preparation in the chamber, sutures were inserted through one side of the intestinal wall at each end of the segment. Care was taken in mounting a segment to insure that its lumen remained open to allow free access of the bath solution. Jejunal preparations were mounted under 1g tension.

Tracheal chains were prepared by the method of Costillo and deBeer (1947) as modified by Akaçasu (1959). After excision from the rabbit and removal of extraneous fatty tissue, the trachea was cut transversely to isolate a series of cartilaginous rings. These rings were tied together to form a chain and each ring in the chain was then opened by a cut through the cartilaginous portion of the ring and the chain mounted in an organ bath under 1g tension.

Measurement of Drug Effect. After an equilibration period of at least two and a half hours, aortic strips were contracted with NE to provide a standard of comparison for subsequent barbiturate-induced contractions. Norepinephrine was added in cumulative increments to achieve final concentrations in the bath solution of 10^{-9}, 10^{-7}, 10^{-5} and

either $3x10^{-5}$ or 10^{-4} molar (M). In some experiments NE concentrations of 10^{-8} and 10^{-6}M were also employed. Since 10^{-5}M NE produced 96% of the response to 10^{-4}M NE, the response to either $3x10^{-5}$ or 10^{-4}M NE was taken as the maximal NE contraction. These maximal contractions varied in amplitude from 20 to 40 mm depending primarily on differences in length of the strips after the period of equilibration. Barbiturate-induced contractions were measured and expressed in terms of the preceding maximal response to NE.

Jejunal segments and tracheal chains were employed primarily for qualitative rather than quantitative comparison of effects of different barbiturates. The parameters of interest were the type of response and the drug concentrations needed to elicit the response; however, all barbiturates that produced contraction in these preparations were subsequently compared to Ach (10^{-4}M) with respect to amplitude of maximal effects.

Physiologic Solutions. Krebs solution was made up in distilled H_2O and contained the following chemicals in mM concentrations; NaCl, 118.9; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4 \cdot 7H_2O$, 0.6; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.1. The Krebs solution was oxygenated with a 5% Co_2, 95% O_2 gas mixture (botained from Liquid Air Inc.). High potassium (K) Krebs solutions were made by substituting KCl for NaCl so that the sum of KCl and NaCl remained 123.6 mM. Alteration of Ca concentration was accomplished by increasing or decreasing the

quantity of $CaCl_2$ without compensation for the resulting slight change in chloride concentration. Krebs solution containing strontium (Sr) was made by adding $SrCl_2$ to a Ca-free Krebs solution.

Krebs solution could not be employed in experiments with lanthanum (La), since a precipitate was formed when La was added to the solution. Therefore, experiments were conducted using a physiological solution buffered with 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic Acid (HEPES) and containing the following substances in mM concentrations; NaCl, 160; KCl, 4.6; $CaCl_2$, 2.5; $MgCl_2$, 1; glucose, 5; HEPES, 10. The HEPES buffered solution (HBS) was adjusted to pH 7.4 with 1N NaOH and oxygenated with 100% O_2. HEPES is a chemically stable compound with little detectable biological activity and a pKa of 7.3 at 25°C, qualities which make it an excellent buffer for use in biological experiments (Shipman, 1969). Solutions containing La were made by adding $LaCl_3$ to the HBS.

Because high concentrations of barbiturate might alter pH of the bath solutions, pH was monitored with a Beckman 76008 pH meter and 46850 micro blood pH assembly mounted in a constant temperature block set at 37°C. A 1 ml aliquot was removed from the bath solution with a tuberculin syringe and immediately injected into the micro blood pH assembly. At concentrations of 10^{-4}M or below, the barbiturates did not alter pH of the HBS or Krebs solution. At barbiturate concentrations of 10^{-3}M, pH was increased by less than 0.08 units. Addition of 1N NaOH in amounts sufficient to increase the bath pH by 0.10 units

did not produce an organ response.

Sweat Response to Intradermal Drug Injection. Four Caucasian males, ranging in age from 26 to 47 years, members of the Department of Pharmacology, University of Oregon Medical School, volunteered to be subjects for these experiments. Just before drug injection, each forearm was washed with 70% alcohol solution and then painted with a solution of iodine and alcohol (3 gm I_2/100 ml EtOH). After the iodine had dried, 0.2 ml of the sterile test solution was injected intradermally and the area covered with an indicator of corn starch suspended in castor oil (50 g starch/100 ml oil; Wada, 1950). If sweating occurred, the iodine in the pores of sweat glands was carried by the sweat into the starch suspension and the starch-iodine reaction (Radley, 1968) caused a dark blue dot to appear directly above each sweat gland. To record the response, photographs of the forearm were taken at 3.0 and 3.2 minutes after the injection, and at later intervals, if indicated by a more slowly developing response.

The vehicle for all injected drugs was HBS at a pH of 7.4. Each of the drug solutions was sterilized by filtration and sterility was checked by innoculation on blood agar plates and in thioglycolate tubes. No growth was observed after incubation for 48 hours at 37°C.

Drug Solutions. Pentobarbital sodium (Abbot), thiopental sodium (Abbot), methohexital sodium (Lilly), phenoxybenzamine (Smith, Kline, and French), norepinephrine (Sigma), isoproterenol (Winthrop), atropine (Sigma), acetylcholine (Calbiochem), nicotine HCl (K&K Laboratories),

and HEPES (Calbiochem) were obtained from the commercial sources indicated in parentheses. Benzylbarbital, CHOMB, and part of the CHEB used in these experiments were synthesized by the late Dr. J.K. Williams, Department of Pharmacology, University of Utah College of Medicine; π DMBB and further CHEB were synthesized by Dr. J.H. Block, School of Pharmacy, Oregon State University. In preliminary experiments, π DMBB, CHEB, CHOMB, and benzylbarbital were administered by intraperitoneal injection to rabbits to demonstrate that these compounds produced the same in vivo activity as described by previous investigators (see introduction). CHEB and π DMBB produced lethal, tonic extensor seizures whereas CHOMB and benzylbarbital caused an exaggerated preanesthetic excitation without lethal, tonic seizures.

All drug solutions were dissolved in 0.9% NaCl prior to addition to organ baths. Barbiturates which were not sodium salts were dissolved in 0.9% NaCl made slightly alkaline with NaOH. Phenoxybenzamine was initially dissolved in a small amount of glycerol and then diluted to the desired concentration with 0.9% NaCl. Drug solutions were added to the bath in volumes of 0.5 ml or less.

RESULTS

Stimulatory Effects of Barbiturates on Smooth Muscle. The effects of the seven barbiturates were tested in each of the three types of smooth muscle with a minimum of three different rabbits used for each combination of drug and preparation. The convulsants, CHEB and π DMBB, elicited contractions in all three types of smooth muscle preparation, whereas the semiconvulsants, CHOMB and benzylbarbital, did not cause contraction in any preparation. Thiopental was the only depressant barbiturate to stimulate smooth muscle and this effect was confined to aortic strips. Figures 2 and 3 show typical responses of aortic strips, tracheal chains and jejunal segments to increasing concentrations of CHEB and show qualitative effects that were typical for both π DMBB and CHEB.

Drug concentration in the bath solution was increased in increments of half log units ($1x10^{-6}$, $3x10^{-6}$, $1x10^{-5}$, up to 10^{-3}M) without washout between drug additions. In all three types of preparation, CHEB and πDMBB produced their maximal effects at a cumulative drug concentration of $4.4x10^{-5}$M. Increasing drug concentration to 1.4 or $4.4x10^{-4}$M led to a gradual depression of contraction (autoinhibition) that is readily apparent in the sample traces. Jejunal segments were the most sensitive of the preparations to CHEB and π DMBB, with some segments responding to concentrations as low as 10^{-6}M (Figure 3B and Figure 7). Tracheal chains were the least sensitive and did not usually respond to concentrations of π DMBB

of less than 1.4×10^{-5}M or concentrations of CHEB of less than 4.4×10^{-5}M.

In aortic strips (Figure 2A), the response to the convulsants was slow in onset and in rate of contraction with at least 15 minutes required to reach the maximal effect of a given concentration. Contractions induced by thiopental occurred slightly more rapidly but still required from 5 to 20 minutes to reach maximal effect (Figure 5C and Figure 10). Figure 4 compares the amplitudes of contractions induced by various concentrations of CHEB, π DMBB and thiopental and shows the difference in both potency and efficacy between thiopental and the two convulsant barbiturates. In most experiments, thiopental produced a just detectable contraction at a concentration of 1.4×10^{-4}M but the amplitudes of such threshold effects were less than 1% of the maximal response to NE and too small to plot in Figure 4. Thereafter, amplitude of thiopental-induced contractions increased with each successive increment in concentration and autoinhibitory effects were not seen.

In tracheal chains, responses to CHEB and πDMBB were also slow in onset and in rate of contraction (Figure 2B) but the magnitude of the response was usually much less than in aortic strips and some tracheal chains showed no response to any concentration of the convulsants. Nine tracheal chains were exposed to CHEB, eight were contracted by the barbiturate and, after washout of the barbiturate, four were subsequently contracted with Ach for comparison of maximal

effects. The maximal CHEB response varied from 2 to 25% (average 9%) of the maximal effect of Ach. A similar variability was also seen in experiments with πDMBB in this preparation. Of three chains exposed to π DMBB and then subsequently contracted with Ach, one chain showed no response to π DMBB and the remaining two showed contractions equivalent to 4% and 49% of the Ach effect.

Jejunal segments responded more rapidly to the convulsants than aortic strips or tracheal chains so that the maximal effects of a given concentration of CHEB or πDMBB were achieved in two or three minutes after drug addition. The convulsants produced two different types of effect in this preparation. More commonly, CHEB and πDMBB produced a sustained shortening of the preparation with a reduction in amplitude of phasic contractions (Figure 3A). This type of effect was similar to that produced by Ach and the extent of convulsant-induced contraction was approximately 50% of the maximal response to Ach. Less commonly, the convulsants elicited high amplitude phasic contractions, as shown in Figure 3C, with relatively little effect on the resting length of the preparation.

Tachyphylaxis to Barbiturate Stimulation. Figure 5 compares two successive contractions of aortic strips induced by CHEB, π DMBB, or thiopental and demonstrates that the second response was reduced in both rate and amplitude of contraction for all stimulant barbiturates. This change in responsiveness termed tachyphylaxis (Hupka et al., 1969) was observed in trachea and jejunum, as well as aorta, and must be

taken into consideration when evaluating the effect of intervening treatment on two successive barbiturate-stimulated contractions.

In aortic strips tachyphylaxis did not reduce the amplitude of contraction during the period of exposure to the drug and contractions induced by the convulsants were maintained for an hour or more with little or no noticeable relaxation. Tachyphylaxis was evident only when the barbiturate was washed from the bath, the tissue allowed to relax and then re-exposed to the barbiturate. Under these conditions four successive exposures of aortic strips to CHEB ($3x10^{-5}$M) resulted in the last CHEB response having only $33\pm7\%$ (mean $\pm$S.E.M.) of the amplitude of the initial CHEB response.

To insure that tachyphylaxis was drug-specific, and not due to general deterioration of tissue responsiveness with time or with repeated contraction, aortic strips were stimulated with K (30mM), which produced a contraction approximately equal in amplitude to an initial CHEB ($3x10^{-5}$M) response. The preparations were repeatedly contracted for the same period of time that had been necessary to reduce the last of a series of CHEB-induced contractions to 33% of the initial CHEB response. In these preparations the amplitude of the last response to K was $109\pm6\%$ of the initial K response so that there was no indication of deterioration of the tissue. Figure 5D shows that two successive K-induced contractions were virtually indistinguishable.

That CHEB tachyphylaxis was an effect of the drug itself, rather

than an effect of time or repeated contraction, was also demonstrated by experiments in which aortic strips were initially contracted with CHEB ($3x10^{-5}$M), then contracted four times with K (30mM) and re-exposed to CHEB. The K-induced contractions were all of equal amplitude; the second CHEB response was 65±14% of the first CHEB response, which was not significantly different than that observed in the absence of any intervening treatment (Figure 5A).

Effect of CHEB Tachyphylaxis on Response to Other Agonists. Aortic strips were initially exposed to one of the agonists, thiopental, NE, or K, then repeatedly contracted with CHEB ($3x10^{-5}$M) until the final CHEB response was about 25% of the initial response, and then re-exposed to the agonist. After this degree of CHEB tachyphylaxis, the response to K (30mM) was moderately reduced (83±5% of initial K response) and the response to thiopental (10^{-3}M) was almost eliminated (9±9% of initial thiopental response). In contrast to either K or thiopental, the response to NE was not affected by CHEB tachyphylaxis. Although there was a shift in the concentration effect curve of NE after tachyphylaxis to CHEB or π DMBB, this shift was similar to that which occurred with repeated exposure to NE without any intervening barbiturate-induced tachyphylaxis (Figure 6).

Depressant Effects of Barbiturates on Smooth Muscle. In jejunal preparations depressant activity was considered to be present if there was (1) an elongation of baseline length, (2) a reduction in amplitude of phasic contractions, (3) both elongation of baseline length and reduction in amplitude of phasic contractions. A reduction in phasic

contractions, accompanied by a shortening in muscle length, was not considered a depressant effect since the amplitude of phasic contractions characteristically diminished whenever the muscle was tonically contracted. By these criteria all barbiturates, convulsants as well as depressants, produced obvious depressant effects if given in high enough concentrations (Figure 3). As depressants the convulsant barbiturates were equipotent with pentobarbital and thiopental (Figure 7); however, the threshold stimulant concentrations of the convulsants were an order of magnitude less than their autoinhibitory or depressant concentrations.

Aortic and tracheal smooth muscle was nearly completely relaxed under control, resting conditions so that drug-induced relaxation could not be measured. Therefore, depressant activity of barbiturates was tested against contractions induced by other agonists. In aortic strips, contractions were induced by NE (3×10^{-6}M) or K (100mM), which caused, respectively, contractions of 90% and 100% of the maximal NE effect. In tracheal chains, contractions were induced with the maximally effective concentration of Ach (10^{-4}M). These agonists produced well-sustained contractions so that any relaxation of contraction following addition of barbiturate was considered to be an indication of depression. Three separate determinations were made for depressant activity of each of the barbiturates with each type of induced contraction. Barbiturate concentrations were increased in cumulative increments of half log units from 10^{-6} to 10^{-3}M and sufficient time was allowed to

establish any change before a subsequent increase in drug concentration. The concentration of barbiturate which first produced a relaxation of induced contraction was considered the threshold concentration for depression.

The bar graph in Figure 8 shows the range of barbiturate concentrations at which depressant effects were first observed. In the series of barbiturates tested, benzylbarbital was the least potent smooth muscle depressant, while methohexital was the most potent depressant. Stimulant concentrations of CHEB or π DMBB when added to aortic strips submaximally contracted with NE ($3x10^{-6}$M), produced more contraction; however, addition of higher concentrations resulted in relaxation. Addition of the convulsant barbiturates to aortic strips maximally contracted with K (100mM) or to tracheal chains maximally contracted with Ach (10^{-4}M) produced only depressant effects. Depressant potency of the convulsants was again very similar to that of pentobarbital and thiopental.

In aortic strips, as in jejunal preparations, the threshold stimulant concentrations of the convulsants were well below depressant or autoinhibitory concentrations. In contrast, the concentration of thiopental required to depress induced contractions of aortic strips were usually less than the concentrations that produced just detectable contraction of relaxed preparations. Addition of thiopental to aortic strips contracted with CHEB or πDMBB (1 to $3x10^{-5}$M) produced depression, whereas strips submaximally contracted with either

CHEB or πDMBB could be further contracted by addition of the other convulsant barbiturate.

Interaction between Barbiturates and Norepinephrine and Phenoxybenzamine. Figures 9 and 10 summarize the results of studies of the interaction between thiopental, phenoxybenzamine, and NE in the aorta. Each point in Figure 9 represents the amplitude of a second thiopental-induced contraction relative to an initial thiopental-induced contraction; sample traces are shown in Figure 10. As indicated previously (Figure 5), without any intervening treatment the second of two successive thiopental-induced contractions averaged 72% of the initial contraction. A threshold concentration of NE ($3x10^{-9}$M), added to the bath after the initial thiopental response, considerably potentiated the second response (Figure 9 and Figure 10B), indicating a synergism between thiopental and low concentrations of NE. As the muscle was further contracted with higher concentrations of NE the overall effect of thiopental reversed, so that aortic strips treated with NE $3x10^{-8}$ to $3x10^{-7}$M showed a biphasic response to thiopental with a small initial contraction, followed by relaxation (Figure 10C). Thiopental added after contractions induced by higher concentrations of NE ($3x10^{-6}$M or greater) produced only depressant effects. Thus the overall effect of thiopental, stimulation or depression, depended on the concentration of NE in the bath.

Administration of phenoxybenzamine (10^{-5}M) for one hour before the second exposure to thiopental caused a reduction in amplitude,

but did not completely block, the second thiopental response (Figure 9 and Figure 10A). Although this concentration of phenoxybenzamine blocked all constrictor response to NE (10^{-4}M), addition of NE (10^{-7} to 10^{-4}M) 15 minutes after phenoxybenzamine, but 45 minutes before the second exposure to thiopental, restored the second thiopental response to control values (Figure 9).

Thiopental was the only barbiturate which acted synergistically with low concentrations of NE. Pretreatment with NE ($3x10^{-9}$M) did not potentiate the stimulant effects of the convulsants in aorta; barbiturates that lacked constrictor effects (CHOMB, benzylbarbital, pentobarbital, and methohexital) also lacked constrictor effects in preparations pretreated with norepinephrine. Pretreatment with phenoxybenzamine (10^{-5}M) had no effect on aortic strip contractions induced by either CHEB or πDMBB. Parenthetically, atropine, a selective parasympathetic blocking agent, did not prevent the contractile effects of convulsant barbiturates in the jejunum (Figure 3B).

Sweat Response to Intradermal Drug Injection. Each forearm of the four subjects was used on two successive days to test the ability of CHEB and thiopental to elicit localized sweating after intradermal injection. The subjects were distributed randomly to the protocol (Table I) by drawing a number I, II, III, or IV from a hat. The protocol was arranged so that two subjects received thiopental and two subjects received CHEB; both barbiturates were administered at concentrations of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4}M. All four

subjects also received vehicle (HBS) as a negative control and nicotine (1 mg/100 ml HBS) as a positive control. Concentrations of barbiturate greater than 10^{-4}M were not injected intradermally in volunteers because preliminary experiments with intradermal injection of higher barbiturate concentrations in rabbits produced evidence of pain and tissue damage.

Neither CHEB nor thiopental stimulated sweating (Figure 11A), whereas nicotine caused sweating at the site of injection and in an area 1-2 cm beyond the radius of the injection wheal (Figure 11B). Nicotine-induced sweating spread into the adjacent wheal raised by previous injection of CHEB or thiopental, so that these barbiturates in the concentrations employed did not block the sweat response to nicotine.

The Relation of Extracellular Calcium to the Stimulant Activity of Barbiturates. Aortic strips did not contract when normal Krebs solution was abruptly replaced with a Ca-free Krebs solution containing a stimulant concentration of CHEB ($3x10^{-5}$M) or πDMBB ($4x10^{-5}$M). However, under similar conditions preparations remained responsive to K and NE (Figure 12). The response of convulsant treated preparations returned when the Ca-deficient Krebs solution was replaced by a series of solutions containing the same concentration of convulsant barbiturate but increasing concentrations of Ca (0.5, 1.0, 2.5, and 4.0 mM Ca). Figure 12C shows that the amplitude of contraction increased with each increment in extracellular Ca concentration, $[Ca]_o$, and the Ca concentration-response relationship in Figure 13

shows that contraction amplitude in CHEB treated preparations was a linear function of the log of $[Ca]_o$ in the bathing solution. Control experiments demonstrated that these concentrations of Ca did not elicit contraction in the absence of CHEB. Calcium concentration could not be increased above 4 mM without formation of a precipitate in the solution.

In aortic strips that had been bathed continuously in 2.5 mM Ca-Krebs solution, CHEB ($3x10^{-5}$M) produced contractions that were $72\pm3\%$ of the maximal response to NE; however, in those preparations exposed to Ca-free Krebs solution, the combination of CHEB ($3x10^{-5}$M) and 2.5 mM Ca produced contractions whose amplitudes were only $53\pm3\%$ of the maximal response to NE (Figure 13). The reduction in CHEB-induced contraction may reflect the effect of prolonged exposure to low Ca solutions, since approximately one hour was required to increase the Ca concentration in steps from 0 to 2.5 mM. In separate experiments with aortic strips that had been exposed for one hour to a Ca-free solution and then stimulated with CHEB ($3x10^{-5}$M) in 2.5 mM Ca solution, CHEB-induced contractions were $43\pm3\%$ of the maximal NE control.

As in experiments with the convulsant barbiturates, aortic strips did not contract when normal Krebs solution was abruptly replaced with a Ca-free Krebs solution containing a stimulant concentration of thiopental (10^{-3}M). However, replacement of Ca, while maintaining the thiopental concentration, did not restore the constrictor effect

of thiopental.

Effect of Strontium on CHEB Treated Aortic Strips. Strontium could substitute for Ca and initiate contraction in aortic strips treated with CHEB in a Ca-free solution, although a higher concentration of Sr was necessary to produce the same amplitude of contraction. In seven preparations pretreated with CHEB ($3x10^{-5}$M) in a Ca-free solution, washing with 1mM Sr solution containing CHEB ($3x10^{-5}$M) produced contraction equivalent to $7\pm3\%$ of the maximal NE control, and washing with 10mM Sr solution produced contraction amplitudes of $61\pm10\%$ of the NE control. Four mM Ca produced approximately the same effect as 10mM Sr (Figure 13).

Lanthanum Blockade of the Constrictor Effect of the Barbiturates. Treatment of aortic strips (4) for 15 minutes with 5mM La completely prevented the contractile response to CHEB ($3x10^{-5}$) or π DMBB ($4x10^{-5}$M). After the same La treatment, preparations remained responsive to NE (10^{-4}M) but did not respond to K (100mM) (Figure 14). Thiopental-induced contractions were not blocked by pretreatment (15 minutes) with 5mM La.

Effect of Calcium Concentration on the Extent of Tachyphylaxis to CHEB. To determine the effect of $[Ca]_o$ on the extent of tachyphylaxis, the dose-response relationship for Ca in a CHEB treated preparation was determined before and after tachyphylaxis to CHEB. CHEB tachyphylaxis was established in the interval between these two determinations by subjecting the aortic strips to four successive

CHEB ($3x10^{-5}$M) induced contractions while $[Ca]_o$ was maintained at one of the following concentrations: 0.5, 1.0, 2.5 or 4.0 mM. Figure 15 shows that the amplitude of contraction elicited by CHEB with each concentration of $[Ca]_o$ was considerably less impaired when tachyphylaxis was established in low Ca solution (0.5 or 1mM) than in high Ca solution (2.5 or 4.0mM).

DISCUSSION

A total of 28 barbituric acid derivatives have been tested for effect on rabbit aortic strips or intestinal segments in this and preceding work by Powell et al., (1943) and Hupka et al., (1969). Table II lists the individual barbiturates with their effects on CNS and smooth muscle. Within this sample of barbiturates, only those derivatives that produced maximal (tonic extensor) seizures in intact animals also had strong stimulant effects on in vitro preparations of aortic and intestinal smooth muscle. The convulsant barbiturates used in the present investigation, CHEB and π DMBB, also contracted tracheal chains but the extent of contraction was much less than in aortic or jejunal preparations. Barbiturates with less intense CNS excitatory effects, the semiconvulsants, failed to produce contraction in any smooth muscle preparation.

Thiopental also caused contraction of smooth muscle; however, this effect was seen only in aortic strips and differed from the effect of the convulsants in nearly every parameter studied. The doses of thiopental required to contract aortic strips were very much higher than those of the convulsants and produced much less intense effects (Figure 4). Aortic strips that were partially contracted with either CHEB or π DMBB could be further contracted by addition of the other convulsant barbiturate but were relaxed by addition of thiopental. Finally, the amplitude of thiopental induced contractions was markedly enhanced by low concentrations of NE and inhibited by phenoxybenzamine,

whereas neither NE nor phenoxybenzamine altered amplitude of contractions induced by CHEB or π DMBB. Therefore the mechanism of constrictor effect of thiopental in aortic strips seems basically different from that of the convulsant barbiturates.

In both the CNS and isolated smooth muscle, the convulsant barbiturates produced stimulant effects at very low concentrations relative to the depressant effects of other barbiturates. In mice, the doses of (+)-DMBB and CHEB that produced maximal seizures in 50% of the animals (CD50) were respectively 3 and 4 mg/kg i.v., whereas the dose of pentobarbital that produced loss of the righting reflex in 50% of the animals (AD50) was 30 mg/kg i.v. (Downes et al., 1970). The intraperitoneal CD50 of π DMBB in mice was only 3 mg/kg (Cain and Kleis, 1959), whereas the i.p. AD50 of pentobarbital is about 60 mg/kg (Barnes and Eltherington, 1966). It has been previously suggested (Downes et al., 1970) that high potency of stimulant activity is a necessary prerequisite for maximal seizures induced by barbiturates and that such seizures occur only when the stimulant effect of a barbiturate is unopposed by depressant effect. In support of this hypothesis, Downes et al., (1970) noted that the maximal seizure can be prevented if animals are treated with low doses of depressant barbiturates prior to injection of a convulsant barbiturate. Analogously, intestinal preparations treated with pentobarbital did not contract in response to racemic DMBB (Powell et al., 1943) and

pentobarbital completely blocked the constrictor effect of CHEB in aortic strips (Hupka et al., 1969).

CHEB and π DMBB at concentrations between 10^{-6} and 10^{-5}M produced just detectable contraction of isolated smooth muscle preparations, and these concentrations are probably very close to those present in blood and brain during maximal seizures induced by these drugs. As the concentration of convulsant barbiturate in the smooth muscle bath was increased above 10^{-4}M, the contraction induced by lower drug concentrations was depressed (autoinhibition). The autoinhibitory concentrations of CHEB and π DMBB in jejunal segments were very similar to threshold depressant concentrations of thiopental or pentobarbital (Figure 3 and Figure 7). In aortic strips or tracheal chains that had been maximally contracted with K or Ach, the effects of CHEB and π DMBB were purely depressant. Against such induced contractions, the threshold depressant concentrations of CHEB and π DMBB were similar to those of thiopental and pentobarbital and varied in individual experiments from 1.4×10^{-5} to 1.4×10^{-4}M. This concentration range is comparable to the CNS concentration that has been reported during anesthesia with thiopental or pentobarbital (Goldstein and Aronow, 1960).

From the standpoint of mechanism of action there is no necessary connection between the maximal seizure pattern in the CNS and stimulant effects in smooth muscle. Tonic extensor seizures simply represent an expression of intense CNS excitation (Esplin, 1959) and can be

elicited by many nonbarbiturate drugs with no known stimulant effect in smooth muscle. The association among barbiturates between ability to produce maximal seizures and ability to contract smooth muscle reflects an excitatory activity, manifest in both CNS and smooth muscle, at concentrations sufficiently low that it is not antagonized by the depressant effects of barbiturates.

In smooth muscle, potency of stimulant activity is not, however, the only determinant of intensity of effect. Thus, in smooth muscle as in the CNS, π DMBB is consistently a more potent stimulant than CHEB. Nevertheless, in aortic strips the maximal effect of CHEB is nearly twice that of πDMBB (Figure 4). Furthermore, the thiobarbiturates are able to produce feeble contraction of aortic strips even though the concentration required to produce maximal effect is much greater than that which depresses smooth muscle response to other agents.

The failure of barbiturates with less intense CNS excitatory effects to produce any contraction in these isolated smooth muscle preparations is open to several interpretations. The structure-activity relationships of barbiturates seem much more rigid for CNS stimulant than for CNS depressant effects (Downes et al., 1970) and receptors responsible for barbiturate-induced contraction of smooth muscle may have an even greater structural specificity. Alternatively, CHEB, πDMBB and (+)-DMBB may activate excitatory mechanism that are entirely distinct from those affected by other excitatory

barbiturates. On the basis of presently available information, these alternatives cannot be resolved but it is probable that in a group of drugs as structurally diverse as barbiturates, more than one mechanism of excitation may be present.

Site and Mechanism of Action of CHEB in Aortic Strips. Intravenous injections of 1 to 2 mg/kg of CHEB in spinal cats produces a 10 to 20 mv depolarization of the dorsal root ganglion cell accompanied by a decrease in the electrical resistance and firing threshold of the cell membrane (Downes and Franz, 1971). At the onset of CHEB-induced depolarization, ganglion cells discharge a burst of action potentials. CHEB also induces bursts of antidromic action potentials (dorsal root reflexes) from intraspinal endings of primary sensory fibers (Downes and Franz, 1971). CHEB might produce similar discharges in the motor nerve terminals of the autonomic nerves, present in isolated smooth muscle, and thus cause smooth muscle contraction. Such a stimulant effect would have to be nonspecific, occurring at both sympathetic and parasympathetic nerve endings, since CHEB induces contraction in a variety of smooth muscles, some of which are contracted by stimulation of sympathetic nerves and others by parasympathetic nerve stimulation.

Nonspecific stimulant effects on autonomic nerve terminals should also affect the nerve endings innervating sweat glands. After intradermal injection, substances that excite autonomic nerve endings of the sweat glands (Ach and nicotine, Coon and Rothman, 1941;

tetraethylammonium, Wada, Kikuchi, Tashiro and Takahashi, 1967), produce sweating by an axon reflex, in an area much larger than the actual site of drug injection. Neither CHEB nor thiopental, in the concentrations employed, produced any evidence of sweating after intradermal injection (Figure 11). CHEB was tested in the complete range of concentrations that produced stimulant effects on in vitro smooth muscle and none of these concentrations produced any evidence of sweating after intradermal injection. Thiopental also failed to produce localized sweating; however, only the lowest in vitro stimulant concentration of thiopental (10^{-4}M) could be injected intradermally.

Studies with phenoxybenzamine and atropine further suggest that neither the convulsant barbiturates nor thiopental produce contraction through release of NE or Ach from nerve endings. Hupka et al. (1969) noted that phenoxybenzamine (10^{-8}M)or atropine (10^{-5}M) did not prevent constriction of aortic strips by CHEB or (+)-DMBB but did block the constrictor effect of 10^{-6}M NE or Ach 10^{-5}M. In the present experiments 10^{-8}M phenoxybenzamine did not prevent contraction of aortic strips exposed to concentrations of NE greater than 10^{-6}M, and for this reason phenoxybenzamine in 10^{-5}M concentrations was used. This concentration of phenoxybenzamine completely blocked all constrictor responses to NE (10^{-4}M) or Ach (10^{-4}M); high concentrations (10^{-5}M) of α-adrenergic blocking agents such as dibenamine and phenoxybenzamine have also been shown to block the effects of histamine and serotonin (Furchgott, 1954; Cook, 1971). Phenoxybenzamine

(10^{-5}M) did not impair the constrictor effect of either CHEB or πDMBB. Similarly in jejunal segments, atropine (10^{-5}M) did not impair contraction induced by either convulsant barbiturate.

Burn (1959) reported that the constrictor effect of thiopental on vascular smooth muscle was a consequence of release of NE; however, Price and Price (1962) suggested that contractions induced by thiopental resulted from a synergistic effect of thiopental with endogenous NE. Results of the present experiments support the latter hypothesis. After pretreatment with phenoxybenzamine (10^{-5}M) aortic strips continued to respond to thiopental although the amplitude of response was reduced. These phenoxybenzamine treated preparations did not respond to NE concentrations as high as 10^{-4}M. If NE (10^{-7} to 10^{-4}M) was added to the bath solution 30 minutes before exposure to thiopental, the response to thiopental was restored to normal, despite the presence of phenoxybenzamine and the absence of any constrictor response to NE itself. These results suggest that contractions induced by thiopental are not a consequence of the α-adrenergic activity of NE, but that NE does potentiate the effect of thiopental.

Experiments with pharmacologic antagonists of specific agonists such as NE or Ach cannot exclude the possibility that the convulsant barbiturates act indirectly by release of some unknown agonist from nerve endings. Release and depletion of an agonist would be consistent with tachyphylaxis; however, such an agonist would have to be present

and pharmacologically active in the autonomic nerve terminals of a number of different types of smooth muscle tissues but not in autonomic nerve terminals innervating the sweat glands. Furthermore, the decrease in the response to K associated with tachyphylaxis to CHEB and π DMBB suggests a functional change in smooth muscle cells rather than depletion of an agonist.

In smooth muscle, as in striated muscle, intracellular free Ca activates the contraction process (Bohr, 1964; Daniel 1964). However, the mechanisms by which different agonists increase activator Ca may be unique for each agonist as well as each type of smooth muscle (Somlyo and Somlyo, 1970). Anatomically, the total tissue Ca is dispersed in a heterogenous milieu and includes extracellular Ca in the interstitial spaces, Ca bound to the surface of the cell membrane and Ca sequestered within the cell. Functionally, total tissue Ca can be divided into various fractions with different importance to the effects of individual agonists. These fractions can be depleted at different rates when tissues are bathed in Ca-free solutions (Hudgins and Weiss, 1968).

NE-induced contractions involve a source of activator Ca that is tightly bound and not rapidly depleted by bathing in Ca-free solution. Acute exposure of aortic strips to Ca-free solutions causes little decrement in the response to NE and four hours in Ca-free solution are necessary to completely block the NE response (Garrett and Carrier, 1971). In contrast, the amplitude of K-induced contractions

is immediately reduced in Ca-free solution and is almost completely eliminated within 45 minutes (Garrett and Carrier, 1971). Thus, K-induced contractions are dependent on a source of activator Ca that is quickly depleted and probably includes extracellular Ca in the bath solution and interstitial spaces and Ca loosely bound to the cell membrane. Ca flux studies (van Breeman and McNaughton, 1970) have demonstrated that K-induced contractions of aortic strips are accompanied by an increased cellular uptake of Ca from the bath solution.

CHEB-induced contractions are even more sensitive to acute Ca depletion than contractions induced by K. Taking into account the lag period which is normally observed between the time of exposure to CHEB and the appearance of contraction (Figure 2 and Hupka et al., 1969), aortic strips became completely unresponsive to CHEB in less than a minute after exposure to Ca-free solution. Free extracellular Ca is the most probable source of an activator Ca that could be so quickly depleted. In the short time involved, it is unlikely that Ca in the interstitial space or bound to cell membranes would be sufficiently reduced to account for the loss of all response to CHEB.

The slow onset and rate of rise of CHEB-induced contractions is consistent with the time necessary for extracellular Ca to diffuse into the cell and initiate contraction. The proportionality of CHEB effect to extracellular Ca concentration is also consistent with this explanation. A similar relationship between extracellular Ca concentration and intensity of effect has been observed for contractions induced by

K (Godfraind and Kaba, 1972).

La, a trivalent cation, has been shown to prevent the influx of extracellular Ca associated with K-induced contractions, possibly by binding to fixed anionic sites in the cell membrane which control Ca permeability (van Breeman et al., 1972). In contrast, the release of intracellular bound Ca during NE induced contractions is not significantly altered by La (van Breeman et al., 1972). The ability of La to block CHEB-induced contractions (Figure 14) indicates that the CHEB effect in aortic strips is primarily, if not completely, dependent on extracellular Ca which must pass through the cell membrane to activate the contractile mechanism.

Ca also appears to be intimately involved with the development of tachyphylaxis to barbiturate effect in aortic strips, as the rate of tachyphylaxis is much greater in higher Ca than in lower Ca solutions (Figure 15). Possibly the loss of responsiveness with repeated CHEB stimulation is a reflection of an accumulation of sequestered Ca within the cells. Some of this Ca might act to limit further accumulation of Ca by stabilizing the permeability of the cell membrane and preventing or retarding Ca-influx with subsequent CHEB stimulation. Such an effect might also explain the reduced response to K in preparations which have undergone tachyphylaxis to CHEB.

SUMMARY AND CONCLUSIONS

Although there is not an exact parallel between intensity of CNS excitation and intensity of constrictor activity in smooth muscle, all barbituric acid derivatives which produced maximal seizures also stimulated isolated smooth muscle. Other barbiturates with less intense CNS excitatory effect, semiconvulsants, did not elicit smooth muscle contraction. The maximal constrictor effect of the convulsant barbiturates was limited by the onset of depression as the convulsant barbiturates had smooth muscle depressant potencies similar to those of other barbiturates. The association between the ability to produce maximal seizures and to stimulate smooth muscle suggests that the convulsant barbiturates have a stimulant action which is manifest in both the CNS and smooth muscle at sufficiently low concentrations that it is not antagonized by the depressant activity of higher drug concentrations.

The constrictor activity of thiopental in aortic strips is basically different than that of the convulsants and is synergistic with NE. However, both thiopental and the convulsant barbiturates probably produce contraction by an action directly on smooth muscle cells rather than on autonomic nerve terminals.

Both the amplitude of CHEB-induced contraction and the extent of tachyphylaxis to CHEB effect are determined by the concentration of Ca in the bath solution suggesting that the constrictor effect of the

convulsant barbiturates in aortic strips probably represents an entry of extracellular Ca into smooth muscle cells. The relationship of extracellular Ca to effect of CHEB is similar to that described for K-induced contractions; however, the effect of CHEB is much more sensitive to acute changes in the concentration of extracellular Ca.

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Weakly, J. N., Esplin, D. W., and Zablocka, B. Criteria for assessing effects of drugs on postsynaptic inhibition. Arch. Int. Pharmacodyn. Ther., 1968. 171, 385-393.

Wynne, R. L. The convulsive properties of thiopentone. Br. Med. J., 1948. 1, 48-49.

	Subject I		Subject II		Subject III		Subject IV	
	Left Arm	Right Arm	Left Arm	Right Arm	Left Arm	Right Arm	Left Arm	Right Arm
Day 1	CHEB 10^{-8} CHEB 10^{-6} HBS	CHEB 10^{-4} Nicotine	CHEB 10^{-5} Nicotine HBS	CHEB 10^{-7} Nicotine	Thiopental 10^{-7} Nicotine	Thiopental 10^{-5} Nicotine HBS	Thiopental 10^{-8} Thiopental 10^{-6} HBS	Thiopental 10^{-4} Nicotine
Day 2	CHEB 10^{-5} Nicotine HBS	CHEB 10^{-7} Nicotine	CHEB 10^{-8} CHEB 10^{-6} HBS	CHEB 10^{-4} Nicotine	Thiopental 10^{-8} Thiopental 10^{-6} HBS	Thiopental 10^{-4} Nicotine	Thiopental 10^{-7} Nicotine	Thiopental 10^{-5} Nicotine HBS

Table I: Protocol for intradermal drug injection.

On each of two experimental days, four subjects received 2 or 3 intradermal injections in each forearm. The drugs and molar concentrations are indicated in the protocol. Nicotine (5×10^{-5} M) was used as a positive control and produced sweating (Figure 11) in all subjects. HBS, the vehicle in which other drugs were dissolved, was injected as a negative control and in no instance caused sweating.

Chemical Formula	Name	CNS Activity	Intestinal Activity	Aortic Activity
5-ethyl-5-R-barbiturate				
R: $-(CH_2)_n CH_3$ where n=1 to 6		D	— (1)	
$-CH(CH_3)(CH_2)_n CH_3$ where n=0,1,3,4 or 5		D	— (1)	
$-CH(CH_3)(CH_2)_2 CH_3$	pentobarbital	D	— (1)	— (2, 3)
$-C_6H_5$	phenobarbital	D		— (2)
$-CH_2C_6H_5$	benzylbarbital	SC	— (3)	— (3)
$-CH_2-O-C_6H_{11}$	CHOMB	SC	— (3)	— (3)
$-CH(CH_3)CH_2CH(CH_3)_2$	(—)-DMBB	SC		— (2)
	(+)-DMBB	C		+ (2)
	dl-DMBB	C	+ (1)	
$-CH(CH_3)CH=C(CH_3)_2$	π DMBB	C	+ (3)	+ (3)
$-CH_2CH=C_6H_{11}$	CHEB	C	+ (3)	+ (2, 3)
5-allyl-5-R-1-methyl barbiturate				
R: $-CH(CH_3)CH=CH\ CH_2\ CH_3$	methohexital	D	— (3)	— (3)
5-ethyl-5-R-thiobarbiturate				
R: $-CH(CH_3)(CH_2)_2\ CH_3$	thiopental	D	— (3)	+ (3)
5-crotyl-5-R-thiobarbiturate				
R: $-(CH_2)_n CH_3$ where n=1 to 3		SC	— (1)	
$-CH(CH_3)(CH_2)_n CH_3$ where n=0 to 2		SC	— (1)	

Table II: Summary of effects of barbiturates on smooth muscle and CNS.

The CNS activity is indicated by D for depressant, SC for semiconvulsant and C for convulsant. CNS activity is that indicated in Doran (1959) or Downes et al. 1970). Smooth muscle activity is indicated by a minus (–) for relaxation and a plus (+) for contraction. The number in parenthesis indicates the source or sources;

(1) Powell et al. (1943)

(2) Hupka et al. (1969)

(3) present thesis investigation.

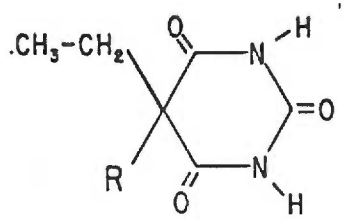
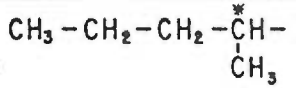
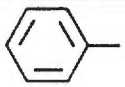
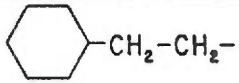
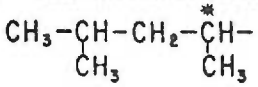
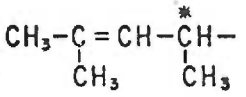
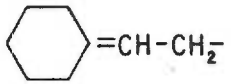
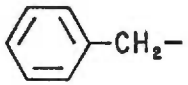
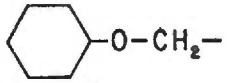
CNS Effect	General Structure (*Optically active carbon)  R		
Depressant	 pentobarbital	 phenobarbital	 5-ethyl-5-cyclohexylethyl barbituric acid
Convulsant	 (+)-DMBB  πDMBB		 CHEB
Semiconvulsant	(-)-DMBB	 benzylbarbital	 CHOMB

Figure 1: Chemical structures of representative depressant, semiconvulsant, and convulsant barbiturates.

The barbiturates are arranged horizontally on the basis of their CNS effect and vertically according to structural similarities of the R side-chain. Names and commonly used abbreviations are given below each structure. (+)-DMBB, a convulsant and (—)-DMBB, a semiconvulsant, are the separate optical isomers of dl-DMBB, a convulsant.

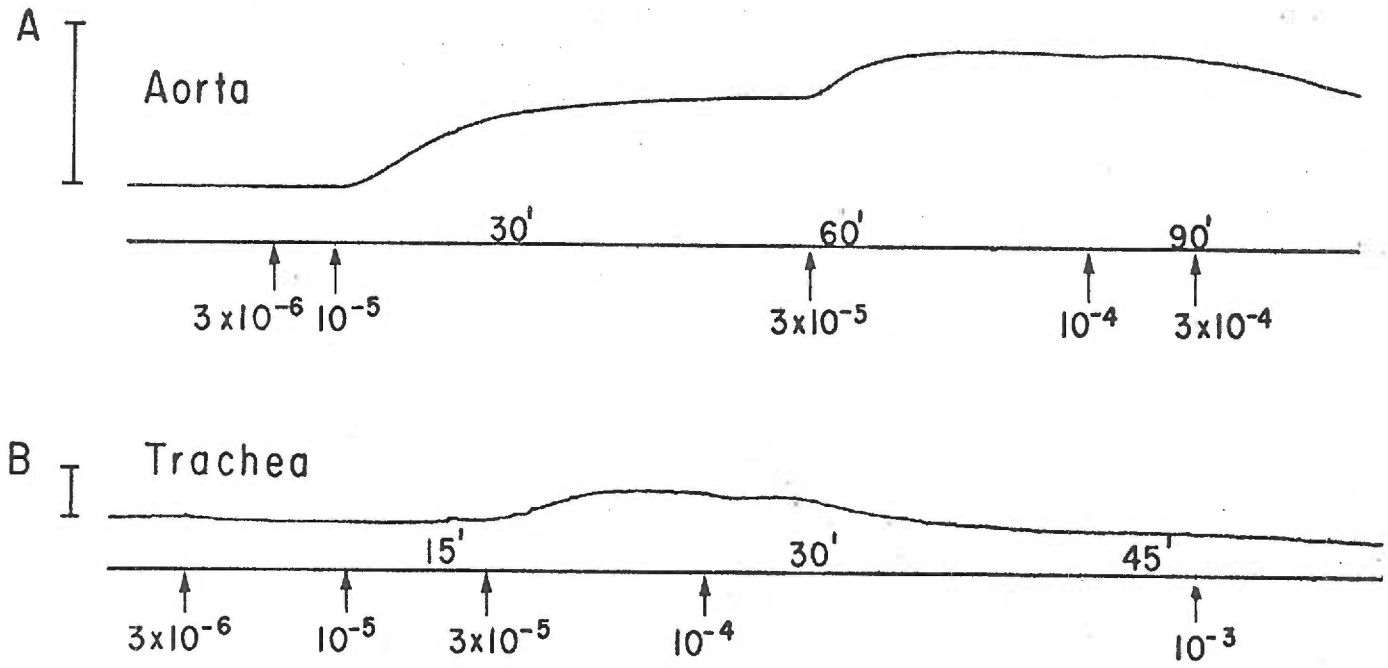

A
Aorta
30'
60'
90'
3x10-6
10-5
3x10-5
10-4
3x10-4
B
Trachea
15'
30'
45'
3x10-6
10-5
3x10-5
10-4
10-3

Figure 2: Typical responses of isolated rabbit aortic strips and tracheal chains to cumulative increases in CHEB concentration.

The calibration line to the left of trace A represents 100% of the maximal response to NE ($3x10^{-5}$M). The calibration line to the left of trace B represents 10% of the maximal response to Ach (10^{-4}M). The elapsed time (minutes) from the beginning of the trace is indicated on the horizontal line below each recording. Addition of each CHEB increment is indicated by an arrow with the molar concentration of the increment given below.

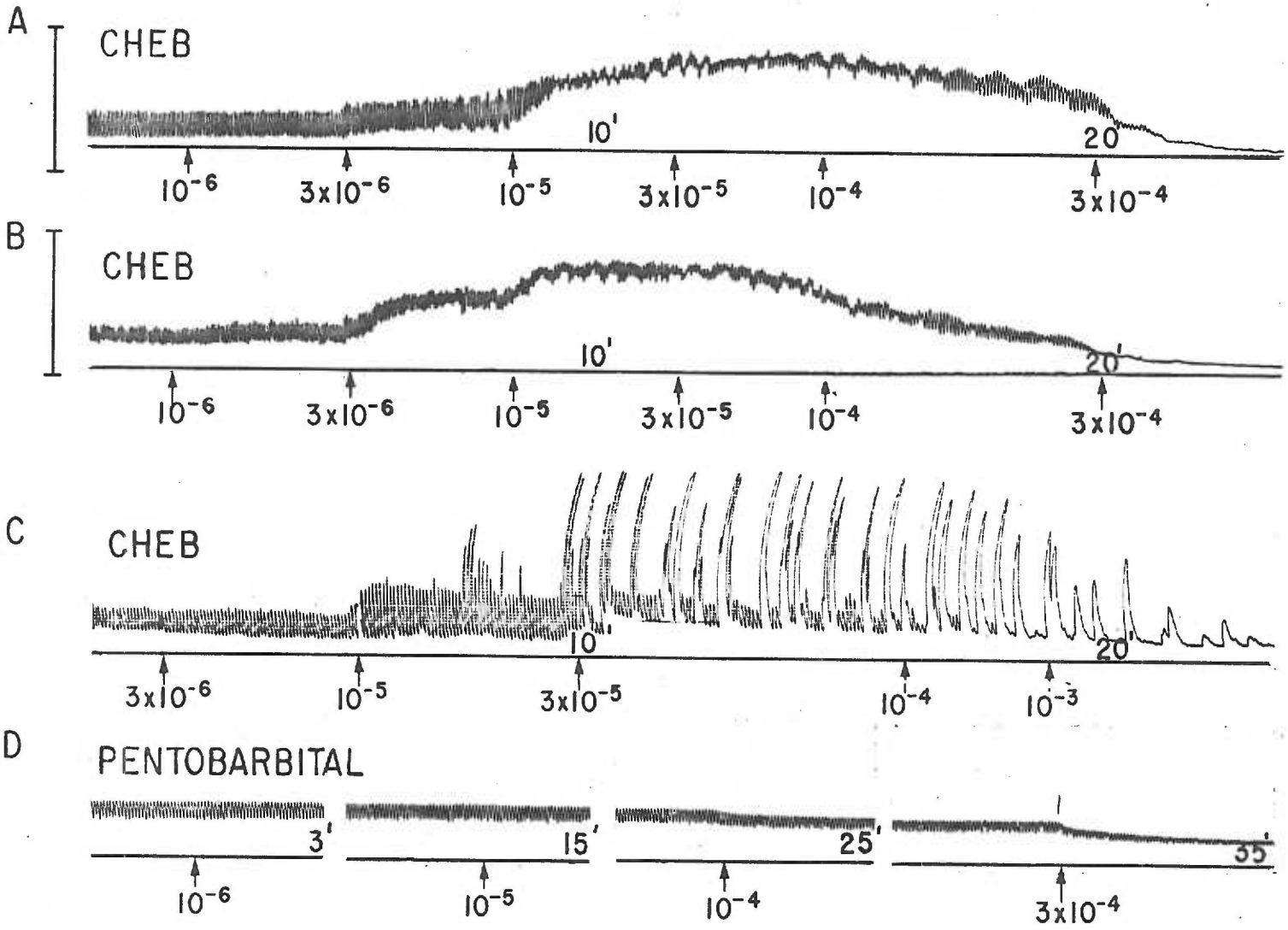

A
CHEB
10'
20'
10⁻⁶
3x10⁻⁶
10⁻⁵
3x10⁻⁵
10⁻⁴
3x10⁻⁴
B
CHEB
10'
20'
10⁻⁶
3x10⁻⁶
10⁻⁵
3x10⁻⁵
10⁻⁴
3x10⁻⁴
C
CHEB
10'
20'
3x10⁻⁶
10⁻⁵
3x10⁻⁵
10⁻⁴
10⁻³
D
PENTOBARBITAL
3'
15'
25'
35'
10⁻⁶
10⁻⁵
10⁻⁴
3x10⁻⁴

Figure 3: Effects of CHEB and pentobarbital on isolated rabbit jejunum.

Addition of each drug increment is indicated by an arrow with the molar concentration of the increment given below. The elapsed time (minutes) from the beginning of the trace is indicated on the horizontal line below each recording. Traces A, B, and C show responses to CHEB. Trace D shows the response to pentobarbital. Traces A and B are recordings of two jejunal segments, obtained from the same animal; however, in the preparation shown in trace B atropine (10^{-5}M) was added to the bath 20 minutes before addition of CHEB. This concentration of atropine completely blocked 10^{-5}M Ach, and the response to 10^{-4}M Ach was barely detectable. The amplitude calibrations to the left of recordings A and B show the maximal contractions subsequently induced by Ach (10^{-4}M) in A and by K (100mM) in B and the maximal relaxation produced by the combined effects of isoproterenol (10^{-5}M) and thiopental (10^{-3}M). Trace C demonstrates phasic, high amplitude contractions occasionally observed with CHEB stimulation.

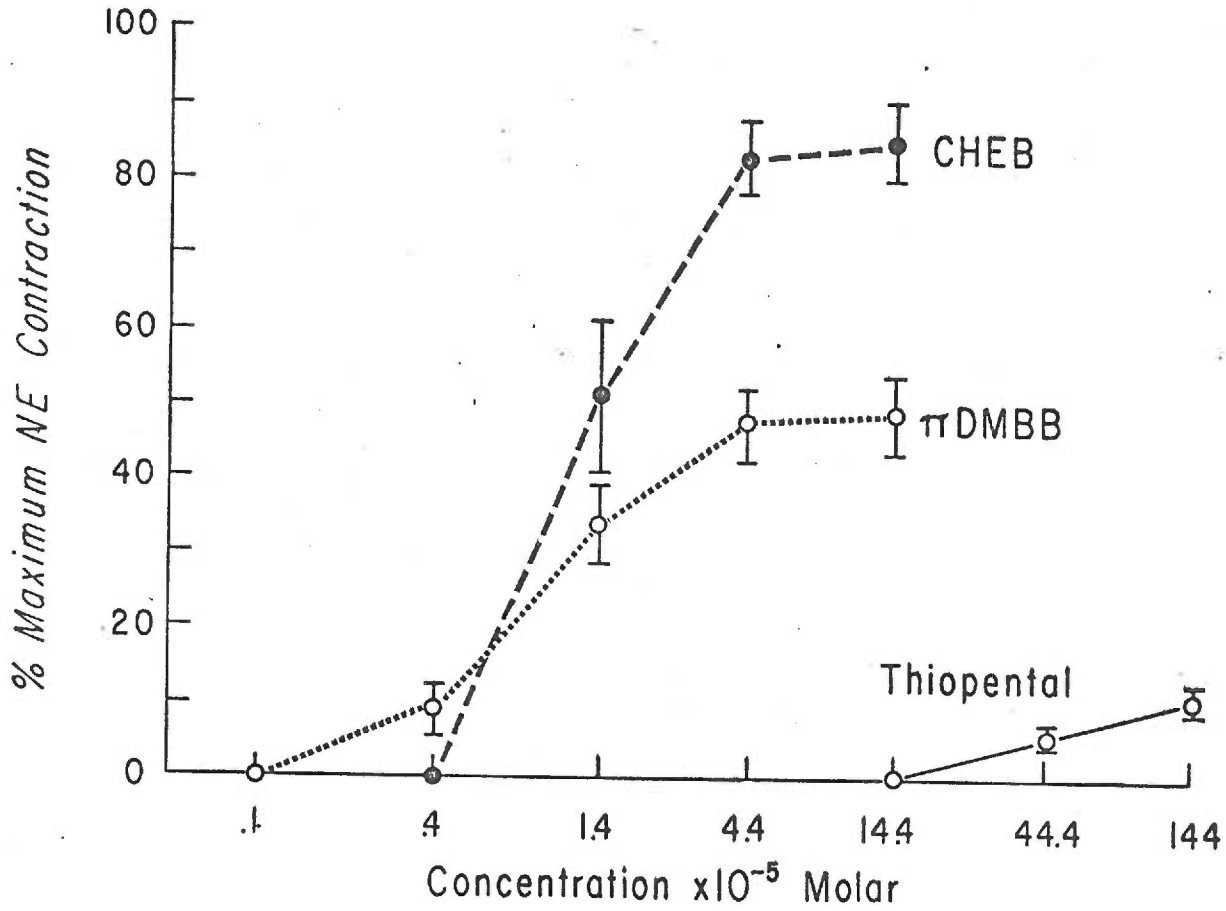

100
80
60
40
20
0
% Maximum NE Contraction
CHEB
πDMBB
Thiopental
.1
.4
1.4
4.4
14.4
44.4
144
Concentration x10⁻⁵ Molar

Figure 4: Cumulative dose-response relationships for barbiturate stimulation of isolated rabbit aortic strips.

Barbiturate-induced contractions were standardized on the basis of a previous maximum response to NE ($3x10^{-5}$ or 10^{-4}M). Each point represents the mean of a minimum of five separate determinations and brackets give the standard error of the mean.

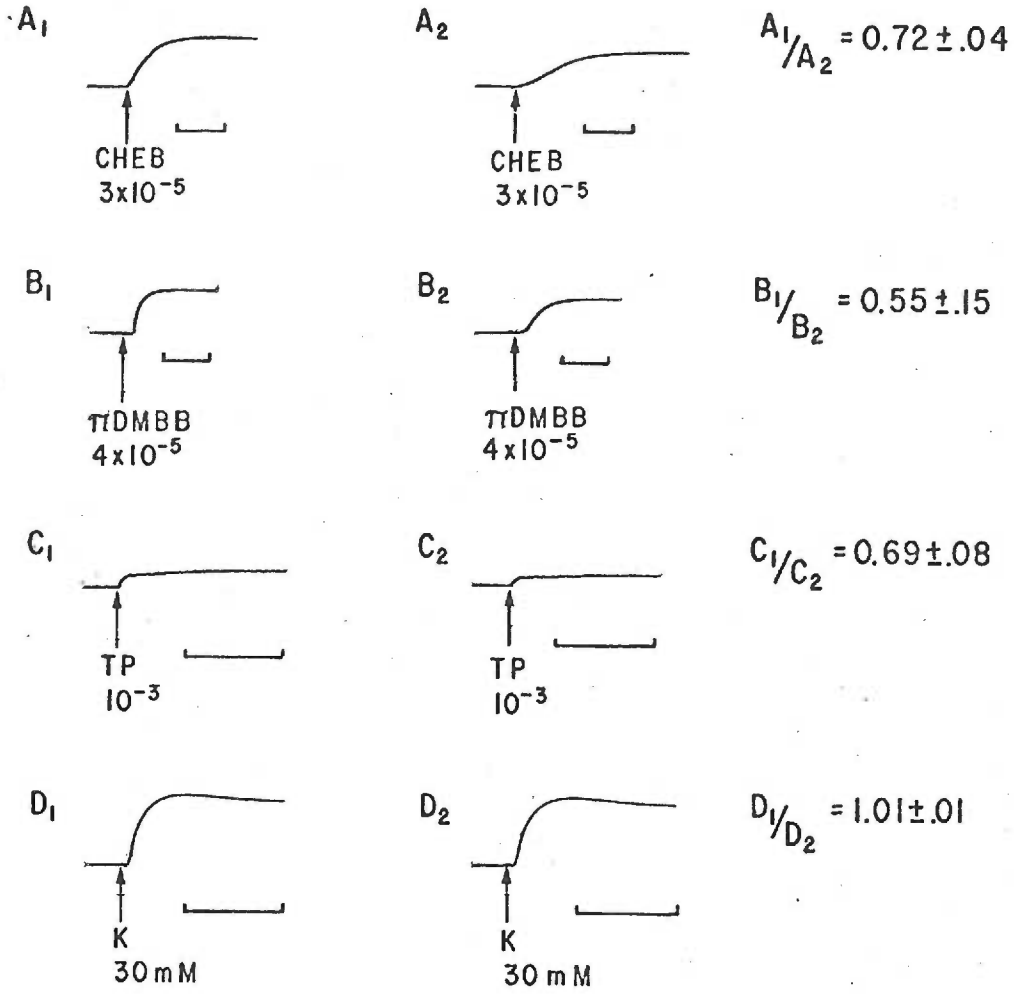

A1
CHEB
3x10-5
A2
CHEB
3x10-5
A1/A2 = 0.72 ± .04
B1
πDMBB
4x10-5
B2
πDMBB
4x10-5
B1/B2 = 0.55 ± .15
C1
TP
10-3
C2
TP
10-3
C1/C2 = 0.69 ± .08
D1
K
30 mM
D2
K
30 mM
D1/D2 = 1.01 ± .01

Figure 5: Comparison of first and second responses of rabbit aortic strip stimulated by CHEB, π DMBB, thiopental, or K.

Each pair of illustrations, as indicated by the subscripts 1 and 2, show two successive drug-induced contractions in a single preparation. After the initial contraction, the preparation was washed and allowed to relax. The time of drug addition is shown by an arrow and the molar drug concentration is given beneath the arrow. The scale below each trace represents 10 minutes. The mean $\pm$S.E.M. of the ratio of the amplitude of the second response to that of the first response is given to the right of each pair of traces. Eight experiments were used to determine mean values for A_1/A_2, B_1/B_2, and C_1/C_2 and five experiments for D_1/D_2.

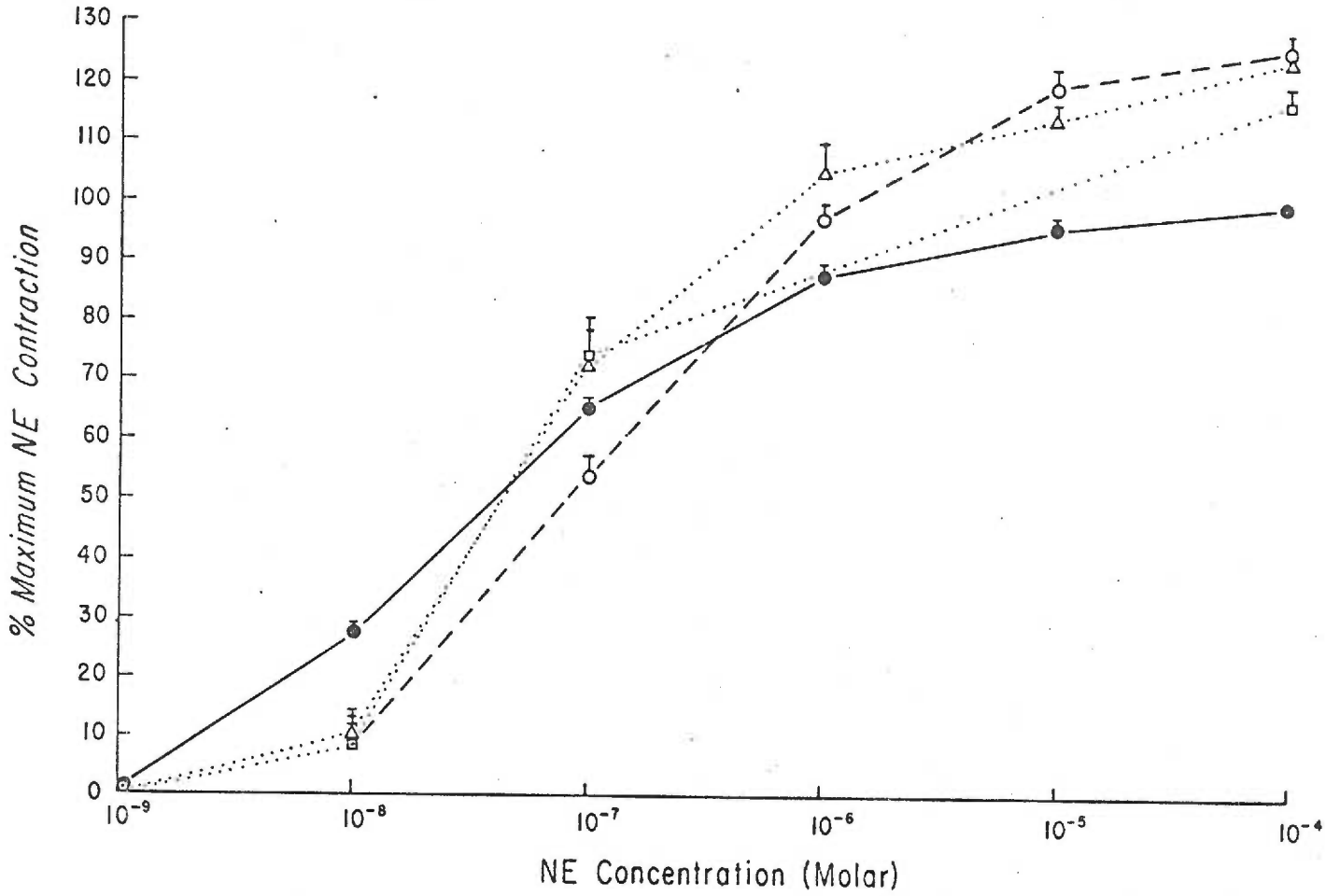

% Maximum NE Contraction
130
120
110
100
90
80
70
60
50
40
30
20
10
0
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
NE Concentration (Molar)

Figure 6: The effect of tachyphylaxis to CHEB and π DMBB on the response of rabbit aortic strips to NE.

The responses to cumulative increments of NE were standardized on the basis of the initial contraction to NE (10^{-4}M). Each point represents the mean of a minimum of three separate determinations and the brackets indicate the mean plus the standard error of the mean. The concentration-effect relationship shown in solid circles and solid lines (●———●) was obtained on the first exposure to increasing concentrations of NE. After the initial contractions the preparations were washed and allowed to relax. The concentration-effect relationship shown in open circles and broken lines (o--------o) was obtained on the second exposure to increasing concentrations of NE without any intervening treatment. The concentration-effect relationship shown in open triangles and dotted lines (△·····△) represents the response to the second exposure to increasing concentrations of NE after an intervening treatment of four successive πDMBB-induced contractions. The concentration-effect relationship, shown in open squares and dotted lines (□·····□) represents the response to the second exposure to increasing concentrations of NE after an intervening treatment of four successive CHEB-induced contractions.

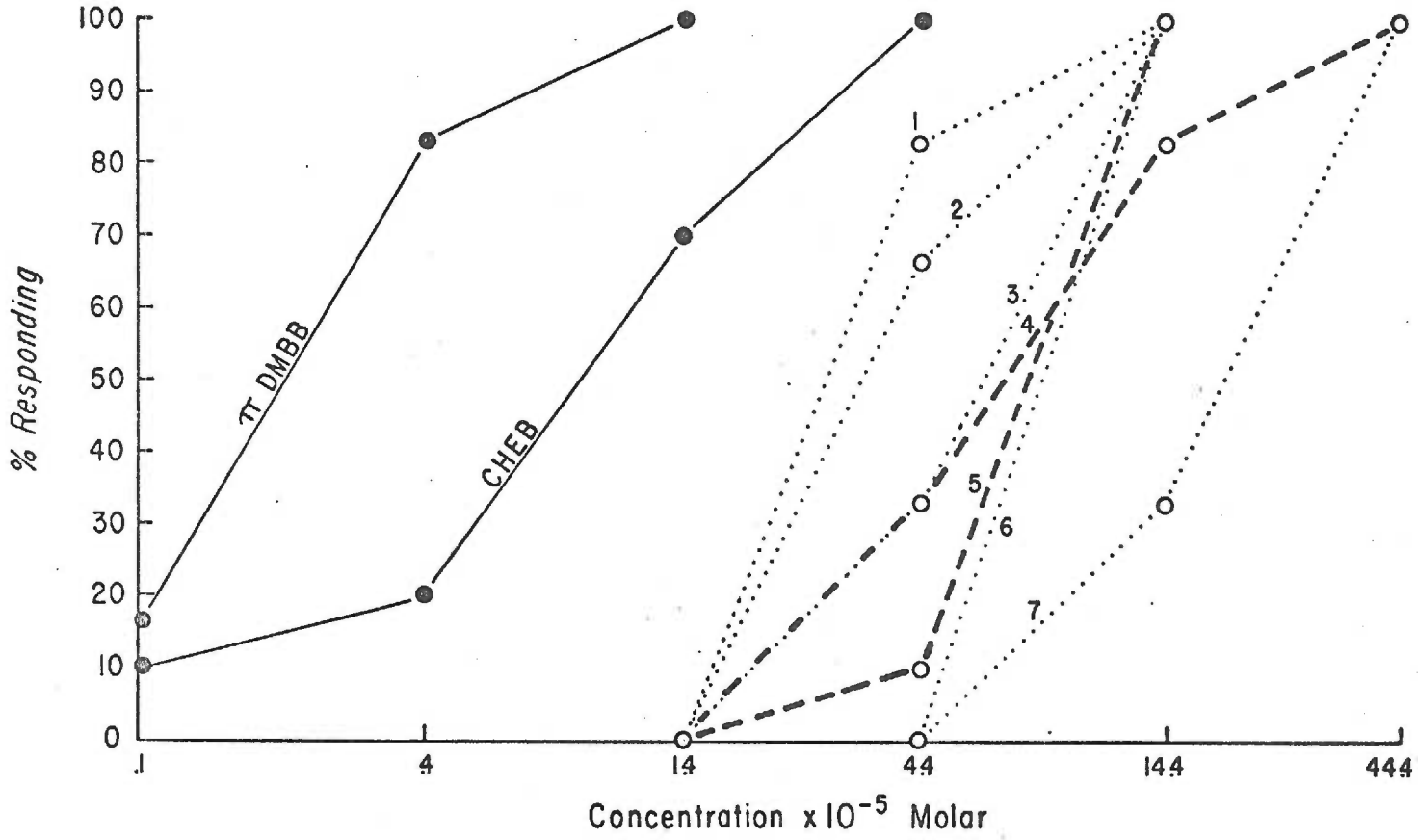

100
90
80
70
60
50
40
30
20
10
0
% Responding
TT DMBB
CHEB
1
2
3
4
5
6
7
.1
4
14
44
144
444
Concentration x 10⁻⁵ Molar

Figure 7: The quantal concentration-effect curves for just detectable (threshold) stimulant and depressant effects on rabbit jejunal preparations.

The ordinate indicates the percent of preparations showing stimulant or depressant effects at the concentrations indicated on the abscissa. CHEB was tested in 10 jejunal preparations and all other barbiturates in 6 preparations apiece. The concentration-effect curves shown in solid circles and solid lines (●———●) represent stimulant effects of CHEB and πDMBB. The concentration-effect curves shown in open circles and broken lines (o- - - - - - - - - o) represent the depressant or autoinhibitory concentrations for π DMBB (4) and CHEB (5). The concentration-effect curves shown in open circles and dotted lines (o·····o) represent the depressant effects of other barbiturates; CHOMB (1), methohexital (2), thiopental (3), pentobarbital (6), and benzylbarbital (7).

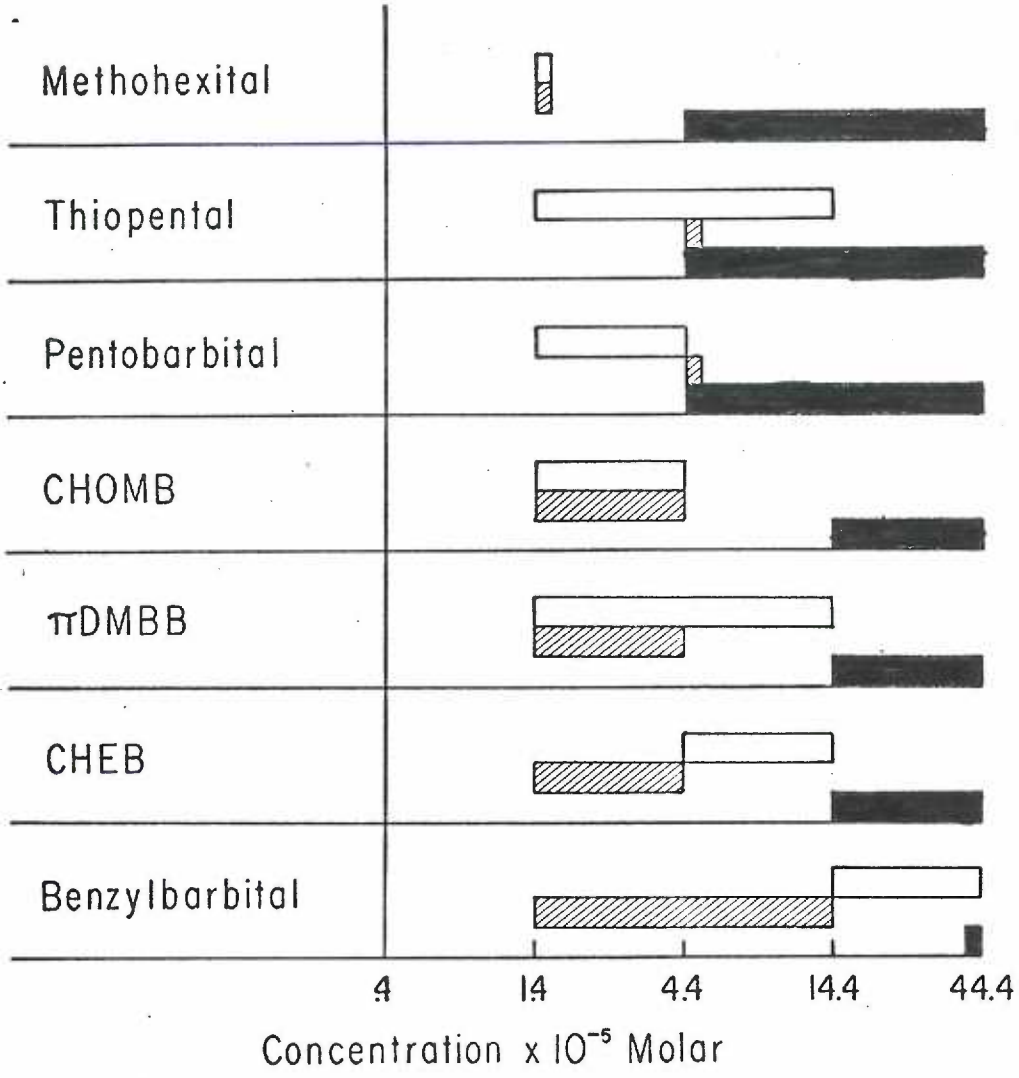

Methohexital
Thiopental
Pentobarbital
CHOMB
πDMBB
CHEB
Benzylbarbital
4
14
44
144
444
Concentration x 10⁻⁵ Molar

Figure 8: Barbiturate concentrations required to produce just detectable depression of induced contractions.

Each bar indicates the range of barbiturate concentrations required to produce depressant effects in three separate experiments. The solid black bars show the depressant concentrations for NE ($3x10^{-6}$M)-induced contractions of aortic strips. The hatched bars show depressant concentrations for K (100mM)-induced contractions of aortic strips. The open bars indicate depressant concentrations for Ach (10^{-4}M)-induced contractions of tracheal chains.

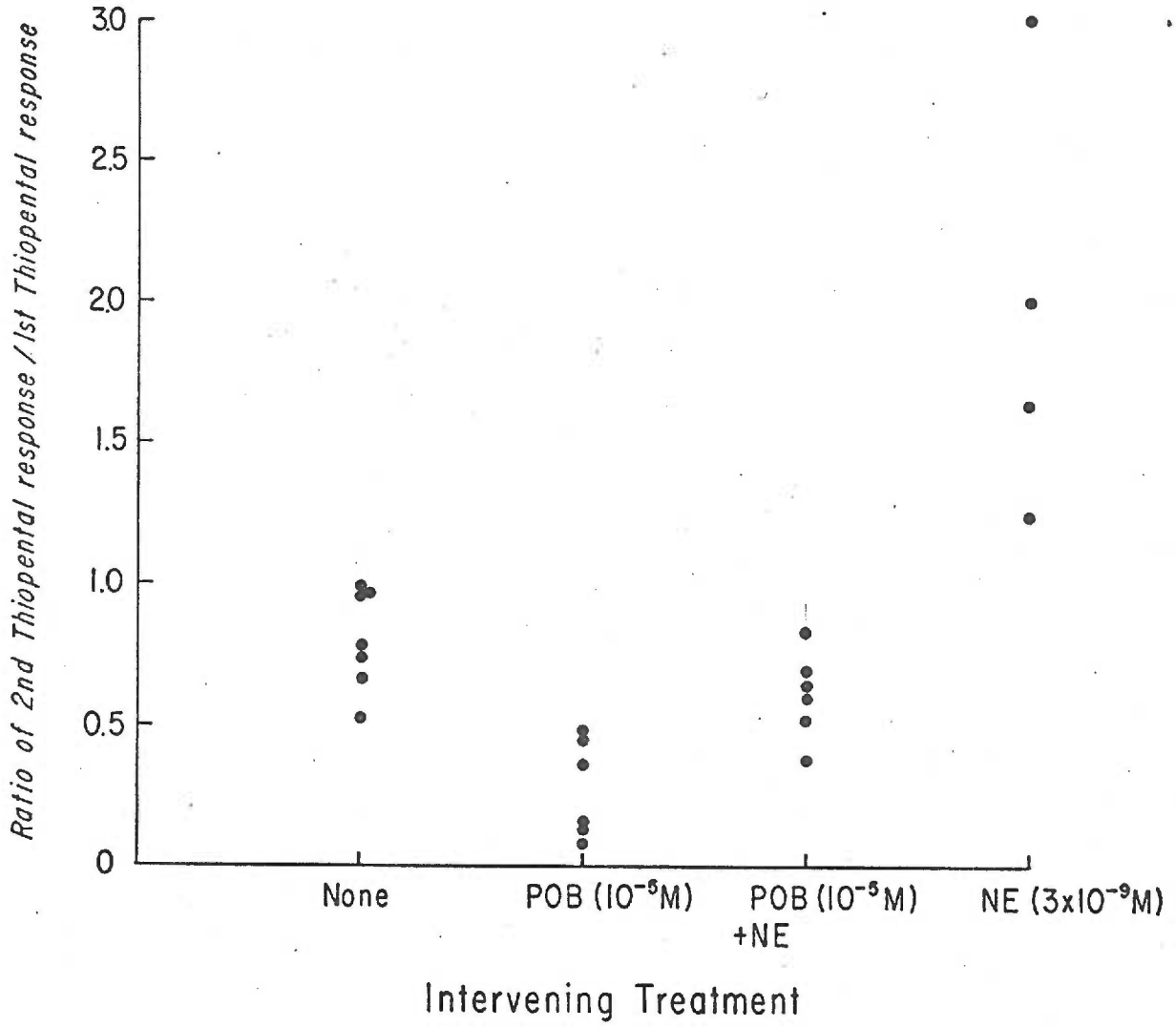

Ratio of 2nd Thiopental response / 1st Thiopental response
3.0
2.5
2.0
1.5
1.0
0.5
0
None
POB (10⁻⁵M)
POB (10⁻⁵M) +NE
NE (3x10⁻⁹M)
Intervening Treatment

Figure 9: Summary of the effects of phenoxybenzamine and NE on the action of thiopental in the rabbit aortic strip.

Each point shows the ratio between second and first response to thiopental (10^{-3}M) obtained in individual experiments. Treatments intervening between the first and second thiopental-induced contractions are indicated below each group of experiments. Experiments labeled "none" had no intervening treatment; those labeled "POB (10^{-5}M)" were exposed to phenoxybenzamine for one hour prior to the second thiopental response; those labeled "POB (10^{-5}M) + NE" were exposed to phenoxybenzamine for one hour and, in addition, treated with NE (10^{-7} or 10^{-4}M) for 45 minutes prior to the second thiopental response; those labeled "NE ($3x10^{-9}$M)" were contracted with this concentration of NE just before the exposure to thiopental.

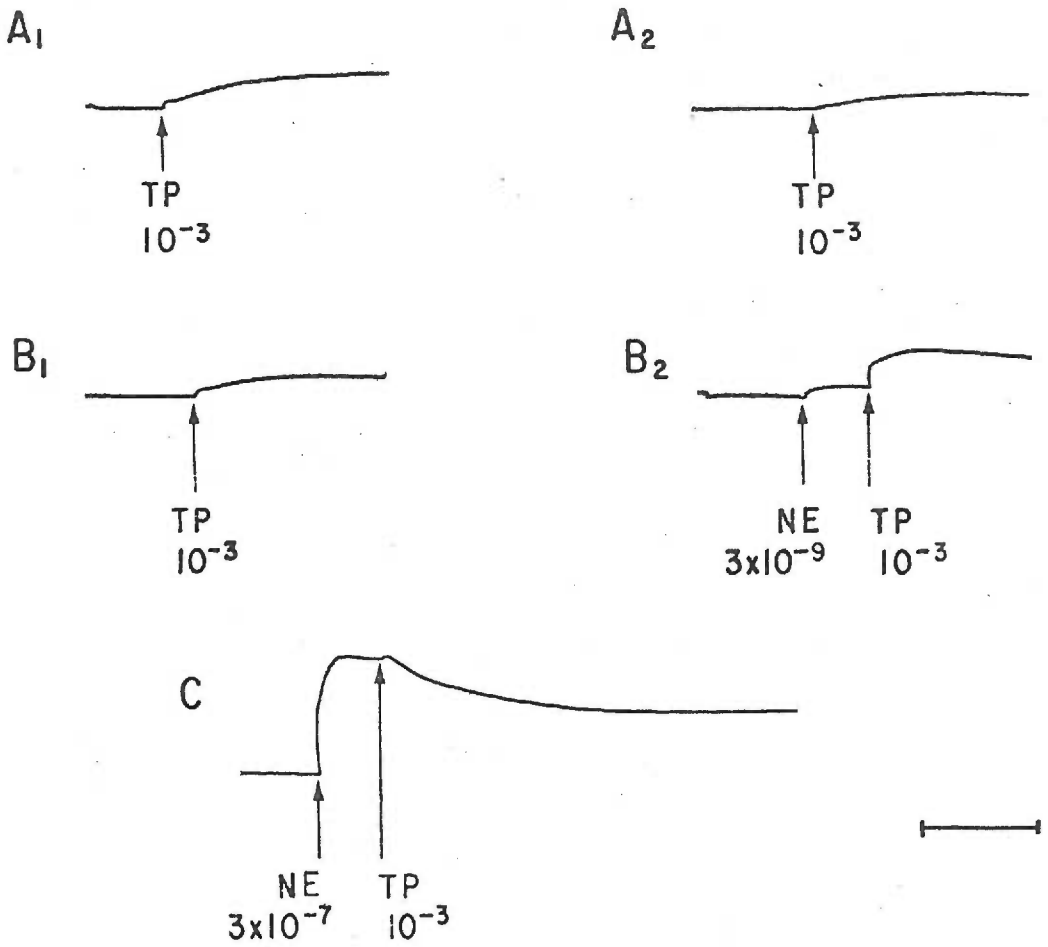
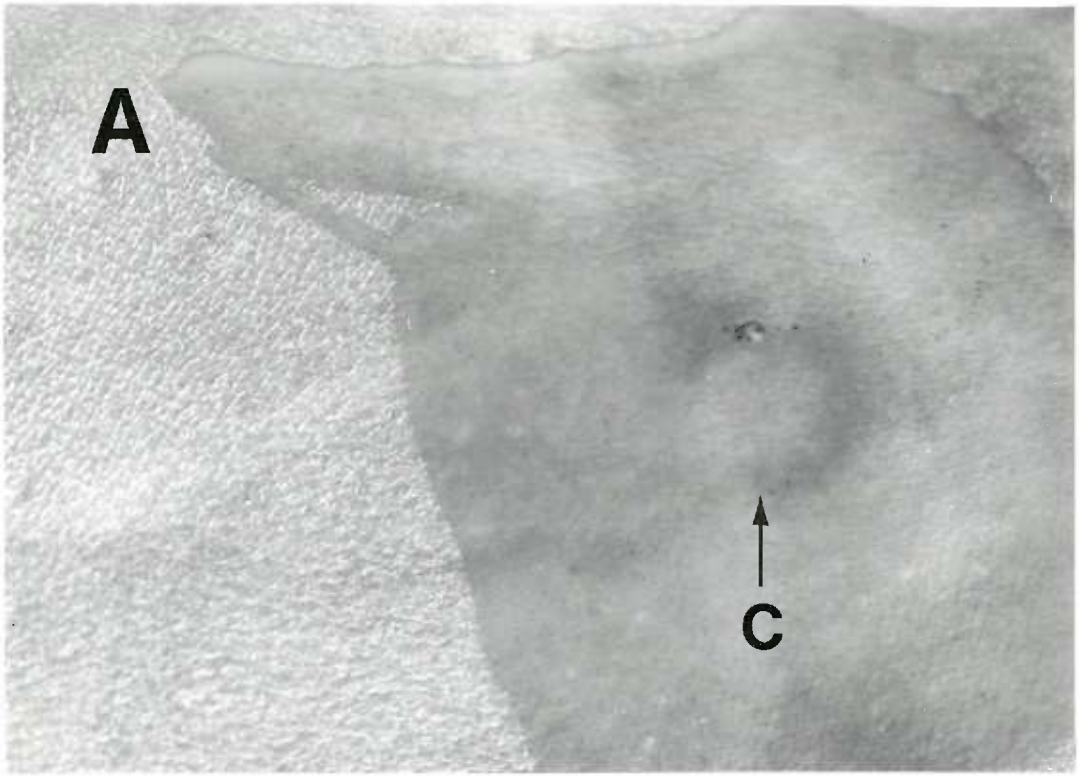

A1
TP
10-3
A2
TP
10-3
B1
TP
10-3
B2
NE
3x10-9
TP
10-3
C
NE
3x10-7
TP
10-3

Figure 10: Interaction between thiopental, phenoxybenzamine, and NE.

Each pair of illustrations show two successive drug-induced contractions in a single preparation. The arrows indicate drug addition with the drug concentration indicated below. A_1 and B_1 indicate the initial response to thiopental (10^{-3}M). After the initial contraction the preparations were washed and allowed to relax. A_2 shows a second thiopental (10^{-3}M)-induced contraction after exposure to phenoxybenzamine (10^{-5}M) for one hour. B_2 shows a second thiopental (10^{-3}M)-induced contraction after the preparation has been contracted with a threshold concentration of NE ($3x10^{-9}$M). C shows the effect of thiopental (10^{-3}M) when added to a preparation previously contracted with NE ($3x10^{-7}$M). The time line represents 10 minutes.


A
C

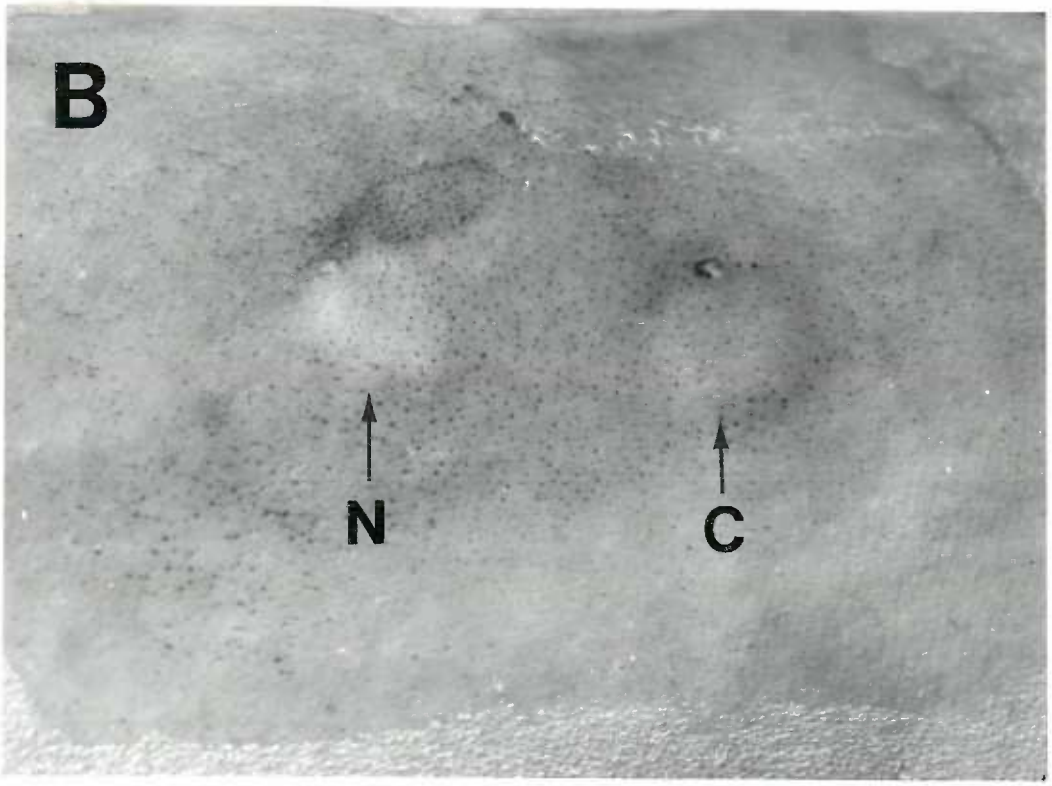

B
N
C

Figure 11: Localized sweating in response to intradermal drug injection.

Photograph A shows an area approximately 2.5 cm wide and 5 cm long surrounding the wheal caused by intradermal injection of CHEB (10^{-5}M). The wheal is indicated by the arrow labeled C. The area immediately over and around the wheal was covered with the indicator and the dark spot at the top edge of the wheal was caused by fluid leaking out of the wheal along the needle track. The picture was taken 3.2 minutes after the injection. The area was observed for an additional 5 minutes without any evidence of sweating. At this time nicotine ($5x10^{-5}$M) was injected intradermally at the site indicated by the arrow labeled N. Photograph B shows the same area as A, 3.2 minutes after the injection of nicotine. The black dots in the area surrounding and directly over the wheals indicate sweating pores.

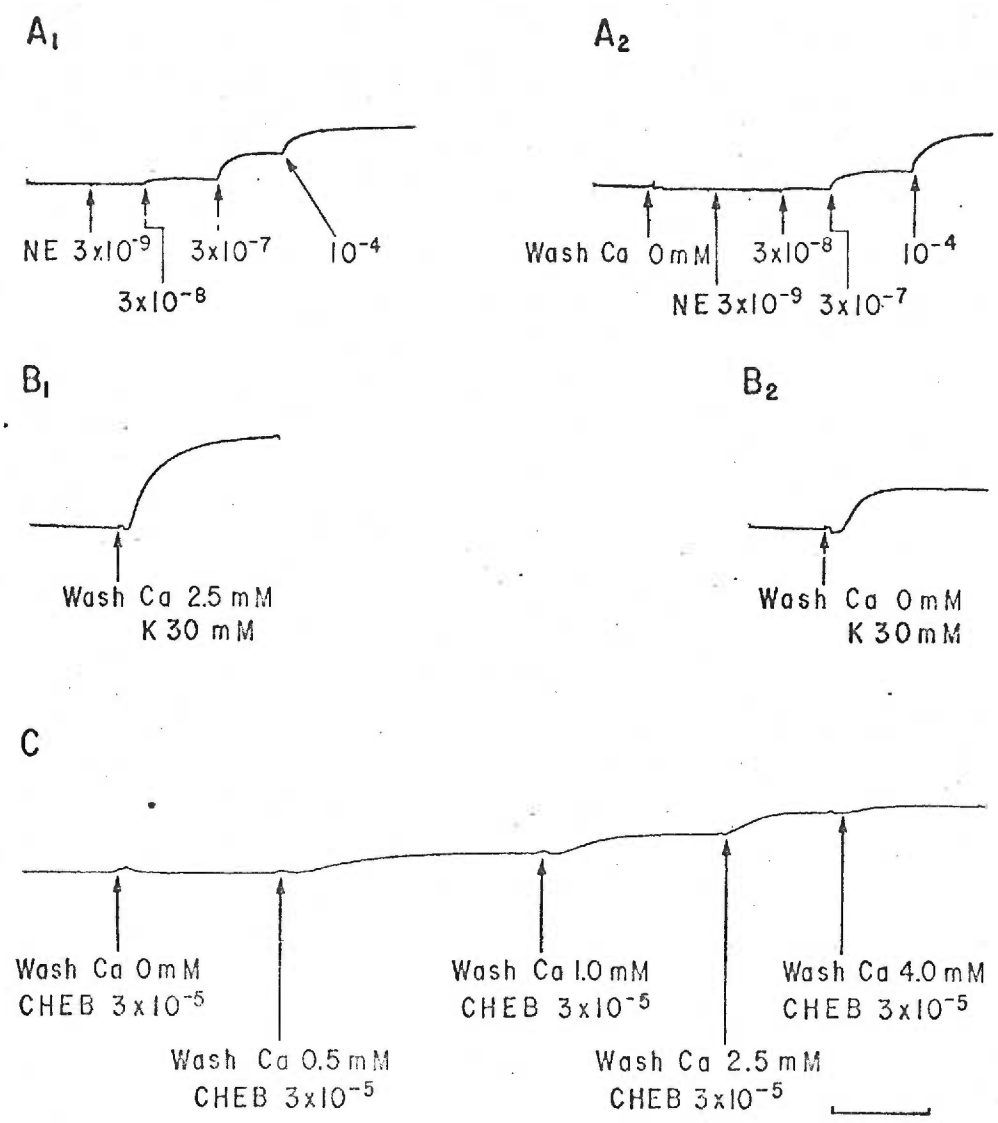

A1
NE 3x10-9
3x10-8
3x10-7
10-4
A2
Wash Ca 0mM
NE 3x10-9
3x10-8
3x10-7
10-4
B1
Wash Ca 2.5 mM
K 30 mM
B2
Wash Ca 0mM
K 30mM
C
Wash Ca 0mM
CHEB 3x10-5
Wash Ca 0.5mM
CHEB 3x10-5
Wash Ca 1.0mM
CHEB 3x10-5
Wash Ca 2.5mM
CHEB 3x10-5
Wash Ca 4.0mM
CHEB 3x10-5

Figure 12: The effect of Ca-free Krebs solution on the response of rabbit aortic strips to NE, K, and CHEB.

Each pair of illustrations show two successive drug-induced contractions in a single preparation. The arrows indicate either drug addition or a "wash" in which the normal Krebs solution was replaced with an altered Krebs solution. A1 and B1 indicate the response of aortic strips bathed in normal Krebs solution (2.5mM Ca) when stimulated with either NE ($3x10^{-9}$ to 10^{-4}M) or K (30mM). A_2 indicates the response of aortic strips to NE ($3x10^{-9}$ to 10^{-4}M) after the preparation was equilibrated for 10 minutes in Ca-free Krebs solution. B_2 indicates the response of a preparation when it was washed with a Ca-free solution containing K (30mM). C indicates the response of a preparation when it was washed with a Ca-free solution containing CHEB ($3x10^{-5}$M) followed by a series of Krebs solutions containing CHEB ($3x10^{-5}$M) and increasing concentrations of Ca (0.5, 1.0, 2.5, and 4.0mM). The time line represents 10 minutes.

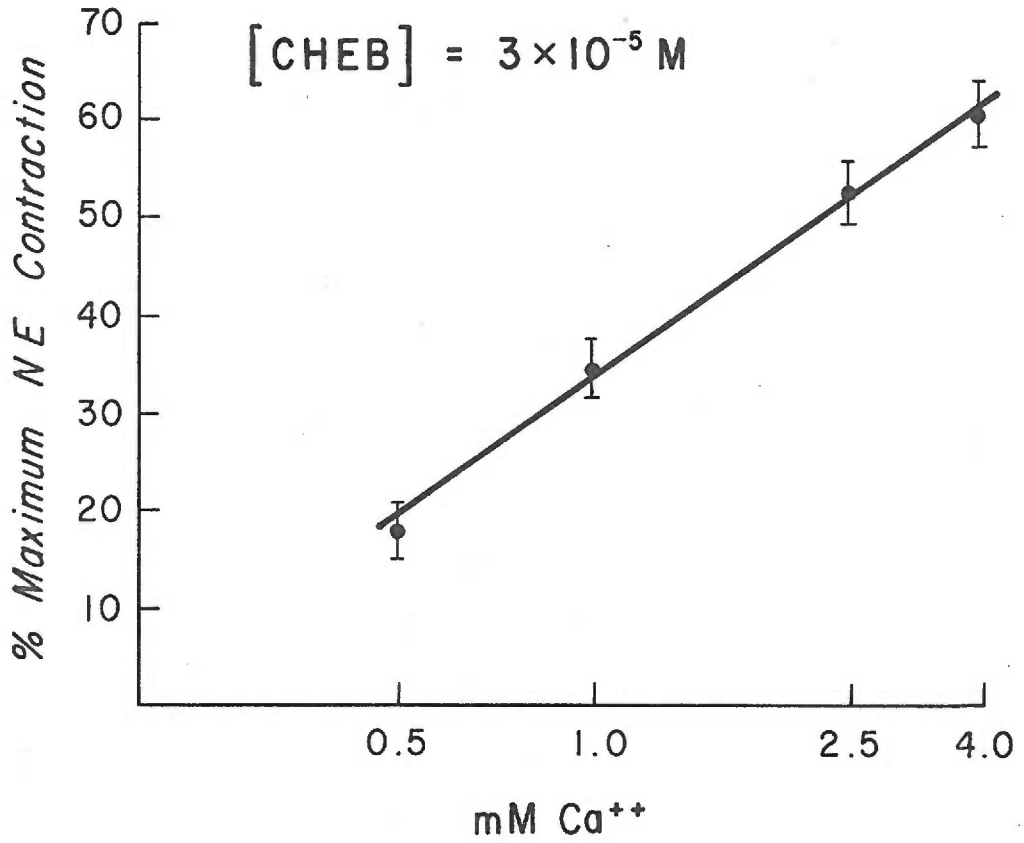

[CHEB] = 3×10⁻⁵ M
% Maximum NE Contraction
70
60
50
40
30
20
10
0.5
1.0
2.5
4.0
mM Ca++

Figure 13: Calcium concentration-response relationship in CHEB-treated rabbit aortic strips.

The amplitude of each contraction was standardized on the basis of a previous maximum response to NE (10^{-4}M). Each point represents the mean of 16 separate determinations and the bracket gives the standard error of the mean.

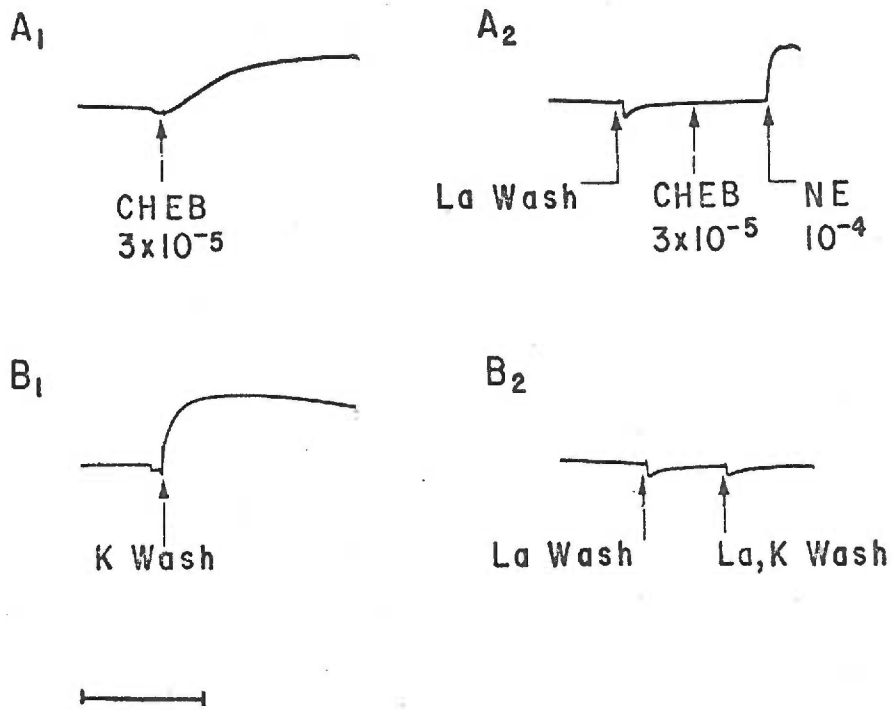

A1
CHEB
3x10-5
A2
La Wash
CHEB
3x10-5
NE
10-4
B1
K Wash
B2
La Wash
La,K Wash

Figure 14: Effect of La on the response of rabbit aortic strips to CHEB, K, and NE.

Each pair of illustrations show the response of a single preparation to two successive drug exposures. The arrows indicate either drug addition or a "wash" in which the normal bath solution (HBS) was replaced by HBS containing La (5mM) and/or K (100mM). A_1 and B_1 show initial contractions induced by CHEB ($3x10^{-5}$M) and K (100mM). After these initial contractions preparations were washed with HBS and allowed to relax. A_2 indicates the effect of CHEB ($3x10^{-5}$M) and NE (10^{-4}M) in a preparation pretreated with La (5mM). B_2 indicates the effect of K (100mM) on a preparation pretreated with La (5mM). The time line represents 20 minutes.

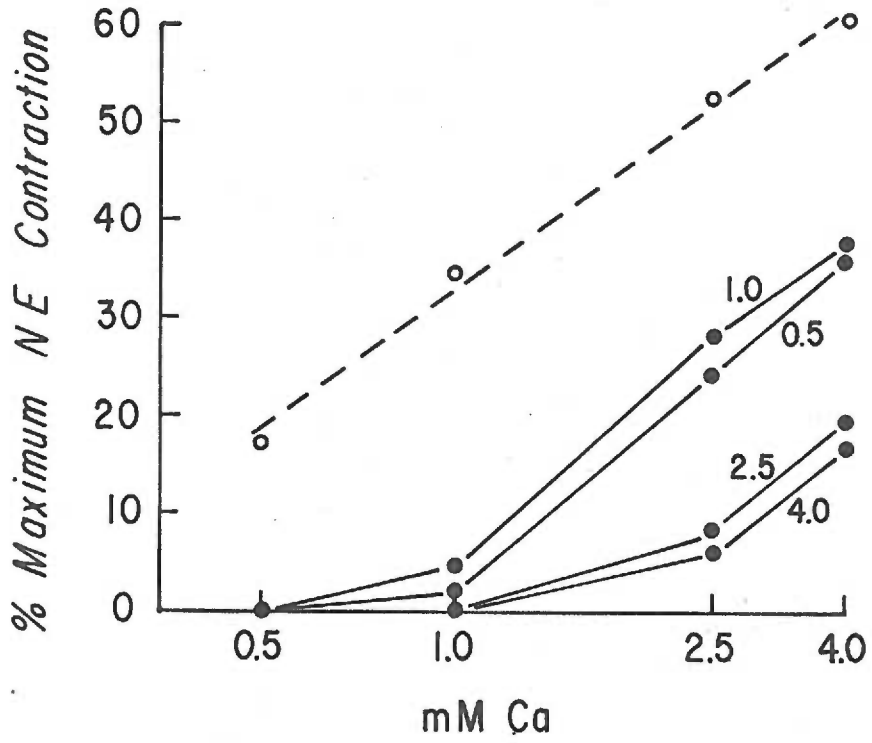

% Maximum NE Contraction
60
50
40
30
20
10
0
0.5
1.0
2.5
4.0
mM Ca
1.0
0.5
2.5
4.0

Figure 15: Effect of $[Ca]_o$ on the extent of tachyphylaxis to CHEB.

The regression line indicated by open circles and a broken line (o---------o) is identical to that shown in Figure 13 and represents Ca concentration-response relationships of aortic strips exposed for the first time to CHEB ($3x10^{-5}$M). Solid circles and solid lines (●————●) represent Ca concentration-response relationships in aortic strips that had undergone tachyphylaxis to CHEB effect. Tachyphylaxis was produced by contracting aortic strips for 4 successive times with CHEB ($3x10^{-5}$M) in bath solutions containing the Ca concentrations (0.5, 1.0, 2.5, and 4.0mM Ca) indicated to the side of each line. Each solid circle represents the mean of 4 experiments. For clarity, the standard errors of the means are not included in the graph but were less than 9% of the maximum NE concentration. Analysis of the data by the nonparametric sign test for paired observations indicates that the points which define the curves labeled 0.5 and 1.0 are significantly different than those points which define the curves labeled 2.5 and 4.0 ($P<0.05$ or 95% level of confidence).