THE ACTION OF SYMPATHOMIMETIC AMINES ON ADRENERGICALLY INHIBITED SMOOTH MUSCLE

by

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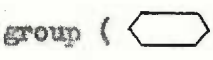
INTRODUCTION

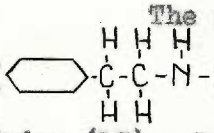
Langley as early as 1901 (30) and Elliot in 1904 (20) concluded that smooth muscle reacts to adrenalin in a similar manner to that following excitation of the sympathetic (thoraco-lumbar) nerves supplying that muscle. On this basis, depending on whether the response to adrenalin is inhibition or excitation, smooth muscle may be separated into two types: Type I and Type E. Adrenergically inhibited smooth muscle or Type I is found in the walls of the intestine, stomach, bladder -- exclusive of sphincters -- and in the bronchi. Type E or adrenergically excited smooth muscle, on the other hand, comprises in part the nictitating membrane, the ureters, the gall bladder, the pilomotor muscles, the retractor penis, and the pyloric sphincter. The uterus, an organ frequently used as an indicator for physiological reactions, whether pregnant or non-pregnant is contracted in many animals; but in the cat, rat, mouse, guinea pig, and human being, the pregnant organ alone is contracted by adrenalin, and the non-pregnant organ is inhibited (12).

Interest in the study of amines related to adrenalin developed rapidly after the discovery by Oliver and Schaefer (32) that extracts of suprarenal glands produced a rise in blood pressure, which led to the subsequent isolation and identification of adrenalin by Abel (1), v. Fürth (48), Aldrich (2), and Takamine (42). Barger and Dale (10) in their classical study found that a large number of amines other than adrenalin simulated the action of sympathetic nerves. They coined the term "sympathomimetic" in describing the action of such amines and designated a benzene ring with a side chain of two carbon atoms, the terminal one

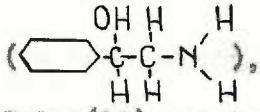
bearing an amino group (), as the fundamental sympathomimetic nucleus. Following this study, various investigators have attempted to establish the potencies of many sympathomimetic amines with regard to smooth muscle. By far the greatest number of experiments have been on blood pressure. At the present time the pressor response is the routine method for comparing potencies of sympathomimetic amines. However, pressor responses involve reactions of several effectors and give no indication of relative potency in single effectors. The determination of the potency of a sympathomimetic amine on a given smooth muscle requires a bioassay on that muscle or other muscle of the same type. Bacq (7, 8) has studied the actions of a series of amines on the nictitating membrane, smooth muscle of the excited type. Tainter and coworkers (14, 35, 46) have completed a study of the actions of amines on the bronchi, smooth muscle of the inhibited type.

The action of phenylethylamine derivatives on adrenergically inhibited smooth muscle will be considered in this paper.

REVIEW OF LITERATURE

The simplest sympathomimetic amine is B-phenylethylamine (), known since 1875 when it was prepared by Columbo and Spica (17). Barger and Dale (10) recognized the compound as a sympathomimetic amine. They found that it produced a rise in blood pressure, dilatation of the pupil, relaxation of the cat's urinary bladder, and inhibition of the tone and rhythm of the virgin cat's uterus. It possesses the minimal skeleton according to their definition for sympathomimicity. Not all the studies, however, are in accord with those of Barger and Dale. Chen et al (16) noticed that in some instances inhibition of the isolated intestine resulted, but that the usual reaction was excitation. The virgin guinea pig uterus was contracted. Patek and Thienes (33) observed that this amine, in concentrations of 1 to 20 mgm. per 100 cc. of perfusing fluid, contracted the isolated intestine of rabbits. The response of the non-pregnant rabbit uterus was contraction or relaxation, while the response of the non-pregnant guinea pig uterus was contraction in concentrations of 1 to 30 mgm. per 100 cc. of perfusing fluid. In experiments on the intact uterus of non-pregnant cats, Barbour (9) discovered that relaxation resulted with a dosage of 10 mgm. or over. Inhibition also occurred with the isolated non-pregnant guinea pig uterus. Alles and Prinzmetal (5) observed the broncho-dilator effect to be about 1/50 that of epinephrine, but Tainter (45) reported the compound ineffective in relaxing bronchial spasm.

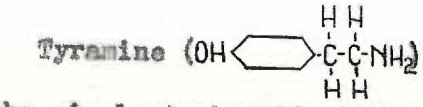
The introduction of a secondary alcoholic hydroxyl group on the first carbon atom of phenylethylamine produces phenylethanolamine,

 , a compound one step closer to adrenalin. Barger and Dale (10) concluded that the activity of this amine did not differ noticeably from phenylethylamine. Tainter (44) reported the action on the bronchial musculature to be irregular and rather ineffective. The intestines of rabbits, cats, and dogs responded with inhibition in vivo and in vitro. He drew the conclusion that phenylethanolamine was less than 1/1000 as active as adrenalin. The response of the isolated non-pregnant cat uterus was contraction. Chen et al (16), likewise, obtained inhibition of the isolated rabbit intestine and contraction of the virgin guinea pig uterus. Alles (3) made similar observations, deciding that the OH group in the side chain decreased the toxicity and increased the activity as indicated by comparison of its actions with those of phenylethylamine. Hoyt et al (27) elicited contraction or inhibition of isolated segments of rabbit ileum and of the non-pregnant rabbit and guinea pig uteri.

Epinine, a compound which differs from adrenalin only in lacking the alcoholic hydroxyl group, illustrates the importance of this group to the smooth muscle-inhibiting function. Barger and Dale (10) found that epinine is a good inhibitor of the non-pregnant cat uterus. Its relative potency as a broncho-dilator has been determined by Tainter et al (46). Hjort (26) discovered that epinine was 1/10 as active as adrenalin on the isolated rabbit intestine and 1/100 as effective as adrenalin on the uterus.

The introduction of phenolic hydroxyl groups in phenylethylamine intensifies sympathomimetic action. Barger and Dale (10) were the first

to investigate the roles of these groups. They obtained maximal activity in 3-4 dihydroxy or catechol derivatives of which adrenalin itself is a member. In comparing the three monohydroxy derivatives of phenylethylamine, they observed that the ortho derivative was no more active than the phenylethylamine itself. The meta and para compounds were equally active and about five times more potent than the parent amine. The non-pregnant cat uterus and blood pressure were used as indicators.

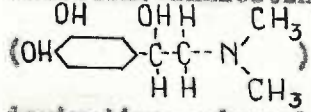
Tyramine  , or p-hydroxyphenylethylamine, is one of the simplest phenolic hydroxy sympathomimetic amines. Like phenylethylamine, its effects on adrenergically inhibited smooth muscle appear to be variable and indefinite. Quagliariello (36) and Nakomura (31) reported that inhibition occurred on the rabbit intestine. Tainter (43) obtained contraction with 18 out of 19 strips of isolated rabbit intestine using 0.0004 to 0.04 per cent solutions. Concentrations higher than these depressed the activity. In the cat, the usual response of the intestine to the amine was inhibition. Intact rabbit and dog bronchi, and the non-pregnant uterus of guinea pigs reacted variably. Seidenfeld and Tainter (39) made observations in which tyramine augmented 28 strips of rabbit intestine and inhibited 6 in concentrations from 1:2500 to 1:100,000. Barbour (9) determined the minimal effective inhibiting dose in cats to be one-tenth that of phenylethylamine.

Monohydroxy phenolic compounds recently introduced for clinical use include synephrin, synephrin ketone, and neosynephrin. In a study of the effects of these amines on the bronchi, Tainter (45) found that

synephrin ketone and synephrin were ineffective. Neosynephrin was rated one-twentieth as active as adrenalin as a broncho-dilator of perfused guinea pig lungs. In dogs neosynephrin was a comparatively poor broncho-dilator (14). For the isolated intestine, synephrin (45), synephrin ketone (45), and neosynephrin (13) have been found to be much weaker inhibitors than adrenalin.

Elphick and Gunn (21) and Epstein et al. (22) methylated the hydroxyl groups of tyramine, phenylethanolamine, synephrin, dihydroxy-phenylethylamine, and adrenalin. They investigated the inhibiting action of these various methoxy amines on the non-pregnant cat's uterus and on the intestine of cats and rabbits, as well as the pressor responses. They discovered that the presence of one nuclear methoxy group did not entirely remove sympathomimetic action. The presence of two or three such groups removed the action entirely. The addition of the methoxy group to the side chain merely weakened the physiological activity without apparent qualitative alteration of actions.

Methylation in the amino group of B-phenylethylamine, tyramine, and phenylethylamine has little effect on the inhibiting action on the non-pregnant cat's uterus (10). A second methyl group, as determined by a comparison of the broncho-dilator action of tyramine with hordenine, works to further disadvantage (4, 25). This is also illustrated when the intestine-inhibiting potency and broncho-dilating potency of methadren



is compared with the potency of its mono-methylamino derivative, adrenalin (50, 41).

That catechol methylamino-bases have a far more pronounced inhibitory action on the virgin and non-pregnant cat uterus than any others, was an observation made by Barger and Dale (10). For the isolated intestine, however, these investigators and others (23) have found that the compound arterenol, which differs from adrenalin only in lacking the methylation of amino group is almost as potent an inhibitor as adrenalin. Tainter and coworkers (45, 35, 14) rank the broncho-dilator potency of arterenol very close to that of adrenalin.

The following study is concerned with quantitative comparisons of the responses of intact (51) and of isolated intestine (6) to a series of six sympathomimetic amines. These amines differ from adrenalin by lacking one or two of the four groups that distinguish adrenalin from phenylethylamine, the fundamental sympathomimetic nucleus. These responses are compared with results of quantitative studies of the same compounds acting as bronchodilators (46, 35, 14) to show the similarity of the two types of muscle. Similarly the results are compared with those obtained from studies of the stimulating action of these compounds on the nictitating membranes (7, 8). Thus, an opportunity is presented for the detection of differences between adrenergically inhibited smooth muscle on the one hand, and adrenergically excited smooth muscle on the other.

METHODS OF STUDY

A. THE INTACT INTESTINE.

The motility of jejunal segments made into Thiry or Thiry-Vella loops was recorded by the balloon-mercury-manometer method described by Krueger (29). A balloon placed in an intestinal fistula was connected by means of rubber tubing to one arm of a mercury manometer arranged in the usual manner to record on a smoked drum. Balloon and tubing were filled with enough water to record the tonus and rhythmic activity of the intestinal segment. Each of three of the six dogs used had one innervated and one denervated Thiry loop. Three dogs had innervated Thiry-Vella fistulae, one of which was subsequently denervated. The denervation was carried out by cutting the mesentery, cleaning the mesenteric blood vessels of nerve fibers, and painting the vessels with phenol.

Intravenous injections at various constant rates were made by means of an electric motor geared to a round shaft on which was mounted a small adjustable drive. This drive rotated a large brass disc, the axle of which was geared to a screw that moved the plunger of a syringe. The speed of rotation could be altered by moving the drive on the shaft. The fastest rotation was obtained by having the drive as near to the center of the disc as possible; the slowest, by having the drive out toward the periphery as far as possible. (See Fig. 1.) In this manner, the rate of injection could be increased by any increment from 1 to 4 cc. per minute. For each compound a dilution was found which was near the threshold for producing a short period of inhibition of the intestine

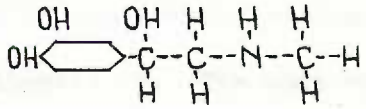
when injected at a rate of 1 cc. per minute. Injections were maintained for a period of from one to eight minutes. Acidified stock solutions of the compounds were kept in the refrigerator. Solutions for injection were prepared by introducing the necessary amount of the stock solutions into 100 cc. volumetric flasks and filling to the mark with physiological saline buffered at a pH of 4.9 to 5. No solutions were used which showed discoloration. No loss of potency was found in the stock solutions which remained colorless. Injection of the diluent alone had no effect on intestinal motility.

Fig. 1 Constant injection apparatus.

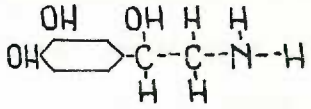
The compounds studied were l-adrenalin (Parke Davis & Co.), dl-arterenol (Winthrop Chemical Co.), epinine (Burroughs-Wellcome & Co.), kephrine (Alba Pharmaceutical Co.), l-neosynephrin (Frederick Stearns & Co.) and dl-synephrin (Frederick Stearns & Co.). The names of the amines,

their variation from adrenalin and the structural formulae are as follows:

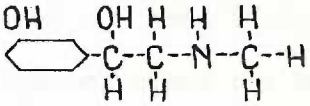
1. l-Adrenalin.



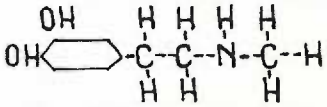
2. Arterenol, lacks the CH_3 group on the amine.



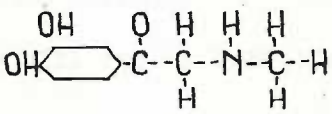
3. Neosynephrin, lacks the para OH on the benzene ring.



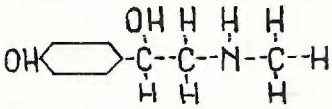
4. Epinine, lacks the OH group on the carbon chain.



5. Kephrine, has a ketone group in place of the OH group on the carbon chain.



6. Synephrin, lacks the meta OH group on the benzene ring.



The ease in preparing the indicator, the regularity of response to equivalent dosage, the great sensitivity, the absence of anesthesia, the ease of recording, and the fact that the same indicator can be used for the entire series of compounds make the intact intestine extremely well suited for the bioassay of sympathonimetic amines.

That these intestinal responses are those of a single effector system, smooth muscle inhibited by its adrenergic nerve supply, is based on the interpretation that the compounds inhibit by direct action on the

smooth muscle. Severe ischemia of the intestine inhibits its motility (11), but several facts indicate that blood supply is not the factor responsible for inhibition of the intestine in these experiments: 1. The most marked ischemia possible, such as may be obtained by obstructing the aorta, requires at least 8 seconds before producing inhibitory effects (11). Ischemia following intravenous injection could not begin to develop before one circulation time, or at least 12 seconds. Therefore, even if maximal, ischemia could not be a factor until at least 20 seconds after the beginning of the injection. However, injections of the amines typically produce maximal inhibitory effects within 12 to 15 seconds. 2. The inhibition of the denervated intestine is produced by some of the compounds when the injection rate is below threshold for blood pressure effects. 3. Plethysmograph records show that the intestinal motility will remain inhibited following an adrenalin injection even after such records indicate that the intestine has become hyperemic. 4. One of the best vasoconstrictors of the group is one of the poorest inhibitors of the intestine. 5. The effects of the compounds are qualitatively the same as the effects of sympathetic nerves to the intestine, an effect shown by Bayliss and Starling (11) to be independent of vasoconstrictor action. 6. The effects of the compounds on the intact intestine are qualitatively identical to the effects of adrenalin on the isolated intestine.

The compounds, with one exception, characteristically produce effects on the intestinal motility within a period of less than two circulation times, indicating that time for building up a concentration in

the blood was not required. All of the compounds are of the general formula which has been shown to be susceptible to rapid enzymatic destruction in tissues (37). Moreover, Rogoff (38) has found that continuous intravenous injections of adrenalin at rates several times as fast as any used in these experiments is not accompanied by an increased adrenalin content of the blood recovered from the adrenal vein. There is nothing to indicate that the differences in injection rates are not an index to the differences in the concentrations reaching the intestine except in the case of the two least potent compounds. These apparently must be injected rapidly enough to exceed the rate of enzymatic destruction.

B. THE ISOLATED INTESTINE.

The motility of isolated segments of the duodenum and jejunum of recently killed rabbits was recorded by a modification of the technic used by Stewart (40). The strips were suspended in oxygenated Locke's solution and responses were recorded by means of a light muscle lever which wrote on a smoked drum. The effect of a certain dilution of adrenalin on the isolated rabbit intestine was determined, and a dilution of each of the other compounds was found that would duplicate the inhibitory effect of the test dilution of adrenalin. The test dilution of adrenalin was usually one part in 20 million or one part in 40 million. The compounds were tested in various orders and in some cases the entire series, except synephrin was tested on the same intestinal segment.

R E S U L T S

A. INTACT INTESTINE.

I. Qualitative Effects of the Compounds. All of the compounds were found to inhibit the rhythmic contractions and decrease the tonus of either the innervated or the denervated intestine when given in a concentration sufficient to produce any effects on the intestine (Figs. 2-7). No injection rates were found which caused an increase in the motility of the intestine during the injection. Hyper-motility commonly occurred during the recovery phase following the end of the injection. This recovery phase may be the most conspicuous feature of the record when the injection time is very short.

The characteristic effects on intestinal motility are as follows: 1. After a short latent period equivalent to one or more circulation times there appears a reduction in the amplitude of rhythmic contractions and a decrease in tonus. The rapidity of onset of the inhibition produced by a given compound is dependent on the injection rate. Of a total of 308 injections, 76 were below the threshold for producing effect on intestinal motility. Of the remaining 234 injections at various rates ranging from barely threshold to five or six times the threshold rate the period from the beginning of the injection to the beginning of inhibition had a duration of 12 to 20 seconds in 51 per cent of the injections, 21 to 30 seconds in 18 per cent of the injections, and longer than 40 seconds in 10 percent of the injections. One-half of the latter occurred with injections of synephrin. A building up of the concentration of this compound in the blood during continuous injections is to be

expected because of the high concentration which must be injected. It is very likely that synephrin and possibly neosynephrin are less potent than the figures obtained by constant injection methods would indicate. 2. If the injection of any one of the compounds, other than synephrin and neosynephrin, be continued at a rate two or three times that required to produce initial complete inhibition, a "breaking through" the inhibition occurs after a few minutes. The intestine tends to recover normal motility during the continuation of the injection. The breaking through appears later with the higher injection rates. Therefore, the duration as well as the degree of inhibition varies with the rate of injection used. Breaking through does not appear as readily with some of the compounds as it does with adrenalin. 3. When the injection is stopped, the intestine begins to recover from the inhibitory effects of the compound within a circulation time or two. The amplitude of rhythmic contractions shows a gradual increase to "normal" or greater. A tonus wave commonly accompanies the hyper-motility. In general, the hypermotile recovery phase is more likely to occur in the denervated than in the innervated intestinal segments. If a given segment shows a hypermotile recovery phase following adrenalin injection, it ordinarily shows it also when recovering from the effects of the other compounds. When a second injection, identical in rate to the first, is begun during the recovery phase, the inhibitory effects resulting from the second injection are less than those resulting from the first.

It has already been shown that the effects on the intestine of reflexly activated adrenergic nerves, or of the sympathin produced by them,

are indistinguishable from the effects of adrenalin (49). However, the intestinal effects of adrenalin are not characteristic of adrenalin alone. Since the mediator of impulses at adrenergic intestinal nerve endings is considered to be adrenalin or a similar compound, it appears unlikely that these nerves could ever perform anything but an inhibitory function.

II. Sensitization by denervation. Hypersensitivity to sympathomimetic amines has been amply demonstrated for smooth muscle of the type that contracts in response to adrenalin (24). Hypersensitivity to adrenalin has been demonstrated in the intact dog for smooth muscle of the type that is relaxed by adrenalin (49). The denervation of intestinal smooth muscle sensitizes it also to the inhibitory effects of each of the compounds studied. Sensitization is illustrated in figures 2 to 7. These records were taken simultaneously from innervated and denervated fistulae in the same animal. For each of the three animals having both innervated and denervated fistulae an injection rate for any one of the compounds could be determined which would produce complete inhibition of the denervated segment without significantly affecting the innervated one. The sensitivity of the intestine is increased by the postganglionic denervation so that it is 2 to 8 times more sensitive than normal. In one animal the sensitivity of a short Thiry-Vella fistula was studied. The loop was then denervated by doing a laparotomy between the two ends, lifting the mesenteric pedicle on a rod, and cutting everything in the pedicle except an artery and two veins. The vessels were then cleaned of nerve fibers and painted with concentrated phenol followed by alcohol and saline. Records taken between 7 and 14 days after this operation showed that

complete sensitization with regard to all of the compounds had been attained, the segment being 4 to 8 times as sensitive as before the denervation. The sensitivity to adrenalin was recorded during the next few weeks, and no further increase in sensitivity occurred. Sensitization of the intestine develops fully as rapidly as the sensitization of the nictitating membrane (24).

III. Absolute and relative intestine-inhibiting potency.

The lowest adrenalin injection rate sufficient to produce a few seconds of complete inhibition in the four denervated segments studied was between 0.0001 and 0.0002 mgm. per kilo per minute. This is about one-half the minimal pressor dose reported by Dragstedt et al. (18), for dogs under simular conditions. The threshold inhibitory dose for the other compounds can be calculated by multiplying the figures in table 1 by these values.

In the last column of table 1, the extremes are listed for the increase in injection rates that were necessary for each of the compounds in order to produce a degree of intestinal inhibition comparable to that produced by test doses of adrenalin. Results are tabulated for six indicators. The reciprocal of the number indicates the potency of the corresponding compound as compared to that of adrenalin.

B. RESULTS ON THE ISOLATED INTESTINE.

Qualitatively, all of the compounds had effects on the isolated rabbit intestine similar to those caused by adrenalin. (See figures 8-9.) There was inhibition of rhythmic contractions and a decrease in tonus.

Hyperactivity was sometimes observed following the inhibition. The hyperactive recovery phase was most marked following neosynephrin, adrenalin and arterenol, and has been previously reported for the latter two compounds (23).

The potency of the compounds as inhibitors of the isolated intestine relative to that of adrenalin is shown in table 2. The figures are tabulated for 10 segments. The extremes obtained from segments of nine rabbits are listed in the last column of the table. Each compound was assayed at least 8 times.

TABLE 1

Number of times the injection rates for each of six compounds must be increased beyond that of a test dose of adrenalin in order to duplicate the degree of inhibition of intestinal motility produced by the adrenalin.

	Dog 1, Den.	Dog 2, Den.	Dog 3, Den.	Dog 4b, Den.	Dog 4a, Inn.	Dog 5, Inn.	Dog 6, Inn.	Extremes
Adrenalin (l)	1	1	1	1	1	1	1	1
Arterenol (dl)	1½-4	1½-3	2	2½-3	2		2-2½	1½-4
Epinine	10	10-20	10	10	10		15-25	10-25
Neosynephrin (l)	100	100	50	25	40	25	25-50	25-100
Kephrine	50-100	50-100	33	25	40		50	25-100
Synephrin (dl)	1,000	1,700	660	2,500	1,500	2,500	1,000	660-2,500

TABLE 2

Relative potency of six amines as inhibitors of the Isolated Rabbit Intestine

Compounds	SEGMENT NUMBERS										Extremes
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	
Adrenalin (l)	1	1	1	1	1	1	1	1	1	1	1
Arterenol (dl)	1-5	1½-2	2	1½-2		1½-2					1½-2
Neosynephrin (l)		9-10		8-10	4-10	4-6	4*	10*	5*		4-10
Epinine		10-12		10-15	15-20	10			10-15*		10-20
Kephrine				30-50	20-40	20-30			25		20-50
Synephrin (dl)								1,000	500-1,000	500-1,000	500-1,000

*Indicates single trials. All other figures are based on two or more assays.

TABLE 3

Extremes for the number of times injection rates for each of five compounds must be increased beyond that of a test dose of adrenalin in order to duplicate the degree of inhibition of intestinal motility in dogs produced by the adrenalin (Col. 1). Relative potency of the compounds as inhibitors of the isolated rabbit intestine (Col. 2). Guinea pig broncho-dilator activity ratio (Col. 3).* Broncho-dilator action in dogs (Col. 4).** Relative potency as contractors of the cat nictitating membrane (Col. 5).*** Pressor Ratio (Col. 6).*

Compounds	(TYPE I)				(TYPE E)	(MIXED)
	Intestine In Situ	Intestine In Vitro	Guinea Pig Bronchi	Dog Bronchi	Nictit. Membrane	Pressor Ratio
Adrenalin (l)	1	1	1	+++ (Very good)	1	1
Arterenol (dl)	1.5-4	1.5-2	7.4	++ (Mod.)	10-30	1.2
Neosynephrin(l)	25-100	4-10	20.4	+ (Poor)	3.5-4	4.3
Epinine	10-25	10-20	50.5	+++	20	12
Kephrine	25-100	20-50			75	150
Synephrin (dl)	660-2,500	500-1,000	Ineffective	Ineffective	30-120	300

*Data from Tainter, Pedden and James (46)
**Data from Pedden, Tainter and Cameron (35)
***Data from Bacq (7, 8)

DISCUSSION

It is well recognized that quantitative biological studies used in evaluating relative potencies of sympathomimetic amines must take into consideration variation in characteristic responses in different species of animals, influence of experimental conditions and procedure, and the mechanism through which a compound produces its effect. It is an advantage, therefore, to study the same series of compounds by more than one method. In the study on the intestine in situ, for example, effects on the indicator may possibly be interpreted as being due to circulatory changes or reflex neurohormonal discharges produced by the injection of the amines. When isolated intestinal segments are used, these factors are eliminated. However, the six compounds studied by the latter method all had effects qualitatively similar to those on the intact innervated and denervated dog intestine. The correspondence both in the order of potency and the potency relative to that of adrenalin was almost complete with the single exception that neosynephrin ranked higher as an inhibitor of the isolated rabbit intestine than as an inhibitor of the intact dog intestine. Such a concordance indicates the similarity of the smooth muscle of the rabbit and dog intestine. It further supports the interpretation that the action of these amines in situ and in vitro is directly on the smooth muscle.

When, as shown in Table 3, the intestine-inhibiting potencies of these compounds are compared with the broncho-dilating potencies, there is concordance in the order of activity of these amines on the isolated rabbit intestine and the perfused guinea pig bronchi. The

same is true in comparing the intact dog intestine with the dog bronchi (46, 35). The relative potencies of these amines on the intestine and bronchi vary, however. This variation illustrates a difficulty in comparing smooth muscle located in different organs. In experiments on the bronchi other drugs along with the amines were employed in arriving at the relative potencies. Broncho-constriction was first produced by pilocarpine, histamine, or barium chloride. After the control constriction produced by a given volume of the constrictor drug alone was ascertained, the same dose of the constrictor drug together with a given amount of the amine was injected, and the dilating potency was calculated. It is possible that such a procedure in which additional drugs were used, may account in part at least for the variations in the potency of the amines on the bronchi and intestine, since in the latter determinations no other drugs were used. The consistent relationship in the order of activity of the amines, in spite of the diversity of procedure is significant, strongly suggesting that the smooth muscle in these two different structures belong to the same type.

The most important group of the adrenalin molecule for intestinal and bronchial smooth-muscle-inhibiting function, is the meta OH group. This is indicated by the extremely low potency of synephrin which differs from adrenalin only in lacking the meta OH group. Next in order of importance are the para OH group and secondary alcohol OH. In the intact dog intestine and bronchi the para OH group plays a greater role in inhibition than in the isolated rabbit intestine and in guinea pig bronchi. The least important group for inhibition of these indicators is the $-CH_3$

group on the nitrogen atom.

When several groups of the adrenalin molecule are removed, but the nucleus is left intact, the inhibitory functions of the resulting compounds are much less than in those with just a single group lacking. Phenylethylamine, a compound lacking four groups, is less active than any of those missing three groups (tyramine and phenylethyanolamine). The ones lacking three groups are weaker inhibitors than any of those having one group removed in the series studied. Whether the potency of a compound, as effected through the removal of groups, is diminished by the sum or by the product of the potency of the respective groups is a problem requiring investigation.

Quantitative ratios representing the effect of these amines on the uterus have not been assigned. Barger and Dale (10) working with the virgin cat uterus, which gives a pure inhibitory response to stimulation of the hypogastric (sympathetic) nerves, found that the CH_3 group on the nitrogen was a relatively important one. This was true also for the non-pregnant uterus. However, the non-pregnant uterus is considered by some to contain a mixture of motor and inhibitory elements, so that the effect produced could be the algebraic sum of two opposite effects. Greer et al (13) have reported a differential effect between uterine responses elicited with l-adrenalin and dl-arterenol which further points to the CH_3 group as an important one in uterine inhibition. In cases where l-adrenalin gave a very marked relaxation, an equal dose of dl-arterenol usually gave no response or slight relaxation. If l-adrenalin produced moderate relaxation, no response or even a slight contraction

was observed with dl-arterenol. Finally, if l-adrenalin gave only a slight relaxation, dl-arterenol frequently caused a definite contraction. Another peculiar situation which the uterus presents is the reversal of response upon the injection of lipoidal extracts of ovary (46) and of progestin (28). Typically the non-pregnant uterus is inhibited by adrenalin. Injection of lipoidal extracts of the mature corpora lutae or progestin will change the response to adrenalin to contraction. The difference in the importance of the CH_3 group and the progestin reversal reactions indicate that the uterus, while similar to other smooth muscle in Type I in many respects, differs in other reactions. The possibility of a sub-type of adrenergically innervated smooth muscle exists, and the uterine responses may warrant including it in this physiological sub-type. Eccles (19) has stated that it seems possible that the transmission of nerve impulses to the uterus is entirely chemical while in the case of other smooth muscle transmission is accomplished both by the action current and by the chemical mediator.

As indicated in table 3, neosynephrin is placed second and arterenol fifth in order of potency as nictitating membrane-contractors. The para OH group is least important and the CH_3 group on the amine highly important for excitation of this type of muscle. Such an inconsistent relationship between the smooth-muscle-relaxing and the smooth-muscle-exciting function is in accord with the postulation of at least two types of smooth muscles (Type I and Type E) with regard to receptive mechanisms. The order of potency for a mixed response, of course, cannot be expected to be consistent with either type of single-effector system. In the last column of table 3 pressor ratios for these compounds are tabulated (44).

Therapeutically, it would be an advantage to establish the physiological classification of smooth muscle and the potency of various amines with regard to these physiological types. A compound could then be chosen to obtain the desired effect on smooth muscle of one type without any marked reactions on the other type.

The results of this study may well have an important bearing on the theories of chemical mediation in the sympathetic nervous system. According to Cannon and Rosenblueth's theory (15), inhibition is brought about by the liberation of adrenalin (M) which combines with a chemical (I) provided by the effector to form "inhibitory sympathin" (MI). Sympathin I acts on smooth muscle locally and may pass into the blood stream to relax smooth muscle of this same type. Excitation is brought about by the release of adrenalin (M) which combines with a substance E provided by this effector to form "excitatory sympathin" (ME). Sympathin E, likewise, acts locally or may enter circulation. This theory implies that there are two and only two physiological types of smooth muscle. It has already been indicated in this paper that subclassification of one of these types is necessary.

Bacq (7, 8) first postulated that several catechol amines, such as epinephrine, dihydroxyphenylethylamine, or arterenol, might serve as mediators. Sympathin I, he suggests, may correspond to adrenalin and sympathin E to partially oxidized adrenalin. Greer et al (23) took up

the suggestion of arterenol as a possible mediator and advanced a theory that either one of the two sympathins may cause relaxation or contraction depending upon the effector smooth muscle cell concerned. The one, however, has a greater intrinsic power of inducing contraction but less intrinsic power of inducing relaxation, than the other sympathin. These are referred to as S_c and S_r respectively. As a working hypothesis, Greer suggested that l-arterenol be assumed to be S_c (corresponding to Cannon's sympathin E) and l-adrenalin to be S_r. In experiments in which the response may be considered to be that of a single type of smooth muscle, arterenol is less potent than adrenalin whether the effector shows a motor response, such as the retractor penis (10) and nictitating membrane (5), or an inhibitory response, such as the non-pregnant uterus (11), the intestine, and the bronchi (5). The difference in potency of adrenalin and arterenol is less with regard to the intestine-inhibiting function in the dog (See table 3) than it is for any of the other single-effector responses. This is especially true if it may be assumed that the laevo form of arternol would show greater intestine-inhibiting potency than the racemic form which was used. If it should be proved that a sympathin is a compound of less potency than adrenalin, it seems more logical to assume in the light of present facts that the mediator is adrenalin, and the less potent sympathin is a stage in the destruction of the mediator.

SUMMARY

A review of the literature pertaining to the action of phenylethylamine derivatives on adrenergically inhibited smooth muscle reveals that only the bronchi and, to some extent, the isolated intestine have been studied quantitatively.

The relative intestine-inhibiting potencies of adrenalin, arterenol, epinine, neosynephrin, Kephrine, and synephrin have been determined in unmedicated dogs and for the isolated rabbit intestine. The similarity of effects on this one organ in two different species, studied by two techniques, indicates the physiological similarity of the smooth muscle used and demonstrates the peripheral and the direct action of these sympathomimetic amines on the smooth muscle of the intestinal wall.

The qualitative effects of the compounds on intestinal motility consist of decreased tonus and inhibition of rhythmic contraction.

Within a period of one to two weeks, the postganglionic denervated intestine becomes from 2 to 8 times more sensitive than normal to each of these amines.

The use of these amines has permitted evaluation of the various groups of the adrenalin molecule in relation to its intestine-inhibiting function. Removal of any one of the groups of the adrenalin molecule that distinguish it from the fundamental sympathomimetic nucleus results in a compound of diminished intestine-inhibiting potency. The meta OH group is the most important single group. The least important in the CH_3 on

the nitrogen atom. The para OH group is more important for inhibitory action on the intact dog intestine than on isolated rabbit intestine.

When the intestine-inhibiting potencies of the compounds are compared with the broncho-dilator potencies, a consistent relationship is observed in the order of activity. This suggests that the smooth muscle of the bronchi and of the intestine belong to the same type.

A comparison of the importance of a given group of the adrenalin molecule to its intestine- or broncho-inhibiting function with the importance of the same group to its nictitating-membrane-stimulating function shows no consistent relationship between smooth-muscle-relaxing and smooth-muscle-contracting potency of these amines.

The relation of the above facts to sympathin theories and the physiological classification of smooth muscle is discussed.

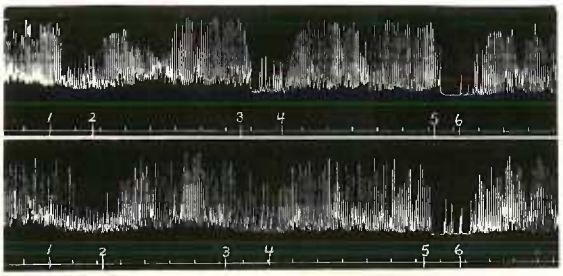


Figure 2.

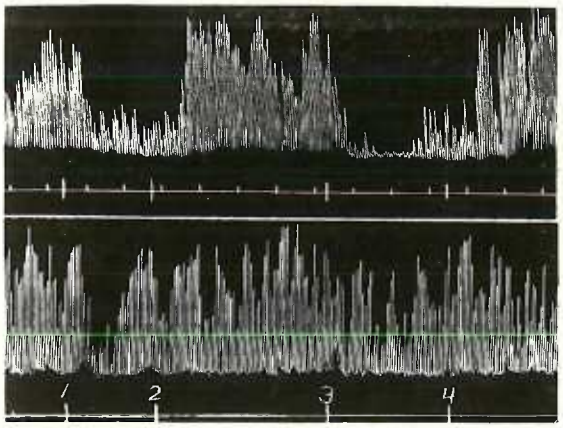


Figure 3.

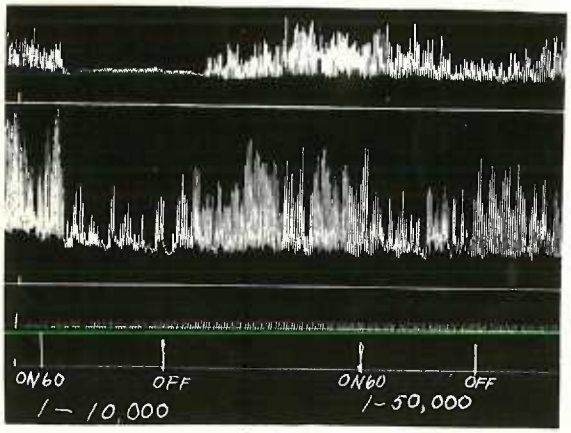


Figure 4.

Fig. 2. Effect of adrenalin and arterenol on the motility of the innervated Thiry-Vella fistula of dog 42. Upper record shows effect of adrenalin 1:250,000 injected at a rate of 1 cc. per minute between 1 and 2, 2 cc. per minute between 3 and 4, and 4 cc. per minute between 5 and 6. Lower record shows effect of arterenol 1:100,000 injected at a rate of 2 cc. per minute between 1 and 2, 1¼ cc. per minute between 3 and 4, and 4 cc. per minute between 5 and 6. Dog weight 9 kilo. Time in minutes.

Fig. 3. Effect of arterenol 1:500,000 on the denervated (upper record) and the innervated (lower record) intestinal segments of dog 1. Injection rate 2 cc. per minute between 1 and 2, 4 cc. per minute between 3 and 4. Dog weight 19 kilo. Time in minutes.

Fig. 4. Effect of epinine on the motility of the denervated (upper record) and the innervated (lower record) intestinal segments of dog 1. Injection rates were 1 cc. per minute of 1:10,000 followed by 1 cc. per minute of 1:50,000. Dog weight 19 kilo. Time in 10 second and 1 minute intervals.

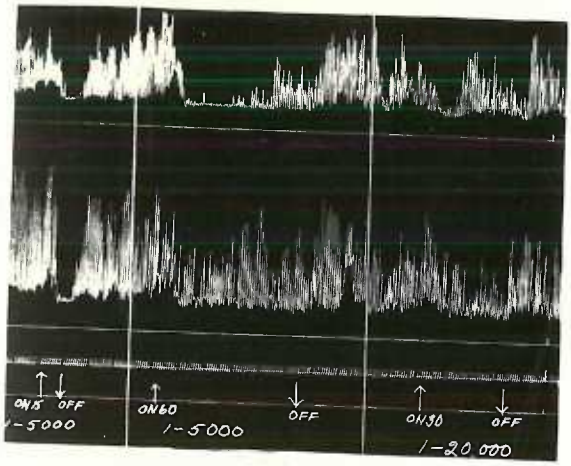


Figure 5.

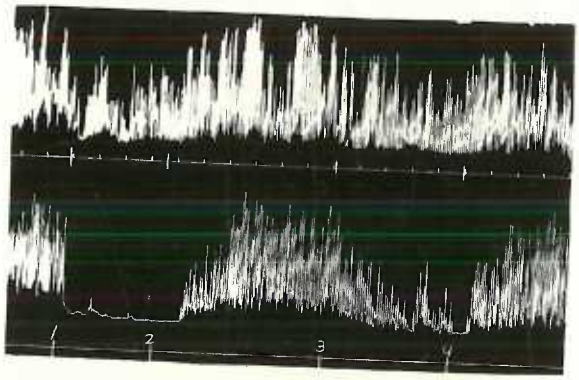


Figure 6.

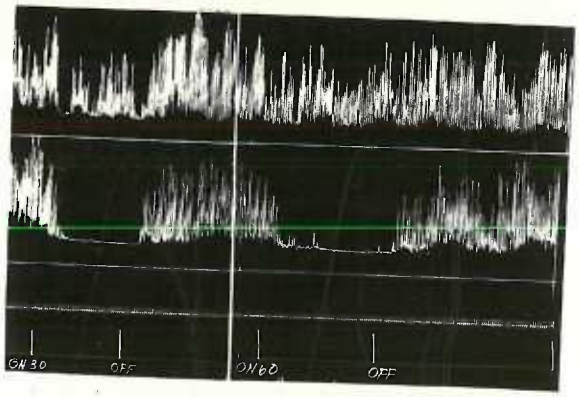


Figure 7.

Fig. 5. Effect of kephrin of the denervated (upper record) and innervated (lower record) intestinal segments of dog 1. Injection rates were 4 cc. per minute of 1:5000 (left), 1 cc. per minute of 1:5000 (middle), and 2 cc per minute of 1:20,000 (right). Dog weight 19 kilo. Time in 10 second and 1 minute intervals.

Fig. 6. Effect of neosynephrin 1:10,000 on the innervated (upper record) and the denervated (lower record) intestinal segments of dog 3. Injection rates were 4 cc. per minute between 1 and 2, and 2 cc. per minute between 3 and 4. Dog weight 14.5 kilo. Time in minutes.

Fig. 7. Effect of synephrin 1:250 on the innervated (upper record) and the denervated (lower record) intestinal segments of dog 3. Injection rates were 2 cc. per minute at ON 30 and 1 cc. per minute at ON 60. Break in record represents a 14 min. interval. Dog weight 14.5 dilo. Time in 10 second and 1 minute intervals.

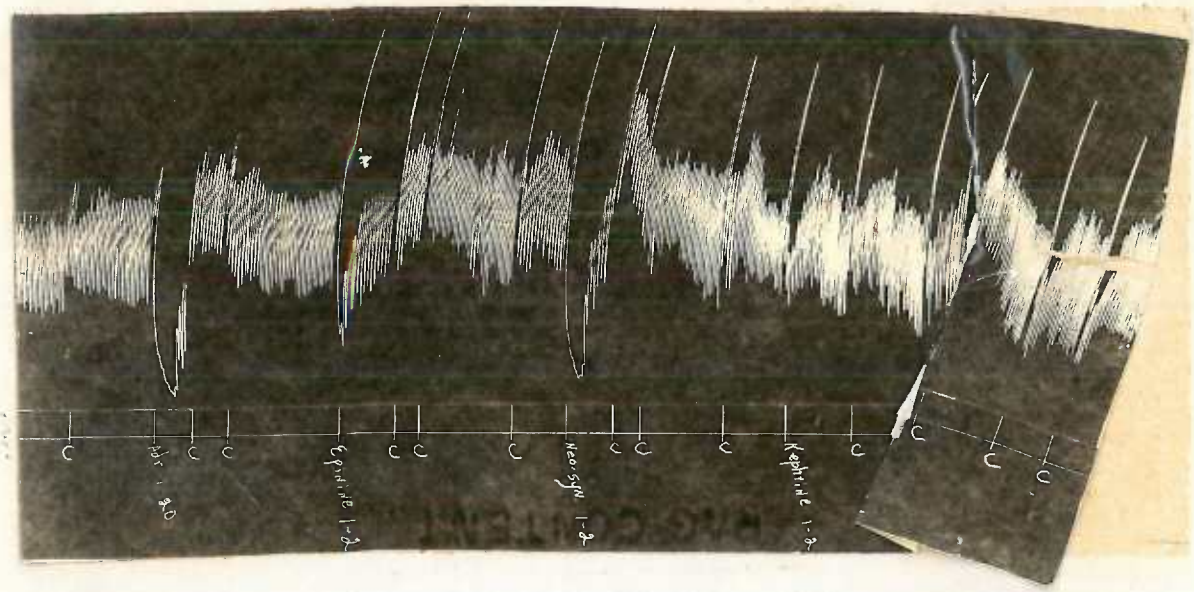


Figure 8. Effect of adrenalin 1:20,000,000, epinine 1:2,000,000 neosynephrin 1:2,000,000, kephrine 1:2,000,000 and kephrine 1:1,000,000 on isolated jejunal segment of rabbit. Dilutions made with Locke's solution. C. represents control change to simple Locke's solution.

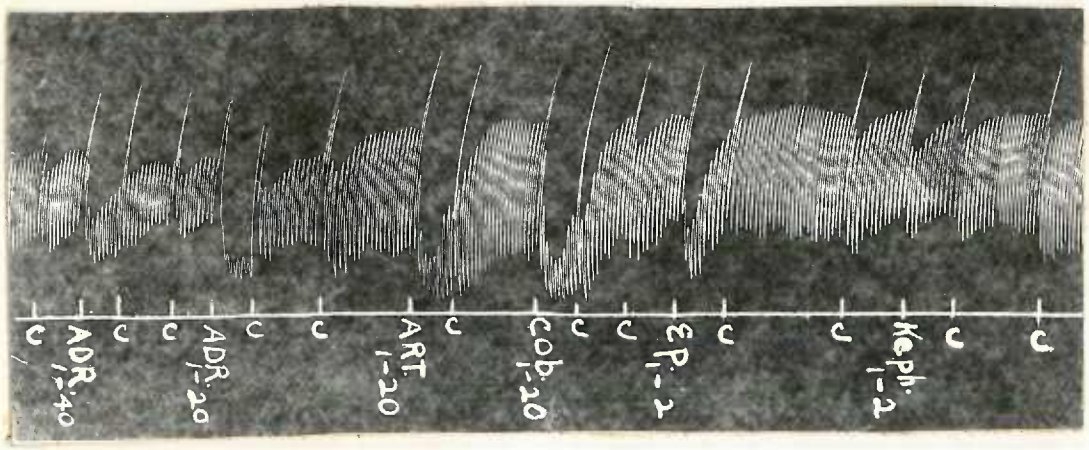


Figure 9. Effect of adrenalin 1:40,000,000, adrenalin 1:20,000,000, arterenol 1:20,000,000, cobefrin 1:10,000,000, epinine 1:2,000,000 and kephrine 1:2,000,000 on isolated jejunal segment of rabbit. Dilutions made with Lock's solution. C. represents control change to simple Locke's solution.

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