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Short Title:

ADRENALINE RECEPTOR ANTAGONISTS

A STUDY OF THE ADRENERGIC RECEPTORS
EMPLOYING ANTAGONISTS

BY

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INTRODUCTION

Antagonists have been used for the quantitative measurement of drug interactions with receptors. Using quantitative measurements based on Gaddum's hypothesis (12) that agonists and antagonists compete for receptors according to the mass law, Arunlakshana and Schild (1) have shown that there are remarkable similarities between the effective concentrations of atropine as an acetylcholine antagonist in such widely differing preparations as the guinea pig ileum, guinea pig lung, and chick amnion. It was concluded the receptor for acetylcholine was similar in all three preparations. Histamine and histamine analogs were also shown to be antagonized to the same degree in the guinea pig trachea, guinea pig lung, guinea pig ileum, and the human bronchi by similar concentrations of mepyramine. Because a similar concentration of antagonist effectively blocked the active drug in all preparations tested, it was concluded histamine acts on a common receptor in those tissues.

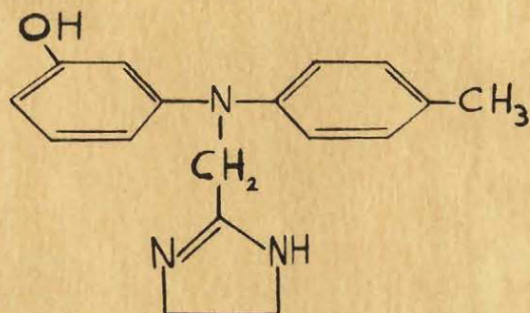
No similar quantitative studies employing antagonists have been carried out to identify adrenergic receptors, although studies concerning the effects of sympathomimetic amines on alpha and beta adrenergic receptors have been performed with various intact and isolated preparations.

The purpose of this investigation was to ascertain whether the alpha and beta adrenergic receptors in the duodenum are

similar to the alpha adrenergic receptor in the aorta and the beta adrenergic receptor in the heart respectively.

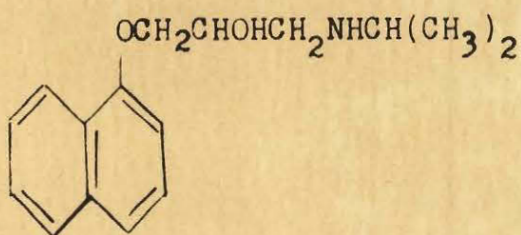
This study attempts to use adrenergic blocking drugs to compare the interactions of alpha and beta adrenergic receptors with selected sympathomimetic amines on isolated rabbit tissues. Phenylephrine and isoprenaline were used as they possess almost entirely alpha or beta effects respectively. Adrenaline and noradrenaline which possess both alpha and beta receptor effects were also studied so that the effects of stimulating both types of receptors as well as a single receptor type in a test organ could be readily compared. Phentolamine (Fig. 1) and Propranolol (Fig. 2) served as alpha and beta receptor antagonists respectively.

The effects of beta adrenergic blockade using propranolol as the antagonist are presented for the isolated rabbit auricles and duodenum. The effects of alpha adrenergic blockade employing the alpha receptor blocking drug phentolamine are presented for the isolated rabbit aorta and duodenum. The effective concentrations of propranolol and phentolamine as antagonists of sympathomimetic amines on the different tissues are then compared.



PHENTOLAMINE
(REGITINE)

Fig. 1.



PROPRANOLOL
(INDERAL)

Fig. 2.

HISTORICAL REVIEW

Receptors

The theory of chemical transmission of the nervous impulse requires that there be present in the effector cell, an area or substance specifically designed to react with the chemical transmitter. To this area the transmitter migrates, at this site it combines with an acceptive material, and in this manner the specific response of the effector cell is called forth.

Langley (3) in 1921 gave the first clear representation of the thesis that nerve impulses and drugs act by combining with a receptive substance. His idea, in fairly simple terms, was propounded thus: The organism, at an early stage, contained the elements of the craniosacral system (parasympathetic), and its cells, as well as the epidermis; these develop certain common characteristics. The thoracolumbar system (sympathetic) and its cells developed later, contained certain characteristics different from the other system. The cells developed therefore, different acceptor substances. Langley's conclusion was that a chemical combination occurs between a drug and a constituent of the cell that he called 'the receptive substance'. In like manner, by extension, the cholinergic or adrenergic transmitter combines chemically with the receptive substance to evoke an effector response.

The intimate nature of the receptive substances has not been determined. Langley conceived of it along the lines of Ehrlich's side-chains theory. Thus a side-chain or portion of a molecule of the cellular substance, exists for which the transmitter or drug, by virtue by one of its side-chains or a polar charge, has an affinity. The receptive substance may, theoretically, exist as an unoccupied or as a loosely occupied side-chain. Beyond this reasoning, which appears probable and sound, we cannot go at present. The receptive substance may be an enzyme itself, or a side-chain of an enzyme or functional groups belonging to some other class of proteins.

Location of receptors. It has been demonstrated clearly that the receptor is part of the effector cell and not part of, or involved with, the nerve producing the transmitter. The best proof of this fact is found in denervation experiments. When the nerve fibre is sectioned it degenerates and is no longer capable of conduction. Despite the fact that the nerve is gone, the effector cells still respond to injected transmitter substance and to drugs. Moreover, the response of the denervated cell to its normal transmitter is greater than his response to the innervated cell. As further proof that the receptor is part of the effector cell, it has been shown that

embryonic heart tissue responds to chemical transmitters or drugs before its innervation has been accomplished. Additionally, the amnion responds to drugs despite the fact that it never receives a nervous supply.

In summary, then, the receptor is part of the effector cell, it is probably part of an enzymatic energy transport process; it may be an enzyme or portion of an enzyme; and it is sensitized to the action of its normal transmitter by denervation.

Since effector cells respond in a characteristic manner to cholinergic- and/or adrenergic-fiber stimulation, it follows that there must be cholinergic and adrenergic receptors. These receptors are specific; a cholinergic one does not respond to the adrenergic transmitter, and an adrenergic one does not respond to the cholinergic transmitter.

Adrenergic receptors. The adrenergic fibers have been classified into one general class, the majority of the thoracolumbar postganglionic fibers. These fibers, like the cholinergic, subserve both excitatory and inhibitory actions.

The concept of two types of adrenergic receptors in vascular smooth muscle was proposed by Dale (4) in 1906. Dale showed adrenaline produced a contraction of smooth muscle in which

the "motor" type of receptor was dominant and a relaxation of smooth muscle in which the "inhibitory" type of receptor was dominant. By selectively blocking the action of adrenaline on "motor" receptors, the ergot alkaloids prevented the contracting effect of adrenaline, and in some vascular smooth muscles actually reversed a contracting effect to a relaxing effect because of the now unmasked effect of adrenaline on the unblocked "inhibitory" receptors. In 1910 Barger and Dale (5) extended the receptor concept in an attempt to characterize and quantify the effects of sympathomimetic amines on blood pressure and smooth muscle. They discussed not only the importance of chemical structure in influencing the preference (affinity) of a drug for the two types of receptors in determining its quantitative and qualitative effect but also the probability that quantitative differences between drugs might be due in part to physical and chemical properties which permitted differences in the partition coefficients between the extracellular fluid and that part of the cell containing the receptors. Following this work, the concept of specific receptors for compounds of specific chemical structure which act upon cells came into general use.

In recent years two noteworthy attempts have been made to modify and extend Dale's original classification of the adrenergic receptors into "motor" and "inhibitory" types.

Ahlquist (6) classified adrenergic receptors mediating specific responses in different effector organs largely on the basis of the order of potency of five sympathomimetic amines in eliciting those responses. Lands (7, 8) objected to certain aspects of Ahlquist's reclassification of motor and inhibitory receptors as alpha and beta types respectively. He preferred to classify adrenergic receptors in the heart as undifferentiated (Acr) since this organ was stimulated by drugs which had a strong affinity for either the excitatory receptors (Ac) or the inhibitory receptors (Ar) of smooth muscle. He criticized Ahlquist's use of the order of the relative potencies of the series of sympathomimetic amines for classifying receptors on the grounds that the relative potencies of different sympathomimetic amines on one type of effector organ often varied considerably with the species of animal and the experimental conditions used.

The simplest method for describing adrenergic receptors would be to make a sharp distinction between excitatory and inhibitory ones. The use of such a technique would be ideal so far as blocking agents are concerned, for there are different blocking agents that prevent excitatory and inhibitory responses to adrenergic stimulation.

Blocking Agents

The most important fact concerning adrenergic or cholinergic blocking drugs in respect of chemical transmission is that the blocking agents do not prevent the access mediators to the receptors. This is probably accomplished in the following way: the blocking agent has an affinity for the chemical structure of the receptor just as does the mediator; the blocking agent forms a chemical combination with the receptor, thus occupying the site normally available to the transmitter; the transmitter, therefore, cannot act at the particular site to produce a characteristic response.

The reactions between blocking agents and receptors and transmitters or drug can be either equilibrium or non-equilibrium reactions. If the reaction is an equilibrium one, the effect of the blocking agent can be overcome by supplying more transmitter or more drug; this type of reaction is also called 'competitive'. If the reaction, however, is not an equilibrium one, the effect is not easily overcome by supplying more transmitter or more drug; this type of reaction is called 'non-competitive'. An example of the first type is the reaction between curare and the receptor and acetylcholine or the reaction between phentolamine, the receptor, and noradrenaline. An example of the second type is the reaction

between dibenamine and the receptor and adrenaline.

It should be remembered that blocking agents are specific for certain receptors, just as transmitters are specific for certain receptors. As prototypes, it will suffice to mention atropine, curare, and tetraethylammonium ion in cholinergic transmission, and ergotoxin, phentolamine, dichloroisoprenaline, and propranolol in adrenergic transmission.

Quantitative Measurement of Drug Antagonism

Clark and Raventos (9) in 1937 suggested a method of estimating antagonistic or blocking power of drugs in terms of "the concentration of antagonist which altered by a selected proportion (e.g. tenfold) the concentration of an active drug needed to produce a selected effect." Schild (2) termed the negative logarithm of this concentration (molar) as the pA_x , where x is the proportion selected. On the guinea pig's ileum the value of pA appears to be independent of the potency of the active drug.

Although drug receptors have not so far been identified by physical or chemical methods, they can be identified pharmacologically by means of antagonists. If two agonists act on the same receptors they can be expected to be antagonized by the same antagonist, and, if the antagonism is competitive, they can be expected to be antagonized by the same concentration

of antagonist and to produce with it the same pA_x (2) or dose ratio (10, 11); pA_x values can thus be used to identify agonists which act on the same receptors. They can also be used to identify receptors in different tissues since tissues with similar receptors would be expected to give a similar pA_x with antagonists.

The mass law equations as applied to drug antagonism refer to events on receptors rather than to observable response, and in applying these equations it is necessary to postulate some relation between receptor activation and response. It has sometimes been assumed that the response is a linear function of the number of activated receptors, but more usually the more limited assumption has been made that equal effects in the absence and presence of antagonist involve equal numbers of receptors. This limited assumption, which does not specify the relation between receptors and response, underlies the use of pA_x (and the dose ratio) in testing for competitive and non-competitive antagonism.

Equation of Competitive Antagonism

Consider the competitive equation

$$y = \frac{K_1 A}{K_1 A + 1} = \frac{K_1 x A}{K_1 x A + K_2 B^n + 1}$$

where y is the fraction of activated receptors, A and B are concentrations of the agonist and antagonist respectively, x is the dose ratio, K_1 , K_2 , and n are constants. Since by definition (1, 2) $pA_x = -\log B$

$$\log (x - 1) = \log K_2 - n pA_x \quad (1)$$

Thus when $\log (x - 1)$ is plotted against pA_x , a straight line results with slope (- n). This line intersects the pA_x axis at a point corresponding to pA_2 . A slope of unity has been shown (79) to be consistent with a bimolecular reaction for agonist and receptor and for antagonist and receptor, and thus provides evidence for a 1:1 agonist-receptor complex, even when the shape of the dose-response curve with the agonist gives no such evidence.

As a practical example of the above theoretical postulates consider the following data from Schild (1).

Figure 3 illustrates an experiment in which a wide range of concentrations of atropine was employed. Only the middle range of the response curve was utilized.

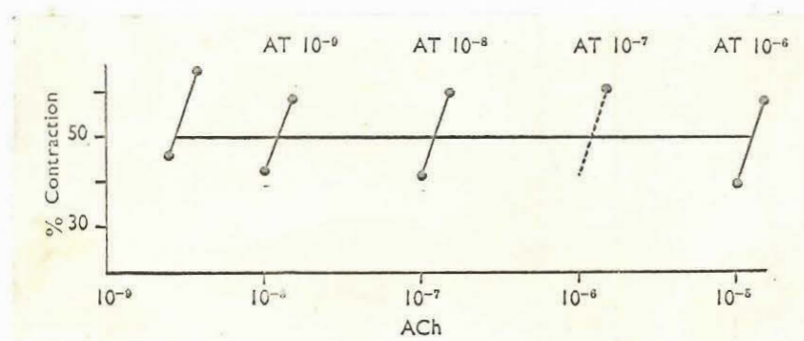


Fig. 3. Guinea pig ileum. Effects of acetylcholine (ACh) in absence and presence of atropine (AT). Hexamethonium 10^{-6} was added to the Tyrode solution.

When plotted by using equation (1) the results can be fitted by a straight line with slope $-n = 1.04$ (Fig. 4).

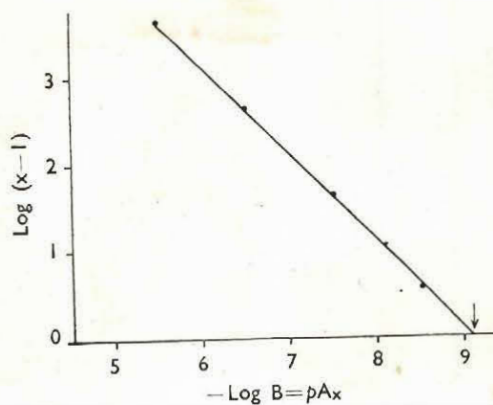


Fig. 4. Results from Fig. 3 plotted by using equation (1) developed in the text $-n = 1.04$. The arrow indicates the pA_2 value.

The various quantitative applications of pA_x here described are all based on the hypothesis which Gaddum (12) formulated mathematically, that agonists and antagonists compete for receptors according to the mass law. This hypothesis, although no doubt over simplified, accounts surprisingly well for a variety of experimental findings. Consider the following two cases in point:

I. Use of pA_x for the Classification of Drugs

Agonists which act on the same receptors can theoretically be expected, then, to produce the same pA_x with competitive antagonists. This is a consequence of the mass law and applies whatever the affinity of the agonist or its intrinsic activity (66), or efficacy (21). If it could be verified experimentally that certain groups of agonists produce the same pA_x (or dose ratio) with antagonists, the receptor theory would be strengthened and a precise method provided of classifying agonists according to the receptors on which they act. By this criterion histamine and pyridylethylamine would be acting on the same receptors, as a single dose of diphenhydramine (see Fig. 5 below) is able to antagonize both these drugs to the same extent.

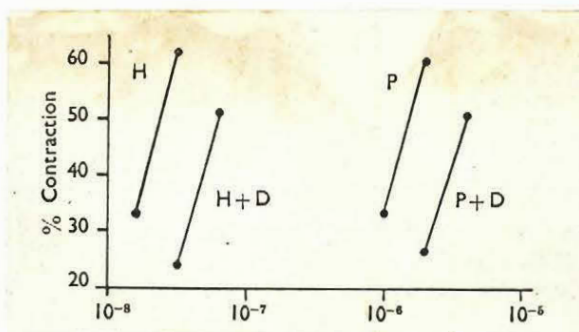


Fig. 5. Guinea pig ileum. Effects of histamine (H) and pyridylethylamine (P) in absence and presence of diphenhydramine (D) $1:300 \times 10^6$. Mean responses of six assays. Abscissa: concentration of agonist. (Data from Schild (1), 1959).

By contrast, histamine and acetylcholine would not act on the same receptor, because although they are both antagonized by atropine their pA_x values are different (see Table 1 below).

Table 1

Data from Schild (1), 1959

COMPARISON OF pA_x VALUES OBTAINED ON THE GUINEA-PIG ILEUM AND THE AIR-PERFUSED LUNG					
The total number of individual determinations is given in parentheses with the standard deviation. The results on the ileum are from Schild (1947a).					
Active Drug	Antagonist	pA_2		pA_{10}	
		Ileum 14 min.	Air-perfused Lung 30 min.	Ileum 14 min.	Air-perfused Lung 30 min.
Histamine	Mepyramine ..	9.46 (5, 0.22)	9.37 (3, 0.16)	8.36 (1)	8.38 (3, 0.28)
	Diphenhydramine ..	8.02 (9, 0.28)	7.80 (4, 0.22)	7.02 (4, 0.31)	6.92 (3, 0.74)
	Pethidine	6.13 (5, 0.46)	6.15 (2, 0.08)	5.00 (5, 0.21)	5.06 (4, 0.23)
	Atropine	5.64 (3, 0.18)	5.91 (4, 0.61)	4.60 (3, 0.24)	5.03 (3, 0.68)
Acetylcholine	"	8.61 (11, 0.15)	8.76 (2, 0.63)	8.05 (3, 0.13)	7.63 (6, 0.64)

II. Use of pA_x for the Comparison of Receptors

Table II gives a summary of pA_x values collected by Schild (1) for atropine and antihistamines in different preparations. The values for different preparations are on the whole remarkably simple. Thus in the case of atropine, similar pA_x values are found in such varied preparations as frog heart, chick amnion, and mammalian intestine, the one exception being the frog rectus which presumably has different nicotinic receptors. The finding that different tissues have receptors with similar affinities for antagonists is interesting since it gives support to the notion that receptors are definite chemical entities.

Table 2

Data from Schild (1), 1959.

pA_x VALUES IN DIFFERENT PREPARATIONS				
(1) Clark and Raventos (1937). (2) De Jalon (after Reuse, 1948). (3) Evans and Schild (unpublished observations). (4) Hawkins and Schild (1951). (5) Marshall (1955a). (6) Marshall (1955b). (7) Reuse (1948). (8) Schild (1947a). (9) Present work. (10) Wilbrandt (1950).				
Preparation	Atropine-acetylcholine		Mepyramine-histamine	Diphenhydramine-histamine
	pA_2	pA_{10}	pA_2	pA_2
Guinea-pig ileum ..	8.8 { 8.6 (8) 9.0 (9) 8.8 (6)	8.1 { 8.1 (8) 8.1 (6)	9.3 { 9.5 (8) 9.3 (7) 9.3 (2) 9.4 (5) 9.3 (9)	8.0 { 8.0 (8) 8.2 (2) 7.8 (10) 8.1 (5)
Rat intestine ..		8.1 (1)		
Guinea-pig trachea ..			9.1 (9)	7.8 (9)
Guinea-pig lung (perfused) ..	8.8 (9)	7.6 (9)	9.4 (9)	7.8 (9)
Human bronchi			9.3 (4)	
Chick amnion	8.8 (3)			
Frog auricle ..		8.3 (1)		
„ rectus ..		4.2 (1)		

Antagonists and Receptor Classification

Antagonists are, to date, as previously stated, the prime tools in the classifications of receptors. Drugs such as phentolamine, dibenamine, phenoxybenzamine, and the ergot alkaloids are alpha adrenergic receptor antagonists. Dichloroisoprenaline, the di-chloro analog of isoprenaline, and more recently pronethalol and propranolol derivatives of naphthalene (13) have been shown to be beta adrenergic receptor antagonists.

The inability of phentolamine, dibenamine, or di-chloro isoprenaline (DCI) to effectively block the inhibitory effects of noradrenaline or adrenaline on the isolated rabbit duodenum led Furchgott (14) to propose a third adrenergic receptor type, for the intestine. Ahlquist and Levy (15) subsequently showed in the intact dog that a combination of phentolamine or dibenamine and DCI could completely block the inhibitory effects of adrenaline and noradrenaline, indicating that activation of either alpha or beta-receptors in the intestine elicit inhibitory responses. Thus, to block an alpha-receptor agonist, such as phenylephrine or a beta-receptor agonist like isoprenaline, only a single alpha or beta-receptor antagonist is necessary; but in the case of agonists such as adrenaline or noradrenaline, which act on both types of

adrenergic receptors, antagonists for both types of receptors must be present simultaneously. Furchgott (16) later confirmed the results of Ahlquist and Levy in a simpler experimental situation using the isolated rabbit duodenum, and withdrew the suggestion of a third type of adrenergic receptor for the intestine. The actual location of alpha and beta-adrenergic receptors in the gut is still speculative. Recently Furchgott (17) has suggested that alpha-receptors may be situated on the nerve cells in the intestinal plexes, while beta-receptors are probably located on the smooth muscle cells.

Harry (74) has attempted to localize the position of the alpha receptors in the circular muscle of the guinea pig isolated ileum. His experiments showed 1) noradrenaline was more active than adrenaline and both these substances were much more active than isoprenaline on the circular muscle strip 2) the inhibitory action of adrenaline but not aminophylline was specifically antagonized by the alpha receptor blocking agent piperoxine, 3) dichloroisoprenaline which antagonizes the beta receptor actions of adrenaline did not modify the inhibitory action of adrenaline on the circular muscle strip. Szerb (76) had previously observed that hexamethonium did not influence periarterial sympathetic stimulation of a segment of isolated guinea pig ileum, suggesting that the efferent

sympathetic chain is not functionally related to the enteric plexuses of the ileum but terminates in the intestinal musculature. Taken together with the results reported above, Harry concluded that the site of action of noradrenaline, adrenaline and isoprenaline on the circular muscle of the guinea pig ileum is located at post-ganglionic neuro-effector junctions in the smooth muscle.

The experiments of Kosterlitz and Watt (75) attempted to localize the sites of both the alpha and the beta receptors. Their first series of experiments was designed to study the action of adrenaline, noradrenaline and isoprenaline on the responses of the longitudinal muscle of the guinea pig isolated ileum stimulated coaxially by the technique of Paton (77). Since hexamethonium was added to the bath, the action of the catecholamines was probably on the neurone innervating the longitudinal muscle or on the muscle itself, or on both. All three catecholamines inhibited the responses; however, while complete inhibition was easily obtained with adrenaline or noradrenaline, the maximum inhibition obtainable after isoprenaline ranged from 20 to 50%. The inhibitory effect of of isoprenaline was unaffected by the alpha blocking agent, phenoxybenzamine, but readily blocked by the beta blocking agent, propranolol. With adrenaline and noradrenaline, on

the other hand the blocking action of phenoxybenzamine and propranolol were additive, the former being more potent than the latter. Their second series of experiments showed that all three catecholamines reduced the size of the contractions of the longitudinal muscle produced by acetylcholine or carbamylcholine. This inhibition was antagonized by propranolol but not by phenoxybenzamine.

These findings suggest that alpha receptors are situated in the neurones innervating the longitudinal muscle and the beta receptors in the muscle itself, although the presence of beta receptors in the neurone cannot be excluded. Catecholamines acting on alpha receptors would appear to interfere with the conduction of the impulse in the nerve fiber and its terminals or with the release of transmitter, while their effect on the beta receptors is depression of the response to agonists acting directly on the muscle.

The question of whether alpha-receptor antagonists can block inotropic and chronotropic effects of adrenaline on mammalian heart tissue was carefully scrutinized by Nickerson and Chan (18). No specific blockade was discovered although non-specific depression occurred with some antagonists in high doses. DCI was observed to be the only antagonist which gave specific blockade. Thus, it was concluded that the adrenergic

receptors in the heart were of the beta type. This recent substantiated the earlier work of Moran and Perkins (20) who concluded, on the basis of the selective blocking action of DCI against the excitatory effects of adrenergic stimuli on the heart, and the inhibitory effects of adrenergic stimuli on the other organs, and the lack of blockade of adrenergic vasopressor action, that the adrenergic receptors of mammalian heart tissue are functionally the same as the adrenergic inhibitory receptors of other tissues.

All these conclusions from experiments employing DCI on the mammalian heart agree with the earlier hypothesis of Ahlquist (6) that the adrenergic receptors in the heart responsible for the chronotropic and inotropic effects are of the beta type, i.e. receptors which serve inhibitory functions in other organs, in contrast to the alpha receptors which serve adrenergic excitatory responses in the vasculature and most other organs. The notable exception being the alpha receptor in the intestine which also serves an inhibitory function (15, 16).

The pharmacology of antagonists used in this study will now be briefly reviewed.

Propranolol

The di-chloro analog of isoprenaline, DCI, was the first beta receptor blocking drug to be discovered. Powell and Slater (19) reported that DCI selectively blocked some inhibitory

effects of adrenaline and isoprenaline. The depressor action of isoprenaline and the secondary depressor action adrenaline were inhibited. The inhibitory effect of adrenaline on isolated rabbit intestine was blocked. On isolated guinea pig tracheal chain, DCI inhibited adrenaline and isoprenaline relaxation of pilocarpine-induced spasm. Moran and Perkins (20) reported the effects of adrenaline, noradrenaline and isoprenaline on the right ventricular contractile force of anesthetized vagotomized dogs following treatment with DCI. Isoprenaline was four times as effective and noradrenaline two times as effective as adrenaline in producing positive inotropic changes. They showed that DCI in cumulative doses of 7-15 mg/kg in anesthetized open-chest dogs completely blocked the increase in the contractile force of the heart brought about by moderate doses of adrenaline, noradrenaline, and isoprenaline. Furchgott (14) has also demonstrated that DCI gives a clear-cut blockade of the inotropic and chronotropic actions of sympathomimetic amines on the heart and the inhibitory actions of these amines on mammalian smooth muscle. This agent, however, was far from an ideal beta-blocking drug, as the concentrations required for blockade often approached those which directly depressed contractility in smooth and cardiac muscle (14). In addition, DCI appeared to have some sympathomimetic activity on the very receptors which it blocks; the degree of activity depending

upon the particular effector organ on which it is tested. Stephenson (21) has suggested that DCI might be classified as a 'weak partial agonist' rather than a 'true antagonist'.

A derivative of naphthalene, 2-isopropyl - 1- (2-naphthyl) ethanol hydrochloride was shown by Black and Stephenson (13) to have beta adrenergic blocking properties. They observed that highly active blockade was found only on those tissues which have been classified as containing adrenergic beta-receptors. Unlike DCI, pronethalol showed little intrinsic sympathomimetic activity. By contrast, Donald, Kvale and Shepherd (22) in anesthetized and conscious dogs, observed different effects of pronethalol on the heart. The augmentation of cardiac output and stroke volume in all of the dogs treated, and the increase in heart rate in conscious dogs with cardiac denervation, during administration of pronethalol demonstrated that the drug itself had stimulatory properties. The difference in effects on the heart observed in these experiments and between the results of Black and Stephenson may be related to the dose used (2.5 mg/kg for 25 min.) by Black et al., and 1 mg/kg for 67 min. by Donald et al.

Thus it appears that pronethalol itself, like DCI, has sympathomimetic activity, but that its intrinsic activity in relation to its specific blocking effects is less than that of DCI.

James and Nadeau (23) demonstrated that this new agent pronethalol was at least as specific as DCI and at least equally as potent. Like DCI, the nature of pronethalol antagonism in smooth muscle was observed to be competitive. Dornhurst and Robinson (24) have shown that the drug blocks heart rate and ECG changes following exercise in patients with cardiac ischemic disease. Clinical trials of pronethalol had to be curtailed in view of the carcinogenic activity discovered in mice (25). That event led to the synthesis of a similar compound, propranolol, 1 - isoamino-3-(1-naphtyl oxy) -2-propranolol hydrochloride, which to date has proved to be free from carcinogenic activity. Propranolol has been shown by Black et al. (26) to be at least ten times more potent than pronethalol in preventing increase in myocardial tension produced by isoprenaline. Both pronethalol and propranolol have been shown to be devoid of cholinergic blocking activity. Thus, these compounds have a greater specificity than alpha-blockers which were shown by Benfey and Grillo (27) to often exhibit simultaneous cholinergic blockade in effective adrenergic blocking doses. Both these compounds, propranolol and pronethalol, exhibit a high degree of specific antagonism to catecholamines.

Gill and Vaughan Williams (28) have recently pointed out that pronethalol and its related compounds, are very closely related to a series of local anesthetic compounds studied

some time ago by MacIntosh and Work (29). It was found that pronethalol is 1.8 times as active as procaine. This raises the question of whether all the actions of pronethalol or propranolol are entirely due to their beta-adrenergic blocking effects.

Phentolamine

The adrenergic blocking action of this imidazoline derivative (Figure 1) was first described by Meier et al. (30) in 1949. They observed that the relaxing effect of adrenaline (.5 ug/ml) on the isolated rabbit ileum was diminished approximately 50% by phentolamine (.5 ug/ml). Higher doses of phentolamine were required to produce an adrenolytic and sympatholytic effect upon the nictitating membrane than upon blood pressure and the salivary gland.

They further observed the isolated vessels of the hind limb of the rabbit showed no reaction to concentrations of phentolamine up to 10 ug. No direct myotropic stimulating effect was observed with respect to the bronchi of the rabbit, nictitating membrane of the cat, isolated ilea of the rabbit, or intact ileum of the dog. As opposed to the majority of adrenolytic substances, phentolamine had no contractile action upon the isolated uterus of the guinea pig or the uterus in vivo of the rabbit. It was concluded that phentolamine was a

potent adrenolytic agent but was much less active as a sympatholytic drug.

Emlet et al. (80) compared the adrenolytic blocking effects of phentolamine and benodaine against adrenaline and noradrenaline in anesthetized dogs. Phentolamine, and benodaine, reduced the pressor response to both sympathomimetics, particularly to adrenaline. Following amounts which effected reversal of adrenaline and blocked or markedly reduced the response to noradrenaline, the action of phentolamine persisted twice as long as the other blocking drug.

Vasodilation produced by phentolamine is due both to adrenergic blockade and to a direct dilator action on the blood vessels (31). Members of this series of imidazoline derivatives have been shown to possess pharmacological properties in common with histamine, antihistamines, the sympathomimetic naphazoline, and the parasympathomimetic pilocarpine.

Leimdorfer (32) observed that in dogs during pentobarbital anesthesia phentolamine alleviated the initial hypotensive phase of nicotic activity. Leimdorfer (33) also showed phentolamine inhibited the effects of peripheral vagal stimulation on the blood pressure and the heart rate of these dogs in converted cardiac arrhythmias induced by high amounts of metacholine to normal rhythm. Since that time phentolamine

has been used extensively for studies of drug antagonism on the alpha adrenergic receptors (16, 35, 34), and more recently in the study of the antagonism of acetylcholine by adrenaline antagonists (27).

Sympathomimetic Amines

Some of the pharmacological properties of sympathomimetic drugs in relation to smooth muscle and to this study will now be discussed. The most prominent effect produced by adrenaline on vascular smooth muscle is contraction (7, 36). The smooth muscles of different vessels may vary considerably in their sensitivity to adrenaline. Zweifach (37) has shown that sensitivity to adrenaline among vessels decreased in the order metarterioles and capillary spinctors, larger arterioles, and venules. Brun (38) was able to produce contraction of small arteries in rat muscle with much lower concentrations of adrenaline than in the case of small arteries in rat omentum.

Cruickshank and Subra Rau (39) illustrated the effect of d,l-adrenaline on isolated rings of ox, dog, and man, coronary and systemic vessels. It was shown that the contraction produced by adrenaline on the larger systemic arteries could be antagonized by ergotoxin. In the smaller arteries, ergotoxin could reverse the contractile response to adrenaline. Wilkie (40) used the isolated sheep carotid artery and showed that in

pharmacological doses the constriction produced by adrenaline was directly proportionate to the log of the dose.

Adrenaline, however, also causes relaxation of the smooth of certain vessels (41, 42, 43). In those vascular beds where adrenaline produces vasodilation at low and vasoconstriction at higher concentrations, and in some vascular beds where it produces only vasoconstriction at all effective concentrations, the vasodilating capacity of adrenaline can be readily demonstrated by selectively blocking the motor receptors of the vascular bed with natural (4) or synthetic adrenergic blocking agents (34). This reversal of the response to adrenaline has been observed in such vascular beds as the rabbit ear, limb muscles, splanchnic region, limb muscles, and lungs (44, 45, 46, 47). In such beds the blocking agent unmasks the presence of sufficient but subordinate inhibitory receptors. There are, however, vascular beds, such as those of the skin and kidney where adrenergic blocking drugs block the vasoconstricting action of adrenaline but do not reverse it (53, 48, 34, 49). Furchgott (50) found that isolated strips of rabbit thoracic aorta were well suited for the studies of drug responses as such strips never exhibited spontaneous contractions. He found adrenaline and noradrenaline to be about equipotent in producing constrictor effects, but that after blockade (51) with dibenamine,

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Adrenaline, however, also causes relaxation of the smooth muscle of certain vessels (41, 42, 43). In those vascular beds where adrenaline produces vasodilation at low and vasoconstriction at higher concentrations, and in some vascular beds where it produces only vasoconstriction at all effective concentrations, the vasodilating capacity of adrenaline can be readily demonstrated by selectively blocking the motor receptors of the vascular bed with natural (4) or synthetic adrenergic blocking agents (34). This reversal of the response to adrenaline has been observed in such vascular beds as the rabbit ear, limb muscles, splanchnic region, limb muscles, and lungs (44, 45, 46, 47). In such beds the blocking agent unmasks the presence of sufficient but subordinate inhibitory receptors. There are, however, vascular beds, such as those of the skin and kidney where adrenergic blocking drugs block the vasoconstricting action of adrenaline but do not reverse it (53, 48, 34, 49). Furchgott (50) found that isolated strips of rabbit thoracic aorta were well suited for the studies of drug responses as such strips never exhibited spontaneous contractions. He found adrenaline and noradrenaline to be about equipotent in producing constrictor effects, but that after blockade (51) with dibenamine,

adrenaline produced slightly greater relaxation than did noradrenaline. Furchgott (52) has shown that adrenaline which constricts strips of rabbit thoracic aorta, has a maximal relaxing effect after blockade with dibenamine in the order of 1/10th the former contractile effect.

Noradrenaline, on the other hand, has been shown to have little or no vasodilatory effects in vascular beds of skeletal muscle after complete inhibition of its vasoconstrictor effects by adrenergic blocking drugs (53, 54, 48). Furchgott (51, 55), on the other hand has suggested that vasodilation in isolated rabbit aortic strips after intense treatment with dibenamine can be observed, and that noradrenaline has only about 1/50th the affinity of adrenaline for inhibitory or beta-receptors in that smooth muscle.

The potency ratio of noradrenaline to adrenaline appears to vary with the vascular bed or blood vessel on which contractile responses are observed (48, 56, 54). In the renal vascular bed where inhibitory receptors are probably too low in number to complicate the situation, adrenaline is several times as potent as noradrenaline (6, 56). Noradrenaline is about twice as potent (34, 54) in the vascular bed of dog limb muscle but here the true vasoconstricting potency of adrenaline is obscured by its simultaneous vasodilating action. Noradrenaline is slightly more

potent in bringing about contractile response on isolated rabbit aortic strips but if allowance is made for the masked beta-receptor relaxing action of adrenaline at low doses, then the two drugs appear to be of about equal potency (51).

West (57) has compared the activity of l-adrenaline with that of d,l-noradrenaline in various isolated tissues and intact animals. Noradrenaline was found to be more effective than adrenaline as a pressor agent on mammalian tissues. It was pointed out that in preparations such as the isolated rabbit ileum noradrenaline can exert strong inhibitory effects. The ratio of the dose of d,l-noradrenaline to the equiactive dose of l-adrenaline, that is (d,l-noradrenaline/l-adrenaline), required to produce similar inhibition on the isolated rabbit ileum preparation is 2. The same ratio for the cat blood pressure preparation has a value of 0.8, for the isolated rat non-pregnant uterus preparation a value of 100. It can be concluded that the potency ratio of noradrenaline/adrenaline can vary widely depending on the species under investigation and the type of preparation.

The subsequent work of Gaddum et al. (58) employing l-noradrenaline in place of the d,l mixture showed that the potency ratio (l-noradrenaline/l-adrenaline) on the isolated rabbit ileum was 3. The ratios reported for other preparations were in general agreement with the results of West.

Ahlquist (67) more recently pointed out that the potency ratio of adrenaline to isoprenaline may vary significantly in the same intestinal strip during the course of a single experiment. Lands (7, 8) has hence criticized Ahlquist's use of the order of potencies of sympathomimetic drugs for the classification of receptors. Both Furchgott (16) and Ahlquist (15) reinvestigated the original classification employing antagonists. Although they used widely different experimental conditions, their results were very similar and served only to substantiate the division of adrenergic receptors into alpha- and beta-types, as was earlier proposed by Ahlquist (6).

Isoprenaline has as its common action a very potent vasodilator effect on vascular smooth muscle (7). This can be attributed to the high affinity of this compound for the inhibitory or beta receptors (8). Kadatz (59) found isoprenaline about 1/200 to 1/300 as potent as adrenaline in constricting the very small cutaneous vessels of the skin. It was later shown that in addition to the relaxing effects of isoprenaline in low concentrations on isolated rabbit aortic strips there exists a high concentration range over which isoprenaline has a marked constricting effect (50). Furchgott (51) used dibenamine in cross protection experiments, that is, experiments in which tests were made on the ability of one sympathomimetic amine to afford protection against dibenamine blockade of a

second sympathomimetic drug, and was able to reasonably demonstrate that the motor receptors with which isoprenaline combine are indeed the same ones with which adrenaline and noradrenaline combine. In conclusion, isoprenaline can activate the motor or alpha-adrenergic receptors but its affinity for those receptors is very low compared with the affinity of isoprenaline for the inhibitory or beta-adrenergic receptors (52).

Practically all sympathomimetic amines which contain a phenolic hydroxy group in the meta position and are derivatives of phenylethylamine produce contraction of vascular smooth muscle primarily by a direct action initiated by their combination with adrenergic receptors (52). Morton and Tainter (60) have shown that derivatives of phenylethylamine with no phenolic hydroxy or only one such group in the para position appear to produce contraction primarily by indirect action. The weak direct action of these latter phenylethylamines might be attributed both to their low affinity for alpha receptors and to a small capacity for activating contraction when in complex with a given fraction of alpha receptors (52). Furthermore, compounds such as ephedrine and amphetamine appear to have a direct rather than indirect contractile effect on the isolated rabbit aortic strip, but the maximal contractile height attainable is about 35% of that obtainable with compounds containing a meta-hydroxy group such as adrenaline, noradrenaline, or phenylephrine (52).

The order of relative potencies of sympathomimetic amines on cardiac tissue has been a subject of controversy for almost two decades. It is now known that there are important species differences in sensitivity to noradrenaline, adrenaline and isoprenaline (61). Lockett (62) reported results on the unanesthetized atropinized dog showing that d,l-noradrenaline equals or exceeds the effects of adrenaline in increasing pulse rate. By contrast, Ahlquist (6) found noradrenaline less stimulating than adrenaline in the anesthetized atropinized dog. The experiments of Garb (63) on the isolated cat papillary muscle suggested that d,l-noradrenaline increased contractile force more than does adrenaline. Nathanson and Miller (64) found a marked and enduring increase in ventricular rate after injection of adrenaline in patients with complete heart block whereas a comparable dose of noradrenaline caused a minimal and transient response. Goldberg (78) and others investigated the effects of sympathomimetic amines in producing inotropic changes upon the heart of open-chest, anesthetized vagotomized dogs. They found that the increase in the force of contraction produced by adrenaline and noradrenaline were of the same order of magnitude but that their effects were of different durations. Phenylephrine was observed to be considerably less potent than adrenaline in producing increases in contractile force.

Lands (61) presented evidence for the order of potencies of the sympathomimetic amines in mammalian cardiac tissue. Adrenaline was less effective than l-noradrenaline when the isolated rabbit auricle or perfused heart was used. Concentrations producing significant changes in rate are lower than for changes in amplitude. Isoprenaline was much more effective than either adrenaline or noradrenaline in producing increases in rate and amplitude of contraction in mammalian tissues. In a recent paper, Lands (65) compared sympathetic beta-receptor activity in the guinea pig heart and lung, and has retracted his earlier statement that l-noradrenaline was more effective than adrenaline in producing chronotropic and inotropic effects on the perfused rabbit heart. He appears to have withdrawn an earlier suggestion (61) that a different receptor mechanism is involved in producing rate changes in the heart than is involved in producing amplitude changes. Lands now suggests that ... "the general class of adrenotropic receptor may consist of a population with somewhat different affinities for structurally varied sympathomimetic amines", and that the notion of a beta-receptor as opposed to an alpha receptor may be simply an expression of this population difference.

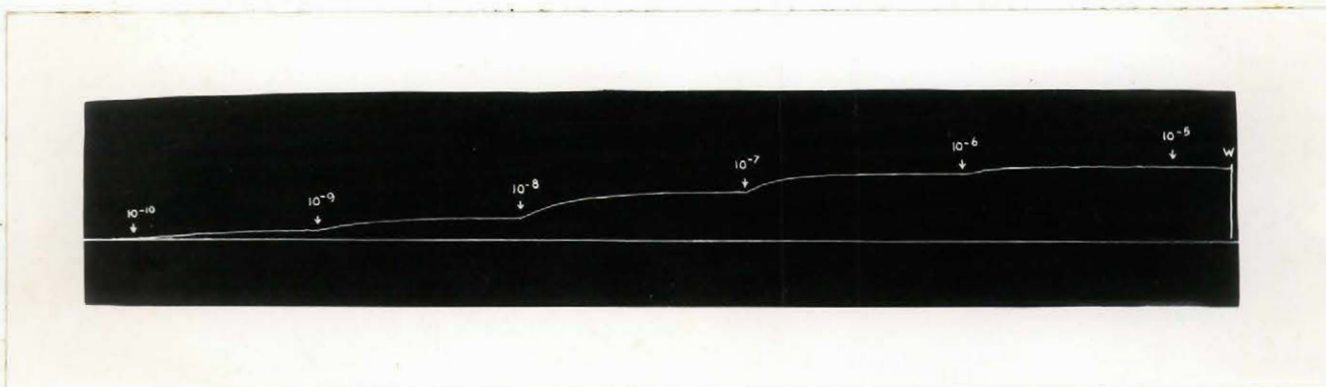
METHODS

1. Isolated Rabbit Aorta

The isolated rabbit aortic strips were prepared by the method of Furchgott and Bhadrakom (50). The aortic strips cut in spiral form, were usually about 0.4 mm. thick, 0.5 cm. wide, and 4 cm. long, prior to mounting in a 100 cc bath maintained at 37°C. Through the bath solution a 95% O₂ - 5% CO₂ mixture was bubbled. Isotonic levers, adjusted to give a nine-fold amplification and counterweighted to give exactly 4 g. tension on the strips, were used. During most of the experiments a small vibrating motor was maintained beside the apparatus to minimize lag in response due to the small amount of friction between writing points and kymograph paper.

Three hours were allowed for the preparation to equilibrate in the bath fluid after mounting. Preparations used before that time had elapsed, appeared to be somewhat more sensitive to small concentrations of sympathomimetic amines. Adrenaline, noradrenaline or phenylephrine were added to the bath in increasing concentrations. Each concentration of drug was permitted to act until the maximum contraction it could produce had been recorded (see Fig. 6), then the next dose of drug (usually greater by a factor of three) was administered and permitted to act until its maximum had passed, and so forth

Fig. 6. Kymograph record showing the effect of increasing doses of noradrenaline on the isolated rabbit aorta. The aortic strip was maximally contracted at a bath concentration of 10^{-5} gm/ml noradrenaline.



until the maximum contraction attainable was produced. Dose-response curve for the above-mentioned sympathomimetic amines were determined in this way.

At the termination of the experiment, the bath was washed out, and then washed out twice again in the succeeding one and one-half hours allowed for the relaxation of the aortic strip. After complete relaxation of the aortic strip, the antagonist phentolamine was added to the bath. After the addition of phentolamine a contraction of about 1 cm. in height was often recorded on the smoked drum. The aortic strip usually required about 15 minutes to relax and for the lever to return to the resting base line that had been established before the addition of the antagonist. The dose/response curve for the sympathomimetic amine under investigation (adrenaline, noradrenaline, or phenylephrine) was then redetermined. The potency of antagonist was calculated in terms of the dose ratio by comparing the effects of the sympathomimetic amines in the presence of the antagonist, with the initial dose/response curve. The dose ratio (10) is the ratio of the concentration of the agonist which has a given effect in the presence of the antagonist, to the concentration of agonist which has the same effect in the absence of antagonist.

2. Isolated Rabbit Duodenum

The isolated rabbit duodenum was suspended in 100 ml. McEwen's solution maintained at 35.5°C. The contractions of the gut were recorded with isotonic levers. The duodenum was allowed to equilibrate for one hour, and the experiment did not proceed until a constant response to a submaximal dose of the sympathomimetic amine to be investigated was recorded. Drugs (adrenaline, noradrenaline, isoprenaline or phenylephrine) were added to the bath in increasing concentrations (see Fig. 7). For all the sympathomimetic amines used except phenylephrine, 100% inhibition of contraction was employed as the end-point of the experiment. In the case of phenylephrine 100% inhibition was difficult to attain so 50% inhibition was used as the end-point for those experiments.

Antagonists were permitted to act in the bath for two minutes prior to the addition of other drugs. The dose ratios were determined as before, from individual experiments.

3. Isolated Rabbit Atria

The isolated rabbit auricles were prepared by quickly removing the entire heart of a 2.5 - 3.5 kg young white rabbit, killed by a blow on the head. The auricles were severed from the rest of the heart and all excess tissue trimmed off by means of small sharp scissors. The beating auricles were then suspended in a 100 ml organ bath containing McEwen solution and maintained at 29°C. Oxygen (95%) - CO₂ (5%) mixture was

Fig. 7. Kymograph records illustrating the blocking action of propranolol against isoprenaline on the isolated rabbit duodenum. The record (control) at the top of the page shows 100% inhibition produced by 3×10^{-7} gm/ml isoprenaline. The lower record shows that 10^{-6} gm/ml isoprenaline is required to produce 100% inhibition in the same preparation following 10^{-6} gm/ml propranolol.

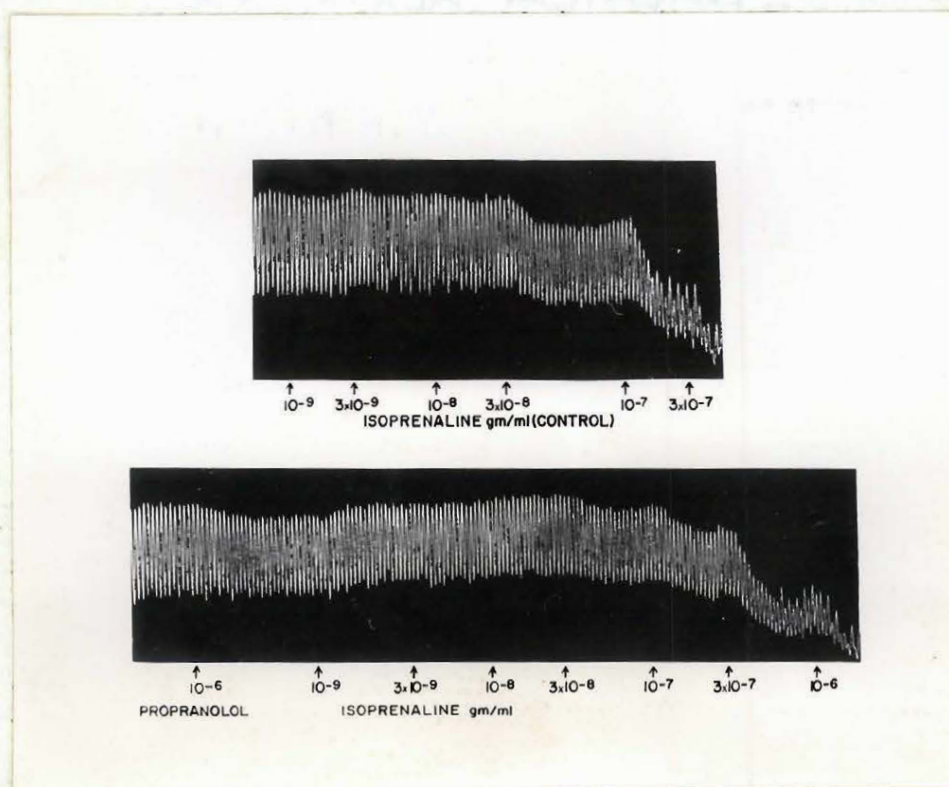
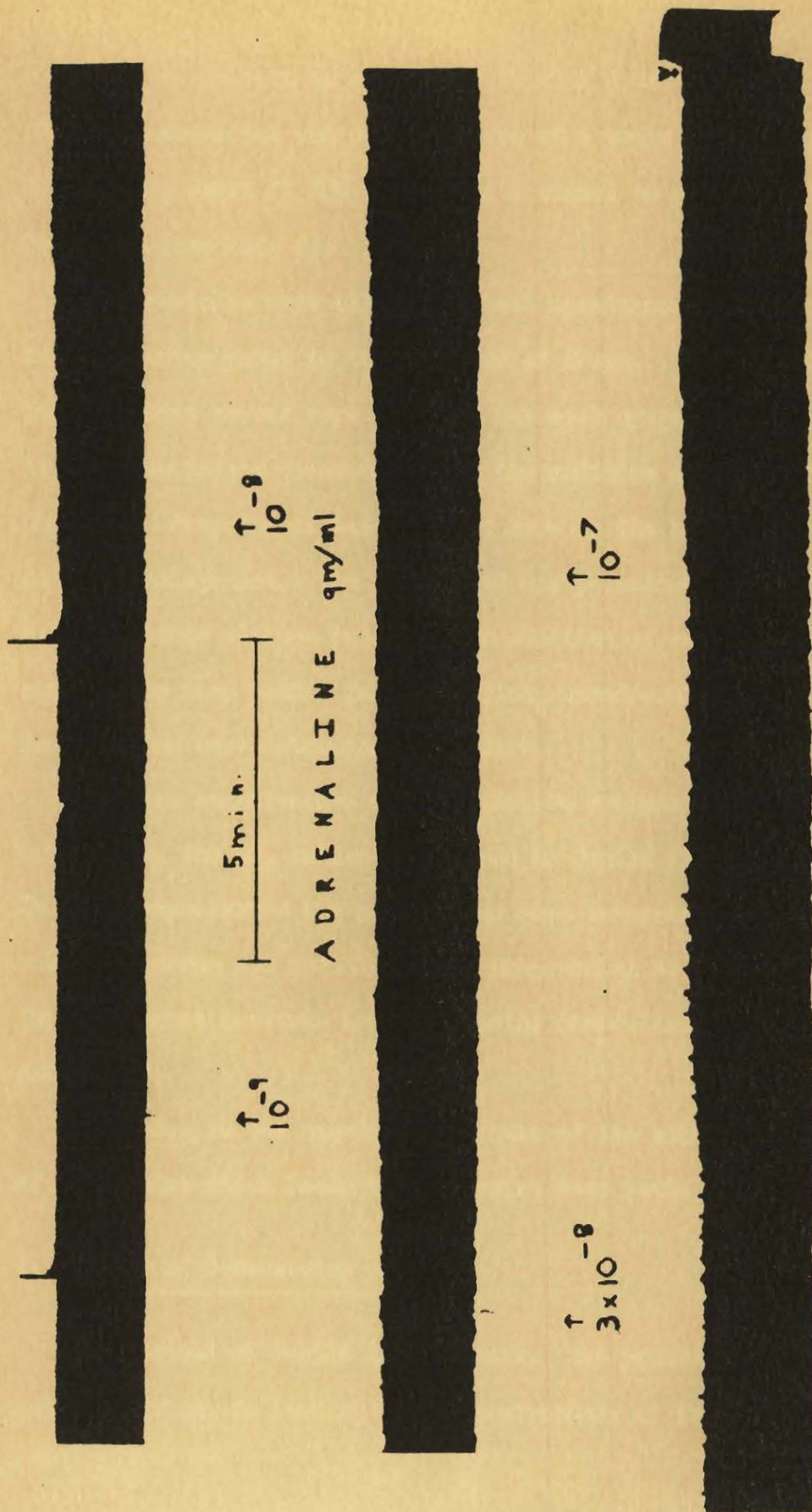


Fig. 8. Effect of increasing concentrations of
adrenaline on contractile force of the
isolated rabbit auricle.

Fig. 8.



supplied to the bath by means of a fritted glass aerator.

Isometric recording was done by means of a Grass Force Displacement Transducer, Model FT 03B, supplying a signal to a 4-Channel Gilson Polygraph (see Fig. 8).

The auricles were allowed to beat spontaneously in the bath for 30 minutes prior to the addition of drugs. Three complete dose-response curves were obtained for each sympathomimetic amine in question before proceeding with the addition of antagonist to the bath. Propranolol was employed here as the beta-adrenergic receptor antagonist. The antagonist was permitted to act in the bath for three minutes prior to the addition of any sympathomimetic amine. The dose ratios for adrenaline, noradrenaline and isoprenaline were determined as before.

For the quantitative and qualitative evaluation of drug antagonism, the pA_x values as defined by Schild (2) was used. In these experiments the pA_{10} values were determined for the rabbit duodenum and auricles using propranolol as antagonist and for the rabbit duodenum and aorta using phentolamine as antagonist.

The pA_{10} is the negative logarithm of the molar concentration of antagonist which leads to an agonist dose ratio of 10.

4. Method of Determining pA_{10}

The method of Schild (2) was used. The principle of the method consists in finding two concentrations of the antagonistic

drug such that one will reduce the effect of ten times the dose of active drug to less and the other to more than the effect of a single dose. The logarithm of the dose ratio of the given agonist-antagonist pair, minus one, is plotted against the negative logarithm of the particular concentration of antagonist used. The concentration corresponding to pA_{10} is then computed by interpolation on the logarithmic scale. For examples, see Figures 9 and 10, and the section dealing with results, Table 4.

The standard errors for all pA_{10} values were determined in the following way:

- (a) The standard errors were affixed to points representing the dose ratios in Figures 9 and 10, taking into account the logarithmic scale.
- (b) The maximum and minimum extremities of the vertical bars representing the standard errors for the dose ratios (points) of a particular agonist-antagonist pair, were then joined by straight lines. These lines cut the pA_{10} axis on both sides of the absolute pA_{10} value (see Figures 9 and 10, Table II) and the standard error was determined by taking the difference between these two pA_{10} values thus obtained in dividing that difference by two. For example, the two pA_{10} values used for this determination for adrenaline-phentolamine, are respectively 5.83, 5.43. The standard error is then equal to $\frac{.40}{2} = 0.20$.

Fig. 9. Relation between the negative logarithm of the molar concentration of propranolol (pA_x) and the logarithm of the dose-ratio of isoprenaline, noradrenaline, and adrenaline -1.

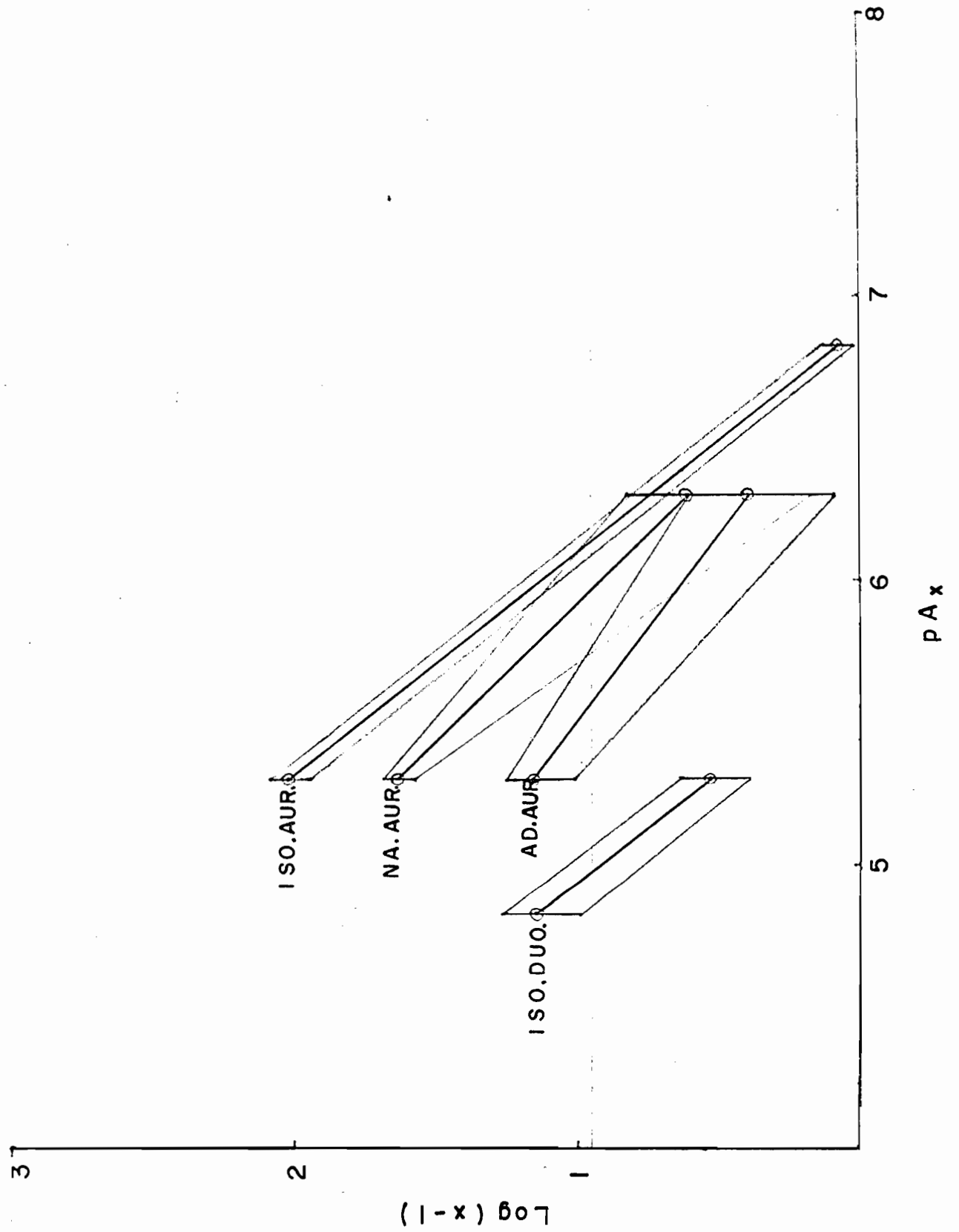
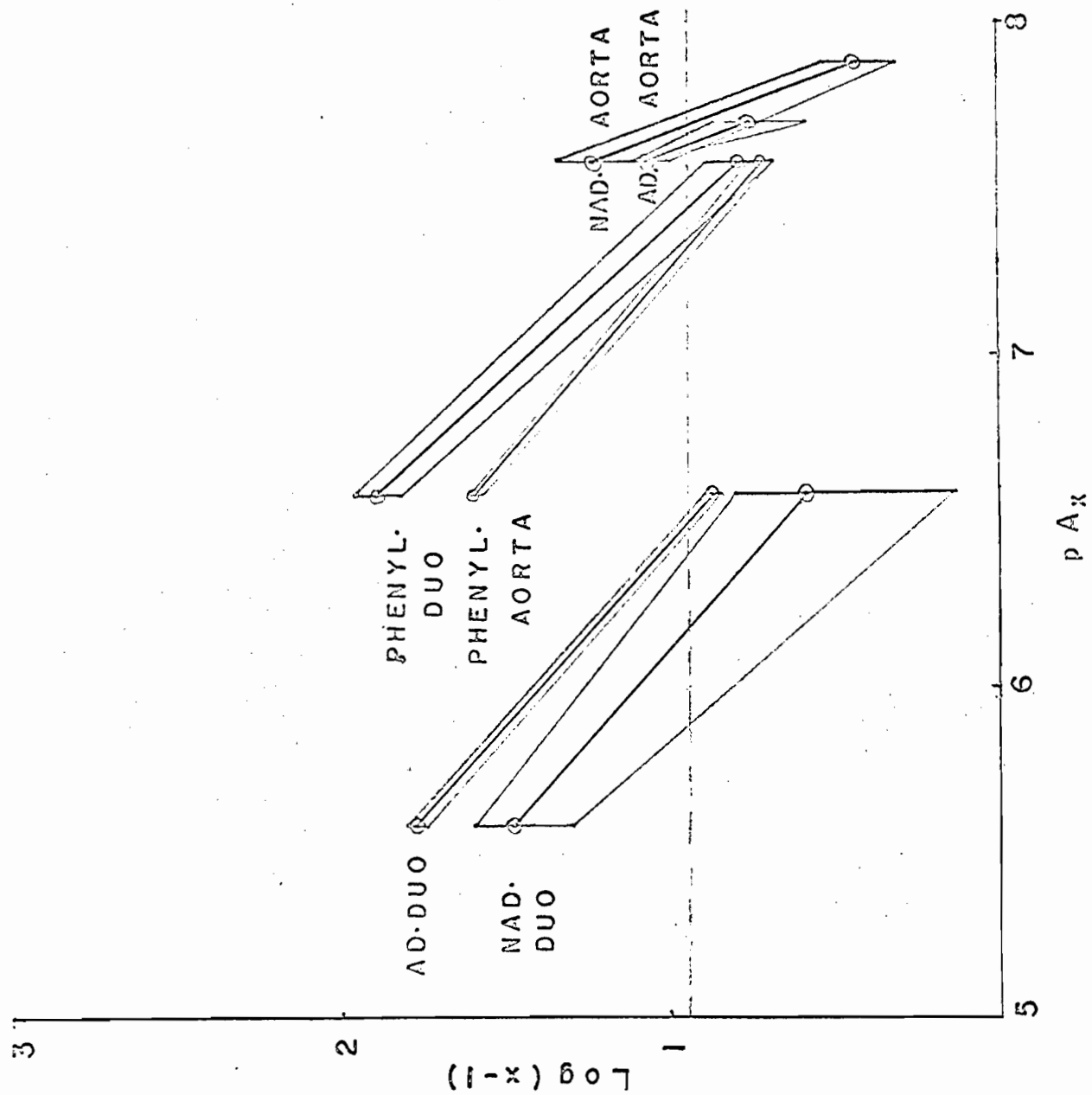


Fig. 10. Relation between the negative logarithm of the molar concentration of phentolamine (pA_x) and the logarithm of the dose-ratio of adrenaline, noradrenaline, and phenylephrine -1.



The standard test for the significance of the difference between two means was used to test for significant differences between values reported in Tables 3 and 4. A value of $P = 0.05$ was used as a rejection value for the null hypothesis.

4. Drugs

McEwen's solution (68) was used throughout this entire work as the physiological medium. This solution has the following composition per liter:

Sodium chloride	7.6 g.
Potassium chloride	0.42 g.
Calcium chloride	0.24 g.
Sodium bicarbonate	2.1 g.
Sodium diphosphate monohydrate	0.164 g.
Glucose	2 g.
Sucrose	4.5 g.

The following drugs were used: phentolamine methyl sulphonate and propranolol hydrochloride as antagonists, l-epinephrine bitartrate, levarterenol bitartrate, isoproterenol bitartrate dihydrate, and phenylephrine hydrochloride as agonists. Stock solutions (10ug/ml) of these drugs were made by dissolving their salts in distilled water. These solutions were stored in the frozen state for periods not longer than five days before use.

RESULTS

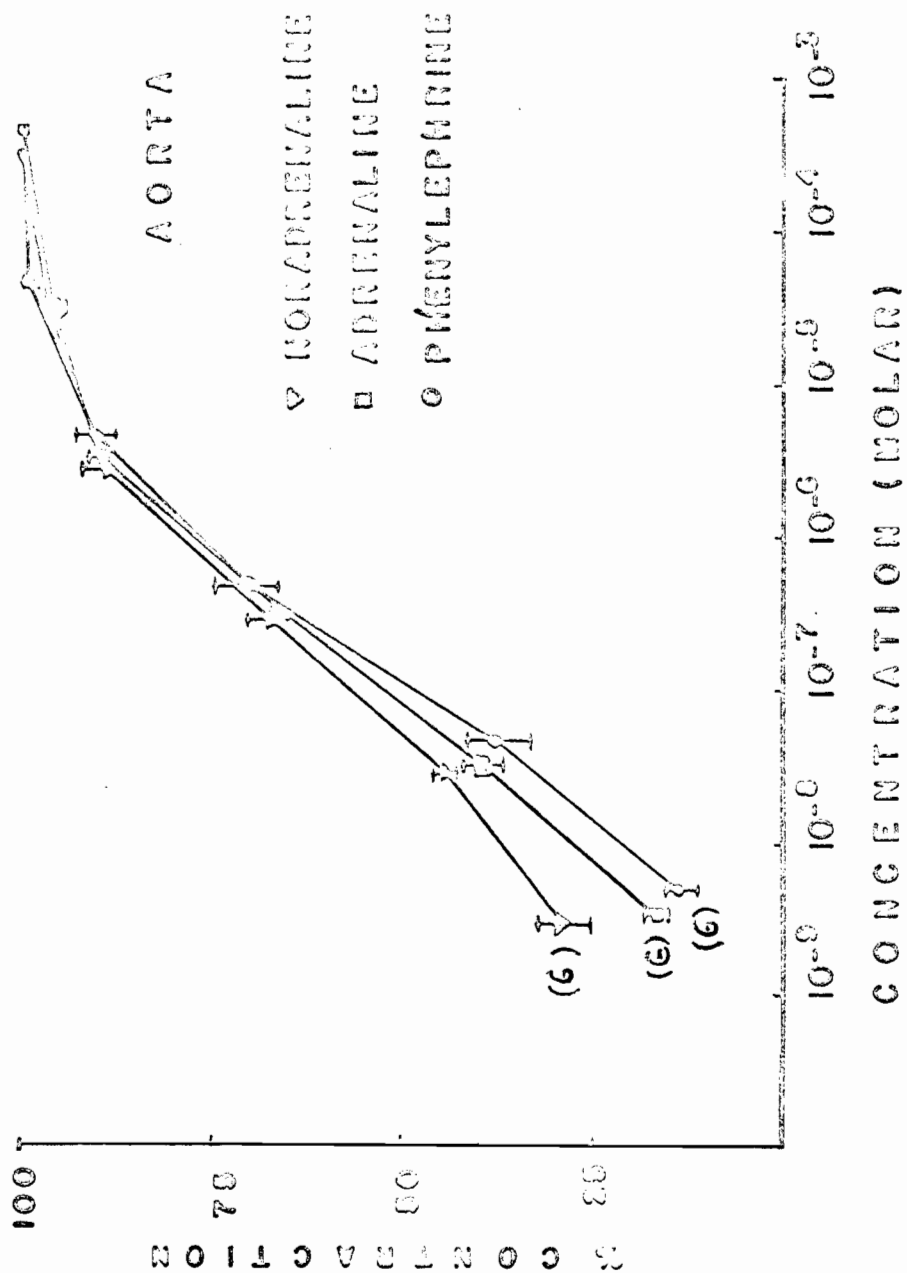
I. POTENCY OF SYMPATHOMIMETIC DRUGS

1. Aorta

Figure 11 and Table 3 illustrate the potencies of adrenaline, noradrenaline, and phenylephrine. From Table 3 it can be seen that noradrenaline is a more potent constrictor agent than phenylephrine. There is no statistically significant difference between the values reported in Table 3 for noradrenaline and adrenaline or between adrenaline and phenylephrine. There is a significant difference between the values reported for noradrenaline and phenylephrine ($P < 0.05$). Thus, adrenaline and noradrenaline are about equipotent as regards constrictor effects.

It should be noted that while Figure 11 represents the mean dose-response curves obtained with the three sympathomimetic amines, the values reported in Table 3 were determined from individual dose-response curves (6 experiments). Hence, Figure 11 (and likewise Figure 12 and Figure 13) merely serve to illustrate the type of experiments used to obtain the data presented in Table 3, and do not actually represent that data.

Fig. 11. Dose-response curves for adrenaline, noradrenaline, and phenylephrine on the isolated rabbit aorta. The number of experiments is shown in parenthesis and the vertical bars represent standard errors.



2. Auricles

Figure 12 illustrates the potencies of isoprenaline, noradrenaline, and adrenaline in producing positive inotropic changes. Isoprenaline is considerably more potent in this respect than the other two sympathomimetic amines tested. Adrenaline appears to be somewhat more potent than noradrenaline in producing inotropic changes (See Table 3, Figure 12). The differences between the values shown in Table 3 for adrenaline and noradrenaline ($P < 0.01$) and isoprenaline and adrenaline ($P < 0.01$) are statistically significant. Thus the order of potencies of these three sympathomimetic amines on the auricles can be stated as $ISO > AD > NAD$.

3. Duodenum

Figure 13, illustrates the potencies of phenylephrine, noradrenaline and adrenaline and isoprenaline. The order of potency from Figure 13 is $ISO > NA > A > PHEN$ in producing inhibitory effects. However, it should be noted that there is no statistically significant difference between the values reported for adrenaline, noradrenaline, and isoprenaline on the duodenum in Table 3. Nevertheless, it is felt that if more experiments were carried out, more significance could be attached to the order of potency of

Fig. 12. Dose-response curves for isoprenaline, adrenaline, and noradrenaline on the isolated rabbit auricles. The number of experiments is shown in parenthesis and the vertical bars represent standard errors.

AURICLES

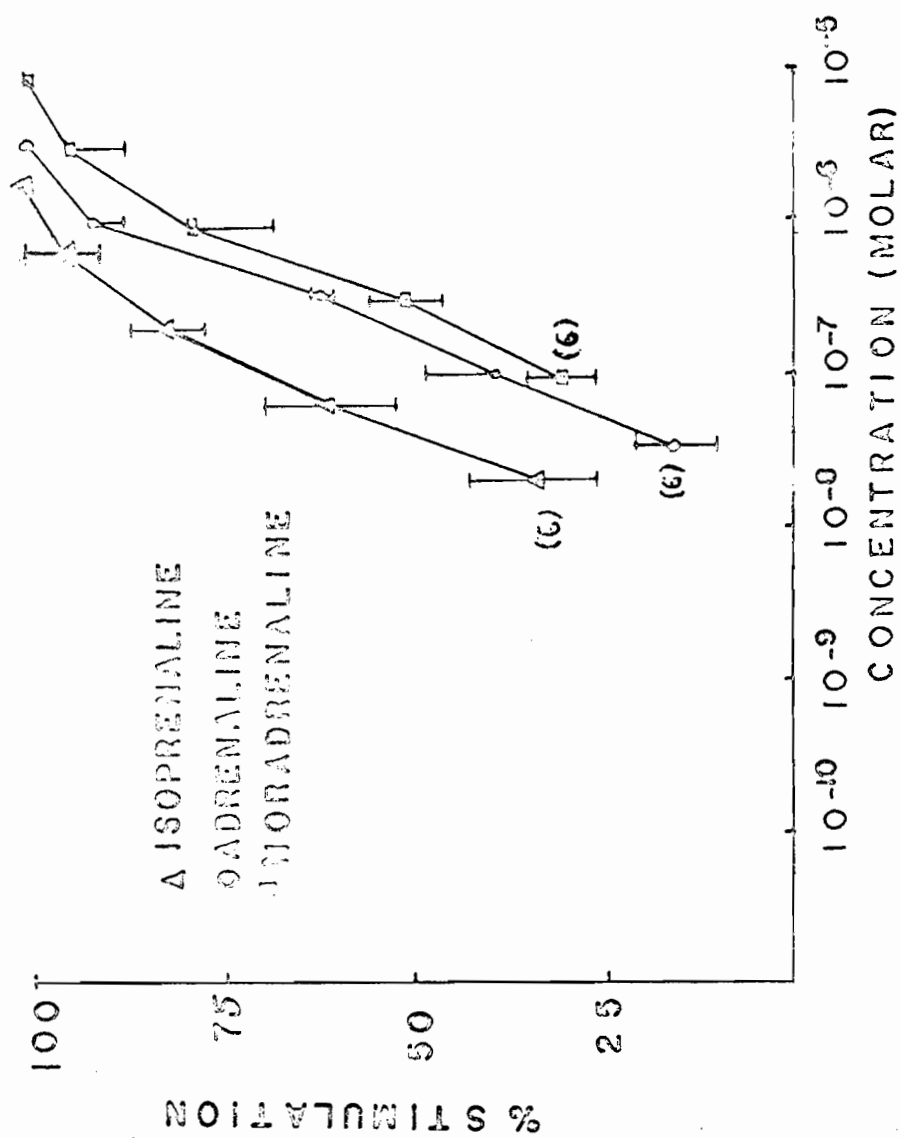


TABLE 3

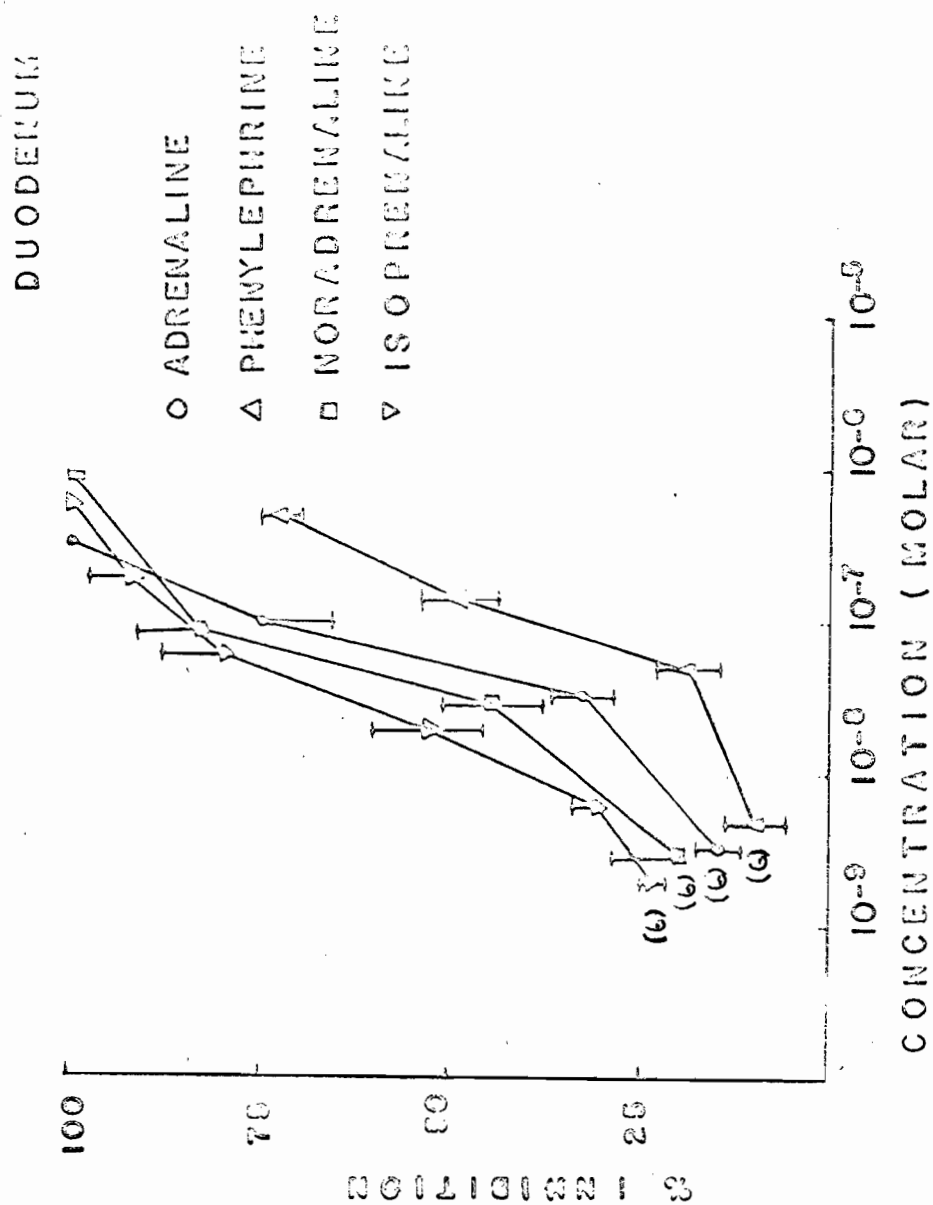
Table showing nanomoles (nM) of drug required to produce 50% of a maximum effect on the isolated rabbit aorta, duodenum, and auricles.

Drug	Test Organ	Effect	No. of Expts.	Dose of Drug to produce 50% effect (nM)
Adrenaline	Aorta	Stimulation	6	93.7 \pm 15
Noradrenaline	Aorta	"	6	59.5 \pm 7 ^x
Phenylephrine	Aorta	"	6	136 \pm 33 ^x
Adrenaline	Duodenum	Inhibition	6	47.8 \pm 5
Noradrenaline	Duodenum	"	6	42.1 \pm 13
Phenylephrine	Duodenum	"	6	176 \pm 26 ^{xx}
Isoprenaline	Duodenum	"	6	49.0 \pm 30 ^{xx}
Adrenaline	Auricles	Stimulation	6	188 \pm 27 ^{/xx}
Noradrenaline	Auricles	"	6	354 \pm 2 ^{xx}
Isoprenaline	Auricles	"	6	51.4 \pm 16 [/]

x, Difference statistically significant ($P < 0.05$).

/,xx Difference statistically significant ($P < 0.01$).

Fig. 13. Dose-response curves for adrenaline, noradrenaline, isoprenaline, and phenylephrine on the isolated rabbit duodenum. The number of experiments is shown in parenthesis and the vertical bars represent standard errors.



these sympathomimetic amines as proposed above. There is a significant difference between the values reported for isoprenaline and phenylephrine ($P < 0.02$), noradrenaline and phenylephrine ($P = 0.001$), adrenaline and phenylephrine ($P < 0.001$), in Table 3. Thus phenylephrine is the least potent of the sympathomimetic amines tested; isoprenaline may be the most potent, and the position of adrenaline and noradrenaline is somewhere between phenylephrine and isoprenaline.

II. POTENCY OF ADRENERGIC BLOCKING DRUGS

1. Effects of Phentolamine and Propranolol Alone

Figures 14 and 15 show the effects of the addition to the bath of phentolamine or propranolol on the isolated rabbit duodenum. The values employed to obtain the graphs illustrated in Figures 14 and 15 were obtained from segments of duodenum used in the actual determination of certain pA values reported herein.

Phentolamine in doses of 2.65×10^{-6} M or less, had no inhibitory effects on the amplitude of contraction of the duodenum. Above that concentration inhibitory effects were readily observed (see Figure 14).

Propranolol in doses of 5×10^{-6} M usually produced a slight inhibitory effect upon the duodenum (see Figure 15), but these inhibitory effects did not increase greatly as the concentration of propranolol was considerably increased. This was in distinct contrast to the effects of phentolamine. It should be noted that concentrations of 5×10^{-6} M propranolol were often employed when studying blockade on the

Fig. 14. Graph illustrating inhibition of contraction in the isolated rabbit duodenum after two (2) minute contact with phentolamine. The number of experiments is given in parenthesis and the vertical bars represent standard errors.

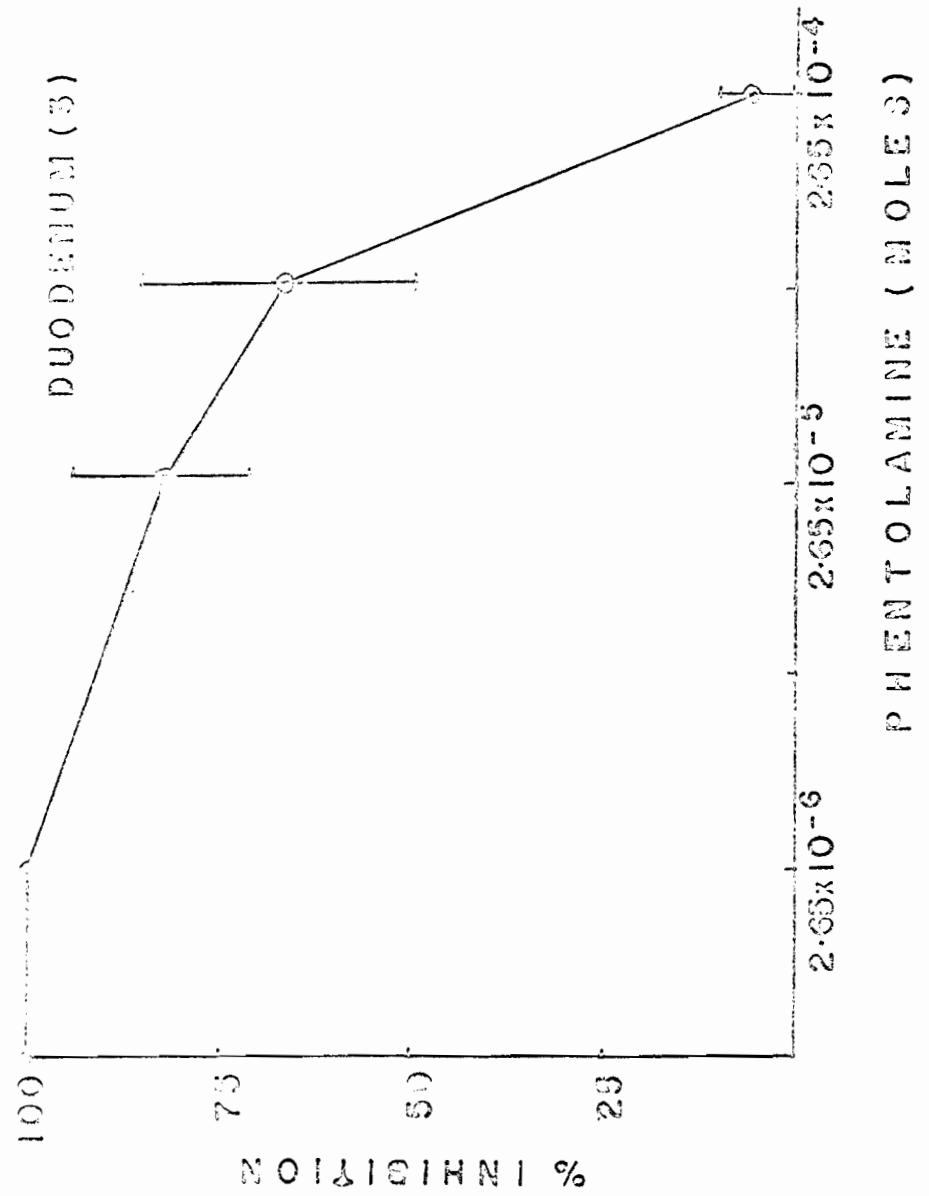
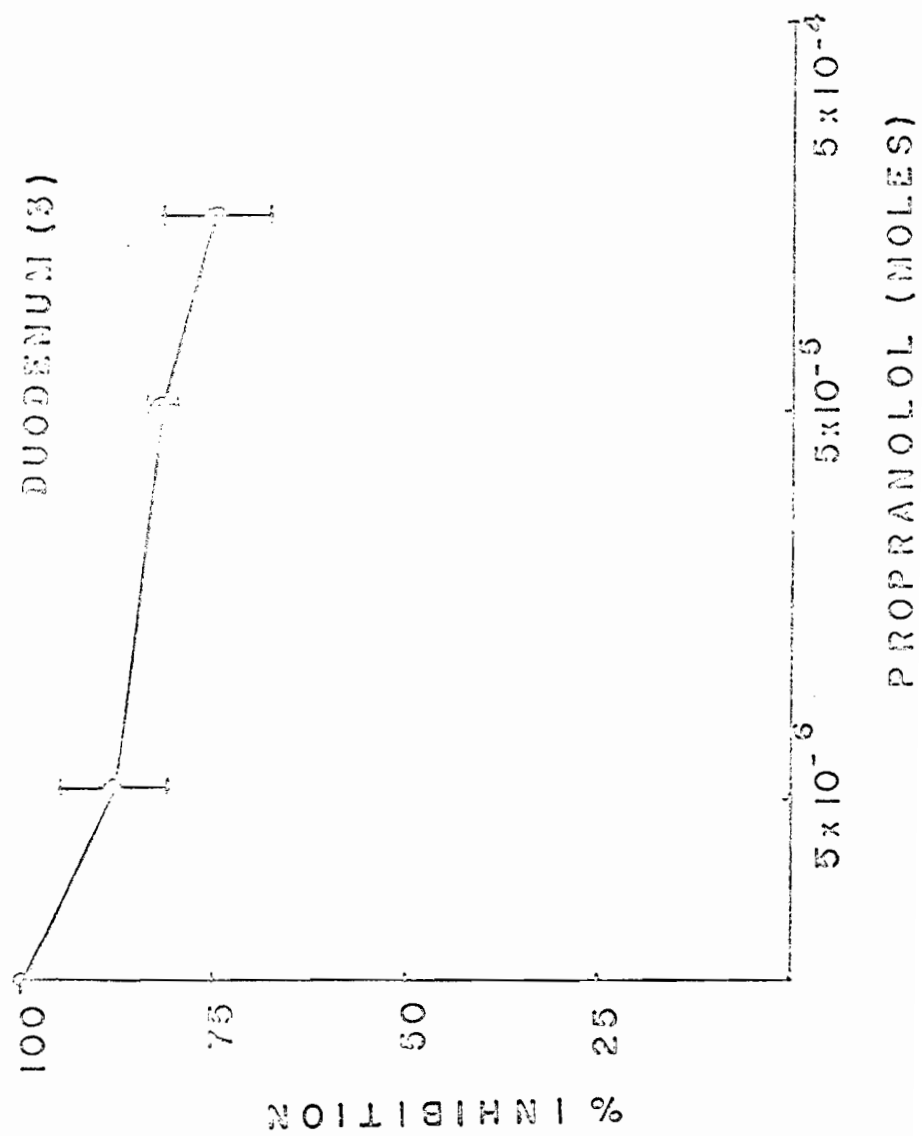


Fig. 15. Graph illustrating inhibition of contraction in the isolated rabbit duodenum after two (2) minute contact with propranolol. The number of experiments is given in parenthesis and the vertical bars represent standard errors.



duodenum and auricles. To achieve a blocking effect of greater than 10 on the duodenum, a concentration of 1.51×10^{-5} M propranolol had to be used. As can be seen from the graph in Figure 15, the addition of that concentration of antagonist to the bath often resulted in the production of considerable inhibitory effects. In this latter case, only the experiments where little or no inhibition of the amplitude of contraction occurred after the addition of the antagonist are reported herein.

After the addition of phentolamine to the isolated rabbit aorta preparation, and the addition of propranolol to the isolated auricles, a mild stimulant effect was often observed. The aorta usually contracted about 5 mm but relaxed to its original length within 15 minutes. The auricles often showed a small increase in the force of contraction of about 30 seconds duration followed by a return to the level of contraction existing before the addition of the antagonist.

In the study to follow, the time of contact with the antagonists was different for the various preparations employed. The time of contact chosen for a particular preparation represents the time it took for any stimulant

or inhibitory effects produced by the addition of the antagonist to the bath to have subsided and a stable base line to be established. The addition of agonists was begun immediately thereafter. For the duodenum this period of time was two minutes, for the auricles three minutes, and fifteen minutes in the case of the aorta.

2. Blocking Effects of Phentolamine on the Aorta and Duodenum

The constrictor action of phenylephrine, noradrenaline, and adrenaline was competitively blocked on the isolated rabbit aorta by small concentrations of phentolamine (see Figure 16). The pA_{10} values obtained from two different concentrations of this antagonist with adrenaline, noradrenaline, and phenylephrine are given in Table 4.

Concentrations of 2.65×10^{-6} M phentolamine were needed to produce a greater than ten fold competitive blockade against phenylephrine, noradrenaline, or adrenaline on the isolated rabbit duodenum (see Figure 18). It was observed that phentolamine in concentrations up to 2.65×10^{-4} M exhibited no blocking action against the inhibitory effects of isoprenaline on the duodenum. The pA_{10} values for the duodenum obtained from two concentrations of phentolamine with various sympathomimetic amines are shown in Table 4.

Fig. 16. Dose-response curves for noradrenaline with different concentrations of phentolamine on the isolated rabbit aorta. The vertical bars represent standard errors and the number of experiments is given in parenthesis.

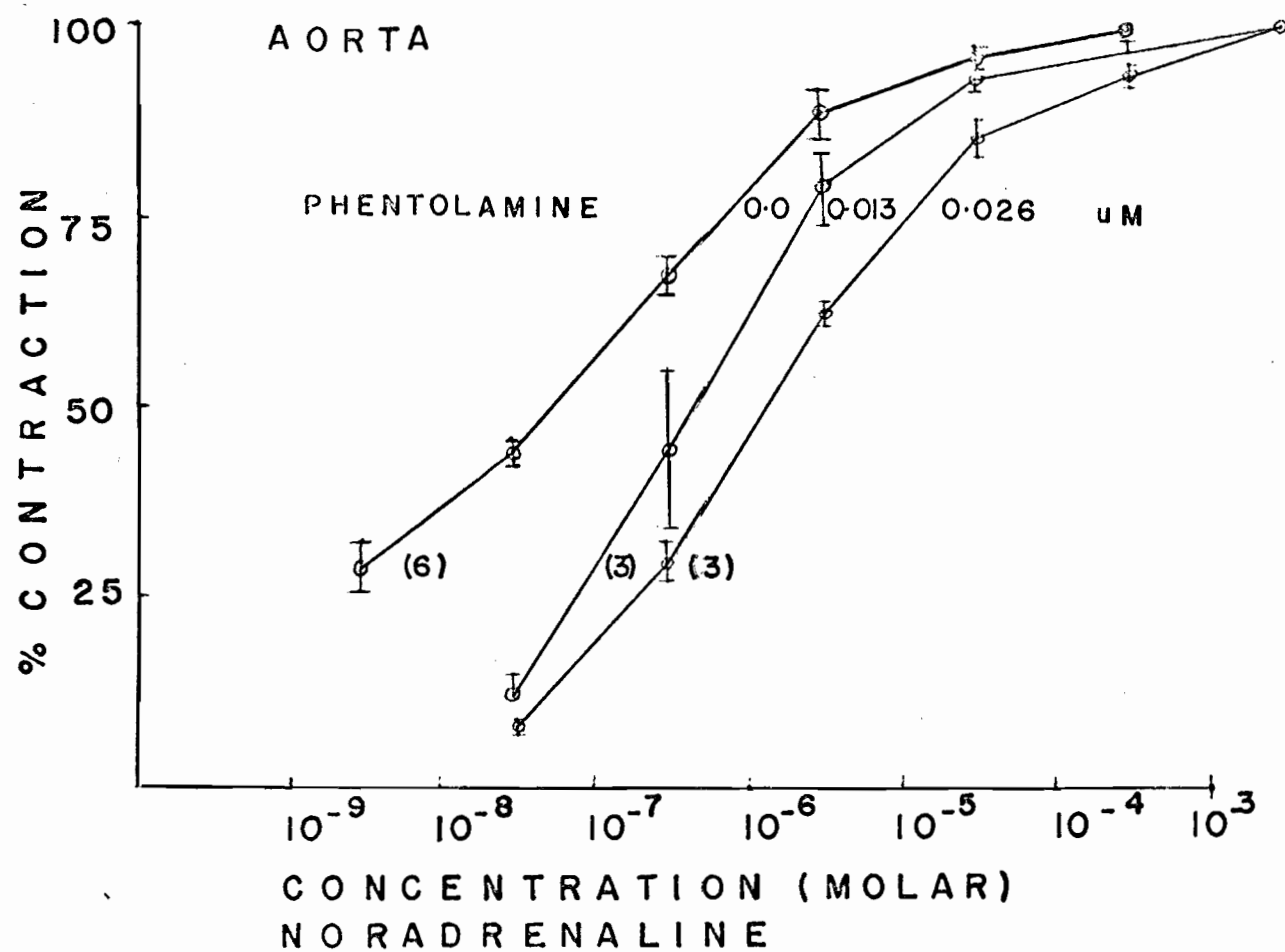


TABLE 4

This table summarizes the pA_{10} values obtained from figures 9 and 10 for different organs and different antagonists on the various isolated rabbit tissues.

Drug	Organ	No. of Expts.	Antagonist	Time of Contact	pA_{10}
Adrenaline	Auricles	3	Propranolol	3 min.	5.63 ± 0.20
Noradrenaline	Auricles	3	"	3 min.	5.97 ± 0.20
Isoprenaline	Auricles	3	"	3 min.	6.14 ± 0.05
Isoprenaline	Duodenum	3	Propranolol	2 min.	4.97 ± 0.10
Adrenaline	Duodenum	3	Phentolamine	2 min.	6.49 ± 0.03
Noradrenaline	Duodenum	3	"	2 min.	6.17 ± 0.25
Phenylephrine	Duodenum	3	"	2 min.	7.43 ± 0.08
Adrenaline	Aorta	3	Phentolamine	15 min.	7.63 ± 0.25
Noradrenaline	Aorta	3	"	15 min.	7.69 ± 0.06
Phenylephrine	Aorta	3	"	15 min.	7.31 ± 0.04

3. Blocking Effects of Propranolol

Large concentrations of propranolol (see Figure 19) were needed to produce effective competitive blockade in the duodenum against isoprenaline. It was observed that propranolol in concentrations up to 5×10^{-4} M exhibited no blocking action against adrenaline, noradrenaline, or phenylephrine on the rabbit duodenum. The pA_{10} value obtained from two different concentrations of propranolol with isoprenaline on the duodenum is shown in Table 4.

Propranolol was a much more effective blocking drug on the isolated rabbit auricles (see Figure 17) than on the duodenum (Figure 19). Lower concentrations of the antagonist were used. Propranolol had no blocking action against the effects of phenylephrine. The pA_{10} values obtained from two concentrations of propranolol on the rabbit auricles are given in Table 4.

4. pA Values

Figures 9 and 10 correlate the molar concentration of phentolamine and propranolol with the dose ratios of phenylephrine, noradrenaline, and adrenaline in the case of phentolamine, and isoprenaline, noradrenaline and adrenaline in the case of propranolol. This plot was used to obtain the pA_{10} values given in Table 4.

Fig. 17. Dose-response curves for adrenaline with different concentrations of propranolol on the isolated rabbit auricles. The vertical bars represent standard errors and the number of experiments is given in parenthesis.

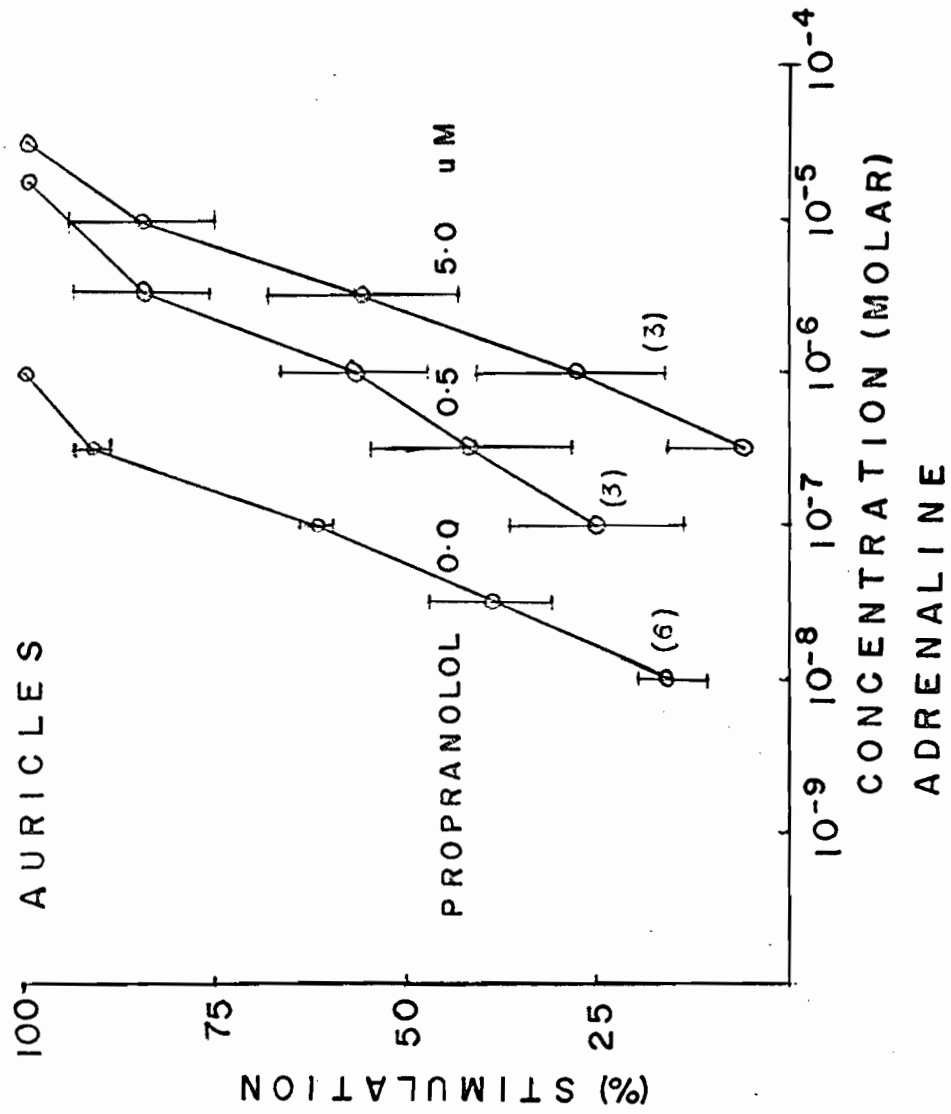


Fig. 18. Dose-response curves for noradrenaline with different concentrations of phentolamine on the isolated rabbit duodenum. The vertical bars represent standard errors and the number of experiments is given in parenthesis.

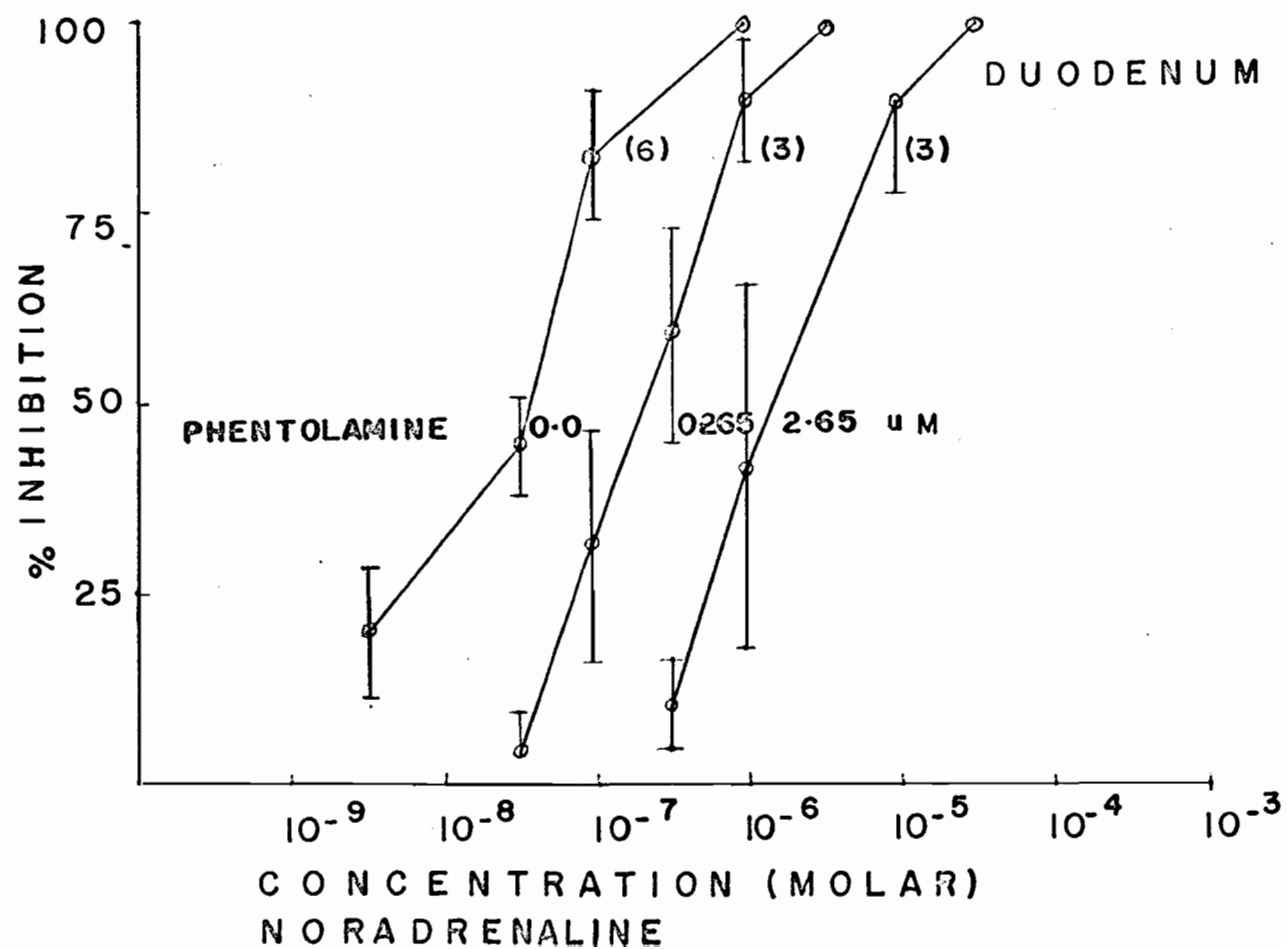
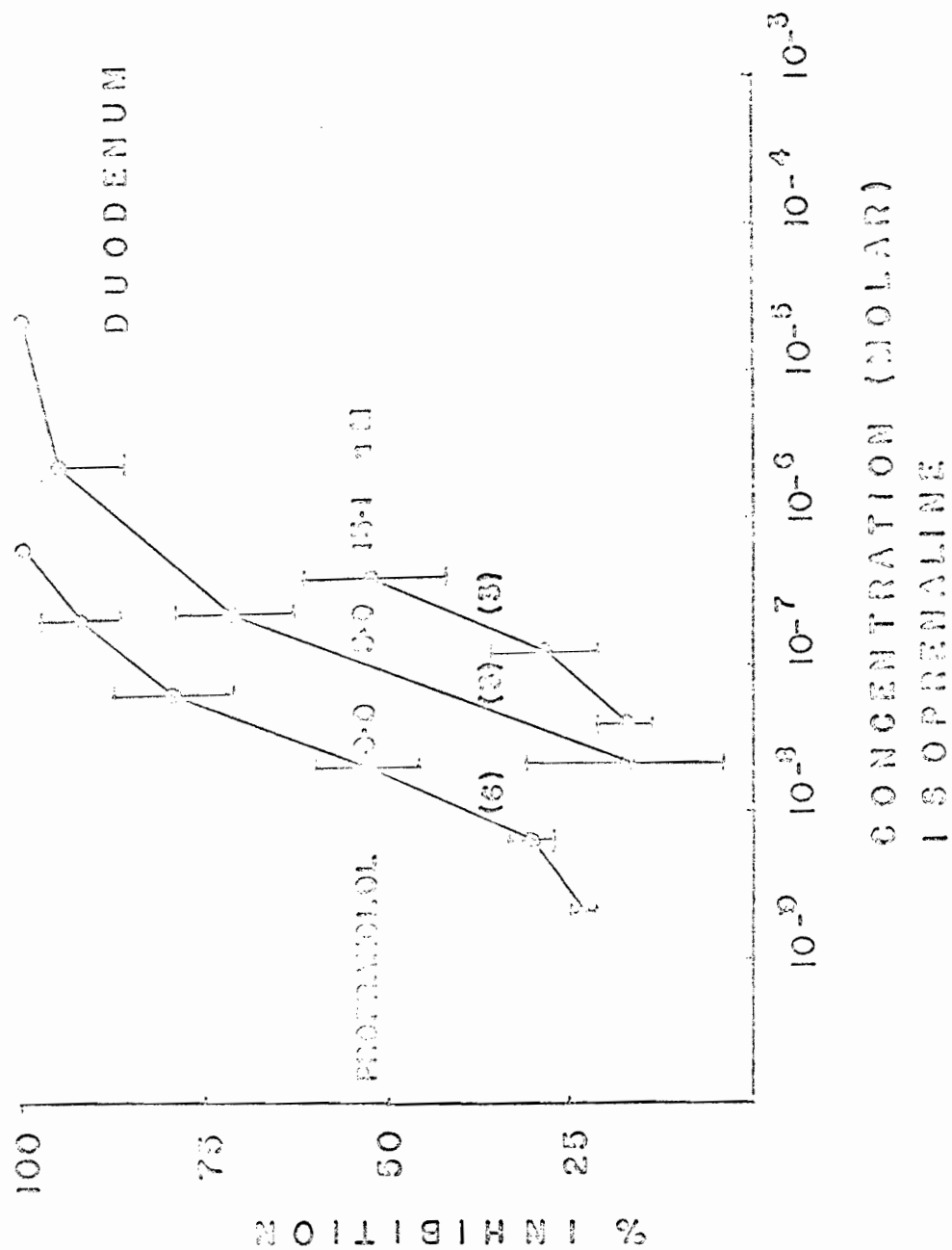


Fig. 19. Dose-response curves for isoprenaline with different concentrations of propranolol on the isolated rabbit duodenum. The vertical bars represent standard errors and the number of experiments is given in parenthesis.



The pA_{10} values in Table 4 were analyzed by means of Student's Test for the significance of the difference between two means. It was found that for propranolol on the isolated rabbit auricles, the pA_{10} values with isoprenaline ($P < 0.001$), adrenaline ($P < 0.05$), and noradrenaline ($P < 0.02$), were significantly different from the pA_{10} value on the isolated rabbit duodenum. The pA_{10} values on the auricles for isoprenaline, adrenaline, and noradrenaline with propranolol were not significantly different from one another.

For adrenaline and noradrenaline with phentolamine on the duodenum, the pA_{10} values were found to be significantly different from the values for adrenaline ($P < 0.001$) and noradrenaline ($P < 0.01$) with phentolamine on the isolated aortic strip. The pA_{10} values obtained for phenylephrine with phentolamine on the duodenum and the aorta were not significantly different ($P > 0.5$).

DISCUSSION

Sympathomimetic Drugs

The order of potencies of the sympathomimetic amines on the isolated rabbit duodenum was similar to that reported by Furchgott (16) using the same preparation and similar experimental conditions. He showed that the relative order of potencies of the sympathomimetic amines was approximately $ISO > NA > A > PE$. The results of the present work as well as of Furchgott's are at variance with those reported by Gaddum (58) and West (57) for the isolated rabbit ileum. They reported that l-adrenaline was approximately twice as potent as l-noradrenaline in bringing about relaxation of that preparation. Different parts of the intestine are known to vary in their sensitivity to catecholamines and this may account for the difference in the results reported herein and the results of Gaddum and West.

Noradrenaline appears to be a more potent constrictor agent on the aorta than either adrenaline or phenylephrine. According to Furchgott (55), individual fibres of rabbit aortic muscle contain both relaxation (beta) and contraction (alpha) receptors which can react with sympathomimetic drugs. Adrenaline has a strong affinity for both types of receptors,

but at all effective concentrations, its contractile effect exceeds and hence masks its relaxing effect. Thus, if this relaxing effect of adrenaline is taken into account, then adrenaline and noradrenaline may be regarded about equipotent as constrictor agents. Helmar (69) has also stated, in his procedure for biological assays employing the isolated rabbit aortic strip, that adrenaline and noradrenaline exert equal constrictor effects upon that preparation. The results herein reported support this observation, as no statistically significant difference was found between the doses of adrenaline or noradrenaline required to produce 50% of maximum contraction of the aortic strip. Phenylephrine was the least potent constrictor agent on the aorta.

Lands (65) recently observed isoprenaline to be approximately ten times more potent than adrenaline and about fifteen times more potent than noradrenaline in producing positive inotropic effects in the perfused rabbit heart. In the present study, isoprenaline was approximately three times more potent than adrenaline and seven times more potent than noradrenaline in bringing about an increase in the force of contraction of the isolated rabbit auricles preparation (see Table 3, Figure 12). Thus, the relative order of potencies of the three agonists on the auricles was the same as the order of potencies reported by Lands, even though the

potency ratios are different. This difference in potency ratios (i.e. 1:3:7 for the auricles versus 1:10:15 for the perfused heart) could be explained by the fact that different tissues and different preparations may vary in their sensitivity to catecholamines.

Adrenergic Blocking Drugs

The relative high concentrations of propranolol used in this study never caused marked inhibition of contraction of the duodenum as reported by Vanov (70) for the beta blocking agent pronethalol. He observed in experiments on the isolated rabbit duodenum and ileum that pronethalol in concentrations of 10^{-6} M to 5×10^{-6} M only partially blocked the relaxation induced by adrenaline, noradrenaline, or isoprenaline. Concentrations higher than 5×10^{-6} M caused depression of tone and reduced the frequency of the rhythmic movements of the intestine. It was observed in the present study that phentolamine, on a molar basis, can produce more inhibition of contraction than propranolol in the isolated rabbit duodenum preparation (see Figures 14, 15). These facts suggest that propranolol is a more suitable agent for blocking the actions of catecholamines on the duodenum than is pronethalol.

If the adrenaline receptors in the auricles, duodenum, and aortic strip were different, one might expect the antagonists to have different affinities towards these receptors and to interact differently with them. It was observed that a significantly different concentration of propranolol was needed to produce a ten fold blockade against the action of isoprenaline on the duodenum than was necessary to produce the same degree of blockade against the action of adrenaline, noradrenaline, or isoprenaline on the auricles. Likewise, significantly different concentrations of phentolamine were required to produce a ten fold blockade against the action of adrenaline and noradrenaline on the duodenum than on the aortic strips. Similar concentrations of phentolamine produced a ten fold blockade against the action of phenylephrine on the duodenum and on the aortic strip (see Table 4).

The fact that the pA_{10} values for isoprenaline with propranolol on the auricles and the duodenum are significantly different from one another indicates that the beta adrenergic receptor in the heart is different from the beta adrenergic receptor in the duodenum. There is also a significant difference between the pA_{10} values for adrenaline and noradrenaline on the auricles and isoprenaline on the duodenum

with propranolol; however, there is no significant difference between the pA_{10} values for isoprenaline, adrenaline, and noradrenaline on the auricles. Thus, this indicates that all three sympathomimetic amines act on the same receptor in the auricles as their pA_{10} values are similar. Hence, it can be stated that isoprenaline, adrenaline, and noradrenaline all mediate positive inotropic effects by means of a common beta receptor in the heart.

The pA_{10} values for adrenaline, noradrenaline, and phenylephrine with phentolamine on the duodenum and aorta seem to indicate that there are also differences among the motor or alpha adrenergic receptors. The alpha receptor for phenylephrine in the duodenum and the aorta are the same as the pA_{10} values are similar, but the alpha receptors for adrenaline and noradrenaline in the aorta and the duodenum appear to be different (see Table 4). According to Lands (65) the class of alpha adrenergic receptors may consist of receptors that differ in their sensitivity towards structural variations of the agonists or possibly the various antagonists as well. Earlier Belleau (72) had tentatively proposed that alpha and beta receptors may actually belong to the same structural entity that can display two or more different types of

catalytic properties and could hence respond in one way or another, depending on which site is actually attacked first by the agonist. In this latter case one would refer to the alpha or beta sites of adrenergic receptors.

SUMMARY

The purpose of this study was to ascertain whether the adrenaline alpha and beta receptors were the same in different tissues. The potency of various sympathomimetic drugs as well as the effective concentrations of alpha and beta adrenergic blocking agents were determined in the isolated rabbit auricles, duodenum, and aortic strip. The effective concentrations of the blocking drugs in different tissues were compared by means of pA_{10} values.

1. It was observed that the relative order of potencies of noradrenaline, adrenaline, isoprenaline, and phenylephrine in different tissues was as follows:

AORTA (Contraction) : NAD \gg AD $>$ PHEN

DUODENUM (Inhibition) : ISO $>$ NAD $>$ AD $>$ PHEN

AURICLES (Stimulation): ISO $>$ AD $>$ NAD

2. On the isolated rabbit auricles, adrenaline, noradrenaline and isoprenaline were antagonized by concentrations of propranolol which were not significantly different. This fact indicated that the receptor in the auricles was the same for adrenaline, noradrenaline, and isoprenaline.

3. The antagonism of isoprenaline on the rabbit duodenum required a larger concentration of propranolol than on the auricles. These concentrations of the antagonist were significantly different; therefore, it was suggested that the beta receptors in the duodenum and in the auricles were different.
4. Adrenaline and noradrenaline required significantly larger concentrations of phentolamine for antagonism in the duodenum as compared with the aortic strip. Thus, the alpha receptor in the duodenum may be different from the alpha receptor in the aorta.
5. On the isolated rabbit aortic strip, adrenaline, noradrenaline, and phenylephrine were antagonized by concentrations of phentolamine which were not significantly different. Hence, the alpha receptor in the aorta was probably the same for all three agonists. The antagonism of phenylephrine in the duodenum and the aorta also required similar concentrations of phentolamine. Although it may be assumed that phenylephrine acts directly on adrenaline receptors it behaved differently from adrenaline and noradrenaline in the isolated rabbit duodenum.

6. It was concluded that the adrenaline alpha and beta receptors in various tissues may be different.

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