THE CARDIAC EFFECTS OF COCAINE AND CERTAIN ANTIHISTAMINES AND ANTIDEPRESSANTS

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ABSTRACT

THE CARDIAC EFFECTS OF COCAINE AND CERTAIN ANTIHISTAMINES AND ANTIDEPRESSANTS

By

Ronald Stephen Davis

Cocaine and certain antihistamines and tricyclic antidepressants will potentiate the cardiovascular effects of norepinephrine. It is generally believed that at least part of the mechanism involved is a blockade of norepinephrine uptake. Recent evidence indicates that these drugs might have stimulating properties of their own as well and that this stimulation might be due to a release of norepinephrine from sympathetic nerve terminals. The present study was undertaken to further investigate this possibility.

Chlorpheniramine, brompheniramine, tripelennamine, triprolidine, desipramine, imipramine, cocaine and tyramine were found to produce marked positive inotropic effects in isolated, spontaneously beating guinea pig right atria. Promethazine, however, depressed the force of contraction. Propranolol was found to block the positive inotropic effects of these drugs. The response was also absent in atria from animals treated with reserpine but could be restored by incubating these atria with norepinephrine. The increase in the force of contraction produced by tyramine and cocaine may have been due partially to an increase in rate. This was not true of the antihistamines and antidepressants as these drugs were found to decrease the spontaneous atrial rate.

Chlorpheniramine, tripelennamine, triprolidine, desipramine, cocaine and tyramine caused an increase in the efflux of H^3-norepinephrine from isolated, perfused guinea pig hearts and from isolated right atria perfused while in a tissue bath. In the latter tissue, this increase was associated with a positive inotropic effect for all drugs tested except desipramine. No increase in the efflux of C^{14}-urea was observed when these drugs were administered to atria previously labeled with the radioisotope. Several of the drugs were tested for their ability to block H^3-norepinephrine uptake at the doses which produced the greatest increase in force. The order of effectiveness from greatest to least was found to be desipramine, triprolidine, tripelennamine and promethazine.

On the basis of these results, it was concluded that chlorpheniramine, tripelennamine, triprolidine and cocaine exert their positive inotropic effects via a tyramine-like mechanism, that is by releasing norepinephrine from sympathetic nerve endings. It is felt, however, that desipramine, at doses which are not depressant to the atria, is acting primarily by blocking the uptake of spontaneously released norepinephrine.

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By

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CHAPTER I

INTRODUCTION

Norepinephrine as the Adrenergic Transmitter

Work during the early 1900's led to the development of the currently held view that certain drugs are capable of releasing norepinephrine from sympathetic nerve endings in the periphery, thereby mimicking the effects of electrical stimulation. The turn of the century marked the beginnings of the study of adrenergic neuro-transmission. Norepinephrine was first synthesized by Stolz in 1904 but received little attention at the time. During this same period, Elliot (1905) and Dixon and Hamill (1909) proposed the concept of chemical transmission at the synapse. It was thought that epinephrine might be the transmitter at the junction of sympathetic nerve endings and their effector organs. However, while studying the effects of various sympathomimetic drugs, Barger and Dale (1910) observed that not all the actions of nerve stimulation were mimicked by epinephrine as would be expected if this were indeed the transmitter. Then, in 1921, Cannon and Uridil in this country and Loewi in Germany provided the first experimental confirmation of the hypothesis that epinephrine was the transmitter by showing similarities between the actions of sympathetic nerve stimulation and administration of epinephrine. It was subsequently demonstrated that extracts of adrenergic nerve fibers could produce many of the same effects as epinephrine both in mammals (Cannon and Lissák, 1939) and in amphibians (Loewi, 1936; Lissák, 1939).

That the adrenergic transmitter might be some substance other than epinephrine was not given much credence in view of the bulk of supportive evidence in favor of this amine. In retrospect, it can be seen how this misconception evolved. First, many of the effects of epinephrine and norepinephrine are quite similar, and early techniques might not have been able to distinguish between the two. For example, early assays lacked the sensitivity to detect the small amounts of norepinephrine present in rabbit adrenals (Euler, 1966). Species variation in the relative tissue content of the two amines resulted in a number of difficulties. In fact, epinephrine is now known to be the sympathetic transmitter in the frog heart (Euler, 1966). Thus, extrapolation of this data to mammals was most unfortunate.

With the exception of the early work of Barger and Dale (1910), significant challenges to the epinephrine hypothesis did not appear for many years. It was in 1933 that Cannon and Rosenblueth proposed their theory of two sympathins. Based on work which confirmed the findings of Barger and Dale (1910), they suggested that a common mediator was released from sympathetic nerve endings and combined with one of two substances at the receptor site. This combination resulted in the formation of either sympathin E (excitatory) or sympathin I (inhibitory). The following year, Bacq (1934) suggested that sympathin E might actually be norepinephrine while sympathin I could be epinephrine. However, Bacq (1935) later came to feel that sympathin E was instead a partially oxidized form of epinephrine.

Bacq's original view was supported by Stehle and Ellsworth (1937) who showed that the effects of sympathetic stimulation more closely resembled the actions of norepinephrine than they did epinephrine. Greer,

et al. (1938) later modified Cannon and Rosenblueth's hypothesis by eliminating the intermediate transmitter substances. They postulated the release of either of two transmitters from sympathetic nerve endings. The excitatory transmitter, they felt, might be norepinephrine.

The evidence implicating norepinephrine in the role of sympathetic transmitter was not well received due to the numerous experiments during this same period which tended to confirm the earlier hypothesis that epinephrine was in fact the transmitter. It was not until the late forties that Euler (1946a,b,c, 1948, 1950) demonstrated that norepinephrine was the predominant catecholamine in peripheral adrenergic nerves and tissues. By more effectively purifying extracts of tissues and adrenergic fibers, he was able to show that the active substance displayed properties more closely resembling those of norepinephrine than epinephrine. In addition, he found that the norepinephrine present in these preparations was the levo-isomer and that amount was closely related to the density of adrenergic fibers in the tissue or nerve. Since small quantities of epinephrine were also detected in the extract, Euler did not rule out the possibility first suggested by Greer, et al. (1938) that there might be two transmitter substances.

Bacq and Fischer (1947) confirmed Euler's findings that norepinephrine was present in nerves and tissues. This amine was also detected in the urine by Holtz, et al. (1947), in the liver by Gaddum and Goodwin (1947), and in the blood vessels of several species by Schmiterlöw (1948).

This work was soon followed by direct evidence that norepinephrine was liberated on stimulation of postganglionic sympathetic nerves. Release of the amine was first shown by Peart (1949) who stimulated the splenic nerve in the cat. Confirmation in other tissues and species was provided

by West (1950, Mann and West (1950, 1951), Outschoorn (1952) and Outschoorn and Vogt (1952). Through the work of these and later investigators, it has become generally accepted that norepinephrine is the only functionally important transmitter found in peripheral adrenergic terminals in mammals (Iversen, 1967).

Release of Norepinephrine by Sympathomimetic Amines

In their classical work in 1910, Barger and Dale showed that a number of amines structurally related to epinephrine and norepinephrine also possessed similar biological actions. The actions of two of these amines, tyramine and ephedrine, were later found to be antagonized by doses of cocaine which caused supersensitivity to the actions of epinephrine (Fröhlich and Loewi, 1910; Tainter and Chang, 1927; Tainter, 1929). Thus originated the term "cocaine paradox". Tyramine stimulates the heart as does epinephrine and cocaine lessens the action of tyramine, yet potentiates that of epinephrine. Similarly, it was noted by Burn and Tainter (1931) that when the nictitating membrane was denervated the actions of tyramine were abolished while those of epinephrine were greatly enhanced. In confirming these observations in the cat foreleg, Burn in 1932 suggested what now seems obvious--that these amines, rather than acting directly on the post synaptic receptor, might depend on the integrity of the adrenergic nerve ending for their effects.

Several investigators in the 1950's (Fleckenstein and Bass, 1953; Fleckenstein and Burn, 1953; Innes and Kosterlitz, 1954; Fleckenstein and Stöckle, 1955) studied the actions of a large number of sympathomimetic amines and concluded that they could be classified into three groups:

1) Direct-acting amines--those whose effects are potentiated by cocaine and denervation--e.g., epinephrine, norepinephrine

2) Mixed-acting amines--those which are only partially affected by either procedure--e.g., ephedrine

3) Indirect-acting amines--those which are definitely less effective after denervation or cocaine--e.g., tyramine

Later, reserpine was also used to distinguish between these three classes. Carlsson, et al. (1957) showed that tyramine activity was absent in the reserpine-pretreated cat. This was subsequently confirmed by Burn and Rand (1958, 1960) who also provided evidence for the possible mechanism when they demonstrated that the peripheral stores of norepinephrine were depleted in reserpine-treated animals and that infusion of norepinephrine temporarily restored the actions of indirect-acting amines. This resulted in their now generally accepted hypothesis that tyramine and other indirectly acting amines exert their sympathomimetic effect by releasing norepinephrine from adrenergic nerve terminals. The extensive substantiation of this work has been reviewed by Trendelenburg (1963) and Iversen (1967).

Evidence that infused tyramine could cause an increase in the amount of norepinephrine in the effluent or venous outflow of an organ was first provided by Stjärne in 1961. In the same year, Burn and Burn showed that tyramine could displace radioactively labeled norepinephrine in the isolated cat atria.

In order to displace norepinephrine from adrenergic neurons, it seems likely that sympathomimetic amines like tyramine must first gain access to the intraneural norepinephrine storage site. It was postulated by Furchgott, et al. (1963) that this process may be mediated by a

cocaine-sensitive transfer mechanism, perhaps the same as that responsible for norepinephrine uptake. This was suggested by the fact that agents that block norepinephrine uptake also prevent the release of norepinephrine by tyramine (Swaine, 1963). In addition, Trendelenburg (1962) has shown that tyramine can act as a potent inhibitor of norepinephrine uptake.

Through the work of Axelrod, et al. (1962a), it was found that C^{14}-tyramine is accumulated by the isolated rat heart. Since the tissue levels of tyramine were several times that of the normal endogenous norepinephrine content, only a fraction of which is available for release, it appeared that the uptake of tyramine was not accompanied by a stoichiometric release of norepinephrine. However, the hearts were removed and assayed immediately after infusion with C^{14}-tyramine. It is now felt that much of the tyramine initially taken up is non-specifically bound (Lee, et al., 1967; McNeill and Brody, 1968), and, therefore, the tyramine levels measured by Axelrod, et al. (1962a) may have little meaning in terms of elucidating norepinephrine release mechanism. In fact, Schümann and Wegmann (1960) and Schümann and Philippu (1962) have shown that the tyramine-induced release of norepinephrine from isolated adrenal medullary storage granules is accompanied by a stoichiometric uptake of tyramine into the granule. The spontaneous release of norepinephrine from such granules as well as the release produced by reserpine is associated with a release of ATP in the molar ratio of 4 NE/ATP. On the other hand tyramine, along with ephedrine, amphetamine, phenylethylamine and methamphetamine, produces a release of norepinephrine without an equivalent release of ATP. This suggests that these amines may be acting simply by taking the place of norepinephrine molecules in their storage sites.

Other Drugs Which Alter the Norepinephrine Transmission Process

Shown in Figure 1 is a schematic representation of an adrenergic nerve ending and its receptor organ. Direct-acting amines, like epinephrine and norepinephrine, exert their effects primarily by a direct action on the receptor, whereas indirect-acting amines, such as tyramine, presumably release norepinephrine from the nerve terminal.

While causing neither a direct nor an indirect stimulation of receptors, a number of compounds have been shown to potentiate the effects of norepinephrine by interfering with its deactivation or removal from the receptor site. Theoretically, this could be accomplished by inhibiting the enzymes which metabolize norepinephrine: catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO). However, it is now considered that the main method of inactivation of norepinephrine in the heart is by uptake of the amine and its subsequent storage in the adrenergic nerve endings.

The importance of uptake in terminating the actions of norepinephrine can explain why the very small amount of norepinephrine release by tyramine and other indirect-acting amines have such potent effects. Since most of these amines block norepinephrine uptake, they will potentiate the effects of any norepinephrine released by interfering with the prime mechanism of norepinephrine inactivation. Among the drugs which have been shown to block uptake are cocaine, tyramine, guanethidine, bretylium, phenoxybenzamine, phentolamine and the tricyclic antidepressants, imipramine and desipramine (Iversen, 1967).

Certain members of another group of compounds, the antihistamines, have been known for more than twenty years to potentiate the cardiovascular effects of epinephrine and norepinephrine (Parrot, 1943; Yonkman,

Figure 1. Possible sites of drug action at the adrenergic nerve ending.

Norepinephrine (NE) exerts its effects by interacting directly with the receptor. The primary means of inactivation is by uptake into the neuron, although monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) may participate to some degree. Drugs which alter these processes will modify the norepinephrine response. The response may be enhanced by drugs which block neuronal uptake of norepinephrine, or it may be mimicked by drugs which release the amine. Depleting norepinephrine by releasing it intraneuronally or by blocking its synthesis will deminish the response to sympathetic stimulation or norepinephrine-releasing drugs. The response may also be blocked by preventing the release of norepinephrine or by preventing interaction of the amine with the receptor.

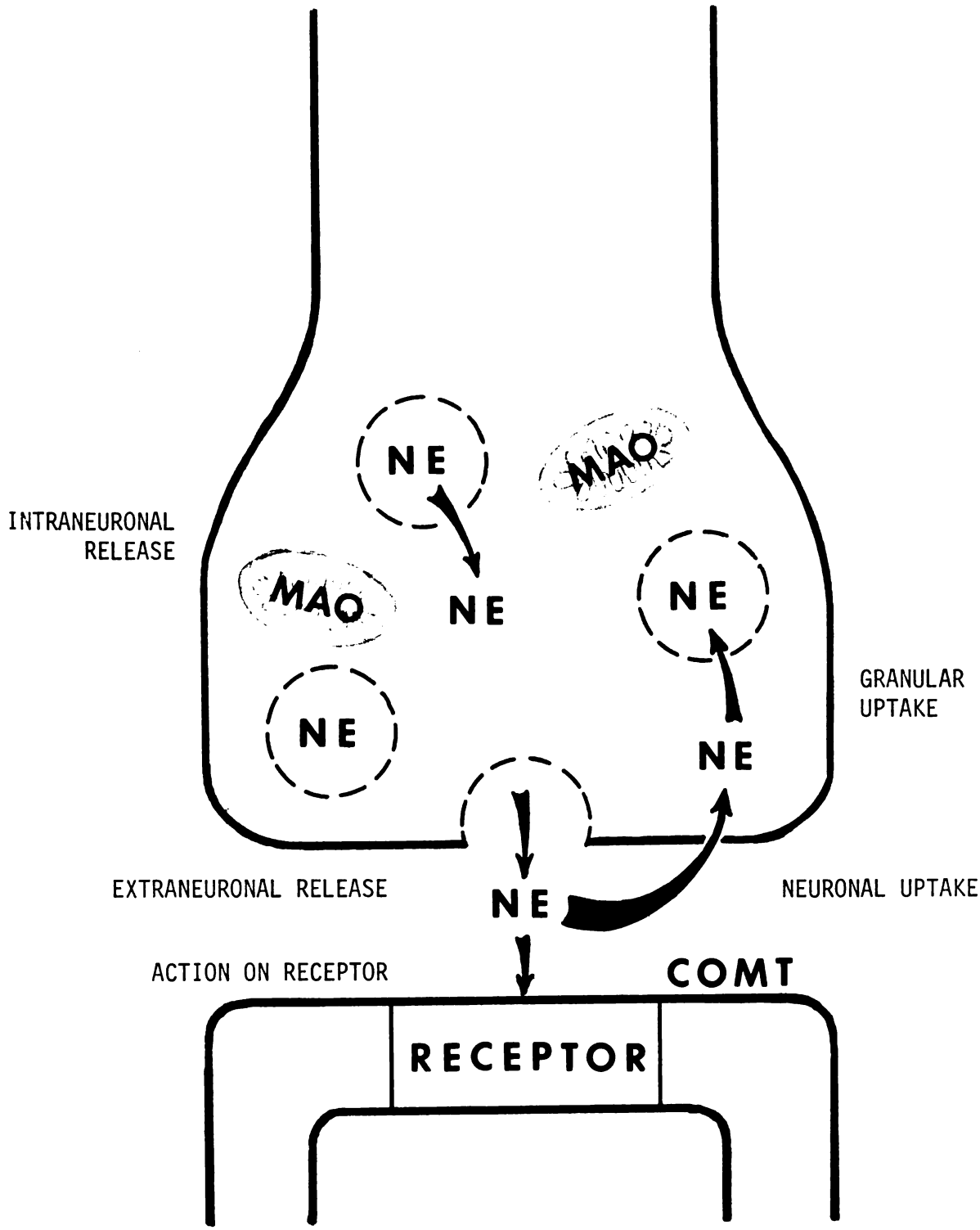

INTRANEURONAL
RELEASE
NE
MAO
MAO
NE
NE
NE
NE
GRANULAR
UPTAKE
EXTRANEURONAL RELEASE
NE
NEURONAL UPTAKE
ACTION ON RECEPTOR
COMT
RECEPTOR

Figure 1

et al., 1946; Sherrod, et al., 1947; Tislow, et al., 1949; Buchholz, et al., 1951; Paasonen, 1953; Kuriaki and Uchida, 1955; Paasonen, et al., 1956; Innes, 1958; Altura and Zweifach, 1965).

More recent work by several investigators indicates that the mechanism of this potentiation is by inhibition of norepinephrine uptake. Isaac and Goth (1965a,b, 1967) showed that tripelennamine, chlorpheniramine, diphenhydramine and phenindamine significantly prevented the uptake of H^3-norepinephrine in the isolated rat atria and the in vivo rat heart. The degree to which this occurred was approximately the same as that found for cocaine. With the exception of phenindamine, these drugs also potentiated the effects of norepinephrine on blood pressure and heart rate. Pyrilamine and promethazine appeared to have no effect either on H^3-norepinephrine uptake or the cardiovascular responses to norepinephrine. However, McNeill and Brody (1966) observed that promethazine could potentiate the norepinephrine-induced activation of rat cardiac phosphorylase though the degree was less than that obtained with chlorpheniramine, tripelennamine or phenindramine. They initially surmised that Isaac and Goth had failed to notice the activity of promethazine because assessing norepinephrine potentiation by measuring the effects of the drug on the rate response might be a less sensitive method than measuring phosphorylase activation. This hypothesis was later modified when promethazine was found to have no significant effect on either norepinephrine (McNeill and Brody, 1968) or tyramine uptake (McNeill and Brody, 1968; Commarato, et al., 1969a); it was suggested that the actions of promethazine might be specific for the enzyme system. A recent report (Muschek and McNeill, 1971) indicating that promethazine can inhibit phosphodiesterase lends support to this suggestion.

In an early report demonstrating the epinephrine-potentiating

properties of the antihistamines Buchholz, et al. (1951) noted that tripelennamine could abolish the bovine carotid constrictor response to tyramine. Later, investigators (Johnson, et al., 1965; Johnson and Kahn, 1966) reported that chlorpheniramine and tripelennamine inhibited the cardiovascular stimulating effects of tyramine and bretylium in open-chest dogs much the same as did cocaine, while triprolidine was without effect. Inhibition of the effects of tyramine and ephedrine by pyrilamine and chlorpheniramine have been observed in the isolated, spontaneously beating rabbit atria by Osterberg and Koppanyi (1969). They, along with Johnson and Kahn (1966) and later, McNeill and Brody (1966, 1968), supported the conclusions of Isaac and Goth, that those antihistamines which enhance the effects of norepinephrine do so by blocking the uptake of the amine. Such drugs will decrease the effect of tyramine by the same mechanism, that is, by blocking amine uptake (Commarato, et al., 1969a, b; McNeill and Commarato, 1969). Similar effects on amine uptake have also been reported for certain tricyclic antidepressant compounds: imipramine and its derivative, desipramine (Axelrod, et al., 1961; Dengler, et al., 1961; Hertting, et al., 1961; Axelrod, et al., 1962b; Titus and Spiegel, 1962; Carlsson, et al., 1963; Shore, et al., 1964; Brodie, et al., 1965; Iversen, 1965; Titus, et al., 1966; Brodie, et al., 1968; McNeill and Brody, 1968; Stjärne, et al., 1968; Commarato, et al., 1969a,b) and amitryptyline and its derivative, protryptyline (Carlsson and Waldeck, 1965; McNeill and Brody, 1968; Commarato, et al., 1969a; McNeill and Commarato, 1969).

Since most norepinephrine-releasing drugs, such as tyramine, are known to prevent norepinephrine uptake as well (Iversen, 1967), it is possible that chlorpheniramine and the other antihistamines which block

uptake may also cause the release of norepinephrine. It was shown by McNeill and Brody (1966) that chlorpheniramine in addition to potentiating the effects of norepinephrine on cardiac phosphorylase, caused a slight increase in the amounts of the active form of this enzyme present. Similar results were noted with tripelennamine (McNeill and Brody, 1969). These effects could have been due to a release of endogenous norepinephrine since the effect was blocked by propranolol. On the other hand, the effect could also have been due to blockade of uptake if sympathetic discharge was causing the release of norepinephrine.

None of the early investigators who studied the catecholamine-potentiating properties of the antihistamines reported any tyramine-like effects when the antihistamines were given alone. However, in pictures of tracings used by Buchholz, et al. (1951) to describe the inhibition of the tyramine response, it appeared that the antihistamine synopen, which structurally resembles chlorpheniramine, also caused dose-related contractions of bovine isolated carotid strips.

Other drugs which block uptake may also release norepinephrine. Furchgott, et al. (1963) and Cervoni, et al. (1966) have reported small to moderate positive inotropic responses to cocaine in the isolated guinea pig left atrium. However, the electrical stimulation used to drive the atria could have released norepinephrine. If so, its effect would have been potentiated by cocaine, due to the ability of this drug to block amine uptake. Recently, however, Trendelenburg (1968) reported positive chronotropic effects of cocaine in the isolated guinea pig right atrium due both to a release of endogenous norepinephrine and to an increase in sensitivity to this amine. Vohra (1969) confirmed the apparent ability of cocaine to release norepinephrine but stated that the amine

must have other actions as well.

The ability to release norepinephrine from sympathetic nerve endings in the heart has also been described for desipramine. The initial work was that of Titus, et al. (1966) who used rather high concentrations of the drug ($2.5\text{-}4.1 \times 10^{-4}$M) in the isolated rabbit heart. Nash, et al. (1968) have shown that 10^{-6}M desipramine caused an increase in the amount of radioactivity in the perfusate effluent of isolated rat hearts treated with H^3-norepinephrine, whereas cocaine did not produce such an increase. A recent more sophisticated study by Leitz and Stefano (1970) indicates that the norepinephrine released by desipramine in rat ventricle slices is primarily in the form of deaminated catechol compounds. This suggests that desipramine in some way causes the displacement of norepinephrine from the storage granules into the neuronal cytoplasm.

On the basis of the work which has been cited, it appeared that chlorpheniramine and tripelennamine might be exerting their effects on cardiac phosphorylase (McNeill and Brody, 1966, 1969) through a release of norepinephrine. The purpose of this study was to determine whether chlorpheniramine and other drugs known to block uptake could be causing such a release. The drugs studied were chlorpheniramine, brompheniramine, tripelennamine, triprolidine, promethazine and imipramine. As a means of comparison to previous work, l-norepinephrine, tyramine, cocaine and desipramine were also used.

CHAPTER II

METHODS

In these experiments, three different types of preparations were used: 1) a tissue bath for isolated atria, 2) a perfused whole heart system, and 3) a perfused tissue bath containing an isolated atria. Each of these will be described individually followed by a discussion of the methods used for preparation of the tissues, drugs and solutions.

Apparatus

A 180 milliliter (ml) tissue bath (Figure 2) was used for the initial experiments with isolated atria. Water was circulated through the outer jacket to maintain the temperature of solutions in the bath at 37°C. A mixture of oxygen (95%) and carbon dioxide (5%) was bubbled through the solutions by means of a fritted glass disk at the bottom of the bath. A small (7 mm x 15 mm) Palmer heart clip or a curved suture needle attached to an L-shaped glass support rod was connected to the apex of the atria. The other end of the tissue was affixed with a similar clip or needle which in turn was connected with a fine silk thread to a Grass force displacement transducer (Model FT03C). A Grass model 7 polygraph was used in these and all other experiments for recording contractions. Tension on the atria was adjusted by means of a Harvard isometric tension clamp.

The isolated heart system (Figure 3) was essentially that described by Fallen, et al. (1967). It consists of a modified Langendorff apparatus

Figure 2. Tissue bath for isolated atria.

A glass 180 ml tissue bath was used for determining the positive inotropic effects of drugs on isolated atria. Contractions were recorded by suspending the atria between one clip attached to the glass support rod and another attached to the force transducer. Tension was adjusted by an isometric tension clamp. Heated water circulated through the outer jacket of the bath was used to maintain solutions and atria at 37°C. Oxygenation was provided by passing a mixture of 95% oxygen and 5% carbon dioxide through a fritted glass disc at the base of the bath.

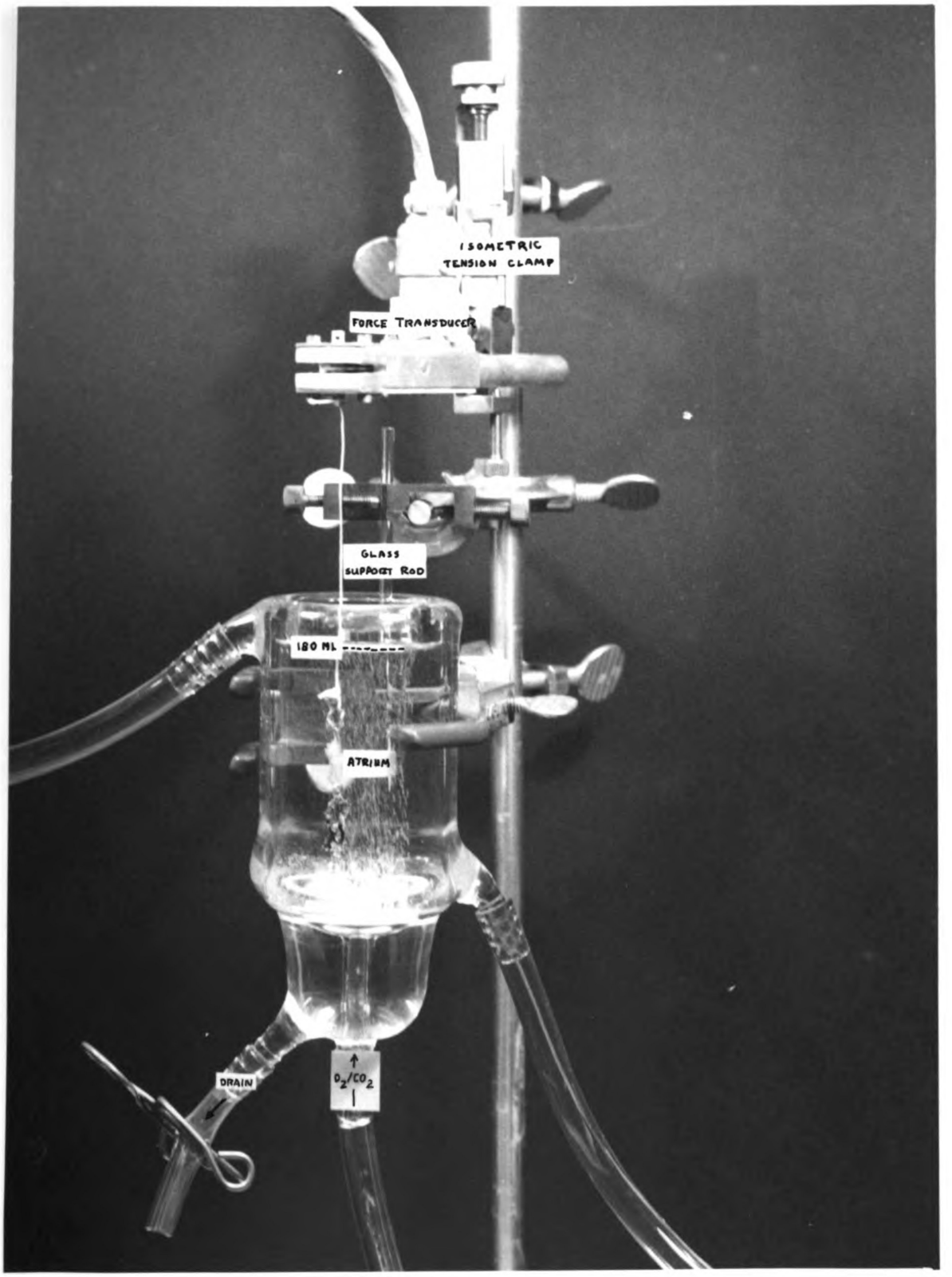

ISOMETRIC
TENSION CLAMP
FORCE TRANSDUCER
GLASS
SUPPORT ROD
180 ML
ATRIUM
DRAIN
O_2/CO_2

Figure 2

Figure 3. Modified Langendorff heart perfusion apparatus.

This system was used for isolated whole hearts. Oxygenated and heated solutions from one of the two reservoirs were pumped into the heart at a constant flow rate. A water jacket around the final length of tubing assured that solutions entering the heart would be at 37°C. A plexiglass chamber (shown with one side removed) also helped to maintain the temperature of the heart. A string attached to the apex of the ventricles was connected to the force transducer via a pair of pulleys. In this way, contractions of the heart were recorded. Tension was adjusted with an isometric tension clamp. A funnel beneath the heart was used for collecting the perfusate effluent.

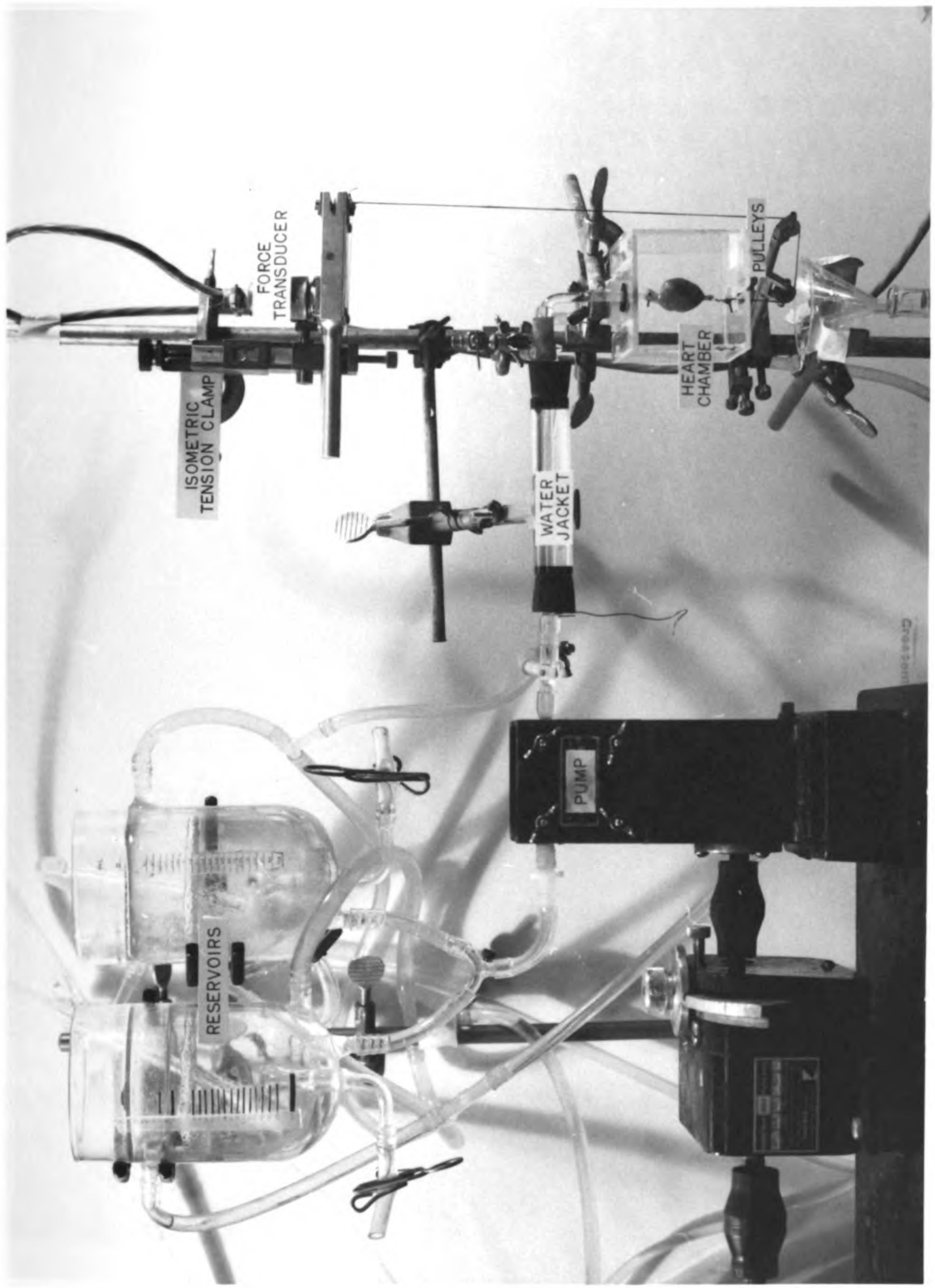

ISOMETRIC TENSION CLAMP
FORCE TRANSDUCER
PULLEYS
HEART CHAMBER
WATER JACKET
PUMP
RESERVOIRS

Figure 3

Figure 4. Heart chamber.

This is a close-up view of the plexiglass chamber around the heart. The front side has been removed. Perfusion of the heart is retrograde via the aorta which has been secured to the tip of the glass cannula. A string from the force transducer is affixed to the apex of the ventricles with a small Palmer heart clip.

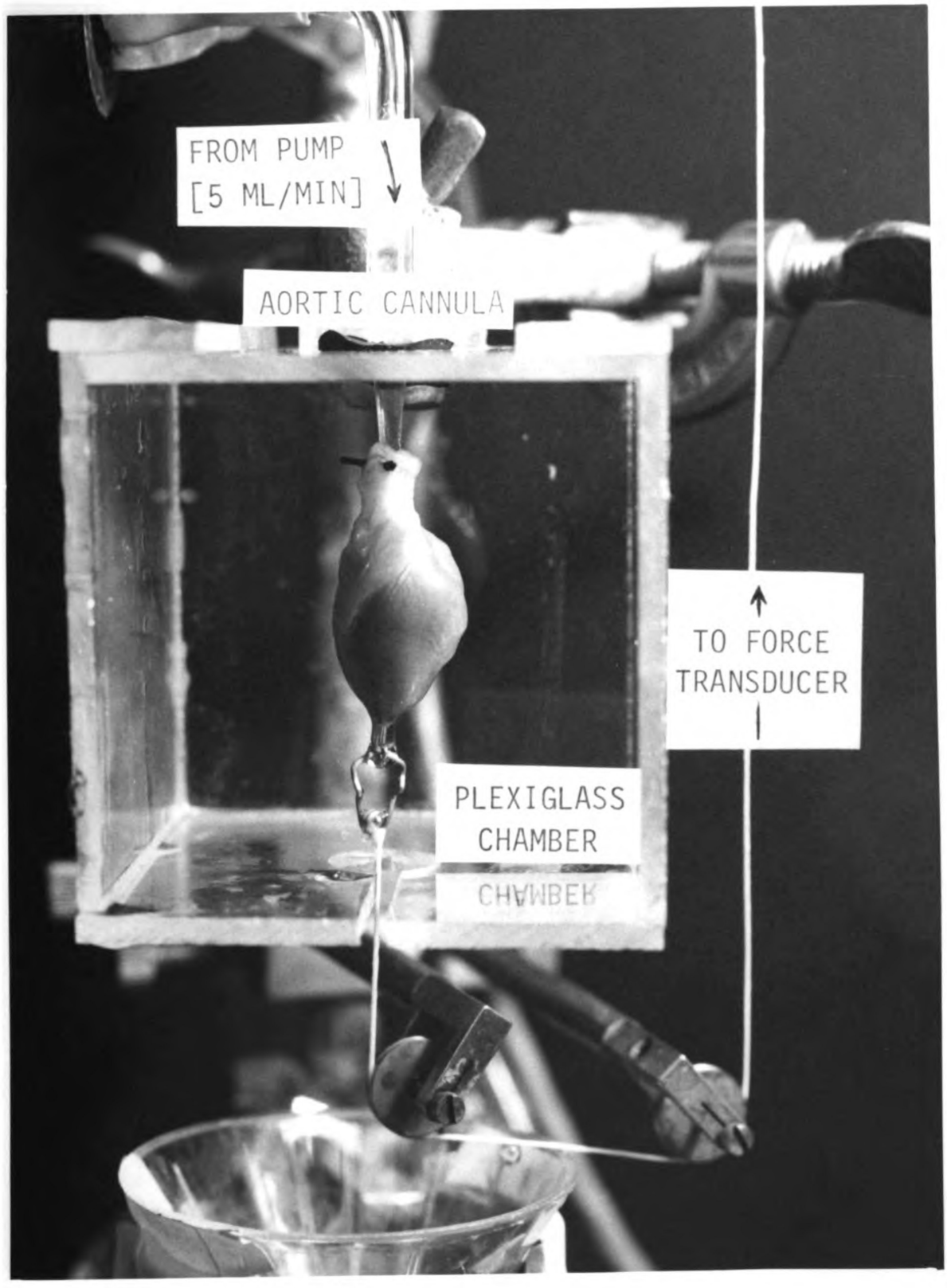

FROM PUMP
[5 ML/MIN]
AORTIC CANNULA
TO FORCE
TRANSDUCER
PLEXIGLASS
CHAMBER

Figure 4

Figure 5. Perfused tissue bath system.

This system was used for similtaneous recording of contractile activity and release of radiolabeled compounds. The apparatus is identical to that shown in Figure 2 except that this bath has a smaller volumn (15 ml) and is perfused and drained at a constant rate. Oxygenated heated solutions were pumped into the bath through the glass cannula at a rate of 5 ml/min. A compression screwclamp was used to adjust the drainage from the bath to the same rate.

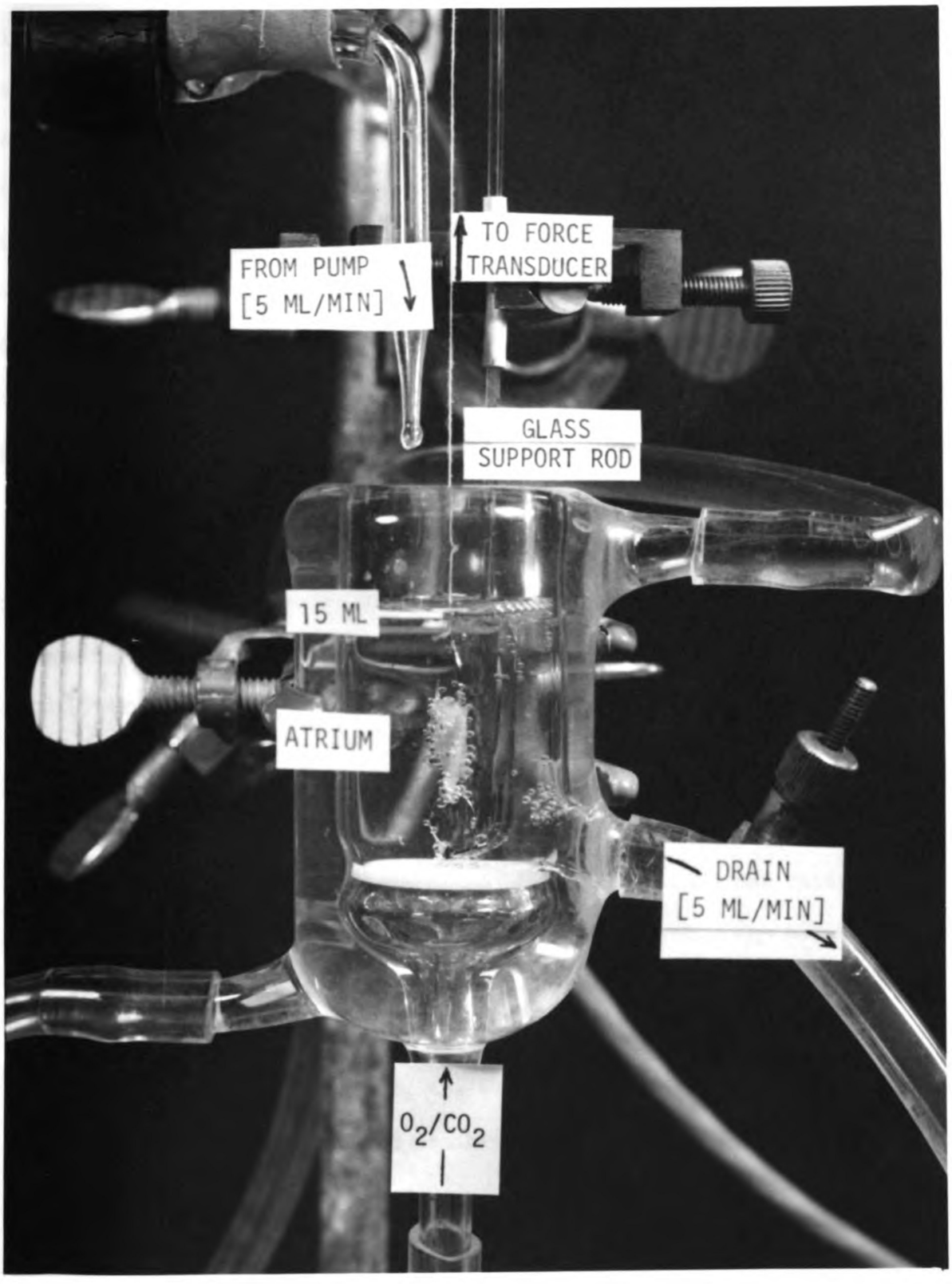

FROM PUMP
[5 ML/MIN]
TO FORCE
TRANSDUCER
GLASS
SUPPORT ROD
15 ML
ATRIUM
DRAIN
[5 ML/MIN]
O_2/CO_2

Figure 5

in which the retrograde perfusion of the heart via the aorta was maintained at a constant flow rather than at a constant pressure. This was achieved by use of a Sigmamotor peristaltic pump. Oxygenated solutions were pumped into the heart from one of the two reservoirs. The water jacket surrounding the final section of tubing and the plexiglass chamber (Figures 3 and 4) helped to maintain the heart and solutions at 37°C. A Grass force displacement transducer was connected to the heart via two small pulleys so that contractions could be monitored. A funnel underneath the plexiglass chamber was used for collection of the perfusate effluent.

The perfused tissue bath system (Figure 5) was designed for the isolated atria experiments in which radioisotopes were used. It differed from the tissue bath previously described only in that it had a smaller volume (15 ml) and was continuously perfused and drained at a constant rate (5 ml/min).

Preparation of Tissues

Guinea pigs ranging in weight from 400 to 700 grams were used in all experiments. They were pretreated with heparin (500 USP units/kg, subcutaneously) at one to two hours prior to sacrifice. The animals were sacrificed by a blow on the head, and the chest cavity was rapidly opened via an incision along the midline of the sternum. In order to wash the blood out of the heart, the vena cava was cut to eliminate venous return, and 1 ml of heparinized saline at 37°C was injected into the left ventricle.

In those experiments using atria, the right atrial appendage and parts of the right ventricle were dissected free from the heart and placed in a tissue bath containing a modified Locke-Ringer solution (see

under Solutions and Drugs). Once in the bath any pieces of ventricle or connective tissue were trimmed away. The atrium was then prepared for recording of contractile activity by suspending it vertically between two small clips or needles as previously described. Using a Harvard isometric tension clamp, tension on the atrium was adjusted to three-fourths of that which produced the maximum force. This was generally in the range of 0.75 to 1.25 grams. Once this was achieved, the atrium was washed several times by draining and quickly refilling the bath with fresh solution. With the perfused bath technique, perfusion was initiated following the washings.

Prior to the addition of any drugs, the atria were allowed to stabilize for periods of thirty to ninety minutes. The preparations were deemed stable when there was no measurable change in the force of contraction for twenty minutes and when the spontaneous rate was constant within ten beats per minute over a fifteen minute period.

For the isolated whole heart preparations, the tissue was first dissected free from all blood vessels and connective tissue attachments. The heart was then quickly removed from the animal and the aorta secured to the perfusion cannula while fluid was being pumped through it at 5 ml/min. This resulted in a strong, viable preparation free of blood clots. A clip or needle was attached to the apical portion of the ventricles for recording contractions, and the plexiglass chamber was placed around the heart. The stabilization criteria used were the same as those described for the isolated atria.

Solutions and Drugs

A modified Locke-Ringer solution described by Chenoweth and Koelle (1946) was used exclusively in all experiments. The buffer was prepared

by dissolving the following amounts of reagents in one liter of distilled water (all weights are expressed as grams of the anhydrous compound): Glucose, 1.9; NaCl, 7.0; KCl, 0.42; $CaCl_2$, 0.24; $MgCl_2$, 0.20. A pH of 7.4 was obtained by adding $NaHCO_3$ (2.0 g/liter) and bubbling a mixture of 95% oxygen and 5% carbon dioxide through the solution. All solutions in contact with cardiac tissue were maintained at 37°C.

The drugs used in this study were chlorpheniramine maleate (Mann Research Laboratories), brompheniramine maleate (A. H. Robins Co., Inc.), tripelennamine hydrochloride (Ciba Pharmaceutical Co.), triprolidine hydrochloride (Burroughs Wellcome & Co.), promethazine hydrochloride (Wyeth Laboratories, Inc.), imipramine and desipramine hydrochloride (Geigy Pharmaceuticals), cocaine hydrochloride (Merck & Co.), tyramine hydrochloride (Calbiochem), l-norepinephrine bitrartrate (Winthrop Laboratories), propranolol hydrochloride (Ayerst Laboratories, Inc.) and reserpine (Aldrich Chemical Co.).

In the isolated atria experiments, concentrated solutions of these drugs were pipetted into the tissue bath in amounts required to achieve the desired molar concentration. The cumulative method of determining dose response curves was used. When the maximum response for a particular dose of a drug was reached, sufficient drug was added to achieve the next highest molar concentration of that drug. In some experiments, propranolol (10^{-7}M) was added to the bath twenty minutes prior to drug administration. Reserpine pretreatment consisted of a dose of 3 mg/kg intraperitoneally forty-eight hours prior to the experiment followed by 2 mg/kg twenty-four hours later.

In the case of isolated whole hearts, drugs dissolved in Chenoweth-Koelle solution were added to one of the reservoirs and pumped into the

heart.

A combination of the above two methods was utilized for the perfused tissue bath technique. When a drug was added to the tissue bath (by the method previously described), it was also added to the perfusate in the same concentration. Therefore, the bath could be instantaneously brought up to a particular drug concentration, and this concentration would be maintained even though the bath was being continuously perfused.

For experiments involving the use of radioisotopes, dl-norepinephrine-7-H^3 (specific activity, 5-13 curies/millimole) and urea-C^{14} (specific activity, 4.7 millicuries/millimole) were obtained from New England Nuclear Corp. The isolated hearts were perfused (at 5 ml/min) with 40 ml of Chenoweth-Koelle solution containing 3×10^{-8}M (0.15-0.39 microcuries/ml) H^3-norepinephrine. Ascorbic acid (0.1 mg/ml) was added as an antioxidant when H^3-norepinephrine was used. The hearts were then washed for a period of ninety minutes with buffer prior to any drug treatments.

The 15 ml perfused bath was employed for the radioisotope experiments with isolated atria. In order to detect any release of radiolabeled compounds, solutions were pumped into and drained from the bath at a constant rate. This technique was somewhat similar to superfusion of a tissue except that the atria remained immersed in a constant volume (15 ml) of solution. Atria were incubated in the bath in 3×10^{-7}M (1.5-3.9 microcuries/ml) H^3-norepinephrine for thirty minutes during which time there was no perfusion. At the end of this period, the tissue was washed several times and the bath refilled with Chenoweth-Koelle solution. Perfusion was started and continued for ninety minutes (at a rate of 5

ml/min) before any drugs were given. Incubations in C^{14}-urea at a concentration of 6×10^{-4}M (3 microcuries/ml) were carried out in the same way.

The washout curves plotted for the radioisotope experiments were obtained by collecting samples of the perfusate effluent over a one minute period. One ml aliquots of these samples plus 1 ml of distilled water were pipetted into 10 ml of a modified Bray's solution (100 g naphthalene plus 6 g of PPO in 1 liter of dioxane). The samples were stored overnight to reduce autoluminescence and then counted in a Beckman liquid scintillation counter (Model LS100).

For determinations of H^3-norepinephrine uptake, atria were incubated as described above. Following this, they were washed with 30 ml of fresh solution at fifteen minute intervals for forty-five minutes. At this time they were removed from the bath, blotted and weighed. They were then homogenized in 5 ml of 0.4N perchloric acid. A 1 ml aliquot of the homogenate plus 1 ml of water were added to 10 ml of Bray's solution and counted for radioactivity.

Calculations

The response of isolated atria to the various drugs is expressed as a percent of their maximal response to norepinephrine. This was determined by giving an initial maximal dose of norepinephrine (10^{-4}M) and washing as soon as the maximum force of contraction had developed. This was followed by further washing until the atrium had again stabilized at its approximate original force and rate. At this time the drug under study was administered. The increase in force in grams obtained

with the drug was measured, and this was divided by the maximum increase in force produced by norepinephrine and multiplied times 100. At the end of the experiment, another maximal dose of norepinephrine was given to compare with the first and thus insure the viability of the preparation. Variability between preparations is minimized when data are calculated in this manner since each atria serves as its own control with regard to the maximum response that can be obtained.

In the radioisotope experiments, the counting efficiency for tritium was 21.4%. This figure was used to calculate disintegrations per minute (DPM). Since the perfusion flow rate was 5 ml/min, the number of DPM for each one minute sample was multiplied by 5 to obtain DPM/min. The drug-induced increases in the efflux of the radioactive component could then be computed by summing the total DPM/min for each minute of the elevated outflow. To obtain the net increase, an extrapolated baseline level of radioactivity was subtracted from the total.

Reliability of Methods

The repeatability of the maximum dose of norepinephrine (10^{-4}M) was tested in four atria. No significant difference in the response of an atrium was found when norepinephrine was given as frequently as four times in thirty minutes. This was further substantiated by the repeatability of the maximum norepinephrine response when given both before and after a series of drug treatments. In addition, the maximum norepinephrine dose did not alter the dose response curves obtained with any of the drugs tested.

The maximum positive inotropic effect which could be obtained with a particular drug was the same regardless of whether the most

effective dose was given by itself or at the end of the series of lower doses.

Statistical Methods

In determining the significance of effects produced by a particular drug treatment on atria, each atrium served as its own control. Data from these experiments were analyzed using Student's "t" test for paired observations (Steel and Torrie, 1960). Student's "t" test for unpaired observations was used for comparing the effect of one drug with another. The level of significance was chosen as P less than 0.05 unless otherwise noted.

CHAPTER III

RESULTS

Positive Inotropic Effect on Isolated Atria

Four of the antihistamines tested--chlorpheniramine, brompheniramine, triprolidine and tripelennamine--produced a marked increase in the force of contraction of isolated, spontaneously beating guinea pig right atria (Figure 6). Over the dose range of 10^{-7}M to 10^{-5}M, they were equipotent with tyramine. However, the average maximum response was approximately half that obtained with tyramine and amounted to increases in force which were 100 to 200 percent above control.

Those increases produced by drug concentrations of 10^{-6}M and above were significant ($P < 0.01$) as shown in Table 1. The differences among the average peak responses produced by chlorpheniramine, tripelennamine and triprolidine were not significant ($P < 0.05$). All drugs were depressant at concentrations above 3×10^{-5}M and above 10^{-5}M for brompheniramine. Promethazine produced no increase in the force of contraction and was depressant at concentrations of 3×10^{-6}M and above.

Desipramine, imipramine and cocaine also caused significant increases in force, with desipramine being the most potent of all drugs tested (Figure 7; Tables 1 and 2). There was no significant difference in the efficacies of desipramine, cocaine and tyramine. Cocaine was approximately equipotent with tyramine as was imipramine with desipramine. Desipramine, however, was significantly more efficacious than imipramine ($P < 0.05$).

Figure 6. Positive inotropic effects on isolated atria: Comparison of tripelennamine, chlorpheniramine, brompheniramine, triprolidine and tyramine.

The increase in force of contraction expressed as a percent of the maximum increase obtained with norepinephrine is plotted against the concentration (M) of the drug in the bath. Increases produced by drug concentrations of 10^{-6}M and above were significantly greater than control ($P < 0.01$).

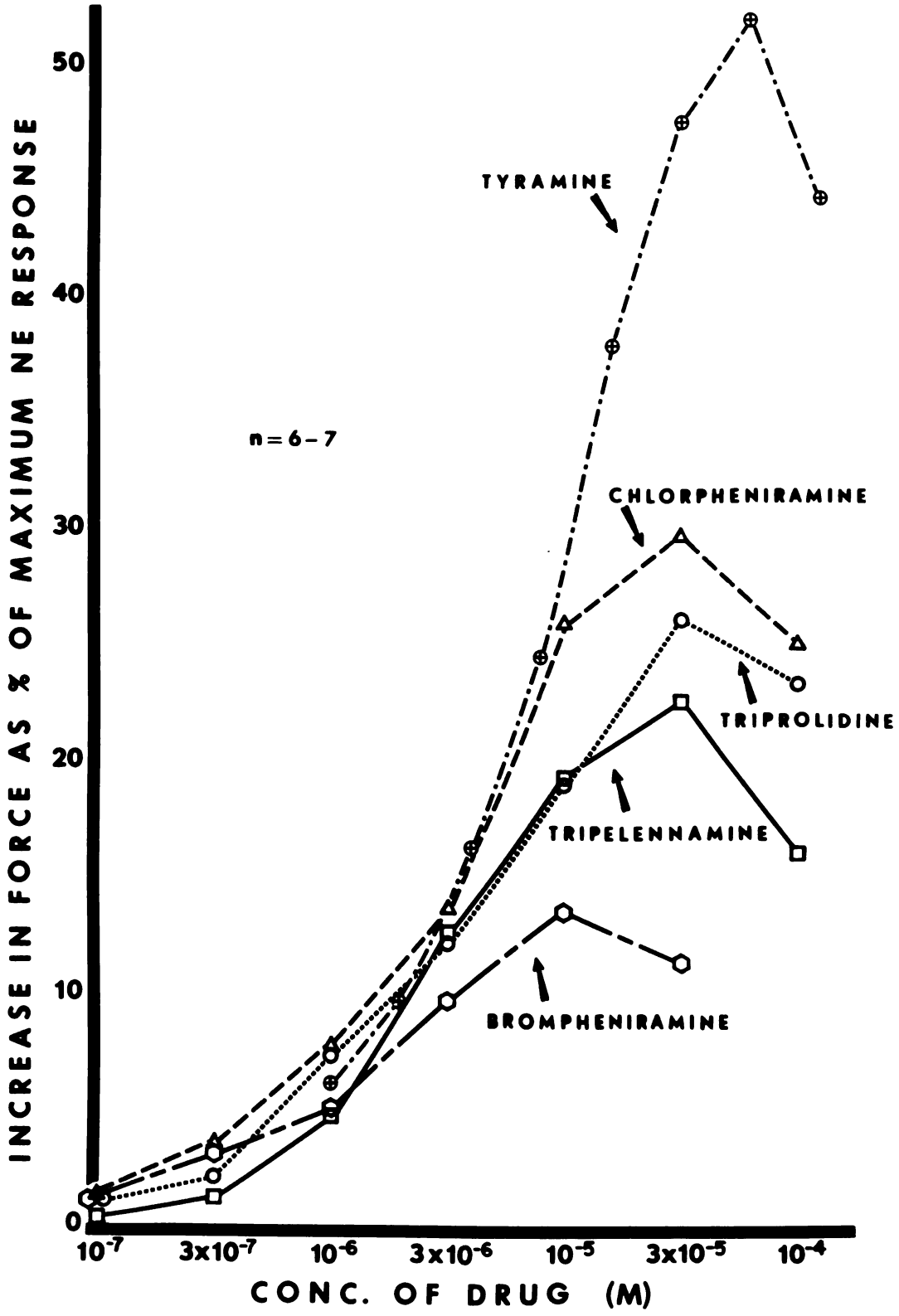

INCREASE IN FORCE AS % OF MAXIMUM NE RESPONSE
50
40
30
20
10
0
n = 6-7
TYRAMINE
CHLORPHENIRAMINE
TRIPROLIDINE
TRIPELENNAMINE
BROMPHENIRAMINE
10⁻⁷
3x10⁻⁷
10⁻⁶
3x10⁻⁶
10⁻⁵
3x10⁻⁵
10⁻⁴
CONC. OF DRUG (M)

Figure 6

Table 1. Increase in force as a percent of maximum response to norepinephrine: Comparison of chlorpheniramine, brompheniramine, tripelennamine, triprolidine, cocaine and tyramine.

The increase in force of contraction expressed as a percent of the maximum increase obtained with norepinephrine is given for each of the drugs at various doses. Each value represents the mean and standard error for at least six experiments. Increases produced by drug concentrations of 10^{-6}M and above were significantly greater than control ($P < 0.01$).

Table 1

Increase in Force as a Percent of Maximum Response to Norepinephrine

Drug	n	Concentration (M) of Drug in Bath						
		10^{-7}	3×10^{-7}	10^{-6}	3×10^{-6}	10^{-5}	3×10^{-5}	10^{-4}
Chlorpheniramine	6	1.1±0.5[a]	3.6±1.0	7.7±1.3	13.6±2.4	25.8±2.8	29.7±4.2	25.0±3.0
Brompheniramine	6	0.9±0.7	2.9±0.6	4.9±0.5	9.5±1.3	13.4±1.3	11.2±2.2	--
Tripelennamine	6	0.2±0.1	1.1±0.6	4.5±1.2	12.5±2.0	19.2±2.0	22.4±2.4	16.0±2.0
Triprolidine	7	1.0±0.6	1.9±0.8	7.1±1.3	11.7±1.9	18.1±3.2	25.8±3.2	23.3±2.5
Cocaine	7	2.4±0.6	4.5±0.7	10.9±1.4	23.1±2.6	37.3±3.8	40.8±4.6	30.3±4.5

Tyramine									
	6.0 ±1.0	9.5 ±1.4	16.2 ±2.0	24.4 ±3.2	37.8 ±3.6	47.5 ±2.3	52.0 ±2.0	44.3 ±2.7	
	10^{-6}	2×10^{-6}	4×10^{-6}	8×10^{-6}	1.6×10^{-5}	3.2×10^{-5}	6.4×10^{-5}	1.3×10^{-4}	

[a]Increase as a percent ± s. e. m.

Figure 7. Positive inotropic effects on isolated atria: Comparison of desipramine, imipramine, cocaine and tyramine.

The increase in force of contraction expressed as a percent of the maximum increase obtained with norepinephrine is plotted against the concentration (M) of drug in the bath. Drugs at the following concentrations produced increases that were significantly greater than control ($P < 0.01$): desipramine, 10^{-8}M and above; imipramine, 3×10^{-8}M and above; cocaine, 10^{-7}M and above; tyramine, 10^{-6}M and above. The bars above or below each point denote the standard error of the mean.

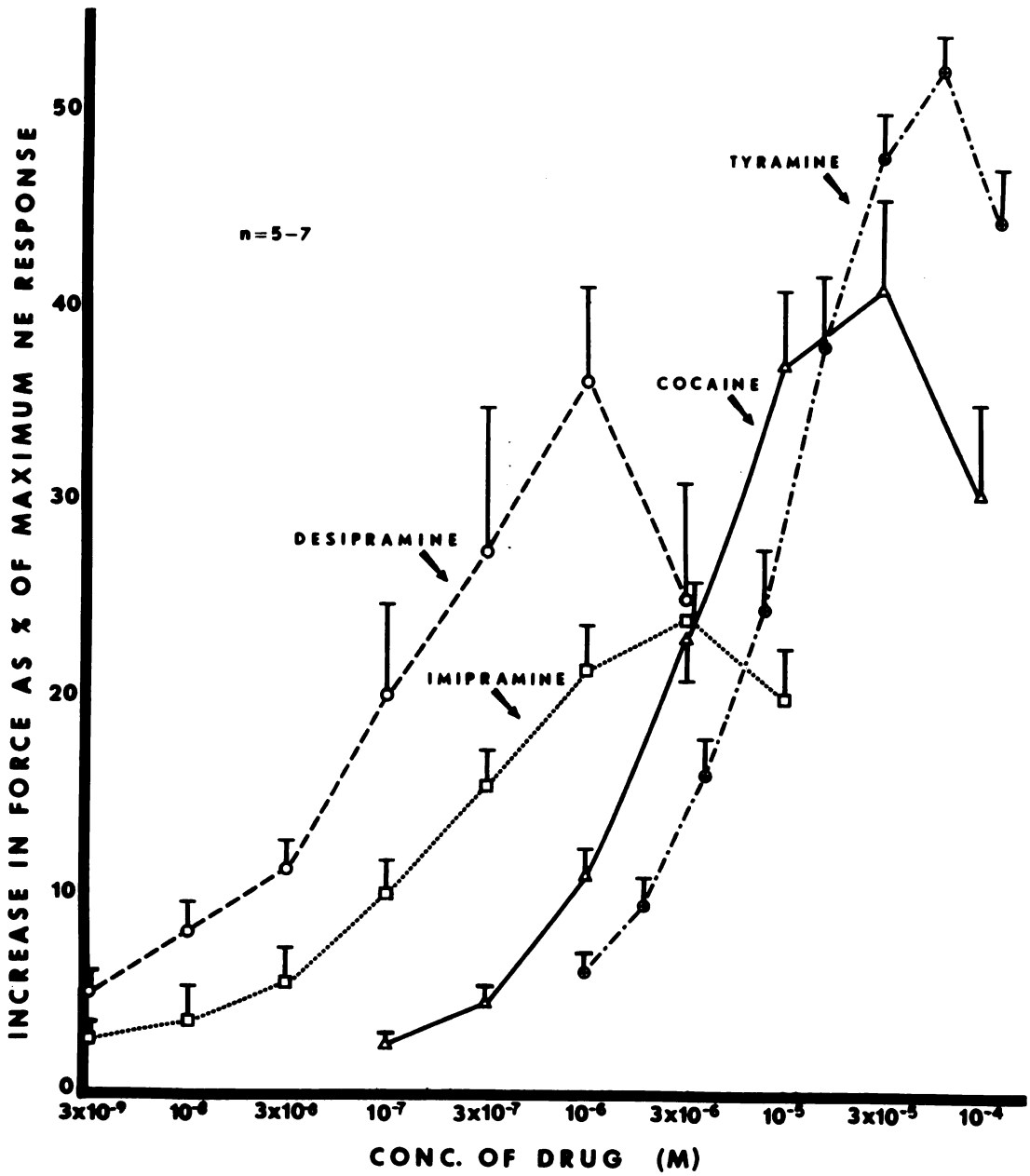

INCREASE IN FORCE AS % OF MAXIMUM NE RESPONSE
50
40
30
20
10
0
n=5-7
TYRAMINE
COCAINE
DESIPRAMINE
IMIPRAMINE
3x10⁻⁹
10⁻⁸
3x10⁻⁸
10⁻⁷
3x10⁻⁷
10⁻⁶
3x10⁻⁶
10⁻⁵
3x10⁻⁵
10⁻⁴
CONC. OF DRUG (M)

Figure 7

Table 2. Increase in force as a percent of maximum response to norepinephrine: Comparison of imipramine and desipramine.

The increase in force of contraction expressed as a percent of the maximum increase obtained with norepinephrine is given for both of the drugs at various doses. Each value represents the mean and standard error for at least five experiments. Increases produced by desipramine concentrations of 10^{-8}M and above and imipramine concentrations of 3×10^{-8}M and above were significantly greater than control ($P < 0.01$).

Table 2

Increase in Force as a Percent of Maximum Response to Norepinephrine

		Concentration (M) of Drug in Bath						
Drug	n	10^{-8}	3×10^{-8}	10^{-7}	3×10^{-7}	10^{-6}	3×10^{-6}	10^{-5}
Imipramine	5	3.5±1.8[a]	5.5±1.7	10.9±1.7	15.5±1.8	21.4±2.2	23.9±2.1	19.9±2.6
Desipramine	5	8.1±1.6	11.3±1.5	20.1±4.6	27.5±7.4	36.2±4.8	22.8±5.0	--

[a]Increase as percent ± s. e. m.

The periods of drug administration utilized to obtain the cumulative dose-response curves for the antihistamines, cocaine and tyramine were under twenty minutes, there being two to five minutes between doses. When the period between doses was greater than fifteen minutes, drug concentrations of 10^{-6}M and above produced no positive inotropic effects and in most cases were depressant to the force of contraction. Periods of five to fifteen minutes were required for the antidepressants to achieve their maximum effect; in addition, they were less depressant than the aforementioned drugs.

Effect of Reserpine and Propranolol

Representative tracings of maximal responses to several of the drugs in the normal atrium are shown in Figures 8 and 9. Also shown are the effects of pretreatment with reserpine, which depletes norepinephrine from the nerve endings, and propranolol, which blocks beta adrenergic receptors. As in the case of tyramine, the positive inotropic effects of chlorpheniramine, triprolidine, tripelennamine (Figure 8), cocaine and imipramine (Figure 9) were all abolished by pretreatment with reserpine (3 mg/kg at forty-eight hours before sacrifice; 2 mg/kg twenty-four hours later) or blocked by propranolol (10^{-7}M). Though sample tracings are not shown, the same results were obtained with desipramine and brompheniramine. The results were confirmed in three out of three experiments for each drug. The effectiveness of 10^{-7}M propranolol in blocking the actions of the same dose of norepinephrine in this preparation is illustrated at the bottom of both Figure 8 and Figure 9. The maximum norepinephrine response was not blocked by this concentration of propranolol.

When the atria from reserpine pretreated animals were incubated in 5 mg/ml norepinephrine (approximately 3×10^{-5}M), the positive inotropic

Figure 8. Effect of reserpine pretreatment and propranolol on the positive inotropic response: Comparison of chlorpheniramine, triprolidine, tripelennamine and tyramine. Effect of propranolol on the response to norepinephrine.

Each pair of tracings represents a control and a treated response for the same atrium. The brackets indicate the time elapsed between tracings, and the arrows denote the points of drug administration. In the left hand column are representative tracings of maximal responses to the drugs. In the center are the effects of reserpine pretreatment, and on the right, the effects of propranolol. Shown at the bottom is the effectiveness of 10^{-7}M propranolol in blocking the same dose of norepinephrine (NE). The maximum norepinephrine response was not blocked by this concentration of propranolol.

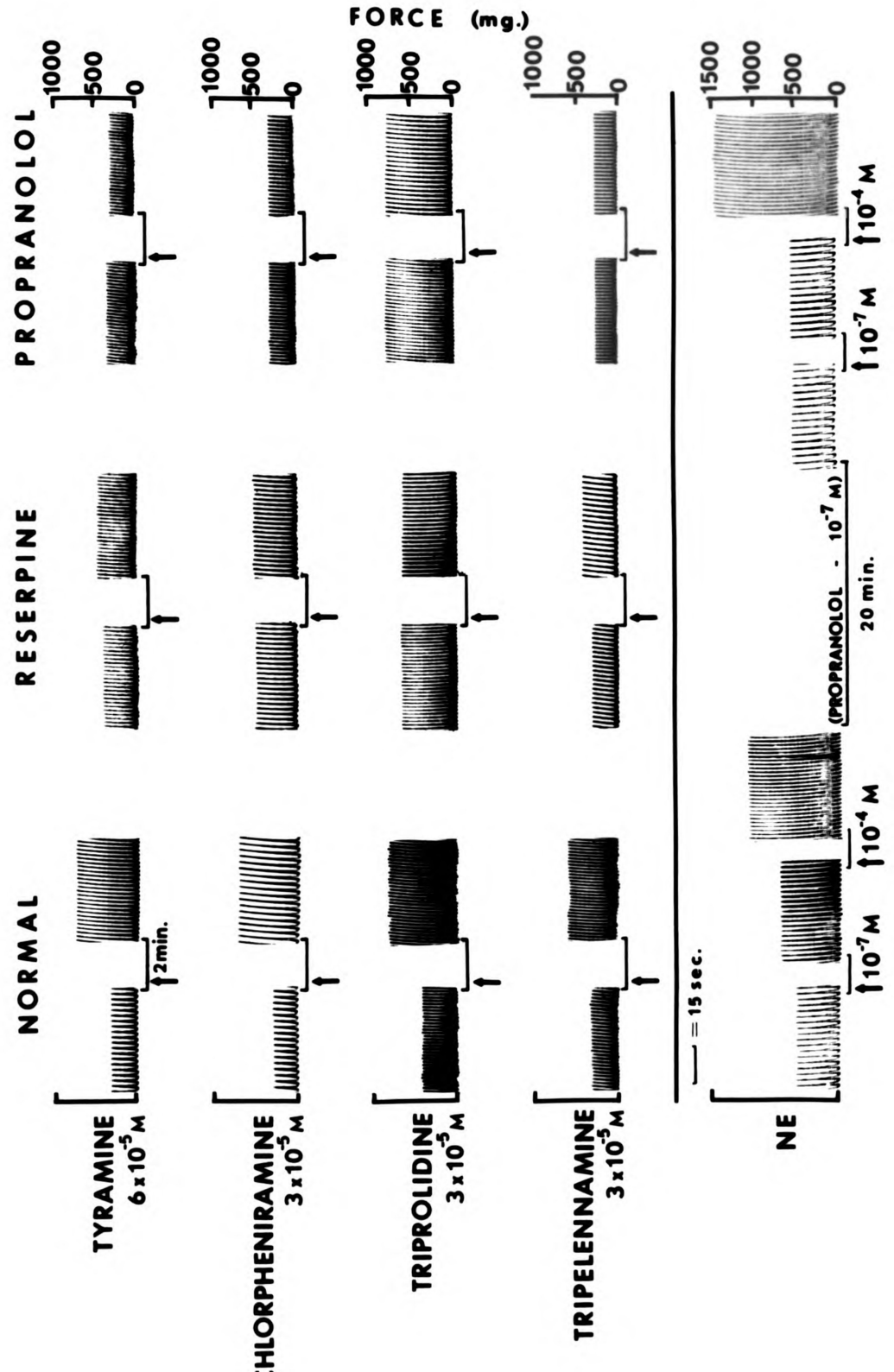

NORMAL
RESERPINE
PROPRANOLOL
FORCE (mg.)
1000
500
0
1500
TYRAMINE
6x10⁻⁵M
2min.
CHLORPHENIRAMINE
3x10⁻⁵M
TRIPROLIDINE
3x10⁻⁵M
TRIPELENNAMINE
3x10⁻⁵M
NE
10⁻⁷M
10⁻⁴M
(PROPRANOLOL - 10⁻⁷M)
20 min.
10⁻⁷M
10⁻⁴M
= 15 sec.

Figure 8

Figure 9. Effect of reserpine pretreatment and propranolol on the positive inotropic response: Comparison of cocaine, imipramine and tyramine. Effect of propranolol on the response to norepinephrine.

Each pair of tracings represents a control and a treated response for the same atrium. The brackets indicate the time elapsed between tracings, and the arrows denote the points of drug administration. In the left hand column are representative tracings of maximal responses to the drugs. In the center are the effects of reserpine pretreatment, and on the right, the effects of propranolol. Shown at the bottom is the effectiveness of 10^{-7}M propranolol in blocking the same dose of norepinephrine (NE). The maximum norepinephrine response was not blocked by this concentration of propranolol.

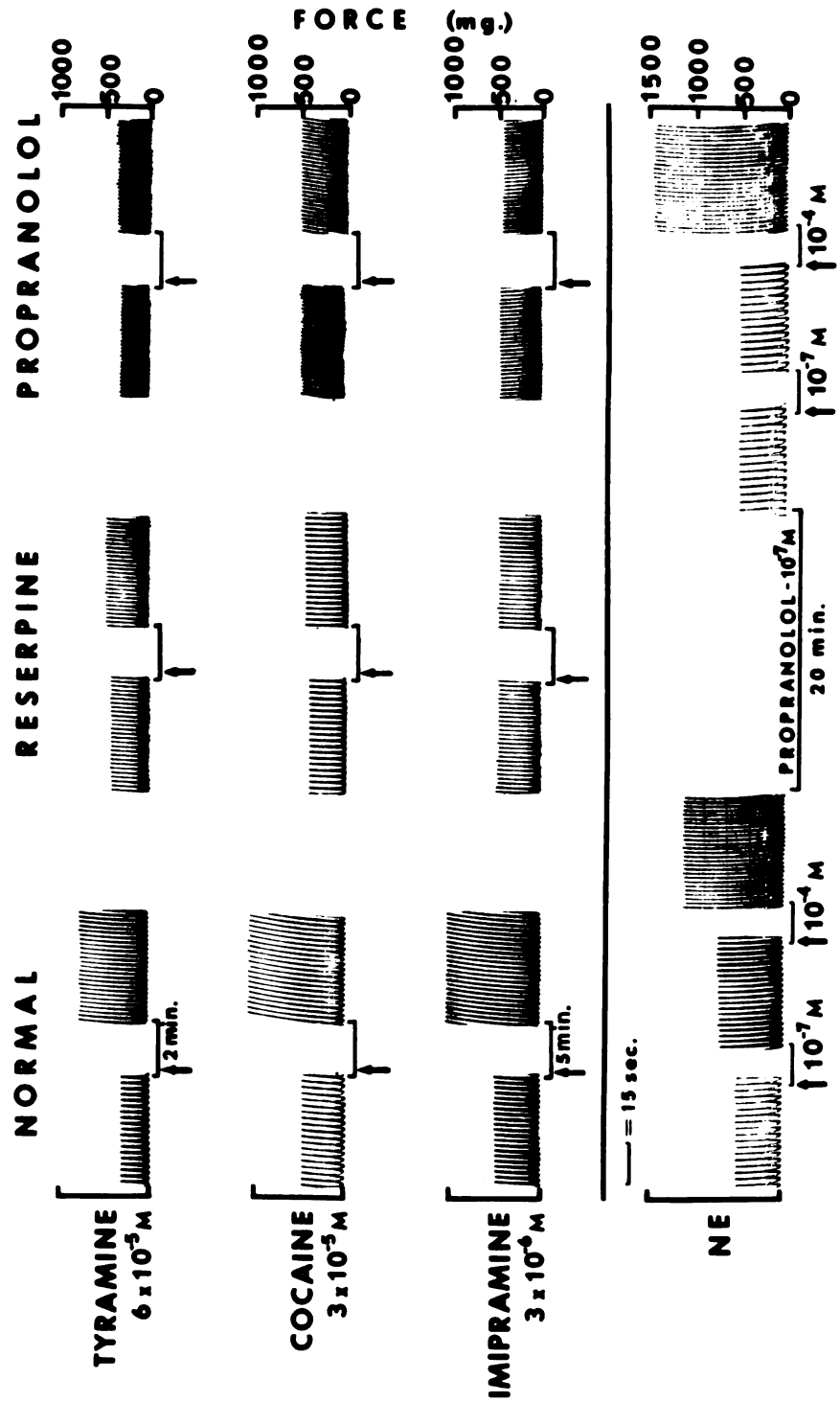

NORMAL
RESERPINE
PROPRANOLOL
FORCE (mg.)
1000
500
0
TYRAMINE 6 x 10⁻⁵ M
2 min.
COCAINE 3 x 10⁻⁵ M
IMIPRAMINE 3 x 10⁻⁶ M
5 min.
= 15 sec.
1500
1000
NE
10⁻⁷ M
10⁻⁴ M
PROPRANOLOL - 10⁻⁷ M
20 min.

Figure 9

Figure 10. Effect of norepinephrine incubation on drug responses in atria from reserpine pretreated guinea pigs.

Each pair of tracings represents a control and a treated response for the same atrium. The brackets indicate the time elapsed between tracings, and the arrows denote the points of drug administration. In the left hand column are representative tracings of maximal responses to the drugs. In the center are the effects of reserpine pretreatment and on the right, responses in the same atria after incubation with norepinephrine (NE).

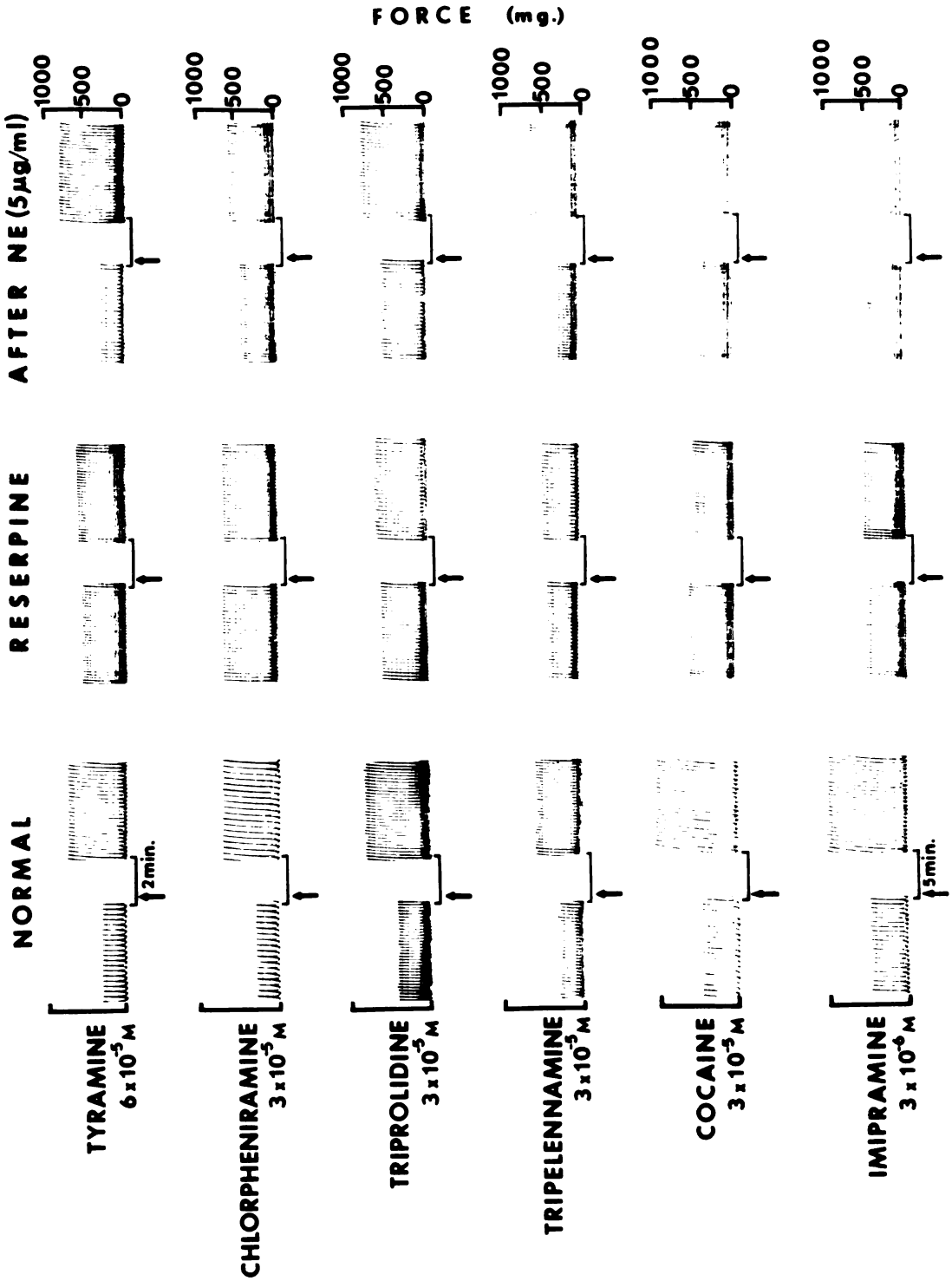

NORMAL
RESERPINE
AFTER NE (5µg/ml)
FORCE (mg.)
1000
500
0
TYRAMINE
6x10⁻⁵M
2min.
CHLORPHENIRAMINE
3x10⁻⁵M
TRIPROLIDINE
3x10⁻⁵M
TRIPELENNAMINE
3x10⁻⁵M
COCAINE
3x10⁻⁵M
IMIPRAMINE
3x10⁻⁶M
5min.

Figure 10

responses to tyramine, chlorpheniramine, brompheniramine, tripelennamine, triprolidine, cocaine, desipramine, and imipramine were at least partially restored (n = 2). Representative tracings for several of the drugs are shown in Figure 10. In the left hand column are typical responses for normal atria; in the center, responses obtained with atria from reserpine pretreated guinea pigs; and on the right, responses in those same atria after incubation with norepinephrine.

Effect on Atrial Rate of Contraction

The increase in force of contraction produced by the antihistamines and antidepressants was not associated with any increase in rate. Cocaine and tyramine produced significant but highly variable increases in rate (10 to 80% above control at a concentration of 3×10^{-5}M) concurrent with their positive inotropic effects. This variation appeared to be due primarily to differences in initial rate. However, in the experiments in which drug concentrations were maintained for fifteen minutes prior to the addition of the next highest dose, cocaine and the antihistamines produced a dose- and time-dependent depression of spontaneous rate (Figure 11). The results obtained with cocaine were in agreement with those of Trendelenburg (1968).

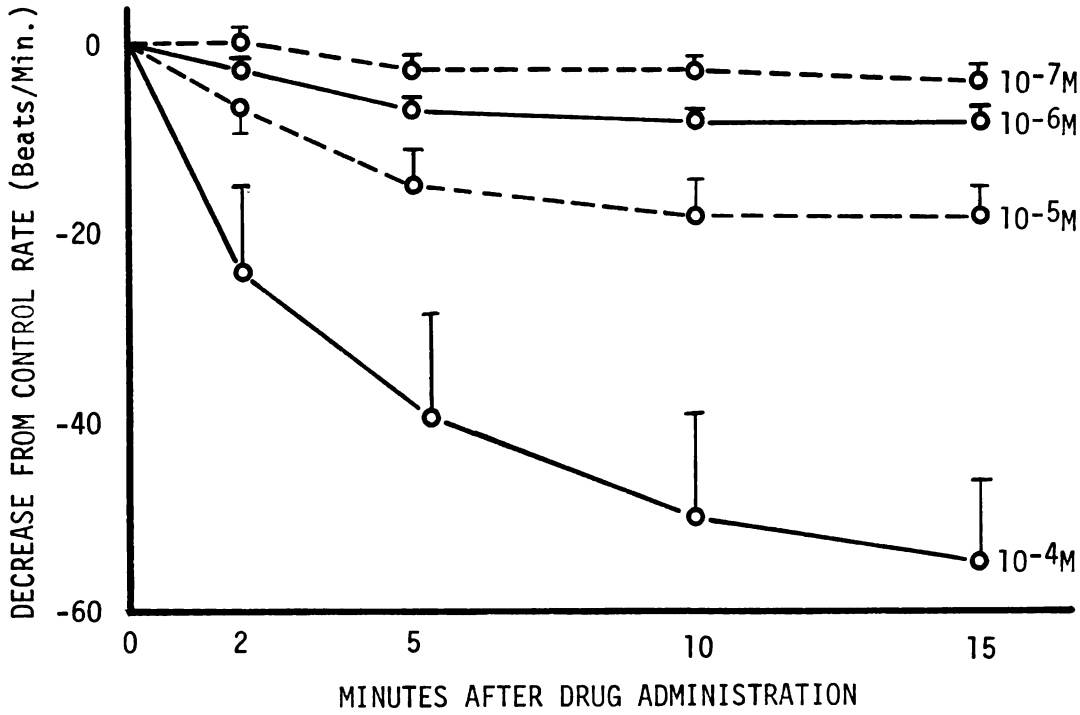
Release of H^3-norepinephrine in Isolated Hearts

Cocaine and the antihistamines and antidepressants under study were tested for their ability to release H^3-norepinephrine from isolated perfused hearts which had been incubated with the radioisotope at a concentration of 3×10^{-5}M for eight minutes. Following a washout period of ninety minutes, the drugs were administered in that concentration which produced the maximum positive isotropic effect in the atria.

Figure 11. Effect of drugs on atrial rate of contraction.

In the upper graph, the decrease from the control rate (in beats per minute) is plotted against the time (in minutes) after drug administration. The curves at the various concentrations represent the averages for chlorpheniramine, tripelennamine and triprolidine (n = 2 for each drug). Standard errors of the mean are denoted by the bars above or below each point.

In the lower graph, the decrease from the control rate in beats per minute is plotted against the concentration (M) of the drug in the bath. The decreases in rate were those reached at fifteen minutes after the administration of the particular drug. The antihistamine curve represents the average decreases obtained with chlorpheniramine, tripelennamine and triprolidine. Promethazine (10^{-7}M) caused a cessation of spontaneous contractile activity.


DECREASE FROM CONTROL RATE (Beats/Min.)
0
-20
-40
-60
0
2
5
10
15
MINUTES AFTER DRUG ADMINISTRATION
10-7M
10-6M
10-5M
10-4M

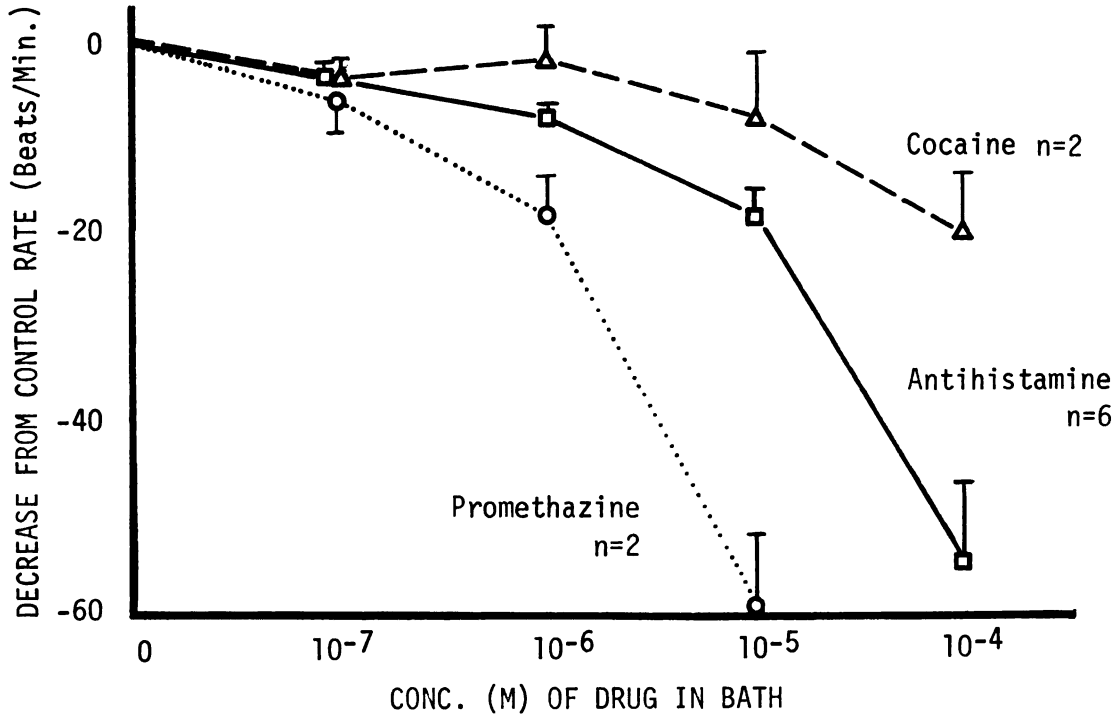

DECREASE FROM CONTROL RATE (Beats/Min.)
0
-20
-40
-60
0
10-7
10-6
10-5
10-4
CONC. (M) OF DRUG IN BATH
Cocaine n=2
Antihistamine
n=6
Promethazine
n=2

Figure 11

Results typical of those obtained with 3 x 10^{-5}M tyramine and tripelennamine are shown in Figure 12. The peak increase in the efflux of the radioactive component was almost five times greater for tyramine than for tripelennamine. Chlorpheniramine, triprolidine and cocaine caused an elevation in radioactivity similar to that of tripelennamine, but a marked degree of variation for each of the drugs precluded precise analysis and quantitation. The slope of the increased efflux seen with tyramine, cocaine and the antihistamines was quite steep. In contrast, the gradual rise observed with desipramine took as long as twenty minutes to reach a peak that was about half the tripelennamine peak. Other drugs were not tested in this system.

The isolated whole heart appeared much more sensitive than the isolated atrium to the depressant effects of these drugs. In fact, no positive inotropic effects were observed with any of the drugs, and it was necessary to limit their perfusion time to four minutes to avoid severe myocardial depression. Chlorpheniramine was the most potent drug in this respect.

The failure to note stimulation with a drug such as tyramine was undoubtedly due in part to the method of measurement. The lack of a single axis of muscle fiber alignment in the heart makes a force transducer connected to the apex of the ventricles an inadequate index of the force of contraction.

Due to the inability to correlate release with increased force, the possibility of myocardial depression causing the release of H^3-norepinephrine and the apparent failure to demonstrate maximum H^3-norepinephrine releasing potential in the isolated heart, the search for a more suitable system was undertaken.

Figure 12: Effect of tyramine and tripelennamine on H^3-norepinephrine efflux in isolated hearts.

The amount of radioactivity in disintegrations per minute (DPM) per minute of effluent flow is plotted against the time in minutes after the end of H^3-norepinephrine (H^3-NE) infusion. The heavy bar on the abscissa denotes the period during which the drug was administered. The beginning of this period was taken as the zero level of radioactivity.

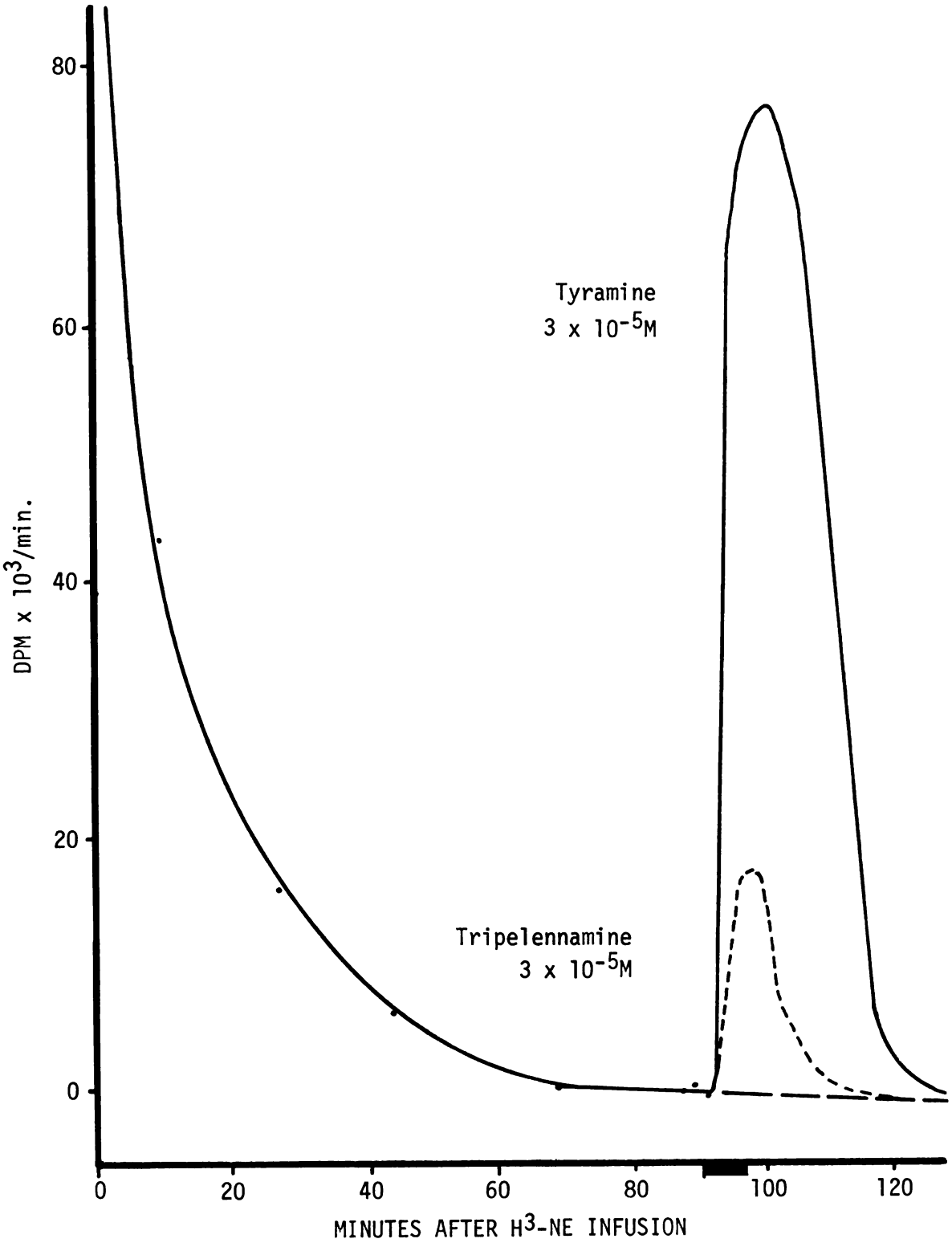

80
60
40
20
0
DPM x 10^3/min.
Tyramine
3 x 10^{-5}M
Tripelennamine
3 x 10^{-5}M
0
20
40
60
80
100
120
MINUTES AFTER H^3-NE INFUSION

Figure 12

Release of H^3-norepinephrine in Isolated Atria

The perfused tissue bath (Figure 5) offered the advantages of a system which utilized the tissue initially studied--the isolated atria--as well as one in which any change in the efflux of radioactivity could be easily and accurately assessed.

The administration of tripelennamine (3×10^{-5}M) to isolated spontaneously beating atria previously incubated in H^3-norepinephrine (3×10^{-7}M) produced a sharp rise in the amount of radioactivity in the perfusate effluent. This was accompanied by an increased force of contraction characteristic of that seen in the initial series of experiments (Figure 13). Chlorpheniramine and triprolidine (3×10^{-5}M) produced a similar increase in the efflux of tritiated compound (Figure 14, Table 3) which again correlated with the positive inotropic effect. These three drugs were significantly less effective ($P < 0.05$) than tyramine (3×10^{-5}M). Desipramine was found to produce only a slight, gradual change in the efflux of H^3-norepinephrine (Table 3) similar to that seen in the isolated heart. However, the marked inotropic effect previously observed with this compound was absent and, unexplainably, could no longer be demonstrated even using the original techniques. Cocaine appeared to be only about half as effective as the antihistamines in displacing H^3-norepinephrine, although this difference was not statistically significant (Figure 14, Table 3).

Effects on H^3-norepinephrine Uptake

Since desipramine did not appear to be releasing H^3-norepinephrine in the guinea pig heart or atrium, experiments were performed to determine if the ability of the drug to block amine uptake was absent as well.

Figure 13. Correlation between positive inotropic effect and increased H^3-norepinephrine efflux in isolated atria.

The amount of radioactivity in disintegrations per minute (DPM) per minute of effluent flow and the increase in force of contraction in milligrams (mg) are plotted against the minutes following the termination of H^3-norepinephrine (H^3-NE) incubation. The arrow on the abscissa indicates the point of administration of 3×10^{-5}M tripelennamine.

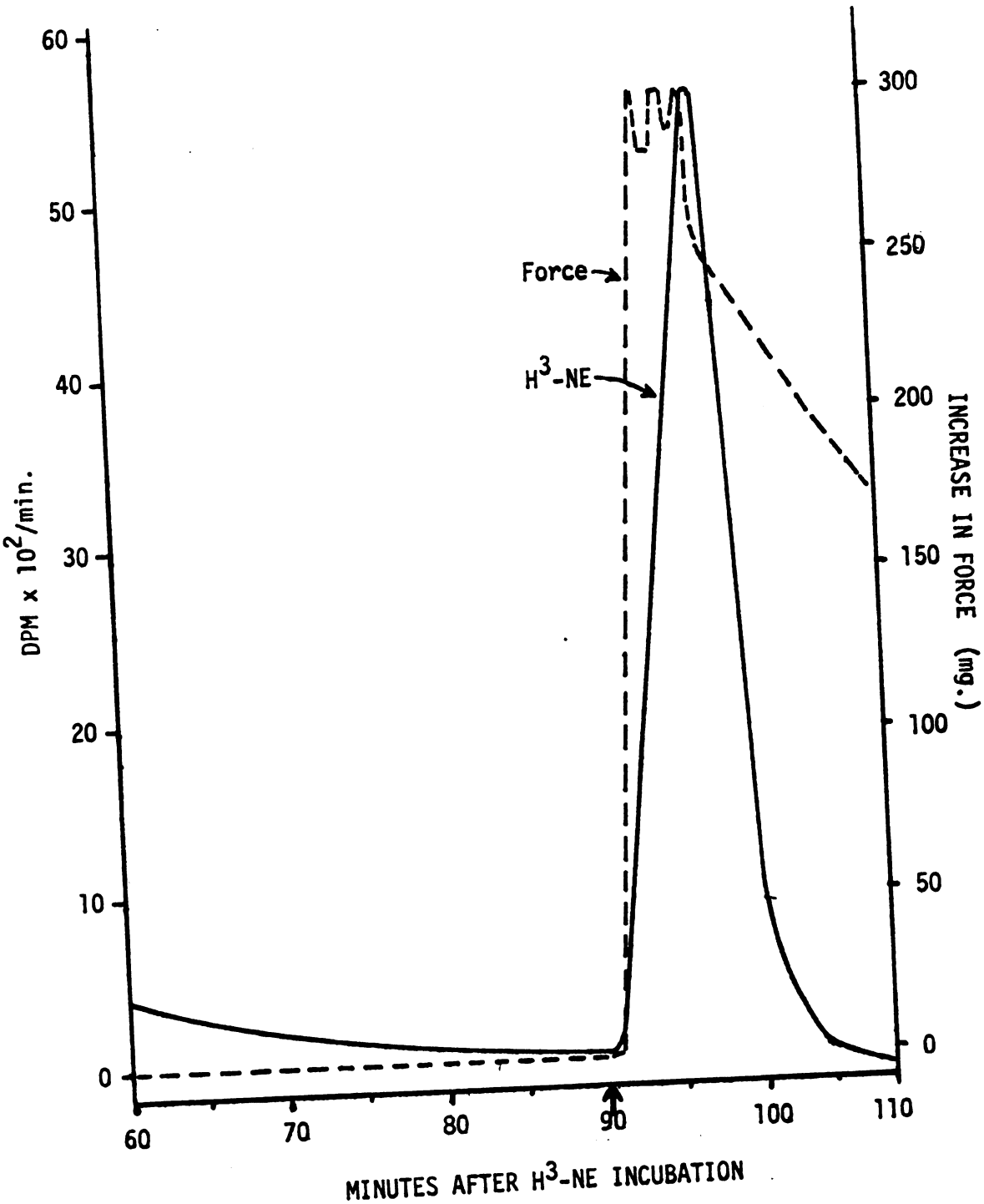

60
50
40
30
20
10
0
DPM x 10^2/min.
300
250
200
150
100
50
0
INCREASE IN FORCE (mg.)
Force
H^3-NE
60
70
80
90
100
110
MINUTES AFTER H^3-NE INCUBATION

Figure 13

Figure 14. Effects of tripelennamine, chlorpheniramine, triprolidine and cocaine on H^3-norepinephrine and C^{14}-urea efflux in isolated atria.

The amount of radioactivity in disintegrations per minute (DPM) per minute of effluent flow is plotted against the time in minutes. The solid lines represent the levels of H^3-norepinephrine, and the dashed lines, the levels of C^{14}-urea. Drugs were administered at ninety minutes after the termination of H^3-norepinephrine incubation. The point of administration was taken as zero time and the zero level of radioactivity.

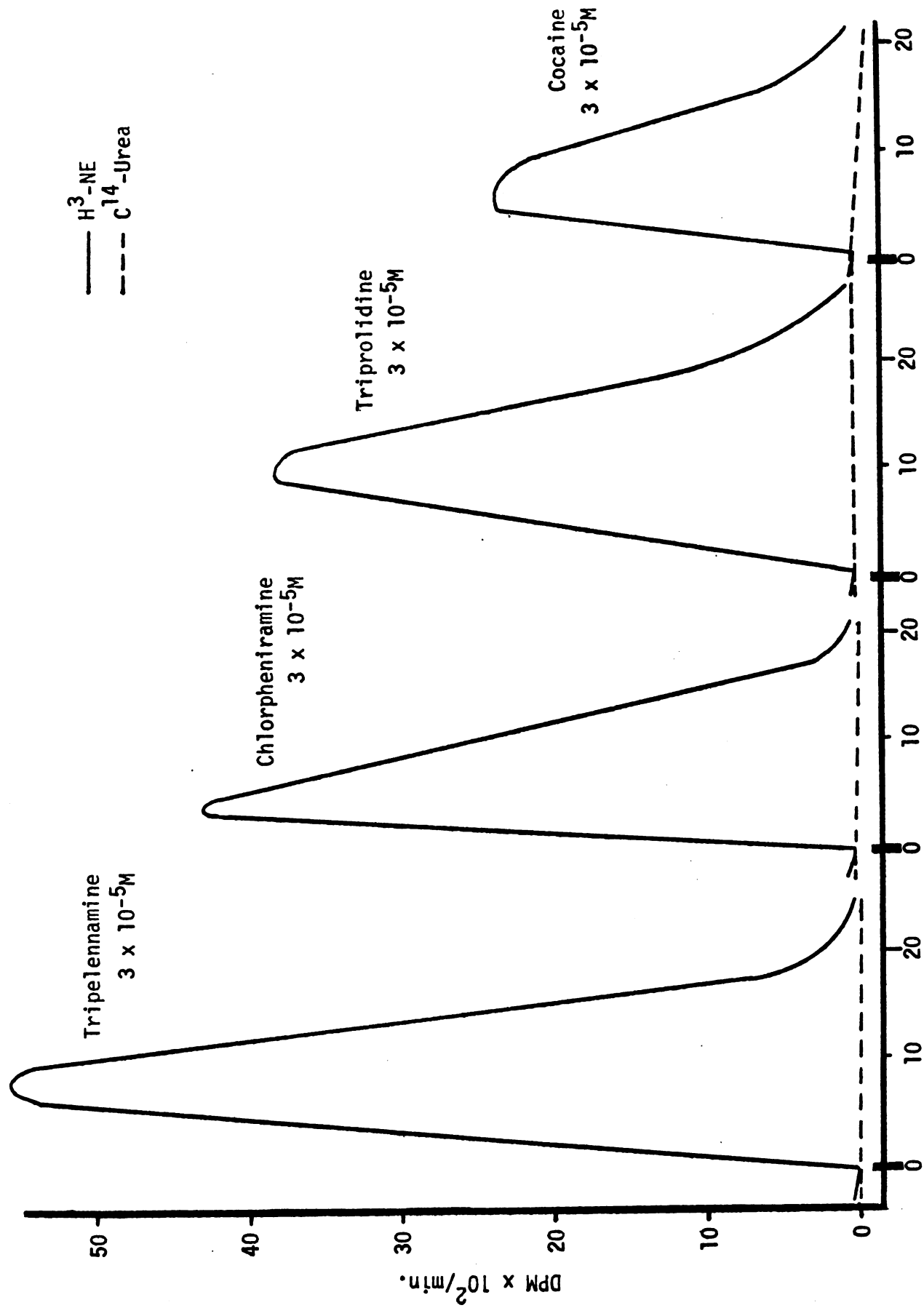

H³-NE
C¹⁴-Urea
Tripelennamine
3 x 10⁻⁵M
Chlorpheniramine
3 x 10⁻⁵M
Triprolidine
3 x 10⁻⁵M
Cocaine
3 x 10⁻⁵M
DPM x 10²/min.
0
10
20
30
40
50
TIME (min.)

Figure 14

Table 3. Drug-induced increases in H^3-norepinephrine efflux in isolated atria.

The total increases in radioactivity elicited by tyramine, tripelennamine, chlorpheniramine, triprolidine, cocaine and desipramine were computed as disintegrations per minute (DPM) per gram of tissue. Each value represents the mean and standard error (s.e.m.) for 4 experiments. Tyramine was significantly more effective ($P < 0.05$) than any of the other drugs.

Table 3

Drug-induced Increases in H^3-norepinephrine Efflux in Isolated Atria

Drug	Conc. (M)	Total Efflux (DPMx10^5/g)
Tyramine	3×10^{-5}	321.0±15.1[a]
Tripelennamine	3×10^{-5}	50.1± 8.0
Triprolidine	3×10^{-5}	43.4± 9.2
Chlorpheniramine	3×10^{-5}	42.0±12.6
Cocaine	3×10^{-5}	22.9±11.6
Desipramine	1×10^{-6}	18.6± 8.9

[a]mean ± s. e. m.

For comparison, tripelennamine, triprolidine and promethazine (all 3×10^{-5}M) were also tested. Desipramine (10^{-6}M) was significantly more potent ($P < 0.05$) than any of the other drugs except triprolidine, producing an 83.2% inhibition of norepinephrine uptake. Tripelennamine and triprolidine blocked uptake by 62.4% and 71.1% respectively. Promethazine was much less effective, inhibiting uptake by only 31.3% (Table 4).

Effects on C^{14}-urea Efflux

Experiments were carried out to determine if the drug-induced increases in radioactivity in the effluent could be due simply to a displacement of non-specifically bound H^3-norepinephrine. Atria were either incubated simultaneously in H^3-norepinephrine (3×10^{-7}M) and C^{14}-urea (6×10^{-4}M) or in C^{14}-urea alone for thirty minutes. When the various drugs were given, the increases in force of contraction and release of H^3-norepinephrine (if present) were not accompanied by any increase in the efflux of C^{14}-urea (Figure 14).

As an additional test, 2×10^{-6}M calcium was administered, bringing the total bath concentration to 4.2×10^{-6}M. This concentration produces an increase in force presumably not mediated by norepinephrine. There was no increase in H^3-norepinephrine or C^{14}-urea efflux associated with the calcium-induced positive inotropic effect.

Table 4. Effect of drugs on the uptake of H^3-norepinephrine in isolated atria.

Drugs were administered to isolated atria thirty minutes prior to incubation with H^3-norepinephrine (3×10^{-7}M for thirty minutes). Forty-five minutes after the end of the incubation period, atria were assayed for radioactivity and the percent inhibition of uptake was calculated. Each value represents the mean and standard error (s.e.m.) for three experiments. Numbers connected by a vertical bar do not differ significantly ($P < 0.05$).

Table 4

Effect of Drugs on the Uptake of H^3-norepinephrine in Isolated Atria

Drug	Conc. (M)	DPMx10^5/g	% Inhibition
None	--	117.8±7.6[a]	--
Promethazine	3 x 10^{-5}	80.9±8.5	31.3
Tripelennamine	3 x 10^{-5}	44.3±6.4	62.4
Triprolidine	3 x 10^{-5}	34.0±7.1	71.1
Desipramine	1 x 10^{-6}	19.8±4.4	83.2

[a]mean ± s. e. m.

CHAPTER IV

DISCUSSION

The ability of cocaine and certain of the antihistamines and antidepressants to potentiate the cardiovascular effects of norepinephrine has been well documented. The mechanism of this potentiation by cocaine and the antidepressants is believed to be, at least in part, a blockade of norepinephrine uptake into sympathetic nerve terminals. This would inhibit the primary means of norepinephrine inactivation at the synapse and thus provide a greater amount of the amine to activate receptors.

Current evidence indicates that a cocaine-like mechanism may be similarly involved in the antihistamine potentiation of the norepinephrine response. Tripelennamine, chlorpheniramine, phenindamine and diphenhydramine, four antihistamines which enhance certain cardiovascular actions of norepinephrine have been shown to block norepinephrine uptake in the heart (Isaac and Goth, 1965a,b, 1967; McNeill and Brody, 1966, 1968).

Since tyramine is thought to utilize the norepinephrine uptake system, one would expect the drugs that block norepinephrine uptake to also inhibit the uptake of tyramine, and this has been observed. Tripelennamine, chlorpheniramine, diphenhydramine, imipramine and desipramine have been shown to block the uptake of both norepinephrine and tyramine in the rat heart. In the same studies, promethazine,

triprolidine and pyrilamine had no effect on the uptake of either amine (Isaac and Goth, 1965a,b; McNeill and Brody, 1966, 1968; Commarato, et al., 1969a,b; McNeill and Commarato, 1969). As would be expected, the cardiostimulatory effects of norepinephrine were enhanced by the drugs which blocked uptake while those of tyramine were inhibited (Isaac and Goth, 1965a,b, 1967; Johnson, et al., 1965; Johnson and Kahn, 1966; McNeill and Brody, 1966, 1968; Commarato, et al., 1969a,b; McNeill and Commarato, 1969).

That certain compounds which block norepinephrine uptake may also release the amine seems to be a distinct possibility. All sympathomimetic amines which release norepinephrine have been shown to be potent inhibitors of its uptake as well (Iversen, 1967). Since these drugs appear to exert their releasing actions intraneuronally, they probably gain access to this site by utilizing the same uptake system as norepinephrine. In doing so, they could block norepinephrine uptake by simple competitive inhibition. Such a mechanism has been proposed for tyramine by Furchgott, et al. (1963).

Recent studies by McNeill and Brody (1966, 1969) suggest that at least two of these antihistamines may be directly releasing norepinephrine in addition to blocking its uptake. While measuring the potentiating effects of some of the antihistamines on norepinephrine-induced cardiac phosphorylase activation, they noted that either chlorpheniramine or tripelennamine was able to increase the amount of the active form of the enzyme glycogen phosphorylase. However, since these experiments were carried out in intact animals, sympathetic discharge could have been releasing norepinephrine in the heart. If this were the case, the effect of norepinephrine would have been enhanced by the antihistamines due to their ability to block uptake.

The recent observation that desipramine releases norepinephrine from the heart (Titus, et al., 1966; Nash, et al., 1968; Leitz and Stefano, 1970) lends support to the idea that drugs which are inhibitors of norepinephrine uptake may also cause release.

Another potent innibitor of norepinephrine uptake, cocaine, has also been shown to block both the uptake and the cardiovascular effects of tyramine (Trendelenburg, 1961: Furchgott, et al., 1963; McNeill and Brody, 1968). In addition, there is some indication that cocaine may be releasing norepinephrine as well (Furchgott, et al., 1963; Cervoni, et al., 1966; Trendelenburg, 1968; Vohra, 1969).

In order to further investigate the possibility that these compounds might be causing release, it seemed essential to have a preparation in which sympathetic discharge would not be a factor. For this reason and because of the large body of work with these drugs in the cardiovascular system, the isolated guinea pig atrium was selected as the preparation to be used. The possibility of catecholamine release by electrical stimulation was avoided by using the right atrium which is spontaneously beating. Another advantage of this tissue for studying norepinephrine release is that it generally has been found to contain a greater amount of the amine per unit weight than either the left atria or the ventricles (Cervoni, et al., 1966).

When tested in isolated atria, tripelennamine, chlorpheniramine, brompheniramine and triprolidine all produced positive inotropic effects that were significant at concentrations of 10^{-6}M and above (Figure 6). Although they were approximately equipotent with tyramine over the lower dose range (10^{-7}M to 10^{-5}M), tripelennamine, chlorpheniramine and triprolidine at 3 x 10^{-5}M generated an average maximum response that was

only about 50 percent of that obtained with an equivalent dose of tyramine. Brompheniramine was somewhat less effective, reaching a maximum at 10^{-5}M that was about one-half the maximum tyramine response, and promethazine was completely ineffective. Since an increase in heart rate per se will cause an increased force of contraction, this parameter was also measured. During the time period in which the positive inotropic effects of the antihistamines were recorded, there were no increases in rate.

In the initial series of experiments, desipramine, imipramine and cocaine also increased the force of contraction in isolated atria with desipramine being the most potent of all drugs tested. The efficacy of cocaine and desipramine was about 80 percent of that of tyramine. Desipramine and imipramine tended to produce much more gradual increases than the other drugs, often taking as long as fifteen minutes to reach maximum. Like the antihistamines, the antidepressants did not alter the spontaneous rate of beating. Cocaine and tyramine, however, did increase atrial rate. The amount of increase appeared to be dependent on the initial rate.

Propranolol (10^{-7}M) and pretreatment with reserpine (3 and 2 mg/kg i.p. forty-eight and twenty-four hours, respectively, prior to the experiment) abolished the positive inotropic effects of all drugs tested. Incubation of atria from reserpine pretreated animals in norepinephrine restored the response.

The results described thus far suggest that those drugs producing positive inotropic effects may do so by a mechanism involving the release of norepinephrine. However, since these drugs are also known to have a number of other properties (e.g., antihistaminic, beta blocking, anticholinergic) which could modify norepinephrine-induced effects, it was

desirable to determine more directly the extent of any amine release.

Attempts were made to measure drug-induced release of norepinephrine in isolated perfused whole hearts labeled with H^3-norepinephrine. Although an increased outflow of tritiated compound was observed when chlorpheniramine, tripelennamine, triprolidine, desipramine, cocaine and tyramine were administered, there were no concurrent increases in the force of contraction. In fact, the antihistamines markedly depressed cardiac contractility. Due to the inability to correlate increases in force with increase in H^3-norepinephrine efflux, and the possibility that myocardial depression might be responsible for releasing norepinephrine, use of the isolated whole heart was discontinued.

Isolated atria placed in a tissue bath that was perfused and drained at a constant rate were found to be more suitable for studying norepinephrine release. When drugs were administered to atria previously incubated in H^3-norepinephrine, it was found that chlorpheniramine, tripelennamine, triprolidine, and cocaine (all 3×10^{-5}M) produced an increase in the amount of radioactivity in the perfusate effluent that was associated with the positive inotropic effect. Each drug was tested in four separate atria. Statistical analysis of the results showed there to be no difference among the four drugs. However, this may not be realistic in view of the small number of animals used and the high degree of variability among atria for the response to a particular drug. All of these drugs were significantly less effective than 3×10^{-5}M tyramine, although the rate of the response and time course were similar. Promethazine produced no increase in the efflux of H^3-norepinephrine.

Desipramine (10^{-6}M) produced an increase in radioactivity that was much more gradual and significantly less in total amount than that obtained

with the antihistamines. In addition, the positive inotropic effects previously seen with this drug were absent.

The various drugs were tested in atria which had been incubated with C^{14}-urea. The purpose of this was to determine if increases in the efflux of radioactivity could have resulted from displacement of unbound norepinephrine caused by increased force of contraction. The results of these experiments showed that there was no change in the influx of C^{14}-urea associated with the increases in force of contraction and efflux of H^3-norepinephrine. In addition, calcium (2×10^{-6}M) was found to produce an increase in force that was not accompanied by an increase in H^3-norepinephrine outflow.

On the basis of this work, it would seem that cocaine and certain of the antihistamines and antidepressants exert their stimulatory actions on the heart by increasing in some way the amount of norepinephrine at the receptor. This could be effected through either a displacement of norepinephrine from storage granules or by an inhibition of the uptake of spontaneously released amine. Isaac and Goth (1965) proposed to distinguish between these two possibilities by administering antihistamines to intact rats after the injection of H^3-norepinephrine. Sympathetic discharge was blocked by prior treatment with a ganglionic blocking agent (chlorisondamine). Their study showed that the administration of antihistamines did not effect the heart H^3-norepinephrine content. The results were interpreted to mean that no H^3-norepinephrine release was occurring, but it seems that another possibility should be considered. The results of the present study show that the increase in efflux of the tritiated compound is small in comparison to the total radioactivity content of the atria. Therefore, it might not be possible to detect such small increases

by measuring the differences in heart radioactivity before and after treatment. The theoretical basis for Isaac and Goth's (1965) method of distinguishing between release and uptake also seems questionable. From the results shown in Figure 14, it is apparent that there is still some spontaneous release of H^3-norepinephrine even in the isolated atria. A similar observation has been made for isolated norepinephrine storage granules by Schümann and Philippu (1962). Although it would eliminate any norepinephrine release resulting from preganglionic sympathetic nerve stimulation, the blocking of sympathetic ganglia should not alter the spontaneous discharge of norepinephrine observed in isolated and denervated preparations. Therefore, whether they are actually releasing norepinephrine or merely blocking the uptake of spontaneously released amine, the antihistamines would be expected to cause a decrease in the radioactivity of H^3-norepinephrine labeled hearts. As pointed out previously, this decrease, expressed as a change in total heart H^3-norepinephrine content, might be too small to be measured accurately.

The present experiments do not positively differentiate between blockade of norepinephrine uptake and release, and a method for doing so is not readily apparent. It is proposed that if the compounds tested in these studies do cause release, they do so by utilizing the norepinephrine uptake system to gain access to the norepinephrine storage granules. Such a mechanism would involve a simultaneous inhibition of norepinephrine uptake. It is therefore quite difficult to determine the relative contribution of the two processes to the inotropic response and increase in amine efflux. What does seem possible is to make a qualitative assessment of the actions of the various drugs on isolated atria. Chlorpheniramine, tripelennamine and triprolidine produced increases in the amount of

radioactivity in the effluent. While the relative amounts of H^3-norepinephrine and metabolites were not determined, it appears that a certain amount was norepinephrine due to the marked positive inotropic effects. The time course of the increases in force of contraction and H^3-norepinephrine efflux obtained with the three antihistamines were similar to those observed for tyramine. Both effects were rapid in the onset and both reached their maximum levels in short periods of time.

Desipramine in the initial series of experiments required as long as fifteen minutes in the bath to develop to maximum effect on the force of contractions. In later experiments no positive inotropic effects were observed and there was only a slight increase in the efflux of radioactivity. It was confirmed that desipramine was blocking norepinephrine uptake in the atria by 83 percent. This leads to the conclusion that the gradual increase in efflux of radioactivity seen with desipramine may have been solely due to an inhibition of the uptake of the spontaneously released amine. Furthermore, in cases where uptake is blocked and the rate of spontaneous release is sufficient to produce a positive inotropic effect, the development of that effect would be slow as seen in the initial experiments with desipramine. It is possible that reported ability of desipramine to release norepinephrine from the heart (Titus, et al., 1966; Leitz and Stefano, 1970) may be due to use of concentrations greater than 10^{-6}M. The concentrations of desipramine used in those studies ranged from 2.5×10^{-5}M to 4.1×10^{-5}M. Doses in this range were found to be depressant to the atria.

Chlorpheniramine and tripelennamine have been previously shown to block norepinephrine uptake but the degree is less than that seen with desipramine (McNeill and Brody, 1968). Similar results were obtained in

these experiments with tripelennamine and triprolidine. It therefore seems unlikely that either tripelennamine, chlorpheniramine, or triprolidine could be exerting their actions solely through a blockade of norepinephrine uptake.

The fact that triprolidine was found to have no effect on norepinephrine or tyramine uptake in the rat heart (McNeill and Brody, 1968) might be explained on the basis of species variation and tissue specificity. In the dog heart, triprolidine potentiates the effect of norepinephrine on the force of contractions but not on heart rate or blood pressure (Johnson and Kahn, 1966). Similar variations have been reported for other antihistamines. Pyrilamine inhibits the effects of tyramine on the rate and force of contraction of the rabbit atrium (Osterberg and Kopannyi, 1969). In the rat, however, pyrilamine neither blocks norepinephrine uptake in the heart or atria nor potentiates the rate and pressor responses to the amine (Isaac and Goth, 1965a,b, 1967). Phenindamine is a potent blocker of norepinephrine and tyramine uptake but does not enhance norepinephrine-induced pressor and rate responses (Isaac and Goth, 1965a,b, 1967; McNeill and Brody, 1966, 1968). Promethazine has been reported to have no effect on either norepinephrine or tyramine uptake. In addition, it does not potentiate norepinephrine pressor and rate responses (Isaac and Goth, 1965a,b, 1967; McNeill and Brody, 1968). Although promethazine was found to inhibit H^3-norepinephrine uptake in the present study, it caused no positive inotropic effect and no increase in H^3-norepinephrine efflux. McNeill and Brody (1968), however, observed that promethazine did significantly enhance norepinephrine-induced cardiac phosphorylase activation. They postulated that the norepinephrine-potentiating effect of promethazine might be limited to this enzyme system.

It seems possible that there might be both tissue and species specificity for all these drugs that have different effects in different systems. In addition, there may be similar variability with regard to sensitivity to other actions of these drugs. This is of importance because some of the actions, such as depression of excitable tissue and blockade of beta receptors, could modify norepinephrine-mediated responses.

Cocaine has long been known to enhance the cardiovascular effects of norepinephrine. Several theories have been put forth to account for this, but current evidence seems to indicate that cocaine acts either by blocking norepinephrine uptake (Whitby, et al., 1960; Hertting, et al., 1961; Farrant, 1963; Furchgott, et al., 1963; Iversen, 1964; Cervoni, et al., 1966) or by facilitating the interaction of the amine with receptors (Maxwell, et al., 1958; Maxwell, et al., 1962; Maxwell, et al., 1966; Nakatsu and Reiffenstein, 1968; Reiffenstein, 1968).

In addition, cocaine has recently been shown to have certain stimulating properties of its own (Furchgott, et al., 1963; Cervoni, et al., 1966; Maegwyn-Davis and Koppanyi, 1966; Reiffenstein, 1968; Trendelenburg, 1968; Vohra, 1969). While it was suggested that these sympathomimetic actions might be due to a release of norepinephrine, this remained to be demonstrated. In the present study, cocaine was found to cause an increased efflux of H^3-norepinephrine in conjunction with a positive inotropic effect. This increase in amine outflow was substantially less than that observed with tyramine, although the two drugs were approximately equipotent and equally efficacious in their effects on force.

If both cocaine and tyramine are actually releasing norepinephrine, then these data would tend to support the idea of a cocaine-induced supersensitivity of beta receptors. Since both produce relatively the

same stimulation of contractility, one would expect to see similar amounts of norepinephrine release. The fact that cocaine produced only about one-tenth the increase in H^3-norepinephrine efflux seen with tyramine indicates that some other mechanism must be significantly contributing to the cocaine response. The current evidence for increased receptor sensitivity would seem to make this a likely explanation.

The results of this study suggest that a number of drugs previously known to block norepinephrine uptake in the heart may also be releasing the amine via a tyramine-like mechanism. Such a mechanism has been recently proposed for cocaine (Trendelenburg, 1968; Vohra, 1969) and desipramine (Titus, et al., 1966; Nash, et al., 1968; Leitz and Stefano, 1970). In addition to these two drugs, chlorpheniramine, tripelennamine and triprolidine were all found to produce marked positive inotropic effects in the isolated guinea pig right atrium. Concurrent with this effect was an increase in the efflux of H^3-norepinephrine. Another antihistamine, promethazine, was ineffective. While the present study does not distinguish between an inhibition of the uptake of spontaneously released amine and actual release, evidence does seem to favor the latter possibility for cocaine, chlorpheniramine, tripelennamine and triprolidine. Though desipramine has been demonstrated to release norepinephrine in other preparations, it did not appear to be doing so in the guinea pig atria at concentrations which produced increases in force. Rather, these increases in force and H^3-norepinephrine efflux seen with desipramine are felt to be due to a blockade of norepinephrine uptake.

CHAPTER V

SUMMARY

1. Tripelennamine, chlorpheniramine, brompheniramine, triprolidine, cocaine, desipramine and imipramine were found to produce marked positive inotropic effects in isolated, spontaneously beating guinea pig right atria. Promethazine was ineffective in this regard.

2. These positive inotropic effects were blocked by propranolol and prevented by reserpine pretreatment. The responses could be restored by incubation of the atria from reserpine treated animals in norepinephrine.

3. Tripelennamine, chlorpheniramine, triprolidine and cocaine produced sharp increases in the efflux of H^3-norepinephrine which were associated with the positive inotropic effects in atria. Desipramine produced a more gradual increase in H^3-norepinephrine outflow.

4. It is suggested that, in the guinea pig atrium, the antihistamines listed above and cocaine exert their stimulating actions by releasing norepinephrine in a tyramine-like manner while desipramine acts primarily by blocking amine uptake.

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