PRESSOR RESPONSES OF CERTAIN ADRENERGIC DRUGS IN HYPOTHERMIC RATS

BY

ROBERT LEWIS CAVANAGH

A thesis submitted in partial fulfillment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY
(Pharmacology)
at the
UNIVERSITY OF WISCONSIN

1967

ACKNOWLEDGMENTS

Appreciation is hereby expressed to:

Dr. Kenneth F. Finger for his continued interest, encouragement and guidance throughout the graduate training program of the author.

Wendy Cavanagh for her help in the preparation of this manuscript.

The Space Science Committee of the University of Wisconsin Graduate School under a grant from the National Aeronautics and Space Administration for their financial support.

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INTRODUCTION

Warm-blooded animals are able to maintain an almost constant body temperature despite environmental temperatures which may range from the +50°C heat of the tropical jungle to the -50°C cold of the polar regions. Since environmental temperatures in certain areas may fluctuate as much as 45°C in a single day, it is apparent that a constant temperature can be achieved only through regulatory mechanisms capable of rapid and sensitive response to environmental change. In order to maintain a constant body temperature, heat loss must be balanced by heat production and a variety of control mechanisms are required for this purpose. Thus, a constant body temperature may be maintained in an elevated environmental temperature by decreasing heat production mechanisms (oxidative processes) and/or by increasing cooling mechanisms such as vasodilation and vaporization of water. The body responds to a lowered environmental temperature by increasing oxidative processes leading to heat production and decreasing heat loss to the environment by such mechanisms as vasoconstriction and piloerection of hair.

However, the body temperature of the homeotherm can be altered by either exposing him to such an unfavorable environment that his control mechanisms are no longer

capable of maintaining his normal temperature or by inhibiting the control mechanisms. Frequently a combination of these two methods is used to alter body temperature both for therapeutic purposes and in order to gain a better understanding of the mechanisms involved in thermoregulation. The remainder of this discussion will be devoted to the mechanisms involved in maintaining euthermia when an animal is exposed to a cold environment.

Hypothermia. The term hypothermia was introduced by Talbott (1) in 1941 to signify the deliberate reduction in total body temperature of man for therapeutic purposes. However, since that time the term has been used more generally to describe the state of the homeothermic animal when his temperature is below normal, regardless of the cause. The terminology describing the various degrees of hypothermia is not clear, however, the following scheme has been suggested by Swan (2) and will be used in this discussion:

Moderate hypothermia	37-28°C
Intermediate hypothermia	28-20°C
Deep hypothermia	20-0°C

Moderate hypothermia permits a reduction of metabolism by about 50% without the danger of ventricular fibrillation. If cooling is deepened into the intermediate range, there is a further reduction in metabolism to about 25% of normal but ventricular fibrillation is almost certain to develop unless inhibited by some specific means. When deep

hypothermia is obtained in homeothermic animals spontaneous rewarming becomes impossible and cardiac activity ceases (2).

Historical Aspects of Hypothermia. Hypothermia has been utilized in medical therapy since ancient times. Because of its analgesic properties and its apparent reduction of hemorrhage, Hippocrates recommended application of cold water or ice for various injuries. During the Renaissance fevers were treated with cold drenching. The first recorded patient to undergo therapeutic general hypothermia was Richard Sutton, Esq. of Liverpool. His physician, Dr. James Currie, reduced his temperature to 83°F by keeping him in a salt water bath for a period of 45 minutes (2). In the Napoleonic era local cooling of the tissues was successfully used for amputations during the retreat from Moscow (3). However, it was not until 1940 that the first major study of the possible clinical value of general hypothermia was made. Smith and Fey (4) were able to maintain more than 100 patients moderately hypothermic for periods as long as eight days and thus demonstrated conclusively the tolerance of man to prolonged temperature reduction.

This entire field of investigation was revitalized by Bigelow, et al. (5) in 1950. They demonstrated that dogs could tolerate temperatures of 20-25°C with cessation of circulation for 15 minutes. The most dramatic use of cold has been in cardiac surgery where opening of the heart

is a procedure limited as to time (from three to eight minutes before there is damage to the central nervous system and risk of life) unless some provision can be made for an alternate circulating system, or a reduction of metabolic requirements. Hypothermia has made possible open heart surgery by direct vision. In addition to cardiac operations, hypothermia, as an adjunct to anesthesia, has also found value in thoracic, vascular, central nervous system, liver and other abdominal operations (6).

Thermoregulation. The process by which animals maintain body temperature in response to cold is called thermogenesis. This phenomenon can be divided into shivering and nonshivering components. Nonshivering thermogenesis may be further subdivided into obligatory and regulatory components (7).

Obligatory nonshivering thermogenesis is the process by which heat is produced to supply the minimal thermal requirements of the animal. It is synonomous with the basal or resting metabolic rate. The magnitude of this response is directly proportional to the concentration of circulating thyroid hormone and, therefore, is related to thyroid activity. Recently it has been shown that it is the concentration of unbound or free thyroxin that is important (8).

Regulatory nonshivering thermogenesis is the process by which heat is produced in response to a lowering of

environmental temperature below some critical level (about 20-30°C for rats) (7). Warm adapted rats, rats raised at 28°C, respond to cold exposure by increasing heat production (shivering) but this is insufficient to maintain their body temperatures if muscular activity is prevented (9,10,11). If the rats are "cold adapted" by placing them in a room at 4°C for a period of time, the potential for regulatory nonshivering heat production is greatly increased and these "cold adapted" rats rely on this process to the exclusion of shivering (7). It has also been shown by a number of investigators (12) that in maintaining a constant body temperature the neonate relies primarily on nonshivering thermogenesis at the exclusion of shivering and in this respect, temperature regulation of the neonate resembles that of the cold adapted rat (13, 14). Thus, within 4 weeks, nonshivering thermogenesis, the prevailing mechanism of heat production in the newborn guinea pig almost totally disappears if the animals are reared at neutral temperatures (30-32°C). Similarly, but in a reverse manner in adult animals, cold exposure results in shivering when an animal is first exposed but nonshivering heat production predominates after several weeks of exposure (7). The processes which bring about these changes in thermoregulation are now under investigation (12).

The role of the thyroid in the regulation of obligatory and regulatory heat production has been studied exten-

sively. Although evidence indicates that thyroid activity controls obligatory heat production, it appears to have little control over regulatory heat production. Thus thyroidectomized rats are still capable of responding to cold stimulus by an increase in nonshivering heat production (15) and cold exposed rats do not become hyperthyroid nor are they hypersensitive to thyroid hormones (16,17). In fact, experiments of Hsieh (7,16,17) indicate that chronically cold exposed rats are not hyperthyroid, if anything they may be hypothyroid. It is the view of Hsieh that the level of circulating thyroid hormone plays an important part in determining the characteristics of the biological engine, but it is the sympathetic nervous system that is in control.

Role of the Sympathetic Nervous System in Thermogenesis. Rats exposed to a cold environment excrete large amounts of norepinephrine (NE) and lesser quantities of epinephrine (18,19,20,21). Johnson (19) has shown that at 4°C inhibition of dopa (dihydroxyphenyl alanine) decarboxylase resulted in a decreased excretion of NE, however, larger amounts of epinephrine were excreted and the animal was able to maintain heat production. This increased production of epinephrine was referred to by Johnson as a "secondary mechanism" and its importance was emphasized by noting that adrenalectomized animals treated with a dopa decarboxylase inhibitor failed to survive three days

exposure at 4°C.

Gilgen et al. (22) have shown that the survival time of adrenal demedullated rats exposed to 4°C was markedly reduced relative to control animals. From oxygen consumption data, these investigators showed that the heat production of the demedullated rats was only about 25% of the heat production of the normal animal. Examination of plasma glucose and free fatty acid levels in the demedullated rats exposed to cold showed no increase in plasma glucose, yet free fatty acid levels were close to normal values. However, if the missing hormone (epinephrine) was administered to the animals, the plasma glucose levels returned to normal and the animals' ability to withstand the cold was restored. It is well known that cold-stressed animals respond by mobilizing fatty acids (22,23, 24,25) and it has been shown also that NE infusions raise plasma free fatty acid levels (26). In addition Wertheimer et al. (27) found that the rise in fatty acids on cold exposure could be blocked by adrenergic blockade. These results were interpreted to indicate that hypermobilization of free fatty acids in response to cold stimulus was mediated by the release of NE at the nerve endings in adipose tissue while the adrenal medullary hormones were responsible for the hypermobilization of glucose. This interpretation was supported by the finding that when adrenal demedullated rats were pretreated with a ganglionic

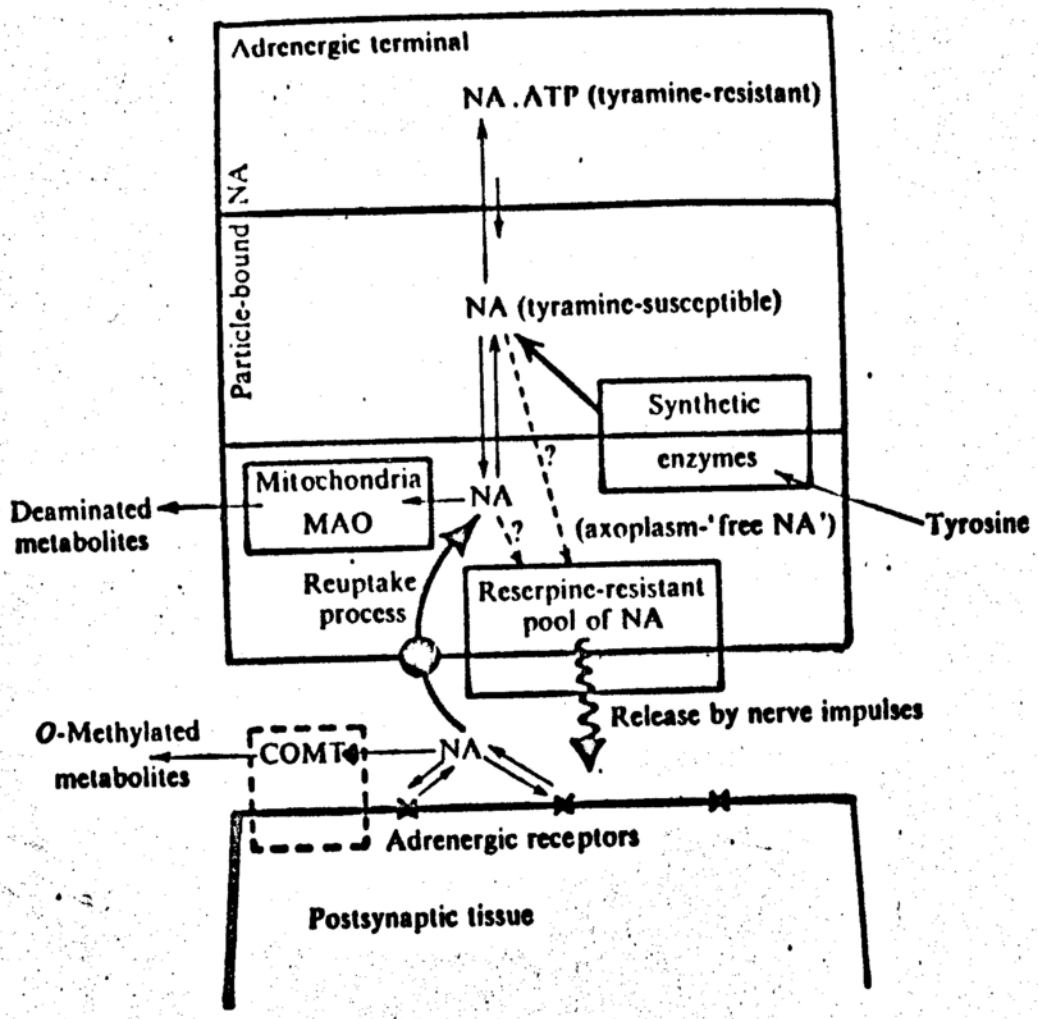
blocking agent and then exposed to cold, their body temperatures fell rapidly and their mean survival time was reduced. There was no significant increase in plasma levels of either glucose or free fatty acids indicating that adrenal demedullation together with ganglionic blockade prevented the mobilization of both energy substrates (24).

It appears that the sympathetic nervous system is the major mechanism by which the homeothermic mammal is capable of mobilizing increased amounts of metabolic substrates in response to an increased need for thermal energy. Furthermore, release of NE from the sympathetic nerve endings in vascular tissues and release of catecholamines, primarily from the adrenal medulla both contribute to heat conservation through vasoconstriction and piloerection (24).

The Adrenergic Synapse. In recent years numerous models of the adrenergic synapse have been proposed, indicating that its exact nature is far from settled. Figure 1, a model proposed by Iverson (30), is based on much of the evidence to be presented in this discussion.

NE is now considered to be the major neurochemical transmitter released from sympathetic nerves (28). The history of the development of this concept and the more recent evidence which substantiates such a claim has been reviewed by a number of authors (29,30) and will not

Figure 1. Schematic representation of norepinephrine stores in an adrenergic terminal. For explanation see text.


Adrenergic terminal
NA . ATP (tyramine-resistant)
NA
Particle-bound
NA (tyramine-susceptible)
Synthetic
enzymes
Mitochondria
MAO
Deaminated
metabolites
NA
?
?
(axoplasm-'free NA')
Tyrosine
Reuptake
process
Reserpine-resistant
pool of NA
Release by nerve impulses
O-Methylated
metabolites
COMT
NA
Adrenergic receptors
Postsynaptic tissue

be discussed here. It is well to bear in mind that the adrenergic synapse is a highly integrated functional unit which consists of many complex anatomical structures and systems within these structures (see Figure 1). In order to gain some insight into the function of this unit the following systems will be considered.

1. The presynaptic terminal contains intracellular storage particles, the biochemical machinery concerned with the synthesis and degradation of the neurotransmitter, and the biochemical or biophysical machinery necessary for the release and uptake of the transmitter.

2. The specialized region of the post synaptic cell (receptors) which is in close proximity to the presynaptic terminal.

Presynaptic Terminal. The existence of specific intracellular storage granules for catecholamines was first demonstrated for adrenal medullary tissue in 1953. That year Blaschko (31) and Hillarp (32) independently demonstrated the localization of pressor amines in intracellular granules from the chromaffin cells of the bovine adrenal medulla. These investigators showed that the granules store 70-90% of the hormones. Electron microscopic studies of adrenal medullary tissue showed densely osmiophilic granules nearly 0.2 u in diameter. These granules were surrounded by a membrane and were easily distinguished from other cell organelles (33). They can be isolated by

differential centrifugation and separated from other cell particles such as mitochondria and microsomes by density-gradient centrifugation. When granules are separated in this manner, they show a double layered membrane surrounding the filaceous ground structure (34).

In addition to catecholamines these granules contain large amounts of adenosine triphosphate (ATP), proteins, lipids and ribonucleic acid (RNA) (35,36,37). It has been shown that the molar ratio of catecholamine to ATP in various species is approximately 4:1 (38). The positive charges of the catecholamines are balanced by the sum of the negative charges of the adenine nucleotide (34). However, Schumann (34) has observed that after osmotic lysis of medullary granules of cattle, all of the ATP is released but only 90% of the catecholamines are released. This evidence and the fact that other investigators find amine-ATP ratios higher than 4:1 argues in favor of a second storage complex within the medullary granule.

Hillarp (35) and Schumann (34) have shown that the granules can be stored at 0°C in isotonic sucrose for several days without losing their catecholamine and ATP content. These findings, and investigations in vitro on catecholamine-nucleotide complexes by nuclear magnetic resonance spectroscopy (39), have led to the hypothesis that catecholamines form together with ATP, and perhaps with intragranular protein as a third component, a non-

diffusible storage-complex within the granule (40). This theory is supported by evidence of other investigators (41,42) who have shown that at 0°C the granules did not exchange their epinephrine with isotopically labeled epinephrine. In addition Prusoff et al. (43) were able to demonstrate that the incorporation of P^{32} into the ATP of the medullary granules is a very slow process in vivo in contrast to that of mitochondria.

Although the storage of catecholamines within the granule is probably independent of metabolic processes, the uptake of these amines appears to be strongly dependent on such processes. Carlsson et al. (42) and Kirshner (44) have shown that the rate of amine uptake increases with the temperature and can be accelerated 5 to 6 times by the addition of ATP and magnesium. Kirshner (41,44) has also demonstrated that the interior surface of the granular membrane shows dopamine-β-hydroxylase activity, indicating that the biosynthesis of NE from dopamine may take place within the storage granule. Thus, the mechanism of uptake of exogenous catecholamines is of importance for the formation of NE. It provides the granules with dopamine, as this amine is taken up to about the same extent as NE and epinephrine. The uptake can be inhibited by small doses of reserpine but is unaffected by ouabain, which has been shown to block transport mechanisms (42,44).

Whether or not an active mechanism for the release

of catecholamines from the storage granule also exists is not yet settled. However, there is much experimental evidence indicating that stored amines are released by a passive diffusional process. In this regard, Schumann (34) in incubation experiments with isolated medullary granules has shown that calcium ions release catecholamines and ATP to the same percentage extent at 37°C as at 0°C. These investigators have also demonstrated that tyramine releases catecholamines from the intragranular pool into the suspension medium. Since ATP is not released, Schumann reasons that there is no available energy for an active transport. Further investigations (34) have shown that during incubation of the granules at 37°C there is also a spontaneous release of catecholamines together with ATP. Since this release is temperature dependent (i.e. it occurs only to a limited extent at 0°C) Schumann (34) has proposed that the release is initiated by an enzymic decomposition of the storage complex and thereafter the amines are released by diffusion.

Studies to prove the possible role of other components in the storage of the ATP-catecholamine complex have shown that during incubation of medullary granules at 37°C, the spontaneous release of amines and ATP is accompanied by a loss of RNA (37). Also, incubation of the granules with purified RNAase preparation produced an increased and dose dependent release of amines, ATP, and RNA to the same degree but did not change the protein content (34). These

investigators (34,37) feel that the depolymerizing effect of the added enzyme on RNA is connected with the release of amines and ATP. They have investigated pure medullary granules obtained from a discontinuous density gradient centrifugation and found that the granular fraction contained sufficient RNAase activity to catalyze the spontaneous release of RNA, catecholamines and ATP at 37°C.

Calcium has also been observed to liberate catecholamines from the intact medullary cell (45). Schumann (34) has shown that amines, ATP, calcium and magnesium are spontaneously released at 37°C while at 0°C none of these substances are liberated. Further work with calcium and magnesium (34) has demonstrated that these bivalent ions are released only if ATP is liberated simultaneously as in the presence of RNAase, prenylamine and reserpine. Tyramine, on the other hand, releases catecholamines but no ATP and therefore no calcium and magnesium. These results are interpreted by Schumann (34) to indicate that bivalent ions as well as RNA or a ribonucleoprotein may participate in the formation of the storage complex.

In 1956 Euler and Hillarp (46) demonstrated the existence of similar catecholamine-storing particles in bovine splenic nerves and in the rat spleen. These granules were found to have a number of properties similar to those found in medullary granules. Thus, they contain large amounts of ATP and again the molar ratio of amine to ATP

is approximately 4:1 (47). Furthermore, the nerve granule membrane is freely permeable to catecholamines at 0°C (48) and the amine uptake mechanism in nerve granules is potentiated by ATP (49) and inhibited by reserpine (50,51), in agreement with previous observations with medullary granules. However, the nerve granules have been found to differ from medullary granules in many respects. They are smaller and more resistant to osmotic changes and to freezing and thawing than the medullary granules (48,52). When incubated in vitro at 0°C they are quite stable, however, at 37°C they lose about 50% of the amines in about ten minutes, indicating a much higher spontaneous release rate than is found with the medullary granules (48,52). Reserpine, known to deplete NE from sympathetically innervated tissues in vivo, surprisingly enough retards the NE release from the nerve granules when incubated in vitro at low concentrations (53). It has also been shown that the catecholamine turnover rate, in vitro, is about ten times higher in the nerve than in the medullary granules (48). Calcium ions had practically no effect on nerve or heart granules (54), in sharp contrast to their action on the medullary granule.

Stjarne (54) has compared the properties of nerve granules isolated from different parts of the neuron and in different tissues and has concluded that with minor exceptions they are similar. However, he warns that it

is as yet premature to state flatly that all kinds of granules in postganglionic sympathetic neurons have identical properties, and differ from those granules of chromaffin cells. Nevertheless, the practice of extrapolating data from chromaffin granules to nerve granules should be abolished since new techniques have made the isolation of nerve granules a relatively simple procedure.

Role of Granules in Norepinephrine Biosynthesis. Dopamine-β-hydroxylase activity has been demonstrated in both rat heart granules (55) and medullary granules (56) indicating that the granules are capable of synthesizing NE from dopamine. However, incubation of nerve granules with tyrosine did not result in the formation of either dopamine or NE (57) although whole nerve homogenates were capable of accomplishing this synthesis (58). These experiments suggest that tyrosine is converted to dopa within the axonal cytoplasm and that the nerve granules are the sites for converting dopa to dopamine and NE (54).

It has also been shown that reserpine inhibits the formation of NE from dopa on incubation of isolated nerve granules (54). These results support the concept that inhibition of NE synthesis may be one of the mechanisms whereby reserpine causes NE depletion (59,60).

Other Norepinephrine Storage Pools. The question as to whether or not other storage pools of NE exist in the

adrenergic nerve terminal has been raised by many investigators. In this regard, it has been found that no more than 77% of the NE of the rat heart (55) or 35% of the NE in splenic "press juice" (52) has been isolated in small particles. The question is whether or not the NE recovered from this high speed supernatant from various tissues corresponds to an extragranular pool of NE in vivo, or if it represents an artifact due to leakage during the homogenization procedure. Prolonged homogenization permits better cellular disruption but also decreases the yield of amine-containing particles(61) indicating that the recovery of NE in particle bound form is clearly dependent on the technique used. However, Iversen (30) has compared the published values for the subcellular distribution of exogenous and endogenous NE in the hearts of various species and has found that several different laboratories have arrived at consistent and closely similar estimates of the ratio of particle-bound to free NE in adrenergic nerves. If the release of particle bound catecholamine into the homogenizing medium were entirely responsible for the existence of supernatant NE, one might expect widely divergent results from different laboratories. Since this was not the case, it may be taken as evidence for the existence of other storage forms in adrenergic nerves (30).

"Compartments" of NE have also been suggested on the

basis of differential drug responses. Depletion of NE stores with tyramine has led to the hypothesis of two stores of NE, one which is readily released by tyramine and the other which is released only when large doses of tyramine are administered. This second store is often referred to as the tyramine resistant store (see Figure 1). Available evidence indicates that these stores of NE are not necessarily two clearly distinguishable pools but rather may be two different storage forms existing within the nerve granules. Evidence for this hypothesis is presented in a recent review on this subject (30). Trendelenburg (62) showed that the diminished responsiveness to tyramine after administration of reserpine could not be observed until a large portion of stored NE had been depleted by reserpine and first suggested that reserpine initially depletes a store which is not important for the action of tyramine. Uptake studies provide further evidence that NE is not stored in a single storage pool in adrenergic nerves. A number of investigators (63,64,65) have demonstrated that following uptake into the heart, either in vivo or in vitro, a multiphasic decrease in the content of NE-H^3 was observed, indicating the presence of at least two pools of NE, each possessing different turnover rates.

It has been shown by many investigators that the rate of reserpine induced depletion of NE in nerve terminals is

dependent on the impulse flow in such nerves. Thus, the disappearance of NE can be delayed by sympathetic decentralization, acute postganglionic denervation or ganglionic blocking drugs (30). Sedvall (66) has noted a two phase disappearance of NE from tissue after treatment with reserpine in the preganglionic denervated gastrocnemius muscle of the cat. Some 85% of the total NE disappeared within the first five hours while the remaining 15% disappeared slowly over the next 19 hours. This second slower phase of disappearance could also be observed in normally innervated muscles. These results have been interpreted to suggest the existence of two storage forms of NE in sympathetic nerves; one a large pool which contains about 85% of the total catecholamine and which is rapidly depleted by reserpine, the remaining 15% represents a reserpine-resistant pool which could be released by nerve stimulation.

Evidence for the existence of an extraneuronal pool of NE which is also resistant to reserpine has been provided by Fischer et al. (68). These investigators have found that in the chronically denervated rat salivary gland, small amounts of NE-H^3 are still accumulated and retained in a store which cannot be depleted by reserpine but which can be partially depleted by tyramine. The authors suggest that it is the filling of this pool which

is responsible for the restoration of tyramine responses in reserpinized tissues on exposure to NE. Further evidence which argues in favor of such a pool may be found in the recent review of this subject by Iversen (30).

In the model in Figure 1 it can be seen that the main stores of NE in the sympathetic nerve terminal are considered to be in the particles, although the exact ratio of total amine to particle bound amine is not known. It is possible that the norepinephrine in the particle is stored in two forms, a fully bound ATP complex and a form which is more readily released by tyramine and is in equilibrium with the extragranular axoplasmic fluid.

Release of Norepinephrine from Adrenergic Nerves. The stimulation of adrenergic nerves causes a release of NE from the terminal regions which then diffuses to adjacent effector cells. Very little is known of the mechanism by which nerve impulses promote the release of NE from adrenergic nerves, however, the primary event that leads to transmitter release is a depolarization of the presynaptic terminal as a result or the action potential wave of the invading nerve impulse. It has been suggested (69) that this depolarization may be followed by an increase in the number of attachment sites on the inner surface of the presynaptic fibre for the preformed packets of transmitter; the transmitter packets attach to these sites and discharge their contents to the exterior into the

synaptic cleft. Kopin (70) in his review of this subject has noted that when an impulse reaches the area from which NE will be released, a chain of events occurs leading to its release. The intermediary steps could involve release of substances other than NE. Thus bretylium, a quarternary amine which is taken up by sympathetic nerves, is capable of blocking the effects of nervous stimulation without interfering with the conduction of the impulse. Furthermore, a substance can be released by nerve stimulation (70) but does not appear to be a false neurotransmitter. From this and other evidence (70) it is apparent that bretylium replaces something other than NE. Burn and Rand (71) have postulated that a cholinergic compound serves as a link between the nerve impulse and the release of the adrenergic transmitter. Although this theory is still highly controversial, it has been proposed that bretylium could act by interfering with this cholinergic compound and thus block transmission (70).

One concept of the mechanism of release of NE from the adrenergic nerves is diagrammed in Figure 1. This figure shows that when NE is released from the terminal in response to nerve impulses, it is released from a small readily available pool which may be resistant to depletion by reserpine; this pool is normally replenished from the larger reservoir of reserpine releasable NE.

Uptake of Norepinephrine. Sympathetic nerves have the

ability to take up and retain large amounts of NE and it appears to be the result of an active transfer process (72,73) quite different from intraneuronal binding. A number of investigators (72,74) have found that the uptake of NE-H^3 in the cat heart and brain slices was saturated at high concentrations of NE, suggesting that the uptake of NE was mediated by a saturable membrane transport process. Iversen (75) found that data from infusion experiments agreed well with the classical Michaelis-Menton equation used to describe saturable enzyme/substrate interactions. Thus values for the maximum rates of NE uptake (Vmax) and the dissociation constant for the interaction between NE and the uptake site (Km) were calculated. These data indicated that the uptake process shows structural and stereochemical specificity in that norepinephrine is taken up more rapidly than its N-substituted derivatives and the l-isomers of NE and epinephrine are accumulated more rapidly than the D-isomers.

Several drugs (eg. cocaine) interfere with this process but do not affect intraneuronal binding to the catecholamine storage sites. On the other hand, reserpine does not appear to interfere greatly with uptake but does interfere with intraneuronal storage (70). Thus it has been proposed that catecholamine uptake is mediated by a membrane transport mechanism located in the axonal membrane of postganglionic sympathetic neurons (30). See Figure 1.

Catecholamine Synthesis. The main pathway for the biosynthesis of catecholamines in animal tissues is now known to be: L-tyrosine ⟶ L-DOPA ⟶ L-dopamine ⟶ L-norepinephrine ⟶ L-epinephrine. This series of reactions was first shown to take place in the adrenal medulla and later the synthesis of NE was demonstrated in peripheral tissues, sympathetic ganglia and axons, and in brain. The rate limiting step appears to be the conversion of tyrosine to DOPA which is catalyzed by tyrosine hydroxylase, see Figure 1. The major portion of the work elucidating this pathway has been done in the last decade and there are a number of reviews on this subject (30).

Catecholamine Metabolism. The excretion of deaminated catechols and O-methylated catecholamines indicated that there were at least two main pathways of catecholamine metabolism. Thus, when NE-H^3 is administered to an animal, a large portion is excreted or rapidly destroyed by O-methylation in the liver and kidney. Catechol-O-methyl transferase (COMT) has been shown to occur in high concentrations in both of these organs (76) and is the enzyme responsible for this inactivation. However, unlike epinephrine which is almost totally metabolized by COMT (77), a fraction of the labeled NE-H^3 is taken into tissues to be released and metabolized more slowly (78,79,80). This portion is destroyed mainly by oxidative deamination by monoamine oxidase (MAO) (79). Radioautography (81,82)

has provided direct evidence for the presence of NE-H^3 in sympathetic nerves and it is believed that NE-H^3 which is taken up by the sympathetic nerves may be considered a valid tracer of endogenous NE.

Reserpine markedly increases excretion of deaminated catechols (83) while tyramine releases unoxidised NE into the circulation. This unoxidised NE, like the injected neurohumor is mainly O-methylated (79,83).

Therefore, it appears that MAO is largely responsible for intraneuronal destruction of NE while COMT acts on extraneuronal NE, either in the tissues or after entry into the circulation (70).

Adrenergic Receptors. The specialised region of the post-synaptic cell which is in intimate contact with the pre-synaptic nerve terminal has received relatively little attention due to the immense interest with uptake, storage, and release mechanisms in the adrenergic neuron. The most direct characterisation of the adrenergic receptors would be the actual isolation of the receptor system to allow precise analysis of drug receptor interaction. However, this has not been accomplished. Indirect methods have been used to characterize adrenergic receptors and most of the present knowledge concerning the characteristics of adrenergic receptors has been obtained from structure activity relationships of agonists and the specificity of blocking drugs (84).

NE, epinephrine and other catecholamines are capable of causing either excitement or inhibition of smooth muscle, depending primarily on the site and, to a lesser extent, on the dose. NE is the most potent excitatory catecholamine and possesses low activity as an inhibitor. Isoproternol exhibits the reverse pattern of activity while epinephrine is relatively potent as both an excitor and as an inhibitor of smooth muscle. On the basis of this type of information, Ahlquist (85) postulated the existence of alpha and beta receptors for adrenoceptive sites on smooth muscles where catecholamines produce excitation and inhibition, respectively. In this scheme, NE was classified as an alpha stimulator, isoproterenol as a beta stimulator while epinephrine was shown to stimulate both alpha and beta receptors (86).

Adrenergic blocking agents have further aided in the characterization of adrenergic receptors. They have been particularly helpful since they can act selectively against either the excitatory or the inhibitory effects i.e. against either alpha or beta receptors. Thus the existence of both alpha and beta adrenergic receptors in the small intestine of the dog has been postulated by Ahlquist and Levy (87). They observed that the inhibitory effect of isoproterenol was blocked by DCI, a beta blocker, and not Dibozane, an alpha blocker, and that the inhibitory effect of NE was blocked by Dibozane and not by DCI. Furthermore,

the inhibitory effects of epinephrine were not completely blocked by either drug alone but were blocked when both drugs were given.

Current concepts in this area have recently been reviewed by Moran (84). In order to help explain the action of the agonist it is necessary to assume that there are constituents of cells, e.g. receptors, which react selectively with certain agonists. The agonist-receptor interaction represents the first step of a multi-step sequential reaction which leads to the response of the cell. Moran has represented this sequential reaction symbolically as: $A + R \longrightarrow AR \longrightarrow a \longrightarrow b \longrightarrow c \longrightarrow n \longrightarrow Ef$ where R is the receptor, a, b, c, n the sequential steps subsequent to the receptor, and Ef the effect. Although the individual steps in most reactions are unknown, Sutherland and Rall (88) have demonstrated that in certain tissues the initial reaction of catecholamines is to activate adenyl cyclase. Furthermore, they have described a stepwise series of reactions that terminate in the contraction of smooth muscle. The assumed relationship between the adrenergic receptor and the neuron is shown diagrammatically in Figure 1.

Because of the increasing therapeutic and other possible medical uses of hypothermia and the possibility of hypothermia becoming a factor in prolonged space travel, the present study was undertaken to elucidate some of its effects on the actions of certain adrenergic drugs.

EXPERIMENTAL

Materials. The chemicals used in this study and their sources are as follows: l-norepinephrine bitartrate (Nutritional Biochemical Corp.); tyramine hydrochloride (Mann Research Laboratories, Inc.); and chlorpromazine hydrochloride.[1]

Methods. Male Sprague-Dawley rats, weighing between 350 and 450 grams, were employed throughout this study. All animals were maintained in their animal quarters for at least one week prior to their use. Purina Lab Chow and tap water were available to them at all times during this period.

The animals were anesthetized with urethane (approximately 1.5 Gm./Kg. i.p.) which was administered in divided doses. The femoral vein was isolated and cannulated with PE 20 Intramedic Polyethylene Tubing, for purposes of drug administration. To facilitate breathing, the trachea was exposed and cannulated with a small piece of PE 240 Intramedic Polyethylene Tubing. The carotid artery was cannulated with PE 60 Intramedic Polyethylene Tubing to record blood pressure and heart rate. They were

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[1]The author thanks Smith, Kline and French Laboratories for the supplies of chlorpromazine used in these studies.

determined from tracings obtained with either the Grass Model 5 Polygraph or the E and M Physiograph both of which were equipped with a Statham P-23 transducer.

Animals were either maintained normothermic by the use of a warming element or allowed to become hypothermic by omitting the warming procedure. Hypothermia was also induced by placing the anesthetized animal in a cold room at 8°C for approximately 20 minutes. This treatment usually lowered the body temperature to about 30°C, after which the animal was removed from the cold room and placed on a warming element where body temperature was maintained at 30°C. All experiments were carried out in an air conditioned room which was maintained at 21-22°C. Rectal temperatures were monitored for all animals utilizing the Yellow Springs Instrument Co. Telethermometer.

In experiments designed to determine the effect of hypothermia on chlorpromazine-induced bradycardia and hypotension, control periods of sufficient length to assure a stabilized blood pressure and heart rate were continuously recorded prior to drug administration. Chlorpromazine, 0.5 mg./Kg., was administered intravenously in a volume of 0.1 ml./Kg. and control animals received 0.1 ml./Kg. of saline. Blood pressures and heart rates were monitored continuously for 20 minutes immediately following drug administration and thereafter for several minutes at 20 minute intervals for approximately 3 hours. At least six animals were employed in each group.

In experiments designed to study the effects of moderate hypothermia on the responses to exogenous amines, blood pressure and heart rate were monitored for approximately 20 minutes after the cooling procedure to assure that the animal had become stabilized. Since norepinephrine and tyramine were administered to the animals over a wide range of doses, various concentrations of each drug were made so that they could be administered in constant volumes. Drugs were infused slowly over a 15-20 second interval and the volume of drug administered during this period never exceeded 0.5 ml. Blood pressures and heart rates were monitored for approximately 1 minute before and 4 minutes after the administration of the drug. In instances where multiple doses were given to the same animal, compounds were administered at 15 minute intervals. In the chlorpromazine studies, compounds were not administered until at least 15 minutes after chlorpromazine had been given.

In both of the above studies at least four to six animals were employed in each group except in the tachyphylaxis experiments where 4 animals were used in each group. The data to be presented here are expressed as the mean value obtained $\pm$ the standard error of the mean.

For the determination of nonesterified fatty acids in plasma, the animals were treated in the manner described above for the cardiovascular experiments except that blood

samples were withdrawn via the carotid cannula and the plasma assayed for fatty acids by a modification of the method of Dole (89).

RESULTS AND DISCUSSION

Effect of Procedures on Body Temperature. The changes in body temperature produced by drug treatment and as a result of the experimental procedures are shown in Figure 2. These data indicate that the procedures employed produced a highly significant difference in rectal temperatures. It is also apparent from this figure that the use of the warming element maintained body temperature at a reasonably constant value, while those animals which were not warmed became hypothermic. These results are in agreement with those of other investigators who have shown that at ambient temperatures below 30°C, homeothermic animals are not capable of maintaining body temperatures if muscular activity is prevented (90).

Effect of Hypothermia on Blood Pressure. The effect of a sustained, moderate hypothermia on the systolic blood pressure of anesthetized rats is shown in Table I. Blood pressures were recorded after the animals had been maintained at 30°C for approximately 30 minutes. The results indicate that moderate hypothermia induced a mild hypertension. This is at variance with the results of some investigators who have reported that moderate hypothermia elicits either no change or a slight reduction in

Figure 2. Rectal temperatures of rats. Arrows indicate temperature data curves for groups of rats, the upper two curves representing groups maintained with a warming element and the lower two curves those which were not heated.

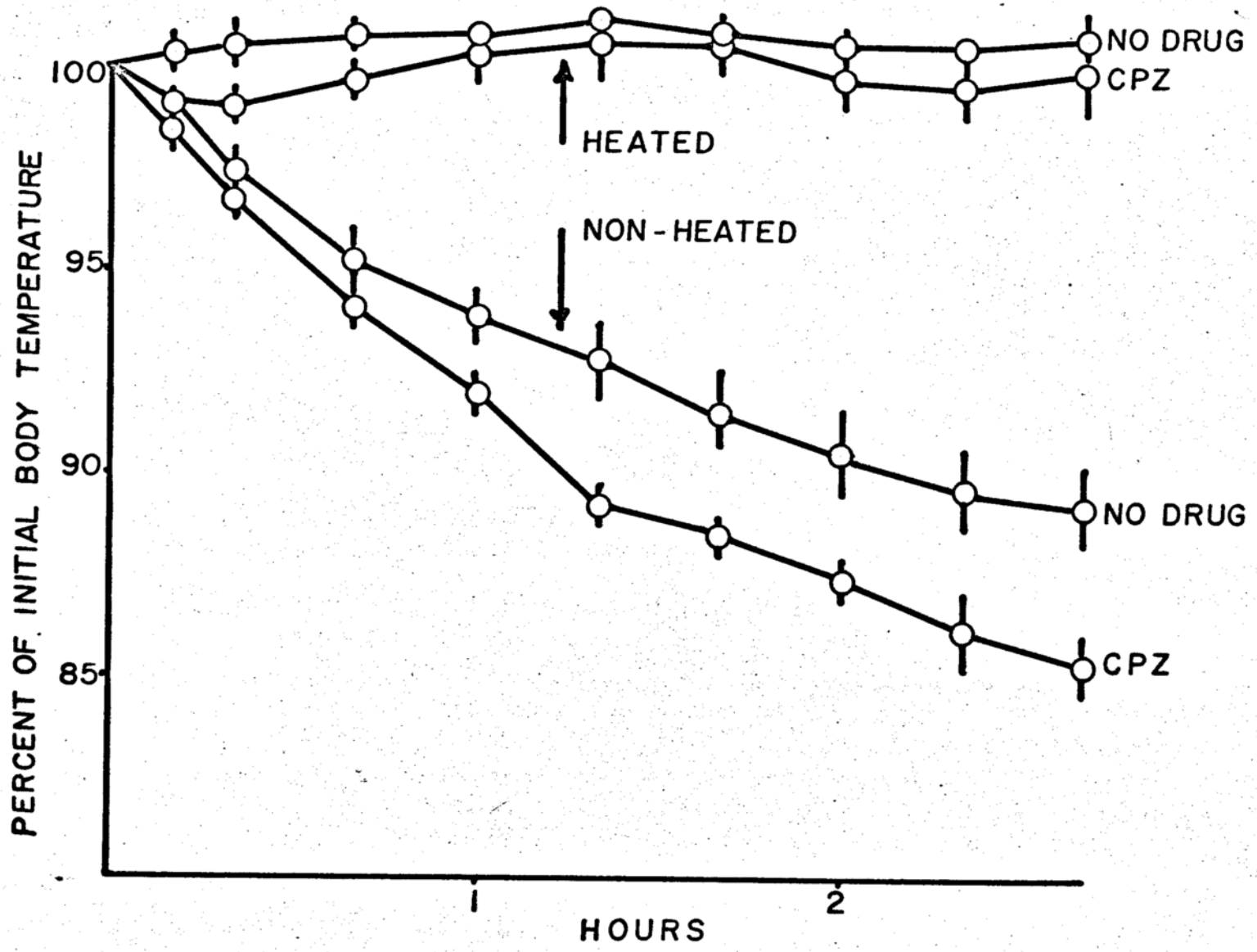

NO DRUG
CPZ
HEATED
NON-HEATED
NO DRUG
CPZ
100
95
90
85
PERCENT OF INITIAL BODY TEMPERATURE
1
2
HOURS

Table I

Effect of Body Temperature and Chlorpromazine (0.5 mg./Kg.) on the Systolic Blood Pressure of Anesthetized Male Rats

Treatment		Blood Pressure	
		mm. Hg.	
Normothermic	No Drug	110 ± 6.3 (40)	P > .001
Hypothermic	No Drug	155 ± 2.5 (36)	
Normothermic	Chlorpromazine	67 ± 1.8 (29)	P > .01
Hypothermic	Chlorpromazine	92 ± 8.1 (29)	

Figures in parentheses indicate the number of animals in each group. P values compare normothermic and hypothermic data.

blood pressure (91,92). The use of different anesthetics may offer a partial explanation for this discrepancy. Many investigators used pentobarbital, an anesthetic capable of producing a moderate hypotensive response (93) and in the present studies, urethane was employed as an anesthetic because its cardiovascular effects are much less pronounced and it is capable of maintaining a more uniform mean blood pressure than is pentobarbital (93). Furthermore, in the other investigations cited above, the animal's body temperature was reduced to 20°C or lower and data pertaining to the moderate hypothermic state were obtained while the animals were losing body heat. The animals in the present study were in a steady state with respect to body temperature, during the period of observation. Since the physiological activities occurring while losing body heat differ from those of animals in a steady state with regard to heat loss and heat production, comparing the data on the basis of body temperature only may not be meaningful.

A large but transient increase in blood pressure upon initiation of the cooling procedure, however, has been reported by many investigators (91,92). It has also been found that catecholamine excretions are grossly increased in non-adapted cold exposed animals (22,94). It can thus be assumed that a mild hypertension may be caused by an increase of epinephrine from the adrenal medulla and norepinephrine (NE) from sympathetic nerve

endings, both resulting from an increase in sympathetic nervous system activity.

Effect of Hypothermia on the Pressor Response of Exogenous Norepinephrine. It has been known for many years that the pressor responses to exogenous epinephrine are diminished in hypothermic animals (95) and more recently it has been shown that the response to many exogenous sympathomimetic amines is also reduced in hypothermic dogs (96). Since there appears to be an increased production but a decreased effectiveness of catecholamines in hypothermic animals, it was decided to quantitate the effects of lowered body temperature on the pressor responses to exogenous NE, the principle neurohumoral transmitter of the sympathetic nervous system.

The results of this study can be seen in Figure 3. Hypothermia antagonized the pressor response to low concentrations of exogenous NE, however, higher doses of NE overcame this antagonism. When these data are plotted, using the double reciprocal method of Lineweaver and Burke, the competitive nature of this antagonism is more evident. The data plotted in this manner are shown in Figure 4. Comparison of the ED_{50} for NE in normothermic and hypothermic animals indicated that hypothermia decreased the potency of NE about two and one-half times. In as much as the initial blood pressure of hypothermic rats was about 45 mm.Hg. above that of normothermic rats (see Table I),

Figure 3. Effect of hypothermia on the norepinephrine-induced increase in blood pressure in non-pretreated rats. Cross hatched bars indicate normothermic rats and black bars indicate hypothermic rats. Vertical lines indicate standard error of the mean. Each bar represents the mean of at least four to six experiments.

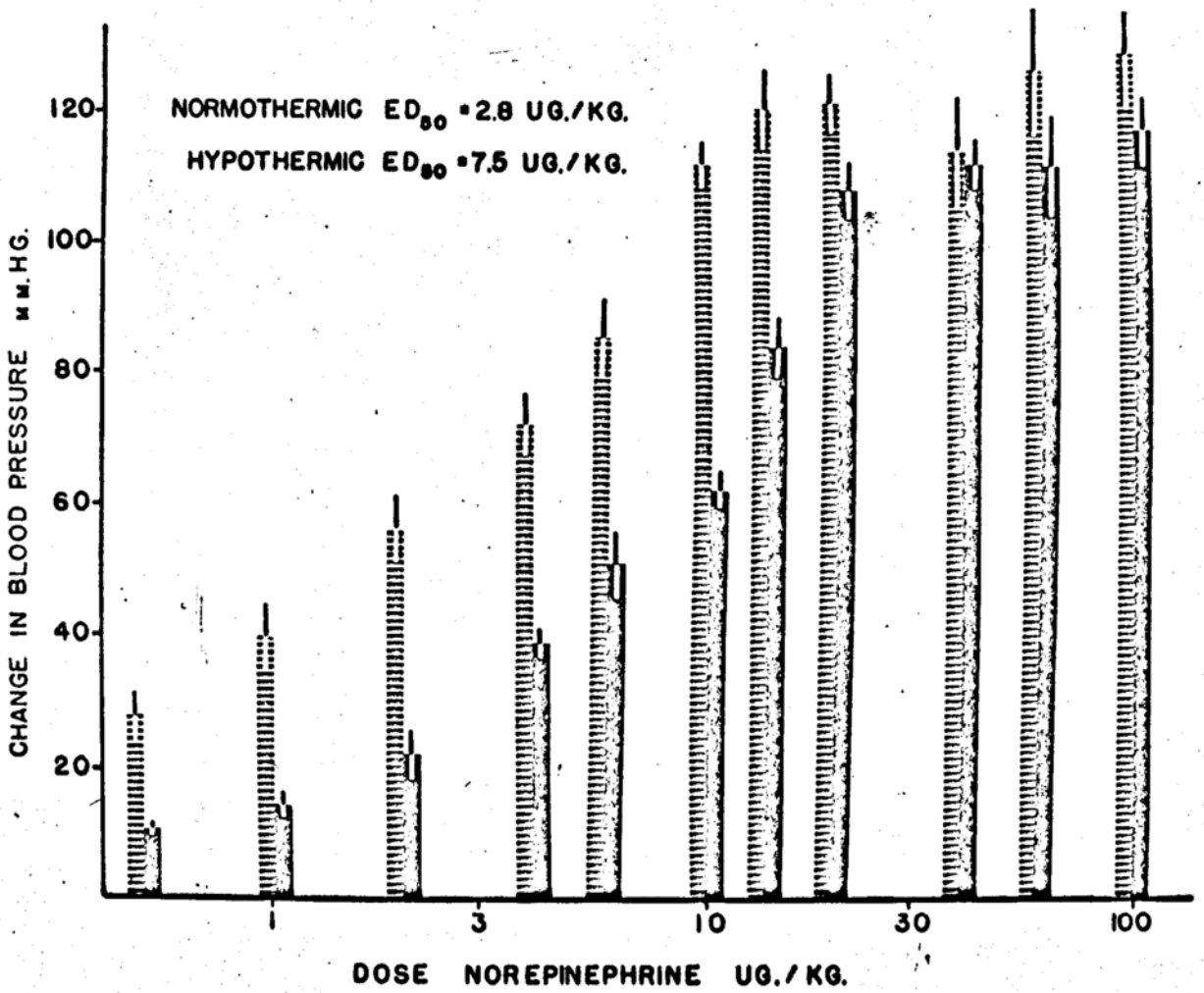
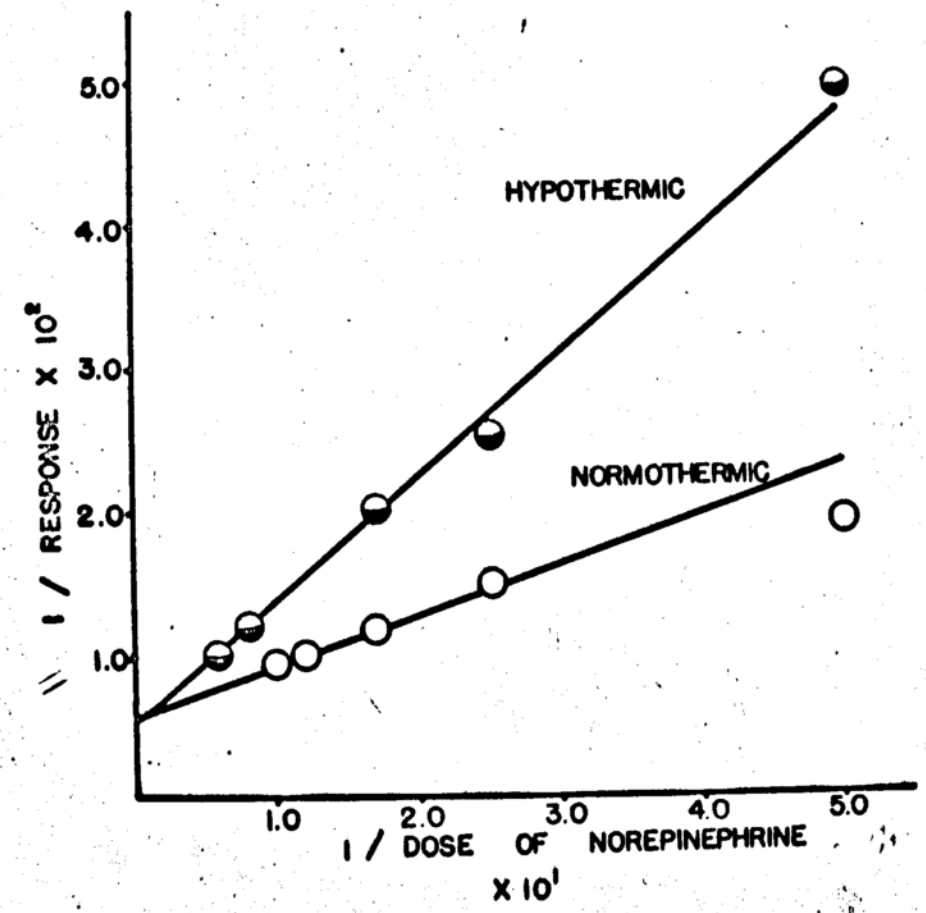

NORMOTHERMIC ED_{50} = 2.8 UG./KG.
HYPOTHERMIC ED_{50} = 7.5 UG./KG.
CHANGE IN BLOOD PRESSURE MM.HG.
120
100
80
60
40
20
1
3
10
30
100
DOSE NOREPINEPHRINE UG./KG.

Figure 4. Double reciprocal plot illustrating the effect of hypothermia on the norepinephrine-induced increase in blood pressure in nonpretreated rats.


5.0
4.0
3.0
2.0
1.0
1 / RESPONSE X 10²
HYPOTHERMIC
NORMOTHERMIC
1.0
2.0
3.0
4.0
5.0
1 / DOSE OF NOREPINEPHRINE
X 10¹

an equal response to administered NE in these two types of animals indicates that the maximum blood pressure obtainable in the hypothermic rats was higher than in the normothermic group (see Figure 3). Thus, the decreased responsiveness of the hypothermic animals is probably not due to differences in initial blood pressures. The reason for higher blood pressure responses in hypothermic animals is not completely understood, however, Furchgott (97), in a recent review, noted that a number of investigators presented mathematical evidence to indicate that a maximum response can occur before all the receptors are occupied. Spare receptors have been postulated and it may be that these are "uncovered" by the stress of hypothermia.

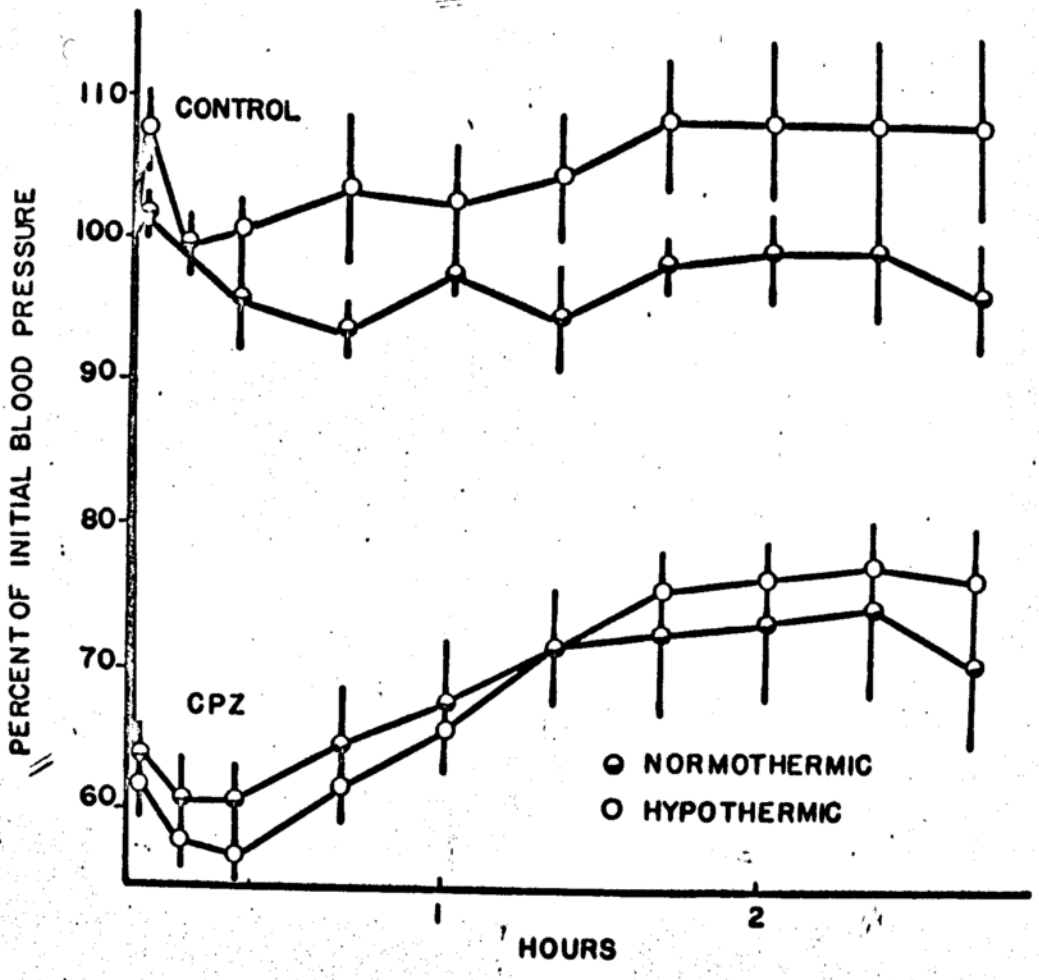
Effect of Chlorpromazine and Hypothermia on Blood Pressure. Chlorpromazine (CPZ), a compound possessing antiadrenergic properties (98), was used to elucidate the mechanisms by which hypothermia antagonized the pressor responses of exogenous NE. CPZ has been shown to act directly on the alpha adrenergic receptors, to inhibit the uptake of amines into the adrenergic neuron (30) and to antagonize the incorporation of amines into the nerve granule (42). The experimental findings of Costa et al. (99) and of Fletcher et al. (100) have shown that the release and/or uptake of biogenic amines is inhibited by CPZ in hypothermic animals but this effect is negated when the animals are maintained normothermic. Costa and co-workers (99) also demonstrated

that CPZ blocked the release of catecholamines by reserpine when the animals were hypothermic but that this action of reserpine was unaffected by CPZ in normothermic animals. Similarly, Fletcher et al. (100), studying the uptake of tryptamine as influenced by drugs, found that body temperature had an influence on CPZ's blockade of tryptamine uptake.

The effect of CPZ on the blood pressure of rats maintained at 30°C and on normothermic animals is shown in Table I. A similar degree of hypotension is noted in both groups indicating that the hypotensive effect of CPZ is not altered by hypothermia. In another experiment, animals were allowed to become hypothermic rather than being maintained at constant temperature. During the period covered in this study, body temperature dropped from 37°C to approximately 30°C. From Figure 5 it is evident that the hypotensive phase of CPZ's action was maintained throughout the period of experimental observation and that hypothermia had no observable effect on this action.

Comparison of the results shown in Table I and Figure 5, indicates that the hypotensive action of CPZ is the same in animals which are losing body heat as it is when animals are maintained either hypothermic or normothermic. Table I shows that CPZ induced a 40% decrease in blood pressure in normothermic and a 41% decrease in hypothermic animals. Figure 5 indicates that CPZ lowered blood pressures to about 60% of their initial values in

Figure 5. Effect of chlorpromazine on blood pressure of hypothermic and normothermic rats. Vertical lines indicate standard error of the mean. Each point represents the mean of at least six experiments.


110
100
90
80
70
60
CONTROL
CPZ
PERCENT OF INITIAL BLOOD PRESSURE
NORMOTHERMIC
HYPOTHERMIC
1
2
HOURS

both normothermic and hypothermic groups. Furthermore, CPZ appears to have little effect on hypothermia-induced hypertension. In control animals hypothermia caused a 40% increase in blood pressure while a 27% increase was noted in the CPZ group.

Effect of Chlorpromazine and Hypothermia on Norepinephrine Induced Pressor Responses. Figure 6 shows that CPZ caused a decrease in the pressor response to administered NE in normothermic animals. It is also apparent from the graph that higher concentrations of NE were not capable of completely overcoming the antagonism of CPZ. The maximum response to NE being slightly less in the CPZ treated animals indicates that CPZ may have caused a noncompetitive type of antagonism of the adrenergic response.

In a similar manner, CPZ seems to antagonize the pressor response to NE in hypothermic animals as is shown in Figure 7. Comparison of these data indicates that CPZ reduced the potency of NE to a slightly greater extent in hypothermic animals than was noted in normothermic animals, however, the curves do not indicate whether or not this antagonism was competitive. A Lineweaver-Burke plot of these data (Figure 8), indicates that the hypothermia-induced antagonism of the NE response in CPZ treated animals is competitive.

These results support the conclusion that CPZ is an

Figure 6. Effect of chlorpromazine on norepinephrine-induced increase in blood pressure in normothermic rats. Vertical lines indicate standard error of the mean. Each point represents the mean of at least four to six experiments.

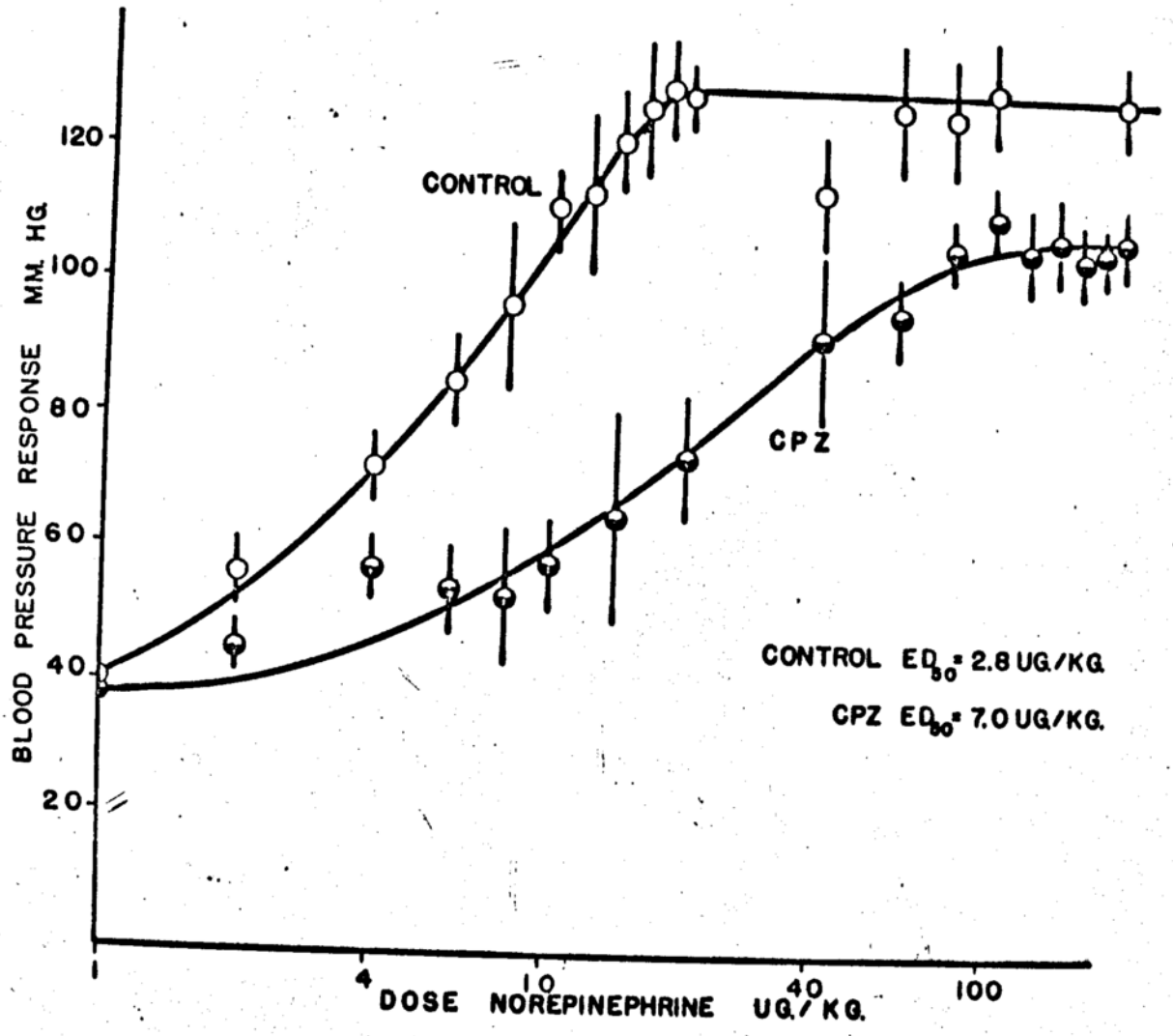

BLOOD PRESSURE RESPONSE MM. HG.
120
100
80
60
40
20
CONTROL
CPZ
CONTROL ED_{50} = 2.8 UG./KG.
CPZ ED_{50} = 7.0 UG./KG.
1
4
10
40
100
DOSE NOREPINEPHRINE UG./KG.

Figure 7. Effect of chlorpromazine on norepinephrine-induced blood pressure response in hypothermic rats. Vertical lines indicate standard error of the mean. Each point represents the mean of at least four to six experiments.

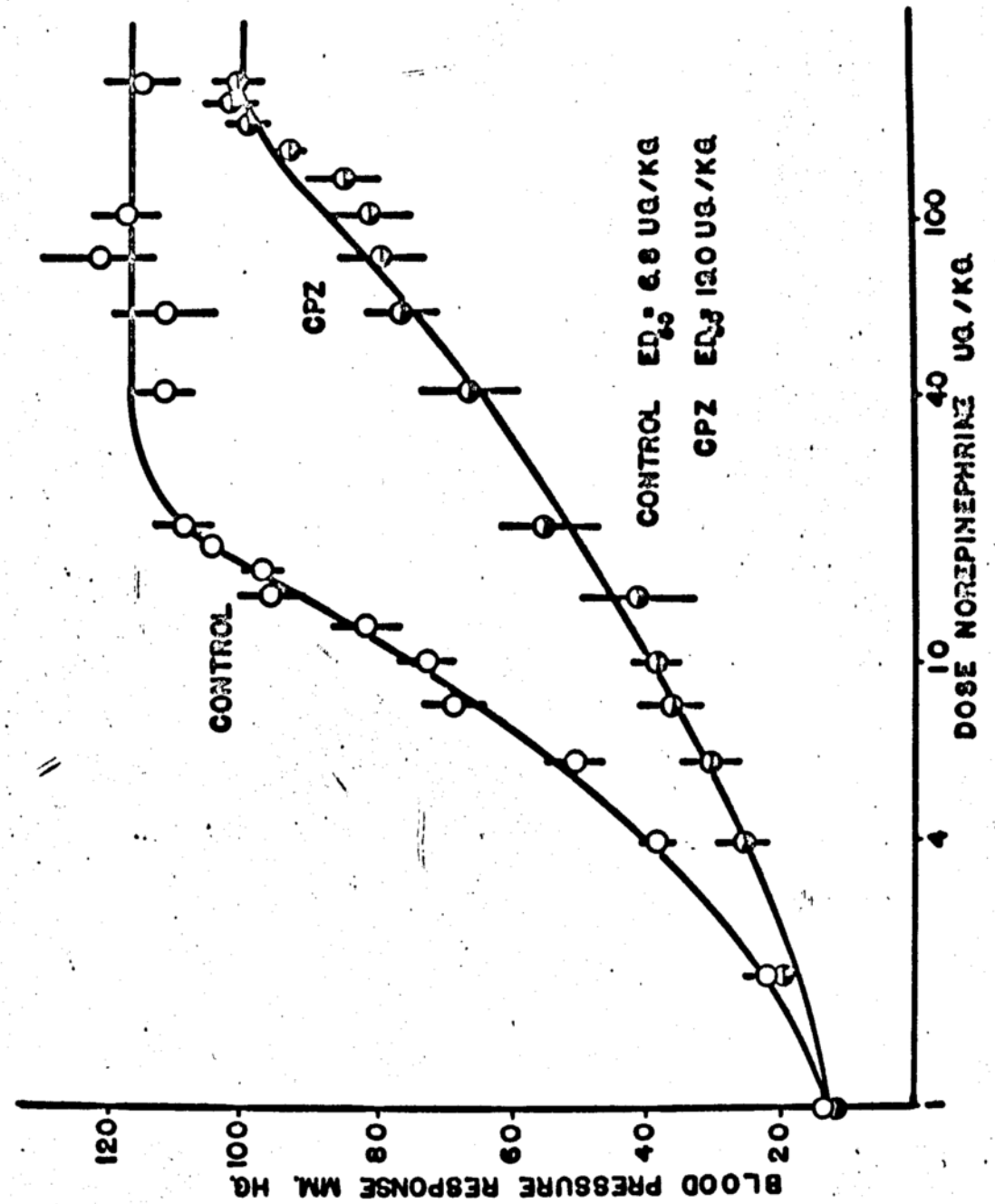
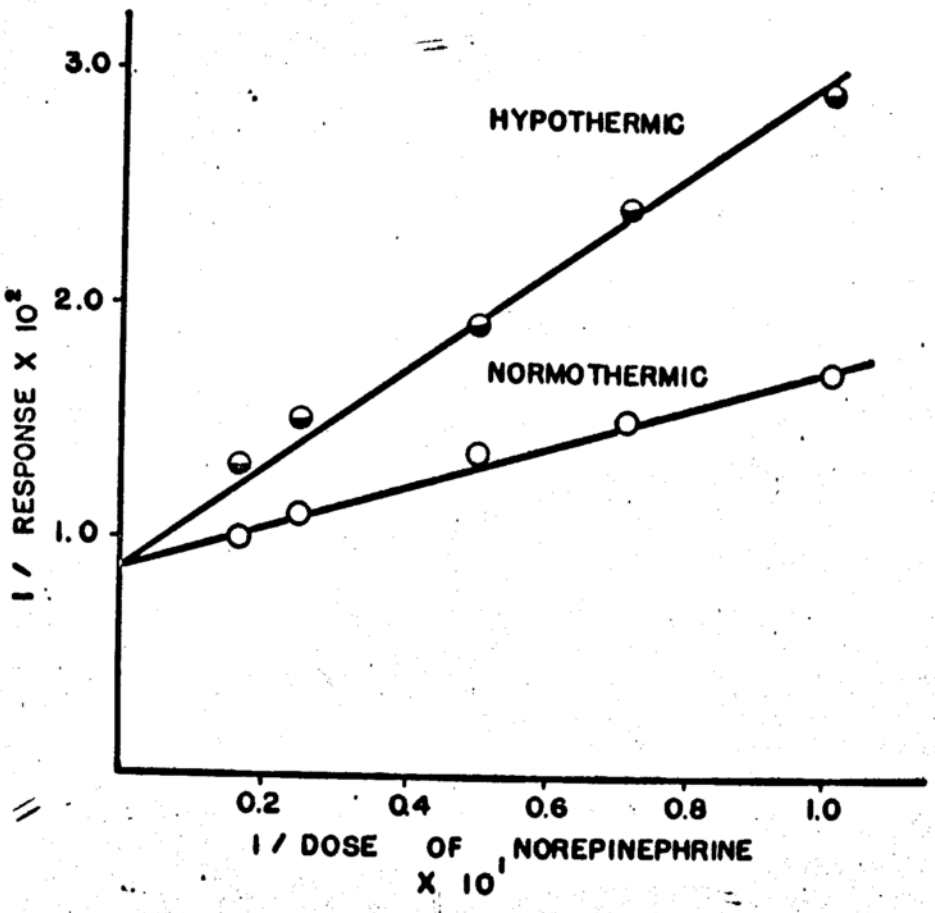

BLOOD PRESSURE RESPONSE MM. HG.
120
100
80
60
40
20
1
4
10
40
100
DOSE NOREPINEPHRINE UG./KG.
CONTROL
CPZ
CONTROL ED50 6.8 UG/KG
CPZ ED50 120 UG/KG

Figure 8. Double reciprocal plot illustrating the effect of hypothermia on the norepinephrine-induced increase in blood pressure in chlorpromazine treated rats.


3.0
2.0
1.0
1 / RESPONSE X 10²
HYPOTHERMIC
NORMOTHERMIC
0.2
0.4
0.6
0.8
1.0
1 / DOSE OF NOREPINEPHRINE
X 10⁻¹

effective antiadrenergic agent. It is capable of decreasing blood pressure in either normothermic or hypothermic animals and, in addition, it antagonized the pressor responses of NE in both groups of animals. Hypothermia potentiated the antiadrenergic effect of CPZ in an apparently competitive manner.

Effect of Hypothermia on Tyramine Induced Pressor Responses. To investigate further the hypothermia-induced antagonism of the adrenergic response, tyramine, a sympathomimetic amine believed to elicit its adrenergic effect through the release of endogenous NE, was employed. Structurally, tyramine differs from NE in containing only one ring hydroxyl group and no hydroxyl group on the beta carbon of the side chain. The classification of tyramine as an indirect acting amine is based upon results of investigations conducted with reserpine, a compound known to deplete catecholamine stores in the adrenergic neuron, and cocaine, a compound which blocks the uptake of catecholamines into adrenergic neurons. It has been shown that in cocaine treated and in reserpinized animals, the adrenergic response to tyramine is abolished while that to NE is potentiated. Thus, it has been postulated that in order to elicit a response, tyramine must enter the adrenergic neuron and displace the neurotransmitter from its storage sites. This is in sharp contrast to the mechanism of

action of exogenous NE which acts directly upon the adrenergic receptor.

Figure 9 shows that hypothermia antagonized the tyramine induced pressor response and increasing the dose of tyramine did not overcome this antagonism. When these data are compared with those for NE in Figure 3, it is apparent that a fundamental difference exists between the effect hypothermia exerts on the action of tyramine and that exerted on the action of NE.

Since these data suggested that the hypothermia-induced antagonism to tyramine was non-competitive, the following experiment was conducted. After recording the response of hypothermic animals to a sub-maximal dose of tyramine at 15 minute intervals, the animals were warmed to 37°C and tyramine was again administered and the degree of response recorded. Figure 10 shows that the response at 37°C was much greater than that seen at 30°C. It was concluded from these data that hypothermia-induced antagonism to tyramine is readily reversible. In experiments (not shown) where the temperature was repeatedly altered between 30°C and 37°C, similar results were obtained.

Effect of Chlorpromazine on the Tyramine Pressor Response. CPZ antagonized the tyramine pressor response in normothermic animals, exerting approximately a 10 fold decrease in potency of tyramine. Figure 11 shows that even at very

Figure 9. Effect of hypothermia on the tyramine-induced increase in blood pressure in nonpretreated rats. Cross hatched bars indicate normothermic rats and black bars indicate hypothermic rats. Vertical lines indicate standard error of the mean. Each bar represents the mean of at least four to six experiments.

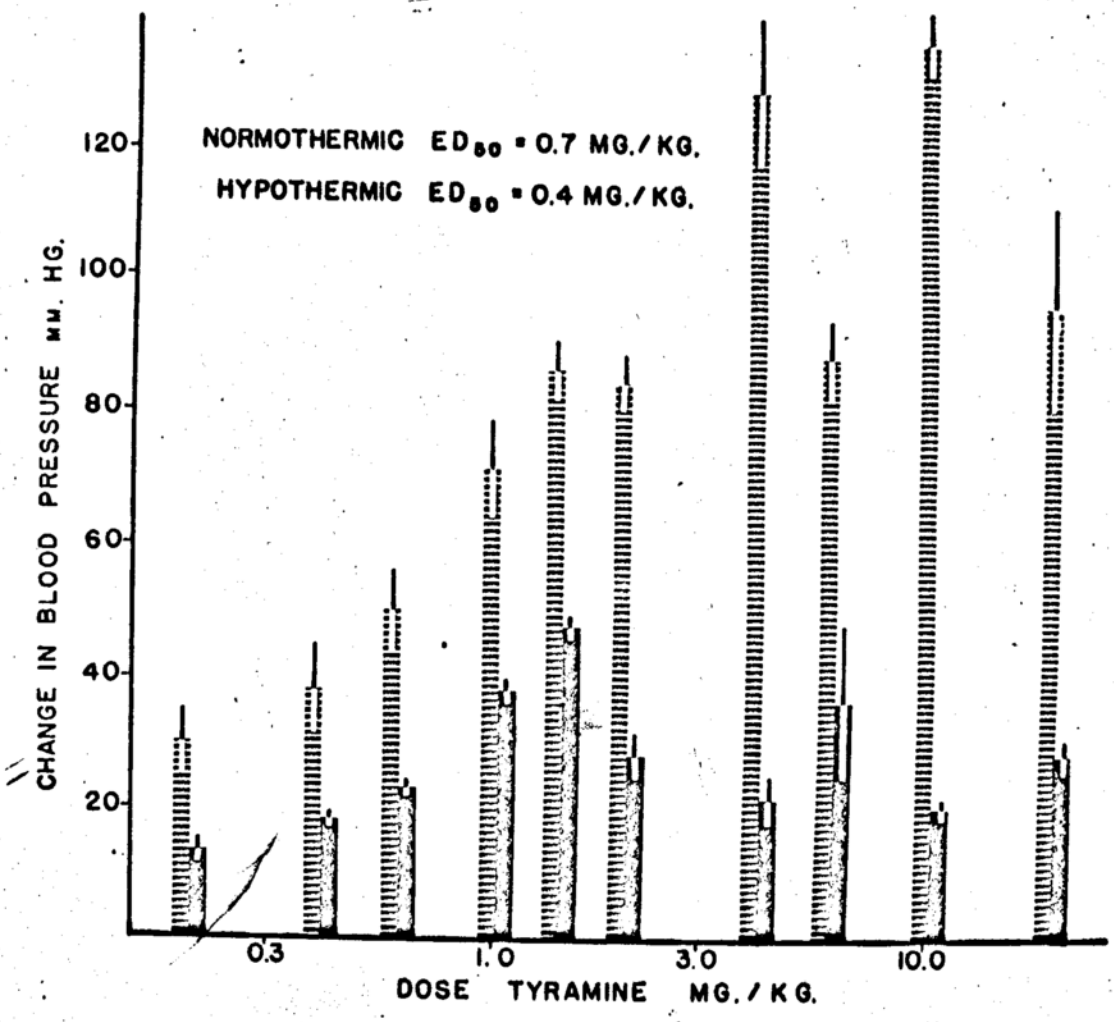

NORMOTHERMIC ED_{50} = 0.7 MG./KG.
HYPOTHERMIC ED_{50} = 0.4 MG./KG.
CHANGE IN BLOOD PRESSURE MM. HG.
120
100
80
60
40
20
0.3
1.0
3.0
10.0
DOSE TYRAMINE MG./KG.

Figure 10. Effect of body temperature on blood pressure response to tyramine. Each experimental point represents mean of four rats.

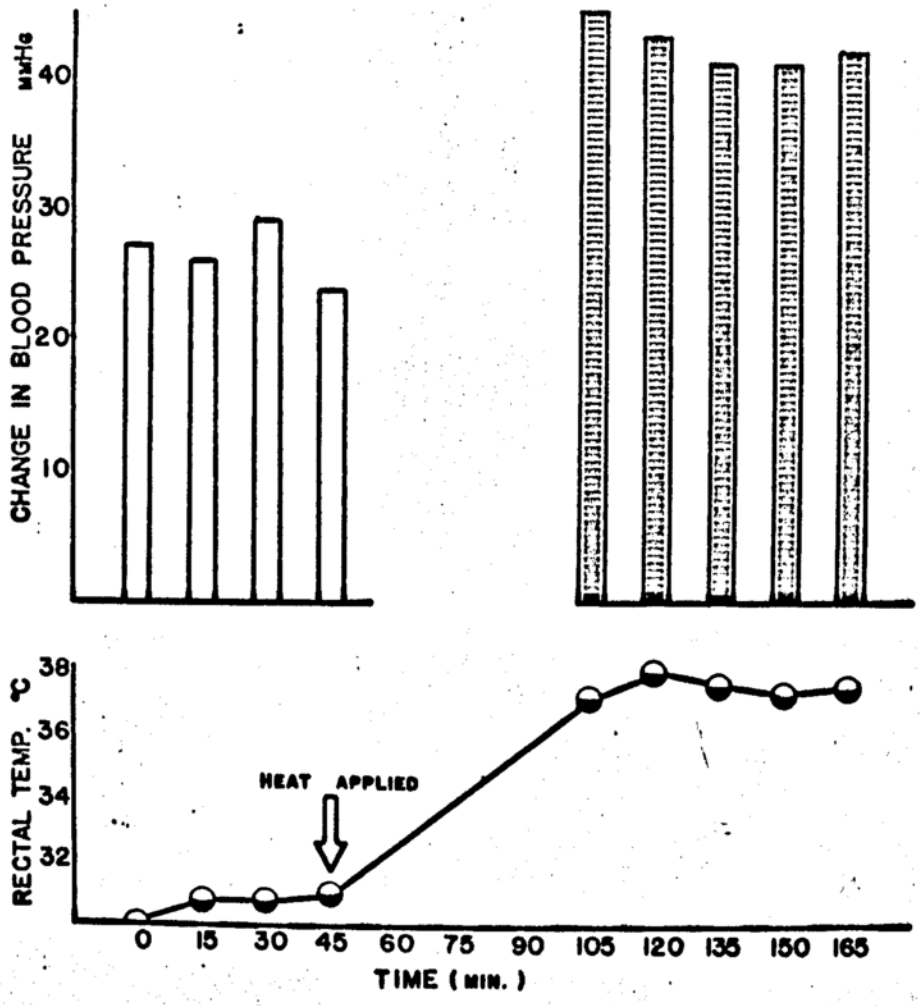
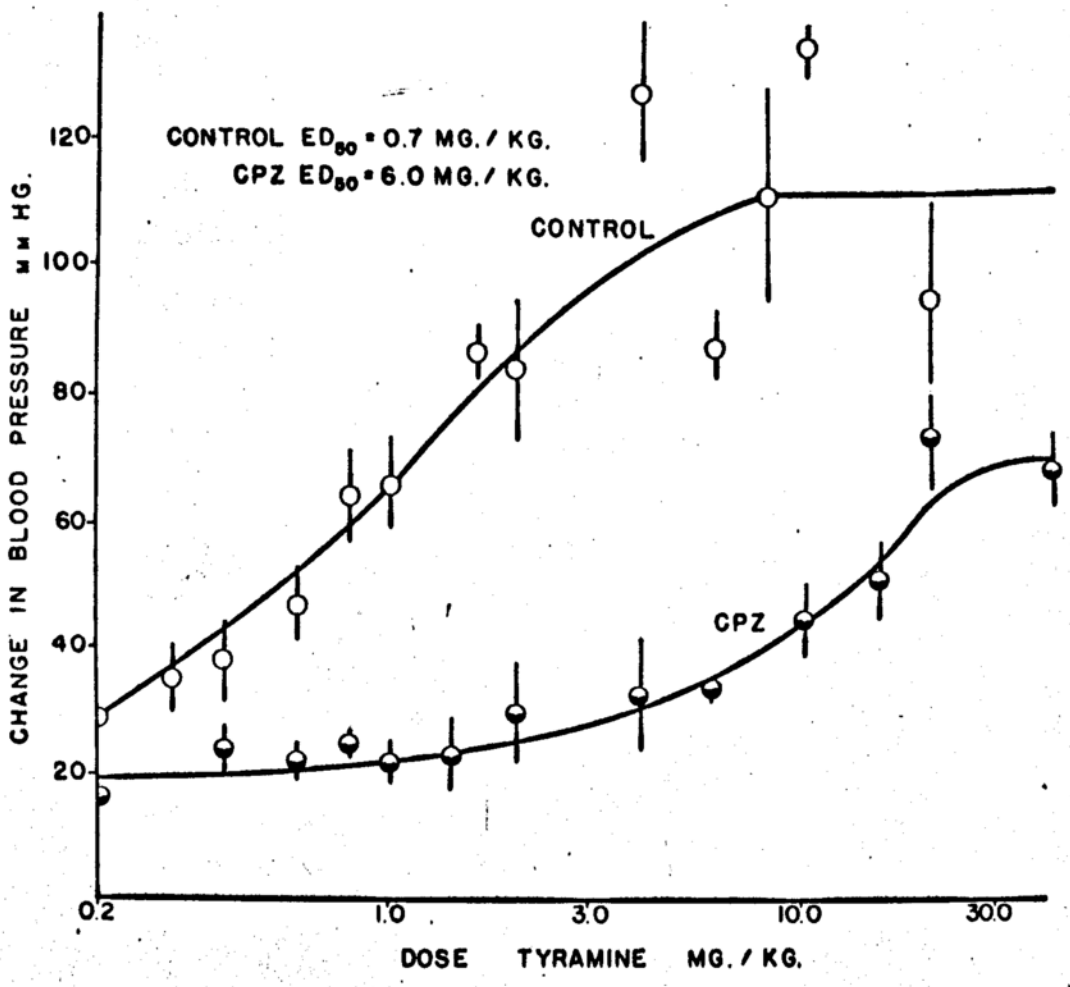

CHANGE IN BLOOD PRESSURE mmHg
40
30
20
10
RECTAL TEMP. °C
38
36
34
32
HEAT APPLIED
0
15
30
45
60
75
90
105
120
135
150
165
TIME (MIN.)

Figure 11. Effect of chlorpromazine on the tyramine-induced increase in blood pressure in normothermic rats. Vertical lines indicate standard error of the mean of at least four to six experiments.


CONTROL ED_{50} = 0.7 MG./ KG.
CPZ ED_{50} = 6.0 MG./ KG.
CONTROL
CPZ
CHANGE IN BLOOD PRESSURE MM HG.
120
100
80
60
40
20
0.2
1.0
3.0
10.0
30.0
DOSE TYRAMINE MG./ KG.

high doses of tyramine, the response was effectively reduced by CPZ. Therefore, not only does CPZ exert a greater antagonism of tyramine's action than it does of NE, but the tyramine antagonism appears to be non-competitive in the normothermic rat.

The effect of CPZ in hypothermic animals can be seen in Figure 12. CPZ effectively antagonized the response to tyramine at low doses but as the concentration of tyramine was increased, the extent of the antagonism diminished. At high doses of tyramine, the pressor response in CPZ treated hypothermic animals was slightly greater than that observed in hypothermic animals without CPZ, however, the response never reached the levels obtained with normothermic control animals.

The comparative effect of CPZ in normothermic and hypothermic animals receiving tyramine is shown in Figure 13. It is apparent that while the antagonistic effects of CPZ in the hypothermic animals are more pronounced at the low doses, at the higher doses of tyramine there is no significant difference between the normothermic and hypothermic animals.

The preceding data have shown that both hypothermia and chlorpromazine are capable of antagonizing the blood pressure effects of NE and tyramine. These data also indicate that the mechanisms of antagonism may be different for these two agonists. In this regard, it has been shown

Figure 12. Effect of chlorpromazine on the tyramine-induced blood pressure response in hypothermic rats. Vertical lines indicate standard error of the mean. Each point represents the mean of at least four to six experiments.

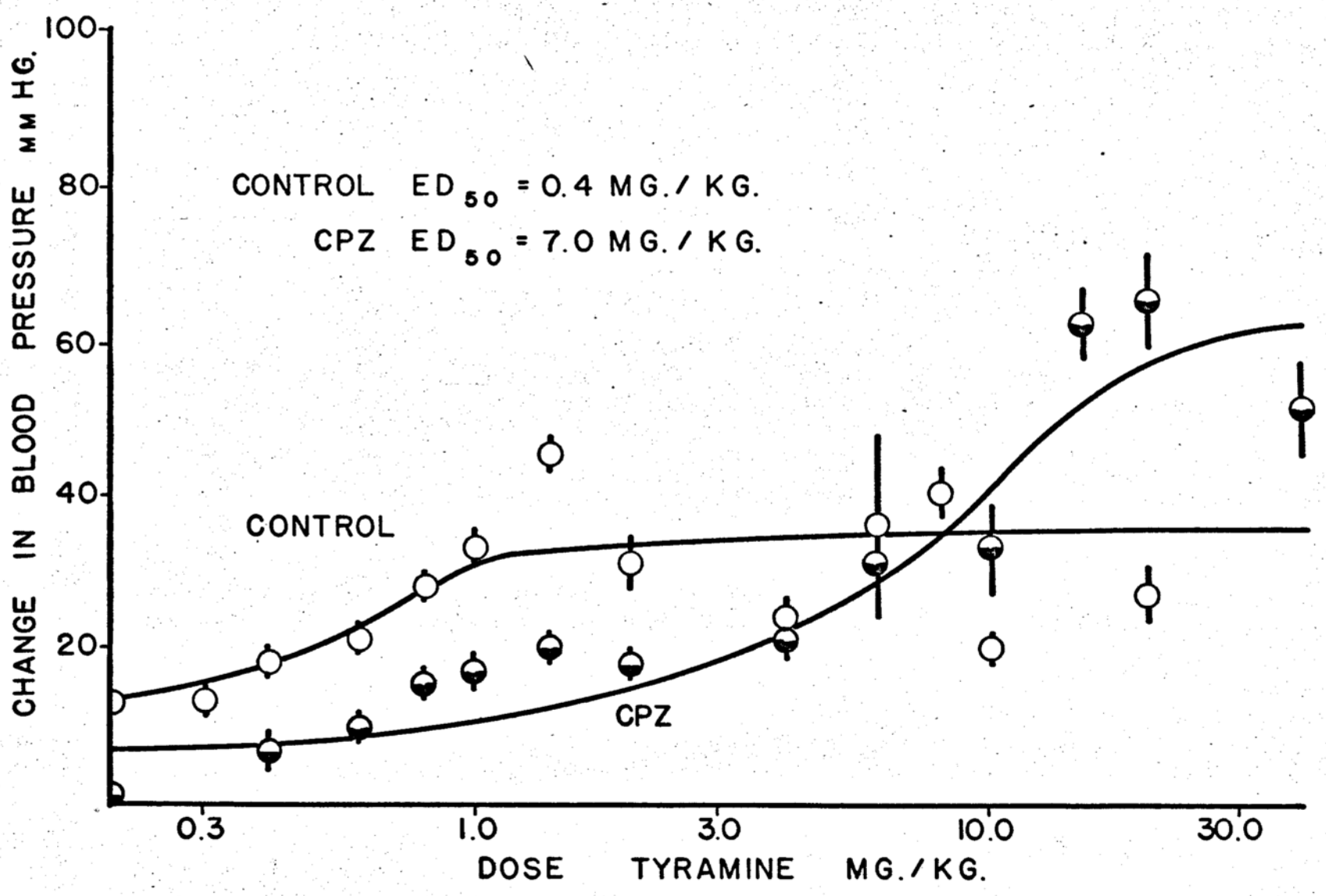
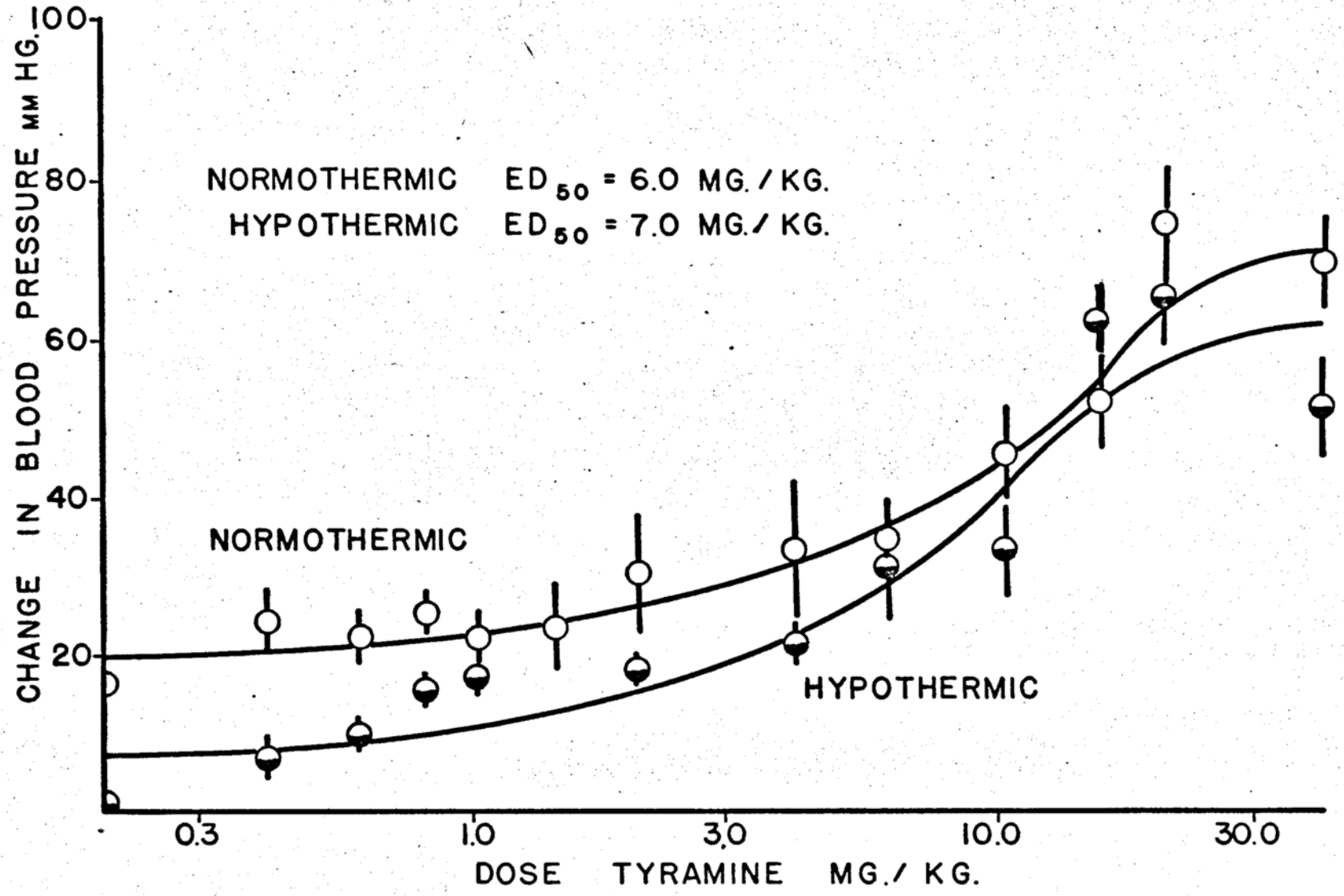

CONTROL ED 50 = 0.4 MG. / KG.
CPZ ED 50 = 7.0 MG. / KG.
CONTROL
CPZ
CHANGE IN BLOOD PRESSURE MM HG.
100
80
60
40
20
0.3
1.0
3.0
10.0
30.0
DOSE TYRAMINE MG. / KG.

Figure 13. Effect of hypothermia on the tyramine-induced increase in blood pressure in chlorpromazine treated rats. Vertical lines indicate standard error of the mean. Each point represents the mean of at least four to six experiments.


NORMOTHERMIC ED50 = 6.0 MG./KG.
HYPOTHERMIC ED50 = 7.0 MG./KG.
NORMOTHERMIC
HYPOTHERMIC
CHANGE IN BLOOD PRESSURE MM HG.
100
80
60
40
20
0.3
1.0
3.0
10.0
30.0
DOSE TYRAMINE MG./KG.

that the hypothermia-induced inhibition of NE was of a competitive nature while that against tyramine could not be overcome by increasing the dose of tyramine. Inasmuch as tyramine elicits its action by releasing endogenous NE, it is difficult to explain the difference solely on the basis of occupation receptor theory, NE being the agonist in both cases. Similarly, if one invokes the concepts attendant in Paton's rate theory of drug-receptor interactions (101,102) the decreased rates of desorption (k_2 in Paton's theory) at any body temperature would be the same irrespective of which agonist was employed, again for the reason that NE acts directly on the receptors and tyramine exerts its actions through the release of endogenous NE. In either case, the active moiety is NE.

The most probable explanation for the differences observed involves the transport mechanisms operative into and within the biophase defining the adrenergic nerve terminal and receptors in addition to receptor effects. It is now well accepted that the transport of NE is controlled to a large extent by active processes (74) and, as a result of lowering the temperature at which these processes must function, the rates of transport would be expected to be reduced. Such a decrease in transport rate would require an increased concentration of NE at the sites of diffusion to provide the receptor area with the same

concentration of agonist molecules as was obtained in the normothermic tissue. Transport of tyramine, on the other hand, is probably of a passive nature although it has been shown that its diffusion into the adrenergic granular tissue may be an active process (42). Decreasing the temperature from 37°C to approximately 30°C would not be expected to cause an appreciable decrease in the passive component of tyramine's transport, however, transport into the granule to release endogenous NE could be appreciably affected by the lowered temperature. It is proposed that this decreased rate of entry may be responsible to some extent for the effect of hypothermia on tyramine's pressor response. Another effect may also be operative in the case of tyramine. Inspection of Figure 9 indicates that as one increases the dose of tyramine, a maximum is reached at approximately 1.5 Mg./Kg. and at higher doses, the blood pressure response appears to decrease rather than increase. One explanation of this type of phenomena is that "auto-inhibition" is being observed. In this case, although tyramine has little or no direct stimulatory action on the adrenergic receptors, it may combine with them in an ineffective manner. That is, the tyramine molecules interact with the receptor molecules but do not initiate the response. Thus, they could act as inhibitors with respect to the active agonist, NE. It would be anticipated that auto-inhibition would be more pronounced in hypothermic

animals than in the normothermic. The results obtained are consistent with such a hypothesis and it is proposed that the autoinhibitory phenomenon is one of the major components of action in the hypothermia-induced antagonism of tyramine.

It is also possible that in hypothermia, a reduced concentration of NE resides in the nerve terminal thus affording fewer molecules of endogenous NE to be released by the entering tyramine. While this possibility cannot be ruled out, it is not consistent with the findings to be discussed below in regard to tachyphylaxis.

Tachyphylaxis. One of the pharmacological properties of tyramine characteristic of most biological systems is the development of tachyphylaxis. This is usually attributed to the tyramine-induced depletion of one of the catecholamine storage pools within the adrenergic neuron. Thus, if tyramine is given at short intervals, the pressor response to a given dose of tyramine will progressively decrease. If sufficient time is allowed for restoration of NE in the storage pools between doses, tachyphylaxis will not be observed.

In the present study the time for the blood pressure to return to control levels after high doses of either tyramine or NE was approximately ten minutes. Therefore, it was decided to administer all drugs at 15 minute intervals to allow sufficient time for the blood pressure to

return to control values prior to the re-administration of tyramine. In order to determine the dose at which tachyphylaxis developed in the system, increasing doses of tyramine were given to both normothermic and hypothermic animals, each dose being administered at 15 minute intervals. No tachyphylaxis was observed when ten successive doses of tyramine at 0.3, 0.6 or 1.0 mg./Kg. were given. Tachyphylaxis was first noticed at a dose of 2 mg./Kg. given every 15 minutes and there appeared to be little difference between normothermic and hypothermic animals in the development of tachyphylaxis at this dose. After the tenth dose, the responses had decreased to about 60% and 40% of their initial values in normothermic and hypothermic groups, respectively.

Administration of higher doses of tyramine elicit a more rapid onset (fewer doses required) and a more pronounced degree of tachyphylaxis. Figure 14 shows the responses after four doses of tyramine at 6 and 10 mg./Kg. It can be seen that the response diminished more rapidly at 10 mg./Kg. than at 6 mg./Kg., and hypothermia appears to have little effect on the onset and degree of tachyphylaxis.

Effect of Chlorpromazine on Tachyphylaxis. The effect of chlorpromazine on tachyphylaxis induced with tyramine is shown in Figures 15 and 16. No tachyphylaxis was observed until doses in excess of 6 mg./Kg. were administered. It

Figure 14. Effect of repeated administration of tyramine (6 mg./Kg. and 10 mg./Kg.) on the tyramine-induced blood pressure response in nonpre-treated rats. Cross hatched bars indicate normothermic rats and white bars indicate hypothermic rats. Each bar represents the mean of four experiments.

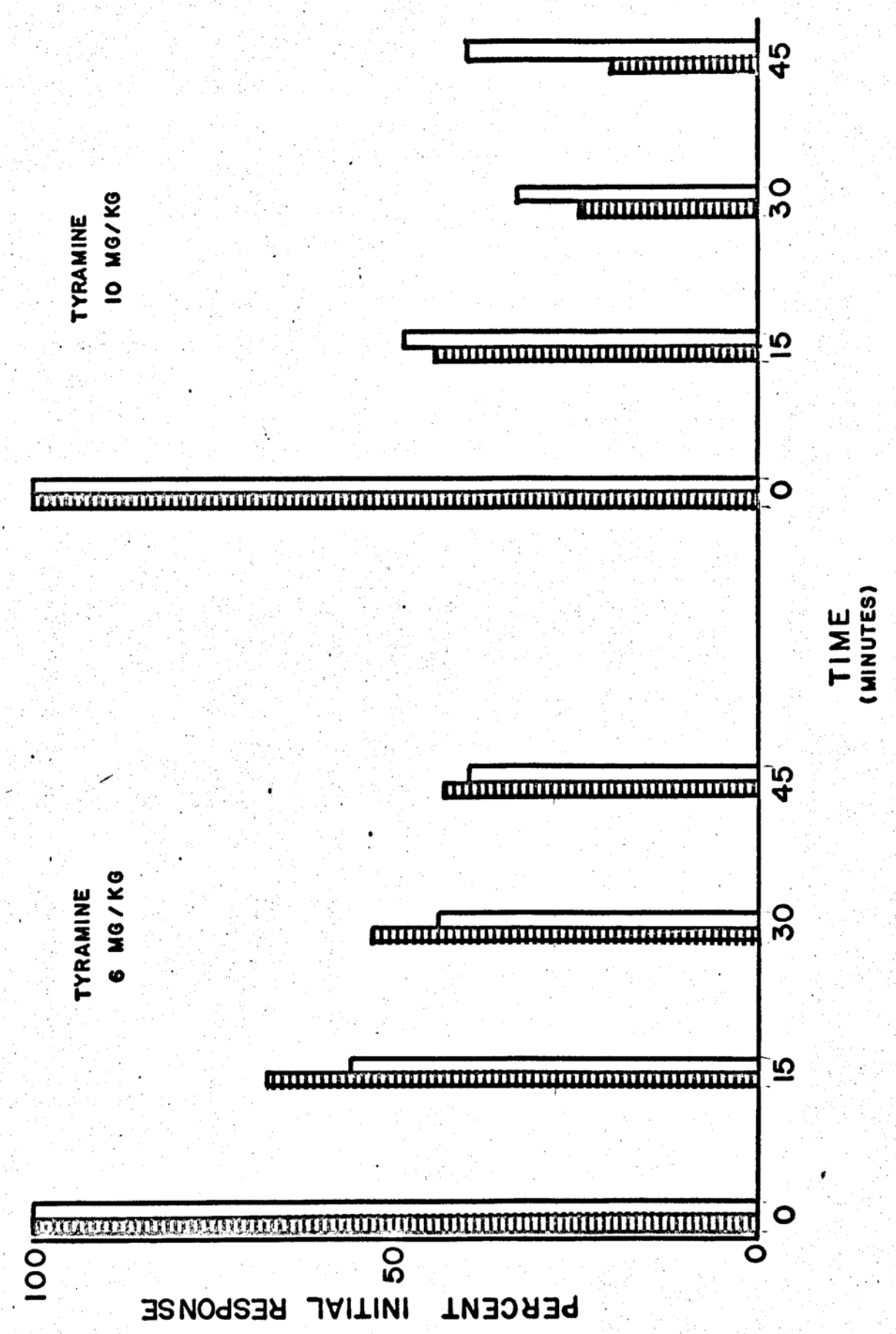

TYRAMINE
10 MG/KG
TYRAMINE
6 MG/KG
0
15
30
45
0
15
30
45
TIME
(MINUTES)
0
50
100
PERCENT INITIAL RESPONSE

Figure 15. Effect of repeated administration of tyramine (6 and 15 mg./Kg.) on the tyramine-induced blood pressure response in chlorpromazine treated rats. Cross hatched bars indicate normothermic rats and black bars indicate hypothermic rats. Each bar represents the mean of four experiments.

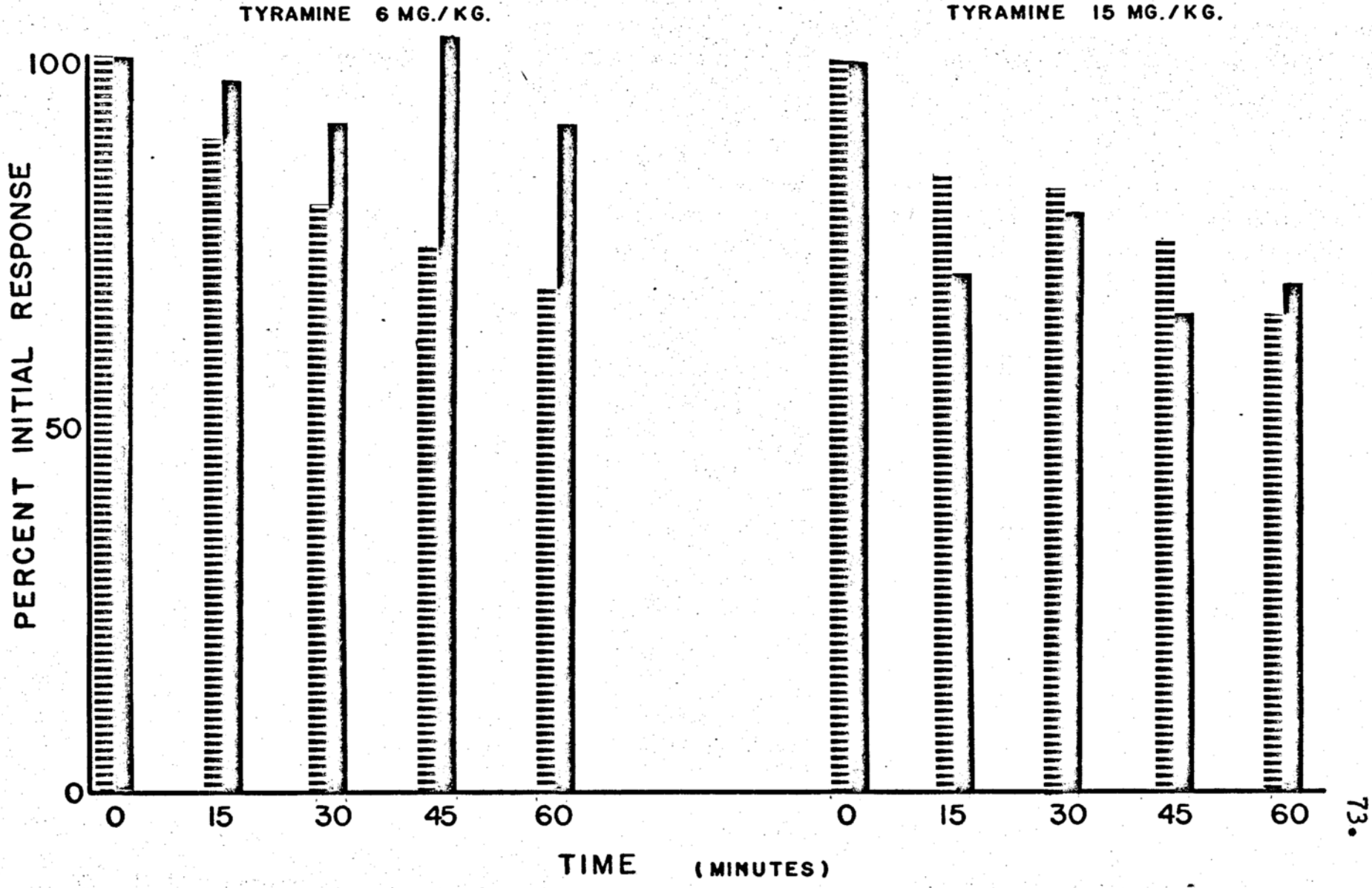
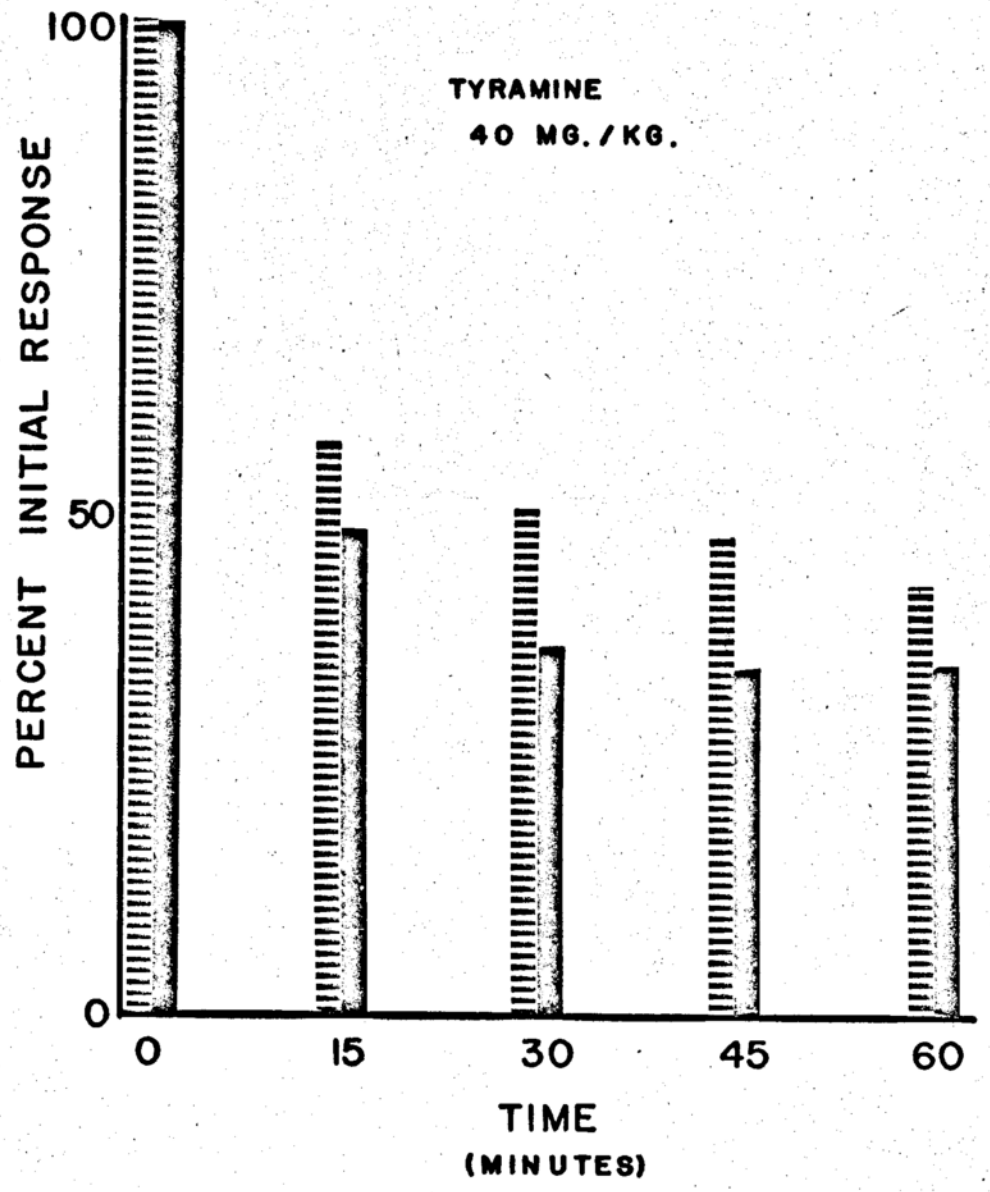

TYRAMINE 6 MG./KG.
TYRAMINE 15 MG./KG.
PERCENT INITIAL RESPONSE
100
50
0
0
15
30
45
60
0
15
30
45
60
TIME (MINUTES)

Figure 16. Effect of repeated administration of tyramine (40 mg./Kg.) on the tyramine-induced blood pressure response in chlorpromazine treated rats. Cross hatched bars indicate normothermic rats and black bars indicate hypothermic rats. Each bar represents the mean of four experiments.


100
50
0
PERCENT INITIAL RESPONSE
TYRAMINE
40 MG./KG.
0
15
30
45
60
TIME
(MINUTES)

It is apparent from Figure 15 that even at a dose of 15 mg./Kg., tachyphylaxis is not pronounced. These results are in sharp contrast to those presented for the control (no CPZ) animals in Figure 14. When a tyramine dose of 40 mg./Kg. was administered, however, tachyphylaxis was clearly evident as can be observed in Figure 16. At this dosage, the second administration of tyramine was only 50% as effective as the first dose. Subsequent doses reduced the response only slightly until after five doses, the response was approximately 40% of the initial response.

It is apparent that CPZ delayed the onset of tachyphylaxis, much higher doses of tyramine being required to produce this phenomenon. It may be concluded, therefore, that CPZ had a pronounced effect on some portion of the mechanism involved in the tyramine-induced release of endogenous NE. In this regard, CPZ has been shown to inhibit the uptake of various amines into the storage granules and also to inhibit their transport through other membranes (30). It is possible, therefore, that CPZ inhibited the uptake of tyramine into the storage pool, thus effectively inhibiting the depletion of NE from the nerve terminal. If the entrance of tyramine into the storage pool were delayed, tyramine may be removed from the biophase by metabolic degradation catalyzed by the enzyme, monoamine oxidase. It is also possible that the transport of tyramine into the biophase was inhibited by

CPZ. Either of these two mechanisms or a combination of the two may serve to protect the storage granule from depletion by tyramine thus interfering with the mechanisms by which tachyphylaxis would be produced.

Effect of Hypothermia on Heart Rate. Increased sympathetic output induced by the stress of hypothermia in control animals appeared to have little effect on the heart rate. From recordings made approximately 15 minutes after the cannulation procedures (Table II), it can be seen that there was no significant difference between heart rates of normothermic and hypothermic animals. This may be explained by the fact that the blood pressure was elevated in hypothermic animals (Table I) reflexly inducing an increase in the vagal tone on the heart. The increased sympathetic activity resulting from cold stress was apparently antagonized or "buffered" by the increased vagal tone.

Effect of Hypothermia on Norepinephrine-Induced Increase in Heart Rate. Figure 17 shows that administration of NE to anesthetized normothermic rats induced an increase in the heart rate. Cardio-acceleration, which lasted from one to three minutes, was usually not observed until one to three minutes after the administration of NE. Reflex mechanisms (see above) complicate heart rate results and probably account for the delayed onset of action noted in

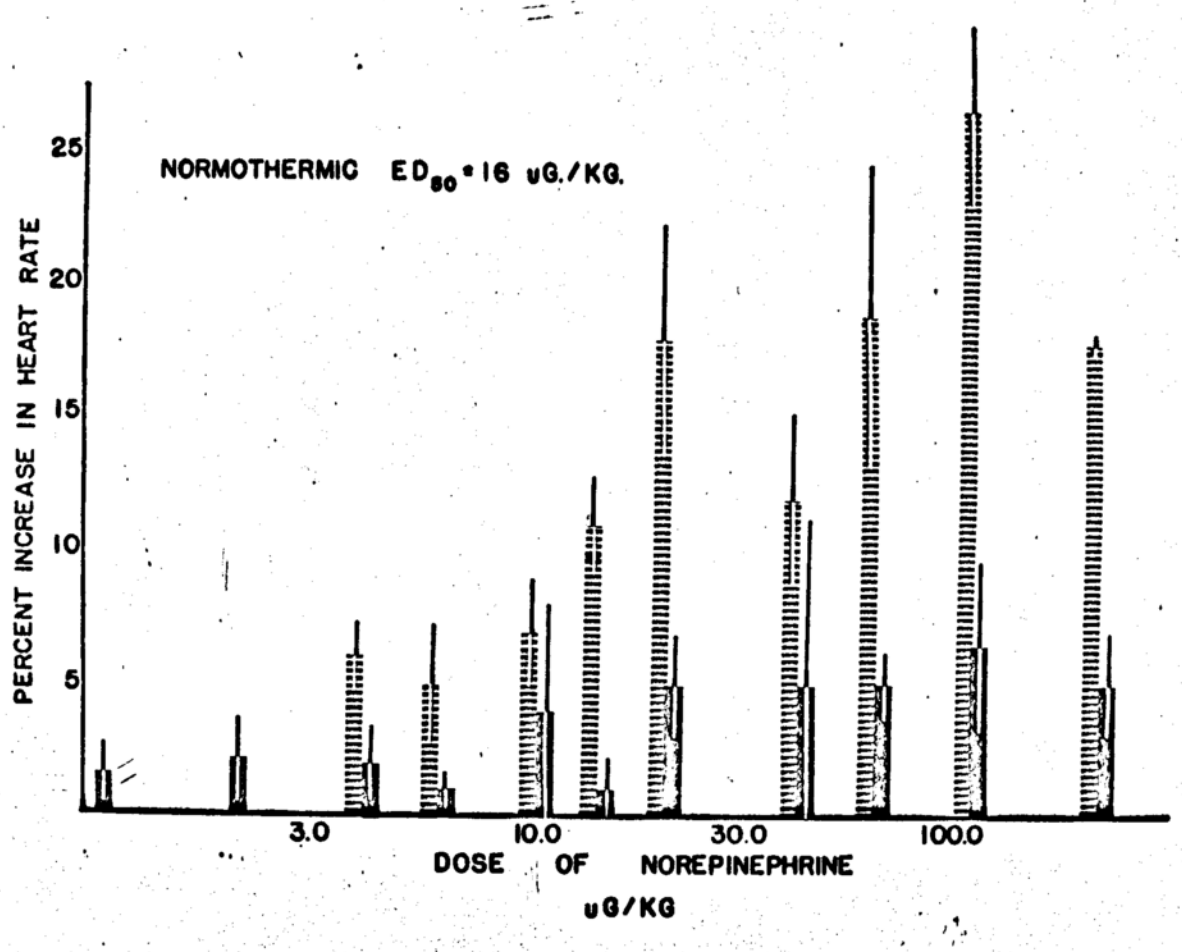
Table II

Effect of Body Temperature and Chlorpromazine (0.5 mg./Kg.) on the Heart Rate of Anesthetised Male Rats

Treatment		Heart Rate (Beats/Minute)
Normothermic	No Drug	282 ± 7.0 (40)
Hypothermic	No Drug	276 ± 4.3 (37)
Normothermic	Chlorpromazine	164 ± 9.2 (28)
Hypothermic	Chlorpromazine	169 ± 12.2 (29)

Figures in parentheses indicate the number of animals in each group.

Figure 17. Effect of hypothermia on the norepinephrine-induced increase in heart rate in nonpretreated rats. Cross hatched bars indicate normothermic rats and black bars indicate hypothermic rats. Vertical lines indicate standard error of the mean. Each bar represents the mean of at least four to six experiments.


NORMOTHERMIC ED_{50} = 16 uG./KG.
PERCENT INCREASE IN HEART RATE
25
20
15
10
5
3.0
10.0
30.0
100.0
DOSE OF NOREPINEPHRINE
uG/KG

these studies. Furthermore, NE is primarily an alpha adrenergic stimulator but is reported to stimulate the beta adrenergic receptors of the heart (86). Thus, the NE-induced increase in blood pressure elicits compensatory vagal reflex activity which slows the heart and antagonizes the direct cardio-accelerator action of NE. Comparison of Figure 3 and Figure 17 reveals that higher doses of NE were required to induce cardio-acceleration than were needed to induce blood pressure responses. The dose of NE needed to elicit 50% of the maximum response for heart rate was about five times that required to elicit a similar response for blood pressure. Thus, the combination of a decreased sensitivity of NE for beta receptors and the increased vagal tone reflexly induced by NE resulted in a decreased effectiveness of NE on the heart.

Figure 17 also indicates that the cardio-accelerator activity of NE was greatly antagonized by hypothermia. Furthermore, this inhibition was not overcome when the concentration of NE was increased, indicating that the antagonism may be non-competitive. Therefore, these data indicate that the hypothermia-induced antagonism on heart rate differs from that observed on blood pressure. Vascular smooth muscle (blood pressure response) is assumed to contain alpha type receptors while receptors in the heart are considered to be of the beta type (86). It is also evident that the heart is more susceptible to the

effects of hypothermia than is the vascular system.

Effect of Hypothermia and CPZ on Heart Rate. Table II shows that CPZ caused about a 40% decrease in heart rate in both normothermic and hypothermic animals indicating that body temperature had no effect on the degree of bradycardia induced by CPZ.

Effect of Chlorpromazine Norepinephrine-Induced Heart Rate Response. CPZ markedly increased the heart rate response to exogenous NE. Figure 18 shows that the response to NE was more than doubled in CPZ treated normothermic animals. An even more dramatic effect was noted in hypothermic animals where CPZ reversed the hypothermia-induced antagonism. It can be seen from Figure 19 that in CPZ treated hypothermic animals, NE caused a response that was about ten times greater than that observed in hypothermic control animals. Therefore, CPZ appears to potentiate the stimulator effect of NE on the heart in normothermic and hypothermic animals.

CPZ exerts a variety of pharmacological actions and included among these are its anticholinergic effects (98). Thus parasympathetic responses, reflexly induced by elevated mean blood pressure would be antagonized by CPZ. This would allow the direct effects of NE on the beta receptors of the heart to manifest themselves at lower concentrations and could account for the apparent

Figure 18. Effect of chlorpromazine on the norepinephrine-induced increase in heart rate in normothermic rats. Vertical lines indicate standard error of the mean. Each point represents the mean of at least four to six experiments.

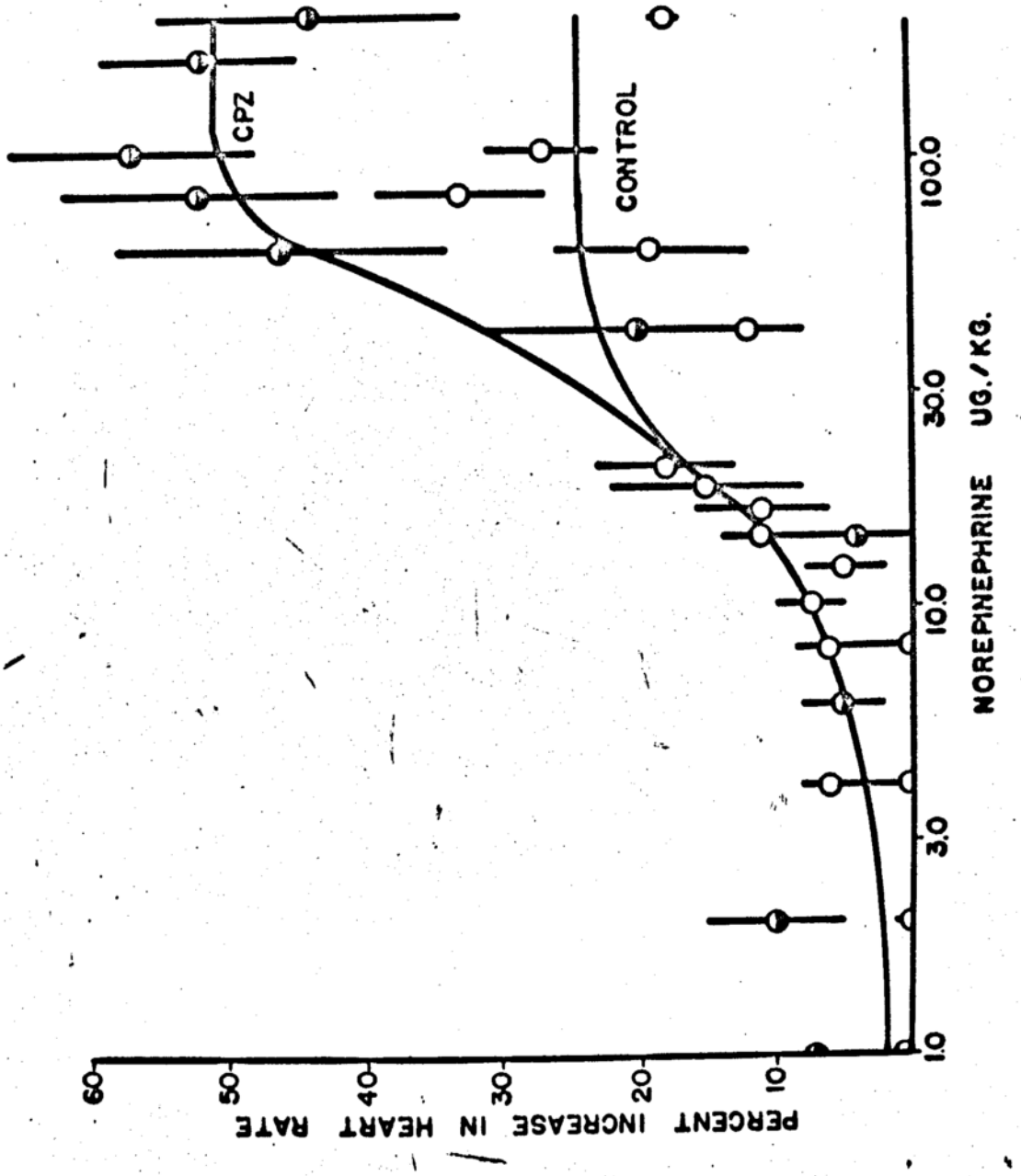
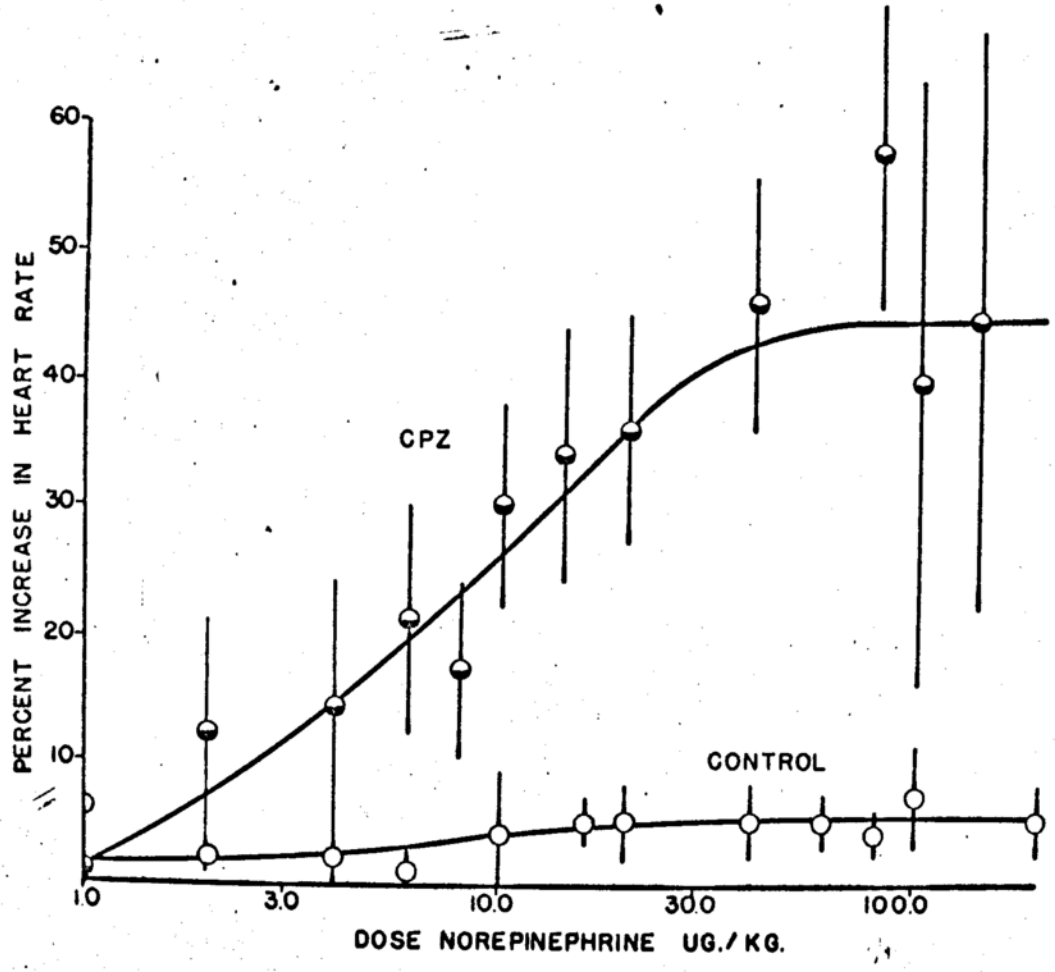

PERCENT INCREASE IN HEART RATE
60
50
40
30
20
10
CPZ
CONTROL
1.0
3.0
10.0
30.0
100.0
NOREPINEPHRINE UG./KG.

Figure 19. Effect of chlorpromazine on the norepinephrine-induced increase in heart rate in hypothermic rats. Vertical lines indicate standard error of the mean. Each point represents the mean of at least four to six experiments.

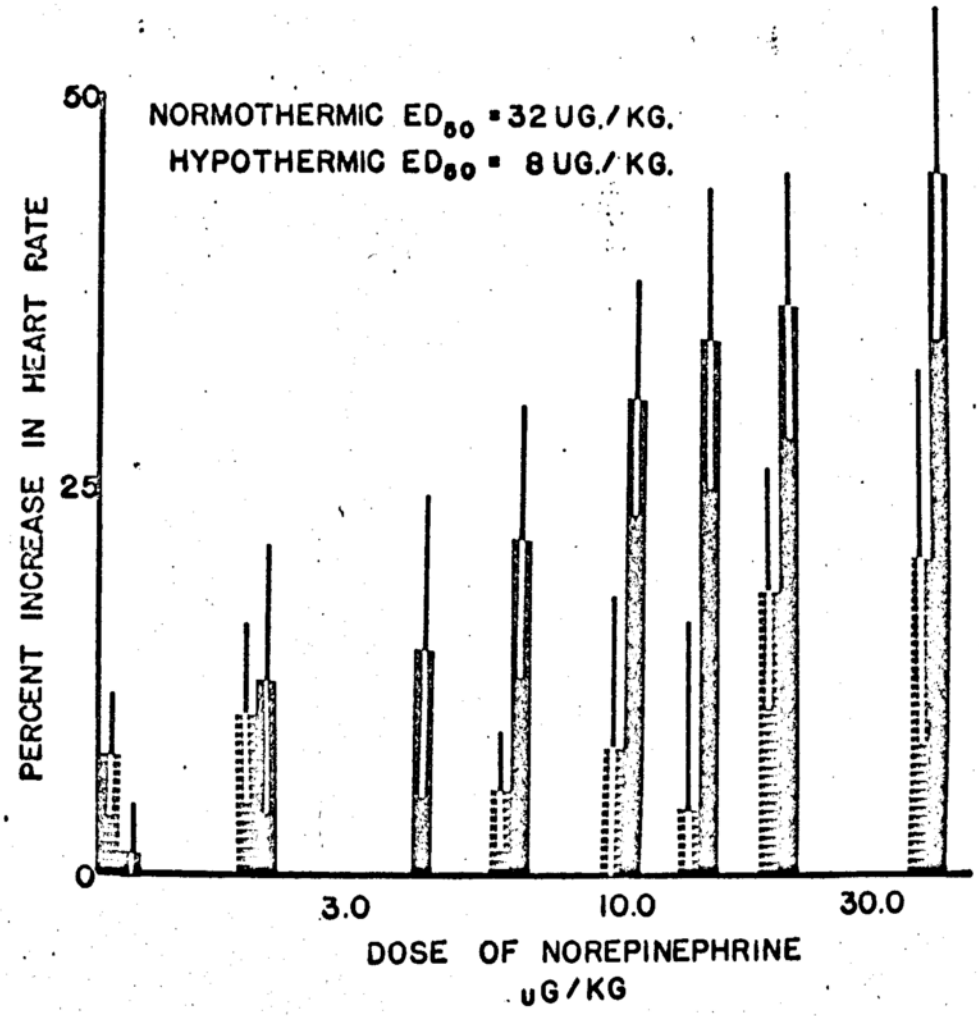

60
50
40
30
20
10
PERCENT INCREASE IN HEART RATE
CPZ
CONTROL
1.0
3.0
10.0
30.0
100.0
DOSE NOREPINEPHRINE UG./KG.

potentiation of NE in CPZ treated normothermic animals. Furthermore, CPZ is also capable of blocking the uptake of exogenous catecholamines into granules and this property could also serve to potentiate the action of exogenous NE, as does cocaine (30).

The effect of hypothermia and CPZ on NE-induced heart rate changes can be seen in Figure 20. This graph indicates that the combination of hypothermia and CPZ actually causes heart muscle to become more sensitive to the effects of exogenous NE. It can be seen that the potency of NE in hypothermic animals was about four times greater than in normothermic animals, however, the same maximum response was observed in both groups.

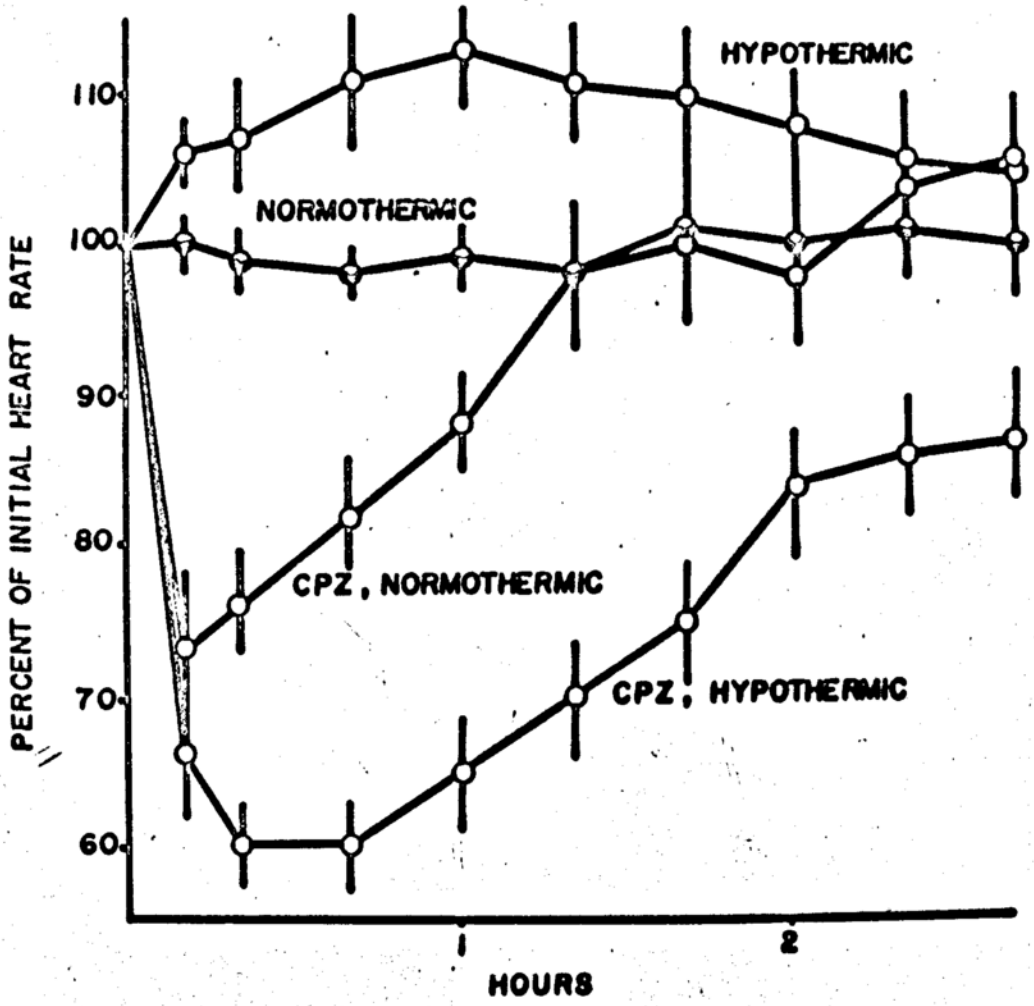
Hypothermia has been reported to induce spontaneous ventricular fibrillation when body temperature falls below 28°C (2) indicating that the heart may become more sensitive to catecholamines at low body temperatures. CPZ is often employed in open heart surgery both to induce hypothermia and to prevent the occurrence of ventricular fibrillation as body temperature decreases (103). The success of CPZ in reducing ventricular fibrillation has been attributed to its antiadrenergic properties. However, in the present study, CPZ appears to have increased the sensitivity of the heart to <u>exogenous</u> NE in both normothermic and hypothermic animals. In the absence of exogenous NE, a bradycardia was observed (see Table II).

Figure 20. Effect of hypothermia on the norepinephrine-induced increase in heart rate in chlorpromazine treated rats. Cross hatched bars indicate normothermic rats and black bars indicate hypothermic rats. Vertical lines indicate standard error of the mean. Each point represents the mean of at least four to six experiments.


NORMOTHERMIC ED_{50} = 32 UG./KG.
HYPOTHERMIC ED_{50} = 8 UG./KG.
PERCENT INCREASE IN HEART RATE
50
25
0
3.0
10.0
30.0
DOSE OF NOREPINEPHRINE
uG/KG

Further studies designed to elucidate the effect of hypothermia on the cardiovascular effects of CPZ, were performed. In these experiments CPZ was administered to a group of normothermic animals and half of these animals were allowed to become hypothermic while the rest were maintained normothermic with a heating element. Heart rates, recorded at 20 minute intervals over a two and one half hour period, are shown in Figure 21. Following the administration of CPZ, a lag time occurred before the effects of CPZ became evident on the heart rate, the lag time varying between two and five minutes. Following this lag period, the heart rate was slowed appreciably in both normothermic and hypothermic animals. Twenty minutes after drug administration, the heart rates of the normothermic animals began to return toward normal values, while those of the hypothermic group decreased to even lower values. Indeed, the minimal value of heart rate in the hypothermic animals was still present 40 minutes after drug administration, while the heart rate in the normothermic group had returned to approximately 85% of normal. In addition, the mean heart rate of the hypothermic group did not return to control values during the period of observation but appeared to plateau out at approximately 85% of the initial value. These results were interpreted to indicate that hypothermia potentiated the antagonism to the adrenergic response reflexly elicited by CPZ's

Figure 21. Effect of chlorpromazine on heart rates of hypothermic and normothermic rats. Vertical lines indicate standard error of the mean. Each point represents the mean of at least six experiments.

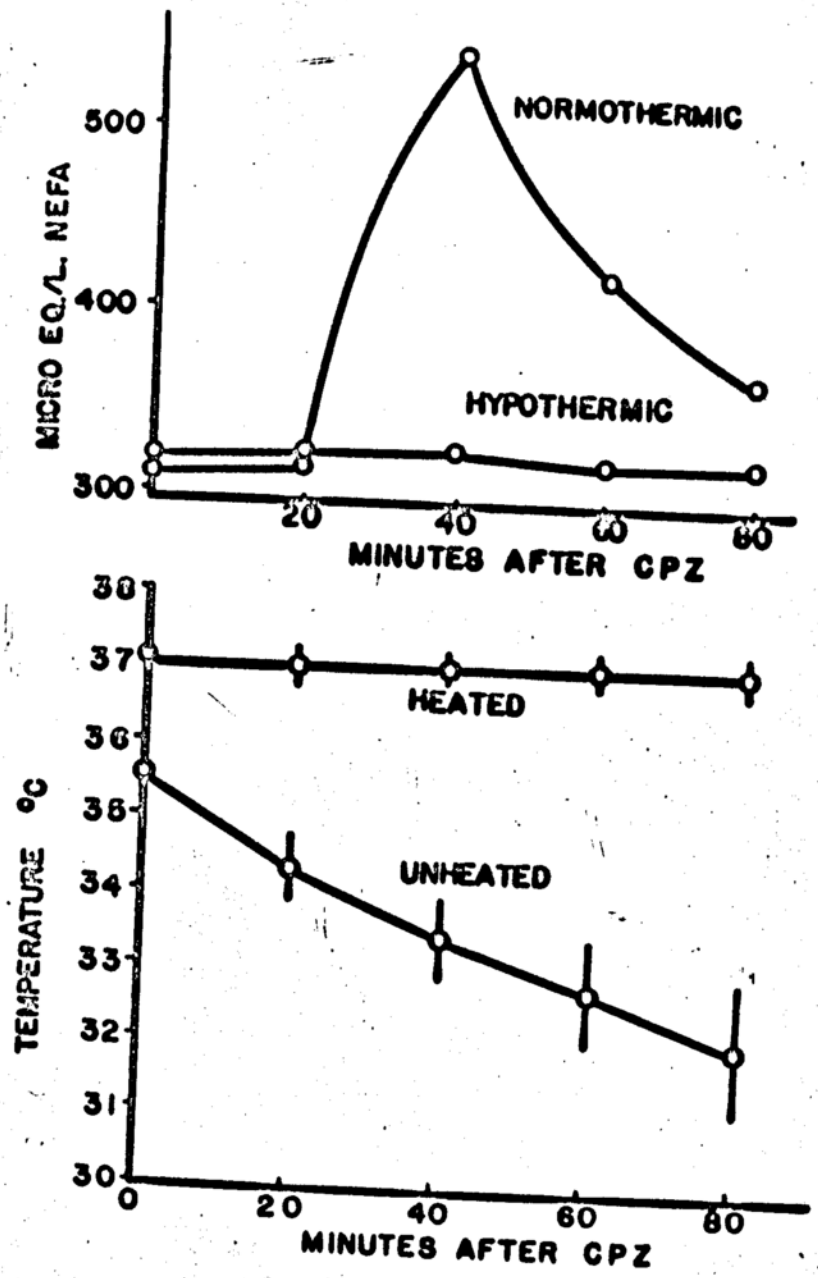

HYPOTHERMIC
NORMOTHERMIC
CPZ, NORMOTHERMIC
CPZ, HYPOTHERMIC
PERCENT OF INITIAL HEART RATE
110
100
90
80
70
60
1
2
HOURS

hypotensive effect.

The work of Costa et al. (99) and Pletscher et al. (100) showed that the release and/or uptake of biogenic amines is inhibited by CPZ in hypothermic animals but not in normothermic animals. To ascertain whether a similar effect was operative in this system, an experimental procedure was sought which was (a) under the primary control of the sympathetic nervous system and (b) sensitive enough to respond to homeostatic, sympathetic mechanisms in the anesthetized animals employed in the present study. Previous work (22,23,104,105) had shown that the mobilization of fatty acids from adipose tissue in vivo satisfied these criteria. Thus, it was decided to investigate the effect of hypothermia and CPZ treatment on the mobilization of fatty acids in vivo.

Effect of Hypothermia and Chlorpromazine on Mobilization of Fatty Acids. The results of this phase of the study are shown in Figure 22. The lower portion of the graph illustrates the change in body temperature observed in the two groups of animals. CPZ was administered to both groups of animals at a dose of 0.5 mg./Kg. It is apparent in Figure 22 that hypothermia effectively antagonized the elevation of plasma fatty acids. These results also show that the dose of CPZ employed was not sufficient to block fatty acid mobilization per se inasmuch as significant elevation in plasma fatty acids were obtained in the CPZ

Figure 22. Top, effect of hypothermia on fatty acid mobilization in chlorpromazine treated rats. Chlorpromazine dose = 0.5 mg./Kg. Bottom, rectal temperature obtained in both heated and unheated chlorpromazine treated rats. Each point represents the mean of at least six experiments.


MICRO EQ/L. NEFA
500
400
300
NORMOTHERMIC
HYPOTHERMIC
20
40
60
80
MINUTES AFTER CPZ
TEMPERATURE °C
38
37
36
35
34
33
32
31
30
HEATED
UNHEATED
0
20
40
60
80
MINUTES AFTER CPZ

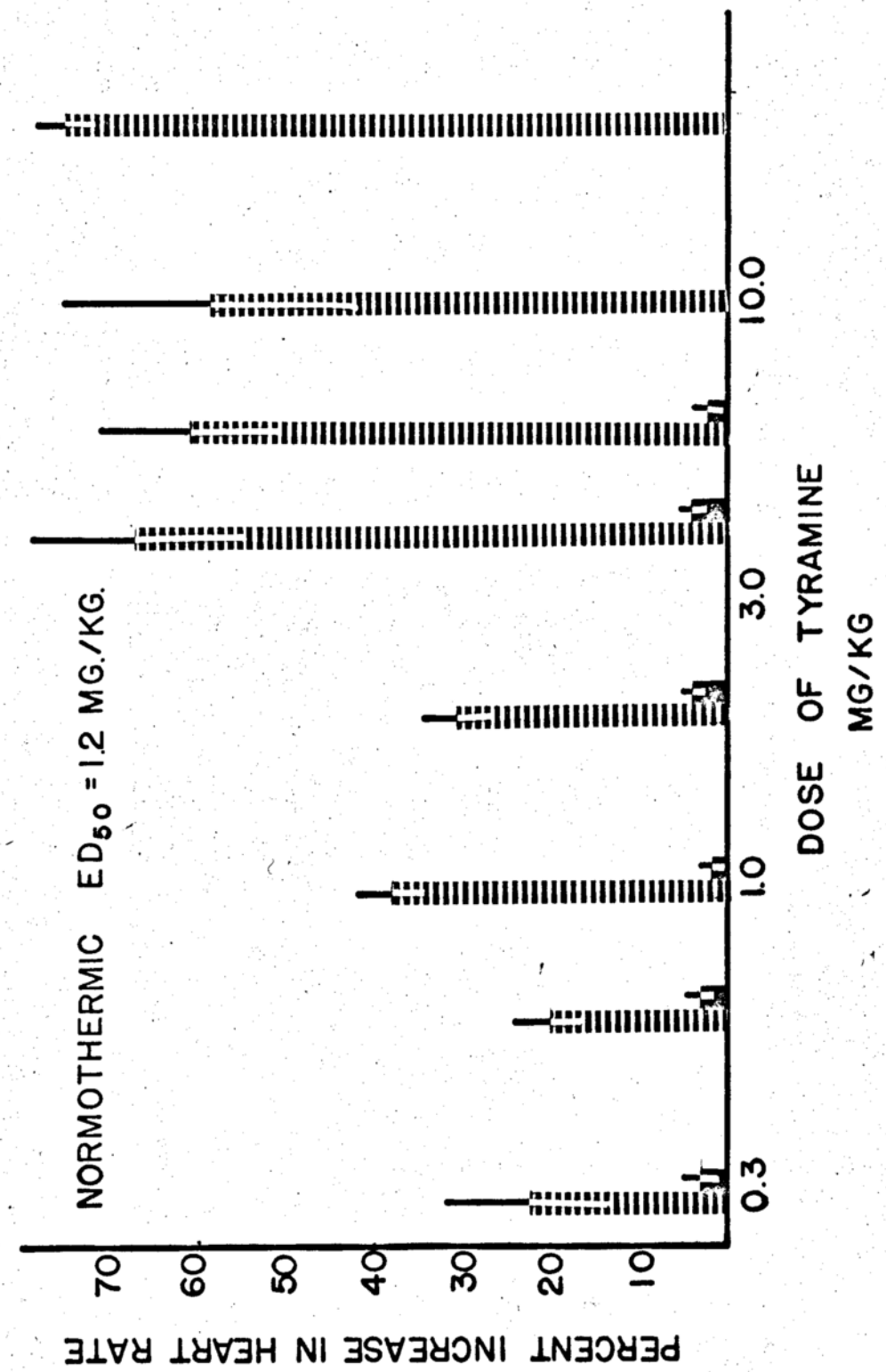
treated, normothermic animals. It would appear that an increased sympathetic outflow occurred as a result of the blood pressure lowering effects of the CPZ-treated group of animals, probably mediated by the release of catecholamines both locally in adipose tissue and systemically via adrenal medullary release. In the hypothermic, CPZ treated animals, however, this manifestation of increased sympathetic tone was antagonized presumably by either inhibiting the release of catecholamines or by a more effective CPZ antagonism of fatty acid mobilization.

It is apparent from these studies that the type of stimulation of the adrenergic receptors determines the response which will be obtained under certain experimental conditions. CPZ appears to potentiate the effects of exogenous NE on the heart in normothermic animals and to overcome the antagonism induced by hypothermia. Yet the combination of CPZ and hypothermia seems to antagonize the effects of NE which has been reflexly released in either the heart or adipose tissue. Therefore, it can be concluded that CPZ and/or hypothermia effect exogenous NE differently than endogenous NE which has been released by sympathetic stimulation. In order to explain these different effects the mechanism of the two stimulatory processes must be separated. Adrenergic responses produced by reflex mechanisms release endogenous NE in the normal physiological manner. It is believed that the impulse causes NE to be released from some storage

pool within the adrenergic neuron, the endogenous NE is transported across the axonal membrane and released into the biophase where it is free to act directly on the receptors while exogenous NE has only to pass from the blood vessel into the tissue biophase. Since it has been demonstrated that CPZ in hypothermic animals inhibits the release and/or uptake of biogenic amines (99,100) it is possible that this release of endogenous catecholamines would be antagonized while exogenous amines could be potentiated.

Effect of Tyramine on Heart Rate. Tyramine was administered to both normothermic and hypothermic animals and the effect on the heart rates were assessed concurrently with the blood pressures. As can be seen in Figure 23, tyramine significantly increased the heart rate in normothermic animals. The observed increase in rate occurred after an initial lag period of approximately one to three minutes, a time lag of the same order of magnitude as observed following the administration of NE. In further comparing tyramine's cardiac effects with those observed with NE, it was found that the maximum response obtainable with tyramine was greater than that found for the doses of NE employed. Thus, tyramine increased the heart rate by 63% while NE caused only a 24% increase in the heart rate. This comparison was made at doses equi-active with respect to their effect on blood pressure. It is also interesting

Figure 23. Effect of hypothermia on tyramine-induced increase in heart rate in nonpretreated rats. Cross hatched bars indicate normothermic rats and black bars indicate hypothermic rats. Vertical lines indicate standard error of the mean. Each point represents the mean of at least four to six experiments.

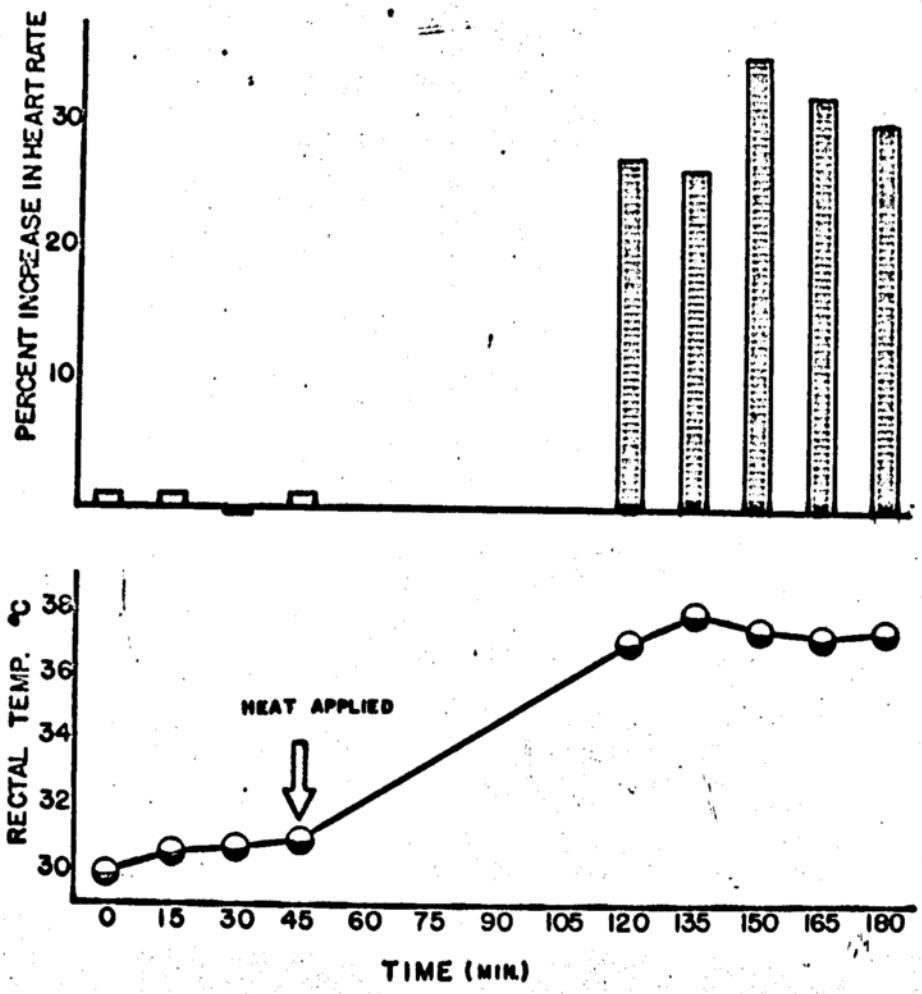

NORMOTHERMIC ED50 = 1.2 MG./KG.
PERCENT INCREASE IN HEART RATE
70
60
50
40
30
20
10
0.3
1.0
3.0
10.0
DOSE OF TYRAMINE
MG/KG

to note that the spread between the dose required to produce an increase in blood pressure equal to 50% of the maximal response and that required to elicit a response equal to 50% of the maximal heart rate response was much less for tyramine than was found for NE.

In hypothermic animals, it was observed (Figure 23) that the lowered body temperature completely abolished the cardio-accelerator action of tyramine. While it was anticipated that hypothermia would effectively reduce this action of tyramine, the magnitude of the antagonism was unexpected. The data illustrated in Figure 24 show that the antagonistic effect of hypothermia in this regard is readily and completely reversible. Thus, when the animal's temperature is returned to normal values, the cardio-accelerator action of tyramine is restored.

The most probable explanation for the effect of hypothermia on the cardio-accelerator action of tyramine involves the so-called "buffer" reflex mechanism initiated by an elevated blood pressure and mediated through an increased activity of the parasympathetic nervous system. Thus, when the blood pressure is elevated as a result of the hypothermia (see Table I), the body responds by increasing the degree of vagal tone (parasympathetic) attendant upon the heart in an attempt to return the blood pressure to normal through its cardiac slowing action. This activity would antagonize the increased adrenergic

Figure 24. Effect of body temperature on heart rate response to tyramine. Each experimental point represents the mean of four rats.


PERCENT INCREASE IN HEART RATE
30
20
10
RECTAL TEMP. °C
38
36
34
32
30
HEAT APPLIED
0 15 30 45 60 75 90 105 120 135 150 165 180
TIME (MIN.)

activity resulting from the administration of tyramine. The buffer reflex mechanism would also be expected to be operative in the normothermic animals but only after the elevation of blood pressure caused by the administration of tyramine and would not be expected to be of the same order of magnitude as in the hypothermic animals.

Effect of Chlorpromazine and Hypothermia on the Tyramine-Induced Changes in Heart Rate. In normothermic animals, CPZ, probably acting through its anti-adrenergic activity, was found to noncompetitively antagonize the cardio-accelerator effects of tyramine. These data are illustrated in Figure 25. This effect of CPZ can be explained either by the inhibitory effect of this molecule on the adrenergic receptor (antagonism of released NE) or by the effect of CPZ on the uptake and/or release of amines (tyramine and NE), or a combination of the two effects.

In hypothermic animals, however, CPZ was found to reverse the hypothermia-induced blockade of tyramine's cardio-accelerator action and to allow tyramine to elicit an increase in the heart rate of the hypothermic animals (see Figure 26). It will be recalled that tyramine was not capable of eliciting an increase in heart rate in hypothermic animals in the absence of CPZ. To explain this apparently paradoxical activity of CPZ, it is necessary to recognize that CPZ possesses many different pharmaco-

Figure 25. Effect of chlorpromazine on tyramine-induced increase in heart rate in normothermic rats. Vertical lines indicate standard error of the mean. Each point represents the mean of at least four to six experiments.

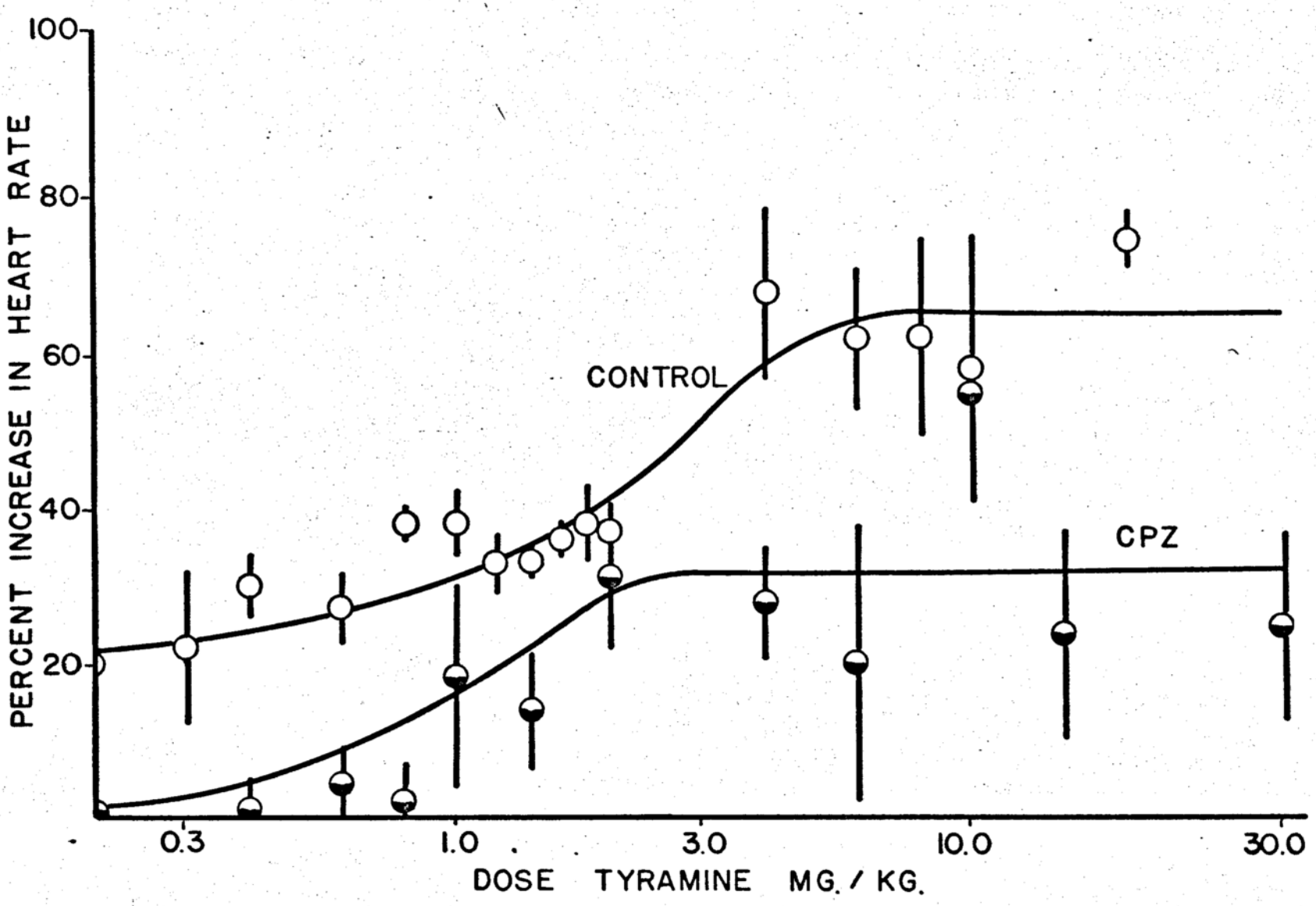
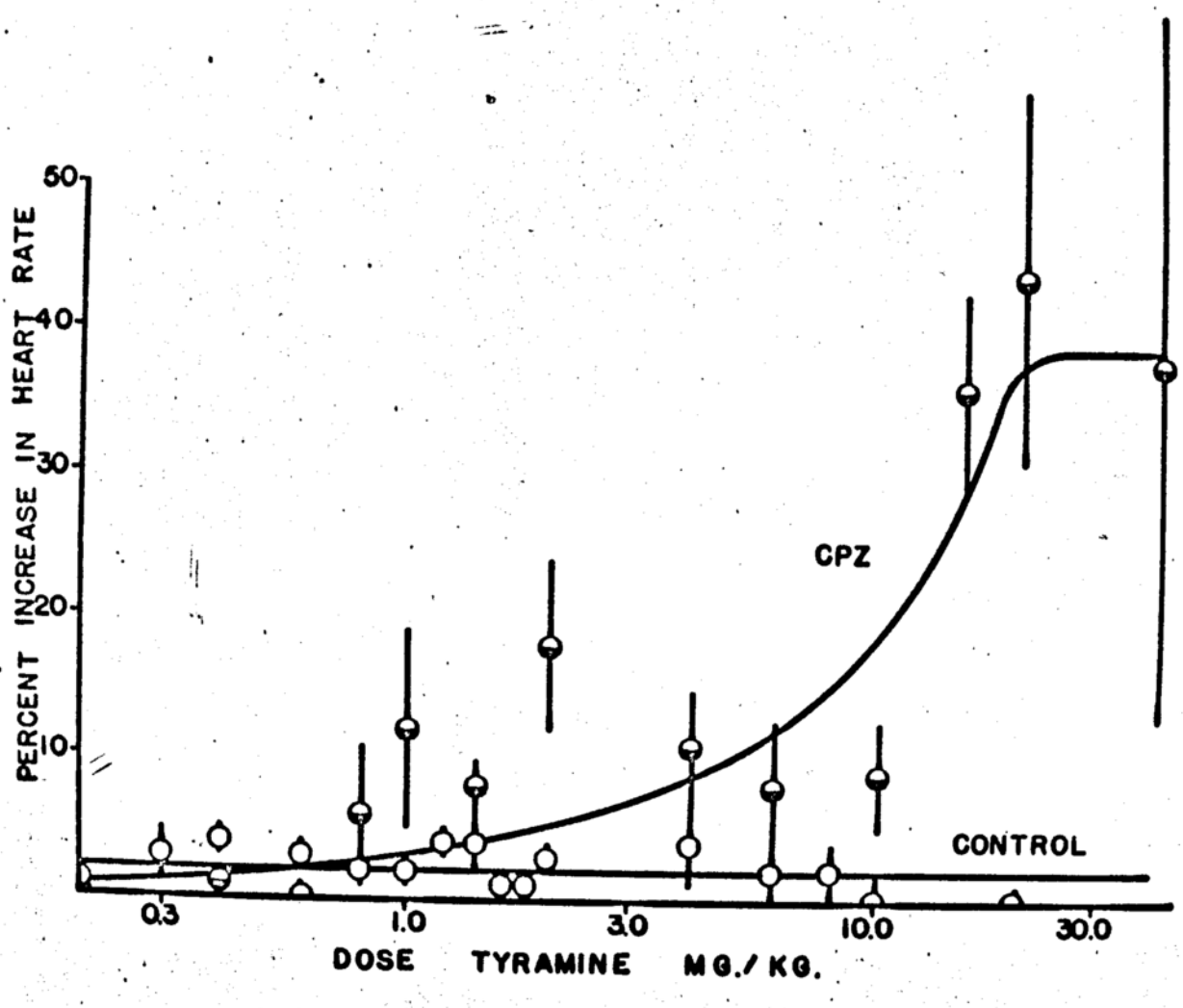

100
80
60
40
20
PERCENT INCREASE IN HEART RATE
CONTROL
CPZ
0.3
1.0
3.0
10.0
30.0
DOSE TYRAMINE MG. / KG.

Fig. 26. Effect of chlorpromazine on the tyramine-induced increase in heart rate in hypothermic rats. Vertical lines represent standard error of the mean. Each point represents the mean of at least four to six experiments.

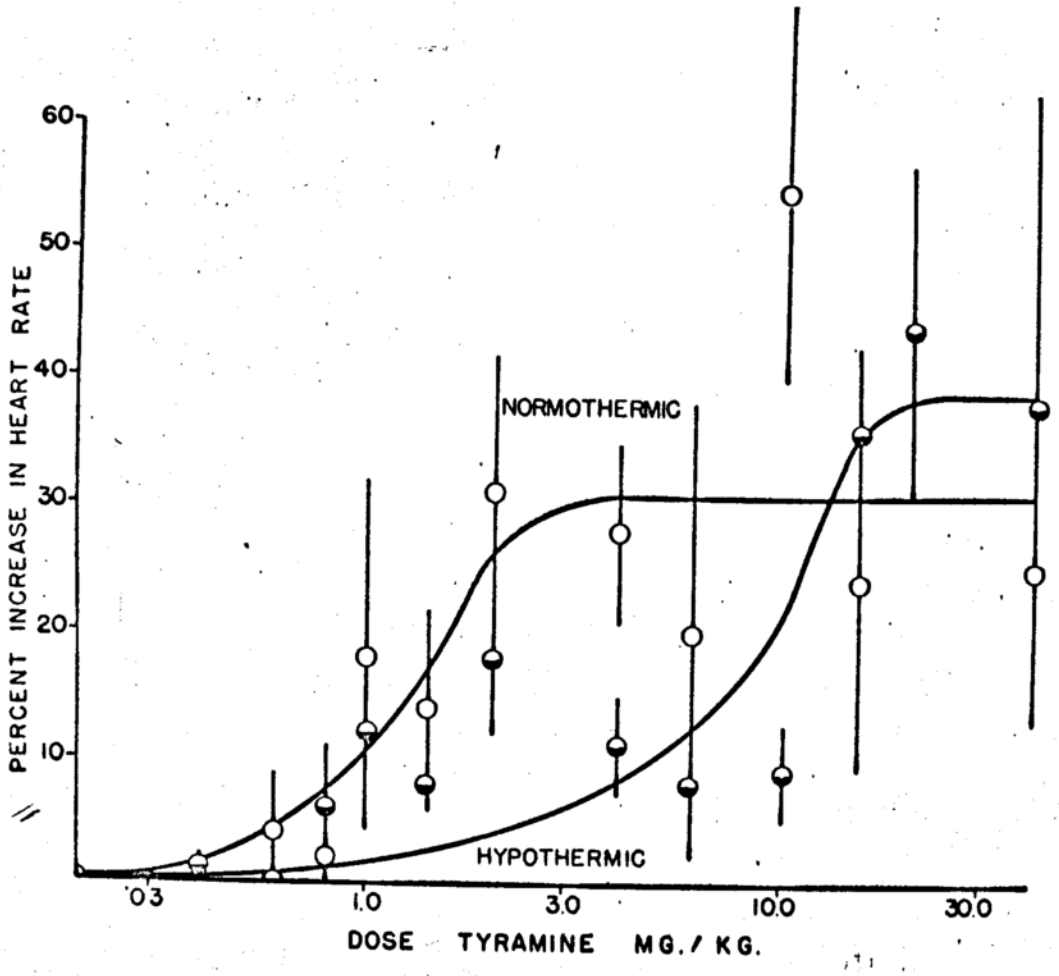

PERCENT INCREASE IN HEART RATE
50
40
30
20
10
CPZ
CONTROL
0.3
1.0
3.0
10.0
30.0
DOSE TYRAMINE MG./KG.

logical properties, one of which is its anti-cholinergic activity. Thus, in the system described above, hypothermia was shown to antagonize tyramine's cardio-accelerator activity probably due to an increase in vagal tone on the heart. When CPZ was administered, the anti-cholinergic action of this compound effectively reduced the degree of vagal tone, thus unmasking the adrenergic effects of the administered tyramine. A similar explanation has been given in the section referring to the effects of NE in hypothermic animals.

The data cited above should not be construed to suggest that CPZ and hypothermia together cancel out their separate antagonisms of tyramine's cardio-accelerator action. Indeed, inspection of Figure 27, which compares the inhibitory action of CPZ in normothermic and hypothermic animals, indicates that CPZ plus hypothermia results in a greater antagonism of tyramine than does CPZ alone. These data suggest that both CPZ and hypothermia influence the adrenergic system responsible for tyramine's action even in the absence of an increased vagal tone.

Development of Tachyphylaxis in the Tyramine-Induced Heart Rate Response. To determine whether or not tachyphylaxis resulted from the repeated administration of tyramine, the drug was administered at 15 minute intervals and the change in heart rate measured. Because tyramine failed to induce an increase in heart rate in hypothermic animals,

Figure 27. Effect of hypothermia on the tyramine-induced increase in heart rate in chlorpromazine treated rats. Vertical lines indicate standard error of the mean. Each point represents the mean of at least four to six experiments.


60
50
40
30
20
10
PERCENT INCREASE IN HEART RATE
NORMOTHERMIC
HYPOTHERMIC
.03
1.0
3.0
10.0
30.0
DOSE TYRAMINE MG./KG.

this phase of the study was limited to normothermic rats. Repeated administrations of tyramine in doses up to 2 mg./Kg., failed to elicit tachyphylaxis. Inspection of Figure 28 indicates, however, that tachyphylaxis was observed at doses of 6 and 10 mg./Kg., the higher dose producing the greater degree of tachyphylaxis. These results are similar to those recorded for tachyphylaxis in the blood pressure experiments, however, the dose required to produce this phenomenon in the heart was higher than that required to obtain a diminished responsiveness in blood pressure.

Effect of Chlorpromazine on the Development of Tachyphylaxis in the Heart. In this phase of the study, it was possible to employ hypothermic rats as well as normothermic inasmuch as CPZ administration reversed the hypothermia-induced blockade of tyramine's cardio-accelerator effects (see above). When tyramine was administered at 15 minute intervals, doses up to 6 mg./Kg. failed to produce tachyphylaxis in either normothermic or hypothermic animals pretreated with CPZ. Figure 29 illustrates the results obtained when tyramine was administered at 15 and 40 mg./Kg., again at 15 minute intervals. Inspection of this graph indicates that tachyphylaxis is evident in the hypothermic animals but that the variability found in the heart rate data preclude making a definitive statement regarding this

Figure 28. Effect of repeated administration of tyramine (6 and 10 mg./Kg.) on the tyramine-induced increase in heart rate in normothermic nonpretreated rats. Each bar represents the mean of four experiments.

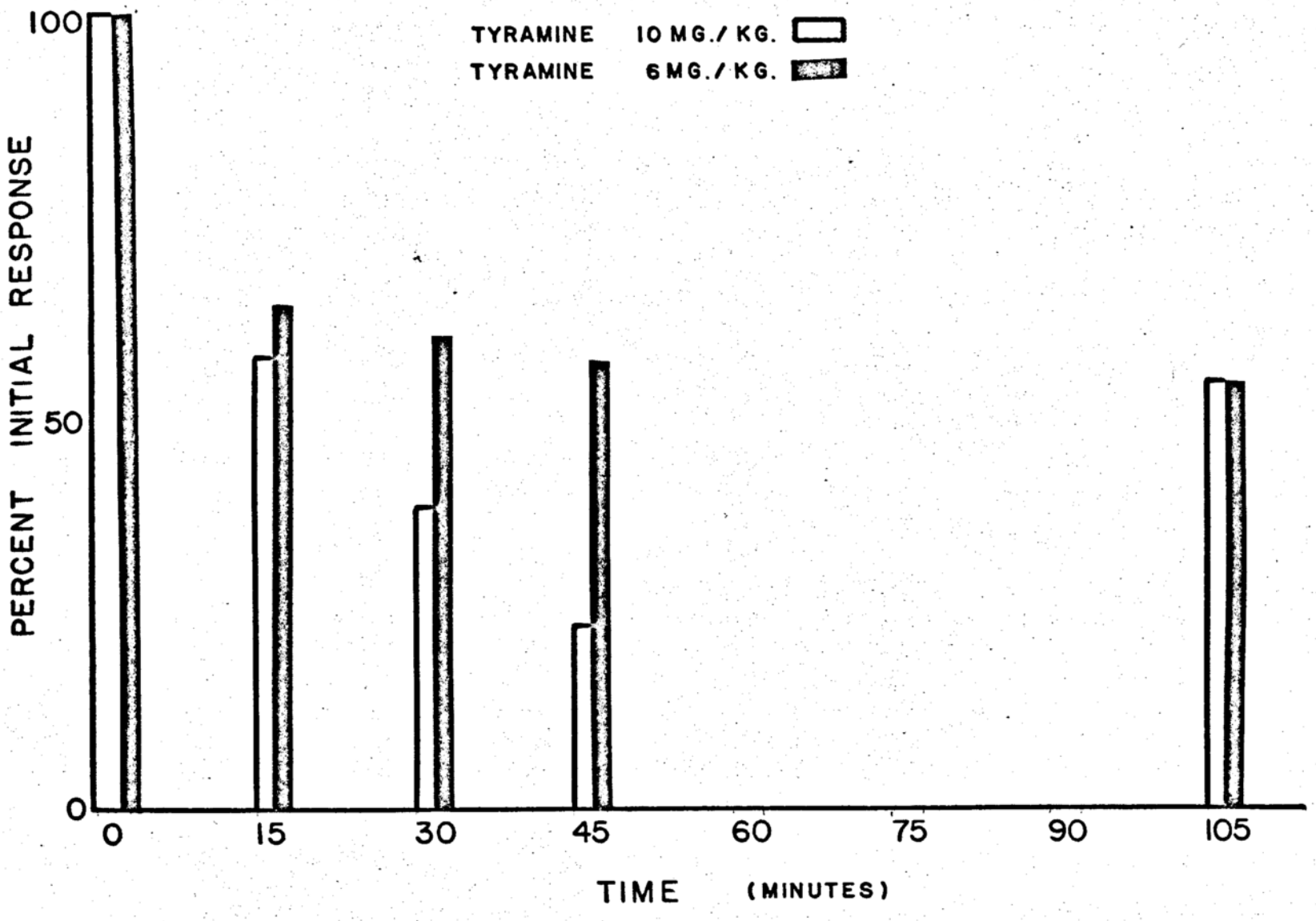
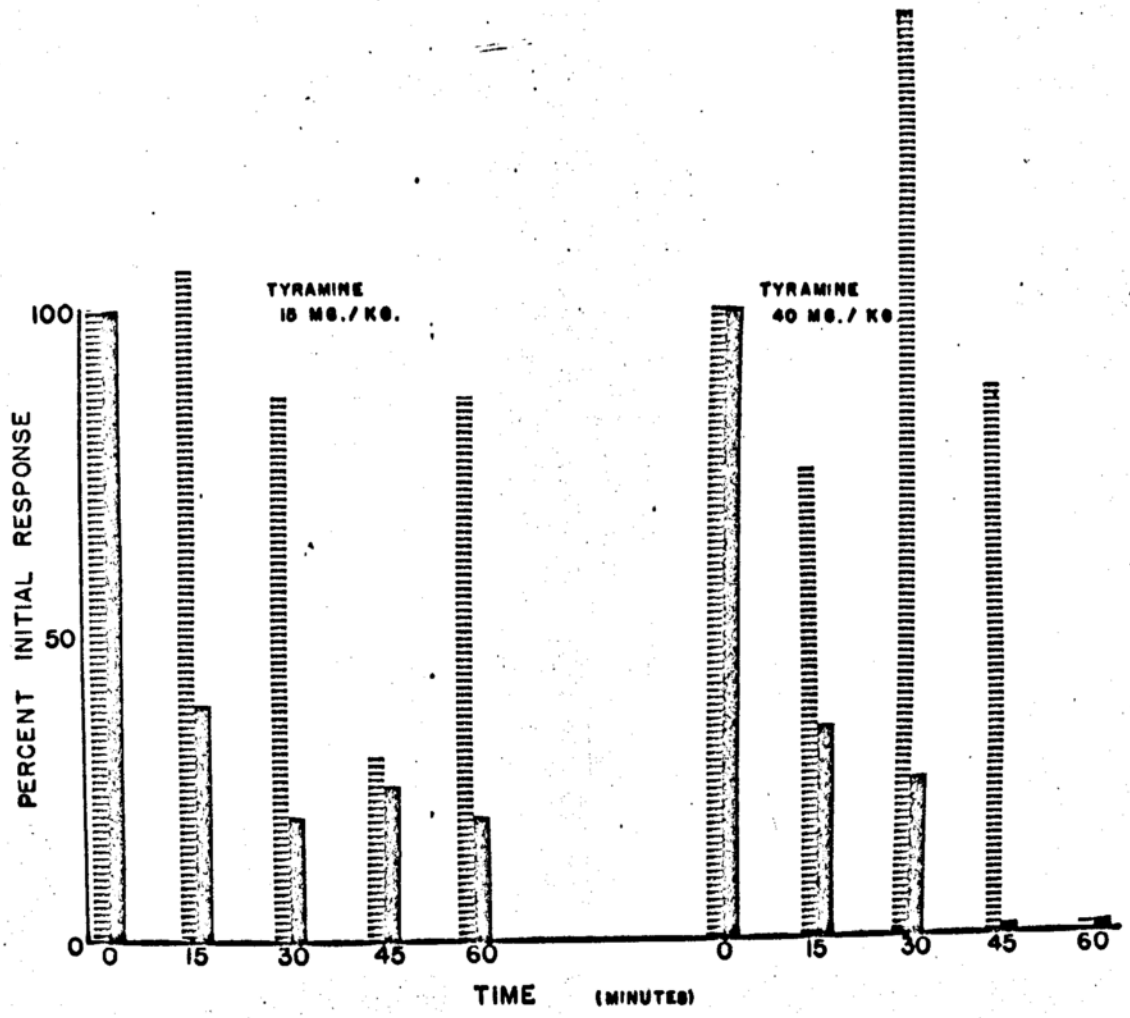

TYRAMINE 10 MG./KG.
TYRAMINE 6 MG./KG.
PERCENT INITIAL RESPONSE
100
50
0
0
15
30
45
60
75
90
105
TIME (MINUTES)

Figure 29. Effect of repeated administration of tyramine (15 and 40 mg./Kg.) on the tyramine induced increase in heart rate in chlorpromazine treated rats. Cross hatched bars indicate normothermic rats and black bars indicate hypothermic rats. Each bar represents the mean of four experiments.


TYRAMINE
15 MG./KG.
TYRAMINE
40 MG./KG.
100
50
0
PERCENT INITIAL RESPONSE
0
15
30
45
60
0
15
30
45
60
TIME (MINUTES)

phenomenon in normothermic rats. It is evident, however, that CPZ exerted a protective effect against depletion of the NE stores by tyramine, a result consistent with those reported for the effect of CPZ on the blood pressure effects of tyramine.

The protective effect of CPZ against the depletion of NE stores in the heart by tyramine again indicates that CPZ exerts an effect on the transport mechanisms operative in the adrenergic neuron. Thus, by decreasing either the rate of uptake of tyramine or the rate of release of endogenous NE, the storage pool of endogenous NE is maintained at an effective level even after the administration of relatively large amounts of tyramine. The results obtained in the CPZ treated animals in which doses of tyramine as large as 40 mg./Kg., failed to elicit a clear-cut example of tachyphylaxis are in sharp contrast to the results found in animals not receiving CPZ in which doses of tyramine as low as 6 mg./Kg. produced tachyphylaxis. The results obtained in the heart rate study are in complete agreement with those obtained in the blood pressure experiments.

CONCLUSIONS

The investigations reported herein were undertaken in an attempt to define and, to some degree, explain the effects of hypothermia on the actions of drugs. Particular emphasis was placed upon the adrenergic drugs because of their role in the maintenance of the homeostatic state in mammals. In this regard, the adrenergic drugs chosen for these experiments were norepinephrine, the neurotransmitter of the sympathetic nervous system and tyramine, an indirect acting adrenergic drug. Through the use of these two drugs it was possible to gain a better insight into the mechanisms operative in the adrenergic system. From the results of these studies it can be concluded that:

1. The lowering of body temperature from a normal of 37°C to approximately 30°C resulted in a moderate elevation of blood pressure, mediated primarily if not solely by an increased degree of sympathetic nervous system activity.
2. Moderate hypothermia (body temperature 30°C) antagonized the NE and tyramine-induced rise in systemic blood pressure. While the effect of hypothermia on the pressor action of these two agonists was similar, the data indicated that the mechanisms by which the antagonism was produced were different. In this regard, it was observed that the hypothermia-induced antagonism of NE's action was

of a competitive nature while that exerted against tyramine could not be overcome by increasing the dose of tyramine. It is postulated that hypothermia decreased the rate of active transport of NE thus effectively reducing the concentration of this agonist reaching the receptor area. In the case of tyramine, an effect on active transport mechanisms may play a role in the antagonism observed, however, inasmuch as the major portion of the transport processes involved with the diffusion of tyramine are believed to be passive in nature, this mechanism is probably secondary in overall importance. It is believed that the hypothermia-induced antagonism to tyramine is due to a property of tyramine itself, the lowered body temperature merely potentiating this characteristic of tyramine. Thus, it is postulated that the phenomenon of auto-inhibition best explains the effects of tyramine observed in hypothermic animals.

3. Hypothermia also was capable of antagonizing the effects of NE and tyramine on the heart rate of rats. While hypothermia effectively reduced the cardio-accelerator effects of NE, it completely abolished the chronotropic action of tyramine. This antagonistic effect characteristic of the hypothermic state is probably due to the increased degree of parasympathetic activity present in the intact animal as a result of a reflex mechanism (buffer reflex) initiated by the rise in systemic blood

pressure. The rise in blood pressure in turn being due to the physiological needs brought about by hypothermia.

4. The administration of CPZ effectively antagonized the blood pressure effects of both tyramine and NE in hypothermic as well as normothermic animals. This property of CPZ has been well documented in many laboratories and the results of the present studies serve to corroborate the work of others. It is postulated that the antagonism exerted by CPZ against the adrenergic agonists has at least two components of action: (1) A direct effect on the adrenergic receptor and (2) an inhibitory effect on the transport processes operative within the adrenergic biophase.

5. While CPZ also exerts an anti-adrenergic effect on the positive chronotropic actions of both NE and tyramine in normothermic animals, its effects on the actions of these two compounds in hypothermic animals is in the opposite direction. Thus, when CPZ is administered to hypothermic animals followed by either tyramine or NE, the increase in heart rate observed is greater than if the adrenergic stimulants were administered to hypothermic animals in the absence of CPZ. Indeed, in regard to tyramine, the effects of CPZ are dramatic. In the absence of CPZ, tyramine is incapable of eliciting an increase in heart rate in hypothermic animals. In the presence of CPZ, tyramine's cardio-accelerator action is present. These

apparently paradoxical results can be explained in the following manner. In addition to its other pharmacological properties, CPZ possesses potent anti-cholinergic properties. Thus, when administered to hypothermic animals, CPZ effectively antagonizes the buffer reflex mechanism (cholinergic) and by this action unmasks the positive chronotropic effects of tyramine. Its effect in NE treated animals is explained in the same way.

6. Tachyphylaxis was observed after the repeated administration of tyramine in both the blood pressure and heart rate experiments. Hypothermia had no pronounced effect on either the rate of onset or the degree of tachphylaxis observed. CPZ, on the other hand, was found to produce a protective effect against the tyramine-induced tachyphylaxis. It is concluded that CPZ either reduces the rate of uptake of tyramine into the adrenergic neurotransmitter storage area or decreases the rate of release of neurotransmitter. By either mechanism, or a combination of the two, CPZ aids in the maintenance of an adequate level of NE within the storage pool, thus preventing the depletion of the storage pool by tyramine.

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Pressor Responses of Certain Adrenergic Drugs in Hypothermic Rats

ROBERT LEWIS CAVANAGH

Under the Supervision of Professor Kenneth F. Finger

The increasing therapeutic and other medical uses of hypothermia in addition to the possibility of encountering lowered body temperatures during prolonged space travel have made it necessary to investigate the effect of hypothermia on the action of drugs. Of particular importance in this regard is knowledge concerned with the action of adrenergic drugs inasmuch as the adrenergic system is intimately involved with the maintenance of the homeostatic state within the animal body. This study was instituted in an attempt to define and explain, if possible, the influence of a lowered body temperature on the actions of two specific adrenergic drugs, namely norepinephrine and tyramine.

Utilizing the anesthetized rat as the experimental animal, blood pressure and heart rate effects of the adrenergic agonists norepinephrine and tyramine were investigated in both normothermic and hypothermic animals. While the knowledge gained concerning the cardiovascular actions of adrenergic drugs is of importance in its own right, it is also believed that this information can be directly extrapolated to other adrenergic systems concerned

with the maintenance of homeostasis.

The results of these studies indicated that hypothermia induced a moderate hypertension in the experimental animal. The results further indicated that this elevation of blood pressure was due to an increased degree of sympathetic nervous system activity resulting from the physiological needs brought about by the hypothermic state. While the blood pressure was increased by hypothermia, no increase in heart rate was observed presumably because of an increase in parasympathetic nervous system activity reflexly induced by the elevation in blood pressure.

During sustained hypothermia, it was observed that both the increase in blood pressure and the increase in heart rate induced by either norepinephrine or tyramine were effectively reduced or abolished by the effects of lowered body temperature. These data can be explained in terms of the effect of lowered body temperature on intrinsic transport processes operative within the adrenergic biophase and the phenomenon of autoinhibition characteristic of compounds such as tyramine. In addition, the "buffer" reflex mechanism manifesting itself in an increased degree of parasympathetic control over the rate of the heart beat probably accounts for a major portion of the antagonistic action of hypothermia on the positive chronotropic effects of the adrenergic agonists.

The administration of chlorpromazine to either normothermic or hypothermic animals effectively inhibited

the elevation of blood pressure induced by either norepinephrine or tyramine. This anti-adrenergic action of chlorpromazine can be explained either through its effects directly on the adrenergic receptor or by its actions on the diffusional processes concerned with the transport of amines into or out of the adrenergic neuron, or a combination of these two effects.

The action of chlorpromazine on the heart rate effects of the adrenergic stimulants, however, differed significantly from those observed in the blood pressure experiments. In hypothermic animals, chlorpromazine was found to potentiate the positive chronotropic effects of both tyramine and norepinephrine. The mechanism by which chlorpromazine accomplished this effect appeared to be due to its relatively potent anti-cholinergic properties which negated the "buffer" reflex mechanisms (see above) thus unmasking the cardio-accelerator actions of the adrenergic stimulants. Despite the potentiation observed in hypothermic animals, it was observed that a significant degree of an anti-adrenergic effect of chlorpromazine was also present in both normothermic and hypothermic animals. Thus, the net effect on heart rate to be obtained with the system employed was dependent upon the physiological state of the animal.

Chlorpromazine was also found to effectively antagonize the onset and degree of tachyphylaxis induced by

the administration of successive doses of tyramine. It was postulated that the protective action of chlorpromazine was due to its effects on the transport mechanisms associated with adrenergic amine transport within the adrenergic biophase.

These studies have shown that a lowered body temperature significantly alters the cardiovascular actions of both norepinephrine and tyramine and has indicated, but not proven, the mechanisms by which these effects occur.

Approved_____
Professor Kenneth F. Finger

Date_____