EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF THIORIDAZINE ON CARDIOVASCULAR RESPONSES

by

Lynn Gourley

A Thesis

Presented to the Department of Pharmacology

and the Graduate Division of the

University of Oregon Health Sciences Center

School of Medicine

in partial fulfillment of the

requirements for the degree of

Master of Science

February 1978

APPROVED:

[redacted]

Hall Downes, M.D., Ph.D.,
Associate Professor in Charge of Thesis

[redacted]

Chairman, Graduate Council

DEDICATION

To my loving parents, Leda Avery Gourley and Donald Earl Gourley, without whose support this work never would have been completed.

ACKNOWLEDGMENTS

I wish to express my deepest thanks to the staff of the Pharmacology Department for training me in their discipline.

I am especially indebted to my mentor, Dr. Hall Downes, who so patiently and consistently offered guidance and encouragement throughout the research and writing of this thesis. I am also indebted to Dr. Herbert Wendel for introducing me to the problem of sudden unexplained death during sustained phenothiazine therapy, to Dr. Ralph D. Tanz for his support and use of his laboratory, and to Dr. Kaye Fox for his concern and encouragement throughout my graduate education.

I owe a special thanks to Ann Kloka for her excellent laboratory assistance and to Dr. Kim Wayson for his work on the thioridazine assay. Their help and friendship was invaluable in this endeavor.

I am grateful to Mrs. Diana Sutton and Mrs. Marion Bergstrom for their patient typing of this manuscript.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure

LIST OF PUBLICATIONS

Gourley, L.A., Kloka, A.G., and Downes, H.: Delayed development of B-adrenergic block during sustained administration of thioridazine. Proc. West. Pharmacol. Soc. 20: 115, 1977.

INTRODUCTION

I. Association of Phenothiazines with Sudden Unexplained Death or Cardiovascular Collapse.

Although the cardiovascular effects of phenothiazines are usually benign, sporadic reports associating phenothiazines with sudden unexplained death have appeared in the literature since the introduction of major tranquilizers in 1952 (1-15). A review of the literature has produced 82 well documented case histories with autopsies. Twenty-four other cases were reported but without detailed case histories or autopsy findings. Even taking into account that in general only a tiny fraction of toxic drug reactions is ever reported, 106 case reports represent a very low incidence considering that an estimated 50 million persons received chlorpromazine between 1955-1965 (16). The reports exhibit a similar clinical picture. An institutionalized psychiatric patient being treated with phenothiazine drugs either collapsed or was found dead without evidence of a stuggle. The death usually occurred in the morning, and there were no apparent premonitory symptoms. Table 1 shows that the greatest incidence of such sudden death was in males, age 25-55, on long term high dose therapy. Table 2 shows the wide variation in dosage and duration of therapy at the time of death or collapse. Chlorpromazine and thioridazine or a combination of the two compounds were implicated most often (Table 1), however, these two drugs were the most commonly prescribed phenothiazines in the overall population. When patients were discovered at the time of collapse, electrocardiograms revealed ventricular tachycardia and/or fibrillation (1, 2, 5, 11-14).

TABLE 1

Incidence of Sudden Death and/or Collapse in Patients Taking Phenothiazine

Age Group	Number of Patients		Chlorpromazine	Thioridazine	Thioridazine and Chlorpromazine	Other Phenothiazine Drugs
15-24	7	5M* 2F	2	1	2	2
25-34	26	13M 14F	10	6	8	2
35-44	20	18M 2F	9	3	6	2
45-54	20	12M 8F	6	5	5	4
55-64	4	3M 1F	2	0	2	0
65	5	3M 2F	3	0	2	0
Totals	82	54M 28F	32	15	25	10

Compiled from references 6-8, 10-15.

*M - males
F - females

TABLE 2

Dose and Duration of Phenothiazine Therapy Received by Patients at the Time of Sudden Death and/or Collapse

	Dose (mg/day)				
	0-99	100-299	300-799	800-1999	2000
Chlorpromazine	3*	3	14	9	1
Thioridazine	0	3	8	4	2
	Duration of Treatment				
	1-29 days	1-5 mo	6-11 mo	1-5 yrs	6-10 yrs
Chlorpromazine	4	4	0	20	5
Thioridazine	4	4	2	3	1

*Tabulated from references 6-8, 10-15.

II. Known Cardiovascular Effects of Phenothiazines.

The most common cardiovascular effect of phenothiazines is postural or orthostatic hypotension (17, 18). Therapeutic doses of chlorpromazine do not usually produce hypotension when given intramuscularly or orally to patients in the recumbent position, but patients frequently become hypotensive upon standing (19). The mechanisms underlying orthostatic hypotension are not well understood but are believed to include decreased central vasomotor tone (17, 18, 26-29), alpha-adrenergic blockade (20, 25), and perhaps an element of myocardial depression (2, 25, 30, 33).

Experimental evidence suggests that depression of central vasomotor tone contributes to phenothiazine-induced orthostatic hypotension. Wang et al. (29) used cross-circulation experiments in dogs to test the effect of chlorpromazine on central vasomotor tone. The head circulation of a recipient dog was isolated from that of its body. The corresponding carotid arteries and jugular veins of a donor dog were anastomosed end-to-end to those of the recipient dog in such a manner that drugs injected intravenously in the donor dog were distributed throughout the body of the donor dog and the head circulation of the recipient dog. Blood pressure and heart rate responses were measured in both the recipient and donor dogs. Chlorpromazine, 0.2 mg/kg, produced a fall in blood pressure in the donor dog, but little or no change in blood pressure in the recipient dog. However, the normal pressor response to electrical stimulation of the vasomotor center in the recipient dog was reduced after pretreatment with chlorpromazine. Since chlorpromazine did not have access to the cardiovascular tree of the recipient

dog, save for the head circulation, the block of the pressor response to vasomotor stimulation could not reflect a peripheral action.

Another characteristic effect of phenothiazines is "epinephrine reversal". Courvoisier et al. (34) in 1953 noted that injection of phenothiazines in anesthetized animals changed the response to epinephrine from a predominately pressor to a predominately depressor effect. Many other authors have noted the phenothiazine antagonism to or reversal of the pressor response to epinephrine (17, 23-27, 35). Studies with isolated aortic strips (20, 21) have shown chlorpromazine to be a competitive antagonist to both epinephrine and norepinephrine. However, alpha adrenergic block may not be the sole explanation of phenothiazine reversal of the pressor response to epinephrine. A number of investigators have noted that the doses of phenothiazines that block the hypertensive response to epinephrine spare that same response to norepinephrine (20, 22, 25). Based on this discrepancy and the observed potentiation by chlorpromazine of the depressor effect of isoproterenol, Gokhale (20) has suggested that beta adrenergic effects might contribute to epinephrine reversal. It has been suggested that enhanced beta adrenergic effects might also give rise to dangerous ventricular dysrhythmias in patients being treated with phenothiazines (7, 27, 36). This possibility is reinforced by animal studies indicating that phenothiazines can impair the reuptake of catecholamines (38-41) and by clinical reports of unusually high blood and urine levels of catecholamines in some patients receiving phenothiazines (42).

The tricyclic antidepressants such as imipramine and amitriptyline are structurally very similar to phenothiazines and share many of their

pharmacological effects. As with the phenothiazines, there have been reports of unexpected deaths during long term therapy (50, 51). Whereas severe cardiovascular toxicity during therapy with phenothiazines is rare, cardiotoxic effects are a prominent feature of acute overdosage with tricyclic antidepressants. The cardiotoxic effects have been observed in many cases of accidental ingestion by children (52-54) or suicidal overdosage by adults (55, 56). Toxic effects include hyper- or hypotension, cardiac conduction deficits (first degree AV block, bundle branch block, and intraventricular disturbances of conduction), tachycardia, and ventricular dysrhythmias. Death from ventricular dysrhythmias is not uncommon during acute overdosage with these drugs. Delayed deaths, after a short period of clinical improvement, have also been reported (52, 56). Tricyclic antidepressants are much more active than the phenothiazines in blocking reuptake of catecholamines (39-41) but are not as active in producing alpha adrenergic blockade (26). In addition, anticholinergic responses of tricyclic antidepressants are prominent in comparison to the weak anticholinergic activity exhibited by phenothiazines (26).

Phenothiazines, like quinidine, decrease intrinsic rhythmicity, conduction velocity and depolarization rates (32). At low doses these effects are manifested as benign ECG changes which consist of prolongation of the PQ and QT interval, flattening of the T wave, appearance of a U wave and depression of the S-T segment (1, 11-15). Laboratory studies using isolated rat hearts (30, 31) have shown that these ECG changes occur at 1.4×10^{-5} M thioridazine. Higher concentrations of thioridazine, 5×10^{-5} M, decrease aortic output and coronary flow.

Electrophysiological studies using guinea pig hearts (32) and canine Purkinje fibers (33) have shown that phenothiazines prolong the plateau and duration of the action potential. The similarity between the myocardial depressant effects of phenothiazines and quinidine may be more than incidental. Sudden death or collapse which has been attributed to ventricular fibrillation has also been associated with quinidine therapy, i.e., "quinidine syncope" (37).

III. Possible Mechanisms Underlying Phenothiazine-associated Cardiovascular Toxicity.

The previous literature review suggests the following mechanisms might contribute to the phenothiazine-associated cardiovascular toxicity:

1. Increased levels of circulating catecholamines;
2. Potentiation of beta adrenergic responses;
3. Loss of opposing parasympathetic activity;
4. Quinidine-like myocardial depression with re-entrant excitation.

IV. Statement of the Problem.

The cardiovascular effects of phenothiazines during acute administration have been extensively studied. However, none of these effects can readily explain the phenothiazine-associated sudden death which occurs predominantly after sustained administration. Thus, the present studies were undertaken to compare the effects of

phenothiazines on cardiovascular responses to autonomic agonists and dysrhythmogenic doses of ouabain during acute and sustained administration.

MATERIALS AND METHODS

I. Animals.

Cats of either sex, 2.5 to 3.5 kg, were obtained from local sources and housed in the Animal Care Department at the University of Oregon Health Sciences Center. Upon arrival the cats received the following prophylactic treatment for the following diseases:

1. Worms: TASK^{R} (Dichlorvos), Shell, San Ramone, Calif.
2. Upper respiratory tract infections: Feline Rhinotracheitis Calici Vaccine (FVR-C), Pitman Moore, Washington Crossing, New Jersey.
3. Panleukopenia: Feline Panleukopenia Vaccine, Norden Labs, Lincoln, Nebraska.
4. Rabies: Rabies Vaccine, Norden Labs, Lincoln, Nebraska.
5. Ear Mites: Canolene^{R} Flea and Tick Spray, Florida Veterinary Supplies, Miami Springs, Florida.

After a general physical examination by a licensed veterinarian the cats were quarantined for ten days. All cats used in these studies received the same pretreatment so that differences between groups were not due to preceding prophylactic treatment.

II. Chronic Treatment

Cats used for the chronic studies were housed in the research laboratory by day to allow for observation and greater freedom of movement. In preliminary experiments five cats received thioridazine 20-30 mg/kg/day p.o. in two divided doses. These dosages were the upper limit of those used in previously published animal studies (21,34).

These animals became excessively depressed and refused to eat; four died before the end of the third week. In subsequent experiments animals were given 10 mg/kg/day for the first week and 20 mg/kg/day for the next two weeks. If the animals lost appreciable weight, they were tube fed twice a day (in conjunction with thioridazine administration) with a 50% solution of Dyne formula (see below). Six of the ten cats developed upper respiratory tract infections during the three week treatment period and were treated with Chloramphenicol, 100 mg/day, until the infection cleared (approximately three days/infection). The chronically treated cats were then used either as intact animals or as sources for isolated preparation studies.

III. Terminology

Cats received thioridazine as an acute i.v. infusion on the experimental day, or as three weeks' pretreatment with an oral preparation, or both. The designation 'pre- or post-thioridazine infusion' refers to the acute i.v. infusion without reference to pretreatment with oral thioridazine. Animals pretreated with oral thioridazine are designated 'exposed'; those not pretreated with oral thioridazine are termed 'naive'. The possible combinations are shown in Table 3.

Similarly, in studies with isolated organs,'pre- and post-thioridazine' refer to addition of thioridazine (1.2 µg/ml) to the bath solution, again without reference to the presence or absence of pretreatment with oral thioridazine.

TABLE 3.

Experimental Conditions under which Cats were Tested

Naive	Animals that had received no thioridazine prior to the experimental day
Naive Pre-Thioridazine Infusion	Responses of naive animals to adrenergic agonists before the infusion of thioridazine
Naive Post-Thioridazine Infusion	Responses of naive animals to adrenergic agonists after the infusion of thioridazine
Naive Post-Saline Infusion	Responses of naive animals to adrenergic agonists after an infusion of normal saline
Exposed	Animals that had received thioridazine, 10-20 mg/kg/day, p.o., for three weeks prior to the experimental day
Exposed Pre-Thioridazine Infusion	Responses of exposed animals to adrenergic agonists before an infusion of thioridazine
Exposed Post-Thioridazine Infusion	Responses of exposed animals to adrenergic agonists after the infusion of thioridazine

IV. Studies in Intact Cats

Cats were anesthetized by i.p. injection of a mixture of equimolar parts of alpha-chloralose and urethane. The morning of the experiment a one molar solution of the anesthetic mixture was prepared in 50% ethanol. Animals were injected with 1 cc/kg of this mixture initially, and 0.5 cc/kg every 30 to 45 minutes until surgical anesthesia was obtained. The maximum dose delivered to any cat was 2 cc/kg.

A midline incision was made along the ventral surface of the neck, the sternomastoid muscles were cut, the sternohyoid muscles were separated to expose the trachea, and an incision was made between two tracheal cartilages. A plastic cannula the size of the trachea was inserted into the trachea and secured with silk sutures. The cannula was attached to a Harvard small animal respirator. The approximate tidal volume for the weight of the animal at a rate of 15 respirations per minute was determined from a ventilation graph (111). The animal was then hyperventilated at 20 respirations per minute, rather than 15, to suppress spontaneous respiration. For subsequent "clamp" procedures both carotid arteries were isolated and marked with ligatures attached loosely to each so as not to occlude the arteries.

An incision was made along the medial surface of the right forelimb to expose the cephalic vein. The vein was cannulated with Clay-Adams #60-90 polyethylene (PE) tubing prefilled with normal saline and advanced until it could be palpated at the shoulder. All drugs were injected through this cannula either as a single rapid injection

or, in the case of ouabain and thioridazine, as slow infusions administered with a Harvard 350 infusion pump. Thioridazine, 10 mg/kg, was infused at 0.5 cc/min over 15 minutes. Ouabain, 5 µg/kg/min, was infused at 0.5 cc/min until cardiac dysrhythmias were induced.

The right femoral artery was palpated and an incision was made along the medial aspect of the right hindlimb just lateral to the artery. The artery was exposed and cannulated with PE tubing prefilled with heparinized (10 units/cc) saline. The cannula was attached to a Statham blood pressure transducer (Model P23AC).

Following surgery the animal was placed on its right side and ECG needle electrodes were inserted in the right forelimb, left hindlimb and right hindlimb (ground) to yield a lead I or II recording. The temperature of the animal was maintained at 38° C by a Thermistep temperature controller (Model 71, Arthur Thomas Co.) connected to a heating plate manufactured by UOHSC Department of Instrument and Safety Services. All drug responses were recorded on a Grass (Model 7) 4-channel polygraph.

V. Studies in Isolated Organs.

Cats were sacrificed by ether inhalation, the thorax opened and aortic strips, tracheal chains and whole hearts rapidly removed and placed in aerated Krebs solution.

A. Aortic Strips: The aortic strips were prepared according to the method of Furchgott and Bhadrakom (65). The thoracic aorta was removed and cut into four spiral strips 0.2 cm wide and 4-5 cm long. The tissues were mounted vertically under 2 gm tension in 50 ml baths

containing aerated Krebs solution (see below) maintained at 37.5° C. Contractions were recorded isotonically by means of Harvard transducers (Model 356) and a Harvard polygraph.

B. Tracheal Chains: The tracheal chains were prepared by the method of Castillo and DeBeer (66). The trachea was removed from the cricoid cartilage to the carina and placed into aerated Krebs solution. Starting at the laryngeal end, the trachea was cut into 16 rings 2-3 mm in width and placed, in turn, into four petri dishes. Each petri dish, therefore, contained four rings taken at approximately equal intervals along the entire length of the trachea. These four rings were tied together with 5-0 silk sutures to form a chain and mounted vertically in 50 ml baths (Fig. 1) containing aerated Krebs solution maintained at 37.5° C. Contractions were recorded with Grass FT .03 isometric transducers on a four channel Grass polygraph. All chains were washed several times with Krebs solution and allowed to equilibrate under 2 gm tension for approximately 2.5 hrs.

C. Isolated Hearts. The whole isolated hearts were prepared and mounted in a moist glass chamber (Fig. 2), as described by Anderson and Craver (67), and perfused with Krebs solution (see below) maintained at 37.5° C and aerated with 95% O_2 and 5% CO_2. A suture attached to the apex of the heart was connected to a Grass FT .03 force transducer for the recording of rate and contractile force and the heart was placed under 1-2 gms tension. Needle electrodes were placed in the right atrium and left ventricular epicardium for an electrical recording of cardiac activity. After a period of

stabilization of 1 to 1.5 hr an epinephrine dose-response curve was obtained. Doses of epinephrine bitartrate, dissolved in Krebs solution, were injected in a volume of 0.5 cc and followed by 1.5 cc of Krebs solution as a flush directly into the coronary arteries by way of a side arm (see Fig. 2). After an injection of a dose of epinephrine the cardiac response was allowed to reach its maximal effect and return to control levels prior to administration of the next higher dose.

D. Addition of thioridazine: Preliminary experiments showed that cat aortic strips and cat tracheal chains could only be maximally contracted once; thereafter, subsequent contractions were diminished. However, from every aorta and trachea three or four strips or chains, respectively, were prepared. Therefore, thioridazine 1.2 µg/ml, was added to the baths of one or two preparations and not added to the other two tissue baths. The response of preparations that had received thioridazine acutely were compared to those that had not been treated.

In the isolated heart preparation two epinephrine dose-response curves were obtained. After the initial dose-response curve thioridazine, 1.2 µg/ml, was added to the perfusate reservoir and continuously recirculated to the heart. At the end of one hour a second dose-response curve was elicited in a manner similar to the first except that each concentration of epinephrine was injected in Krebs solution containing thioridazine · HCl, 1.2 µg/ml.

VI. Thioridazine Assay

Whole blood was obtained by aspiration from the chest cavity of chronically treated cats immediately after their hearts had been excised. Each sample of blood was allowed to stand for 10 min in glass tubes which were inverted several times to facilitate clotting. The clots were "rimmed" and the blood centrifuged at 2,000 rpm for 10 min. The serum was separated after centrifugation and frozen (-11° C). The extraction of thawed serum was adapted from the phenothiazine assay developed by Dinovo et al. (68) as follows: 2.5 ml of 1N NaOH was added to 5 ml of serum and this solution was extracted twice with 10 ml of N-heptane/iso-amyl alcohol solvent mix (100:1.5). The two aliquots of solvent-mix were combined and 5 ml were extracted with 4 ml of 1N HCl; 3.5 ml of the HCl extract was alkalinized with 2.5 ml of 10 N NaOH and extracted with 1 ml of the N-heptane/iso-amyl alcohol solvent mix. A total of 0.85 ml of the final solvent-mix extract was transferred in 50 µl aliquots, to a 0.3 ml vial. Each aliquot (50 µl) was evaporated to dryness under a gentle stream of N_2 before the next was transferred. After the transfer and evaporation of the total 0.85 ml, the residue was redissolved in 25 µl of the solvent-mix containing prochlorperazine as the internal standard and was analyzed by gas chromatography. The gas chromatographic analyses were performed using a Varian 2700 instrument equipped with a 1.7 mm glass column (2 mm = internal diameter) packed with 3% OV-17 on Chromsorb Q. The conditions of the instrument were as follows: injector temperature 300° C, detector temperature 300° C, oven temperature 275° C (isothermal), carrier gas (N_2) flow 46 ml/min.

VII. Solutions and Drugs.

A. Solutions and emulsions used in chronic studies: Dyne High Calorie, Liquid Diet Formula (Biolab Corp., Norborne, Miss.), which was used to supplement the diet of cats receiving thioridazine for three weeks, contained per ml: Fat 50%, carbohydrates 20%, protein 3%, Vitamin A, 500 I.U.; Vitamin D, 40 I.U.; thiamin, 0.15 mg; riboflavin, 0.17 mg; pyridoxine, 0.2 mg; ascorbic acid, 6 mg; nicotinamide, 2 mg; pantothenic acid, 2 mg; folic acid, 0.04 mg; milk ash, 0.4%; and sodium benzoate, 0.1%.

B. Solutions used in isolated organ studies: Solutions used in isolated organ studies were diluted in distilled H_2O. Krebs solution had the following composition (in mM): NaCl, 118.9; KCl, 4.7; $CaCl_2$, 2.5 $MgSO_4 \cdot 7H_2O$, 0.6; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11.1. The Krebs solution was aerated with a mixture of 5% CO_2 and 95% O_2.

C. Solutions used in thioridazine assay: N-heptane was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. Iso-amyl alcohol was obtained from Baker Laboratories, Miami, Florida.

D. Drugs used throughout the investigation: Phenylephrine HCl was obtained from Winthrop Laboratories, New York, N.Y. L-epinephrine bitartrate, norepinephrine HCl, dl-isoproterenol HCl, acetylcholine chloride, carbamylcholine chloride, alpha-chloralose, and urethane were obtained from Sigma Chemical Co., St. Louis, Mo. Prochlorperazine was obtained from Smith Kline and French, Philadelphia, Pa.

Thioridazine HCl crystals obtained from Sandoz, East Hanover, N. J., were first used in preliminary experiments and in the blood assay but were difficult to dissolve, therefore the prepared solution was used. Thioridazine HCl stock solution used in *in vivo* and *in vitro* studies studies was obtained from Sandoz, East Hanover, N. J. The solution contained thioridazine HCl, 30 mg/ml and ethanol, 3.0% by volume. To prepare the thioridazine solution used for in vivo experiments, 0.33 ml/kg of thioridazine stock solution was added to 7.5 cc of normal saline. The solution used in *in vitro* experiments was prepared by diluting 0.4 cc of the thioridazine stock solution, 30 mg/cc, to 100 ml with distilled H_2O to give a concentration of thioridazine of 3×10^{-4} M. Addition of 0.5 ml of this concentration to a 50 ml bath chamber produced concentrations of 3×10^{-6} M thioridazine and 2.6×10^{-6} M ethanol.

Carbamylcholine chloride was dissolved in Krebs solution. All other drugs were dissolved in 0.9% NaCl with 10^{-4} M ascorbic acid. All drugs were prepared immediately prior to addition to baths or prior to injection into the animals, with the exception of the commercial thioridazine solution.

RESULTS

I. Behavioral Effects of Thioridazine.

Cats that received oral thioridazine were lethargic during the first week of treatment and lost an average of 0.3 kg each. In the second week sedative effects were much less pronounced and food intake was increased. If food intake in the second week was still inadequate, the animals (5 of 10) were fed with Dyne formula through an oral gastric tube. During the second week of therapy the dose of thioridazine was increased from 10 mg/kg to 20 mg/kg. This increase produced sedation, from which the animals recovered within 24 hrs. Figure 3 shows the weights of the exposed animals throughout the treatment period and shows the times of tube feeding.

II. Studies in Intact Cats.

A. Effects of Thioridazine on Baseline Heart Rate and Blood Pressure Values.

Three weeks' pretreatment with thioridazine did not change baseline values of heart rate or blood pressure (Table 4). Similarly, an infusion of saline equivalent in volume to the acute thioridazine infusion did not alter baseline values of heart rate or blood pressure (Table 4). Thioridazine given as an infusion in naive animals produced a decrease in blood pressure of $-18 \pm 6/-9 \pm 4$ mmHg (systolic $\pm$ S.E./diastolic $\pm$ S.E.) and a decrease in heart rate of -24 ± 7 beats per minute. In exposed animals, a thioridazine infusion produced a decrease in blood pressure of $-24 \pm 14/-14 \pm 1$ mmHg and a decrease in heart rate of -15 ± 9 beats per minute.

Blood pressure and heart rate responses to autonomic agonists were measured at the peak of the drug effect. After each injection

TABLE 4.

Blood Pressure and Heart Rate Baseline Values
Prior to Administration of Autonomic Agonists
in the Various Experimental Conditions

Condition	N[a]	Blood Pressure ± S.E.[b]	Heart Rate ± S.E.[c]
Prior to Administration of Epinephrine			
Naive Pre-Thioridazine	10	129 ± 6/91 ± 4	218 ± 13
Naive Post-Saline	5	134 ± 6/89 + 3	215 ± 13
Naive Post-Thioridazine	5	115 ± 8/87 + 10	214 ± 20
Exposed Pre-Thioridazine	5	134 ± 16/98 + 15	226 ± 24
Exposed Thioridazine	4	115 ± 11/90 + 11	213 ± 19
Prior to Administration of Isoproterenol			
Naive Pre-Thioridazine	10	138 ± 6/91 ± 5	220 ± 10
Naive Post-Saline	5	137 ± 10/92 ± 8	208 ± 14
Naive Post-Thioridazine	5	119 ± 6/93 ± 8	214 ± 21
Exposed Pre-Thioridazine	5	138 ± 12/100 ± 12	264 ± 30
Exposed Post-Thioridazine	4	124 ± 7/99 ± 10	233 ± 15
Prior to Administration of Phenylephrine			
Naive Pre-Thioridazine	10	135 ± 6/94 ± 5	219 ± 10
Naive Post-Saline	5	140 ± 11/94 ± 9	212 ± 9
Naive Post-Thioridazine	5	122 ± 7/94 ± 9	214 ± 19
Exposed Pre-Thioridazine	5	137 ± 10/101 ± 12	272 ± 28
Exposed Post-Thioridazine	4	124 + 12/97 ± 14	218 ± 15

a Number of experiments

b Measured in mm Hg, systolic ± S.E./diastolic ± S.E.

c Measured in beats per minute.

the heart rate and blood pressure were allowed to stabilize for 10-15 minutes. The readings at the end of the stabilization period served as a control for the next injection. Responses were calculated as change from control. Student's t-test was used to determine significant differences between points.

B. Pressor and Depressor Responses to Epinephrine. Epinephrine (3 µg/kg) was administered in two sets of experiments. In the first series four naive animals received epinephrine at 10, 30, 60, 90 and 120 minutes after thioridazine infusion in order to test the duration of thioridazine effect, as manifested by "epinephrine reversal" (Fig. 4). Two other naive animals received epinephrine at similar intervals after an infusion of saline. These six animals received no other autonomic drugs. Table 5 shows that epinephrine reversal of approximately equal magnitude persisted for at least two hours after the thioridazine infusion.

Epinephrine (3 µg/kg) was also administered as part of the standard battery of adrenergic agonists tested in naive and exposed animals before and after infusion of thioridazine. Figures 5 and 6 show the effect of epinephrine on blood pressure in naive as contrasted to exposed animals. In the naive animals epinephrine produced its typical biphasic response, with an initial pressor response followed by a delayed depressor response (Fig. 5). The three-week treatment with oral thioridazine produced a statistically insignificant decrease in the pressor response but almost total block of the subsequent depressor response (Fig. 5). In contrast, the acute infusion of thioridazine (Fig. 6) blocked the pressor response to epinephrine in

naive animals but left the depressor response intact. This is the characteristic "epinephrine reversal". In exposed animals, however, the infusion of thioridazine greatly reduced the extent of epinephrine reversal; furthermore, in exposed animals epinephrine produced a pressor response even after an infusion of thioridazine.

C. Pressor Response to Phenylephrine. Phenylephrine produced only a pressor response. The dose-response curves in naive and exposed animals are shown in Figure 7 and responses are tabulated in Table 6. In naive animals (Fig. 7a) responses to phenylephrine were not significantly different before and after infusion of saline, but the infusion of thioridazine (Fig. 7b) shifted the dose-response curve to the right. Three weeks' pretreatment with oral thioridazine produced a shift to the right in the phenylephrine dose-response curve (Figs. 7c, 7d) similar to that produced by the acute infusion.

D. Depressor Responses to Isoproterenol. In naive animals, an injection of isoproterenol, 0.1 µg/kg produced only a depressor response (Fig. 8). Three weeks' pretreatment with oral thioridazine produced almost complete block of the depressor response to isoproterenol (Fig. 8). An infusion of thioridazine in naive animals (Fig. 9) produced a slight antagonism of the depressor response to isoproterenol, 0.1 µg/kg but did not alter the extent of the block of that response in exposed animals (compare Figs. 8 and 9).

Table 7 shows the responses to different doses of isoproterenol. Three weeks' pretreatment with thioridazine produced a marked shift to the right of the isoproterenol dose response curve, whereas an acute infusion of thioridazine had little or no effect (Table 7).

3	32 ± 5.1 20 ± 3.3	27 ± 7.8 18 ± 8.3	8 ± 3.9 6 ± 5.1	14 ± 3.9 9 ± 3.3	13 ± 5.2 13 ± 7.5
10	56 ± 6.5 35 ± 6.9	48 ± 9.4 30 ± 13.4	21 ± 6.2 10 ± 6.1	29 ± 7.5 26 ± 7/8	14 ± 7.5 9 ± 3.1
30			39 ± 10.8 23 ± 8.5	38 ± 5.9 23 ± 4.3	

---

a Naive Pre-Thioridazine

b Naive Post-Saline

c Naive Post-Thioridazine

d Exposed Pre-Thioridazine

e Exposed Post-Thioridazine

f Systolic

Diastolic

* Mean ± S.E.

TABLE 7

Change from Baseline Values in Heart Rate and Blood Pressure Responses to Isoproterenol in the Various Experimental Conditions

Agonist, µg/kg	N Pre-T[a] (n=10)	N Post-S[b] (n=5)	N Post-T[c] (n=5)	E Pre-T[d] (n=4-5)	E Post-T[e] (n=4)
Heart Rate, change in beats per minute					
Isoproterenol					
0.01	8 ± 2.1	8 ± 3.8		2 ± 1.9	-3 ± 4.8
0.03	22 ± 7.4	35 ± 7.1		10 ± 7.7	3 ± 10.3
0.1	25 ± 4.9	46 ± 7.5	38 ± 3.7	16 ± 6.8	25 ± 2.9
0.3	43 ± 5.9	63 ± 14.4	40 ± 5.5	49 ± 14.1	50 ± 20
1.0	40 ± 11.8		52 ± 9.7	53 ± 21.9	
Blood Pressure, change in mm Hg					
0.01	-17 ± 6.9[f]	-23 ± 9.4		-5 ± 6.1	-1 ± 3.1
	-18 ± 6.9	-33 ± 9.4		-7 ± 4.4	-5 ± 3.5
0.03	-38 ± 3.1	-32 ± 9.0		-15 ± 7.4	0 ± 3.5
	-36 ± 6.9	-43 ± 8.5		-6 ± 2.9	-4 ± 2.4

0.1	-48 ± 2.5 -57 ± 3.5	-41 ± 5.6 -52 ± 3.4	-38 ± 5.1 -44 ± 5.6	-17 ± 6.4 -11 ± 5.3	-29 ± 3.5 -26 ± 88
0.3	-53 ± 3.7 -59 ± 3.7	-46 ± 5.1 -57 ± 3.4	-47 ± 8.3 -52 ± 3.7	-19 ± 8.3 -25 ± 9.5	-29 ± 6.6 -30 ± 0
1.0	-50 ± 4.7 -63 ± 4.1		-47 ± 3.0 -63 ± 3.5	-28 ± 8.5 -33 ± 15.9	

a Naive Pre-Thioridazine

b Naive Post-Saline

c Naive Post-Thioridazine

d Exposed Pre-Thioridazine

e Exposed Post-Thioridazine

f Systolic

Diastolic

* Mean ± S.E.

TABLE 8

Change from Baseline Values in Heart Rate and Blood Pressure Responses to Bilateral Carotid Clamp in the Various Experimental Conditions

	n	HR[1]	Systolic[2]	Diastolic[2]
Naive Pre-Thioridazine	5	-3.2 ± 9.4	53 ± 15.7	27 ± 12.9
Naive Post-Saline	5	2 ± 6.6	47 ± 18	32 ± 18.5
Naive Post-Thioridazine	1	0	40	35
Exposed Pre-Thioridazine	5	-16 ± 14	22 ± 10	19 ± 5.4
Exposed Post-Thioridazine	4	25 ± 20.5	28 ± 13.8	26 ± 2.5

* Mean ± S.E.

1. Change in beats per minute

2. Change in mm Hg

difference in the PQ, QRS and QT intervals in the five experimental conditions (analysis of variance).

During the ouabain infusion (see Methods) no change in the PQ and QRS intervals occurred until after the animals had received 75 µg/kg. At that time, both the PQ and QRS intervals in naive post-saline animals and the PQ intervals in exposed post-thioridazine animals began to broaden and were significantly longer at the endpoint (occurrence of three extrasystoles per minute) than at the beginning of the infusion (Table 10). However, in naive and exposed animals that had received an infusion of thioridazine, the QRS interval remained unchanged throughout the ouabain infusion. Comparing the PQ and the QRS intervals at each concentration of ouabain shows that there are no significant differences between naive post-saline, naive post-thioridazine and exposed post-thioridazine conditions (analysis of variance; Table 10).

After administration of an adequate dose ouabain consistently produced premature ventricular contractions (PVC, Fig. 10). Three PVC occurring within one minute were arbitrarily chosen as marking the onset of ventricular dysrhythmias. Table 11 shows that the mean onset of ventricular dysrhythmias in naive post-saline, naive post-thioridazine and exposed post-thioridazine animals was not significantly different. However, there was much more individual variation in time to onset of dysrhythmias among the animals receiving thioridazine (exposed post-thioridazine and naive post-thioridazine) than in animals not receiving thioridazine (naive post-saline).

TABLE 11

Dose of Ouabain (µg/kg) that elicited three Ventricular Contractions in One Minute after a Thioridazine Infusion in Naive and Exposed Animals

	n	Dose, µg/kg ± S.E.
Naive Post-Saline	5	108 ± 7.9
Naive Post-Thioridazine	5	119 ± 21.9
Exposed Post-Thioridazine	4	107 ± 20.9

III. Studies in Isolated Organs.

A. Responses of Aortic Strips to Norepinephrine and Tyramine.

Figures 11, 12 and 13 show the responses of aortic strips to cumulative increases in the concentration of norepinephrine and tyramine. Norepinephrine (Fig. 11) elicited a greater maximal contraction ($p < 0.05$) in strips obtained from exposed animals than in strips obtained from naive animals. In both types of strips addition of 1.2 µg/ml of thioridazine produced a marked but surmountable antagonism of the contractile response to norepinephrine. The concentration of norepinephrine needed to produce a half-maximal response ($NE_{0.5\ max}$) and its 95% confidence limits were as follows:

naive pre-thioridazine	0.4 µM/L (0.05 µM/L to 1 µM/L)
naive post-thioridazine	10.0 µM/L (2 µM/L to 20 µM/L)
exposed pre-thioridazine	1 µM/L (0.2 µM/L to 2 µM/L)
exposed post-thioridazine	5.5 µM/L (1 µM/L to 50 µM/L)

Comparing responses in strips obtained from the same animal, addition of thioridazine produced a significant increase in the $NE_{0.5\ max}$ (paired t-test; $p < 0.05$) in both naive and exposed animals (Table 12). Comparing responses in strips obtained from different animals (t-test for non-paired data; $p < 0.05$), however, did not show any significant differences in $NE_{0.5\ max}$ between strips obtained from naive and exposed animals before addition of thioridazine (naive and exposed pre-thioridazine) or after addition of thioridazine (naive and exposed post-thioridazine).

The responses to tyramine (Fig. 13) were identical in naive pre-thioridazine and exposed pre-thioridazine strips. Tyramine responses

TABLE 12

Comparison of Paired* Responses to Norepinephrine in Cat Aortic Strips

Animal	- Log $NE_{0.5\ max}$		
	Naive Pre-Thioridazine	Naive Post-Thioridazine	Difference (Pre-Post)
1	5.40	4.64	0.76
2	6.55	5.10	1.45
3	6.34	5.21	1.13
4	6.14	5.00	1.14
5	5.68	4.82	0.86
	Exposed Pre-Thioridazine	Exposed Post-Thioridazine	Difference (Pre-Post)
1	5.70	5.55	0.20
2	6.64	5.82	0.82
3	6.74	5.14	1.60
4	6.12	6.00	0.12
5	5.40	4.85	0.55

* Responses of aortic strips taken from the same animal

were markedly diminished (Fig. 13) in naive post-thioridazine strips and the maximal responses could not be restored by increasing the concentration of tyramine up to 10^{-3} M/L. Tyramine responses were not tested in exposed post-thioridazine strips.

B. Responses of Tracheal Chains to Carbamylcholine

Figure 14 shows the dose-response curves for carbamylcholine in the four experimental conditions. Since the maximal tension elicited by carbamylcholine was similar for all four conditions, the contractile response is expressed as percent of the maximum response. The concentration of carbamylcholine needed to produce a half-maximal response ($Carb_{0.5\ max}$) and its 95% confidence limits were as follows:

naive pre-thioridazine	0.16 μM/L (0.05 μM/L to 0.4 μM/L)
naive post-thioridazine	1.0 μM/L (0.33 μM/L to 3.2 μM/L)
exposed pre-thioridazine	0.33 μM/L (0.1 μM/L to 1.1 μM/L)
exposed post-thioridazine	2.6 μM/L (0.82 μM/L to 8 μM/L)

Comparing responses in strips obtained from the same animal, addition of thioridazine produced a significant increase in the $Carb_{0.5\ max}$ (paired t-test; $p < 0.05$) in both naive and exposed animals (Table 13). Comparing responses in strips obtained from different animals (t-test for nonpaired data; $p < 0.05$), however, did not show any significant differences in $Carb_{0.5\ max}$ between strips obtained from naive and exposed animals before addition of thioridazine (naive and exposed pre-thioridazine) or after addition of thioridazine (naive and exposed post-thioridazine).

C. Responses of Isolated Hearts to Epinephrine

Figures 15 through 18 show the effects of thioridazine on the chronotropic and inotropic responses of the isolated perfused

TABLE 13

Comparison of Paired* Observations to Carbamylcholine in Tracheal Chains

Animal	- Log $NE_{0.5\ max}$		
	Naive Pre-Thioridazine	Naive Post-Thioridazine	Difference (Pre-Post)
1	6.77	6.10	0.67
2	7.12	6.15	0.97
3	6.64	6.00	0.64
4	6.85	6.10	0.75
5	6.80	5.89	0.91
	Exposed Pre-Thioridazine	Exposed Post-Thioridazine	Difference (Pre-Post)
1	6.70	6.32	0.38
2	6.10	5.68	0.42
3	6.89	5.96	0.93
4	6.60	5.64	0.96
5	6.48	6.05	0.43

* Responses of tracheal chains taken from the same animal

heart (Langendorff preparation). Two epinephrine dose-response curves were obtained in each heart. After the initial dose-response determination, 1.2 µg/ml of thioridazine or an equivalent volume of saline, was added to the Krebs perfusate and allowed to circulate for one hour prior to the second epinephrine dose-response determination. To produce the same response higher concentrations of epinephrine were required in the second than in the first dose-response determinations. The effect of chronic treatment can be seen by comparing the responses to epinephrine in the first dose-response determinations (naive pre-thioridazine exposed pre-thioridazine; Figs. 15 and 17). The effect of acute treatment can be seen in second dose-response determinations (naive post-saline, naive post-thioridazine, exposed post-thioridazine; Figs. 16 and 18).

Chronotropic response, measured as a change in beats per minute (Fig. 15) was reduced in exposed pre-thioridazine hearts as compared to naive pre-thioridazine hearts. Similarly, the chronotropic response was reduced in exposed post-thioridazine hearts as compared to naive post-saline hearts (Fig. 16). There is no difference between naive post-saline and naive post-thioridazine responses (Fig. 16). In other words, acute exposure to thioridazine did not impair the positive chronotropic response to epinephrine, whereas, the response was markedly reduced in hearts obtained from animals pretreated with thioridazine for three weeks.

Studies of the inotropic response yielded results similar to those of the chronotropic studies. Acute thioridazine treatment (naive post-thioridazine) did not change the inotropic response to epinephrine

(Fig. 18). However, the inotropic response to epinephrine was reduced in the exposed pre-thioridazine condition as compared to the naive pre-thioridazine condition (Fig. 17).

High concentrations of epinephrine often produced dysrhythmias in isolated hearts. In these experiments, the heart was considered dysrhythmic if two of the following four criteria appeared for more than one minute: (1) irregular rate, (2) irregular force, (3) irregular QRS height, (4) A-V dissociation. All hearts taken from exposed animals exhibited dysrhythmic patterns at high concentrations of epinephrine. Two hearts that received thioridazine acutely (naive post-thioridazine) and two hearts that received no thioridazine (one naive pre-thioridazine, one naive post-saline) exhibited dysrhythmic patterns at high concentrations of epinephrine (Table 14).

D. Pathology

In cats that had been treated for three weeks with thioridazine, specimens of kidney, liver and small intestine were fixed with 10% formalin within sixty minutes after sacrifice. Hearts studied as isolated perfused organs were similarly fixed after the experiments. Pathologist, Dr. Don Houghton, reviewed the specimens and determined that there were no consistent findings, and that the existing pathology was not drug related. Table 15 shows the pathology reported in the various organs.

TABLE 14

Total Number of Isolated Hearts that Became Dysrhythmic During the Epinephrine Dose-Response Curve in the Various Experimental Conditions

	N+	Number of Hearts Meeting Criteria* for Dysrhythmias
NAIVE PRE-THIORIDAZINE	5	1
NAIVE POST-SALINE	5	1
NAIVE POST-THIORIDAZINE	5	2
EXPOSED PRE-THIORIDAZINE	5	3
EXPOSED POST-THIORIDAZINE	5	5

* Hearts considered to be dysrhythmic exhibited two of the following abnormalities: irregular rate, irregular force of contraction, irregular QRS height, and A-V dissociation.

\+ Number of isolated hearts in each condition.

TABLE 15

Pathology Report on Organs Taken From 10 Exposed Cats

Organ	Number of specimens showing pathology	Pathology
Heart	1	Coronary artery shows perivascular fibrosis
	1	Intramuscular perivascular fibroblast proliferation
	1	Mild acute pericarditis
Liver	1	Lymphocyte aggregation
	1	Possible early fatty changes
	1	Mild nonspecific hepatitis (probably secondary to interstitial pneumonia)
Lung	1	Interstitial pneumonia (probably viral)
Kidney		No obvious pathology
Small Bowel		No obvious pathology

DISCUSSION

I. Acute Effects of Phenothiazines

A. Alpha Adrenergic Blockade

Acute treatment with thioridazine produces a marked alpha adrenergic blockade. This was demonstrated in intact animals as a fivefold shift to the right of the phenylephrine dose-response curve and in isolated aortic strips as a twenty-fivefold shift to the right of the norepinephrine dose-response curve. In both preparations, the blockade could be overridden by increasing the concentration of the agonist without changing the slope of the dose-response curve. In isolated aortic strips, there was no alteration in the maximal effect but no attempt was made to elicit maximal responses in the intact preparation. These results suggest that thioridazine is acting as a competitive alpha adrenergic antagonist. Gokhale et al. (20) previously found that chlorpromazine acted as a competitive antagonist of norepinephrine in rabbit aortic strips.

Concentrations of norepinephrine, 10^{-6} M and above, were required to initiate contraction in aortic strips after thioridazine. This concentration of norepinephrine is probably greater than any that can be released from endogenous stores, and thioridazine produced an insurmountable and virtually complete block of the tyramine response. Tyramine is an indirectly acting sympathomimetic amine whose effects reflect catecholamine release from endogenous stores. If alpha adrenergic blockade were as intense throughout the entire vascular tree, it is doubtful that sympathetic reflexes could be elicited. However, the block of pressor responses to phenylephrine *in vivo* was

not as pronounced as that of the norepinephrine response *in vitro*, and, in intact preparations, a reflex sympathetic response to bilateral carotid clamp was consistently obtainable.

B. Beta Adrenergic Responses

Reversal of the pressor response to epinephrine is one of the most characteristic effects of phenothiazines (17, 23-27, 34, 35) and was a consistent effect in the present experiments. Epinephrine reversal has been variously explained on the basis of alpha adrenergic blockade and potentiation of beta adrenergic vasodilation. However, since acute treatment with thioridazine produced little or no effect on the depressor response to epinephrine or isoproterenol, potentiation of beta adrenergic effects played little or no role in epinephrine reversal in the present experiments. Similarly, in isolated hearts, acute administration of thioridazine did not alter the chronotropic or inotropic response to epinephrine.

C. Cholinergic Blockade

In addition to alpha adrenergic blockade, thioridazine has antimuscarinic effects when given acutely. Studies in tracheal chains showed that thioridazine produced a sixfold shift to the right of the carbamylcholine dose-response curve. Since neither the slope nor the maximal contraction were altered, thioridazine again appeared to act as a competitive antagonist.

Such atropine-like effects of thioridazine may account for block of phenylephrine-induced reflex bradycardia. However, phenothiazines have been shown to depress the central vasomotor center resulting in decreased autonomic outflow, and block of the reflex bradycardia is

probably due to a combination of the interaction of thioridazine with the cholinergic receptor and depression of the central vasomotor centers.

D. Responses to Ouabain Infusion

Thioridazine (33), like quinidine (70), has been shown to prolong the duration of the cardiac action potential. This effect is seen in the EKG as an increase in QT interval and flattening of the T wave at therapeutic doses (1, 11-15) and a widening of the QRS interval at higher doses. In the present experiments, the acute infusion of thioridazine did not significantly alter the QRS or QT interval (Table 9). The PQ interval was slightly prolonged by thioridazine. Similar effects have been reported for quinidine (70).

The ouabain infusion was administered as a dysrhythmogenic stimulus to determine the effects of thioridazine on cardiac excitability. Digitalis toxicity is characterized by atrial, junctional, and ventricular dysrhythmias. The mechanisms underlying these dysrhythmias appear to be related to an increase in automaticity, shortening of the refractory period and alterations in conduction velocity. At the low doses of ouabain, the EKG effect is seen as a decrease in the QT interval and ST wave changes reflecting a shortening of the duration of electrical systole and accelerated repolarization. At higher doses, the PR interval increases reflecting both direct effects on the AV node and vagal stimulation (71-78). Ventricular excitability is also increased at high doses with frequent premature contractions. The basis for the increased excitability is thought by some (79) to result from inhibition of the electrogenic sodium-potassium pump with consequent decrement in resting membrane potential.

In the present experiments, the effects of digitalis (Table 10) were seen as (1) prolongation of the PQ interval, (2) prolongation of the QRS complex, and (3) premature ventricular contractions. The prolongation of the QRS complex was not expected on the basis of previous studies with ouabain infusion but was a consistent finding in the present experiments. Rosen et al. (80) has reported slowing of conduction in Purkinje fibers during digitalis toxicity which could account for the prolongation of the QRS complex. Comparing ouabain responses in naive post-thioridazine and naive post-saline animals showed that the thioridazine infusion did not alter the effect of ouabain on the PQ interval (Table 10) or significantly alter the time to onset of ventricular ectopic contractions (Table 11); thioridazine did, however, prevent the ouabain-induced increase in QRS interval. Thus, these studies show little evidence of either dysrhythmic or antidysrhythmic effects of acute administration of thioridazine.

II. Three Weeks' Treatment

During the first week of thioridazine treatment, the cats slept for most of the day and ate very little, which usually resulted in a loss of weight. By the end of the second week of treatment, the animals were more active and were eating enough to maintain a stable weight. Such a change in activity was evidence of the development of tolerance to the sedative effects of thioridazine. Similar tolerance is seen in patients on phenothiazine therapy (16, 19).

At the end of three weeks, the animals were used either for intact preparations or as a source for isolated organs. Control blood pressure and heart rate parameters measured before initiation of

dose-response curves for autonomic agonists and immediately after the thioridazine infusion were not significantly different in naive and exposed animals. Specimens of liver, kidney and heart were taken from the chronically treated animals used for isolated organ studies and were examined by a pathologist who found no consistent pathology that could be related to phenothiazine therapy. These results indicate that, after three week pretreatment with thioridazine, the cats were generally in good health and altered responses to autonomic agonists were due to the direct effect of thioridazine and not due to pre-existing alterations in physical status.

III. Responses in Exposed Preparations

A. Alpha Adrenergic Blockade

Chronic treatment with thioridazine produced alpha adrenergic blockade of the same magnitude as acute treatment. In intact animals, this was demonstrated as a sixfold shift to the right of the phenylephrine dose-response curve. In isolated aortic strips, the norepinephrine dose-response curve in the exposed pre-thioridazine condition was not significantly different from the naive pre-thioridazine condition. This may have been due to washout of thioridazine during the equilibration period. Addition of thioridazine to the bath produced a sixfold shift to the right of the norepinephrine dose-response curve in strips obtained from exposed animals and a twenty-fivefold shift in strips obtained from naive animals (Fig. 11). Thus, thioridazine remains a potent competitive alpha adrenergic antagonist during sustained administration, although results with isolated aortic strips suggest that some tolerance does develop.

Although the dose-response relationships were similar in naive and exposed conditions the absolute amplitude of the response was greater in aortic strips obtained from exposed animals. The mechanisms underlying the increased contractile response are unclear but may be due to an increased mobilization of free intracellular calcium by norepinephrine. Chlorpromazine and the free radical of chlorpromazine, which is a major metabolite, have been shown to inhibit the calcium pump of the sarcoplasmic reticulum (81-85). In striated muscle, this inhibition leads to an increased calcium concentration in myoplasm reflected as an increased contraction. Similar results have been explained on the basis of other studies (86-89) which have shown chlorpromazine to compete with calcium for membrane binding sites. The increased contractile response to chlorpromazine may be due to accumulation of chlorpromazine or a metabolite within the sarcoplasmic reticulum and consequent changes in calcium binding and uptake.

B. Beta Adrenergic Blockade

Three weeks' pretreatment with thioridazine produced beta adrenergic blockade as evidenced by a decrease in cardiac responses to epinephrine in the isolated hearts and in depressor responses to epinephrine and isoproterenol in intact animals. The dose-response curve for epinephrine-induced tachycardia in hearts taken from exposed animals was shifted threefold to the right. As in aortic strips, a greater maximal response was obtained with epinephrine, 10^{-4} M, in the exposed pre-thioridazine condition. Shifts to the right in the inotropic dose-response curves were even greater. In contrast, addition

of thioridazine to the bath had no effect on either the chronotropic or inotropic response.

The mechanisms underlying beta adrenergic blockade may include changes involving cyclic 3',5' adenosine monophosphate (cyclic 3',5' AMP), an accumulation of metabolite with beta blocking activity or a change in the adrenergic receptor. Considerable evidence has accumulated demonstrating an increased formation of cyclic 3',5' AMP in response to catecholamines in various tissues. Sutherland et al. (92-93) and others (94) have shown that the ability of catecholamines to stimulate cyclic 3',5' AMP formation was closely associated with the inotropic potencies of these compounds. Such increased synthesis has been correlated with stimulation of adenyl cyclase which is present in cells that respond to epinephrine. Dichloroisoproterenol (100), a beta adrenergic blocker, has been shown to inhibit the stimulatory effects of catecholamines on adenyl cyclase in conjunction with its ability to block their inotropic response. Similarly, pronethalol blocks the accumulation of cyclic 3',5' AMP and the inotropic response induced by epinephrine. Thioridazine may produce its beta blocking effects by inhibiting formation of cyclic 3',5' AMP mediated through adenyl cyclase. Lack of beta blockade after acute treatment suggests that the parent compound is not responsible for such effects.

The delayed onset of beta blockade may reflect the time necessary to achieve high plasma concentrations of a metabolite of thioridazine that has beta blocking activity. Phenothiazines are thought to have over a hundred metabolites, of which only a few have been identified (95, 97). Even though the pharmacology of the known metabolites has not been well worked out, it is possible to speculate on the chemical

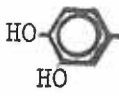
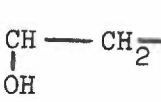
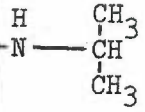
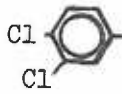
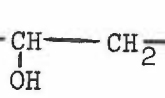
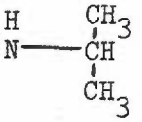
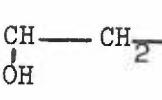
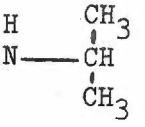
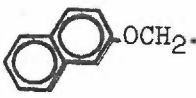
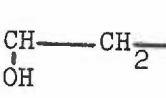
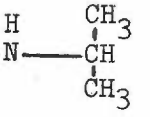
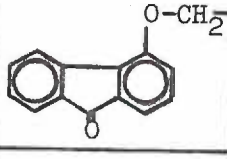
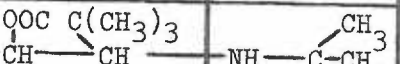
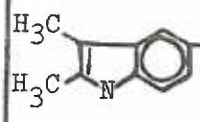
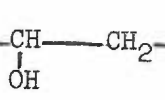
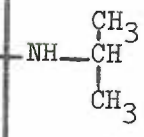
structure of a possible beta blocking metabolite. The basic structure of beta adrenergic blockers contains (a) a bulky aromatic or heterocyclic ring, (b) a two or three carbon alkyl side chain with a hydroxyl group on the beta carbon, and (c) a secondary amine (96, 98, 99). The beta hydroxy and secondary amine group of the alkyl side chain are believed to determine affinity of the compound for the beta receptor whereas the intrinsic activity of the compound is governed by structure of the aromatic or heterocyclic ring. Table 16 shows the structure of a number of beta adrenergic antagonists.

A metabolite of thioridazine (Fig. 19) could fulfill the structural requirements for beta blockade through demethylation, which would yield a secondary amine, and hydroxylation of the beta carbon of the alkyl side chain. Cyclization of the amino group probably would not affect the affinity of the compound for the receptor since it would maintain a tetrahedral configuration. The bulky tricyclic structure is not inconsistent with beta blocking activity as evidenced by the last two compounds shown in Table 16. Structure B (Fig. 19) might be expected to have beta blocking activity. The demethylated metabolite, northioridazine (95), has been identified in both man and animals after administration of thioridazine, but a beta hydroxylated metabolite has not yet been reported.

Several investigators (99-102) have proposed a close association between alpha and beta receptors. In 1964, Robinson et al. (101) suggested that alpha and beta receptors might represent different sites for hormone-enzyme interaction on the adenyl cyclase molecule. This suggestion was based on studies that demonstrated adenyl cyclase to be

TABLE 16

Chemical Structures of Isoproterenol and β-adrenergic Antagonists

Drug of Compound	Aromatic or Heterocyclic Ring	Alkyl side-chain with a β-hydroxyl group	Secondary Amine	Effect
Isoproterenol				β-agonist
Dichloroisopro-terenol				β-antagonist
Pronethalol				β-antagonist
Propranalol				β-antagonist
LL21945 (98)				β-antagonist
Compound 5 (112)				β-antagonist

equivalent to the beta receptor, but that similar responses mediated by alpha and beta receptors varied from species to species and with changes in physiological or pathological conditions (103). In line with this hypothesis, Kunos et al. (99,100) have suggested that the alpha and beta receptors might represent allosteric configurations of the same active site. This speculation is based on studies which showed that alpha adrenergic antagonists and not beta adrenergic antagonists block chronotropic and inotropic responses of frog hearts at low temperatures, whereas beta adrenergic antagonists and not alpha adrenergic antagonists blocked these same responses at high temperatures. It was previously suggested that slight chemical alterations of the thioridazine molecule may convert an alpha to a beta adrenergic blocking compound. Such a suggestion emphasizes not only specific chemical requirements for drug interactions with the beta or alpha adrenergic receptor, but also points out the similarity between the receptors. Thus, beta adrenergic blockade during chronic administration might result from allosteric conversion of alpha to beta receptors.

C. Cholinergic Blockade

Tolerance to the anticholinergic effects of thioridazine did not develop during chronic administration. In isolated tracheal chains the initial carbamylcholine dose-response curve in the exposed pre-thioridazine condition was not significantly different from the naive pre-thioridazine condition, but this may have been due to washout of thioridazine during the equilibration period. However, in chains taken from exposed animals, addition of thioridazine to the bath produced an eightfold shift to the right of the carbamylcholine dose-response curve

compared to a sixfold shift to the right in chains taken from naive animals.

Block of the reflex bradycardia to phenylephrine-induced pressor response may be due to cholinergic blockade or to central vasomotor depression. In the exposed pre-thioridazine condition, the phenylephrine induced rise in arterial pressure reflexly slowed the heart but this reflex bradycardia was blocked in the naive post-thioridazine condition. Since tolerance to cholinergic blockade after three weeks' treatment with thioridazine was not evident in experiments with tracheal chains, the reflex bradycardia may have been due to the development of tolerance to central vasomotor center depression. Similarly, such tolerance was reflected in the behavior of the cats during the treatment period. However, after an infusion of thioridazine in exposed animals, the reflex bradycardia to phenylephrine pressor response was blocked to the same extent as that seen in the naive post-thioridazine condition. These results suggest that by increasing the plasma concentration of thioridazine, tolerance to vasomotor center depression could be overcome, or there occurred an increased cholinergic blockade at the end organ.

D. Responses to Ouabain Infusion

Three weeks' pretreatment with thioridazine did not alter the PQ, QRS or QT intervals. In contrast, the QT interval is usually prolonged in patients on long term phenothiazine therapy (43, 44). The mechanisms underlying phenothiazine induced prolongation of the QT interval have been based on electrophysiology studies which have shown phenothiazines to prolong phase 3 and slow upstroke velocity of cardiac action potentials (104). Arita

et al. (105) have reported that propranolol, a beta receptor antagonist, corrects phenothiazine induced T wave abnormalities. Electrophysiology studies have shown propranolol to accelerate repolarization and shorten action potential of Purkinje fibers (69). The lack of QT prolongation seen in these studies may reflect enhanced ventricular repolarization due to beta adrenergic blockade.

Beta adrenergic blocking agents have been shown to protect against ouabain-induced dysrhythmias (106-108). The mechanism underlying this effect has been attributed to their beta blocking properties and not to their local anesthetic properties. In these experiments, beta adrenergic blockade which developed after three weeks' pretreatment with thioridazine did not alter the time to onset of ouabain-induced dysrhythmias. Thus, the studies with ouabain infusion gave no evidence of antidysrhythmic or dysrhythmic effects in association with sustained treatment with thioridazine.

IV. Cardiotoxicity of Thioridazine

Although the cardiovascular effects of phenothiazines have been extensively studied, little is known about the mechanisms underlying the cardiotoxicity. The possible causes include an imipramine-like block of reuptake of catecholamines with exaggerated beta adrenergic responses and a quinidine-like depressant effect on the myocardium. Several studies have shown phenothiazines to block reuptake of catecholamines (38-41) and catecholamine blood levels of up to 7 µg/ml have been reported in patients receiving phenothiazines (63).

The quinidine-like actions of thioridazine are manifest as prolongation of the Q-T interval and U waves (109). Quinidine, like thioridazine,

has been associated with sudden cardiovascular collapse secondary to ventricular tachycardia and fibrillation (37). Both quinidine and thioridazine delay ventricular repolarization and prolong ventricular recovery possibly permitting reentry excitation. According to the theory of reentrant excitation, nonuniform or delayed ventricular repolarization results in varying velocities of impulse conduction in the myocardium such that an impulse from a more slowly conducting portion may stimulate an adjacent portion that is in its relative refractory period. Such reentrant impulses can result in ventricular tachycardia or fibrillation (110).

The present studies give some evidence of enhanced ventricular excitability during sustained administration. Hearts taken from exposed cats showed a greater incidence of dysrhythmias to high concentrations of epinephrine than hearts taken from naive cats. Similarly, in the intact preparation, one exposed cat died of ventricular dysrhythmias during the bilateral carotid clamp procedure. Nonetheless, there was no evidence for exaggeration of beta adrenergic responses or for quinidine-like effects in the electrocardiogram. The most characteristic electrocardiographic changes were a prolonged PQ interval in the naive post-thioridazine condition and no change in the QT interval in the naive post- and exposed pre-thioridazine conditions rather than prolongation of the QT interval and U waves typical of quinidine-like effects.

The most characteristic effect of prolonged treatment with thioridazine in cats was beta adrenergic blockade rather than exaggeration of beta responses. The beta block may have no direct relation to dysrhythmias during sustained treatment with thioridazine, but may

indicate underlying abnormalities of cellular function possibly involving sequestration of calcium or control of intracellular levels of cyclic AMP. In addition, a beta blocking metabolite might serve as a partial agonist to increase ventricular excitability. Several beta adrenergic antagonists have been shown to be partial agonists (96).

SUMMARY AND CONCLUSIONS

These experiments were conducted in order to compare responses to autonomic agonists and ouabain after acute and sustained treatment with thioridazine. Three weeks' pretreatment produced only slight alterations in the alpha adrenergic and cholinergic blockade seen after acute treatment, and there was no difference in time to onset of ouabain-induced dysrhythmias in the two conditions. Chronic treatment did, however, produce marked beta adrenergic blockade and an increased contractile response to norepinephrine in aortic strips that was not apparent after acute treatment. The mechanisms postulated to underlie beta adrenergic blockade include a metabolite with beta blocking activity, a change in the adrenergic receptor, changes involving cyclic 3',5' AMP independent of an action on specific beta receptors, or changes in binding of intracellular calcium.

REFERENCES

1. Kelly, H.G., Fay, J.E., and Laverty, S.G.: Thioridazine hydrochloride (Mellaril): Its effect on the electrocardiogram and a report of two fatalities with electrocardiographic abnormalities. Can. Med. Assoc. J. 89: 546, 1963.

2. Desautels, S., Filteau, C., and St-Jean, A.: Ventricular tachycardia associated with administration of thioridazine hydrochloride (Mellaril). Can. Med. Assoc. J. 90: 1030, 1964.

3. Hollister, L.E., and Kosek, J.C.: Sudden death during treatment with phenothiazine derivaties. J. Am. Med. Assoc. 192: 93, 1965.

4. Richardson, H.L., Graupner, K.L., and Richardson, M.E.: Intramyocardial lesions in patients dying suddenly and unexpectedly. J. Am. Med. Assoc. 195: 114, 1966.

5. Schoonmaker, F.W., Osteen, R.T., and Greenfield, J.C.: Thioridazine (Mellaril^{R})-induced ventricular tachycardia controlled with an artificial pacemaker. Ann. Intern. Med. 65: 1076, 1966.

6. Leestman, S.E., and Koenig, K.L.: Sudden death and phenothiazines. Arch. Gen. Psychiatry 18: 137, 1968.

7. Alexander, C.S., and Nino, A.: Cardiovascular complications in young patients taking psychotropic drugs. Am. Heart J. 78: 757, 1969.

8. Guillan, R.A., Zelman, S., Reinert, R.E., and Smalley, R.L.: Electron microscopy. Sudden death in patients under phenothiazine therapy; study of three cases. Kansas Med. J. 71: 213, 1970.

9. Guillan, R.A., Smalley, R.L., and Zelman, S.: Electrocardiographic and ultrastructural cardiac effects of phenothiazines in rabbits. Chest 62: 62, 1972.

10. Isac, M., Stern, S., and Edelstein, E.L.: The treatment of phenothiazine-induced tachycardia by propranolol. Isr. Ann. Psychiatry 10: 272, 1972.

11. Aherwadkar, S.J., Efendigil, M.C., and Coulshed, N.: Chlorpromazine therapy, and associated acute distrubances of cardiac rhythm. Br. Heart J. 36: 1251, Dec. 1974.

12. Levine, D., and Marshall, A.J.: Letter: Cardiac arrhythmia induced by phenothiazines. Lancet 2: 990, 1975.

13. Fowler, N., McCall, D., Chou, T., Holmes, J., and Hanenson, I.: Electrocardiographic changes and cardiac arrhythmias in patients receiving psychotropic drugs. Am. J. Cardiol. 37: 223, 1976.

14. Thornton, W.E., and Pray, B.J.: Ventricular arrhythmia and thioridazine: a case report. Am. J. Nurs. 76 (2): 245, 1976.

15. Goodson, W., and Litkenhous, E.: Sudden unexplained death in psychiatric patients taking thioridazine. South Med. J. 69: 311, 1976.

16. Goodman, L.S., and Gilman, A. (Eds.): The pharmacological basis of therapeutics. 5th Edition. New York: MacMillan Company: Robert Byck 1975 (pages 157-166).

17. Foster, C.A., O'Mullane, E.J., Gaskell, P., and Churchill-Davidson, H.C.: Chlorpromazine: A study of its action on the circulation in man. Lancet 2: 614, 1954.

18. Feldman, S., and Eliakim, M.: Observations on the mechanism of blood pressure changes following chlorpromazine administration in the cat. Arch. Int. Pharmacodyn. Ther. 116: 340, 1958.

19. Moyer, S.H.: The pharmacology of chlorpromazine. Int. Rec. Med. 168: 301, 1955.

20. Gokhale, S.D., Gulati, O.D., and Parikh, H.M.: An investigation of the adrenergic blocking action of chlorpromazine. Br. J. Pharmacol. 23: 508, 1964.

21. Martin, W.R., Riehl, J.C., and Unna, K.R.: Chlorpromazine III. The effects of chlorpromazine and chlorpromazine sulfoxide on vascular responses to L-epinephrine and levarterenol. J. Pharmacol. Exp. Ther. 130: 37, 1960.

22. Kopera, J., and Armitage, A.K.: Comparison of some pharmacological properties of chlorpromazine, promethazine and pethidine. Br. J. Pharmacol. 9: 392, 1954.

23. Spurr, G.B., Horvath, S.M., and Farrand, E.A.: Cardiovascular effects of chlorpromazine in the dog. Am. J. Physiol. 186: 525, 1956.

24. Duff, R., and Ginsburg, J.: Antagonism between chlorpromazine and noradrenaline in blood vessels of the hands. Br. J. Pharmacol. 11, 318, 1956.

25. Madan, B.R.: Some cardiovascular actions of thioridazine hydrochloride. Arch. Int. Pharmacodyn. Ther. 144: 299, 1963.

26. Hollister, L.E.: Human pharmacology of antipsychotic and antidepressant drugs. Annu. Rev. Pharmacol. Toxicol. 8: 491, 1968.

27. Ginsburg, J., and Duff, R.: Effect of chlorpromazine on adrenaline vasoconstriction in man. Br. J. Pharmacol. 11, 180, 1956.

28. Dasgupta, S.R., and Werner, G.: Inhibition of hypothalamic medullary and reflex vasomotor responses by chlorpromazine. Br. J. Pharmacol. 9: 389, 1954.

29. Wang, H.H., Kanai, T., Markee, S., and Wang, S.C.: Effects of reserpine and chlorpromazine on the vasomotor center in the medulla oblongata of the dog. J. Pharmacol. Exp. Ther. 144: 186, 1964.

30. Landmark, K., Glomstein, A., and Oye, I.: The effect of thioridazine and promazine on the isolated contracting rat heart. Acta Pharmacol. Toxicol. 27: 173, 1969.

31. Langslet, A.: Changes in coronary flow and ECG in the isolated perfused rat heart induced by phenothiazine drugs. Acta Pharmacol. Toxicol. 27: 183, 1969.

32. Hollander, P.B., and Cain, R.M.: Effects of thioridazine on transmembrane potential and contractile characteristics of guinea pig hearts. Eur. J. Pharmacol. 16: 129, 1971.

33. Arita, M., and Surawicz, R.: Electrophysiologic effects of phenothiazines on canine cardiac fibers. J. Pharmacol. Exp. Ther. 184: 619, 1973.

34. Courvoisier, S., Fournel, J., Ducrot, R., Kolsky, M., and Koetschet, P.: Proprietes pharmacodynamiques du chlorhydrate de chloro-3 (dimethylamino-3' proply)-10 phenothiazine (4.560 R.P.) Arch. Int. Pharmacodyn. Ther. 92: 305, 1953.

35. Huidobro, F.: Some pharmacological properties of chloro-3 (dimethylamine-3' proply)-10-phenothiazine or 4.560 R.P. Arch. Int. Pharmacodyn. Ther. 98: 308, 1954.

36. Ebert, M.H., and Shader, R.I.: Cardiovascular effects of psychotropic drugs. Conn. Med. 33: 695, 1969.

37. Selzer, A., and Wray, H.: Quinidine syncope: Paroxysmal ventricular fibrillation occurring during treatment of chronic atrial arrhythmias. Circulation 30: 17, 1964.

38. Axelrod, J., Hertting, G., and Potter, L.: Effect of drugs on the uptake and release of H^3-norepinephrine in the rat heart. Nature 194: 297, 1962.

39. Carlsson, A., and Waldeck, B.: Inhibition of H^3-metaraminol uptake by antidepressive and related agents. J. Pharm. Pharmacol. 17: 243, 1965.

40. Iversen, L.L.: Inhibition of noradrenaline uptake by drugs. J. Pharm. Pharmacol. 17: 62, 1965.

41. Maxwell, R.A., Keenan, P.D., Chaplin, E., Roth, B., and Eckhardt, S.B.: Molecular features affecting the potency of tricyclic antidepressants and structurally related compounds as inhibitors of the uptake of tritiated norepinephrine by rabbit aortic strips. J. Pharmacol. Exp. Ther. 166: 320, 1969.

42. Carlsson, C., Dencker, S.J., Grimby, G., and Haggendal, J.: Noradrenaline in blood-plasma and urine during chlorpromazine treatment. Lancet 1: 1208, 1966.

43. Huston, J.R., and Bell, G.E.: The effect of thioridazine hydrochloride and chlorpromazine on the electrocardiogram. J. Am. Med. Assoc. 198: 134, 1966.

44. Jeeva, R., and Benson, R.: Phenothiazines and the electrocardiogram. Postgrad. Med. J. 51: 65, 1975.

45. Crane, G.: Cardiac toxicity and psychotropic drugs. Dis. Nerv. Syst. 31: 534, 1970.

46. Graupner, K.I., and Murphree, O.D.: Electrocardiographic changes associated with the use of thioridazine. J. Neuropsych. 5: 344, 1963-1964.

47. Ban, T.A., and St. Jean, A.: The effect of phenothiazines on the electrocardiogram. Can. Med. Assoc. J. 91: 537, 1964.

48. Ban, T.A., and St. Jean, A.: Electrocardiographic changes induced by phenothiazine drugs. Am. Heart J. 70: 575, 1965.

49. Wendkos, M.: Cardiac changes related to phenothiazine therapy with special reference to thioridazine. J. Am. Geriatr. Soc. 15: 20, 1967.

50. Williams, R.B. and Sherter, S.: Cardiac complications of tricyclic antidepressant therapy. Ann. Intern. Med. 74: 395, 1971.

51. Moir, D.C., Cornwell, W.B., Dingwall-Fordyce, I., Crooks, E.J., O'Malley, K., Turnbull, M.J., and Weir, R.D.: Cardiotoxicity of amitriptyline. Lancet 2: 561, 1972.

52. Steel, C.M., O'Duffy, J., and Brown, S.S.: Clinical effects and treatment of imipramine and amitriptyline poisoning in children. Br. Med. J. 3: 663, 1967.

53. Sacks, M.H., Bonforte, R.J., Lasser, R.P., and Dimich, I.: Cardiovascular complications of imipramine intoxication. J. Am. Med. Assoc. 205: 116, 1968.

54. Crocker, J., and Morton, B.: Tricyclic (antidepressant) drug toxicity. Clin. Toxicol. 2 (4): 397, 1969.

55. Rasmussen, J.: Amitriptyline and imipramine poisoning. Lancet 2: 851, 1965.

56. Noble, J., and Matthew, H.: Acute poisoning by tricyclic antidepressants: Clinical features and management of 100 patients. Clin. Toxicol. 2 (4): 403, 1969.

57. Masters, A.B.: Delayed death in imipramine poisoning. Br. Med. J. 3: 866, 1967.

58. Rasmussen, E.B., and Kristhansen, P.: ECG changes during amitriptyline treatment. Am. J. Psychiatry. 119: 781, 1963.

59. Cairncross, K.D., Gershon, S.: A pharmacological basis for the cardiovascular complications of imipramine medication. Med. J. Aust. 49: 372, 1962.

60. Sigg, E.B., Osborne, M., and Korol, B.: Cardiovascular effects of imipramine. J. Pharmacol. Exp. Ther. 141: 237, 1963.

61. Osborne, M., and Sigg, E.B.: Effects of imipramine on the peripheral autonomic system. Arch. Int. Pharmacodyn. Ther. 129: 273, 1960.

62. Barth, N.: The effects of the tricyclic antidepressants dismethylimipramine, doxepine and iprindole on the isolated perfused rabbit heart. Naunyn Schmiedebergs Arch. Pharmacol. 277 Supp: R2, 1973.

63. Leading article: Cardiovascular complications from psychotropic drugs. Br. Med. J. 1: 3, 1971.

64. Babulova, A., Bareggi, S.R., Bonaccorsi, A., Garattini, S., Morselli, P.L., and Pontarotto, C.: Correlation between desimipramine levels and (-)- noradrenaline uptake and chronotropic effect in isolated atria of rats. Br. J. Pharmacol. 48: 464, 1973.

65. Furchgott, R.F., Bhadrakom, S.: Reactions of strips of rabbit aorta to epinephrine, isoproylarterenol, sodium nitrite and other drugs. J. Pharmacol. Exp. Ther. 108: 129, 1953.

66. Castillo, J.C., and DeBeer, E.J.: The tracheal chain. I. A preparation for the study of antispasmodics with particular reference to bronchodilator drugs. J. Pharmacol. Exp. Ther. 90: 104, 1947.

67. Anderson, F.F., and Craver, B.N.: A pyrex apparatus for the perfusion of the coronary circulation of mammalian hearts. J. Pharmacol. Exp. Ther. 93: 135, 1948.

68. Dinovo, E.C., Gottschalk, L.A., Noble, E.P., and Biener, R.: Isolation of a possible new metabolite of thioridazine and mesoridazine from human plasma. Res. Commun. Chem. Pathol. Pharmacol. 7: 489, 1974.

69. Wit, A.L., Hoffman, B.F., and Rosen, M.R.: Electrophysiology and pharmacology of cardiac arrhythmias. IX: Cardiac electrophysiologic effects of beta-adrenergic receptor stimulation and blockade. Part A. Am. Heart J. 90: 521, 1975.

70. Hoffman, B.F., Rosen, M.R., and Wit, A.L.: Electrophysiology and pharmacology of cardiac arrhythmias. VII. Cardiac effects of quinidine and procaine amide. B. Am. Heart J. 90: 117, 1975.

71. Mendez, C., Aceves, J., and Mendez, R.: Anti-adrenergic action of digitalis on the refractory period of the A-V transmission system. J. Pharmacol. Exp. Ther. 131: 199, 1961.

72. Rosen, M.R., Gelband, H., Merker, C., and Hoffman, B.F.: Mechanisms of digitalis toxicity: Effects of ouabain on phase four of canine purkinje fiber transmembrane potentials. Circulation 47: 681, 1973.

73. Mclain, P.L., Kruse, T.K., and Ridick, T.F.: The effect of atropine on digitoxin bradycardia in cats. J. Pharmacol. Exp. Ther. 126: 76, 1959.

74. Ten Eick, R.E., and Hoffman, B.F.: Chronotropic effect of cardiac glycosides in cats, dogs and rabbits. Cir. Res. 25: 365, 1969.

75. Krueger, E., and Unna, K.: Comparative studies on the toxic effects of digitoxin and ouabain in cats. J. Pharmacol. Exp. Ther. 76: 282, 1942.

76. DeMello, W.C. (Ed.): Electrical phenomena in the heart. New York and London: Academic Press, Inc.: Theodore C. West, 1972 (pages 208-211).

77. Cranefield, P.F.: The conduction of the cardiac impulse. Futura Publishing Co., New York, 1975.

78. Rouse, W.: Effects of propranolol and ouabain on the conducting system of the heart in dogs. Am. J. Cardiol. 18: 406, 1966.

79. Dreifus, L.S., and Ukoff, W. (Eds.): Cardiac arrhythmias. New York and London: Greene and Stratton, Inc.: Mason, D.T., Zelus, R., Amsterdam, E.A., and Massumi, R.A., 1973 (pages 501-503).

80. Rosen, M., Gelband, H., and Hoffman, B.F.: Correlation between effects of ouabain on the canine electrocardiogram and transmembrane potentials of isolated purkinje fibers. Circulation 47: 65, 1973.

81. Godfraind, T., and Verbeke, N.: Inhibition by chlorpromazine of Ca^{2+}- activated ATPase in brain microsomes. Arch. Int. Pharmacodyn. Ther. 203: 400, 1973.

82. Lee, C-Y., Akera, T., and Brody, T.M.: Comparative effects of chlorpromazine and its free radical on membrane function. Biochem. Pharmacol. 25: 1751, 1976.

83. O'Callaghan, M.A., and Duggan, P.F.: Differential effects of chlorpromazine and chlorpromazine free radical on calcium transport in sarcoplasmic reticulum vesicles. Biochem. Pharmacol. 24: 563, 1975.

84. Balzer, H., Makinose, M., and Hasselbach, W.: The inhibition of the sarcoplasmic calcium pump by prenylamine, reserpine, chlorpromazine and imipramine. Naunyn Schmiedebergs Arch. Pharmacol. 260: 444, 1968.

85. Balzer, H., and Makinose, M.: The inhibition of the sarcoplasmic calcium pump produced by agents which react with its lipid component: reserpine, prenylamine, chlorpromazine, imipramine. Naunyn Schmiedebergs Arch. Pharmacol. 259: 151, 1967-1968.

86. Balzer, H., and Hellenbrecht, D.: Beeinflussung des Calcium-Austauschs und der Muskelfunktion des M. rectus and sartorius des Frosches durch Chlorpromazin, Prenylamin, Imipramin und Reserpin. Naunyn Schmiedebergs Arch. Pharmacol. 264: 129, 1969.

87. Balzer, H., and Hellenbrecht, D.: "Über die kontraktionsauslösende" Wirkung von Hemmstoffen des aktiven Calciumtransports (Chlorpromazin, Prenylamin, Reserpin) am Froschrectus und -sartorius. Naunyn Schmiedebergs Arch. Pharmacol. 260: 93, 1968.

88. Godfraind, T., and Kaba, A.: Blockade or reversal of the contraction induced by calcium and adrenaline in depolarized arterial smooth muscle. Br. J. Pharmacol. 36: 549, 1969.

89. Godfraind, T., and Kaba, A.: Inhibition by cinnarizine and chlorpromazine of the contraction induced by calcium and adrenaline in vascular smooth muscle. Br. J. Pharmacol. 35: 354P, 1969.

90. Burks, T.F., and Cooper, T.: Enhancement of peripheral alpha-receptor stimulation by blockade of "silent" beta receptors. Circ. Res. 21: 703, 1967.

91. Patil, P.N., Tye, A., May, C., Hetey, S., and Miyagi, S.: Steric aspects of adrenergic drugs. XI. Interactions of dibenamine and beta adrenergic blockers. J. Pharmacol. Exp. Ther. 163: 398, 1968.

92. Sutherland, E.W., and Rall, T.W.: The relation of adenosine 3', 5', phosphate and phosphorylase to the actions of catecholamines and other hormones. Pharm. Rev. 12: 265, 1960.

106. Duce, B.R., Garberg, L., and Johansson, B.: The effect of propranolol and the dextro and laevo isomers of H 56/28 upon the ouabain-induced ventricular tachycardia in unanaesthetized dogs. Acta Pharmacol. Toxicol. 25, Suppl. 2: 41, 1967.

107. Florez, J., and Arconada, J.A.: Comparison of the anti-arrhythmic action and beta-blocking potency of LB 46 and (-)-propranolol. Arch. Int. Pharmacodyn. Ther. 190: 199, 1971.

108. Mosey, L., and Tyler, M.D.: The effect of diphenylhydantoin sodium (Dilantin), procaine hydrochloride, procaine amide hydrochloride, and quinidine hydrochloride upon ouabain-induced ventricular tachycardia in unanaesthetized dogs. Circulation 10: 65, 1954.

109. Reynolds, E.W., and VanderArk, C.R.: Quinidine syncope and the delayed repolarization syndromes. Mod. Concepts Cardiovasc. Dis. 45: 117, 1976.

110. Wit, A.L., Rosen, M.R., and Hoffman, B.F.: Electrophysiology and pharmacology of cardiac arrhythmias. 1. Relationship of normal and abnormal electrical activity of cardiac fibers to the genesis of arrhythmias B. Re-entry. Section 1. Am. Heart J. 88: 664, 1974.

111. Kleenman, L.I., and Radford, E.P.: Ventilation standards for small animals. J. Appl. Physiol. 19: 360, 1964.

112. Chodnekar, M.S., Crowther, A.F., Hepworth, W., Howe, R., McLoughlin, B.J., Mitchell, A., Rao, B.S., Slatcher, R.P., South, H., and Stevens, M.A.: β Adrenergic blocking agents. II. Heterocyclic analogs of pronethalol, [2-Isopropylamino-1-(2-naphthyl) ethanol]. J. Med. Chem. 15: 49, 1972.

Figure 1: Isolated Organ Bath

A. Isometric pressure transducer

B. Organ bath
   1. Inlet for constant temperature water
   2. Outlet for constant temperature water

C. Glass rod which was bent under heat to form base support for tracheal chain

D. Aerator for delivery of 95% O_2 and 5% CO_2 to Krebs solution

E. Outlet for Krebs solution

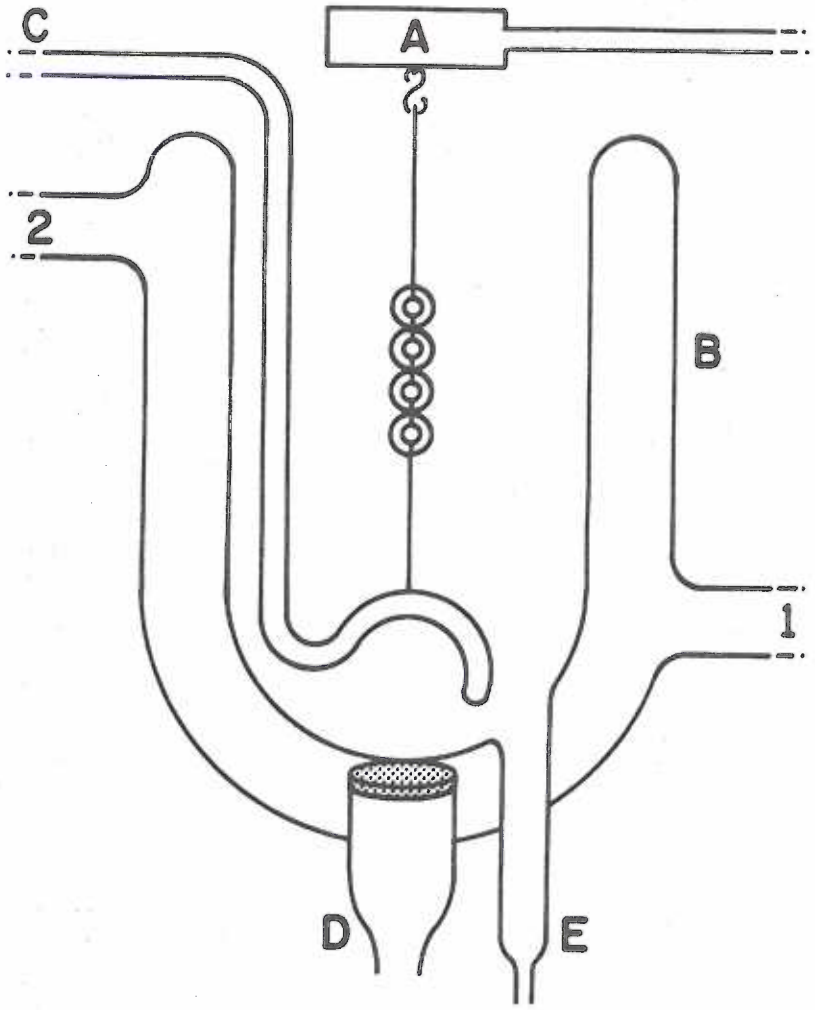

C
A
2
B
1
D
E

Figure 2: Heart Perfusion Apparatus

A. Perfusate Reservoir

B. Constant Level Unit

C. Upper Chamber

1. Standpipe for release of gas bubbles in perfusate and for maintenance of constant pressure
2. Outlet for constant temperature water to lower chamber D.
3. Injection nipple which is attached by a short length of pure gum rubber tubing to male connection of a B and D stopcock.
4. Nipple with short length (3 to 5 cm) of pure gum rubber tubing which serves as the point at which the cannulated heart is placed.
5. Inlet for perfusate from the constant level unit
6. Inlet for constant temperature water (G-1) from water pump.

D. Lower Chamber

1. Outlet for constant temperature water from jacket to water pump at G-2
2. Support for clamp holding the glass pulley assembly (D-4)
3. Opening through the double wall for insertion of glass pulley assembly (D-4) and for thread to heart lever from hook in ventricle of heart.
4. Glass pulley assembly attached to stainless steel, L-shaped rod which is clamped to D-2
5. Outflow of perfusate effluent to coronary flow drop counter
6. Inlet for constant temperature water to jacket from C-2.

E. Coronary Flowmeter

1. Air inlet
2. Male stopper with air outlets
3. Float in body of flowmeter
4. Discharge line for perfusate to perfusate pump at F-5
5. Inlet for perfusate from lower chamber at D-5

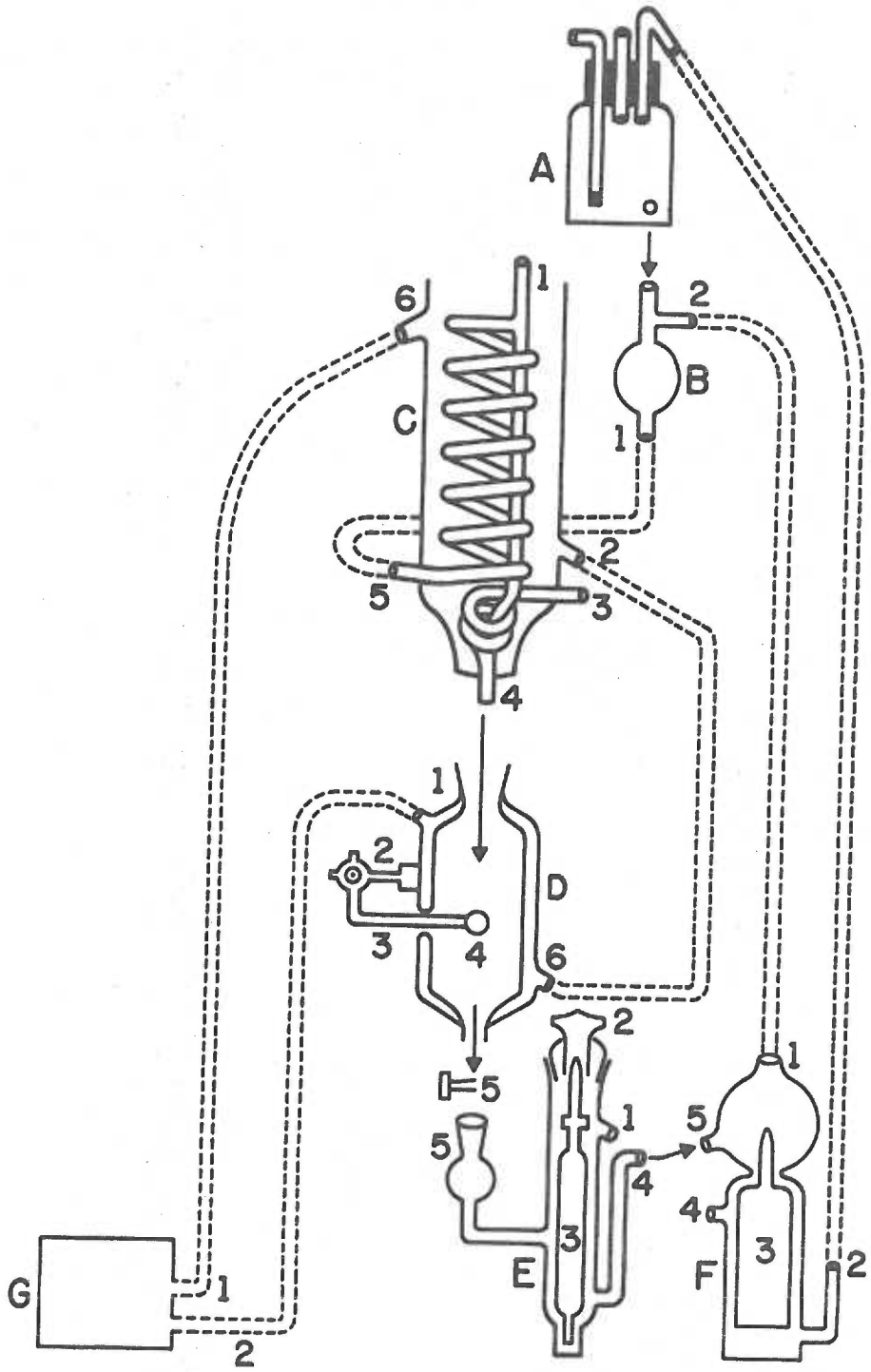

A
B
C
D
E
F
G
1
2
3
4
5
6

Figure 3: Change in weight of cats during the treatment period

Individual graphs of the weight of the experimental cats during the three weeks pre-treatment with thioridazine, 10-20 mg/kg/day. Circled weights indicate days when the cats were tube fed with dosing.

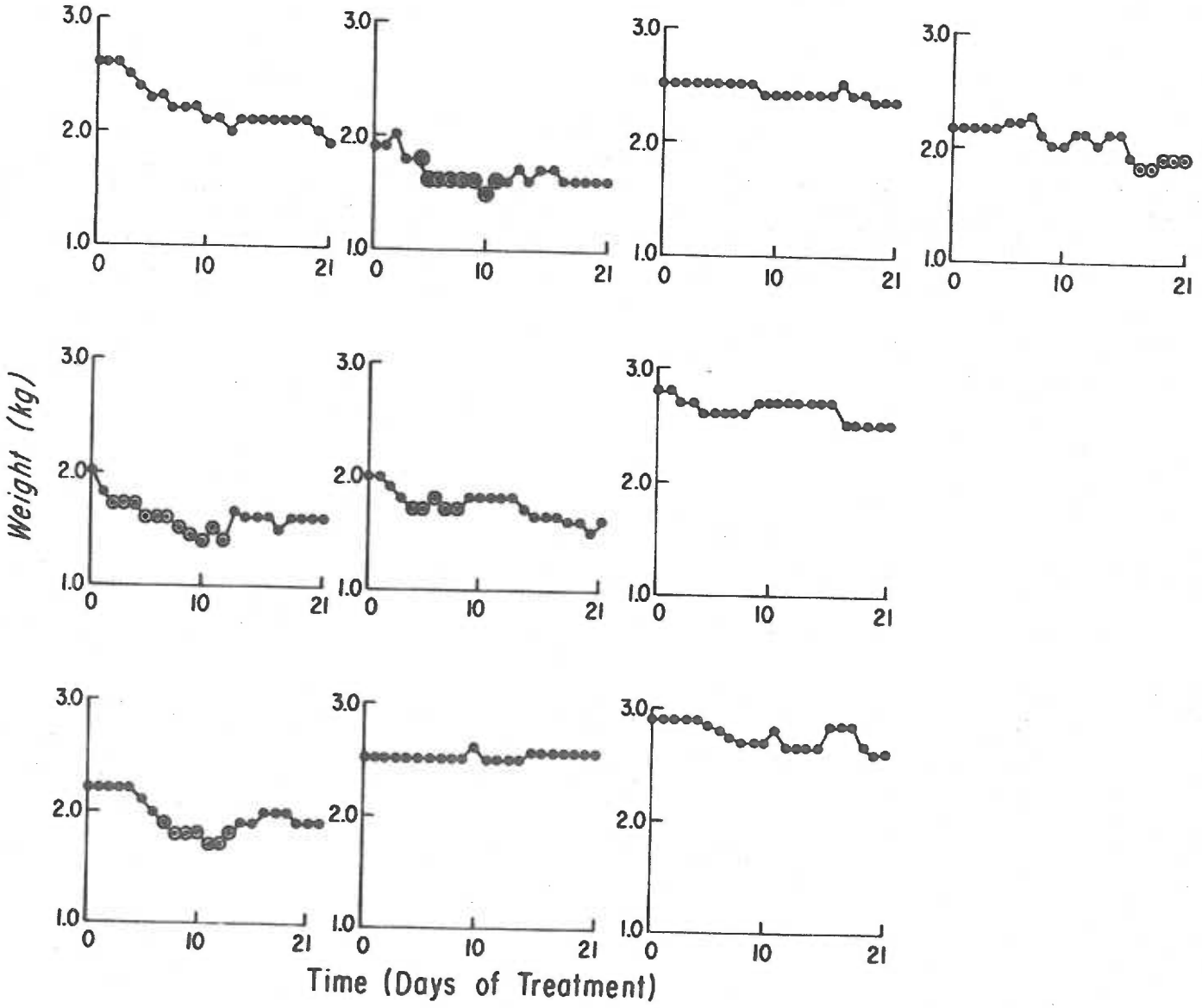

Weight (kg)
3.0
2.0
1.0
0
10
21
Time (Days of Treatment)

Figure 4: Epinephrine Reversal

Effect of epinephrine, 3 µg/kg, in naive animals before (A) and after (B) an infusion of thioridazine, 10 mg/kg.

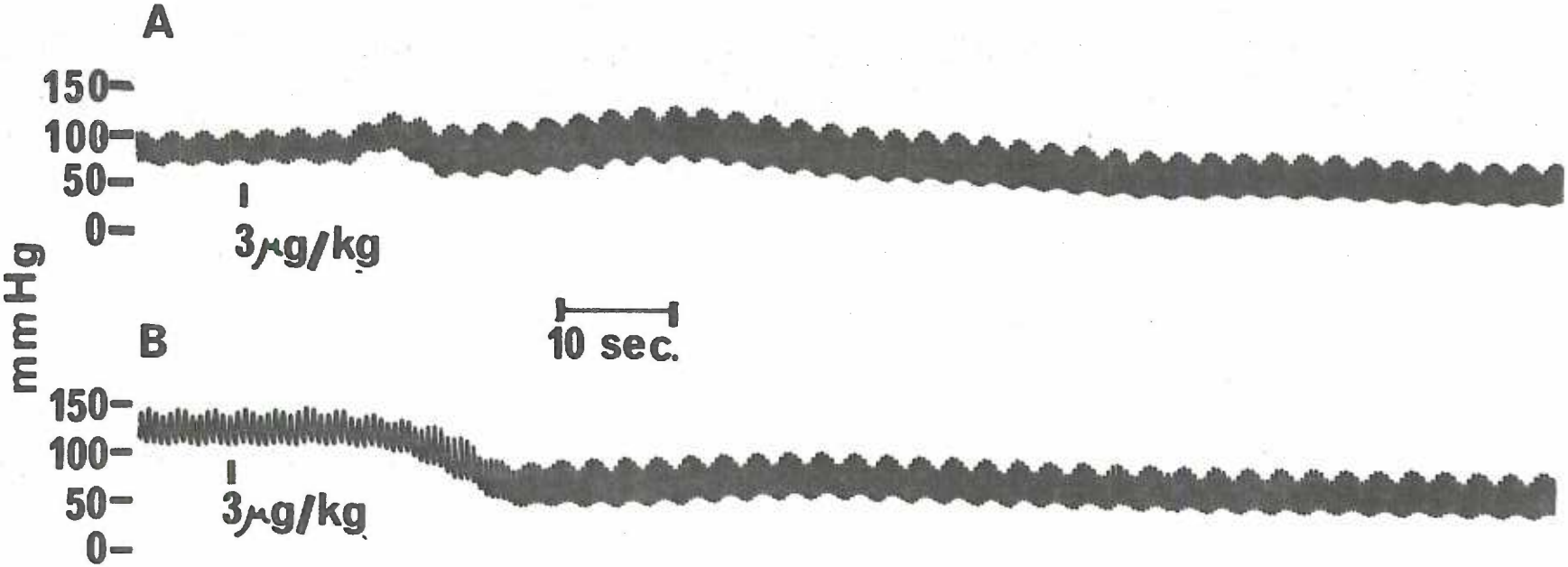

A
150
100
50
0
3μg/kg
mm Hg
10 sec.
B
150
100
50
0
3μg/kg

Figure 5: Epinephrine-induced changes in blood pressure in naive and exposed animals

Points show the means of five experiments. Brackets give the S.E. and the direction of the bracket indicates systolic or diastolic blood pressure. Blood pressure in the control period averaged 129/91 $\pm$ 6/4 ($\pm$ S.E. systolic/diastolic) mm of Hg in naive animals and 134/98 $\pm$ 16/15 in exposed animals so that block of the hypotensive response was not due to preexisting hypotension.

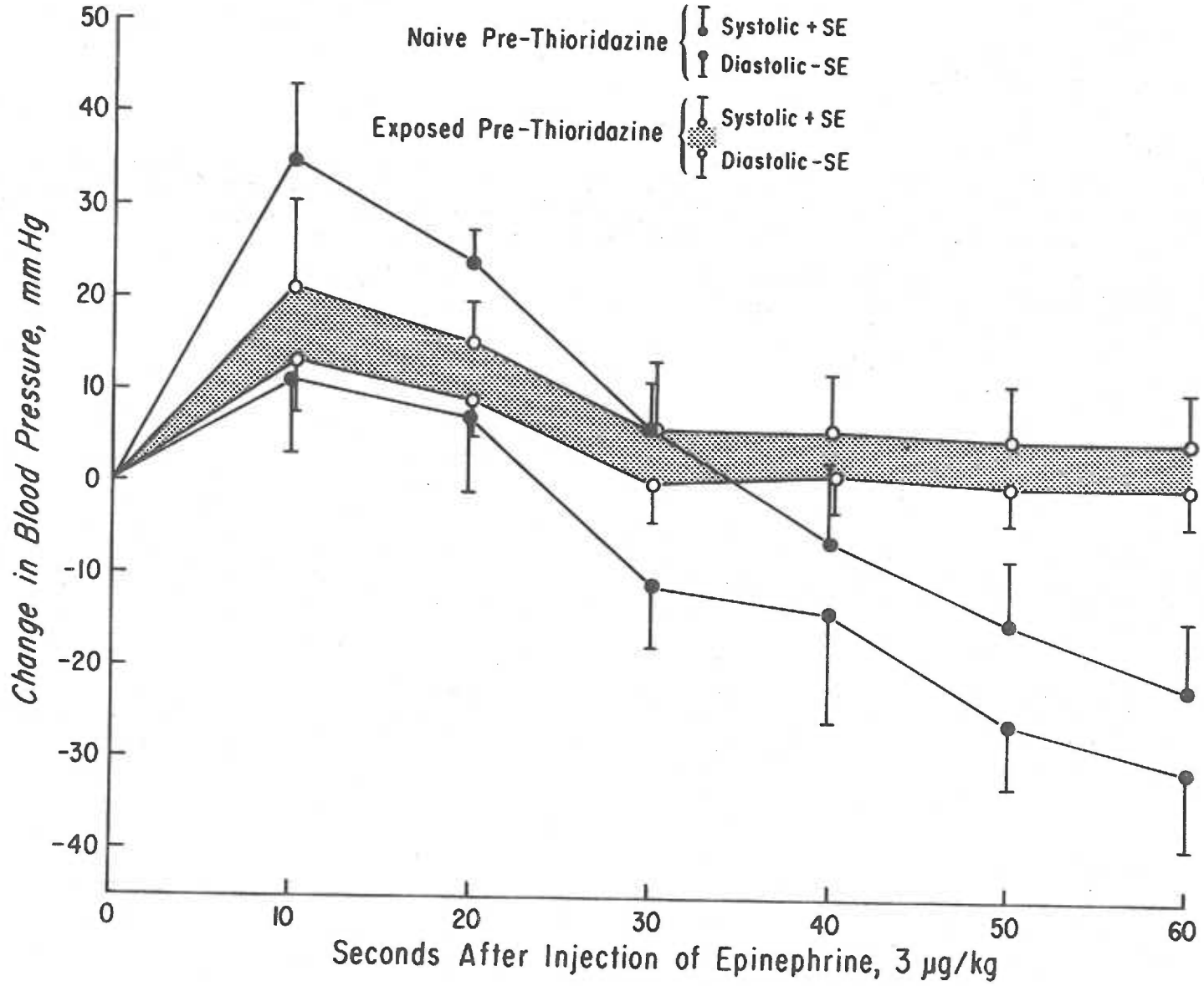

Naive Pre-Thioridazine
Systolic + SE
Diastolic - SE
Exposed Pre-Thioridazine
Systolic + SE
Diastolic - SE
Change in Blood Pressure, mm Hg
50
40
30
20
10
0
-10
-20
-30
-40
0
10
20
30
40
50
60
Seconds After Injection of Epinephrine, 3 µg/kg

Figure 6: Block of epinephrine reversal in exposed animals

Points show the means of four experiments in exposed animals and five experiments in naive animals. Brackets give the S.E. and indicate systolic or diastolic pressure. Blood pressure before injection of epinephrine averaged 115/87 $\pm$ 8/10 mmHg ($\pm$ S.E. systolic/diastolic) in naive post-thioridazine animals and 115/90 $\pm$ 11/11 in exposed post-thioridazine animals.

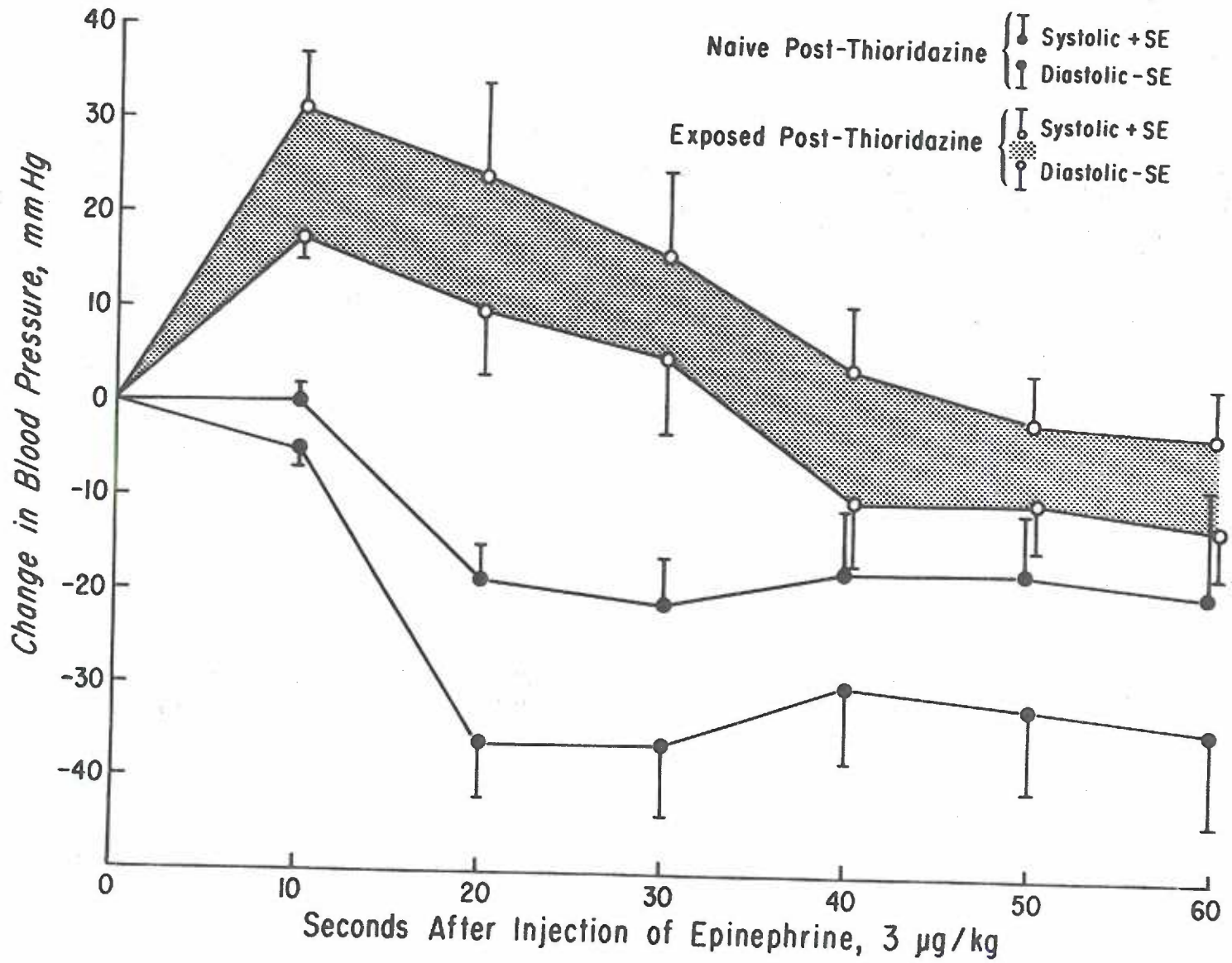

Naive Post-Thioridazine
Systolic + SE
Diastolic - SE
Exposed Post-Thioridazine
Systolic + SE
Diastolic - SE
Change in Blood Pressure, mm Hg
40
30
20
10
0
-10
-20
-30
-40
0
10
20
30
40
50
60
Seconds After Injection of Epinephrine, 3 µg/kg

Figure 7: Comparison of the systolic blood pressure responses to phenylephrine in the various experimental conditions

Points show the means of ten experiments in the naive pre-thioridazine condition, means of four experiments in the exposed post-thioridazine condition, and means of five experiments in the naive post-thioridazine, naive post-saline and exposed pre-thioridazine condition. Brackets show S.E.

A. Phenylephrine responses in naive animals before an infusion of thioridazine or saline.

B. Phenylephrine responses before and after an infusion of thioridazine in naive animals.

C. Phenylephrine responses in naive and exposed animals before an infusion of thioridazine.

D. Phenylephrine responses in naive and exposed animals after an infusion of thioridazine.

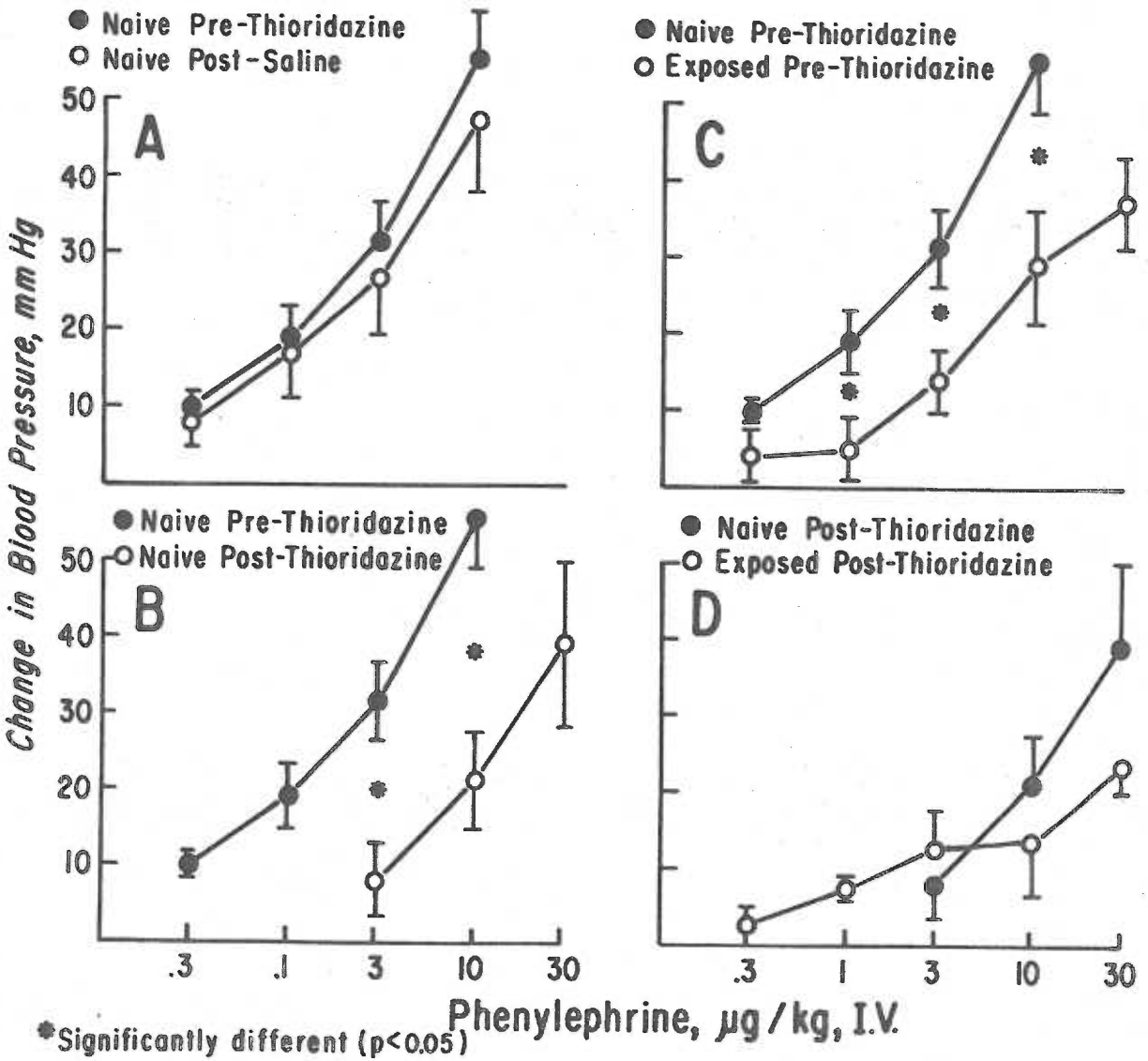

● Naive Pre-Thioridazine
○ Naive Post-Saline
A
● Naive Pre-Thioridazine
○ Exposed Pre-Thioridazine
C
● Naive Pre-Thioridazine
○ Naive Post-Thioridazine
B
● Naive Post-Thioridazine
○ Exposed Post-Thioridazine
D
Change in Blood Pressure, mm Hg
50
40
30
20
10
.3
.1
1
3
10
30
Phenylephrine, µg/kg, I.V.
*Significantly different (p<0.05)

Figure 8: Isoproterenol-induced changes in blood pressure in naive and exposed animals

Points show the means of ten experiments for naive pre-thioridazine and the means of five experiments for exposed pre-thioridazine. Brackets give the S.E. and the direction of the bracket indicates systolic or diastolic blood pressure. Blood pressure in the control period averaged 138/91 $\pm$ 6/5 ($\pm$ S.E. systolic/diastolic) mmHg in naive animals and 138/100 $\pm$ 12/12 in exposed animals, so that block of the depressor response was not due to preexisting hypotension.

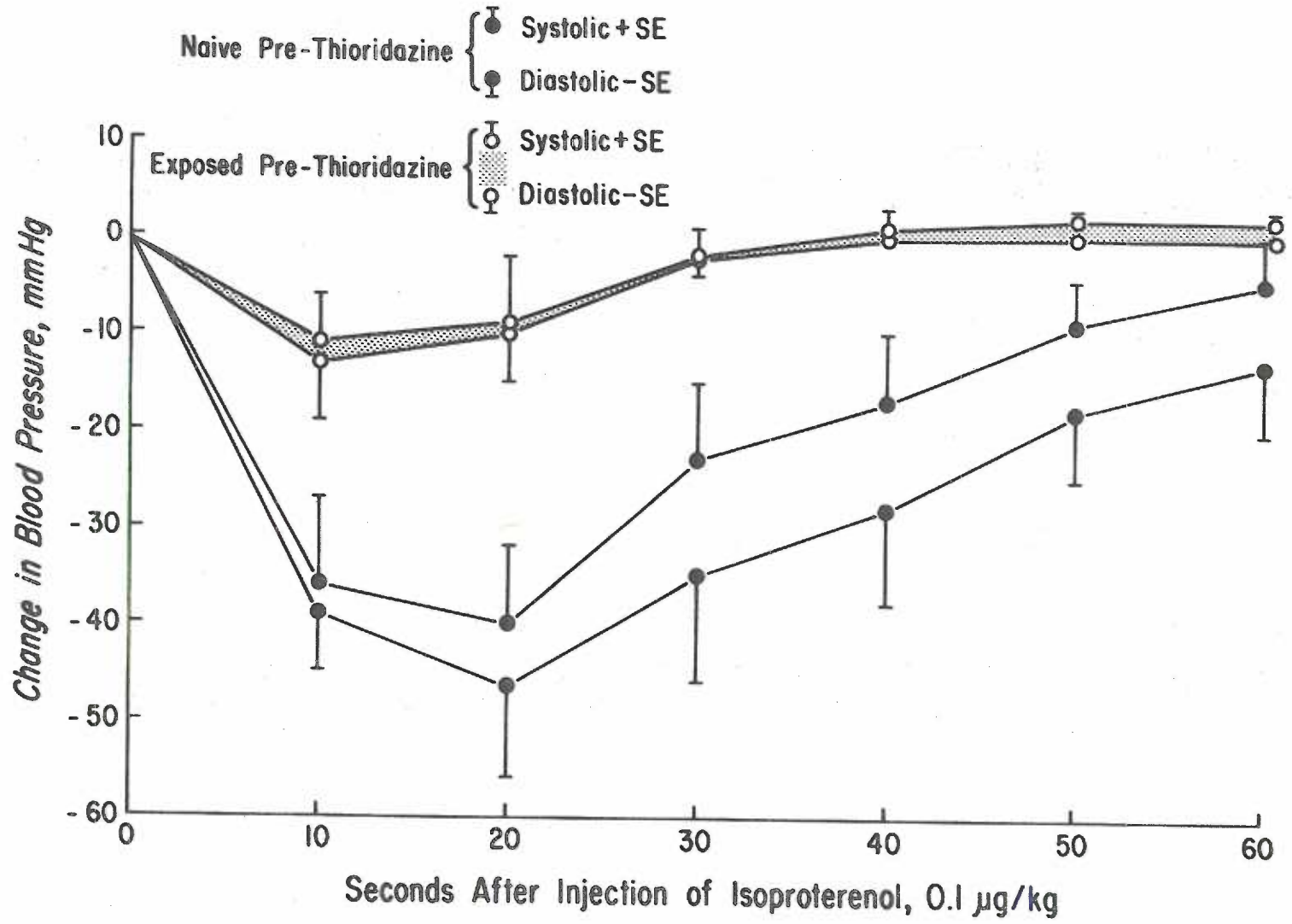

Naive Pre-Thioridazine
Systolic + SE
Diastolic - SE
Exposed Pre-Thioridazine
Systolic + SE
Diastolic - SE
Change in Blood Pressure, mm Hg
10
0
-10
-20
-30
-40
-50
-60
0
10
20
30
40
50
60
Seconds After Injection of Isoproterenol, 0.1 µg/kg

Figure 9: Isoproterenol-induced changes in blood pressure after an infusion of thioridazine in naive and exposed animals

Points show the means of four experiments in the exposed animals and five experiments in naive animals. Brackets give the S.E. and the direction of the brackets indicate systolic or diastolic pressure. Blood pressure before injection of isoproterenol averaged 119/93 ± 6/8 (± S.E. systolic/diastolic) in naive post-thioridazine and 124/99 ± 7/10 in exposed post-thioridazine animals.

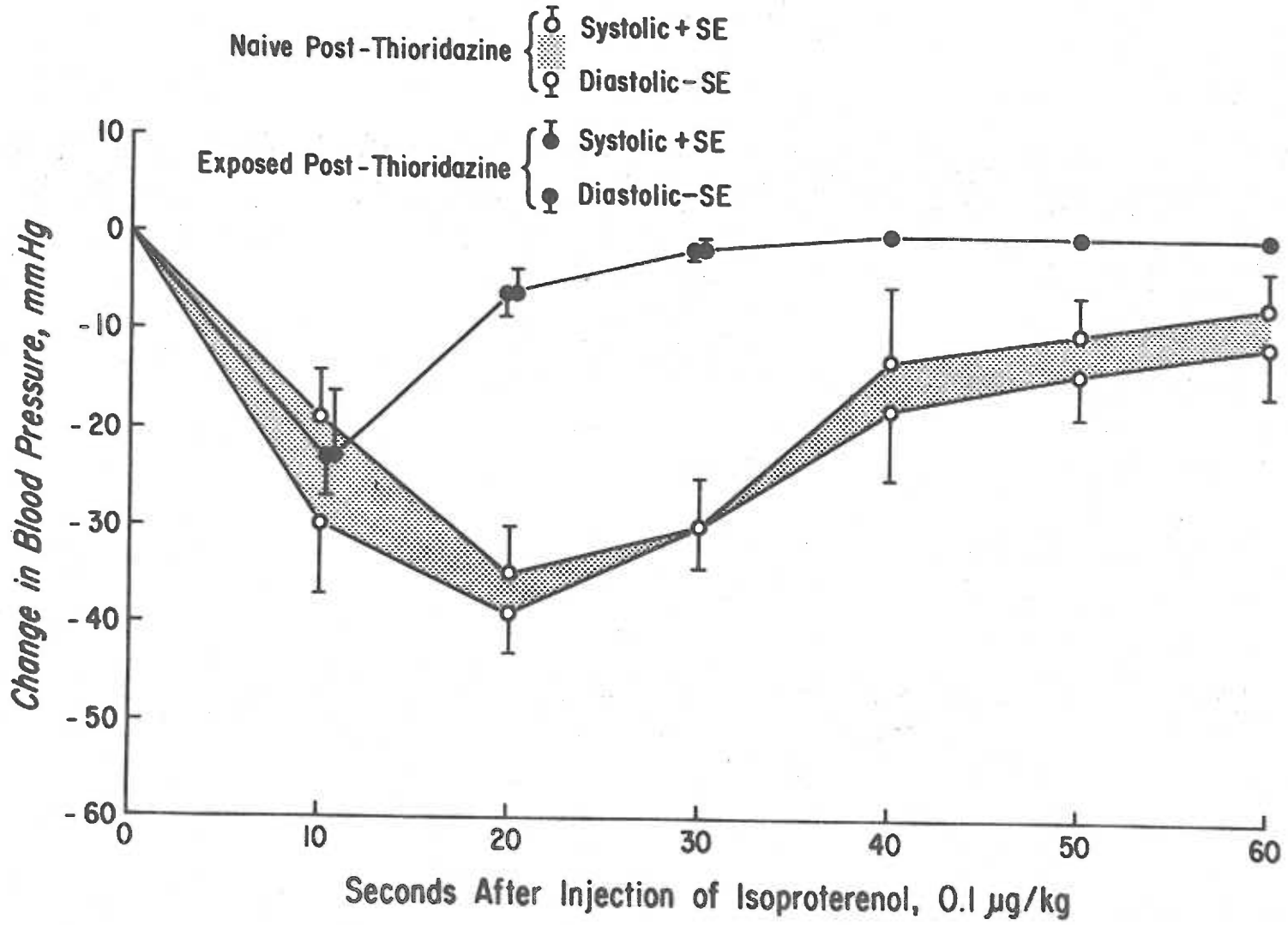

Naive Post-Thioridazine
Systolic + SE
Diastolic - SE
Exposed Post-Thioridazine
Systolic + SE
Diastolic - SE
Change in Blood Pressure, mmHg
10
0
-10
-20
-30
-40
-50
-60
0
10
20
30
40
50
60
Seconds After Injection of Isoproterenol, 0.1 µg/kg

Figure 10: Effect of a ouabain infusion on the EKG in naive post-saline animals

Paper speed for the three recordings is 50 mm/sec.

A. EKG just before the beginning of the ouabain infusion. Heart rate, 144 bpm; QRS, .08 sec.; PR, .13 sec.

B. Effect of ouabain, 100 µg/kg, on EKG. Heart rate 132 bpm; QRS, .08 sec.; PR, .13 sec.

C. Effect of ouabain, 125 µg/kg, on the EKG. Heart rate irregular, approx. 180, QRS, .14 sec. PR, .22 sec. Ectopic ventricular contractions are occurring every third or fourth beat and the atrial focus is variable.

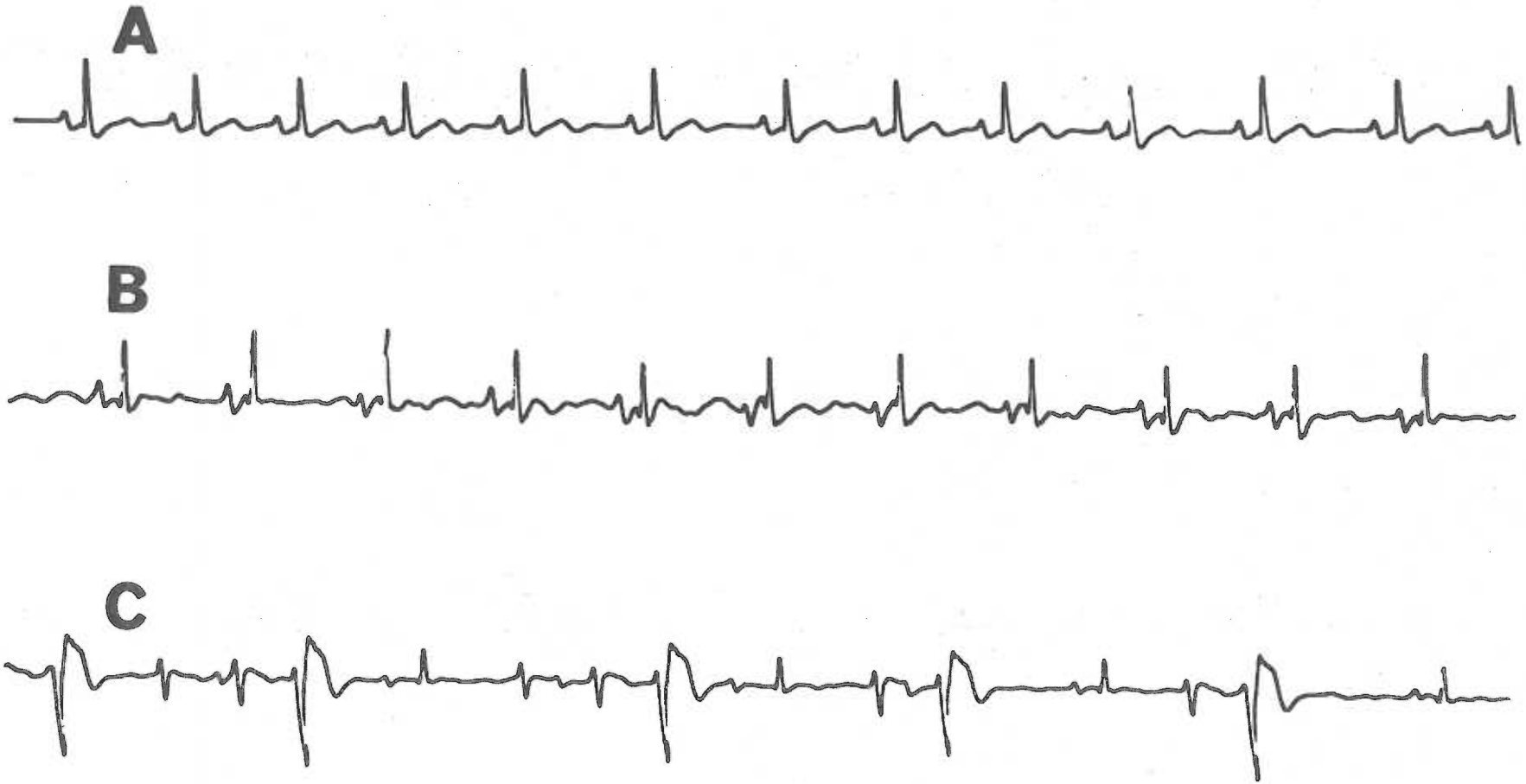

A
B
C

Figure 11: Norepinephrine-induced contraction in isolated aortic strips

Points are the means of five experiments. Brackets show the S.E. Responses of aortic strips to increasing concentrations of norepinephrine are calculated as mm contraction.

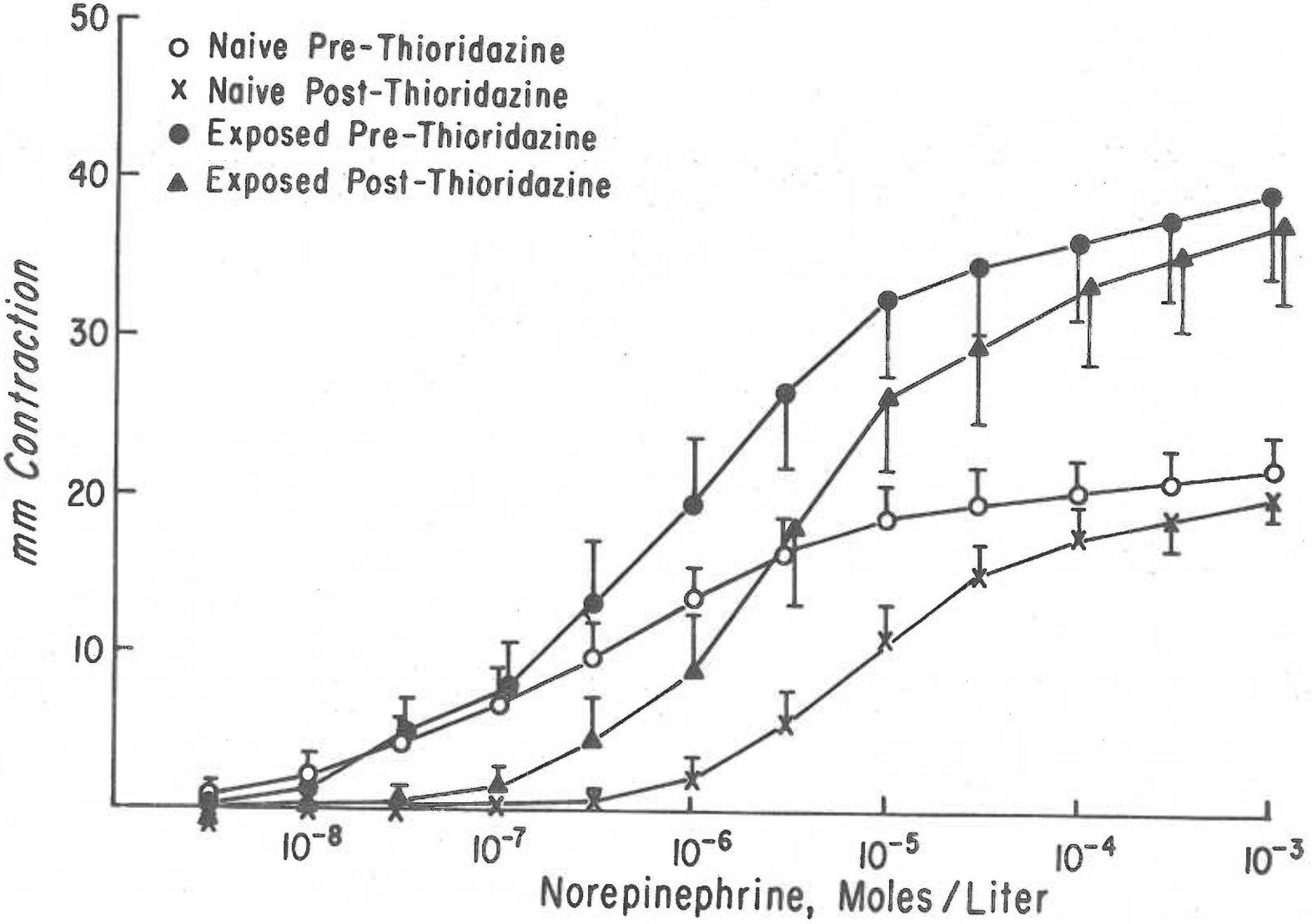

50
40
30
20
10
mm Contraction
o Naive Pre-Thioridazine
x Naive Post-Thioridazine
● Exposed Pre-Thioridazine
▲ Exposed Post-Thioridazine
10-8
10-7
10-6
10-5
10-4
10-3
Norepinephrine, Moles / Liter

Figure 12: Norepinephrine-induced contraction in isolated aortic strips

Points are the means of five experiments. Brackets show the S.E. Responses of aortic strips to increasing concentrations of norepinephrine is calculated as percent of maximum contraction.

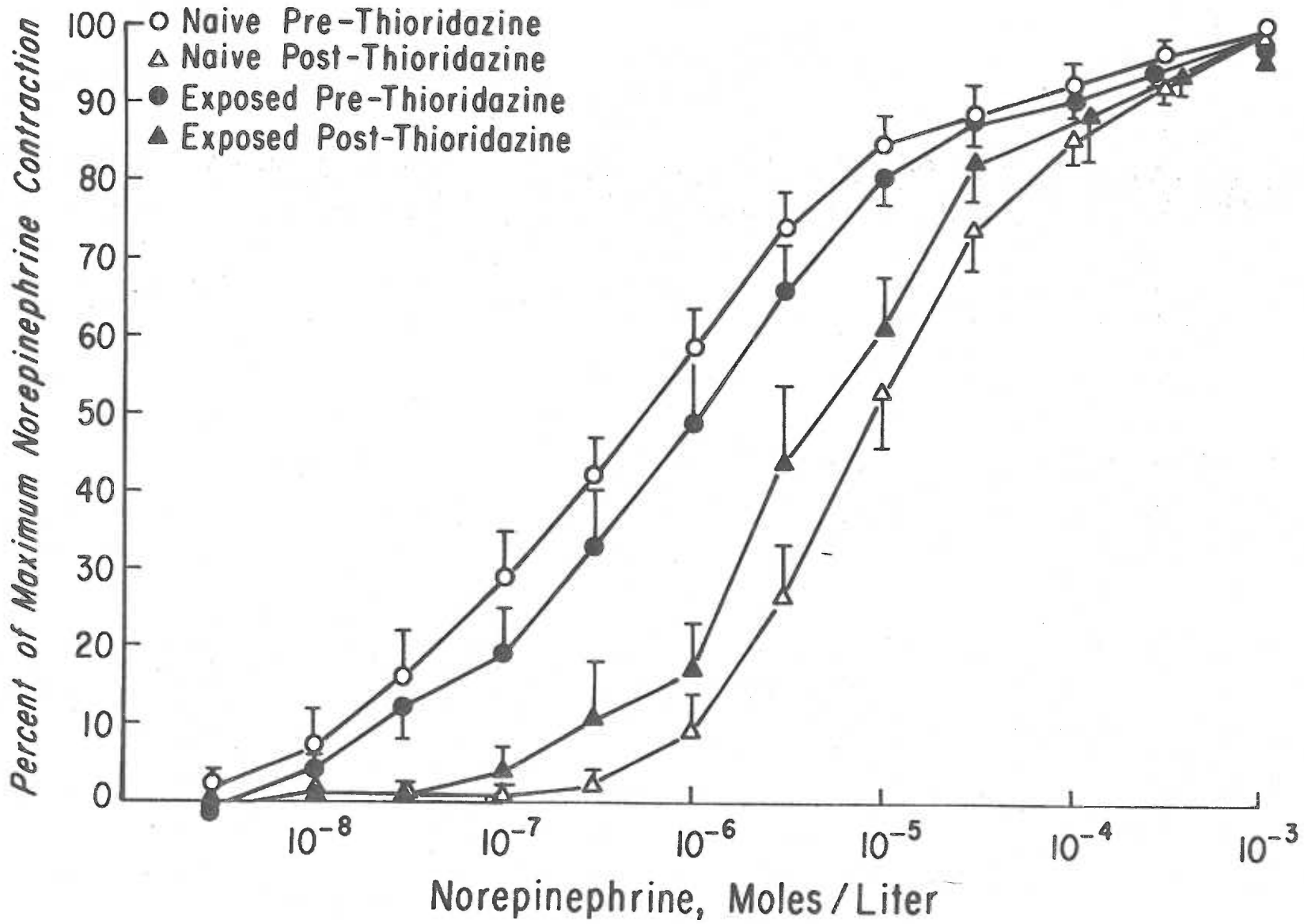

Naive Pre-Thioridazine
Naive Post-Thioridazine
Exposed Pre-Thioridazine
Exposed Post-Thioridazine
Percent of Maximum Norepinephrine Contraction
100
90
80
70
60
50
40
30
20
10
0
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
Norepinephrine, Moles / Liter

Figure 13: Contractile responses of isolated aortic strips to tyramine

Points are means of five experiments and brackets show S.E. Contractile responses to increasing concentrations of tyramine are in mm of contraction.

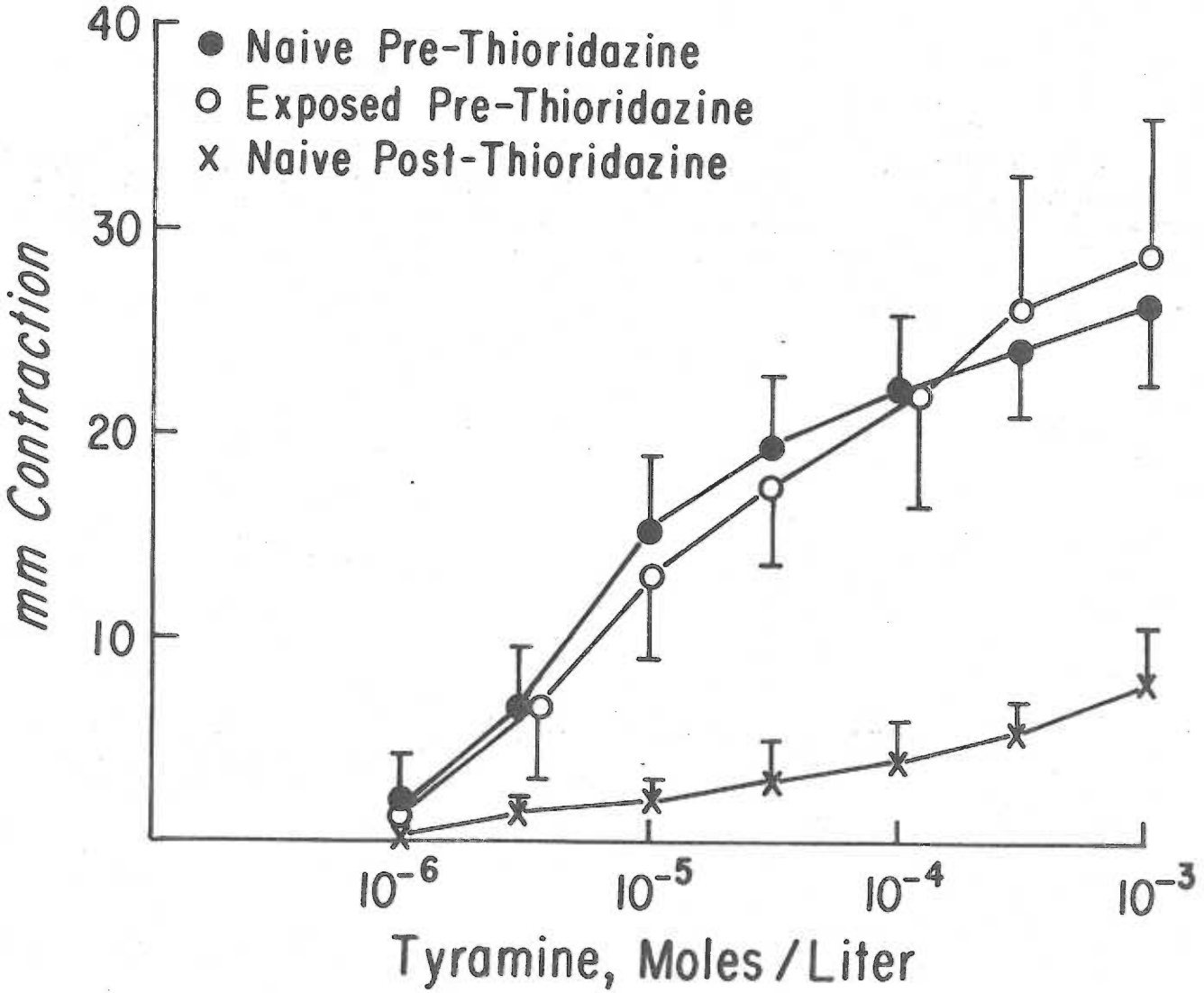

40
30
20
10
mm Contraction
● Naive Pre-Thioridazine
○ Exposed Pre-Thioridazine
× Naive Post-Thioridazine
10⁻⁶
10⁻⁵
10⁻⁴
10⁻³
Tyramine, Moles / Liter

Figure 14: Carbamylcholine-induced contraction in isolated tracheal chains

Points are the means of five experiments. Brackets show the S.E. Response of tracheal chains to increasing concentrations of carbamylcholine is calculated as percent of maximum contraction.

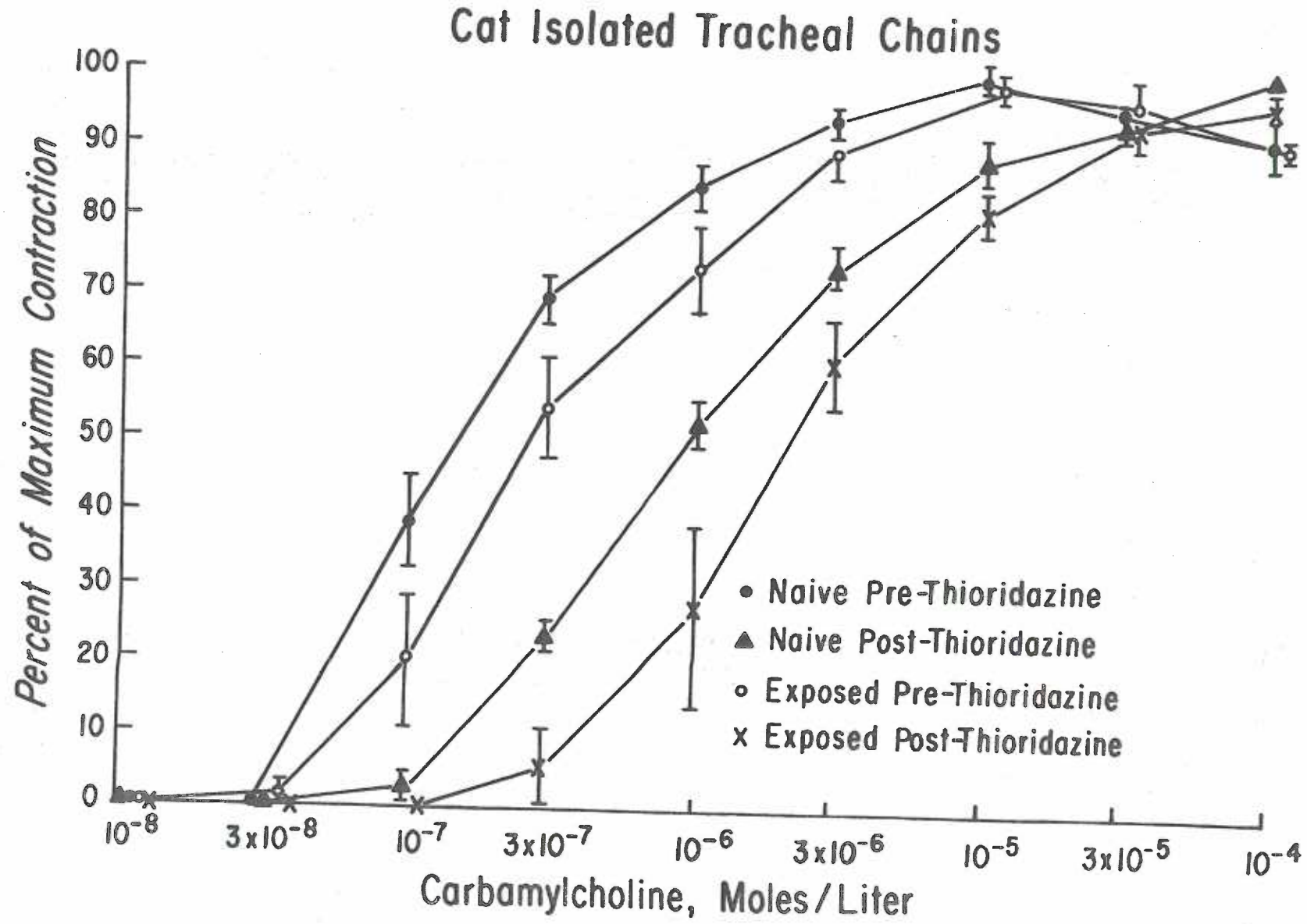

Cat Isolated Tracheal Chains
Percent of Maximum Contraction
100
90
80
70
60
50
40
30
20
10
0
10⁻⁸
3x10⁻⁸
10⁻⁷
3x10⁻⁷
10⁻⁶
3x10⁻⁶
10⁻⁵
3x10⁻⁵
10⁻⁴
Carbamylcholine, Moles/Liter
Naive Pre-Thioridazine
Naive Post-Thioridazine
Exposed Pre-Thioridazine
Exposed Post-Thioridazine

Figure 15: Effect of three weeks' pretreatment with thioridazine on the heart rate response to epinephrine in the isolated heart preparation

Heart rate is measured in beats per minute. Points are means of five experiments and brackets show S.E.

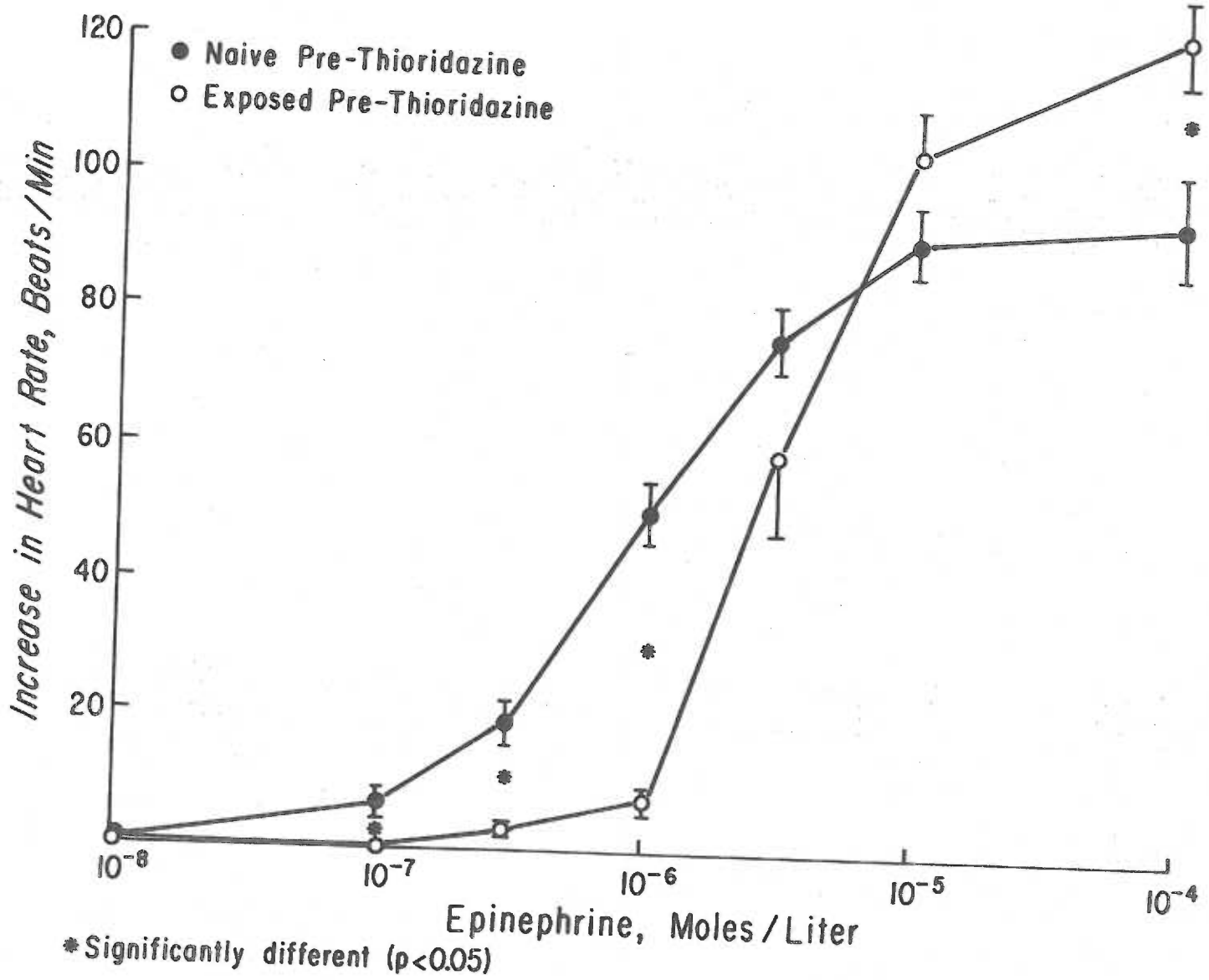

120
100
80
60
40
20
Increase in Heart Rate, Beats/Min
● Naive Pre-Thioridazine
○ Exposed Pre-Thioridazine
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
Epinephrine, Moles/Liter
*Significantly different (p<0.05)

Figure 16: Effect of epinephrine on the heart rate response in the isolated heart preparation after thioridazine, 1.2 µg/ml, has been added to the perfusion fluid

Points are means of five experiments and brackets show S.E. Heart rate response is measured in beats per minute. An asterick indicates significant differences between heart rate responses in the exposed post-thioridazine condition and heart rate responses in the naive post-saline and naive post-thioridazine.

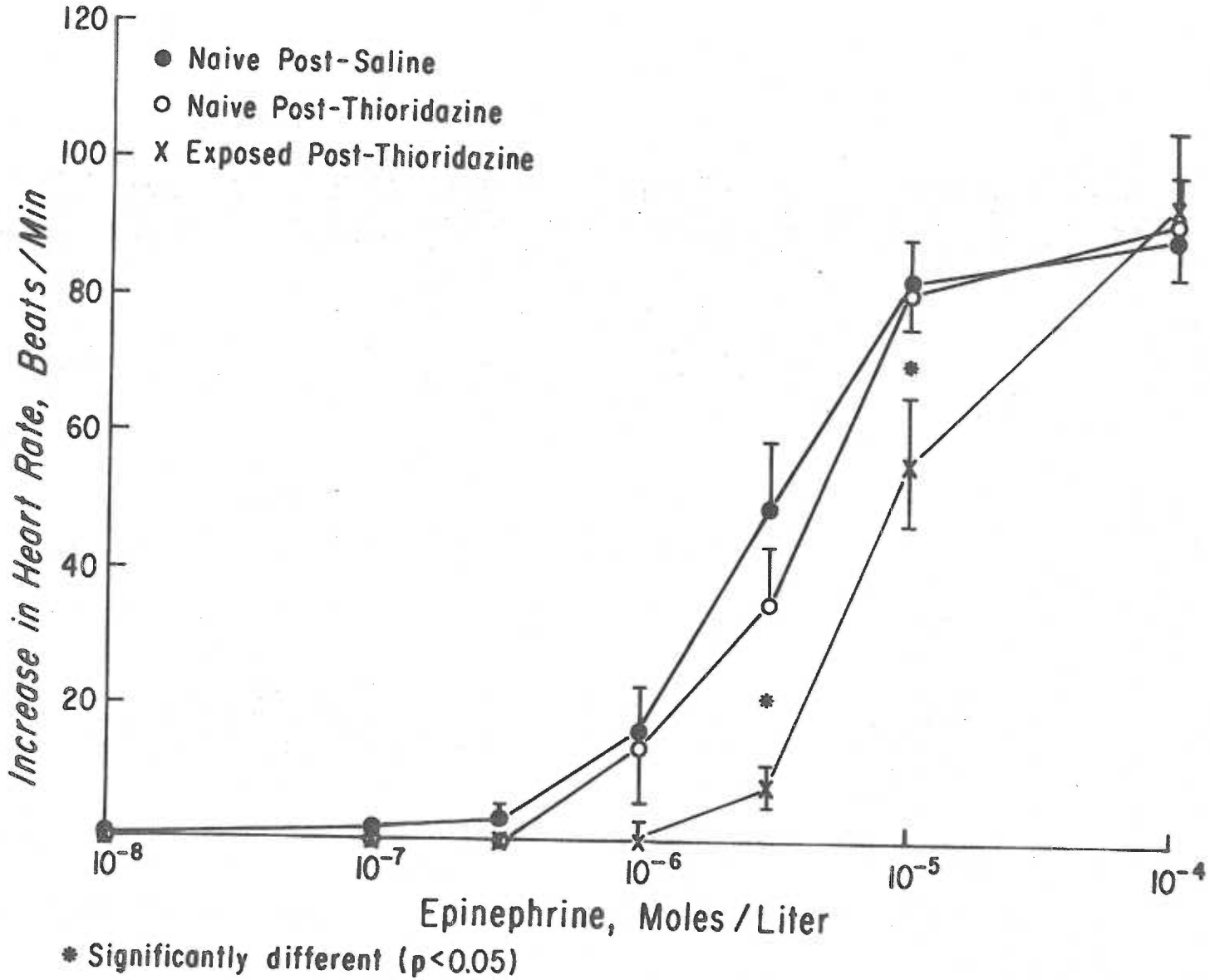

120
100
80
60
40
20
● Naive Post-Saline
○ Naive Post-Thioridazine
X Exposed Post-Thioridazine
Increase in Heart Rate, Beats/Min
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
Epinephrine, Moles / Liter
* Significantly different (p<0.05)

Figure 17: Effect of three weeks' pretreatment with thioridazine on the contractile force response to epinephrine in the isolated heart preparation

Contractile force is measured in grams tension developed. Points are means of five experiments and brackets show S.E.

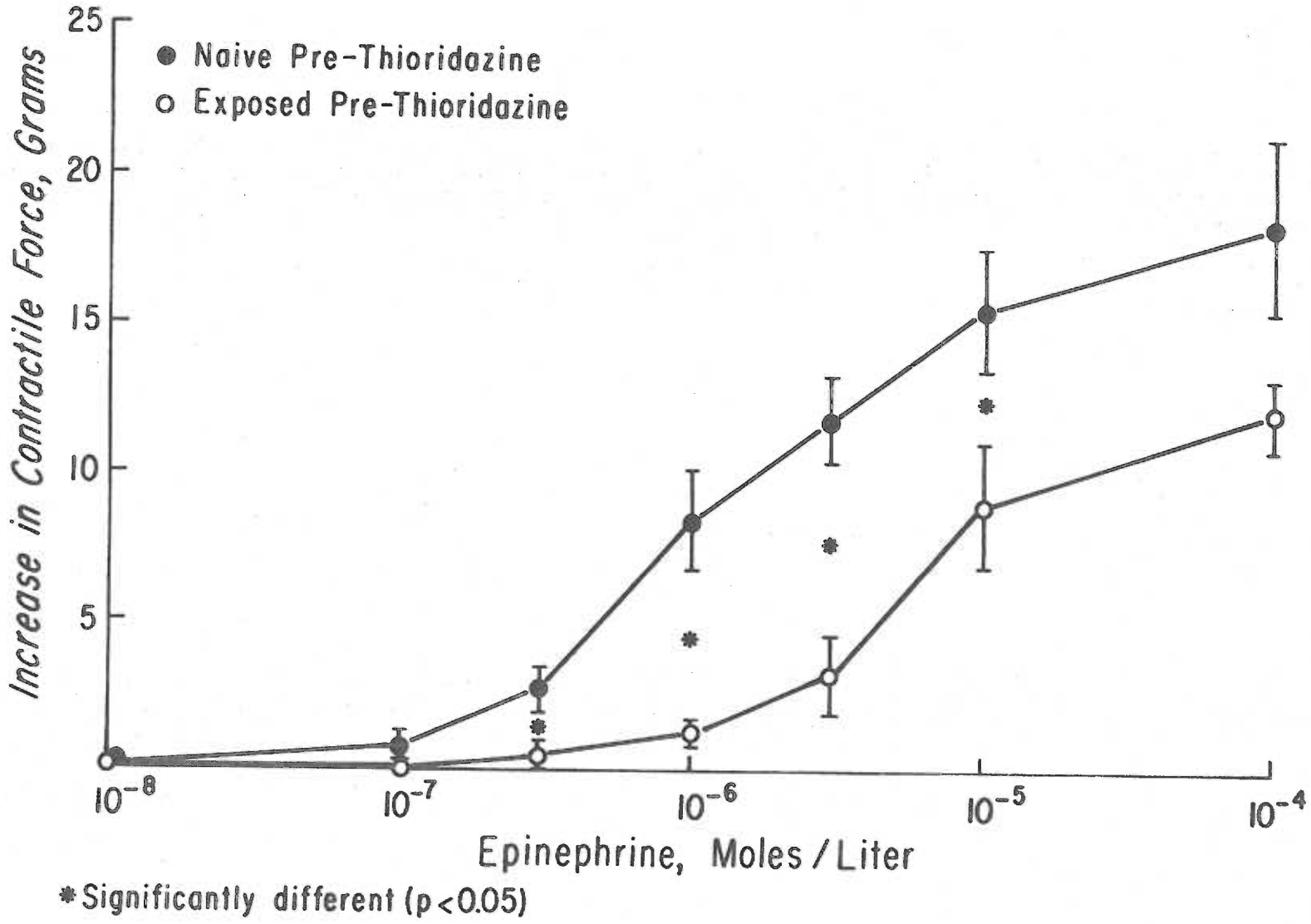

Naive Pre-Thioridazine
Exposed Pre-Thioridazine
Increase in Contractile Force, Grams
25
20
15
10
5
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Epinephrine, Moles / Liter
* Significantly different ($p < 0.05$)

Figure 18: Effect of epinephrine on the contractile force response in the isolated heart preparation after thioridazine, 1.2 µg/ml, has been added to the perfusion fluid

Contractile force is measured in grams tension developed. Points are means of five experiments and brackets show S.E. An asterisk indicates significant differences between contractile force responses in the exposed post-thioridazine condition and contractile force responses in the naive post-saline and naive post-thioridazine condition.

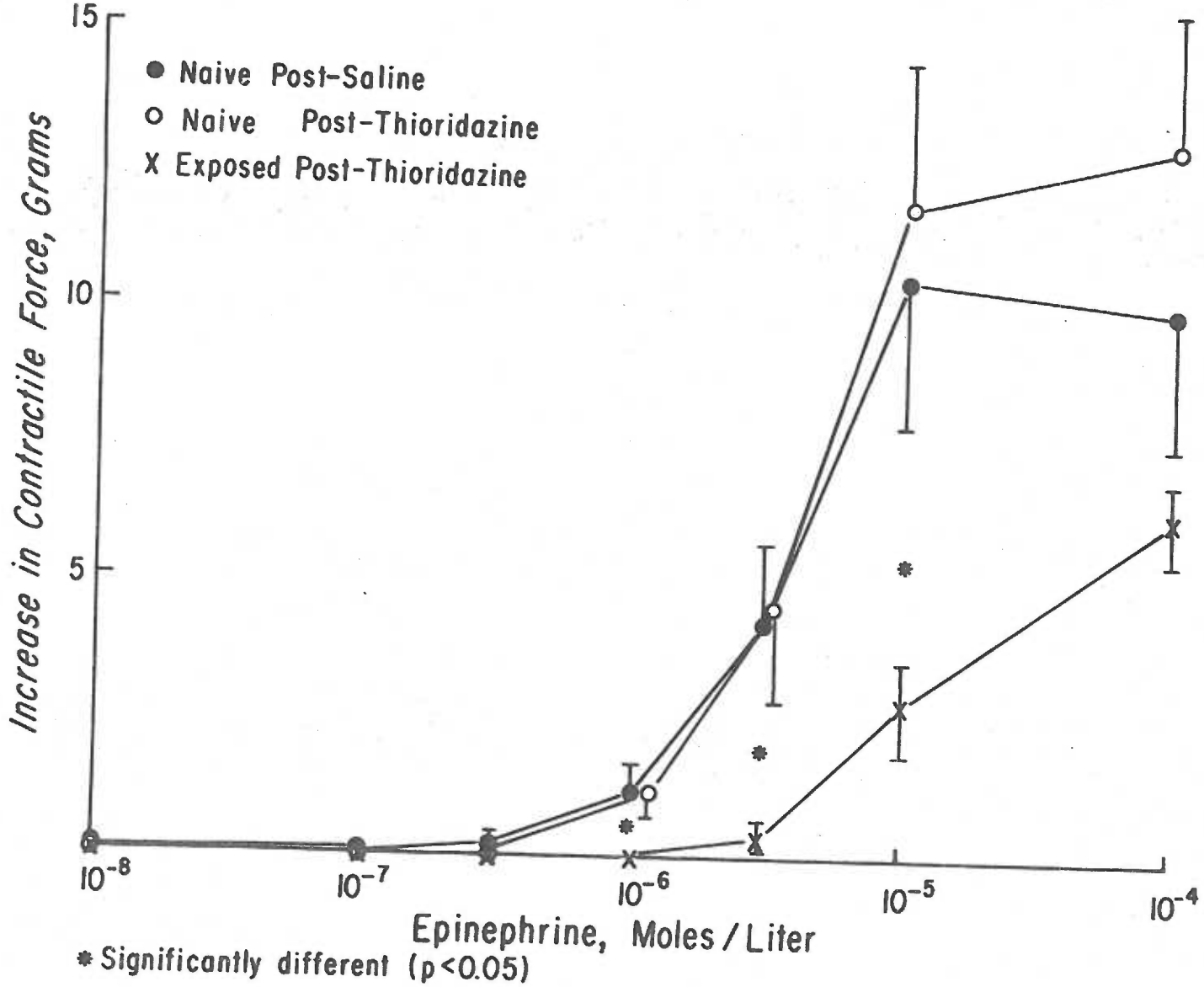
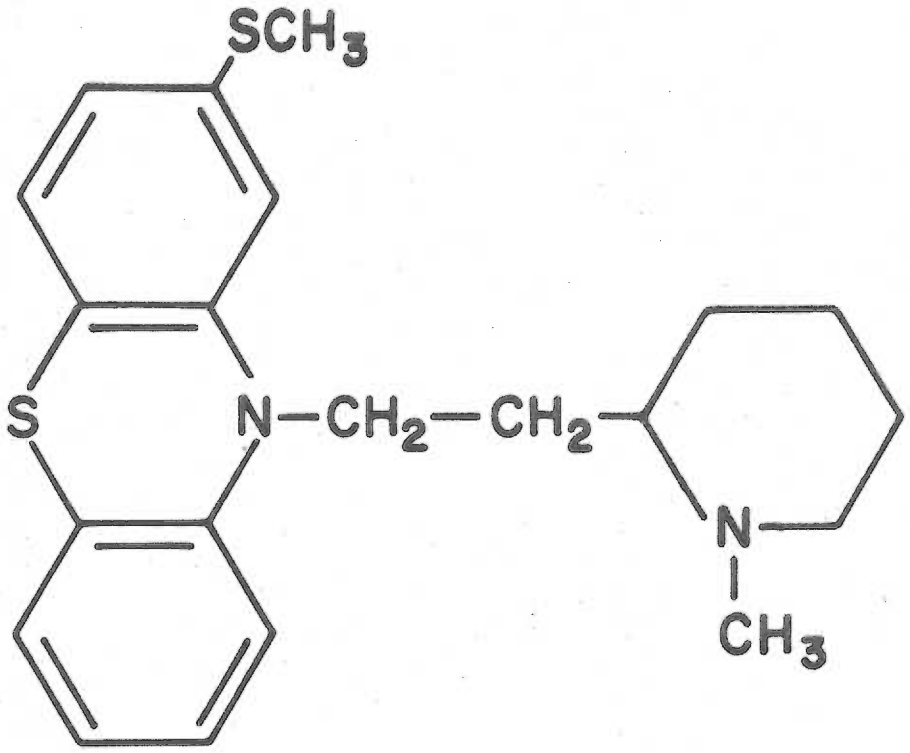

15
10
5
Increase in Contractile Force, Grams
Naive Post-Saline
Naive Post-Thioridazine
X Exposed Post-Thioridazine
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Epinephrine, Moles / Liter
* Significantly different ($p<0.05$)

Figure 19: Chemical structures of thioridazine (A) and a possible metabolite (B) with beta blocking activity

A.



B.