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A proposed mechanism for the thermotropic effects of pipradrol in the rabbit

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A PROPOSED MECHANISM FOR THE THERMOTROPIC EFFECTS OF PIPRADROL IN THE RABBIT

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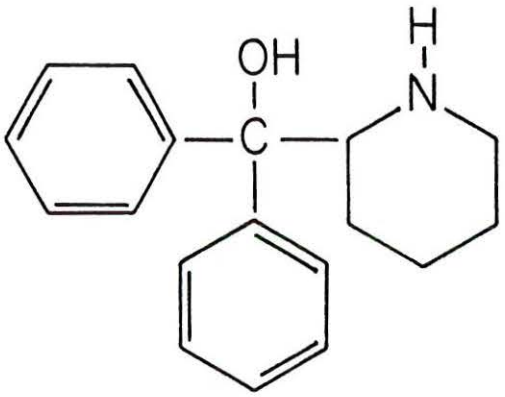
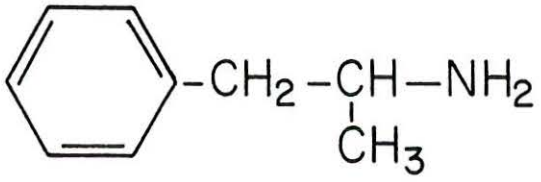
INTRODUCTION

Pipradrol HCl was developed in the early 1950s by the Wm. S. Merrell Co., Cincinnati, Ohio, as a new chemical-type stimulant. Designated by the company as MDR-108, it was shown to induce changes in the activity of laboratory animals, specifically an acceleration of reaction time to environmental stimuli. Small doses were administered to pigs, mice, guinea pigs, rats and rabbits and were observed to induce extremely rapid but highly coordinated movements such as licking, chewing, eating, drinking and scratching. These observations closely resembled those seen by the administration of amphetamine (1).

Pipradrol HCL is alpha-(piperidyl)benzhydrol hydrochloride. The hydrochloride salt is a white odorless powder with a slightly bitter taste. One part dissolves in about 60 parts of hot water. Pipradrol is also known by trade names such as Alertol, Gadexyl, Ceptidrol, Meratonic and

Metratran (2).





Pipradrol Amphetamine

Considering the similarity between the action of pipradrol and amphetamine on laboratory animals, it was questioned as to whether or not the stereochemical enantiomers of pipradrol would also have different pharmacological actions. Therefore, Portoghese and co-workers (3) conducted studies on rat locomotor activity using enantiomers of pipradrol synthesized from (R)- and (S)- pipecolic acid. It was found that all of the central stimulant activity was due to the (R)- pipradrol enantiomer while the more active enantiomer of amphetamine possessed the opposite configuration. Because of these steric differences in activity, it was suggested that these compounds may be acting on different receptors in the central nervous system.

The administration of lethal doses of pipradrol intravenously to mice, rats, guinea pigs and rabbits caused

tremors and convulsions with death occurring during the convulsive stage due to respiratory failure. Lethal doses given orally to dogs caused an increase in motor activity (circling and rolling) with the animals becoming less coordinated but rarely experiencing convulsions. Death occurred suddenly during the hyperactivity stage.

Early observations in dogs given pipradrol showed it to differ behaviorally from amphetamine in that the animals did not become irritable and remained amenable to handling whereas animals treated with amphetamines were easily aggravated (1). With these observations, pipradrol became classified pharmacologically as a psychomotor stimulant.

Psychomotor stimulants are known to increase locomotor activity and to induce stereotyped behavior (4). Locomotor activity in rats can be measured by means of a photocell cage utilizing two parallel photocell beams directed across the long axis of the cage. The number of interruptions of the photocell beam indicates the degree of activity of the test animals. Stereotypic behavior in rats is usually manifested by continuous sniffing, licking or biting, grinding of the jaws, grooming, rearing and occasionally by backward locomotion (5). Sahakian et al. (6) postulated that these two behaviors might be mediated by different mechanisms.

Psychomotor stimulation can also facilitate operant performance such as that reinforced by electrical stimulation

of the brain (7-9). Therefore, it has been suggested that the increase in behavioral activity produced by psychomotor stimulants results from an increase in the value of the rewarding stimuli. This effect may be mediated by the potentiation of a brain reinforcement mechanism (10). Robbins (11) believed that this "reward enhancing" property and the stereotypic effects of psychomotor stimulants could arise from a common mechanism.

Pipradrol, when given to rats, has been shown to cause an increase in both locomotor activity and stereotypic behavior (12, 13). The concept of the facilitation of conditioned reinforcement may be thought of as a sensitization of the neural systems involved in the processing of rewards. This would result in increased effectiveness of the stimuli controlling behavior. Pipradrol may be viewed, therefore, as a drug which serves to either facilitate or inhibit the various motor responses that make up the total behavior of the animal.

Clinical Considerations

Pipradrol has been used clinically in treating conditions of mild to moderately severe mood disorders of reactive depression (14). It has been used to elevate mood and to alleviate psychomotor retardation, attention defect, loss of interest, and withdrawal from social intercourse (15). The above conditions may be construed as situations in which a lack of sufficient reward exists for the patient to

function at normal behavioral levels. In the geriatric patient, pipradrol has also been used as an activity booster. When administered in a vitamin-containing tonic, it has the effect of making simple tasks such as gardening, shopping and other household activities much more pleasurable, thus increasing the quality and productivity of life for the elderly patient (16). The effectiveness of pipradrol in alleviating these conditions may be attributed to its ability to increase the effectiveness of the conditioned reinforcer. This property may also be responsible for its decrease in clinical utility. When the disorder is not merely a depressed emotional state but associated with undesirable behavioral patterns, the effect of pipradrol may be to aggravate the condition. Thus the manic patient becomes more manic, the agitated patient becomes more agitated and the anxious patient becomes more anxious (15).

With the marketing of more effective antidepressant drugs in recent years, pipradrol has fallen from clinical favor and is no longer commercially available for use. In recent clinical trials (double-blind, placebo-controlled studies), physicians reported no indication of any superiority of pipradrol over placebo in treating depression. On the other hand, pipradrol caused significantly more anorexia and weight loss than placebo (17).

Temperature Regulation

In the homeothermic or "warm-blooded" animal, it is essential that body temperature be maintained within a very narrow range to insure optimal conditions for enzymatic reactions. Normal body temperature is maintained by mechanisms involving either heat production or heat loss.

One major source of heat production is derived from skeletal muscle in processes such as locomotion, manual work and shivering. In some animal species and in infants (but not adult humans), a considerable source of heat may be derived from the metabolism of a special type of fat located around the scapulas and called "brown fat."

The chief means by which heat loss occurs involves the radiation or conduction of body heat to the surrounding environment. These processes utilize the dilation of cutaneous blood vessels channeling warmer blood from the interior of the body to the surface thus allowing dissipation of heat to occur. For this process to be effective, it is necessary that the environmental temperature be cooler than the body temperature. Heat loss is also mediated by the vaporization of sweat. In those animals that do not sweat, respiration becomes an important means of heat loss. In this instance, respiration has the effect of vaporizing water in the respiratory passages, nasal sinuses and mouth creating an effect similar to that of sweating (18).

The regulation of body temperature is a very complex phenomenon most likely involving the integration of various reflex neuronal pathways and neurotransmitters. It has been well documented that these thermoregulatory processes are co-ordinated in the central nervous system at the level of the hypothalamus (19). The nature of the neurotransmitters involved appears to vary from species to species.

In cats, dogs and monkeys, the intracerebroventricular (I.C.V.) administration of norepinephrine produces hypothermia (20, 21). In rats, the response was dose-dependent with the smaller doses resulting in hyperthermia and the larger doses producing a pronounced hypothermia (22). In both sheep and rabbits, I.C.V. norepinephrine resulted in hyperthermia (23).

Thermotropic studies with the neurotransmitter dopamine also showed a species variation in its thermic response. Dopamine was shown to cause hypothermia when given I.C.V. to cats, mice, rats, pigeons, hens, goats and sheep (24 - 30). However, in rabbits dopamine I.C.V. resulted in a delayed hyperthermia which was attributed to its probable conversion to norepinephrine (31).

Species variation has also been shown with serotonin or 5-hydroxytryptamine (5-HT). I.C.V. administration raises the body temperature in cats, dogs and monkeys (32). In the rabbit, there are inconsistent reports regarding the nature of the thermic response -- ranging from a weak and

inconsistent hypothermia (32), to a pronounced hypothermia (30), to an initial fall followed by a rise in body temperature (33). The administration of various drugs thought to be 5-HT agonists resulted in a hyperthermic effect which was blocked by 5-HT antagonists (34). In goats and oxen, the I.C.V. dosage of 5-HT produced a pronounced hypothermic effect (23, 35, 36).

Histamine has been recognized as a neurotransmitter (37) and studies have been conducted to determine its role in central thermoregulation. Rats given I.C.V. histamine (as well as the H_2-receptor agonists dimaprit and impromide) show a rise in body temperature which can be blocked by the H_2-receptor blocker cimetidine (38). Other studies have been conducted showing that the administration I.C.V. of histamine to rats will produce hyperthermia rather than hypothermia (39). However, more recent work by Dhawan (40) has indicated a dose-dependent response to histamine in rats with the lower dose resulting in hyperthermia and the higher doses in hypothermia. Central histamine (H_2- receptor) activity has also been shown to be associated with the administration of the antihypertensive drug clonidine (41). Intraperitoneal injection of clonidine in rats results in a significant decrease in body temperature. This effect was attenuated by the I.C.V. injection of the alpha-adrenergic blocker phentolamine and also by the I.C.V. administration of cimetidine indicating involvement of both

adrenergic and histaminergic mechanisms in the hypothermic response to clonidine (42).

Hypothalamic cholinergic mechanisms have been associated with the control of body temperature in rats (43), sheep, goats, rabbits (44), monkeys and cats (45). While earlier works of Bligh et al. (44) and Cooper et al. (23) failed to show any thermal effect of I.C.V. acetylcholine in the rabbit, a more recent study by Tangri and co-workers (46) did show a significant hyperthermic response to acetylcholine when administered I.C.V. to conscious rabbits. In this study, evidence for both nicotinic and muscarinic components of acetylcholine was observed to be associated with thermoregulation. The hyperthermic response to norepinephrine was reported to be mediated by nicotinic receptors and the hypothermic response to 5-HT receptors. It was also suggested that the hyperthermic adrenergic response was related to a "cold-sensitive" mechanism and the hypothermic serotonergic response related to a "heat sensitive" mechanism. Other studies also have shown evidence indicating monoaminergic and cholinergic mechanisms of thermoregulation in the monkey (47) and noradrenaline and 5-HT interaction on thermoregulation in the rat (48).

Statement of the Problem

Since pipradrol in this laboratory consistently has been shown to elevate the core temperature of unanesthetized

rabbits, it was thought possible to study the mechanisms of this thermotropic response (presumably mediated via the central nerous system) by administering before the pipradrol certain pharmacologic agents for which the central mechanisms of action are reasonably well defined. Therefore, an agent blocking one of the pipradrol mechanisms should lower core temperature. Since the behavioral responses of pipradrol are distinctive in the rabbit and closely resemble those seen with amphetamine, this study also was set up to observe (but not quantitate) the effect of the test drugs on the behavioral parameters normally seen in the rabbit with pipradrol alone.

Haloperidol is generally considered to be a dopamine receptor blocker. In the rabbit, haloperidol has been reported to inhibit the hyperthermic response to dopamine given I.C.V. (49) and inhibit apomorpine- and amphetamine-induced hyperthermia which is thought to be mediated by dopaminergic mechanisms (50). Pimozide, a selective dopamine receptor antagonist has also been shown to block the dopamine-mediated hyperthermia seen with apomorphine (51).

The involvement of alpha-adrenergic receptors in the central thermoregulatory process has been clearly shown to be present in the rabbit (31), and the alpha-adrenergic blocker phenoxybenzamine has been reported to block the hyperthermic response to I.C.V. norepinephrine in this animal (46). The present study will also investigate two

other alpha-blockers: chlorpromazine and HEAT. Chlorpromazine is not very specific since it has also been shown to possess significant antihistaminic, anticholinergic and antidopaminergic properties (52). HEAT (2-[beta-(4-hydroxyphenyl)ethylaminomethyl]-tetralone) has been reported to be an effective alpha-adrenergic receptor blocker comparable to chlorpromazine in its ability to block the central response to norepinephrine. However, it also has very weak antagonistic properties for central dopamine receptors (stated to be 500 times less potent than haloperidol in blocking apomorpine responses) (53).

The mediation of hyperthermia in rabbits also has been associated with serotonin (5-HT) (34). The 5-HT receptor blockers cyproheptadine and cinanserin have been shown to antagonize the hyperthermic effect of fenfluramine which is reported to act through 5-HT mechanisms (54). Cyproheptadine also has significant antihistaminic effects and some anticholinergic capacity in addition to its antiserotonin activity.

Dhawan and Dua (31) have suggested that histamine may be involved in the thermoregulatory process seen in certain species of rats; therefore, the present study will investigate the effects of diphenhydramine, a potent inhibitor of H_1 histamine receptors. Diphenhydramine also possesses significant anticholinergic capacity (55).

Tangri et al. (46) have reported a possible cholinergic

mechanism in the rabbit for the control of body temperature; therefore, the present study will investigate atropine, an agent highly selective for muscarinic receptors (55).

The work of Dhawan and Dua (31) in the rabbit failed to show a role for the beta-adrenergic receptors in the response to I.C.V. administered norepinephrine; however, there is evidence that, in rats, body temperature regulation is involved with beta-adrenergic receptors. In higher doses, the I.C.V. injection of norepinephrine can produce a dose-dependent thermic response with the hyperthermia being blocked by the beta-adrenergic receptor blocker propranolol (56).

In the present study, the influence of various drug pretreatments will be investigated in the rabbit in an attempt to (a) define the pharmacological mechanism of action of pipradrol in its mediation of hyperthermia and (b) observe the effects of these pretreatments on the behavioral effect of pipradrol. Although pipradrol is no longer of clinical utility, it is hoped that the results of this study may provide more information for its use in future studies as a pharmacological tool.

MATERIALS AND METHODS

Male New Zealand rabbits (Fredrick's Rabbitry, Modesto, California) weighing between 2.0 and 3.0 kg were used for this study and housed in an animal room with a controlled temperature of between 21° C and 23° C. The animals were allowed to acclimate with free access to food and water for a period of not less than 7 days prior to experimentation. On the day of the experiment, the animals were taken off food and restrained in wooden stanchions (57) for a conditioning period of at least 2 hours or until body temperature stabilized. Animals with a temperature of greater than 40° C were not used.

Colonic temperatures of all animals were measured by means of a thermistor probe (Yellow Springs Instruments Model 401) and recorded continuously on a Honeywell automatic recorder. The thermistor probles were inserted rectally to a depth of 15 cm and secured to the base of

the tail by adhesive tape. The animals were allowed water throughout the conditioning and recording sessions.

The following drugs were used in these experiments: pipradrol HCl (Merrell); chlorpromazine HCl (Smith Kline and French); pimozide (McNeil); phenoxybenzamine HCl (Smith Kline and French); 2-[beta-(4-hydroxyphenyl)-ethylamino-methyl] tetralone (HEAT) (Merck); pentobarbital Na (Abbott); clonidine HCl (Boehringer-Ingelheim); cinanserin HCl (Squibb); cyproheptadine HCl (Merck Sharp and Dohme); apomorphine HCl (Merck Sharp and Dohme) and haloperidol (McNeil). Aqueous solutions of the above were used with the exception of haloperidol and chlorpromzaine HCl which came as pre-prepared solutions. Pimozide required acidification with glacial acetic acid for solubility. Drug solutions were administered into the marginal ear vein except for pimozide which was given intraperitoneally and HEAT which was given both intravenously and intracerebroventricularly. Pre-treatment times were 30 minutes with the exception of phenoxybenzamine HCl, pimozide and cinanserin HCl (given 60 minutes prior to the experiment) and HEAT (given 15 minutes intracerebroventricularly prior to the experiment).

HEAT was administered into the lateral ventricle in the following manner. Twenty-four hours prior to the experiment, the animals to be used were anesthesized with pentobarbital Na (40 mg/kg) intravenously and restrained in open wooden stanchions. Following the loss of the corneal

reflex, the area on the top of the head between the ears and eyes was shaved using electric clippers. A 2-cm incision was made midline exposing the bregma. Using a dental drill, a hole the size of a 25-gauge needle was drilled through the skull at a point 2 mm lateral to the sagittal suture and 2 mm posterior to the coronal suture (33, 58). The incision was closed with a wound clip and the animals given penicillin 600,000 units (Wyeth) intramuscularly and allowed to recover. After 24 hours, these animals were again restrained in wooden stanchions and colonic temperatures recorded for at least 2 hours. Animals with temperatures greater than 40^{o} C were sacrificed because of possible systemic infection. The incision was then reopened and the area cleansed with sterile normal saline. HEAT dissolved in sterile normal saline was administered by means of a microburet and 3-ml syringe calibrated to give a precise volume. The volume was delivered through PE-20 tubing with a 5/8-inch, 26-gauge needle attached. The needle was inserted into the skull to a depth of 6 mm. A plastic disc was fixed to the needle 6 mm from the tip of the bevel and this assured the proper depth for implantation as well as stabilizing the inserted needle in a perpendicular manner. The drug was administered over a period of 1 minute at a fixed total volume of 50 µl. At the conclusion of the experiment, 50 µl of 2% gentian violet was injected in the same manner and the animals were sacrificed. Examination

of the lateral ventricles was conducted to note placement of the injected dye.

Tabular values are expressed as the arithmetic mean (Tables I through VI) ± one standard error of the mean (SEM). Areas-under-the-curve for the response *versus* time data were calculated as shown in Appendix A. Test for significance between test groups was verified using Dunnett's *t* test or Student's *t* test adapted to the Burroughs 6700 computer by Namba (59). Sample printout of the program is shown in Appendix B.

RESULTS

The intravenous (I.V.) administration of pipradrol HCl produced a dose-dependent increase in the colonic body temperature of rabbits (Figure I, Table I). At 10.0 mg/kg I.V., two of the four test animals had to be sacrificed due to a body temperature in excess of 44^{o} C. Behavioral effects were assessed on an all-or-none basis. An increase in locomotor activity was observed consisting of occasional vocalization and excessive leg-thumping episodes. Mydriasis and an increase in respiratory rate were also observed as well as a high incidence of compulsive gnawing. Behavioral effects in this and subsequent testing were not strictly quantitated, so such comments must be regarded as subjective observations.

Effects of Pipradrol in Rabbits Pretreated with Chlorpromazine.

The administration of chlorpromazine HCl I.V. 0.5 mg/kg

30 minutes prior to pipradrol HCl 5.0 mg/kg I.V. (Figure 2, Table I), resulted in a significant ($P < 0.05$) reduction in the hyperthermic effect of pipradrol as measured by area-under-the-curve data (Table IV). A decrease in the locomotor activity was observed with no apparent change in the incidence of compulsive gnawing.

Effects of Pipradrol in Rabbits Pretreated with Dopamine Receptor Blockers.

Haloperidol 0.5 mg/kg I.V. given 30 minutes prior to pipradrol I.V. 5.0 mg/kg (Figure 3, Table I) failed to antagonize the hyperthermic effect. The area-under-the-curve data (Table IV) showed the two curves to be not significantly different ($P > 0.05$). However, haloperidol at this dose appeared to reduce the incidence of compulsive gnawing. Pimozide 4.0 mg/kg intraperitoneally (I.P.) 60 minutes prior to pipradrol 5.0 mg/kg I.V. (Figure 4, Table I) also failed to significantly ($P > 0.05$) block the hyperthermic response of pipradrol (Table IV). Unlike haloperidol, pimozide did not appear to reduce the incidence of compulsive gnawing.

Effect of Pipradrol in Rabbits Pretreated with Alpha Adrenergic Receptor Blockers.

The I.V. administration of phenoxybenzamine HCl 1.0 mg/kg 60 minutes prior to pipradrol 5.0 mg/kg I.V. (Figure 5, Table I) resulted in a significant ($P < 0.05$) attenuation of

the hyperthermia seen with pipradrol (Table IV). The pretreatment with HEAT 0.125 mg/kg I.V. 30 minutes prior to pipradrol 5.0 mg/kg I.V. (Figure 6) also showed a significant reduction in the hyperthermia of pipradrol ($\underline{P} < 0.05$) (Tables I and IV). Compulsive gnawing was not noticeably affected by phenoxybenzamine but appeared potentiated by HEAT. HEAT at a dose of 0.25 mg/kg given I.V. 30 minutes prior to pipradrol 5.0 mg/kg I.V. (Table I) also significantly reduced the hyperthermic effect ($\underline{P} < 0.05$) of pipradrol (Table IV). The animals given HEAT showed a decrease in locomotor activity which appeared to be dose-dependent with the higher dose of HEAT at 0.25 mg/kg resulting in prostration of many of the animals.

The intracerebroventricular (I.C.V.) administration of HEAT 0.15 mg 15 minutes prior to pipradrol 5.0 mg/kg I.V. (Figure 7) resulted in a significant ($\underline{P} < 0.05$) attenuation in the hyperthermic response to pipradrol (Tables II and V). HEAT I.C.V. 0.15 mg alone produced a slight decrease in the colonic body temperature which was significantly ($\underline{P} < 0.05$) different from the saline control (Tables II and V). The degree of increased locomotor activity due to pipradrol was observed to be decreased with the administration of HEAT I.C.V. in a manner similar to the administration of HEAT I.V.

Effects of Pipradrol in Rabbits Pretreated with Serotonin Receptor Blockers.

Cinanserin HCl 5.0 mg/kg I.V. given 60 minutes prior to pipradrol HCl 5.0 mg/kg I.V. (Figure 8) did not significantly block ($P > 0.05$) the hyperthermic response of pipradrol (Tables I and IV). The I.V. pretreatment of cyproheptadine HCl 5.0 mg/kg (Figure 9) did not block the hyperthermic effect of pipradrol 5.0 mg/kg I.V.; in fact, the effect seen was a significant ($P < 0.05$) potentiation of the hyperthermic response to pipradrol (Table IV).

Effect of Pipradrol in Rabbits Pretreated with Histamine Receptor Blockers.

While cyproheptadine has significant affinity for both serotonin and histamine receptors and some affinity for muscarinic receptors, diphenhydramine has affinity for only histaminic receptors (and to a lesser extent cholinergic receptors). Diphenhydramine HCl 6.0 mg/kg I.V. given 30 minutes prior to the I.V. administration of pipradrol 5.0 mg/kg (Figure 10) was ineffective ($P > 0.05$) in blocking the hyperthermia induced by pipradrol (Table I). The effect appeared to be potentiated but this effect was not shown to be statistically significant ($P > 0.05$) (Table IV). One of the animals had to be sacrificed due to excessive body temperature.

Effects of Pipradrol in Rabbits Pretreated with Cholinergic Receptor Blockers.

Atropine has little to no affinity for serotonergic and histaminergic receptors, but has high affinity for muscarinic acetylcholine receptors (and to a lesser extent nicotinic acetylcholine receptors). Atropine SO_4 2.0 mg/kg given I.V. 30 minutes prior to pipradrol HCl 5.0 mg/kg (Figure 11) was shown to significantly potentiate the hyperthermic effect of pipradrol ($P < 0.05$) (Tables I and IV). The locomotor activity due to pipradrol seemed to be also potentiated with the animals showing signs of extreme agitation, leg-thumping and vocalization.

Effects of Pipradrol in Rabbits Pretreated with a Beta-Adrenergic Receptor Blocker.

Pretreatment with propranolol HCl 1.0 mg/kg I.V. 30 minutes prior to pipradrol HCl 5.0 mg/kg I.V. (Table I) had little effect on the hyperthemic response of pipradrol (Figure 12). The area-under-the-curve data (Table IV) showed no significant ($P > 0.05$) difference between the two curves. However, two of the animals had to be sacrificed due to excessive body temperatures.

Effects of Pipradrol in Rabbits Pretreated with a Central Nervous System Depressant.

Pentobarbital Na 10.0 mg/kg I.V. given 30 minutes before pipradrol HCL 5.0 mg/kg I.V. (Figure 13, Table III)

had no significant effect ($P > 0.05$) on the hyperthermic capacity of pipradrol (Table V). The effect of pentobarbital greatly attenuated the locomotor activity, with the animals remaining quiescent throughout the experiment. A dose of 10.0 mg/kg I.V. is not sufficient to produce loss of the righting reflex and barbiturate anesthesia.

Effects of Clonidine in Rabbits Pretreated with HEAT.

Clonidine HCl 2.5 mg/kg I.V. (Figure 14) was able to produce an increase in the colonic body temperature similar in nature to that of pipradrol (Table III). Pretreatment with HEAT I.V. 0.5 mg/kg 30 minutes before clonidine was able to completely block ($P < 0.05$) the hyperthermic response of clonidine (Figure 14, and Table VI).

Effects of Apomorphine in Rabbits Pretreated with Pimozide.

Apomorphine alone was shown to have hyperthermic properties in rabbits (Table II). When given to rabbits pretreated with pimozide 4.0 mg/kg (-60 minutes) I.P. at a dose of 5.0 mg/kg, the hyperthermia was completely abolished (Tables III and VI).

Effects of Successive Treatments of Pipradrol.

The re-administration of pipradrol 5.0 mg/kg. I.V. 6 hours after the initial treatment with pipradrol 5.0 mg/kg I.V. showed no significant ($P > 0.05$) difference in the hyperthermic response (Figure 15, Table III).

Table I. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with Various Known Receptor Blockers.

Treatment, Dosage, (Injection Time, Min.)	N	-5 Min. Colonic Temperature °C ± SEM	Zero-Time Challenge Dosage I.V.
-	3	39.20 ±0.07	Saline 1 ml/kg
-	8	39.20 ±0.01	Pipradrol HCl 2.5mg/kg
-	12	39.33 ±0.06	Pipradrol HCl 5.0 mg/kg
-	4	39.00 ±0.24	Pipradrol HCL 10.0 mg/kg
Chlorpromazine HCl I.V. 0.5 mg/kg (-30)	4	39.03 ±0.02	Pipradrol HCl 5.0 mg/kg
Chlorpromazine HCl I.V. 1.0 mg/kg (-30)	5	38.76 ±0.15	Pipradrol HCl 5.0 mg/kg
Haloperidol I.V. 0.5 mg/kg (-30)	7	39.20 ±0.24	Pipradrol HCl 5.0 mg/kg
Pimozide I.P. 4.0 mg/kg (-60)	6	38.80 ±0.15	Pipradrol HCl 5.0 mg/kg
Phenoxybenzamine HCl I.V. 1.0 mg/kg (-60)	5	38.23 ±0.15	Pipradrol HCl 5.0 mg/kg
HEAT I.V. 0.125 mg/kg (-30)	6	39.28 ±0.05	Pipradrol HCl 5.0 mg/kg
HEAT I.V. 0.25 mg/kg (-30)	4	38.90 ±0.05	Pipradrol HCl 5.0 mg/kg
Cinanserin HCl I.V. 5.0 mg/kg (-60)	6	39.16 ±0.15	Pipradrol HCL 2.5 mg/kg

Change in Colonic Temperature with Time °C ± SEM							
+15	+30	+45	+60	+75	+90	+105	+120 Min.
+0.03 ±0.03	-0.10 ±0.06	+0.03 ±0.03	+0.03 ±0.03	+0.03 ±0.03	0.00 ±0.00	0.00 ±0.03	+0.03 ±0.03
+0.70 ±0.08	+0.90 ±0.11	+1.00 ±0.12	+1.10 ±0.14	+1.10 ±0.11	+1.10 ±0.11	+1.10 ±0.11	+0.90 ±0.09
+0.80 ±0.15	+1.20 ±0.13	+1.50 ±0.15	+1.60 ±0.13	+1.70 ±0.15	+1.60 ±0.17	+1.60 ±0.20	+1.30 ±0.22
+1.20 ±0.24	+2.80 ±0.21	+3.00 ±0.26	+3.30 ±0.23	-	-	-	-
+0.50 ±0.20	+0.80 ±0.20	+1.00 ±0.27	+1.10 ±0.23	+1.20 ±0.20	+1.20 ±0.20	+1.30 ±0.20	+1.30 ±0.23
+0.20 ±0.05	+0.20 ±0.05	+0.20 ±0.05	+0.40 ±0.10	+0.40 ±0.10	+0.40 ±0.10	+0.40 ±0.09	+0.40 ±0.07
+1.00 ±0.20	+1.40 ±0.20	+1.80 ±0.20	+1.90 ±0.26	+2.00 ±0.35	+2.10 ±0.30	+2.00 ±0.40	+2.00 ±0.40
+1.20 ±0.19	+1.50 ±0.20	+1.50 ±0.27	+1.60 ±0.22	+1.70 ±0.22	+1.70 ±0.19	+1.50 ±0.19	+1.40 ±0.18
+0.40 ±0.10	+0.70 ±0.06	+0.80 ±0.08	+0.90 ±0.08	+0.80 +0.12	+0.80 ±0.13	+0.80 ±0.12	+0.60 ±0.12
+0.40 ±0.09	+0.60 ±0.09	+0.70 ±0.10	+0.80 ±0.10	+0.70 ±0.09	+0.70 ±0.09	+0.70 ±0.10	+0.60 ±0.13
+0.10 ±0.05	+0.10 ±0.03	+0.10 ±0.05	+0.30 ±0.12	+0.30 ±0.08	+0.40 ±0.10	+0.50 ±0.08	+0.60 ±0.12
+0.50 ±0.09	+0.80 ±0.10	+0.87 ±0.16	+0.97 ±0.21	+0.88 ±0.16	+1.08 ±0.16	+1.05 ±0.15	+1.08 ±0.15

continued

Table I. (continued)

Treatment, Dosage, (Injection Time, Min.)	N	-5 Min. Colonic Temperature °C ± SEM	Zero-Time Challenge Dosage, I.V.
Cyproheptadine HCl I.V. 2.0 mg/kg (-30)	7	39.90 ±0.29	Pipradrol HCl 5.0 mg/kg
Diphenhydramine HCl I.V. 6.0 mg/kg	6	39.70 ±0.20	Pipradrol HCl 5.0 mg/kg
Atropine SO_4 I.V. 2.0 mg/kg (-30)	4	39.37 ±0.31	Pipradrol HCl 5.0 mg/kg
Propranolol HCl I.V. 1.0 mg/kg (-30)	3	39.20 ±0.20	Pipradrol HCl 5.0 mg/kg

Change in Colonic Temperature with Time °C ± SEM							
+15	+30	+45	+60	+75	+90	+105	+120 Min.
+1.28 ±0.33	+1.70 ±0.31	+2.23 ±0.32	+2.45 ±0.30	+2.60 ±0.28	+2.88 ±0.35	+3.03 ±0.47	+3.08 ±0.47
+1.30 ±0.20	+1.80 ±0.14	+2.20 ±0.12	+2.30 ±0.20	+2.30 ±0.20	+2.10 ±0.30	+1.90 ±0.30	+1.90 ±0.40
+1.00 ±0.10	+1.90 ±0.10	+2.20 ±0.20	+2.30 ±0.20	+2.70 ±0.20	+2.60 ±0.20	+2.70 ±0.20	+2.80 ±0.30
+1.20 ±0.15	+1.90 ±0.05	+2.20 ±0.20	+2.30 ±0.20	+2.20 ±0.20	+2.30 ±0.20	+2.10 ±0.20	+2.00 ±0.30

Table II. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated by Intracerebroventricular Administration of HEAT.

Treatment, Dosage I.C.V. (Injection Time, Min.)	N	-5 Min. Colonic Temperature °C ± SEM	Zero-Time Challenge Dosage, I.V.
Sterile, Pyrogen-Free Normal Saline 100 (-15)	4	39.25 ±0.05	Saline 1 ml/kg
HEAT 0.15mg (-15)	4	39.32 ±0.08	Saline 1 ml/kg
HEAT 0.15mg (-15)	6	39.12 ±0.05	Pipradrol HCl 5.0 mg/kg

Change in Colonic Temperature with Time °C ± SEM							
+15	+30	+45	+60	+75	+90	+105	+120 Min.
+0.05 ±0.03	+0.13 ±0.05	+0.10 ±0.06	+0.13 ±0.09	+0.08 ±0.09	+0.13 ±0.08	+0.15 ±0.03	+0.20 ±0.04
+0.05 ±0.03	+0.05 ±0.20	0.00 ±0.04	+0.05 ±0.04	-0.03 ±0.09	-0.13 ±0.09	-0.15 ±0.03	-0.18 ±0.03
+0.32 ±0.05	+0.43 ±0.07	+0.40 ±0.09	+0.60 ±0.10	+0.60 ±0.16	+0.60 ±0.16	+0.56 ±0.19	+0.56 ±0.19

Table III. Effects of Various Drugs and Pretreatments on the Colonic Body Temperature of Rabbits.

Treatment, Dosage, (Injection Time, Min.)	N	-5 Min. Colonic Temperature °C ± SEM	Zero-Time Challenge Dosage, I.V.
-	3	39.20 ±0.07	Saline 1 ml/kg
-	3	39.20 ±0.06	Clonidine HCl 2.5 mg/kg
HEAT I.V. 0.125 mg/kg (-30)	3	38.40 ±0.28	Clonidine HCl 2.5 mg/kg
-	1	39.10 -	Apomorphine HCl 5.0 mg/kg
Pimozide I.P. 4.0 mg/kg (-60)	1	38.00 -	Apomorphine HCl 5.0mg/kg
Pentobarbital Na I.V. 10.0 mg/kg (-30)	3	38.50 ±0.18	Pipradrol HCl 5.0 mg/kg
Pipradrol HCl I.V. 5.0 mg/kg (-120)	3	39.33 ±0.06	Pipradrol HCl 5.0 mg/kg

Change in Colonic Temperature with Time °C ± SEM							
+15	+30	+45	+60	+75	+90	+105	+120 Min.
+0.03 ±0.03	-0.10 ±0.06	+0.03 ±0.03	+0.03 ±0.03	+0.03 ±0.03	0.00 ±0.00	0.00 ±0.03	+0.03 ±0.03
+0.60 ±0.00	+0.70 ±0.03	+0.80 ±0.05	+1.00 ±0.06	+1.00 ±0.12	+1.30 ±0.14	+1.50 ±0.14	+1.50 ±0.15
-0.20 ±0.00	+0.10 ±0.03	0.00 ±0.12	0.00 ±0.15	-0.10 ±0.24	-0.20 ±0.26	-0.30 ±0.26	-0.40 ±0.33
+0.40 -	+1.10 -	+1.50 -	+1.40 -	+1.20 -	+0.90 -	+0.80 -	+0.60 -
0.00 -	0.00 -	0.00 -	0.00 -	0.00 -	-0.10 -	-0.20 -	-0.10 -
+0.60 ±0.12	+1.00 ±0.17	+1.30 ±0.23	+1.60 ±0.17	+1.80 ±0.18	+1.90 ±0.15	+2.10 ±0.27	+2.00 ±0.34
+0.97 ±0.19	+1.17 ±0.22	+1.40 ±0.27	+1.40 ±0.25	+1.40 ±0.25	+1.30 ±0.22	+1.20 ±0.15	+1.07 ±0.07

Table IV. Evaluation of the Area-Under-the-Curve Data for the Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with Various Known Receptor Blockers.

Treatment, Dosage (Injection Time, Min.)	Challenge I.V. Dosage	N	Area Under the Curve ± SEM	P
-	Saline 1.0 ml/kg	3	0.75 ± 0.1	> 0.05
-	Pipradrol HCl 5.0 mg/kg	10	175.5 ± 18.6	-
-	Pipradrol HCl 2.5 mg/kg	8	126.4 ± 12.8	< 0.05
Chlorpromazine HCl I.V. 0.5 mg/kg (-30)	Pipradrol HCl 5.0 mg/kg	4	124.9 ± 25.4	< 0.05
Chlorpromazine HCl I.V. 1.0 mg/kg (-30)	Pipradrol HCl 5.0 mg/kg	5	41.8 ± 9.7	< 0.05
Haloperidol I.V. 0.5 mg/kg (-30)	Pipradrol HCl 5.0 mg/kg	7	212.6 ± 32.6	> 0.05
Pimozide I.P. 4.0 mg/kg (-60)	Pipradrol HCl 5.0 mg/kg	5	183.6 ± 25.1	> 0.05
Phenoxybenzamine HCl I.V. 1.0 mg/kg (-60)	Pipradrol HCl 5.0 mg/kg	4	85.1 ± 9.1	< 0.05
HEAT I.V. 0.125 mg/kg (-30)	Pipradrol HCl 5.0 mg/kg	6	78.9 ± 10.7	< 0.05

Table IV. (continued)

Treatment, Dosage (Injection Time, Min.)	Challenge I.V. Dosage	N	Area Under the Curve ± SEM	P
Cyproheptadine HCl I.V. 2.0 mg/kg (-30)	Pipradrol HCL 5.0 mg/kg	3	294.7 ± 42.7	<0.05
Cinanserin I.V. 5.0 mg/kg (-60)	Pipradrol HCl 2.5 mg/kg	6	110.0 ± 16.0	>0.05
Diphenhydramine HCl I.V. 6.0 mg/kg (-30)	Pipradrol HCl 5.0 mg/kg	5	236.2 ± 26.2	>0.05
Atropine SO_4 I.V. 2.0 mg/kg (-30)	Pipradrol HCl 5.0 mg/kg	4	261.3 ± 12.8	<0.05
Propranolol HCl I.V. 1.0 mg/kg (-30)	Pipradrol HCl 5.0 mg/kg	3	235.0 ± 27.9	>0.05

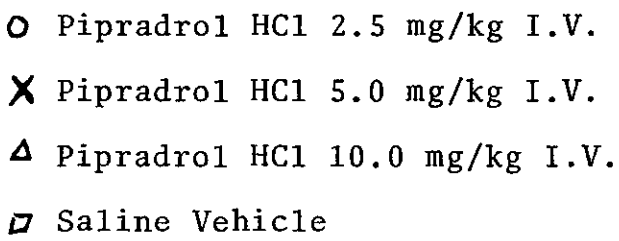
Table V. Evaluation of the Area-Under-the-Curve Data for the Colonic Temperature Effects of Pipradrol in Rabbits Pretreated by the Intracerebroventricular Administration of HEAT.

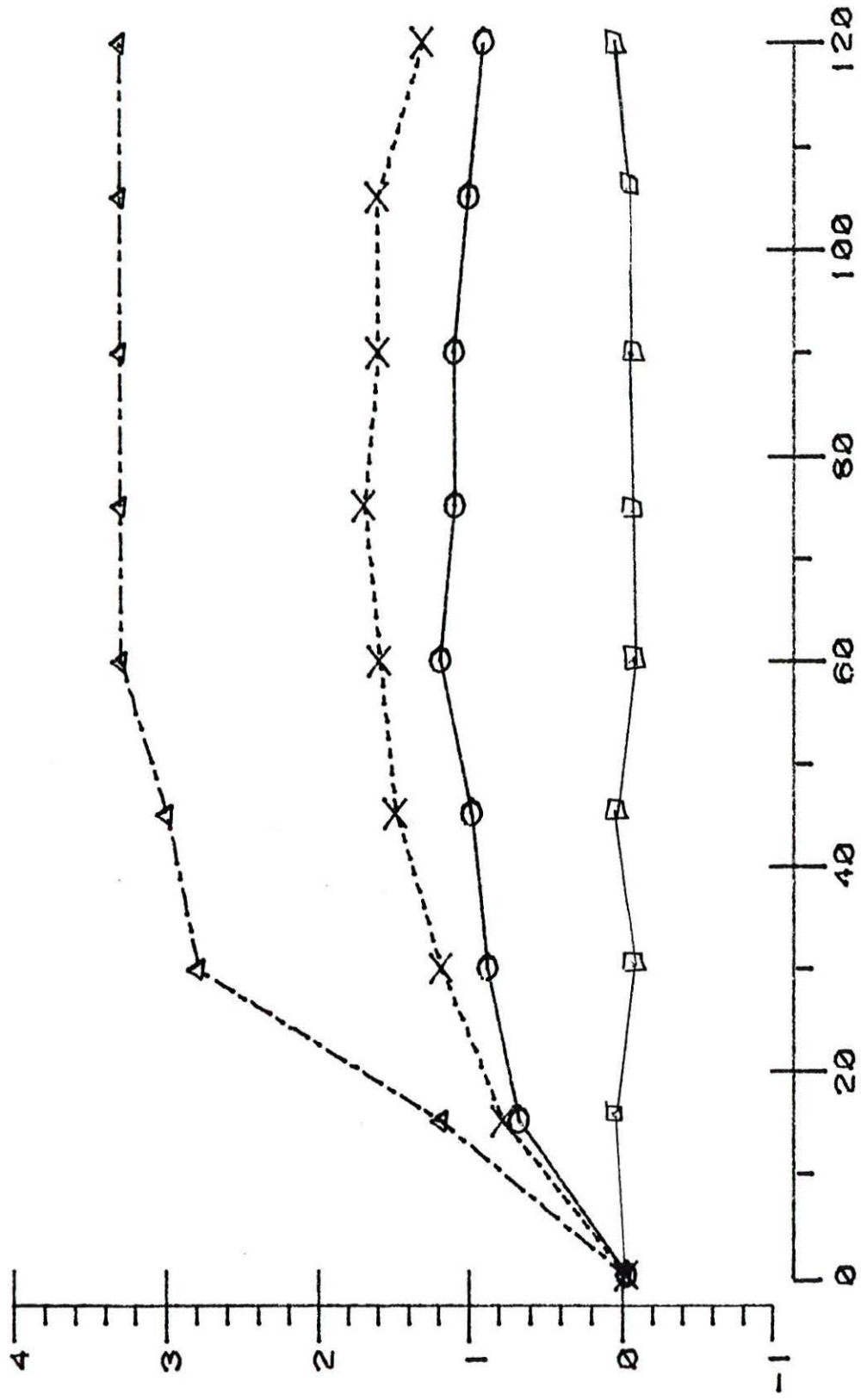
Treatment, Dosage I.C.V. (Injection Time, Min.)	Challenge I.V. Dosage	N	Area Under the Curve ± SEM	P
Sterile, Pyrogen-Free Normal Saline 100 (-15)	Saline 1 ml/kg	4	14.5 ± 0.1	< 0.05
-	Pipradrol HCl 5.0 mg/kg	10	175.5 ± 18.6	-
HEAT 0.15 mg (-15)	Saline 1 ml/kg	4	-5.1 ± 0.1	< 0.05
HEAT 0.15 mg (-15)	Pipradrol HCl 5.0 mg/kg	6	62.0 ± 12.5	< 0.05

Table VI. Evaluation of the Area-Under-the-Curve Data for the Effects of Various Drugs and Pretreatments on the Colonic Body Temperature of Rabbits.

Treatment, Dosage (Injection Time, Min.)	Challenge I.V. Dosage	N	Area Under the Curve ± SEM	P
-	Saline 1.0 ml/kg	3	0.75 ± 0.1	< 0.05
-	Clonidine HCl 2.5 mg/kg	3	137.00 ± 3.8	-
HEAT I.V. 0.5 mg/kg (-30)	Clonidine HCl 2.5 mg/kg	3	-18.00 ± 6.8	< 0.05
-	Pipradrol HCl 5.0 mg/kg	10	175.50 ± 18.6	-
Pentobarbital Na I.V. 10.0 mg/kg (-30)	Pipradrol HCl 5.0 mg/kg	3	172.00 ± 22.3	> 0.05
-	Apomorphine HCl 5.0 mg/kg	1	118.50 -	-
Pimozide HCl I.V 4.0 mg/kg (-60)	Apomorphine HCl 5.0 mg/kg	1	-5.00 -	-
Pipradrol HCl I.V. 5.0 mg/kg (-120)	Pipradrol HCl 5.0 mg/kg	3	149.00 ± 18.5	> 0.05

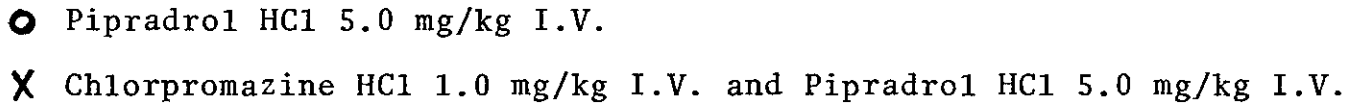
Figure 1. The Dose Response of the Colonic Body Temperature in Rabbits given Pipradrol.

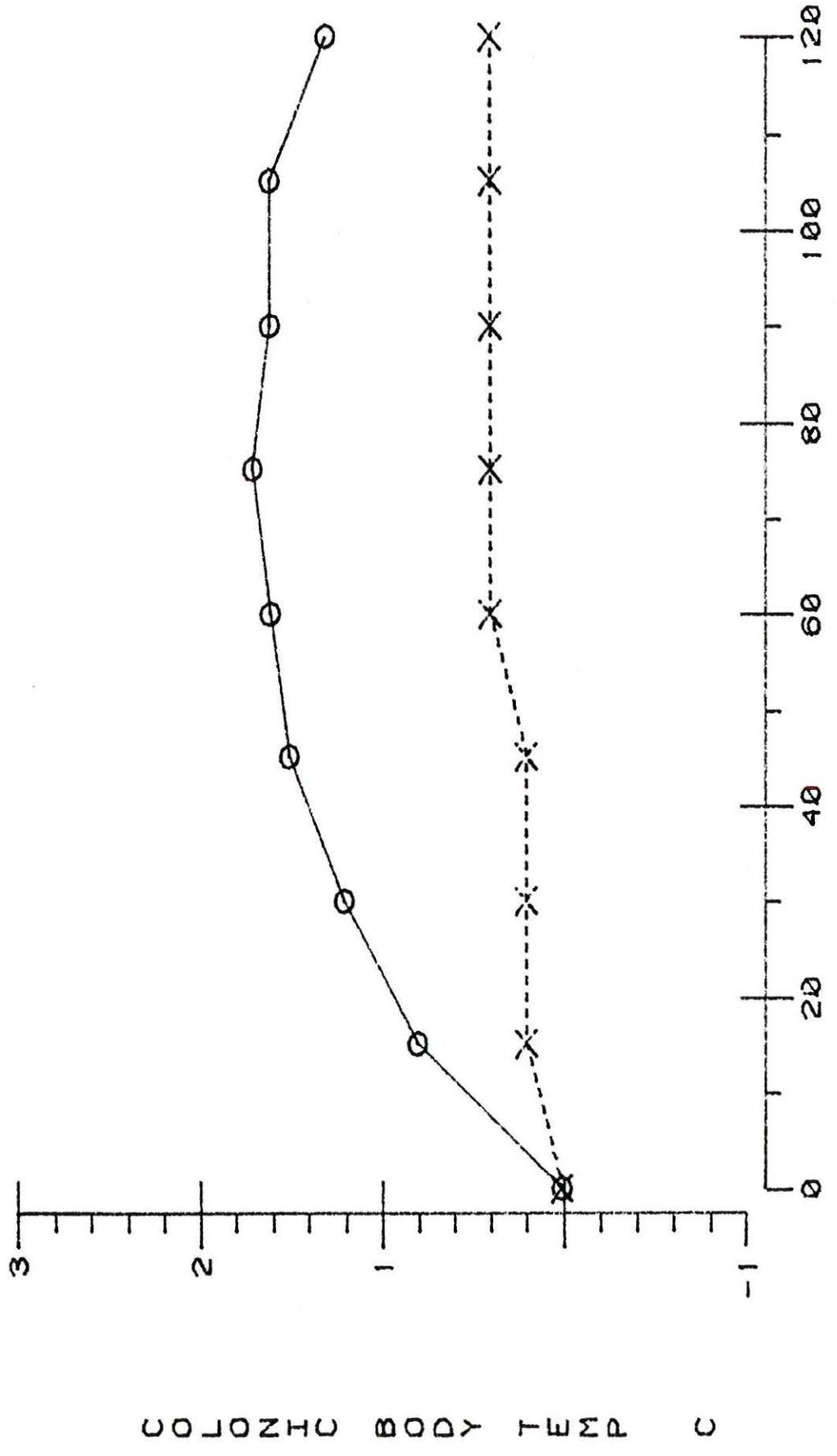



4
3
2
1
0
-1
0
20
40
60
80
100
120
TIME (MIN)
CORRECTED BODY TEMP C

X O

Figure 2. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with Chlorpromazine (-30 minutes).




COLONIC BODY TEMP C
3
2
1
-1
0
20
40
60
80
100
120
TIME (MIN)

X O

Figure 3. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with Haloperidol (-30 minutes).

O Pipradrol HCl 5.0 mg/kg I.V.

X Haloperidol 0.5 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.

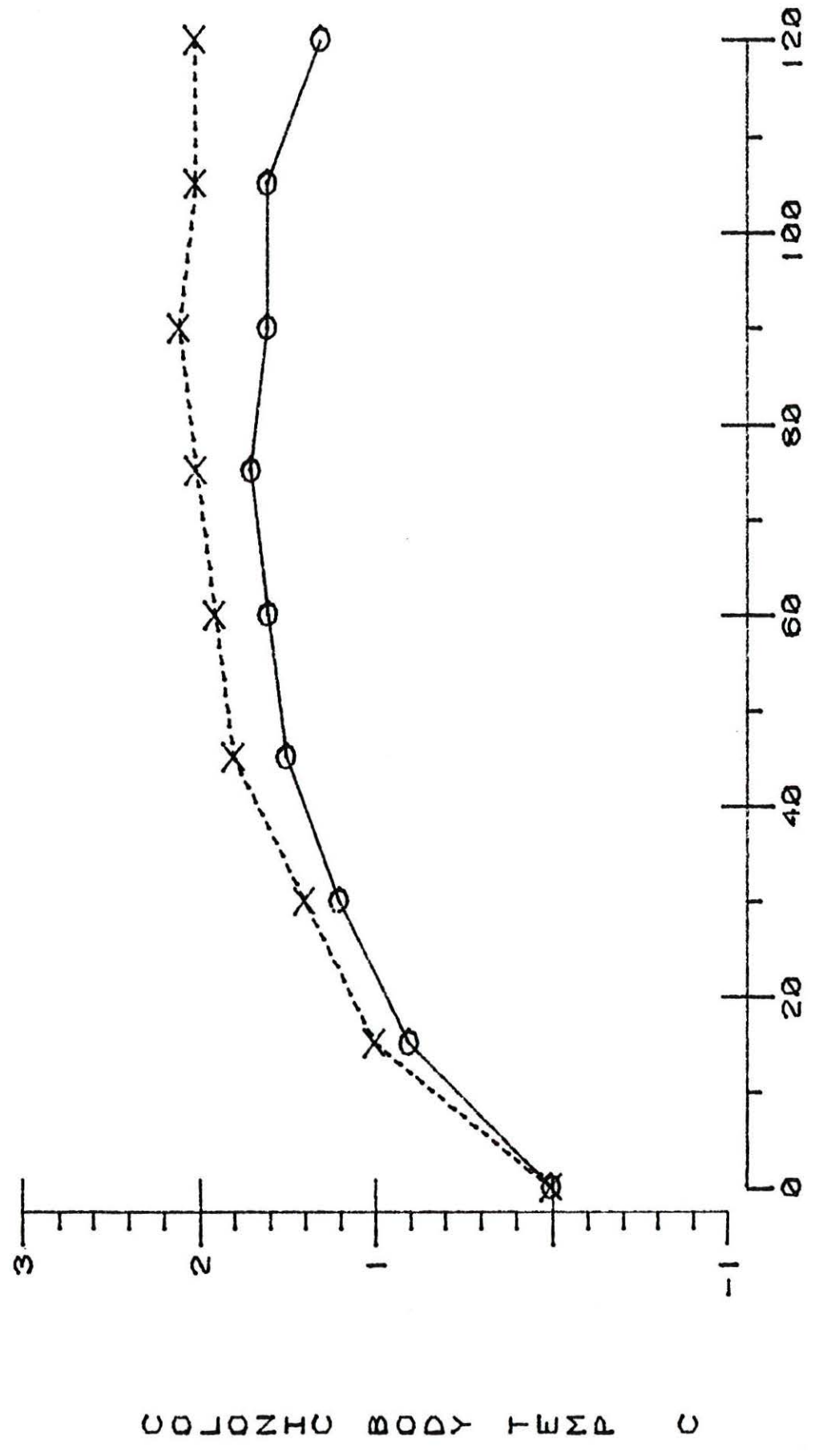

CORRECTED BODY TEMP C
3
2
1
-1
0
20
40
60
80
100
120
TIME (MIN)

Figure 4. The Colonic Temperature Effects of Pipradrol and Apomorpine in Rabbits Pretreated with Pimozide (-60 minutes).


Pipradrol HCl 5.0 mg/kg I.V.


Pimozide 4.0 mg/kg I.P. and Pipradrol HCl 5.0 mg/kg I.V.


Pimozide 4.0 mg/kg I.P. and Apomorphine 4.0 mg/kg I.V.

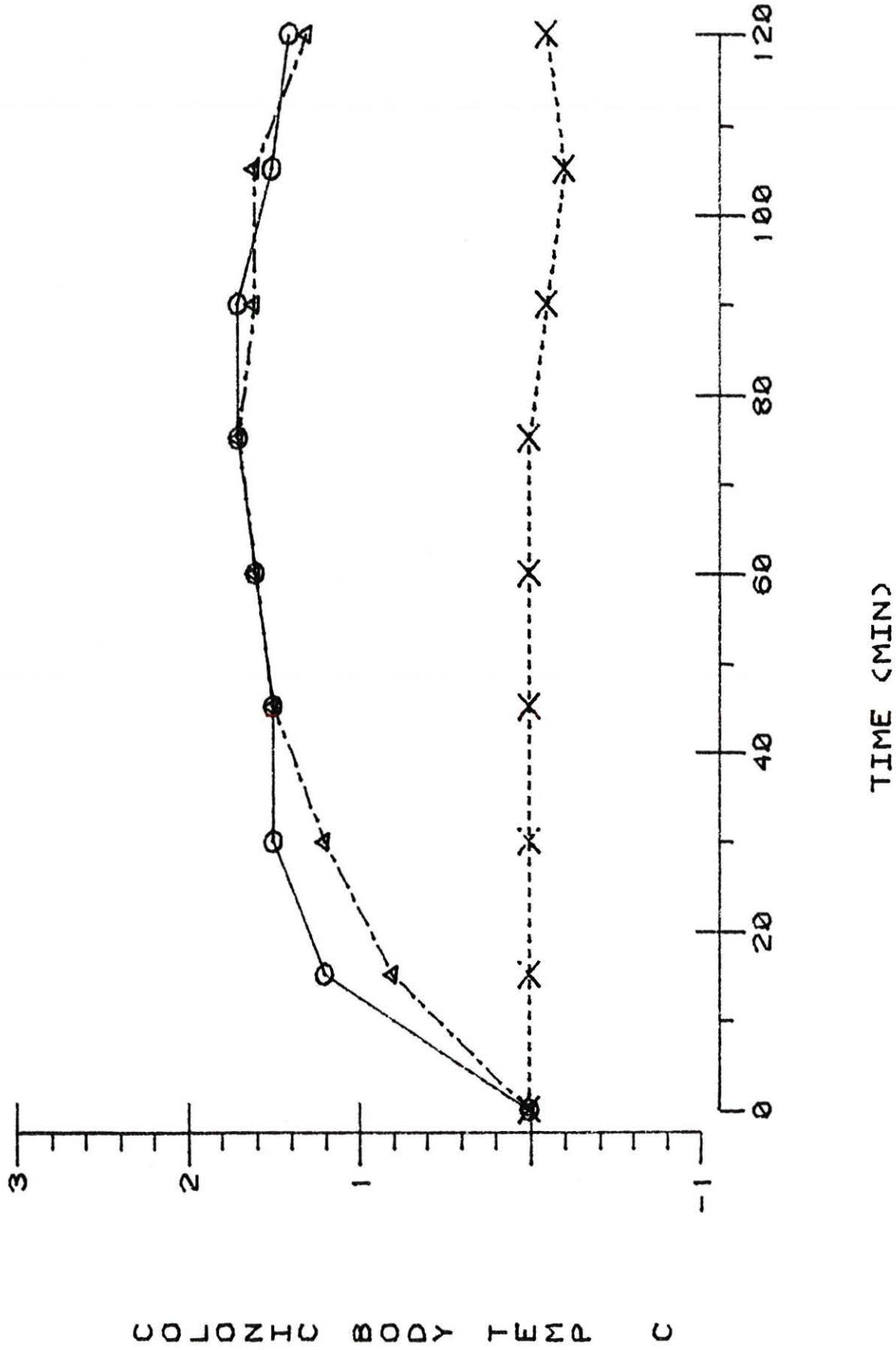

3
2
1
-1
0
20
40
60
80
100
120
TIME (MIN)
ORAL BODY TEMP C

Figure 5. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with Phenoxybenzamine (-60 minutes).

O Pipradrol HCl 5.0 mg/kg I.V.

X Phenoxybenzamine HCl 1.0 mg/kg and Pipradrol HCl 5.0 mg/kg I.V.

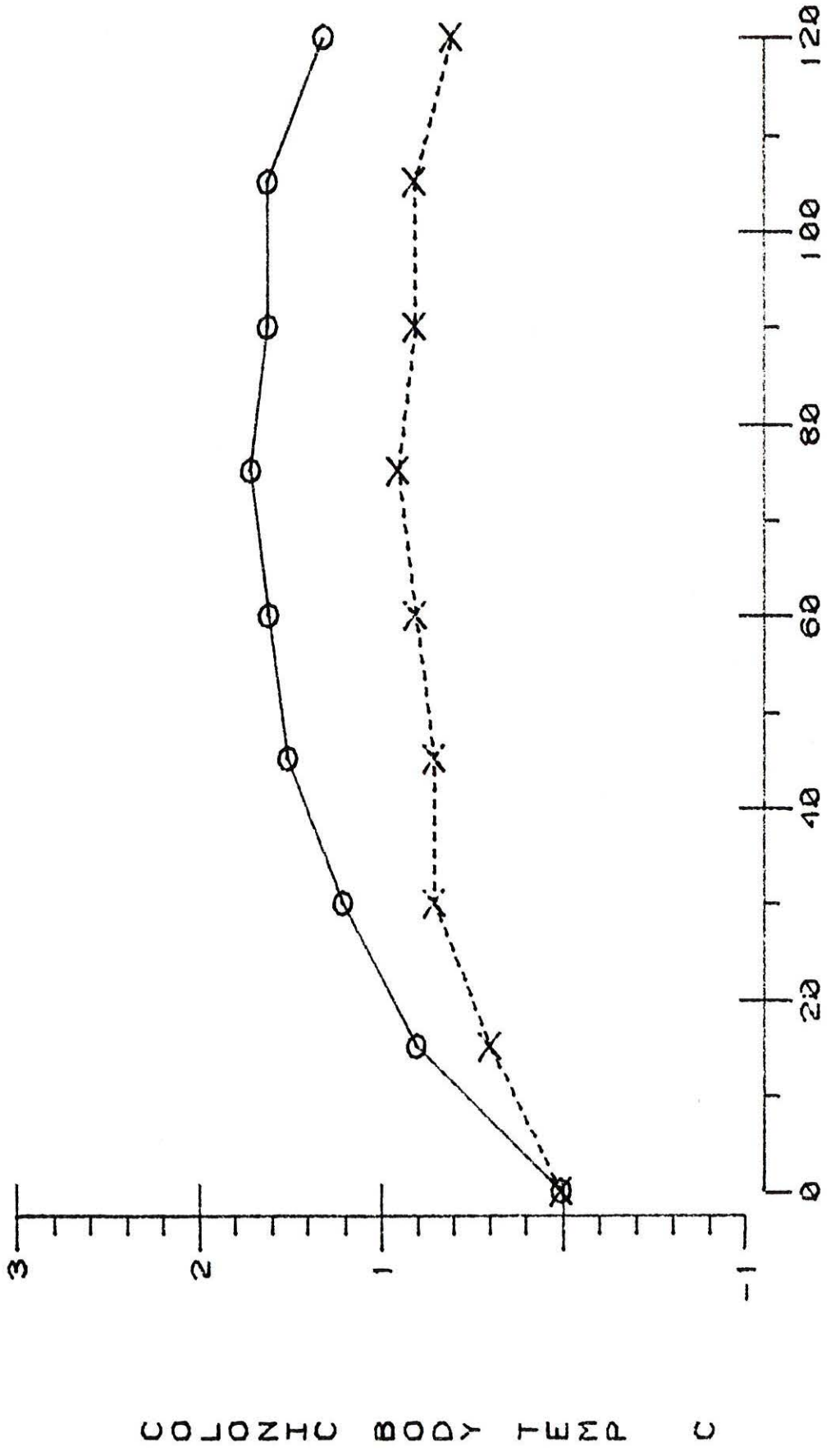

3
2
1
-1
0
20
40
60
80
100
120
TIME (MIN)
ORAL BODY TEMP C

Figure 6. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with HEAT (-30 minutes).

o Pipradrol HCl 5.0 mg/kg I.V.

x HEAT 0.125 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.

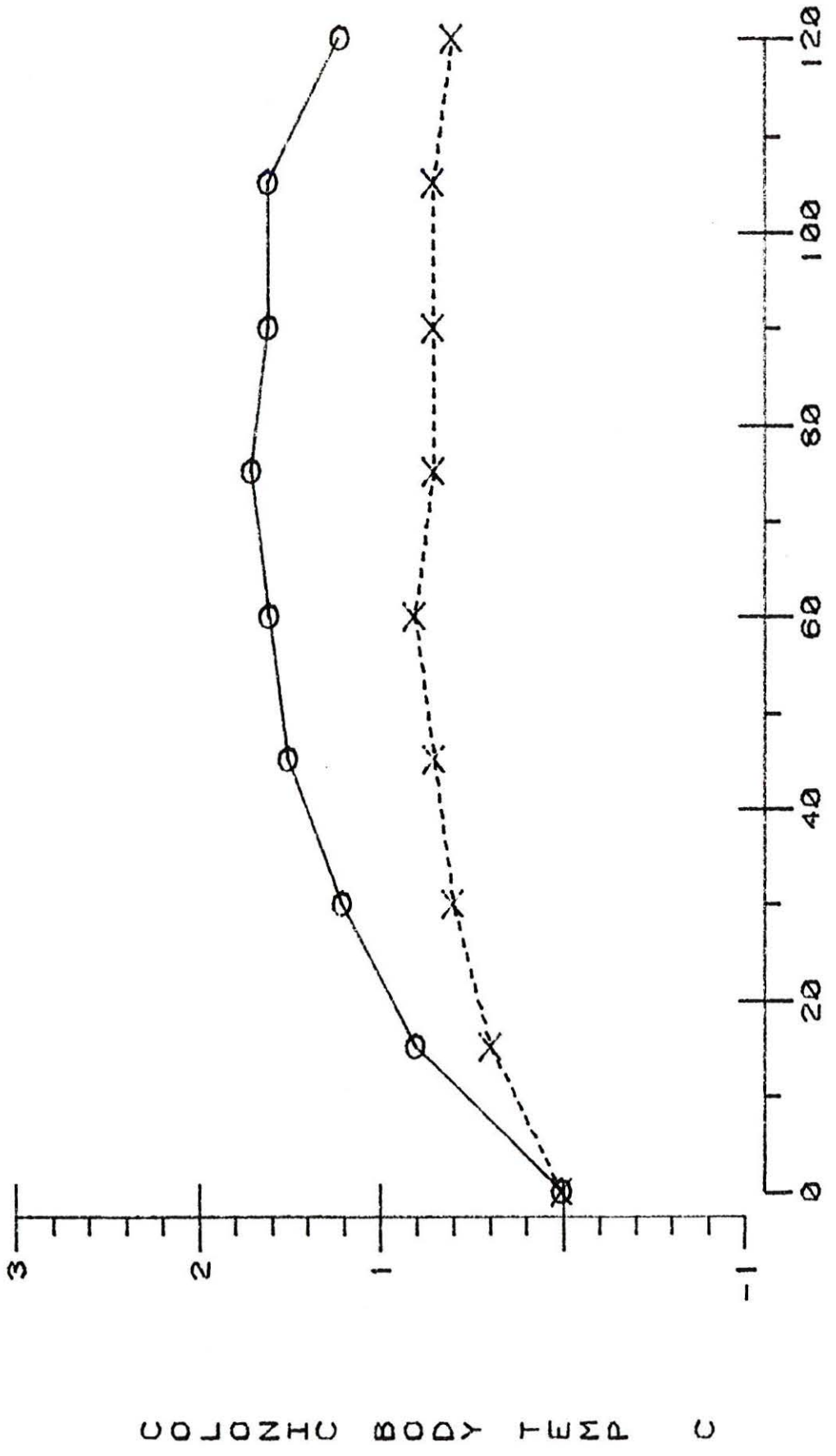
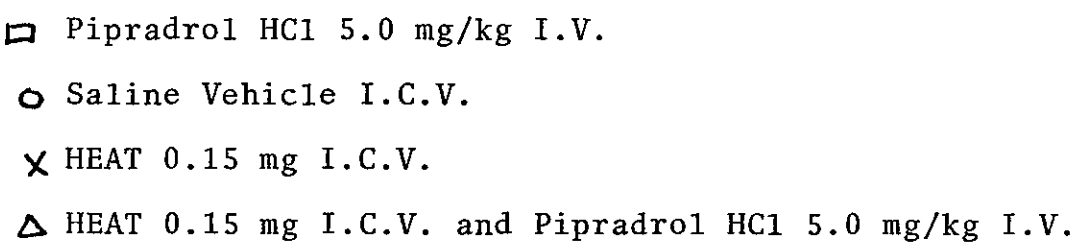

3
2
1
-1
0
20
40
60
80
100
120
TIME (MIN)
CORRECTED BODY TEMP C

Figure 7. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with HEAT (-15 minutes).



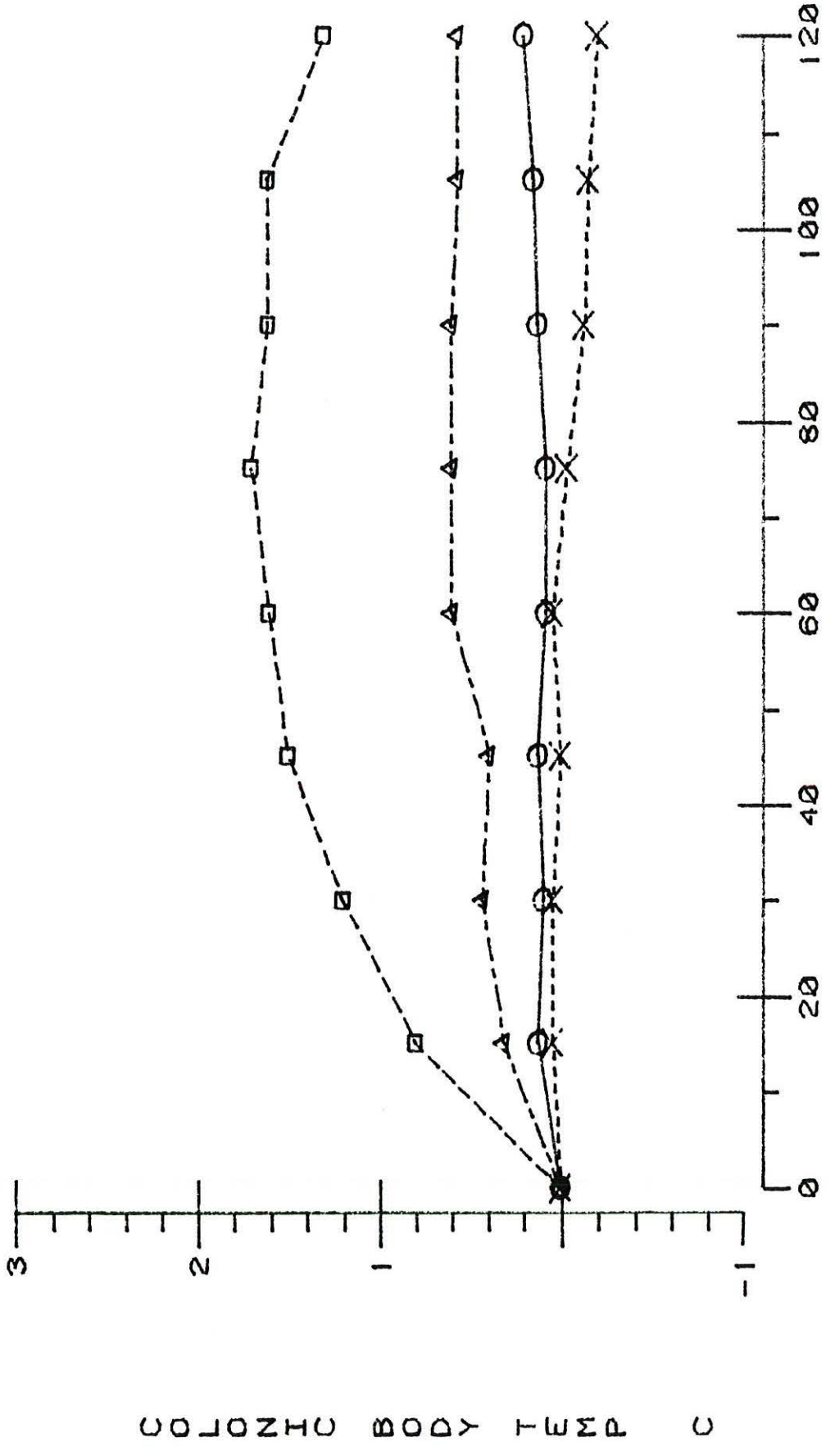

3
2
1
-1
0
20
40
60
80
100
120
TIME (MIN)
CHANGE BODY TEMP C

Figure 8. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with Cinanserin (-60 minutes).

X Pipradrol 2.5 mg/kg I.V.

O Cinanserin HCl 5.0 mg/kg and Pipradrol HCl 2.5 mg/kg

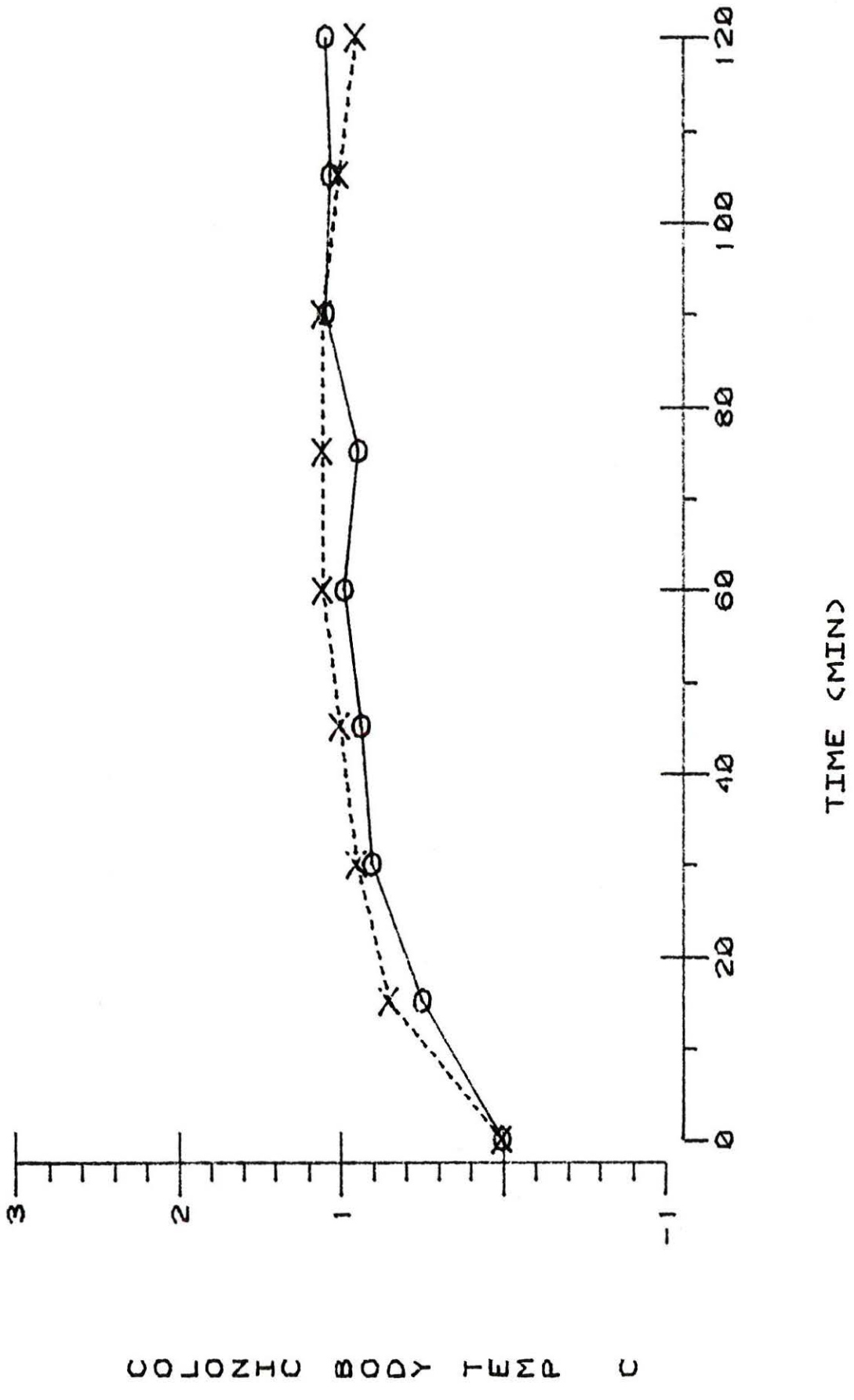

CORRECTED BODY TEMP C
3
2
1
-1
0
20
40
60
80
100
120
TIME (MIN)

Figure 9. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with Cyproheptadine (-30 minutes).

X Pipradrol HCl 5.0 mg/kg I.V.

O Cyproheptadine HCl 2.0 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.

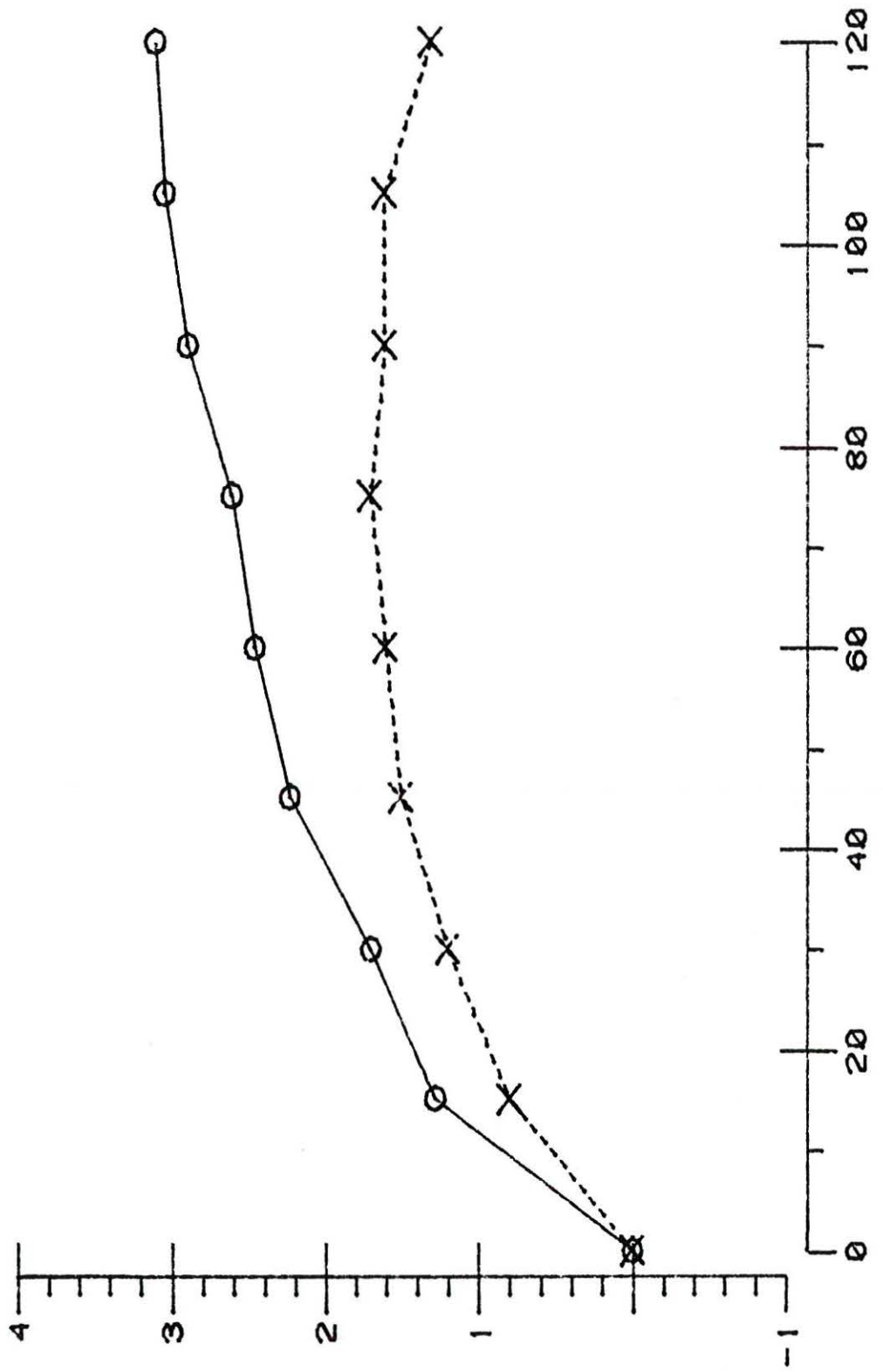

COLONIC BODY TEMP C
4
3
2
1
-1
0
20
40
60
80
100
120
TIME (MIN)

Figure 10. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with Diphenhydramine (-30 minutes).

X Pipradrol HCl 5.0 mg/kg I.V.

o Diphenhydramine HCl 6.0 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.

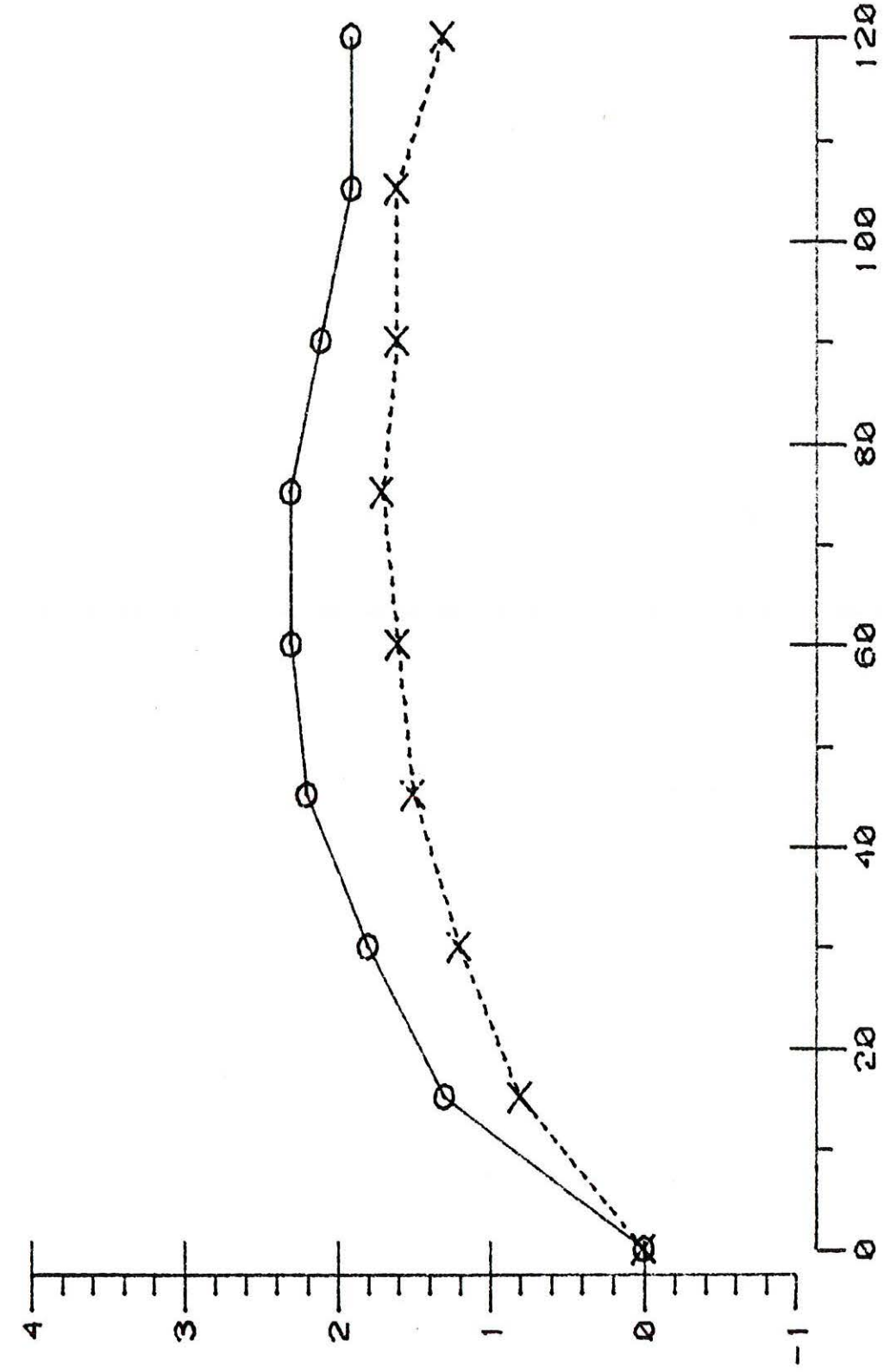

4
3
2
1
0
-1
0
20
40
60
80
100
120
TIME (MIN)
CORRECTED BODY TEMP C

Figure 11. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with Atropine (-30 minutes).

× Pipradrol HCl 5.0 mg/kg I.V.

o Atropine Sulfate 2.0 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.

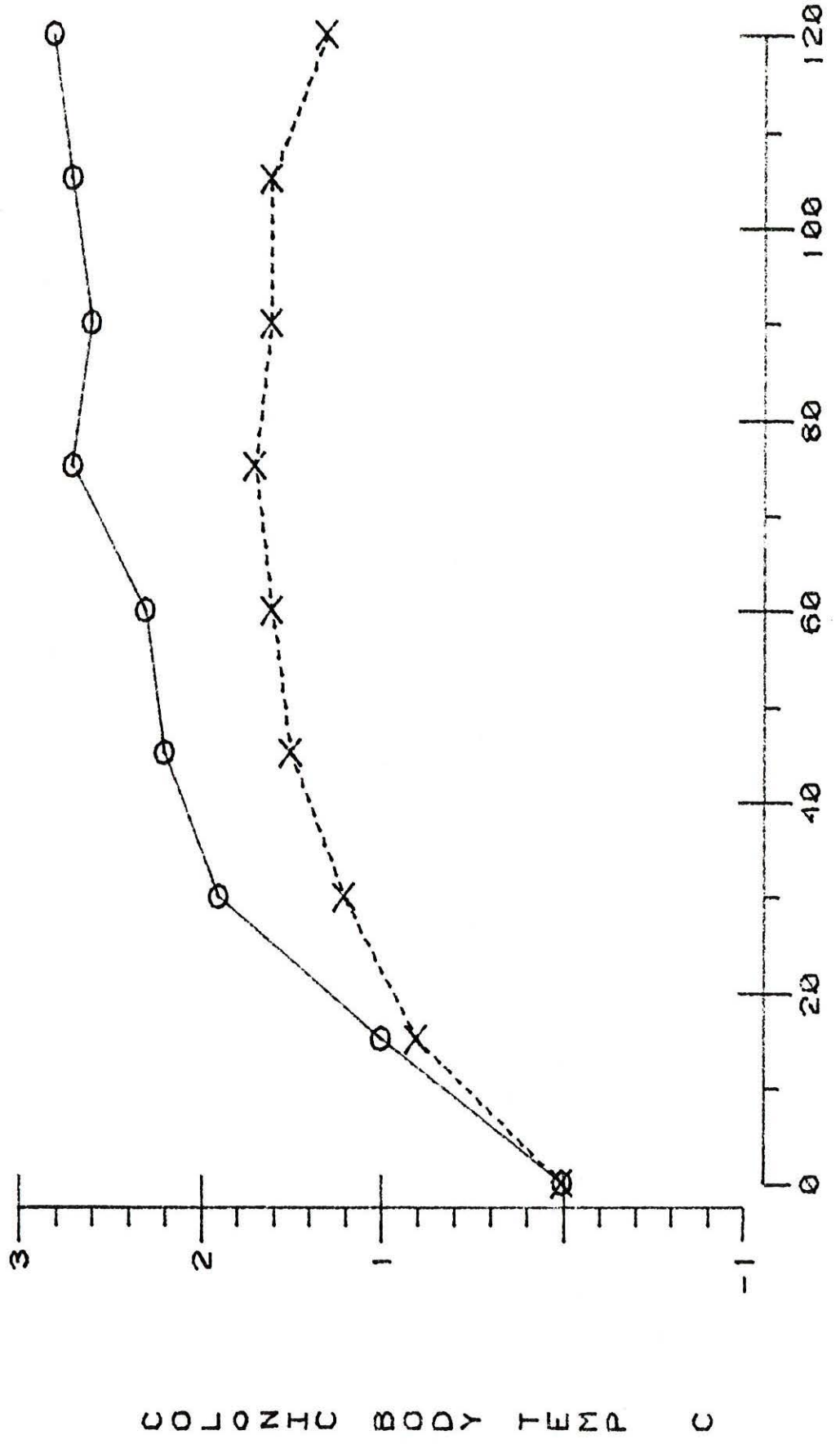

COLONIC BODY TEMP C
3
2
1
-1
0
20
40
60
80
100
120
TIME (MIN)

Figure 12. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with Propranolol (-30 minutes).

X Pipradrol HCl 5.0 mg/kg I.V.

O Propranolol HCl 1.0 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.

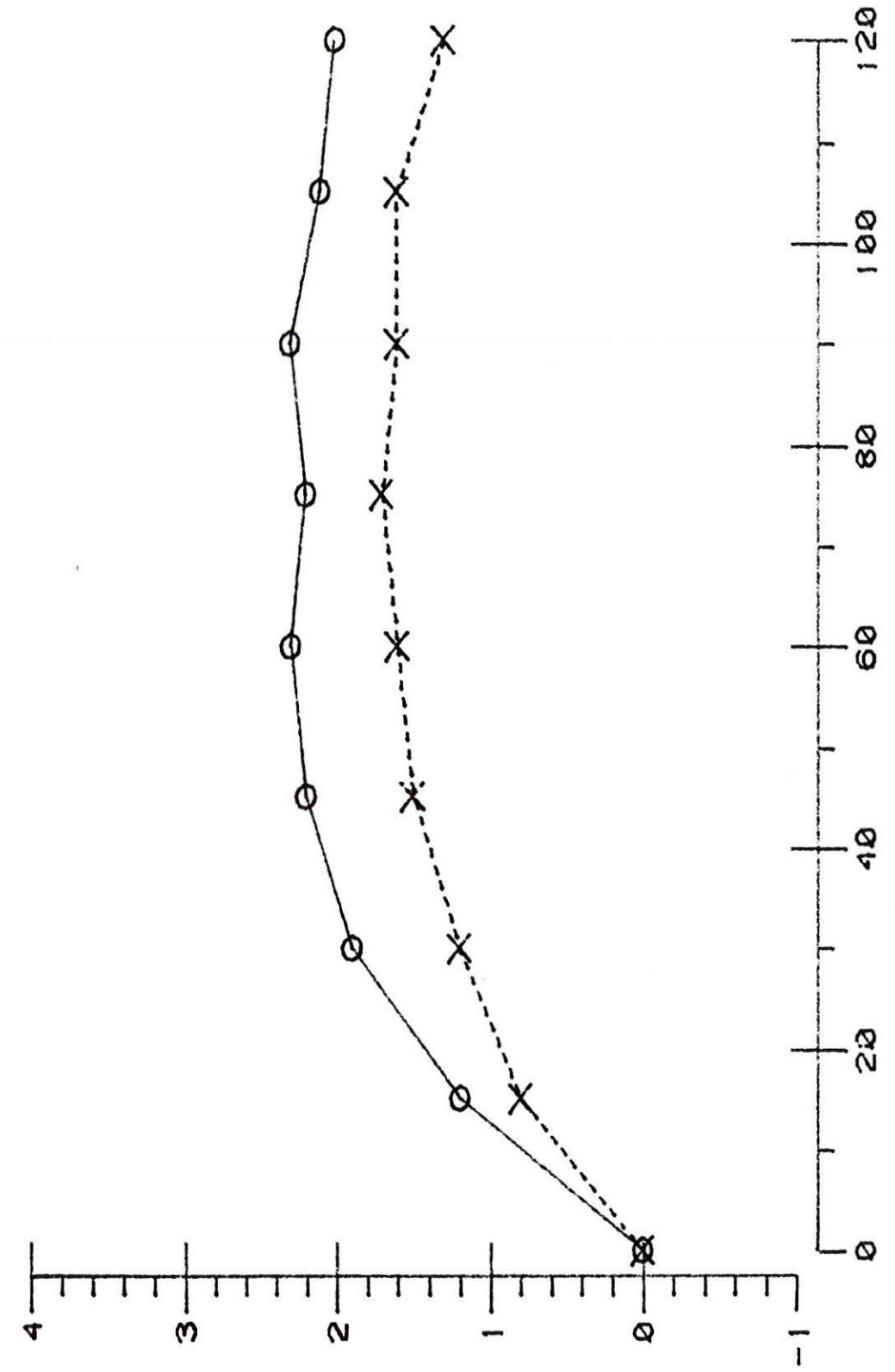

CORRECTED BODY TEMP C
4
3
2
1
0
-1
0
20
40
60
80
100
120
TIME (MIN)

Figure 13. Colonic Temperature Effects of Pipradrol in Rabbits Pretreated with Pentobarbital (-30 minutes).

X Pipradrol HCl 5.0 mg/kg I.V.

O Pentobarbital Na 10.0 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.

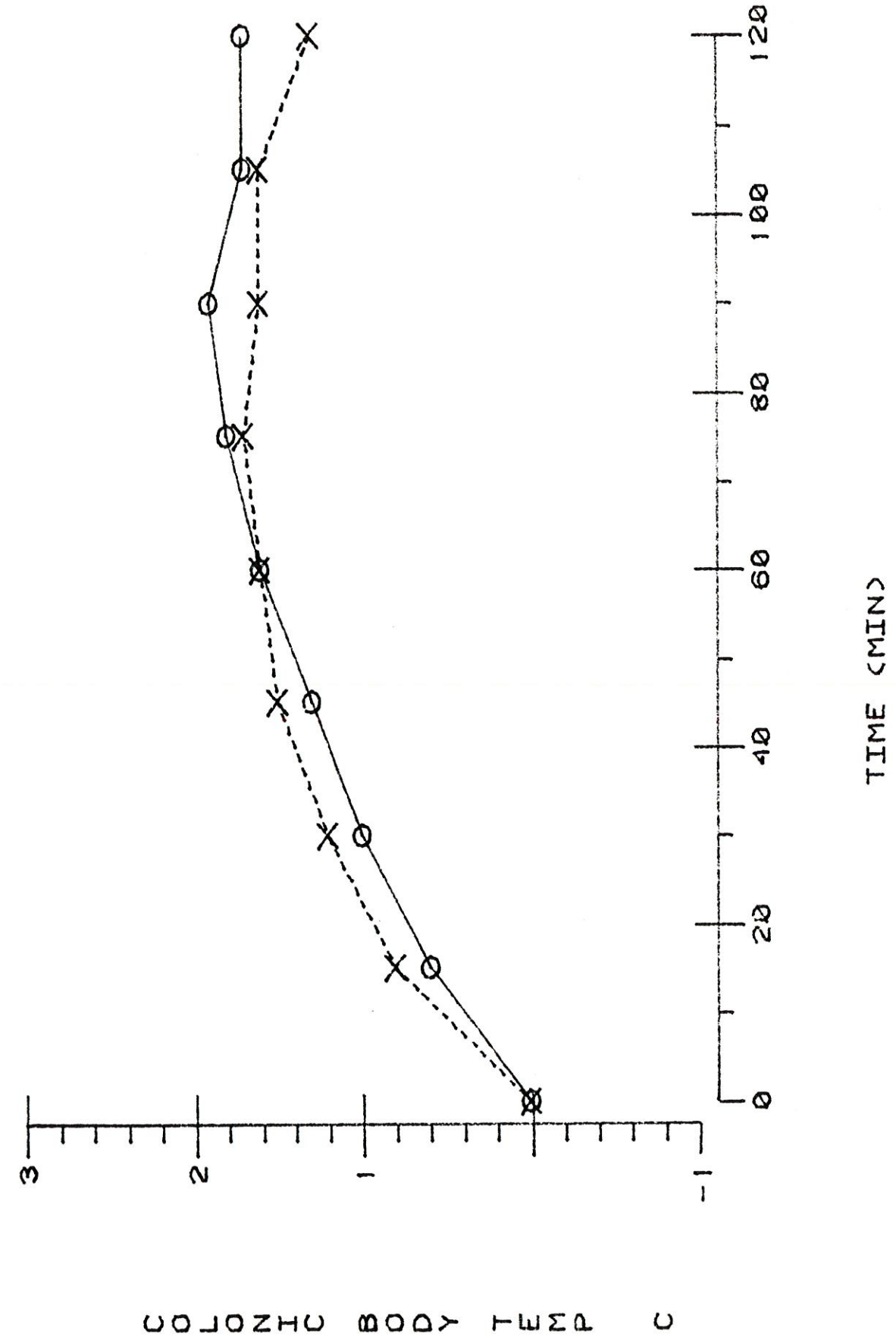

3
2
1
-1
0
20
40
60
80
100
120
TIME (MIN)
ORAL BODY TEMP C

Figure 14. Colonic Temperature Effects of Clonidine in Rabbits Pretreated with HEAT (-30 minutes).

X Clonidine 2.5 mg/kg I.V.

O HEAT 0.5 mg/kg I.V. and Clonidine 2.5 mg/kg I.V.

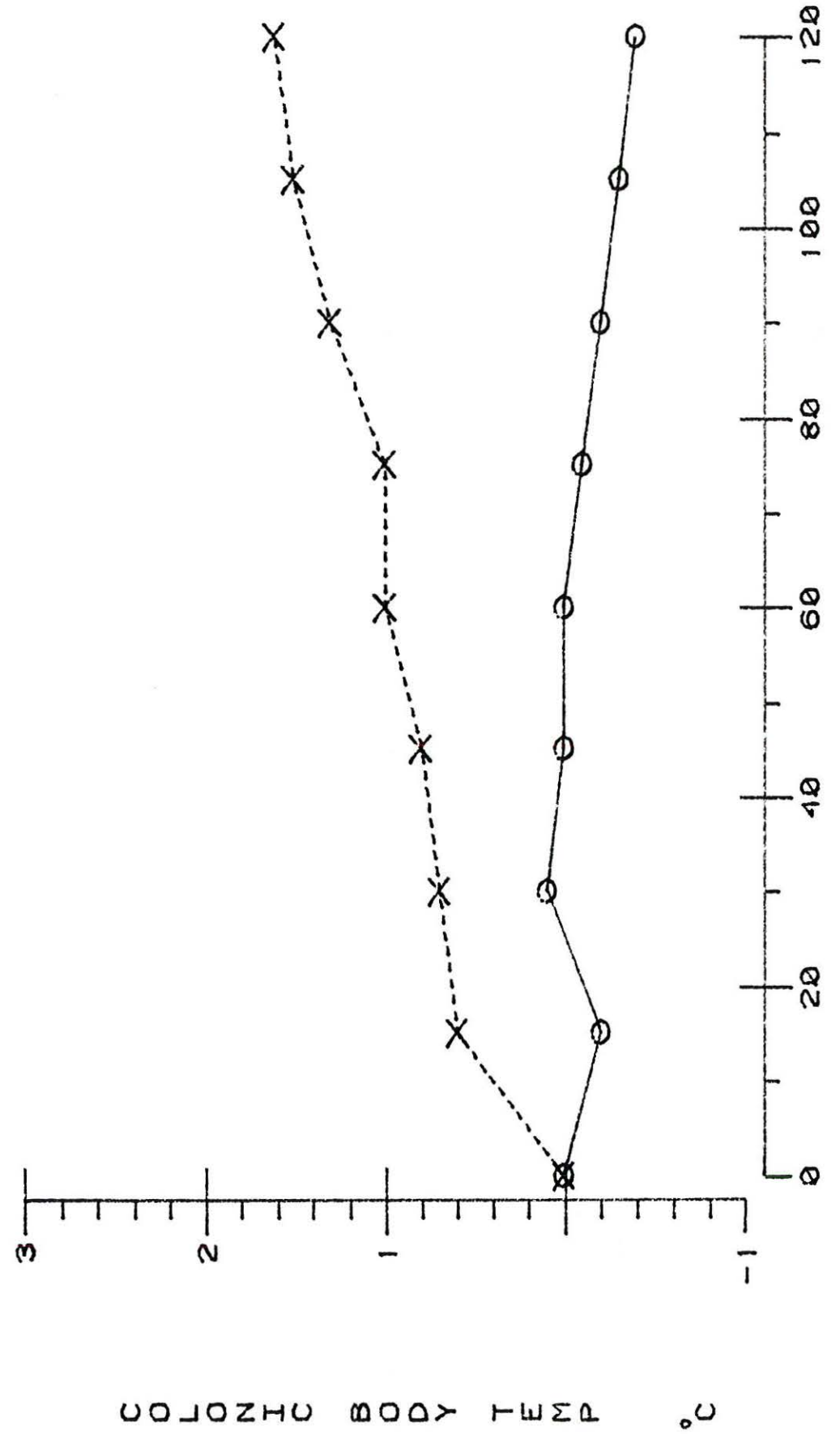
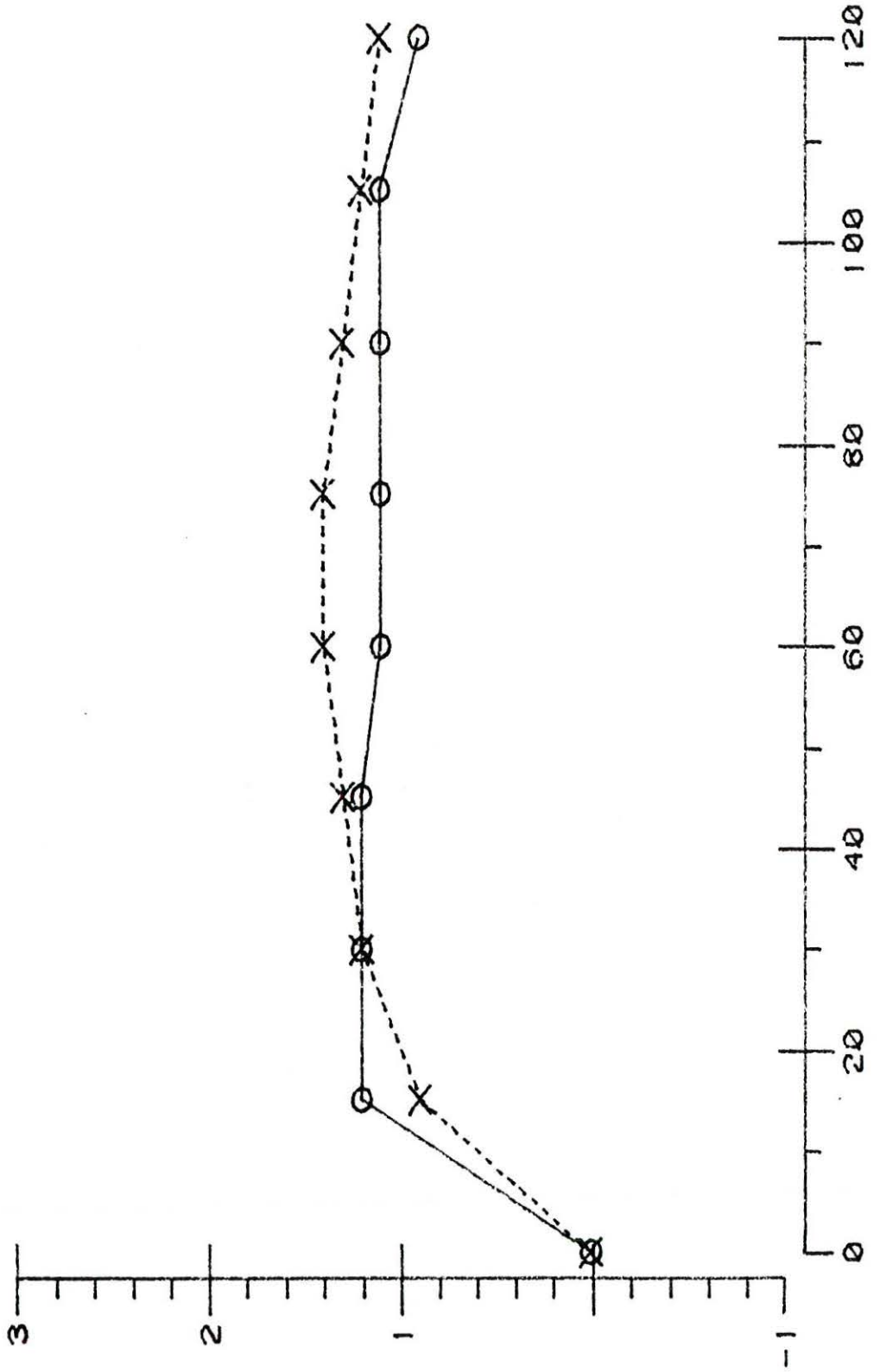

3
2
1
-1
0
20
40
60
80
100
120
TIME (MIN)
°C

Figure 15. The Effect of Successive Treatments of Pipradrol on the Colonic Body Temperature of Rabbits.

X Pipradrol HCl 5.0 mg/kg I.V. 8:00 A.M.

O Pipradrol HCl 5.0 mg/kg I.V. 3:00 P.M.


3
2
1
-1
0
20
40
60
80
100
120
TIME (MIN)
CORRECTED BODY TEMP C

DISCUSSION

The results of this study show that the I.V. administration of pipradrol causes dose-related hyperthermia in the rabbit as well as behavioral changes characterized by both compulsive gnawing and increased locomotor activity. Compulsive gnawing was manifested by either grinding the teeth or by biting the metal water trough continuously. The locomotor activity consisted of signs of tenseness associated with occasional episodes of leg-thumping and attempts to escape from the restraining stanchion. After the administration of pipradrol, vasodilation was also observed to occur in the ear veins with the ears becoming very warm to the touch. The ears are normally cool to cold at the ambient temperature of 23^{o} C.

A dose-response curve was established using I.V. doses consisting of 2.0, 5.0 and 10.0 mg/kg. The dosage 5.0 mg/kg resulted in a consistent hyperthermic response

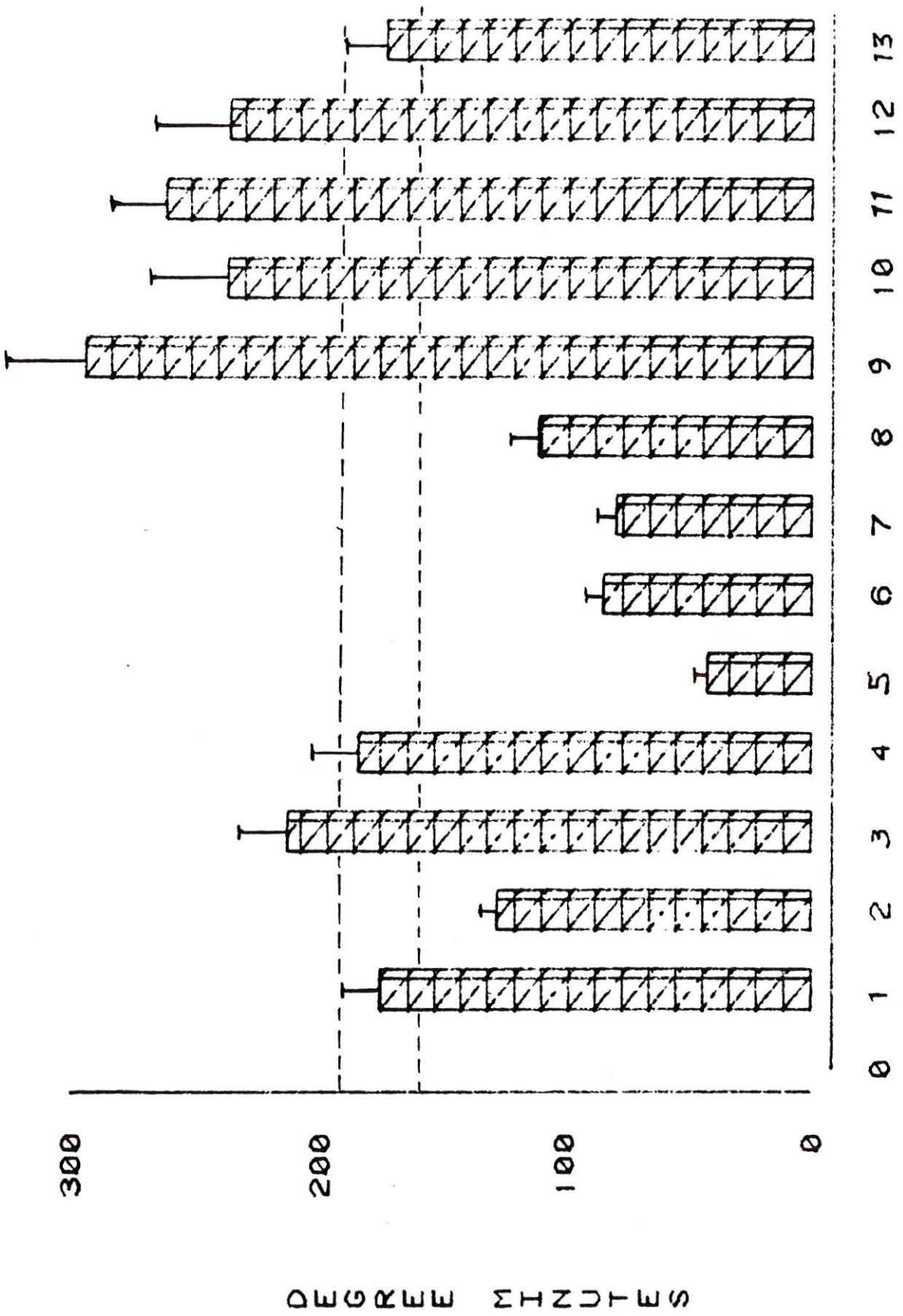
which was tolerated well by all of the animals tested. At the 10.0 mg/kg dosage, death occurred in 50% of the tested animals. The cause of death appeared to be respiratory failure occurring during tonic-clonic convulsions.

While the effects of pipradrol appear to parallel those effects reported to be seen with amphetamine, there are questions as to whether these two drugs are acting by the same mechanisms. It has been reported that the behavioral excitatory effects of pipradrol are dependent on catecholamines stored in a reserpine-sensitive pool while those of amphetamine are produced independently of this catecholamine source (22). The behavioral action of both these drugs, however, ultimately appears to depend on the synthesis of catecholamines. There also has been shown to be no stereochemical relationship between the more active enantiomers of pipradrol and amphetamine in studies conducted on rat locomotor activity (53).

In an effort to investigate the nature of the receptors involved in mediating the responses seen with pipradrol, various receptor antagonists were utilized. Chlorpromazine, which is known to antagonize alpha-adrenergic, histamine, dopamine and acetylcholine receptors, was found to block the hyperthermia seen with pipradrol (Figure 16) and this effect was dose-dependent. Chlorpromazine also inhibited both compulsive gnawing and locomotor activity and the animals generally appeared to be depressed. This effect

Figure 16. Comparative Evaluation of Area-Under-The-Curve Data for the Colonic Body Temperature Effect of Intravenous Pipradrol in Rabbits Pretreated with Various Known Receptor Blockers (bars represent one S.E.M.; dashed lines represent the range betweeen the mean $\pm$ 1 S.E.M. for pipradrol HCl 5.0 mg/kg I.V.).

1. Pipradrol HCl 5.0 mg/kg I.V.
2. Pipradrol HCl 2.5 mg/kg
3. Haloperidol 0.5 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.
4. Pimozide 4.0 mg/kg I.P. and Pipradrol HCl 5.0 mg/kg I.V.
5. Chlorpromazine HCl 1.0 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.
6. Phenoxybenzamine HCl 1.0 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.
7. HEAT 0.125 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.
8. Cinanserin HCl 5.0 mg/kg I.V. and Pipradrol HCl 2.5 mg/kg I.V.
9. Cyproheptadine HCl 2.0 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.
10. Diphenhydramine HCl 6.0 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.
11. Atropine SO_4 2.0 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.
12. Propranolol HCl 1.0 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.
13. Pentobarbital Na 10.0 mg/kg I.V. and Pipradrol HCl 5.0 mg/kg I.V.


0 1 2 3 4 5 6 7 8 9 10 11 12 13
0
100
200
300
DEGREE SEARCHES

is in agreement with its clinical utility as a major tranquilizer.

The failure of the dopamine receptor blockers haloperidol and pimozide to block pipradrol-induced hyperthermia (Figure 16) indicates that, unlike amphetamine, the involvement of dopaminergic pathways is not a significant component of this response. Haloperidol eliminated the incidence of compulsive gnawing caused by pipradrol; however, pretreatment with pimozide appeared to have little effect on compulsive gnawing. Comparison of the temperature indexes (Figure 16) of haloperidol and pimozide indicates that (at the doses used) haloperidol has slightly more influence than pimozide on the hyperthermic effect of pipradrol. While not statistically significant, the effect of haloperidol appeared to potentiate the hyperthermic response of pipradrol.

With I.V. pretreatment with alpha-adrenergic blocking agents phenoxybenzamine and HEAT, both were shown to inhibit the hyperthermic response to pipradrol (Figure 16). These results suggest the involvement of alpha-adrenergic receptors in the thermotropic response to pipradrol. The alpha-adrenergic component fits the model (Figure 17) proposed by Tangri et al. (46) which suggests that by stimulation of alpha-adrenergic receptors in the preoptic area of the hypothalamus a resultant hyperthermia occurs. This

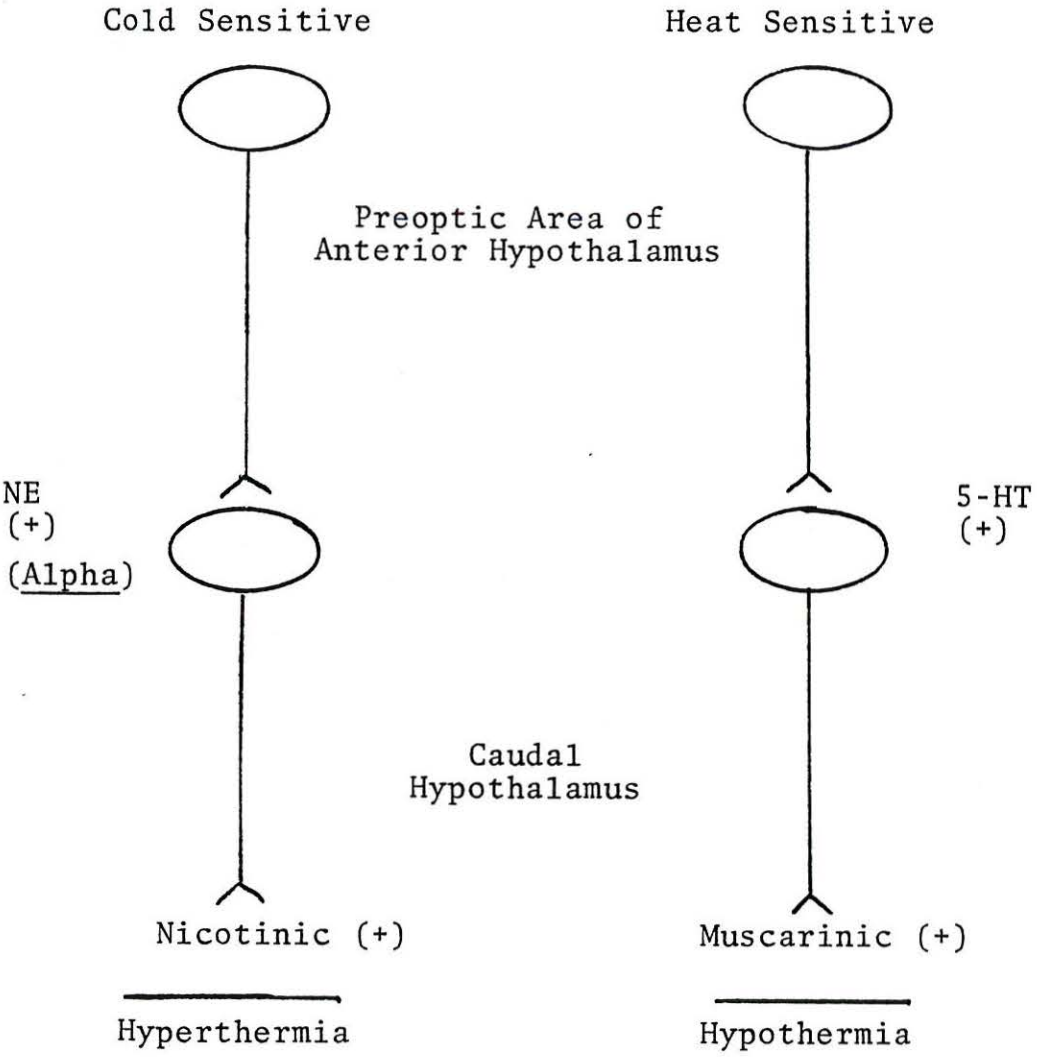


Figure 17. A proposed model for thermoregulation involving NE (norepinephrine), alpha-adrenergic receptors, nicotinic acetylcholine receptors and muscarinic acetylcholine receptors (after Tangri et al.) (46).

response is thought to be mediated through nicotinic acetylcholine receptors in the caudal hypothalamus. It is also suggested that this pathway is mediated through the stimulation of "cold sensitive" receptors. In an effort to establish the involvement of pipradrol centrally in mediating hyperthermia, HEAT was administered I.C.V. 15 minutes prior to I.V. pipradrol which resulted in attentuation of the hyperthermia (Figure 7). Substances introduced into the lateral cerebral ventricles are known to have ready access to the preoptic area of the anterior hypothalamus which is thought to be the area involved in temperature regulation (60).

The antihypertensive drug clonidine is known to be a potent alpha-adrenergic receptor stimulant in both the peripheral and central nervous systems (61). The I.V. administration of clonidine was shown to cause a hyperthermic response (similar to that seen with pipradrol) which was inhibited by pretreatment with HEAT administered I.V. These data give evidence that in the rabbit HEAT is capable of inhibiting hyperthermia induced by a known alpha-adrenergic stimulant. The pretreatment with HEAT I.V. also appeared to attenuate the locomotor activity seen with pipradrol but an increase in compulsive gnawing was observed. These observations are in conflict with those reported by Clineschmidt (53) whose findings showed an inhibition of

apomorphine-induced gnawing in the mouse indicating antagonism of central dopaminergic receptors.

The I.V. pretreatment with the serotonergic receptor blocker cyproheptadine resulted in a significant ($P < 0.05$) potentiation of the hyperthermia seen with pipradrol. The animals were extremely agitated during the course of the experiment and three of the six animals had to be sacrificed due to excessive body temperatures (> 44^{o} C). Since cyproheptadine has antihistaminic and weak anticholinergic activity as well as antiserotonin properties, it was not clear as to which of these actions is responsible for the potentiation. However, information collected later in this study showed that while the antihistamine diphenhydramine also appeared to elevate the hyperthermic response to pipradrol, this response was not significant ($P > 0.05$). These results tend to indicate that the potentiation of hyperthermia may reside within the antiserotonin or anticholinergic capabilities of cyproheptadine. These results agree with the thermoregulatory model presented by Tangri et al. (46) which suggests a role for serotonin in mediating hypothermia through muscarinic receptors in the rabbit (Figure 17). Inhibition of these serotonin receptors would impair the "heat sensitive" mechanism for thermoregulation, thus allowing for uninhibited hyperthermia to occur. The antiserotonin drug cinanserin failed to show

a significant block of the thermic response to pipradrol. However, this may be dose-dependent and higher doses of cinanserin could produce different results.

The hyperthermic effect of pipradrol was not significantly altered by pretreatment with the antihistaminic compound diphenhydramine. This effect appeared to be slightly potentiated but was not statistically significant ($P > 0.05$). Diphenhydramine also possesses anticholinergic activity and it may be this component which is responsible for the tendency for potentiation of the pipradrol-induced hyperthermia.

The anticholinergic agent atropine has high affinity for the muscarinic receptor and very little for the nicotinic receptor. Pretreatment with atropine resulted in a significant ($P < 0.05$) potentiation of the pipradrol-induced hyperthermia. These results also agree with the model (Figure 17) proposed by Tangri *et al*. (46). It is suggested that the muscarinic receptor which is "downstream" from the serotonin receptor is also associated with the thermoregulatory process -- more specifically with the mediation of hypothermia due to stimulation of "heat-sensitive" receptors. It may be that blockade of either muscarinic receptors or serotonin receptors would result in inhibition of the compensatory mechanism involved in controlling the hyperthermic response to pipradrol, thus resulting in potentiation of this

effect. The pretreatment with atropine also appeared to potentiate the locomotor activity due to pipradrol. It is not clear as to the nature of involvement of muscarinic receptors in mediating locomotor activity in the rabbit.

The involvement of the beta-adrenergic receptor in the mediation of hyperthermia by pipradrol was studied by pretreatment with the beta-adrenergic blocker propranolol. The failure of propranolol to influence the thermic response of pipradrol suggests that these receptors are not closely associated with this event. The work of Dhawan and Dua (31) also failed to substantiate a basis for the involvement of beta-adrenergic receptors in the hyperthermia associated with norepinephrine.

Pipradrol has been shown to cause excitation and increased locomotor activity in the rabbit. To investigate whether or not this activity influences the body temperature of rabbits treated with pipradrol, the central nervous system depressant pentobarbital was given as an I.V. pretreatment at a dose which rendered the animals quiescent but not anesthetized throughout the experiment. The failure of pentobarbital to influence the hyperthermia seen with pipradrol (Figure 16) suggests that this effect is not directly related to excessive locomotor activity or to excitation. However, this does not rule out a peripheral component of hyperthermia since pentobarbital is known to

act only centrally in its depressant action.

It has been stated many times that "no drug has a single effect." The events presented in this study represent only a small window in the vast picture of the possible pharmacological activities of pipradrol.

CONCLUSIONS

From this study it can be concluded that there is a characteristic dose-related hyperthermia produced in response to I.V. administered pipradrol. This thermotropic activity may be associated with different neuronal receptors, the most important of which appears to be alpha-adrenergic in nature. The antagonism of hyperthermia by the alpha-adrenergic blockers HEAT (administered both peripherally and centrally), chlorpromazine and phenoxybenzamine lends support for an alpha-adrenergic mechanism which is at least in part centrally mediated.

The failure of dopamine receptor blockers haloperidol and pimozide to attenuate the hyperthermia seen with pipradrol suggests that dopaminergic pathways are not directly involved in this response. These data indicate a different thermotropic mechanism of action for pipradrol than the dopamine agonists apomorphine and amphetamine which are

thought to mediate hyperthermia in the rabbit via dopaminergic pathways.

The results of this study also give support for the controversial thermoregulartory model proposed by Tangri et al. (46), since both the antiserotonin drug cyproheptadine and anticholinergic drug atropine potentiate pipradrol's hyperthermic activity.

The involvement of beta-adrenergic receptors has been shown to be not significant in this study.

Histamine receptors which have been shown to be thermotropic in other species have not been shown to be unequivocally significant or not significant in this study. Further work will be needed to allow a definite statement to be made.

The more apparent behavioral components of pipradrol administration consist of increased locomotor activity and compulsive gnawing. These may be produced by two different mechanisms as evidenced by the attenuation of locomotor activity and potentiation of compulsive gnawing by the administration of HEAT. Since these behavioral aspects of pipradrol were not statistically evaluated, this conclusion may only be stated as a general observation.

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APPENDIX A

CALCULATION OF AREA UNDER THE CURVE

The area-under-the-curve data (A) was calculated using a TI-58C hand-held calculator.

$$A = \frac{[S\ (\Delta t\ I)_1 + S\ (\Delta t\ I)_2 \ldots + S\ (\Delta t\ I)_N]}{N}$$

S = sum of

Δt = change in temperature (degrees, centigrade)

I = time interval (15 min.)

N = test group number

A = area under the curve expressed as the mean (degree min.)

APPENDIX B

PROGRAM AND ILLUSTRATIVE CALCULATIONS

```
#B6700:417 CANDE 31.280; YOU ARE ACAD/3(23)
#ENTER USERCODE PLEASE
H00182801
#ENTER PASSWORD PLEASE.
■■■■■■■■■■■■■■■■■■
#SESSION 7168  15:33:58  03/19/83

GET DUNNETTS;L;
#WORKFILE DUNNETTS: SEQ, 157 RECORDS, SAVED
10      (* THIS PROGRAM WILL PERFORM A DUNNETT'S T-TEST IN WHICH THE
20         DIFFERENCES OF MEANS BETWEEN A CONTROL AND SEVERAL EXPERIMENTAL
30         GROUPS CAN BE STATISTICALLY COMPARED.  THE CONTROL GROUP VALUES
40         MUST BE ENTERED FIRST, FOLLOWED BY THE EXPERIMENTAL VALUES. \
           \ THE
50         PROGRAM WILL THEN PERFORM THE NECESSARY CALCULATIONS AND\
           \ PRINTOUT
60         THE T-VALUES.  IT CAN HANDLE A MAXIMUM OF 20 GROUPS WITH UP TO
70         30 MEASUREMENTS IN EACH GROUP. *)
80
90      (* WRITTEN BY MIKE M. NAMBA, NOVEMBER 1982, AT THE UNIVERSITY OF
100        OF THE PACIFIC SCHOOL OF PHARMACY. *)
110
120     (* THIS PROGRAM IS DEDICATED TO MY GOOD FRIEND STEVE SMALL WHO
130        SPARKED THE FIRES OF MY CURIOSITY AND KNOWLEDGE.  SHORTLY
140        AFTER MY RETURN TO U.O.P., ON AN AUTUMN DAY, STEVE CALLED ME
150        AND ASKED ABOUT AVAILABLE COMPUTER PROGRAMS.  THE RESULT OF
160        THAT CONVERSATION IS THIS PROGRAM.  HERE AT U.O.P., THOSE
170        FIRES GLOW LIKE A SUPERNOVA IN THE NIGHT SKY.  *)
180
190     PROGRAM DUNNETTS (INPUT,OUTPUT);
200
210     TYPE    LIST = ARRAY[1..30,1..20] OF REAL;
220             TOTALNUMBER = ARRAY[1..30] OF INTEGER;
230             MEANVALUE = ARRAY[1..20] OF REAL;
240
250     LABEL 1;
260
270     VAR     VALUE : LIST;
280             NGRP : TOTALNUMBER;
290             SEM,XGRP,TVALUE : MEANVALUE;
300             NUMGRP,NUM,NTOT,I,J,AN,NAP : INTEGER;
310             DFBETGRP,DFWTHGRP : INTEGER;
320             SSG,SX2,TXT,S2,F,TX : REAL;
330             MEANSQUARE,FG,SD,C : REAL;
340             SX2T,SSGFIN : REAL;
350             CH : CHAR;
360
```

```
370   BEGIN (* THE PROGRAM DUNNETTS *)
380   WRITELN;
390   WRITELN('NOTE: THE MAXIMUM NUMBER OF GROUPS THAT THIS PROGRAM');
400   WRITELN('CAN HANDLE IS 20.  THE MAXIMUM NUMBER OF MEASUREMENTS');
410   WRITELN('WITHIN EACH GROUP IS 30.  DO NOT EXCEED THESE LIMITS');
420   WRITELN('OR ERRORS WILL RESULT IN THE T-VALUES THAT ARE GENERATED\
        \.');
430   WRITELN;
440   WRITELN;
450
460   (* READ IN CONTROL VALUES *)
470  1:WRITELN('ENTER THE TOTAL NUMBER OF GROUPS.');
480   READ(NUMGRP);
490   WRITELN;
500   WRITELN('ENTER THE NUMBER OF VALUES OF THE CONTROL GROUP.');
510   READ(NUM);
520   WRITELN;
530   WRITELN('ENTER THE VALUES.');
540   FOR I:= 1 TO NUM DO
550     BEGIN
560     READ(VALUE[I,1]);
570     END;
580   NGRP[1]:= NUM;
590   WRITELN;
600
610   (* READ IN EXPERIMENTAL VALUES *)
620   FOR J:= 2 TO NUMGRP DO
630     BEGIN
640     NAP:= J - 1;
650     WRITELN('ENTER THE NUMBER OF VALUES OF EXPERIMENTAL GROUP ',NAP\
         \:2);
660     READ(NUM);
670     WRITELN;
680     WRITELN('ENTER THE VALUES.');
690     FOR I:= 1 TO NUM DO
700       BEGIN
710       READ(VALUE[I,J]);
720       END;
730   NGRP[J]:= NUM;
740   WRITELN;
750   END;
760
770   (* CALCULATE TOTAL NUMBER OF VALUES *)
780   NTOT:= 0; SX2T:= 0; SSG:= 0; TXT:= 0;
790   FOR J:= 1 TO NUMGRP DO
800     BEGIN
810     TX:= 0; SX2:= 0;
820     NTOT:= NTOT + NGRP[J];
830
840   (* CALCULATE TOTAL OF VALUES AND SQUARED VALUES *)
850     FOR I:= 1 TO NGRP[J] DO
860       BEGIN
870       TX:= TX + VALUE[I,J];
880       TXT:= TXT + VALUE[I,J];
```

```
890         SX2:= SX2 + SQR(VALUE[I,J]);
900         SX2T:= SX2T + SQR(VALUE[I,J]);
910         END;
920       SSG:= SSG + SQR(TX)/NGRP[J];
930       XGRP[J]:= TX/NGRP[J];
940       SEM[J]:= SQRT((SX2-(SQR(TX)/NGRP[J]))/(NGRP[J]-1))/SQRT(NGRP[J]\
           \);
950       END;
960
970    (* CALCULATE C AND FINAL SSG *)
980    C:= SQR(TXT)/NTOT;
990    SSGFIN:= SSG - C;
1000
1010    (* CALCULATE F VALUES *)
1020    DFBETGRP:= NUMGRP - 1;
1030    DFWTHGRP:= NTOT - NUMGRP;
1040    MEANSQUARE:= SSGFIN/DFBETGRP;
1050    S2:= (SX2T - C - SSGFIN)/DFWTHGRP;
1060    F:= MEANSQUARE/S2;
1070    FG:= NTOT/NUMGRP;
1080    SD:= SQRT(2*S2/FG);
1090
1100    WRITELN('**********************************************************\
          \);
1110    WRITELN('**********************************************************\
          \);
1120    WRITELN;
1130    WRITELN('THE D.F. BETWEEN GROUPS = ',DFBETGRP:4);
1140    WRITELN;
1150    WRITELN('THE D.F. WITHIN GROUPS =  ',DFWTHGRP:4);
1160    WRITELN;
1170
1180    WRITELN('THE F-VALUE IS ',F:11:4);
1190    WRITELN;
1200
1210   (* WRITE THE VALUES FOR THE CONTROL *)
1220   WRITELN('THE VALUES FOR THE CONTROL GROUP:');
1230   WRITELN('    N       = ',NGRP[1]:11);
1240   WRITELN('    MEAN    = ',XGRP[1]:11:4);
1250   WRITELN('    S.E.M.  = ',SEM[1]:11:4);
1260   WRITELN;
1270
1280    (* CALCULATE THE DUNNETT'S T VALUES AND WRITE THE VALUES FOR THE
1290       EXPERIMENTAL GROUPS *)
1300    FOR J:= 2 TO NUMGRP DO
1310      BEGIN
1320      TVALUE[J]:= (XGRP[1]-XGRP[J])/SD;
1330      AN:= J-1;
1340      WRITELN('THE VALUES FOR EXPERIMENTAL GROUP ',AN:2,':');
1350      WRITELN('    N       = ',NGRP[J]:11);
1360      WRITELN('    MEAN    = ',XGRP[J]:11:4);
1370      WRITELN('    S.E.M.  = ',SEM[J]:11:4);
1380      WRITELN('    T-VALUE = ',TVALUE[J]:11:4);
1390      WRITELN;
1400      END;
```

```
1410
1420  WRITELN('*************************************************************');
1430  WRITELN('*************************************************************');
1440  WRITELN;
1450
1460  (* ASK USER IF HE/SHE WANTS TO GO AGAIN *)
1470  WRITELN('DO YOU WISH TO DO ANOTHER DUNNETT''S T-TEST?');
1480  READ(CH);
1490  WHILE CH = ' ' DO READ (CH);
1500  IF CH = 'Y' THEN
1510     BEGIN
1520     WRITELN;
1530     WRITELN;
1540     GOTO 1;
1550     END;
1560
1570  END. (* THE PROGRAM DUNNETTS *)
#


GET STEVE
#NO FILE:STEVE.
BYE
#END SESSION 7168  ET=5:51.8 PT=1.2 IO=0.2
#USER = H00182801  15:39:50  03/19/83


#B6700:417 CANDE 31.280; YOU ARE ACAD/3(23)
#ENTER USERCODE PLEASE
H00182701
#ENTER PASSWORD PLEASE.
@@@@@@@@@@@@@@@@@@
#SESSION 7208  15:40:44  03/19/83
GET DUNNETTS;R; FILE INPUT(KIND=DISK,TITLE=STEVE)
#WORKFILE DUNNETTS: SEQ, 157 RECORDS, SAVED
#OBJECT FILE PRESENT, SAVED
#RUNNING 7211

NOTE: THE MAXIMUM NUMBER OF GROUPS THAT THIS PROGRAM
CAN HANDLE IS 20.  THE MAXIMUM NUMBER OF MEASUREMENTS
WITHIN EACH GROUP IS 30.  DO NOT EXCEED THESE LIMITS
OR ERRORS WILL RESULT IN THE T-VALUES THAT ARE GENERATED.


ENTER THE TOTAL NUMBER OF GROUPS.

ENTER THE NUMBER OF VALUES OF THE CONTROL GROUP.

ENTER THE VALUES.

ENTER THE NUMBER OF VALUES OF EXPERIMENTAL GROUP  1

ENTER THE VALUES.
```

```
THE VALUES FOR EXPERIMENTAL GROUP  1:
    N       =          7
    MEAN    =    213.6429
    S.E.M.  =     32.5881
    T-VALUE =     -0.8597

THE VALUES FOR EXPERIMENTAL GROUP  2:
    N       =          4
    MEAN    =    124.8750
    S.E.M.  =     25.3922
    T-VALUE =      1.8451

THE VALUES FOR EXPERIMENTAL GROUP  3:
    N       =          5
    MEAN    =     41.8000
    S.E.M.  =      9.7386
    T-VALUE =      4.3765

THE VALUES FOR EXPERIMENTAL GROUP  4:
    N       =          5
    MEAN    =    183.6000
    S.E.M.  =     25.1189
    T-VALUE =      0.0558

THE VALUES FOR EXPERIMENTAL GROUP  5:
    N       =          4
    MEAN    =     85.1250
    S.E.M.  =      9.0907
    T-VALUE =      3.0563

THE VALUES FOR EXPERIMENTAL GROUP  6:
    N       =          6
    MEAN    =     78.9167
    S.E.M.  =     10.6571
    T-VALUE =      3.2455

THE VALUES FOR EXPERIMENTAL GROUP  7:
    N       =          3
    MEAN    =    172.5000
    S.E.M.  =     22.6991
    T-VALUE =      0.3940

THE VALUES FOR EXPERIMENTAL GROUP  8:
    N       =          6
    MEAN    =    110.0000
    S.E.M.  =     16.0888
    T-VALUE =      2.2984

THE VALUES FOR EXPERIMENTAL GROUP  9:
    N       =          4
    MEAN    =    261.2500
    S.E.M.  =     12.7631
    T-VALUE =     -2.3103
```

```
ENTER THE NUMBER OF VALUES OF EXPERIMENTAL GROUP  2

ENTER THE VALUES.

ENTER THE NUMBER OF VALUES OF EXPERIMENTAL GROUP  3

ENTER THE VALUES.

ENTER THE NUMBER OF VALUES OF EXPERIMENTAL GROUP  4

ENTER THE VALUES.

ENTER THE NUMBER OF VALUES OF EXPERIMENTAL GROUP  5

ENTER THE VALUES.

ENTER THE NUMBER OF VALUES OF EXPERIMENTAL GROUP  6

ENTER THE VALUES.

ENTER THE NUMBER OF VALUES OF EXPERIMENTAL GROUP  7

ENTER THE VALUES.

ENTER THE NUMBER OF VALUES OF EXPERIMENTAL GROUP  8

ENTER THE VALUES.

ENTER THE NUMBER OF VALUES OF EXPERIMENTAL GROUP  9

ENTER THE VALUES.

ENTER THE NUMBER OF VALUES OF EXPERIMENTAL GROUP 10

ENTER THE VALUES.

ENTER THE NUMBER OF VALUES OF EXPERIMENTAL GROUP 11

ENTER THE VALUES.

**********************************************************
**********************************************************

THE D.F. BETWEEN GROUPS =   11

THE D.F. WITHIN GROUPS =    51

THE F-VALUE IS     10.3804

THE VALUES FOR THE CONTROL GROUP:
    N       =          10
    MEAN    =    185.4300
    S.E.M.  =     16.4862
```

```
THE VALUES FOR EXPERIMENTAL GROUP 10:
    N       =           5
    MEAN    =    236.2000
    S.E.M.  =     26.2172
    T-VALUE =     -1.5470

THE VALUES FOR EXPERIMENTAL GROUP 11:
    N       =           4
    MEAN    =    294.7500
    S.E.M.  =     42.7368
    T-VALUE =     -3.3310

**********************************************************
**********************************************************

DO YOU WISH TO DO ANOTHER DUNNETT'S T-TEST?
#ET=2:30.1 PT=1.4 IO=1.0
GET STEVE;L
#WORKFILE STEVE: DATA, 26 RECORDS, SAVED
100     12
200     10
300      175.8  157.5 159 154.5 268.5 180 120 204 153 282
400     7
500     199.5 316.5 316.5 234 97.5 117 214.5
600     4
700     60 117 141 181.5
800     5
900     33 18 72 56 30
1000    5
1100    185 249 221 104 159
1200    4
1300    103.5 73.5 66 97.5
1400    6
1500    115.5 63 84 71 42 98
1600    3
1700    139.5 162 216
1800      6
1900      70.5 94.5 72 174 123 126
2000    4
2100    284 282 234 245
2200     5
2300      267 285 150 278 201
2400      4
2500       309 250.5 408 211.5
2600       N
#
BYE
#END SESSION 7208  ET=4:01.5 PT=1.7 IO=1.0
#USER = H00182701  15:44:46  03/19/83
```