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Alterations of central dopamine receptor sensitivity in the spontaneously hypertensive rat

John Richard Martin
University of the Pacific

ALTERATIONS OF CENTRAL DOPAMINE RECEPTOR SENSITIVITY IN THE SPONTANEOUSLY HYPERTENSIVE RAT

John Richard Martin

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This thesis, written and submitted by

John Richard Martin

is approved for recommendation to the Committee on Graduate Studies, University of the Pacific.

Department Chairman or Dean:

Carl O. Pound

Thesis Committee:

Raymond M. Zurek Chairman

T C West

Katherine Knapp

James W. Blankenship

Dated July 23, 1979

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Abstract of Thesis

Since the time of the introduction of the spontaneously hypertensive rat (SHR) into medical research, several neuropharmacological studies have been performed on these animals which have been unrelated to their cardiovascular systems. A few of these studies have indicated possible abnormalities in the dopaminergic systems of SHR. Therefore the present study was undertaken to examine more closely the sensitivity of SHR to stimulation of their dopamine (DA) receptors.

Three rat strains were utilized in this study: 1) SHR; 2) Wistar-Kyoto rat (WKY); and 3) Wistar rat (NWR). Three parameters were chosen on the basis of their appearance after stimulation of DA receptors in three different brain regions: 1) stereotyped behavior via stimulation of DA receptors in the corpus striatum; 2) locomotor activity via stimulation of DA receptors in the mesolimbic forebrain; and 3) hypothermia via stimulation of DA receptors in the hypothalamus. Apomorphine (APO) was chosen as the DA agonist.

No significant difference in APO-induced stereotyped behavior was found to exist between the three rat strains suggesting that there is no alteration in the sensitivity of DA receptors in the corpus striatum of SHR.

No significant difference was found to exist between the three rat strains with respect to either spontaneous locomotor activity or APO-induced locomotor activity suggesting that there is no alteration in the sensitivity of DA receptors in the mesolimbic forebrain of SHR.

SHR was found to be more sensitive than NWR to the hypothermic effect of APO. From parallel log-dose response curves of the three rat strains, APO and a second DA agonist, L-DOPA, were calculated to be more potent in producing hypothermia in SHR than NWR. WKY showed a hypothermic response intermediate to SHR and NWR. NWR and WKY were more susceptible than SHR to antagonism of APO-induced hypothermia by haloperidol. Chronic lithium treatment had no effect on APO-induced hypothermia in NWR and WKY. Lithium was found to significantly attenuate APO-induced hypothermia in SHR and to alter this response to one similar to those shown by NWR and WKY. Chronic hydralazine treatment in SHR had no effect on APO-induced hypothermia. Since lithium has the ability to "stabilize" supersensitive DA receptors, and APO-induced hypothermia in SHR, it is proposed that DA receptors in the hypothalamus responsible for mediating hypothermia are supersensitive in SHR.

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INTRODUCTION

A. Spontaneously Hypertensive Rat

Development of a genetically pure strain of rat in which there occurs 100 percent spontaneous hypertension was reported in 1963 by Okamoto and Aoki. These investigators isolated this strain by mating a male Wistar rat showing spontaneous hypertension to a female of the same strain displaying a blood pressure slightly above average. This mating produced F_1 rats. F_2 rats were obtained from brother-sister matings of F_1 rats which had been selected for their persistent hypertension. Pairs of F_2 rats which showed persistent hypertension were then selected for brother-sister matings to obtain F_3 rats. This procedure of matings was carried on to the sixth generation of offspring. It was noted that the incidence of occurrence of spontaneous hypertension and the development of hypertension occurred at younger ages from generation to generation so that by the third generation all rats developed spontaneous hypertension within fifteen weeks of age. Since the incidence of occurrence of spontaneous hypertension was 100 percent, these researchers suggested that this strain of Wistar rat be named "spontaneously hypertensive rat (Okamoto and Aoki)" (Okamoto and Aoki, 1963).

Being that this research was carried on at the Kyoto University in Japan, this particular colony of Wistar rat strain from which the spontaneously hypertensive rat was isolated became known as Wistar-Kyoto.

As an increased sympathetic tone quite often corresponds with hypertension, and may possibly be the cause in the spontaneously hypertensive rat (SHR), many investigations have tried to determine sympathetic activity in SHR. Earlier studies, utilizing biochemical techniques, showed a decreased norepinephrine (NE) turnover in cardiac tissue of SHR. Since NE uptake and endogenous levels were found to be the same in SHR as in the control rat strain, it was concluded that there is a decreased release of NE from heart tissue (Louis et al., 1968; Sjoerdsma, 1972). Further studies on this organ indicated a reduced rate of synthesis of NE in SHR. These differences could not be attributed to differences in endogenous levels of NE since these levels were the same in both strains (Louis et al., 1969, 1970; Sjoerdsma, 1972). Nakamura et al. (1971) confirmed the reduction in cardiac turnover in SHR but found that the endogenous levels were significantly lower. Furthermore, these researchers found that the higher the blood pressure the slower the NE turnover. These results led to the conclusion that the decrease in peripheral NE turnover represents a decrease in sympathetic nerve activity. This decrease in sympathetic activity was suggested to be an attempt to compensate for an increased peripheral vascular resistance.

In contrast to the biochemical evidence of a decrease in

sympathetic nerve activity in SHR, an increasing amount of literature suggests an augmented sympathetic tone. Okamoto and coworkers (1967) found that the discharge rate of the left splanchnic nerve of SHR was significantly greater than that of control Wistar rat. Application of square pulses (Iriuchijima, 1973) of voltage to the peripheral cut ends of the left or right splanchnic nerve indicated that the number of stimulations, or frequency, required to restore arterial pressure to the level prior to nerve severance was significantly greater in SHR than control Wistar rat. Assuming that this frequency represents the average discharge rate of the sympathetic vasoconstrictor fibers in the splanchnic nerve, it appears that SHR has a significantly greater discharge rate.

Direct measurements of sympathetic nerve activity had shown that there is a two- to three-fold increase in sympathetic activity in SHR. This increase in sympathetic activity was shown to be generalized to the nerves supplying the abdominal viscera and cervical sympathetic chain. A high correlation was found between the age of SHR and their corresponding sympathetic nerve activity. Blockade of sympathetic ganglia with hexamethonium lowered the blood pressure to identical levels in SHR and Wistar rat (Judy et al., 1976).

Inhibition of sympathetic nerve activity by reflex mechanisms indicated that the pressure-sensitive baroreceptors were not able to inhibit sympathetic nerve activity as effectively in SHR as in normotensive Wistar rat when stimulated by rises in systemic arterial pressure of equal magnitude and

duration (Judy et al., 1976; Coote and Sato, 1977). This reduction in effectiveness of the baroreceptors to completely inhibit sympathetic nerve activity decreases as a function of age (Judy et al., 1976). A high sympathetic activity in SHR has been suggested as the reason for the reduction in ability of the cardiovascular pressure receptors to inhibit ongoing sympathetic activity. In other words, the excitatory drive onto sympathetic neurons can better overcome baroreceptor inhibition in SHR than in Wistar rat. It was hypothesized that there is throughout life a continual imbalance in the pressure-receptor-sympathetic neuron control system which cannot be counteracted. This imbalance was suggested to be a consequence of increased sympathetic activity (Coote and Sato, 1977). Transection experiments by Okámoto et al. (1967) indicated that the increased sympathetic vasoconstrictor activity may originate in the ponto-bulbar portion of the brainstem.

Since baroreceptor sinus reflex appears to be less effective in inhibiting sympathetic nerve activity in SHR, alterations in baroreceptor function had been suggested as a causative factor for the hypertension. Nosaka and Wang (1972) found that the threshold pressure required for the carotid sinus to elicit hypotensive responses was significantly higher in SHR than in normotensive Wistar rat. Pressures required to stimulate maximum hypotensive responses were also significantly higher. These results indicate that baroreceptor sensitivity, at least of the carotid sinus, in SHR is shifted towards operating at higher pressures. No alterations in sensitivity of the baroreceptor response to changes in pressure

occur with this resetting. Similar results were found for the aortic sinus (Sapru and Wang, 1976). Additionally, it was found that resetting of the baroreceptors could be prevented by antihypertensive therapy of 11-week old SHR. Treatment of older SHR resulted in reversal of resetting in some but not all animals. Histological examination of vessel walls revealed that reversal or prevention of aortic hypertrophy correlated with reversal or prevention of baroreceptor resetting whereas partial reversal of baroreceptor resetting occurred with partial reversal of hypertrophy. It was suggested that high sympathetic tone results in irreversible hypertrophy of the tunica media of the vessel walls. This thickening of the aorta causes a resetting of the baroreceptors at a higher pressure level without altering the normal sensitivity of the baroreceptor.

Nakamura and Nakamura (1977) reported that an enhanced sympathetic activity in young SHR is not responsible for the development of hypertension. These investigators noted that young pre-hypertensive SHR have elevated activities of choline acetyltransferase and tyrosine hydroxylase (TH) in the celiac ganglia as compared to Wistar-Kyoto rat strain. Elevated activities of these enzymes were not found in the superior cervical ganglia, stellate ganglia or adrenal glands. This rise in activity of both enzymes in the celiac ganglia disappeared in the adult SHR. Bilateral removal of the celiac ganglion did not alter the development of hypertension. It was suggested that the faster maturation of the celiac ganglion in SHR is causally related to activation of the

peripheral sympathetic nervous system. Therefore, it was concluded that peripheral sympathetic activation in young SHR be regarded as a warning sign, but that this activation does not trigger the development of hypertension.

In contrast to Nakamura and Nakamura (1977), Vapaatalo et al. (1974) found that 6-hydroxydopamine (6-OHDA) given to newborn SHR prevented the development of hypertension. Since 6-OHDA is known to destroy peripheral and central adrenergic nerve terminals (Malmfors and Sachs, 1968; Thoenen and Tranzer, 1968; Tranzer and Thoenen, 1968; Bloom et al., 1969; Uretsky and Iversen, 1969, 1970), it was concluded that the sympathetic nervous system and/or adrenergic structures play an important part in the development of hypertension in SHR. It appears that interference with either of these systems shortly after birth can prevent the development of the biochemical and/or histopathological changes responsible for the development and/or maintenance of spontaneous hypertension. This interference could be due to either central or peripheral mechanisms. It may be a central mechanism as it had previously been shown (Haeusler et al., 1972) that administration of 6-OHDA into the lateral brain ventricle of 7-week old SHR prevented the development of hypertension. Furthermore, the dose-response curves of vascular reactivity to NE of animals that developed hypertension were steeper and displayed a higher maximum whereas the dose-response curves of SHR treated with 6-OHDA were virtually identical to those of normotensive controls. It was proposed that there is a central trigger mechanism for the development of hypertension.

The search for a central trigger mechanism has centered on central catecholamine levels, catecholamine turnover, and enzyme activities. Early studies indicated a decreased NE synthesis in the brainstem of SHR whereas the endogenous levels were similar between SHR and Wistar rat (Louis et al., 1970). Nakamura et al. (1971) found that NE levels in the hypothalamus and medulla-pons of 6- and 12-week old SHR were the same as Wistar rat. Wistar-Kyoto rat was later found to also have similar NE contents in these brain regions (De Jong et al., 1975). No change in NE content or turnover was found to exist in the brainstem of SHR in relation to changes in blood pressure (Nakamura et al., 1971). Yamori and co-workers (1971) found that the NE content of the brainstem and hypothalamus was significantly lower in SHR than in Wistar rat. It was noted in 6-week old SHR that DOPA decarboxylase activity was 50 percent of that of Wistar rat. This reduction in activity was not attributed to depression secondary to the increased blood pressure (Yamori et al., 1970; Nagatsu et al., 1972). Other studies also revealed a lower NE content and a slower rate of synthesis in the brainstem of SHR than Wistar rat (Sjoerdsma, 1972). However, Wistar-Kyoto rat (when this strain became available for study) was found to have a NE content similar to that of SHR (Yamabe et al., 1973; Osumi et al., 1974). Similar results were found with respect to NE turnover when SHR were compared to Wistar-Kyoto rat. Enzyme studies showed that Wistar rat had significantly higher levels of activity of TH, DOPA decarboxylase, and dopamine-beta-hydroxylase (DBH) than either SHR or Wistar-Kyoto rat. It

appears that the variations in catecholamine levels, catecholamine turnover, and enzyme activities arise from genetic factors that may not be related to the pathogenesis of hypertension.

It had been suggested that these earlier studies, because of the gross areas investigated, failed to detect changes in small brain regions and that it is likely that central cardiovascular control is mediated by individual brain areas. Thus Versteeg and coworkers (1976, 1978) studied smaller regions and found variations in SHR in regions thought to be involved in regulation of blood pressure. Elevated levels of dopamine (DA) were found in the nucleus interstitialis striae terminalis and frontal cortex of SHR as compared to Wistar-Kyoto rat. No differences were found in NE and DA levels in the hypothalamic nuclei. Elevated levels of epinephrine (EPI) were found in the hypothalamic nuclei investigated. However, only the nucleus paraventricularis of SHR showed a significantly higher EPI level. Many areas of SHR brainstem had elevated levels of NE, including the A2-region of the nucleus tractus solitarii. Elevations of DA and EPI were also found in this region. It appears that SHR has elevated catecholamine levels in individual brain regions which are supposedly involved in the central control of arterial blood pressure. How these changes in brain catecholamine levels are related to the evolution of hypertension in SHR has yet to be determined.

However, Nakamura and Nakamura (1978) recently reported observing selective activation of A2-region noradrenergic

neurons in young SHR as indicated by an increase in DBH activity. Elevated activity of this enzyme was also found in the locus coeruleus and thoracic intermediolateral cell area of the spinal cord. This elevation of DBH activity was not apparent in adult SHR (Nakamura and Nakamura, 1978). Young SHR also had significantly elevated levels of NE and DBH, confirming earlier studies, which was interpreted as an increase in peripheral sympathetic nerve activity (Roizen et al., 1975; Nagaoka and Lovenburg, 1976; Nakamura and Nakamura, 1978). These investigators concluded that the selective activation of noradrenergic neurons of the A2-region, locus coeruleus, and thoracic intermediolateral cell area, in combination with the activation of the sympathetic nervous system, initiates hypertension in young SHR (Nakamura and Nakamura, 1978).

Several other studies have indirectly implicated a central involvement in the hypertension of SHR. L-DOPA administered to SHR significantly decreased blood pressure. Coupling the L-DOPA with a peripheral DOPA decarboxylase inhibitor potentiated the depressor response. Examination of brainstem NE levels showed that a depressor response occurred only when there was a concomitant rise in brainstem NE levels (Yamori et al., 1972). Judy et al. (1978) recently confirmed these results. Additionally, they found that SHR were more sensitive to sympathetic nerve activity inhibition by L-DOPA combined with a peripheral DOPA decarboxylase inhibitor. Greater distribution of L-DOPA to the central nervous system of SHR was eliminated as a possible explanation for these results as the amount of L-DOPA in the cerebral parenchyma

was equivalent in both rat strains (Judy et al., 1978). It appears that there may be a highly significant inverse relationship between brainstem NE levels and blood pressure (Yamori et al., 1972).

Like the brainstem, the hypothalamus has also been implicated in the development of hypertension in SHR. Yamori and Okamoto (1969) discovered that when the neural connection between the posterior hypothalamus and mesencephalon was separated, a significantly greater fall in blood pressure was elicited in SHR as compared to Wistar rat. Other separations in the hypothalamus or between the hypothalamus and other brain structures were ineffective in producing depressor responses. These results provide evidence of hypothalamic tonic influence on blood pressure and its participation in pathogenesis of hypertension in SHR.

Various studies have shown that the posterior hypothalamus mediates pressor responses (Karpus and Kreidl, 1918, 1927) while the anterior hypothalamus mediates depressor responses (Folkow et al., 1959, 1964; Phillippu and Schartner, 1976). EPI and NE may be the neurotransmitters involved in the depressor response (Struyker Boudier and Bekers, 1975; Struyker Boudier, 1975). Intrahypothalamic increases in cyclic-AMP (cAMP) have been shown to result in pressor responses. Conversely, decreases in cAMP result in depressor responses (Walland, 1975). Weak electrical stimulations of the posterior hypothalamus, which were ineffective in Wistar rats, were found to elicit measurable responses in SHR. Currents above threshold always elicited greater pressor responses in SHR.

Hypersensitivity of the posterior hypothalamic pressor areas of SHR was considered as an explanation for these results. However, possible hypersensitivity of other sites in the efferent neural pathway could not be excluded (Buñag et al., 1975). Hypothalamic concentrations of cAMP has recently been shown to be lower in SHR than in Wistar-Kyoto rat whereas cortical levels of cAMP did not differ. It was suggested that reduced levels of hypothalamic cAMP may be related to the elevated blood pressure of SHR. In any case, these results indicate a possible alteration of the adenylate cyclase-cAMP-phosphodiesterase system in hypothalamic structures of SHR (Schmid et al., 1978).

Because of the possible variations of catecholamine levels within certain brain regions of SHR, several behavioral studies have been performed on this particular rat strain. Open field tests had indicated that SHR were at a lower emotional state than Wistar rat (Shimamoto and Nagaoka, 1972; Pappas et al., 1974). A greater excitement of the cortex of SHR had been postulated from their apparently greater spontaneous activity (Shimamoto and Nagaoka, 1972; Pappas et al., 1974; Myers et al., 1977). Greater cortical excitement may be related to the finding that SHR were more sensitive to electroshock seizures. This increased sensitivity was correlated with a slower turnover of NE in the brainstem and cortex and of DA in the striatum (Goldberg et al., 1975).

Behavioral responses induced by methamphetamine, apomorphine and L-DOPA have been assessed by scoring. Methamphetamine caused greater behavioral excitement in SHR as indicated

by an increase in peak effect and duration of action. Subcutaneous administration of apomorphine resulted in similar stereotyped gnawing behavior in both SHR and Wistar rat. L-DOPA also resulted in similar behavioral responses. It was concluded from these drug-induced behavioral changes that SHR are more susceptible to release of DA from corpus striatum (Shimamoto and Nagaoka, 1972).

Sidman avoidance response rates showed that the average avoidance response in SHR increased after the fifth test and remained significantly higher than that shown by Wistar rat (Takaori et al., 1972). Rosecrans and Adams (1976) also observed higher avoidance rates which they interpreted as being indicative of a lower emotional state. Treatment with para-chlorophenylalanine (p-CPA) resulted in an increase in avoidance in both strains, but the increase in SHR was not significant due to the higher initial level. L-5-hydroxytryptophan (5-HTP) markedly lowered the frequency of avoidance responses in SHR whereas only a high dose lowered the frequency in Wistar rat. Treatment with alpha-methyltyrosine had no effect on the avoidance rates. L-DOPA produced a slight increase in avoidance response in Wistar rat, whereas the same dose caused a marked decrease in frequency in SHR. Since p-CPA depleted brain serotonin and 5-HTP increased brain serotonin, these results indicate that there may be an abnormality in serotonin metabolism in SHR. Additionally, the response to L-DOPA may indicate an alteration in SHR brain catecholamine metabolism as there were observable increases in central catecholamine levels (Takaori et al., 1972).

In keeping with the possibility of abnormal serotonin metabolism in SHR, Rosecrans and Adams (1978) attempted to correlate changes in serotonin metabolism to changes in behavior. These investigators found enhanced serotonin turnover in cerebral cortex, diencephalon, and brainstem, with significantly increased turnover occurring in the limbic forebrain. SHR were found to be less active than the Wistar rat upon initial testing of spontaneous motor activity, an observation inconsistent with previous studies. When SHR were subjected to repeated testing situations they were found not to habituate as readily as Wistar rat, as evidenced by an eventual higher spontaneous activity than Wistar rat. Similar results were obtained with respect to rearing. It was concluded that habituation is an active process which is retarded in SHR because of hyperfunctional serotonergic systems.

Body temperature studies by Tanaka and others (1972) showed that the body temperature response of SHR to L-DOPA differed from that displayed by Wistar-Kyoto rat. These investigators found that L-DOPA alone was able to cause hypothermia in SHR. L-DOPA administered alone to Wistar-Kyoto rat resulted in a hyperthermic response. Only when L-DOPA was coupled with a peripheral DOPA decarboxylase inhibitor pretreatment was a hypothermic response in Wistar-Kyoto rat observed. Since L-DOPA-induced hyperthermia is mediated peripherally and hypothermia centrally, these researchers concluded that this difference in temperature response was due to the presence of a more permeable blood-brain-barrier in

SHR.

B. Apomorphine-Induced Stereotypic Behavior in the Rat

Administration of apomorphine to rats results in its accumulation within minutes in all brain regions (Butterworth and Barbeau, 1974). As a consequence of its presence, a behavioral syndrome is elicited which follows a time course that parallels this accumulation (Butterworth and Barbeau, 1974). This behavioral syndrome is known as "stereotyped behavior", and consists largely of purposeless, repetitive, compulsive movements. These movements begin as discontinuous sniffing which progresses to continuous sniffing concomitant with repetitive head and forelimb movements. Increased locomotor activity then occurs in the presence of this continuous sniffing giving the impression of exploratory activity. This behavior is eventually replaced by continuous gnawing, biting, or licking as the rat restricts its motor activity, if any, to a single area. The purposeless, repetitive, compulsive nature of this activity justifies its "stereotyped" label (Di Chiara and Gessa, 1978).

The importance of the corpus striatum in the mediation of the apomorphine-induced gnawing behavior was demonstrated very early (Harnack, 1874; Amsler, 1923). Ernst and Smelik (1966) narrowed down the location by implanting microquantities of DOPA or apomorphine into various regions of the brain. The gnaw-syndrome was elicited only when these chemicals were administered to the dorsal part of the caudate nucleus and globus pallidum. No change in behavior was observed when

either of these drugs was implanted into the ventral caudate nucleus, nucleus lateralis septi, subthalamic structures, or the substantia nigra. It was concluded that the specific area of the caudate nucleus that is involved in this syndrome is the neostriatum. It was suggested that accumulation of DA in this structure, as shown by Bertler and Rosengren (1959), is responsible for the gnawing behavior and that apomorphine, because of its structural similarity to DA, is able to stimulate DA receptors thus mimicking the effects of DA (Ernst and Smelik, 1966). Dose-dependent increases in stereotyped behavior were observed when apomorphine was injected into the caudate nucleus while bilateral electrocoagulation of this region inhibited the response to intraperitoneally-administered apomorphine (Nagy and Decsi, 1977). The course of study on the putative dopaminergic agonistic activity of apomorphine has taken two routes: 1) those which have employed biochemical techniques, and 2) those which have utilized whole animals.

Several studies have determined the amount of DA depletion after inhibition of TH with alpha-methyltyrosine. These studies have consistently shown that apomorphine causes a retardation of DA depletion (Andén et al., 1967; Butcher and Andén, 1969). Unilateral transection of the nigrostriatal pathway interrupts the concentration of DA at striatal receptor sites. Treatment with the DA receptor blocker, haloperidol, after DOPA decarboxylase inhibitor pretreatment, was found to have no effect on the lesioned side while the intact side showed an increase in DOPA concentration (Kehr et al., 1972). Apomorphine treatment resulted in a bilateral decrease

in DOPA concentrations which could be antagonized by haloperidol. Measurement of DA levels indicated that apomorphine reduced DA to similar levels on the intact side (Kehr et al., 1972). Further experiments demonstrated that apomorphine was able to significantly decrease the extraneuronal metabolite of DA, homovanillic acid (Roos, 1969). From these results it was concluded that apomorphine is able to reduce the activity of DA neurons via a negative-feedback mechanism due to stimulation of DA receptors (Andén et al., 1967; Butcher and Andén, 1969), and that this feedback mechanism controls the rate of tyrosine hydroxylation (Kehr et al., 1972).

Apomorphine was also demonstrated to decrease TH activity in synaptosomes while haloperidol had little or no effect. Haloperidol did partially attenuate the inhibitory action of apomorphine on TH. It was postulated that haloperidol may be able to block only one of two actions that apomorphine may have on TH activity (Christiansen and Squires, 1974). Besides apparently reducing TH activity by a receptor-mediated feedback mechanism as a result of direct DA receptor stimulation, apomorphine may also directly inhibit this enzyme. Goldstein and coworkers (1970) found that apomorphine could inhibit TH as well as inhibit the synthesis of DA from tyrosine in striatal slices. Haloperidol added to the striatal slices did not affect the inhibitory action of apomorphine. Thus it may be that apomorphine, besides decreasing TH activity indirectly via a feedback mechanism, may also be able to directly inhibit TH activity. This was considered to be in agreement with its cathechol stucture (Golstein et al., 1970).

Structural examination of the apomorphine molecule also suggests that it is capable of possessing dopaminergic activity. By studying the gnaw-syndrome in rats, Ernst (1964, 1967) determined a phenylethylamine configuration with a hydroxy-group at the para-position, or at the para- and meta-positions of the phenol-ring is necessary for the gnaw-syndrome. Other studies implicated the requirement of the dihydroxytetrahydroaminonaphthalene moiety for the induction of the gnaw-syndrome (Pinder et al., 1971, 1972), which is supported by molecular orbital calculations using the extended Hückel theory (Rekker et al., 1972). These data indicate that the active structures of dopamine and apomorphine are extremely similar.

The specificity of apomorphine for inducing changes in DA neuron activity was shown by Anden and others (1967). Apomorphine was found to be ineffective on the flexor reflex in spinalized rats in contrast to noradrenergic receptor stimulants. They also found that apomorphine did not influence the disappearance of NE from the brain or spinal cord after TH inhibition with alpha-methyltyrosine. Furthermore, haloperidol, a rather specific DA receptor blocker (Van Rossum, 1966), blocked the effects of apomorphine (Anden et al., 1967).

Demonstration of direct DA receptor stimulation action by apomorphine has also been shown in animals pretreated with enzyme inhibitors or catecholamine depletors. Pretreatment of rats with alpha-methyltyrosine or alpha-methyldopa did not block the stereotypic action of apomorphine (Ernst, 1967). Costall and Naylor (1973a) found that reserpine, as well as

alpha-methyltyrosine, enhanced apomorphine-induced stereotyped behavior. 6-OHDA administered intraventricularly to rats was found to enhance some of the stereotypic effects (Schoenfeld and Uretsky, 1972) while a separate study showed potentiation of all stereotypic effects (Price and Fibiger, 1974). Although finding no significant difference in the log-dose response curves between rats treated intraventricularly with 6-OHDA and untreated rats, Jalfre and Haefely (1971) demonstrated that haloperidol completely blocked the gnawing response to apomorphine. 6-OHDA-induced lesions of the caudate nucleus also resulted in enhanced apomorphine-induced stereotyped behavior (Kelly et al., 1975; Iversen et al., 1975). An earlier study indicated that bilateral lesions of the substantia nigra with 6-OHDA resulted in furious compulsive gnawing which was far more violent than after the same dose of apomorphine administered to a normal rat (Ungerstedt, 1971). These results provide further evidence that apomorphine is capable of directly stimulating DA receptors.

Besides observing an enhancement of stereotypy in intraventricularly 6-OHDA-treated rats, Schoenfeld and Uretsky (1972) noted that this treatment abolished gnawing suggesting that intact dopaminergic neurons are necessary for full expression of apomorphine-induced stereotypy. Oxyperitine and Ro 04-1284 were also found to reduce or abolish the stereotypy (Costall and Naylor, 1973a). Electrolytic bilateral ablation of the substantia nigra or lesions of the ascending dopaminergic fibers of the nigrostriatal pathway abolish or reduce apomorphine-induced stereotypy (Costall et al., 1972;

Costall and Naylor, 1973b). From these findings it appears that the action of apomorphine depends to a great extent on intact dopaminergic neurons.

Studies which have utilized electrolytic lesions have been criticized on the basis that this technique not only disrupts dopaminergic pathways, but also interrupts the function of other pathways. Unilateral intranigral injection of kainic acid produces rotation of the treated animal to the side away from the lesion, or contralateral turning. Similarly, unilateral electrolytic lesions of the substantia nigra also result in contralateral turning. In contrast, unilateral lesion of the nigrostriatal dopaminergic pathway by intranigral injection of 6-OHDA, which is just as effective in destroying dopaminergic neurons as electrolytic lesions, results in turning towards the side of the lesion, or ipsilateral turning. Since it was shown that kainic acid produced contralateral turning independently of dopaminergic nigrostriatal function, it was postulated that non-dopaminergic neurons exist in the substantia nigra which control turning behavior in a manner opposite to that of the nigrostriatal dopaminergic neurons. These results were suggested to provide an explanation for some turning responses obtained from electrolytic lesions of the substantia nigra or nigrostriatal pathway which are not readily explained by an action on the nigrostriatal DA system (Di Chiara et al., 1977; Olianas et al., 1978). Some of these other non-dopaminergic pathways, which could easily be disrupted by electrolytic lesions, may be necessary for complete expression of apomorphine-induced

stereotyped behavior (Di Chiara and Gessa, 1978).

In addition to these postulated pathways, other neurotransmitters and brain structures have been implicated in the mediation of the stereotypic effects of apomorphine. Thus it was found that electrocoagulation of the noradrenergically-innervated nucleus amygdaloideus lateralis abolished the gnawing, biting, and licking behavior induced by apomorphine while intensifying the sniffing behavior and the repetitive movements. It was suggested that the nucleus amygdaloideus lateralis, and therefore noradrenergic neurons, are involved, either directly or indirectly, in the mediation of the gnawing, biting, and licking components of apomorphine-induced stereotyped behavior (Costall and Naylor, 1972). These observations were similar to those of Schoenfeld and Uretsky (1972). Intraventricular administration of 6-OHDA may have depleted the noradrenergic stores of the nucleus amygdaloideus lateralis thereby abolishing the gnawing, biting, and licking components of apomorphine-induced stereotypy while allowing the enhancement of the sniffing and repetitive movements.

It is becoming apparent that some of the dopaminergically-innervated structures of the mesolimbic forebrain are also important in the mediation of apomorphine stereotypy. It was found that bilateral destruction of the tuberculum olfactorium markedly reduced apomorphine-induced stereotyped behavior in the rat (McKenzie, 1972; Costall et al., 1973, 1975). Destruction of the nucleus accumbens was less effective than tuberculum olfactorium lesions in reducing the effects of apomorphine while both of these regions were found to be essential

for the development of sniffing and repetitive head movements (Costall and Naylor, 1973). Similarly, lesions of the ascending dopaminergic fibers to the mesolimbic forebrain reduced apomorphine-induced stereotyped behavior. In contrast to reductions, Kelly and coworkers (1975) found that 6-OHDA lesions of the nucleus accumbens enhanced the sniffing behavior upon subsequent treatment with apomorphine. These results indicate that the mesolimbic forebrain is important in mediating the sniffing and repetitive movements induced by apomorphine.

A detailed examination (Costall et al., 1975) of the stereotypic effects induced by apomorphine revealed that these effects can be differentiated into two dose-dependent components. Low doses of apomorphine result in the appearance of the low-intensity component which consists of sniffing, and repetitive head and limb movements. Higher doses of apomorphine result in the high-intensity component consisting of gnawing, biting, or licking. By various central lesions and subsequent administration of apomorphine, these two components were found to be mediated by different brain regions. Bilateral lesions of the caudate putamen (neostriatum) failed to have any effect on apomorphine-induced stereotypy (Divac, 1972; McKenzie, 1972; Costall et al., 1973, 1975). Intracaudate administration of apomorphine resulted in the development of periodic biting without sniffing and repetitive head and limb movements. Lesions of the substantia nigra abolished the biting response without affecting the low-intensity component. Lesions of the nucleus accumbens abolished the sniffing

response to apomorphine while enhancing the intensity of the biting component. Injection of apomorphine into this nucleus resulted in sniffing behavior that was dose-dependent. Biting was also observed but did not appear to be dose-dependent. Lesions of the tuberculum olfactorium also abolished the low-intensity component. However, the biting component was also reduced. Administration of apomorphine into the tuberculum olfactorium resulted in the development of both high- and low-intensity components of stereotypy. As with the nucleus accumbens, the intensity of sniffing appeared to be dose-dependent whereas the biting response remained periodic at all doses. It appears that the high-intensity component of apomorphine-induced stereotyped behavior is mediated largely by the extrapyramidal systems, while the low-intensity component is mediated by the mesolimbic system (Costall et al., 1975).

Recently, Ljungberg and Ungerstedt (1977a) reported observing two independent behavioral patterns elicited by subcutaneous (s.c.) injections of apomorphine. These two behavioral patterns seem to follow the low- and high-intensity components described by Costall et al. (1975). Low-intensity behavior was observed when apomorphine (as the base) was dissolved by heating and injected s.c. into the neck, or when it was dissolved by heating together with a high concentration of ascorbic acid and injected s.c. into the flank or neck. High-intensity behavior resulted when apomorphine, dissolved by heating, was injected s.c. into the flank. Additionally, it was found that these results were not merely due

to differences in dose as the low-intensity responses could not be obtained by lowering the dose of the high-intensity treatment, and the high-intensity responses could not be obtained by increasing the dose of the low-intensity treatment. Failure of being able to change the intensity of the response by changing the dose of apomorphine suggests that the sniffing and repetitive head and limb movements are not of a lower intensity than compulsive gnawing as previously suggested.

Additional studies showed that apomorphine-induced gnawing is greatly affected by such factors as test environment and experimental design. Locomotion, which usually occurs simultaneously with the apomorphine-induced sniffing behavior, was shown to be essentially independent of these factors. It was found that restricting an animal's ability to locomote by restricting its area in which to move did not increase its gnawing behavior. However, it was shown that pretreatment of these rats with a dose of clozapine that is able to antagonize apomorphine-induced locomotion without affecting gnawing behavior could change the locomotor behavior to gnawing behavior. It was suggested that these results indicate that apomorphine-induced locomotion is more compulsive than the so-called "compulsive gnawing" (Ljungberg and Ungerstedt, 1978).

C. Dopaminergic Involvement in Control of Locomotor Activity in Rat

A role for DA has been suggested in the control of locomotor activity in the rat. Marked increases in both locomotor

activity and brain levels occurred when rats were peripherally administered L-DOPA coupled with a DOPA decarboxylase inhibitor pretreatment. Although NE levels remained unchanged, increases in motility only occurred in the presence of sufficient NE (Ahlenius and Engel, 1971; Maj et al., 1971; Friedman and Gershon, 1972). These results agreed with an earlier study (Everett and Weigand, 1962) which indicated that both DA and NE were important in L-DOPA-induced locomotor activity. L-DOPA coupled with a monoamine oxidase inhibitor (MAO-I) pretreatment resulted in an increase in brain levels of both DA and NE, as well as stimulating locomotor activity (Green and Kelly, 1976; Heal et al., 1976). Rats pretreated with a DBH inhibitor known to reduce brain NE concentration had no effect on the MAO-I/L-DOPA-induced locomotor activity (Green and Kelly, 1976). Dopaminergic receptor blockers were found to antagonize the increase in activity (Heal et al., 1976). Thus it appears that DA receptor stimulation is necessary for locomotion while the role of NE remains equivocal.

Intraventricular injection of DA was found to result in an increase in locomotor activity that was dose-dependent. Examination of brain levels of DA and NE indicated that a low dose of DA did not result in significant changes whereas a high dose resulted in significantly higher levels of DA which gradually decreased as NE levels increased (Malec and Kleinrok, 1972). Costall and coworkers (1975, 1976a) observed dose-dependent hyperactivity after bilateral injection of DA into the nucleus accumbens. Nialamide (Pijnenburg and Van Rossum, 1973; Costall and Naylor, 1975; Costall et al., 1976a)

or reserpine/nialamide pretreatments (Andén and Jackson, 1975) were found to strongly enhance locomotor activity. Bilateral administration of NE to this nucleus after nialamide pretreatment only slightly enhanced locomotor activity (Pijnenburg and Van Rossum, 1973). Bilateral injection of DA into the tuberculum olfactorium resulted in periodic increases in locomotor activity (Costall and Naylor, 1975) whereas only a slight to moderate increase occurred after administration to the caudate nucleus (Pijnenburg and Van Rossum, 1973). Although it appears that dopaminergic receptors of the nucleus accumbens are involved in the control of locomotor activity, the possible involvement of NE could not be conclusively excluded by these studies.

Later studies performed by Costall and Naylor (1976a) showed that the dose-dependent increases in locomotor activity produced by bilaterally-applied DA to the nucleus accumbens of nialamide pretreated rats could be antagonized by intraperitoneal administration of the so-called typical DA receptor blockers (haloperidol, fluphenazine, pimozide, clothiapine) and atypical DA receptor blockers (clozapine, sulpiride, thioridazine). Systemic administration of pimozide to reserpine/nialamide pretreated animals effectively antagonized the locomotor activity produced by DA injected bilaterally into the nucleus accumbens (Jackson et al., 1975). The alpha-adrenergic antagonist, phenoxybenzamine, and the beta-adrenergic antagonist, propranolol, were both ineffective in blocking the hyperactivity (Jackson et al., 1975). These studies provide further evidence that dopaminergic receptor mechanisms,

while not necessarily noradrenergic receptor mechanisms, are involved in the control of locomotor activity.

Several other studies have attempted to determine the importance of DA and NE in the control of locomotor activity. Pijnenburg and coworkers (1975a) used amphetamine to differentiate the contribution of these two amines to the control of locomotor activity. Haloperidol administered bilaterally into the nucleus accumbens blocked amphetamine-induced hyperactivity while administration of the alpha-adrenergic antagonist, phentolamine, or propranolol, did not significantly inhibit locomotor stimulation. Bilateral administration of haloperidol into the caudate nucleus did not inhibit amphetamine-induced locomotor activity. Haloperidol antagonized the locomotor stimulation by both DA and NE injected into the nucleus accumbens of nialamide pretreated rats (Pijnenburg et al., 1975b). Phentolamine, on the other hand, potentiated the hyperactivity produced by both of these amines, suggesting that the stimulation of locomotor activity induced by NE is the indirect result of dopaminergic mechanisms (Pijnenburg et al., 1975b). NE and the alpha-adrenergic agonists, clonidine and phenylephrine, injected into the nucleus accumbens of non-pretreated animals produced depression rather than stimulation while DA resulted in a significant increase in locomotor activity which could be antagonized by haloperidol (Pijnenburg et al., 1976). These results agree with the earlier studies which implicate dopaminergic receptors of the nucleus accumbens in the control of locomotor activity. Although the results on NE involvement remain

equivocal, this neurotransmitter may be indirectly involved via dopaminergic systems in the control of locomotor activity.

Apomorphine unexpectedly produced both stimulant and depressant effects when applied bilaterally into the nucleus accumbens, but produced locomotor stimulation when applied bilaterally into the tuberculum olfactorium (Pijnenburg et al., 1976). In contrast, Jackson and coworkers (1975) found apomorphine induced locomotor activity similar to that produced by DA applied bilaterally into the nucleus accumbens.

Systemic administration of apomorphine was shown by Maj et al. (1972) to stimulate an increase in locomotor activity. Haloperidol and spiroperidol blocked the increase induced by lower doses of apomorphine while higher doses were able to overcome the blockade. Phenoxybenzamine antagonized the higher doses suggesting the involvement of NE in apomorphine-induced hyperactivity. Furthermore, alpha-methyltyrosine, dimethyldithiocarbamate, and disulfiram partly inhibited the locomotor effect of apomorphine. It was concluded that both DA and NE are involved in the control of motility in rats, and that apomorphine induces hyperactivity in rats by direct stimulation of DA receptors (Maj et al., 1972). In a following report, Maj and coworkers (1973) found that alpha-methyltyrosine did not significantly affect the locomotor response, although the motility induced by apomorphine was reduced by this treatment.

Systemic administration of apomorphine to rats with bilateral 6-OHDA lesions of the nucleus accumbens resulted in

an enhanced locomotor response which could be blocked by pimozide (Iversen et al., 1975).

In contradiction to their results obtained with DA, Costall and coworkers (1975) found that bilateral injections of apomorphine directly into the nucleus accumbens resulted in activity that was indistinguishable from that exhibited by animals receiving solvent injections. In later studies, Costall and Naylor (1976b) discovered that systemically-administered apomorphine in substereotypic doses was able to antagonize, in a dose-dependent manner, the hyperactivity induced by DA administered directly into the nucleus accumbens. Furthermore, apomorphine administered directly into the nucleus accumbens was able to antagonize the locomotor stimulant effect of directly applied DA. It was suggested that these results may indicate that while apomorphine has affinity for the DA receptors in the nucleus accumbens, it may lack intrinsic activity. These authors also speculated on the possible activity that apomorphine may have on serotonin mechanisms in the nucleus accumbens (Costall and Naylor, 1976b).

Earlier studies had shown that lesions of the midbrain raphe nucleus resulted in marked decreases in the levels of serotonin and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in the forebrain. A concomitant increase in locomotor activity was observed suggesting that serotonin may play an inhibitory role in the control of locomotor activity (Kostowski et al., 1968). Electrolytic lesions of the medial raphe nucleus resulted in an enhanced locomotor response to apo-

morphine (Grabowska, 1974) while pretreatment of animals with the serotoninolytics, methysergide and brom-lysergic acid diethylamide, also enhanced apomorphine-induced hyperactivity (Grabowska and Michaluk, 1974). It was hypothesized that serotonin plays an inhibitory role in apomorphine-induced locomotor stimulation in rats. Since it had also been observed that the decline of apomorphine-induced hyperactivity was inversely related to the elevation of 5-HIAA concentration, it was suggested that apomorphine activates central serotonin neurons and thereby attenuates its own stimulatory effect on locomotor activity (Grabowska et al., 1973a). Indirect support for this hypothesis was provided by Costall et al. (1976b) when they showed that serotonin injected into the nucleus accumbens resulted in dose-dependent reductions in the hyperactivity induced by prior injections of DA. Larger doses completely abolished the DA effect. When forebrain concentrations of serotonin were reduced by lesions of the medial raphe nucleus, it was found that the hyperactivity response to DA was markedly enhanced. These results were suggested to indicate that the facilitatory effect of DA on locomotion may be modulated by an inhibitory influence from serotonergic mechanisms (Costall et al., 1976b). It was also noted that the antagonism of the DA-induced locomotor activity by serotonin after injections of these putative neurotransmitters into the nucleus accumbens was similar to that produced by apomorphine administered in the same manner (Costall and Naylor, 1976b).

D. Apomorphine- And L-DOPA-Induced Hypothermia in Rat

Apomorphine has been consistently reported to cause a decrease in the core body temperature of the rat (Kruk and Brittain, 1972; Kruk, 1972; Yehuda and Wurtman, 1972; Hansen and Whisaw, 1973; Grabowska et al., 1973b; Grabowska, 1974; Scheel-Kruger and Hasselager, 1974; Chiel et al., 1974; Przewlocki, 1976, 1977; Ary et al., 1977; Cox and Lee, 1977a, 1977b). Various injection methods have been used in these numerous studies. Kruk and Brittain (1972) found that intraventricular injection of apomorphine resulted in a fall in core temperature along with a rise in skin temperature. Kruk (1972) reported a dose-dependent decrease in core body temperature and an increase in skin temperature after intraventricular administration of apomorphine. Pretreatment with pimozide antagonized the activity of apomorphine. Similarly, several other studies have shown that pimozide and spiroperidol can block the hypothermic action of systemically-administered apomorphine (Yehuda and Wurtman, 1972; Grabowska et al., 1973b). It appears that apomorphine mediates hypothermic responses through activation of DA receptors.

Development of tolerance to the hypothermic effects of apomorphine has been shown to occur with repeated administration. Chiel and coworkers (1974) showed that weekly injections of apomorphine resulted in a gradual decline in the hypothermic response. It was suggested that the development of tolerance was due at least in part to alterations in the sensitivity of DA receptors.

The site of action of apomorphine has been demonstrated by Cox and Lee (1977a, 1977b) to reside within the preoptic-anterior hypothalamus. Administration of apomorphine into the preoptic-anterior hypothalamus and the lateral ventricle resulted in hypothermic responses. The response elicited from intrahypothalamic administration was of greater magnitude and took significantly less time to develop. A separate study (Ary et al., 1977) reported similar results. In addition it was found that hypothermia induced by systemic injections of apomorphine could be antagonized by pretreatment with pimozide injected into the rostral hypothalamus. Intraventricular administration of pimozide did not block the hypothermic response to systemically-administered apomorphine. Tolerance was discovered to occur to repeated injections of apomorphine given both systemically and intrahypothalamically. Repeated injections of apomorphine into the lateral ventricle did not result in the development of tolerance. It was suggested that apomorphine can induce hypothermia via two different types of DA receptors at two different sites: 1) in the rostral hypothalamus where the receptors develop tolerance and where the temperature is due to a downward shift in the set point as determined by behavioral thermoregulation studies; and 2) in areas surrounding the lateral ventricle where receptors do not develop tolerance (Ary et al., 1977). The possibility of a relay from the lateral ventricular sites to the hypothalamus was also suggested.

Since serotonin has been shown to be involved in the control of locomotor activity, the possible involvement of

this putative neurotransmitter in apomorphine-induced hypothermia has also been investigated. Grabowska (1974) found that lesions of the medial raphe nucleus attenuated the hypothermia caused by apomorphine. Contradictory results were reported by Przewlocki (1977) who found that electrolytical and chemical lesions of both the dorsal and medial raphe nuclei prevented apomorphine-induced hypothermia. Lesions of the medial raphe nucleus by both methods did not affect the fall in body temperature whereas lesions of the dorsal raphe nucleus attenuated the hypothermia. Additionally, bilateral electrolytic lesions of the caudate nucleus, substantia nigra, or nucleus accumbens did not affect or potentiated the hypothermic response. It was concluded that mesolimbic dopaminergic neurons were not involved in the hypothermia elicited by apomorphine, whereas the neurons of the dorsal raphe nucleus may be involved in this action of apomorphine (Przewlocki, 1977). On the basis of these results, it has been suggested that part of the hypothermic action of apomorphine may possibly be the result of indirect activation of serotonergic neurons which originate in the midbrain (Grabowska, 1974; Przewlocki, 1976).

The results of locomotor activity studies have suggested that L-DOPA-induced increases in locomotion in the rat are related to the conversion of L-DOPA to DA with subsequent activation of DA receptors. L-DOPA has also been shown to decrease body temperature which could be assumed to also be due to the formation of DA and stimulation of DA receptors in the preoptic-anterior hypothalamus. However, Reichenburg and

Vetulani (1973) reported that hypothermia induced by intraventricular injection of L-DOPA was unaffected by spiroperidol. Maj and Pawlowski (1973) confirmed these results by showing that L-DOPA administered with a peripheral DOPA decarboxylase inhibitor also elicited a hypothermic response which was unaffected by spiroperidol or pimozide. Additionally, the L-DOPA treatment caused a decrease in brain serotonin levels while increasing 5-HIAA levels. Since the serotonin depletor p-CPA attenuated both the fall in body temperature and the changes in brain serotonin and 5-HIAA levels, it was suggested that the release of serotonin may be responsible for L-DOPA-induced hypothermia.

E. Statement of the Problem

Several studies have indicated possible alterations in the levels and metabolism of brain monoamines in SHR (Yamori et al., 1970; Nakamura et al., 1971; Shimamoto and Nagaoka, 1972; Sjoerdsma, 1972; Goldberg et al., 1975; Rosecrans and Adams, 1976; Versteeg et al., 1976, 1978). Several of these have been neuropharmacological in nature (Shimamoto and Nagaoka, 1972; Rosecrans and Adams, 1976) and only one has attempted to examine a central neurotransmitter system in any detail. This study (Rosecrans and Adams, 1976) attempted to correlate alterations in behavior of SHR to alterations in serotonergic mechanisms. This study found an increased turnover of serotonin and a decreased ability to habituate to stimuli which was suggested to represent a hyperfunctional serotonergic system in SHR. Neither of the catecholaminergic

systems have been studied in any detail. However, several studies utilizing L-DOPA have indicated changes in catecholamine metabolism in SHR (Shimamoto and Nagaoka, 1972; Tanaka et al., 1972). These L-DOPA-induced changes rely largely on presynaptic events as the L-DOPA must be converted to DA and/or NE, or release of serotonin before it can exert its effect. Changes in presynaptic levels and metabolism of the catecholamines could lead to alterations in postsynaptic events such as changes in sensitivity of postsynaptic receptors.

There are extremely few studies which have examined postsynaptic receptors in SHR by using direct acting catecholamine receptor agonists. One study used the direct acting DA agonist, apomorphine, to compare drug-induced behavior between SHR and Wistar rat (Shimamoto and Nagaoka, 1972). This study found no difference in response between the two rat strains indicating no change in receptor sensitivity of striatal DA receptors. However, receptor sensitivity of two other central dopaminergic systems remain unstudied. Therefore there seems to be little information on DA receptor sensitivity in SHR.

Since body temperature and Sidman avoidance responses to L-DOPA were reported to differ significantly from normotensive controls (Shimamoto and Nagaoka, 1972; Tanaka et al., 1972), suggesting possible alterations in dopaminergic metabolism and/or DA receptor sensitivity, the present study was undertaken to examine central DA receptor sensitivity of SHR. Apomorphine was selected as the dopaminergic agonist as it is postulated to be a direct acting agonist, and it is

capable of inducing three responses: 1) stereotyped behavior; 2) locomotor activity; and 3) hypothermia. As stereotyped behavior is mediated via the neostriatum and mesolimbic forebrain, locomotion via the mesolimbic forebrain, and hypothermia via the hypothalamus, determination of possible alterations in central DA receptor sensitivity in SHR will be attempted by comparing the three apomorphine-induced responses obtained from SHR to those obtained from normotensive rats.

MATERIALS AND METHODS

A. Animals and Blood Pressure Measurement

Three rat strains were utilized in this study: 1) the spontaneously hypertensive rat (SHR); 2) the Wistar-Kyoto rat (WKY); and 3) the Wistar rat (NWR). The SHR and WKY were obtained from the breeding colony at the University of the Pacific School of Pharmacy. Both the SHR and WKY colonies were descendants of the original colonies in Japan. The breeding stock was obtained from Dr. J.V. Levy of the Pacific Medical Center, San Francisco, Calif. SHR has been maintained by brother-sister matings of animals selected for their high systolic blood pressure (greater than 180 mmHg). WKY has been maintained by randomized matings of animals selected for their normal blood pressure (a systolic blood pressure no higher than 145 mmHg). NWR were obtained from Simonsen Laboratories, Gilroy, Calif. Upon arrival, these rats were housed in groups of not more than 20 per cage in an animal room which was not under a 12 hour light-dark cycle but which was maintained at 22-26 ^{o}C until they reached proper age and weight (longer than one week). At this time their blood pressures were measured. SHR and WKY were housed in the same animal room which was maintained at 22-26 ^{o}C under a 12 hour light-

dark cycle until their blood pressures were measured. Each strain was given food (Simonsen Laboratories Custom Lab Diet) and water ad libitum up until the time of the experiments. All animals used were drug-naive males ranging from 12 to 15 weeks of age. At this age the weights of the animals ranged from 250 to 350 grams. Animals were selected before the experiments were performed on the basis of their systolic blood pressures.

Systolic blood pressures were determined in unanesthetized rats by the previously described plethysmographic tail-cuff method (Williams et al., 1939; Del Greco et al., 1953; Buñag, 1973). Before measuring, the rats were pre-warmed in a metal cage floated in a hot water bath (Precision Scientific, Company) for 10 min at 37 ^{o}C for the purpose of increasing blood flow in the tail (Rand et al., 1965). After warming, the animals were placed in a rat holder connected to a temperature control unit (Narco Biosystems) which was set at "8". A pressure cuff (E & M Instrument Company) was placed on the base of the tail behind which a bulb for the pneumatic pulse transducer (E & M Instrument Company) was placed. The bulb was held in place by the barrel of a 5 cc syringe which had been cut down to the same length as the bulb. The pneumatic pulse transducer was connected to an electrosphygmograph (E & M Instrument Company) which was in turn connected to the pressure cuff and a desk model physiograph (E & M Instrument Company; type DMP-4A). The cuff was inflated by a blood pressure cuff pump (E & M Instrument Company) which also automatically deflated the cuff. Upon

release of the pressure, blood flowed back into the tail. This blood flow set up pulsations which could be picked up by the pneumatic bulb which transmitted it to the pneumatic pulse transducer. The signals from the pneumatic pulse transducer were amplified by an amplification unit (Narco Biosystems) in the physiograph. The unit was calibrated before each session of blood pressure recordings by setting the deflection of the tracing stylus to 2.5 cm on the graph paper (Narco Biosystems) for every 100 mmHg pressure. The 100 mmHg pressure was provided by pressing the "Calibrate" button on the electrosphygmograph. A minimum of three pressure measurements were taken on each rat. After their blood pressures were measured, the animals were divided into groups of three and maintained on a light-dark cycle of 800 to 2000 hr light for approximately one week before being used for an experiment.

B. Stereotyped Behavior

Animals were placed individually into clear plastic cages (Maryland Plastics; 44 cm x 23 cm x 20 cm) and allowed to acclimate one hour before injection. The bottoms of the cages were filled approximately 1-1/2 cm deep with bedding material (KC Pharmacal) upon which the animals could gnaw or bite. Screens constructed of cardboard were placed between each of the cages to avoid the possibility of the animals influencing each others behavior.

Animals were injected with apomorphine at 5 min intervals with the first injection being made at 1300 hr. The apomor-

phine-induced stereotyped behavior was then assessed by scoring every 5 min according to the rating scale shown in Table I. Scores were then cumulated into 15 min-intervals for each rat for statistical analysis. All observations and scoring were made by several experienced observers who were not blind to the treatment. They assessed stereotyped behavior at two doses of apomorphine. The susceptibility of the rat strains to antagonism of apomorphine-induced stereotyped behavior was also assessed after pretreatment with haloperidol.

E. Locomotor Activity

After the animals had been on the light-dark cycle of 800 to 2000 hr for one week, they were weighed in the morning and placed in a clear plastic cage of the same make as those used for the stereotyped behavior experiments. Unlike the stereotyped behavior experiments, these experiments were run with three animals per group in order to allow for normal social interactions. During these experiments the animals were allowed access to food (which was placed within the cage), but were not allowed water as drinking interfered with the activity counts recorded by the activity meter. At 1600 hr of the first day each of the animals was injected with 1 ml/kg of vehicle. At this time, the water bottle was removed from the cage. The spontaneous activity of the animals was measured overnight with the lights going off at 2000 hr and on at 800 hr. Each animal was again weighed on the morning of the second day. Water was given to the animals ad libitum at this time. At 1600 hr of the second day the

TABLE I

Stereotyped Behavior Rating Scale

Assigned Score:	Behavior Displayed:
0	Behavior indistinguishable from that displayed by untreated animals.
1	Periodic sniffing, and/or repetitive head and limb movements.
2	Continuous sniffing with exploratory activity, and repetitive head and limb movements.
3	Occasional or periodic gnawing, biting, or licking, and exploratory activity with continuous sniffing.
4	Persistent and intense gnawing, biting, or licking with locomotion restricted to a small area.
5	Compulsive gnawing, biting, or licking at one location.

(This rating scale was modified from those of Ernst (1965, 1967), and Costall and Naylor (1973)).

animals were injected with a 2.0 mg/kg dose of apomorphine, the water was removed, and the activity recorded for 2 hr. Recordings were begun each day immediately after the third rat had been injected.

Activity was measured by a Model "S" Selective Activity Meter (Columbus Instruments Company) with its sensitivity set at "5.0". The meter was always within a quiet room, and was allowed a warm-up period of at least 1/2 hr before the beginning of an experiment. Cumulative activity counts were recorded automatically every six min by a PC-1 printing counter (Columbus Instruments Company).

The activity counts from the first day (overnight locomotor activity) were divided into 2 hr-intervals for each group of three rats for the purpose of determining whether spontaneous locomotor activity differs between the three rat strains. To determine the effect that apomorphine had on locomotor activity, the activity counts obtained for the first 1-1/2 hr from each group of three rats for each day were divided into 18 min-intervals. The activity counts obtained during each 18 min-interval of the first day (vehicle-injected) were then subtracted from the activity counts obtained from the corresponding 18 min-interval of the second day (apomorphine-injected). This difference was considered to be the locomotor activity induced by apomorphine.

D. Body Temperature

Animals were placed individually into metal cages (30.0 cm x 23.5 cm x 15.0 cm) covered on the bottom with

bedding material. The animals were allowed to acclimate to the cages for 1 hr before beginning the experiment. Body temperature was determined rectally by use of a Model 43 TA telethermometer (Yellow Springs Instruments Company) with flexible thermistor probe (number 402). The probe was inserted 4 cm into the rectum and the temperature was allowed to stabilize before being recorded (at least 30 sec). Experiments were started at 1100 hr when body temperature was first measured and recorded for each rat. Temperatures were then taken every 15 min for the entire duration of the experiment. The rats remained untreated for the first 2 hr of the experiment thereby allowing their body temperatures to equilibrate. Apomorphine or L-DOPA was injected immediately after the 1300 hr body temperature measurement. This temperature was used as the baseline temperature. Carbidopa was administered as a pretreatment 1 hr prior to the injection of L-DOPA and was injected immediately after the 1200 hr temperature reading. Haloperidol, in the experiments in which blockade of apomorphine-induced hypothermia was attempted, was administered immediately after the 1230 hr temperature recording. Temperatures were taken for another 2-1/2 hr after the injection of apomorphine or L-DOPA. Changes in body temperature were determined for each rat by subtracting the baseline temperature from the temperature obtained every 15 min after the administration of apomorphine or L-DOPA.

E. Drugs

Apomorphine (APO) (Merck, Sharp, and Dohme Research

Laboratories) was used and weighed out as the hydrochloride salt. This drug was dissolved in double-distilled water immediately prior to injection. Concentrations of 0.25 and 0.35 mg/ml of APO were prepared by diluting a volume of 0.5 mg/ml concentration of APO with an equal volume of double-distilled water, and by diluting 7.0 ml of the 0.5 mg/ml concentration with 3.0 ml of double-distilled water, respectively. APO was administered in a volume of 1 ml/kg.

L-DOPA (ICN Pharmaceuticals; and Sigma Chemical Company) and carbidopa (Merck, Sharp, and Dohme Research Laboratories) were used in their base form, and were prepared by a modification of a previously described method (Ahlenius and Engels, 1971). The 50 mg/kg and 100 mg/kg doses of L-DOPA, and carbidopa (50 mg/kg dose was used in all experiments) were prepared in the following manner: 1) 75 mg or 150 mg of L-DOPA, or 75 mg of carbidopa was dissolved in 0.4 ml of approximately 2N HCl; 2) 2.2 ml of a propylene glycol solution was added to this acidic solution; 3) this solution was in turn neutralized by 0.4 ml of approximately 2N NaOH. A 25 mg/kg dose of L-DOPA was prepared by: 1) dissolving 37.5 mg of L-DOPA in 0.2 ml of approximately 2N HCl; 2) to which was added 2.6 ml of the propylene glycol solution; and 3) this solution was in turn neutralized with 0.2 ml of approximately 2N NaOH. These drug preparations totaled 3.0 ml and were injected immediately after their preparation as the L-DOPA or carbidopa would begin to precipitate. The particles that did precipitate out were held in suspension by the propylene glycol. Both the L-DOPA and carbidopa were injected in a volume of 2 ml/kg.

The propylene glycol solution was prepared by diluting 3.0 ml of propylene glycol (U.S.P., Fisher Scientific Company) with 7.0 ml of double-distilled water. 2N HCl was prepared by diluting concentrated hydrochloric acid (Mallinckrodt) while the 2N NaOH was prepared by dissolving the proper amount of sodium hydroxide pellets (J.T. Baker Chemical Company) in double-distilled water.

Haloperidol (HAL) was obtained in pre-prepared ampuls of 5 mg/ml in a volume of 1 ml (Haldol^{R} by McNeil). Nine-tenths ml of the pre-prepared haloperidol solution was diluted with double-distilled water to a total of 15 ml to obtain a concentration of 0.3 mg/ml haloperidol. A concentration of 0.5 mg/ml haloperidol was prepared by diluting 0.9 ml of the pre-prepared haloperidol solution with double-distilled water to a total of 9.0 ml. These solutions were kept refrigerated after being placed in vials wrapped with aluminum foil. HAL was administered in a volume of 1 ml/kg.

A lithium chloride solution was prepared from lithium carbonate (J.T. Baker Chemical Company, Reagent Grade) by dissolving 7.389 gm to neutrality with concentrated hydrochloric acid. This solution was brought to 100 ml with double-distilled water. The resulting concentration of lithium was 2 mEq/ml. A sodium chloride solution was prepared by dissolving 11.700 gm of sodium chloride (J.T. Baker Chemical Company, Reagent Grade) in 100 ml of double-distilled water. The resulting concentration of sodium was 2 mEq/ml. These solutions were used for chronic treatment of the three rat strains. The treatment schedule consisted of daily administration of

2 mEq/kg lithium or sodium at 1000 hr for 14 consecutive days (modified from Friedman and Gershon (1973)). On the 14th day a body temperature experiment using a 0.5 mg/kg dose of APO was performed on these chronically treated animals.

A hydralazine HCl solution was prepared by diluting 5 ampuls of 1 ml ampuls containing 20 mg hydralazine HCl (CIBA) to 1000 ml so that the resulting concentration of hydralazine HCl was 100 mg/1000 ml. This solution was given to a group of SHR ad libitum in place of their drinking water for 4 weeks. At the end of the 4 week period, a body temperature experiment was performed on these SHR. The average volume of solution consumed by each rat during the course of the antihypertensive therapy was determined to be 25 ml/24 hr. From this information it was calculated that each rat received approximately 2.5 mg hydralazine HCl for each 24 hr.

All drugs were administered intraperitoneally (except for the hydralazine HCl) slightly to the right of the abdominal midline approximately half-way between the sheath and the sternum. One-milliliter tuberculin syringes equipped with 25 G, 5/8-inch long needles (Becton-Dickinson) were used for all injections.

F. Determination of Serum Lithium Levels

A group of animals was selected for the purpose of determining serum lithium levels. These animals were chronically treated with lithium or sodium in the same manner as those animals used for the temperature experiments. At 1300 hr of the 14th day these animals were decapitated and bled into open-

ed vacutainer tubes. The cells were allowed to coagulate and settle out. The sample was then centrifuged to obtain the serum to be analyzed for lithium. This analysis was performed by medical technologists at the Stockton Medical Pathology Group. As a result of sending the samples out, the analysis was performed by three different individuals on three separate occasions. These individuals were blind to the treatment schedule and therefore to whether the samples contained lithium or sodium. They analyzed the samples according to the following procedure:

A Varian Model 1000 atomic absorption spectrophotometer was used to analyze the serum for lithium. A light source of 671 millimicrons was supplied by a Varian lithium hollow cathode lamp with an operating current of 0.5 milliamps. The slit width was set at 0.5 nanometers. The burner was supplied with acetylene at 12 pounds per square inch with a flow of 3 units on the instrument meter and air at 43 pounds per square inch with a flow of 5 units on the meter. The lithium standard (Instrumentation Laboratories) contained 1.0 mEq/l $\pm$ 1.5 %. A stock solution of this standard was prepared by diluting 1:10 with a diluent containing 14.0 mEq/l sodium and 0.5 mEq/l potassium. Two levels of control serum (by Hyland division of Travenol Laboratories) were reconstructed according to the manufacturer's instructions and assayed with each group of unknown serum. Lithium control values were 0.5 $\pm$ 0.1 mEq/l for level I and 1.5 $\pm$ 0.1 mEq/l for level II. The unknown and control sera were diluted volumetrically with distilled water. Serum lithium concentration was determined by

the following procedure: 1) distilled water was aspirated first at a rate of approximately 4.5 ml/min and the percentage absorbance adjusted to 0.00 on the visual scale and 000 on the digital indicator; 2) the diluted standard was aspirated and the digital indicator set to 010 by the concentration mode; 3) diluted controls and unknowns were then assayed and the initial concentrations of the unknowns were calculated by multiplying the number of units on the digital indicator by 0.1 mEq/unit (Hansen, 1968).

G. Ambient Temperature

All experiments were carried out at an ambient temperature of 23 ± 1 °C.

H. Statistics

The data obtained for the following: 1) the cumulative stereotyped behavior scores for each 15 min-interval; 2) the overnight spontaneous locomotor activity counts for each 2 hr-interval; 3) the APO-induced locomotor activity counts for each 18 min-interval; and 4) the change in body temperature, were averaged and compared between SHR and the two normotensive rat strains by Student's t-test. Slopes of the log-dose response curves obtained during the body temperature experiments were compared by ANOVA linear regression.

RESULTS

A. Blood Pressures

An elevated systolic blood pressure that was very highly significantly different from those of the normotensive strains existed in the SHR used for the present study. In this rat strain the blood pressure averaged 202 ± 1 mmHg (arithmetic mean ± standard error of the mean) (n = 157). Systolic blood pressures for the two normotensive rat strains averaged 135 ± 1 mmHg for WKY (n = 157), and 131 ± 1 mmHg for NWR (n = 148).

B. Stereotyped Behavior

Two doses (0.5 and 2.0 mg/kg) of APO were utilized for the assessment of drug-induced stereotyped behavior in these three rat strains. It was found that a 2.0 mg/kg dose of APO induced stereotyped behavior within a few minutes after injection and that at this dose the stereotypy lasted for approximately 90 min. Gnawing behavior without motor activity, which is the most intense behavior, occurred mainly during the 15-30 min interval, although gnawing with motor activity occurred during the first 15 min after injection and also during the 30-45 min interval. These results are illustrated in Figure 1.

This figure also shows, except for a single difference in significance during the 4th interval, that the six 15-min intervals of cumulated stereotyped behavior scores did not differ significantly over the 90 min. SHR, however, appeared to recover from the stereotypic effects of APO faster than either WKY or NWR. The smaller cumulated score during the 45-60 min interval in SHR illustrates this possibility.

Since the 2.0 mg/kg dose of APO may have been a supra-maximal dose required for the induction of gnawing, the stereotypic effects of APO at a second dose of 0.5 mg/kg were also assessed. This dose also induced stereotyped behavior within minutes of administration, but the duration of stereotypy was only about half as long, 45 min, as with 2.0 mg/kg APO. The intensity of the behavior observed at this lower dose was much less than that observed with the 2.0 mg/kg dose. Occasional to continuous sniffing with exploratory type activity were the most intense behaviors induced by the 0.5 mg/kg dose of APO, and these behaviors occurred within the first 15 min as illustrated by Figure 2. The 15-min intervals of cumulated stereotyped behavior scores again did not differ signifcantly between each of the three rat strains. It can also be seen that SHR again appeared to display a quicker recovery from these effects of APO than either WKY or NWR as no stereotyped behavior was observed in SHR during the 30-45 min interval.

Total stereotyped behavior scores covering the entire 45 min for the 0.5 mg/kg dose if APO, and the entire 90 min for the 2.0 mg/kg dose of APO show no significant dif-

ference between the three rat strains as summarized in Table II. A significant difference may have been expected as suggested by the more rapid recovery from stereotypy by SHR.

To determine whether each of the rat strains was as susceptible to attenuation of the stereotyped behavior induced by APO as the other two strains, a 0.3 mg/kg dose of HAL was used to antagonize this stereotypy. As shown in Figure 3, this dose of HAL antagonized the stereotyped behavior induced by 2.0 mg/kg APO to approximately the same degree in each of the three rat strains.

C. Locomotor Activity

As rats are nocturnal animals, their spontaneous locomotor activity was assessed during the night while they were most active. Figure 4 graphically represents the overnight locomotor activity counts obtained from each of the three rat strains when their locomotor activity counts are divided into 2-hr intervals. No significant differences were found to exist between each of these three rat strains with respect to their overnight spontaneous locomotor activity. It can also be seen from Figure 4 that the locomotor activity increased significantly after the lights were turned off. As the locomotor activity counts did not differ significantly between the rat strains before the lights went off, the sudden increase in locomotor activity counts after the lights were turned off indicates that the one week light-dark schedule of 800-2000 hr was successful.

Figure 1--Stereotyped behavior induced by 2.0 mg/kg APO. (For n values see Table II.)

Key: CUM S B SCORES = Cumulated Stereotyped Behavior Scores; Time Interval 1 = 0-15 minutes after injection with APO; 2 = 15-30 min; 3 = 30-45 min; 4 = 45-60 min; 5 = 60-75 min; 6 = 75-90 min.

* = Significantly different from SHR ($p<0.05$).

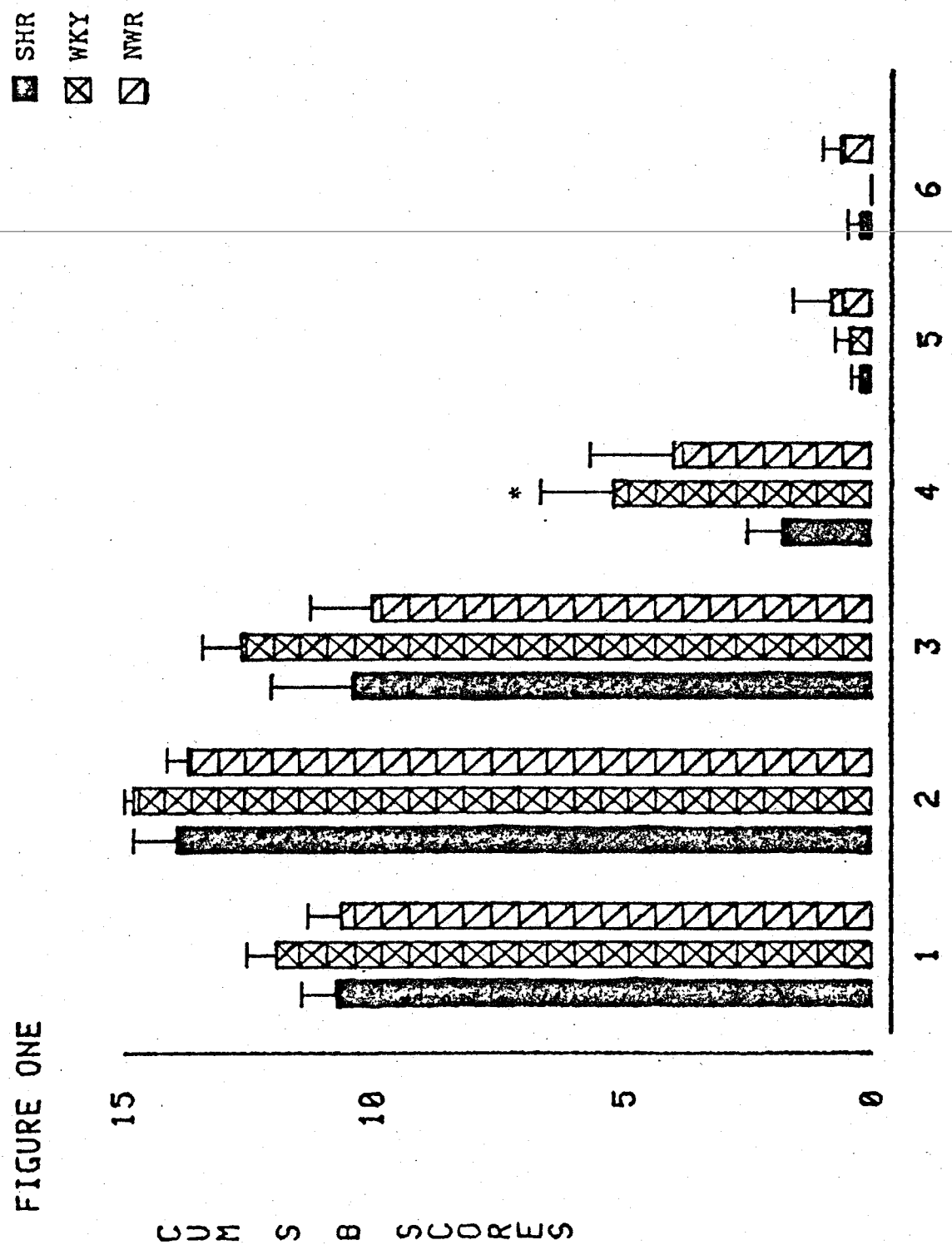


FIGURE ONE

Figure 2--Stereotyped behavior induced by 0.5 mg/kg APO. (For n values see Table II.)

Key: CUM S B SCORES = Cumulated Stereotyped Behavior Scores; Time Interval 1 = 0-15 minutes after injection with APO; 2 = 15-30 min; 3 = 30-45 min; 4 = 45-60 min.

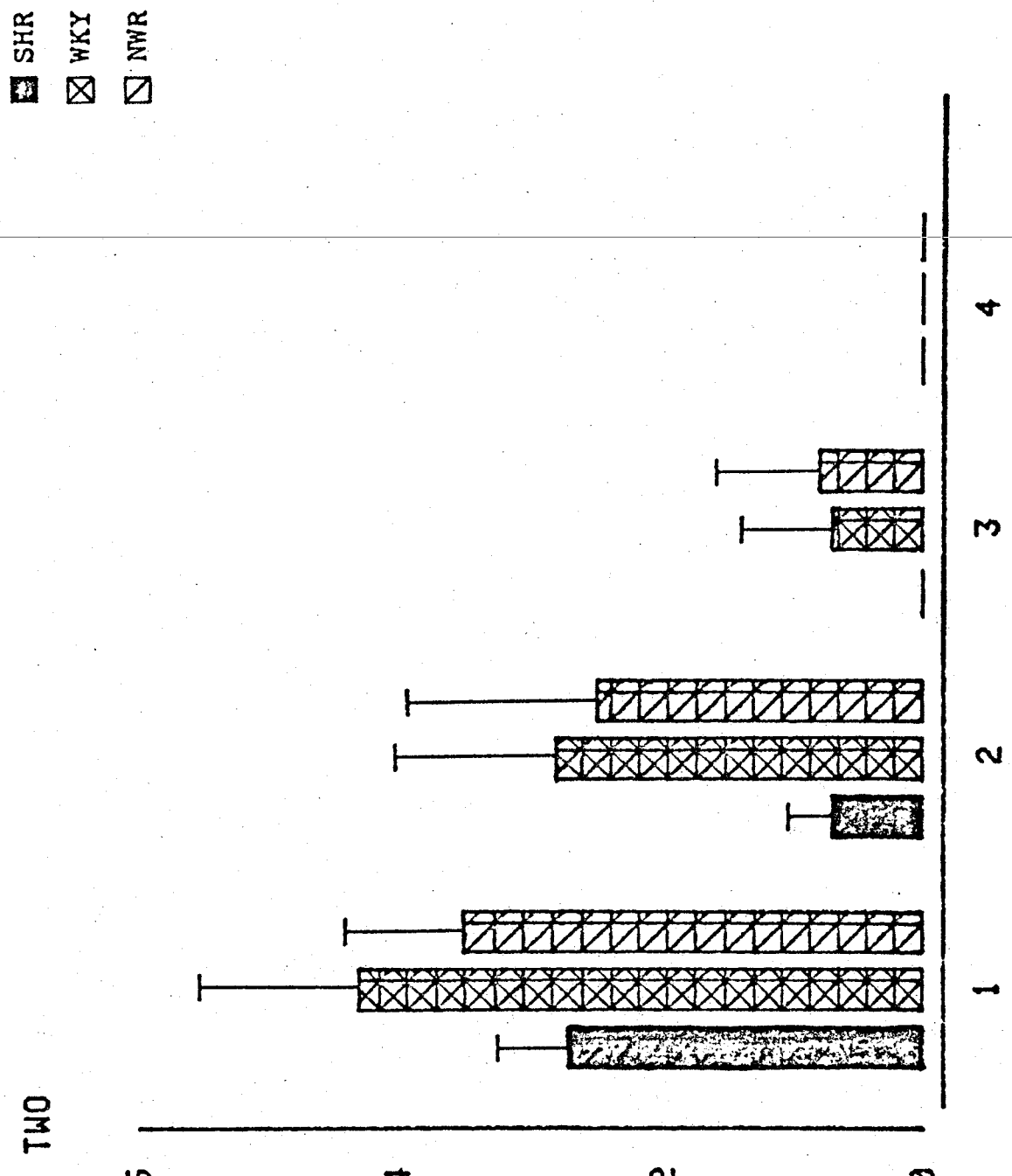


FIGURE TWO

TABLE II

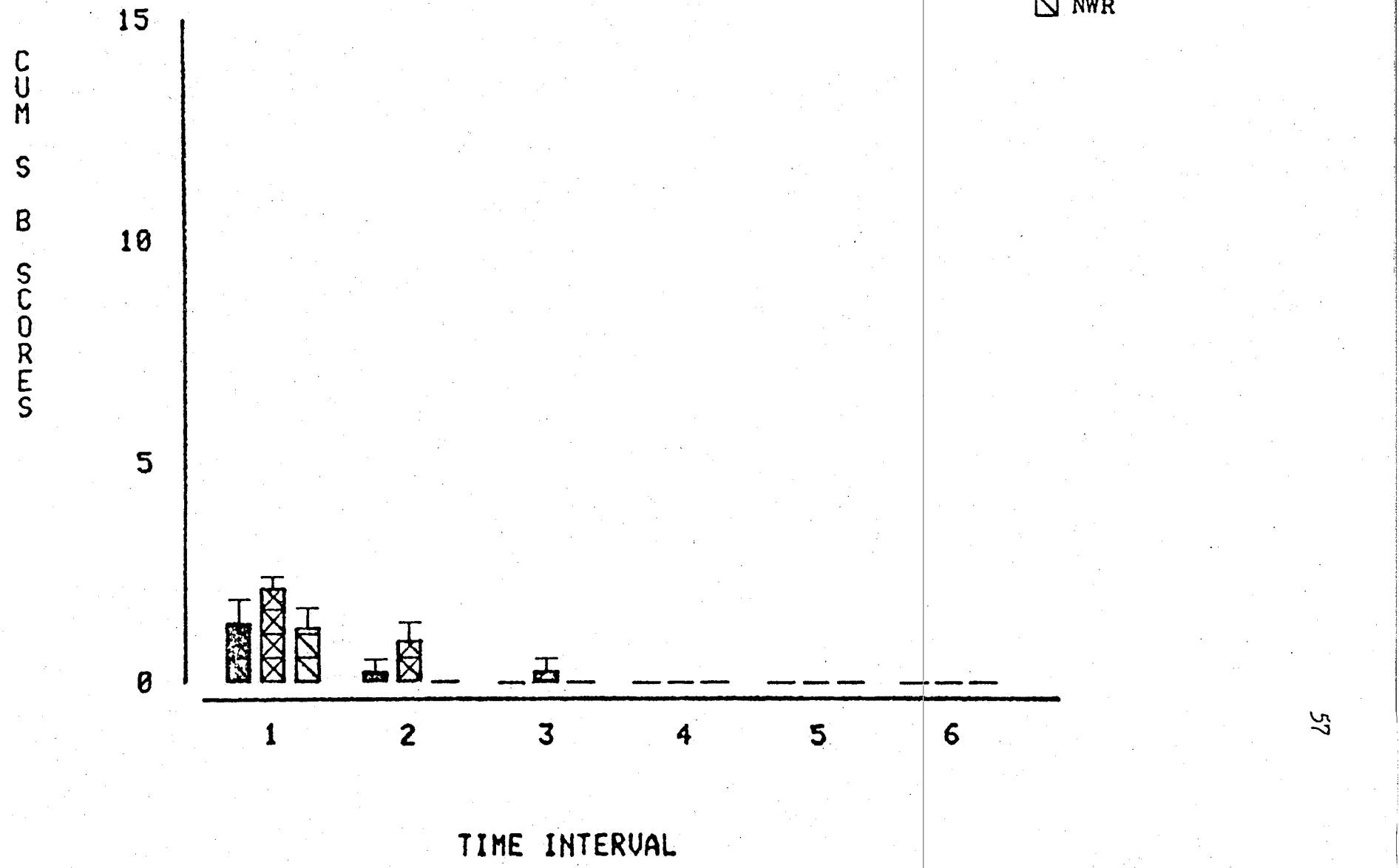
Total Cumulated Stereotyped Behavior Scores

Dose Apomorphine		0.5 mg/kg	2.0 mg/kg
Total Time		45 min	90 min
N		6	9
Rat Strain	NWR	6.8 ± 2.8	39.6 ± 2.8
	WKY	7.8 ± 2.9	44.9 ± 2.4
	SHR	3.3 ± 0.7	33.8 ± 5.1

Figure 3--Antagonism of 2.0 mg/kg APO-induced stereotyped behavior by 0.3 mg/kg HAL. (SHR = 6 rats; WKY = 8 rats; NWR = 6 rats.)

Key: CUM S B SCORES = Cumulated Stereotyped Behavior Scores; Time Interval 1 = 0-15 minutes after injection with APO (HAL pretreatment time = 30 min); 2 = 15-30 min; 3 = 30-45 min; 4 = 45-60 min; 5 = 60-75 min; 6 = 75-90 min.

FIGURE THREE



The locomotor activity counts obtained after administration of APO were compared to the spontaneous locomotor activity counts obtained after administration of vehicle for each rat strain. Figure 5 shows the results obtained for SHR. In this figure it can be seen that the locomotor activity counts obtained after APO during the time interval of 36-54 min was significantly greater than the spontaneous (vehicle-injected) locomotor activity counts of the same time interval. A significantly greater number of locomotor activity counts during the time intervals of 36-54 min and 54-72 min was obtained after injection of APO in WKY when compared to locomotor activity counts of vehicle-injected animals (Figure 6). Although not significantly greater, the locomotor activity counts obtained from APO-treated WKY were much greater during the 18-36 min interval than those obtained from vehicle-injected WKY. The locomotor activity counts obtained from APO-treated NWR were significantly greater than the counts obtained from vehicle-injected NWR during the time intervals of 18-36 min and 36-54 min as shown in Figure 7.

By subtracting the locomotor activity counts obtained after vehicle-injection from those obtained after APO-injection (Figures 5-7) one can construct Figure 8 which illustrates the actual degree of locomotor stimulation due to APO. This subtraction was done in order to determine the increase in locomotor activity induced by APO which, if APO truly induces locomotor activity, should be greater than normal, or spontaneous, locomotor activity. As can be seen from

Figure 8, APO-induced locomotor activity did not significantly differ between the rat strains. However, the induced increase was much smaller in SHR than either WKY or NWR. Even though the increase in locomotor activity was much smaller in SHR than either WKY or NWR. Even though the increase was much smaller for each of the six 18-min intervals in SHR, the total increase in locomotor activity counts for the entire 90 min was not significantly different between the three strains as summarized in Table III.

D. Body Temperature

Baseline rectal temperatures were not significantly different between SHR, which displayed an average core temperature of 36.6 $\pm$ 0.1 ^{o}C (n = 114), and NWR, which showed an average body temperature of 36.7 $\pm$ 0.1 ^{o}C (n = 118). WKY displayed a baseline body temperature significantly lower than either SHR or NWR as its rectal temperature averaged 36.2 $\pm$ 0.1 ^{o}C (n = 139).

The initial body temperature experiments performed utilized an intraperitoneal dose of 2.0 mg/kg APO. It was discovered that the hypothermia produced by this dose of APO, as shown in Figure 9, was of greater magnitude and more prolonged in SHR than in NWR. The decrease in body temperature was significantly greater in SHR during the entire duration of the hypothermic response when compared to NWR. WKY, on the other hand, displayed a hypothermic response similar in magnitude to that of SHR. Return to baseline temperature was faster, however, in WKY as there was a significant differ-

Figure 4--Overnight spontaneous locomotor activity.

Experiment begun at 1600 hr. Lights off at 2000 hr.

(For n values see Figures 5, 6, and 7.)

Key: Time Interval 1 = 0-2 hours after injection with vehicle; 2 = 2-4 hr; 3 = 4-6 hr; 4 = 6-8 hr; 5 = 8-10 hr; 6 = 10-12 hr; 7 = 12-14 hr.

FIGURE FOUR

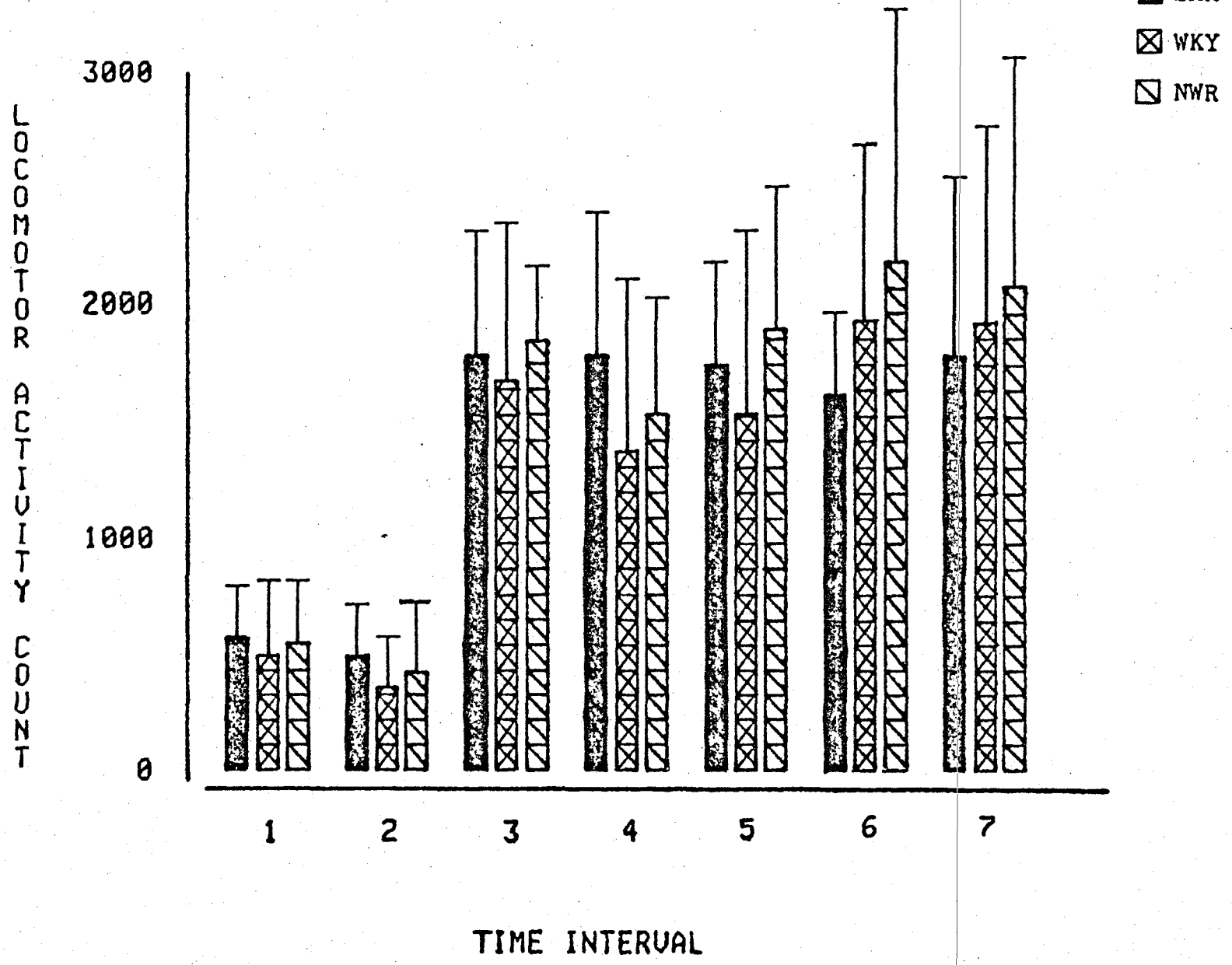

SHR
WKY
NWR
3000
2000
1000
0
LOCOMOTOR ACTIVITY COUNT
1
2
3
4
5
6
7
TIME INTERVAL

Figure 5--Locomotor activity counts obtained after vehicle-injection compared to locomotor activity counts obtained after APO-injection: SHR (n = 11 groups of 3 rats).

Key: Time Interval 1 = 0-18 minutes after injection with vehicle or APO; 2 = 18-36 min; 3 = 36-54 min; 4 = 54-72 min; 5 = 72-90 min.

* = Significantly different from vehicle-injected animals ($p<0.05$).

FIGURE FIVE

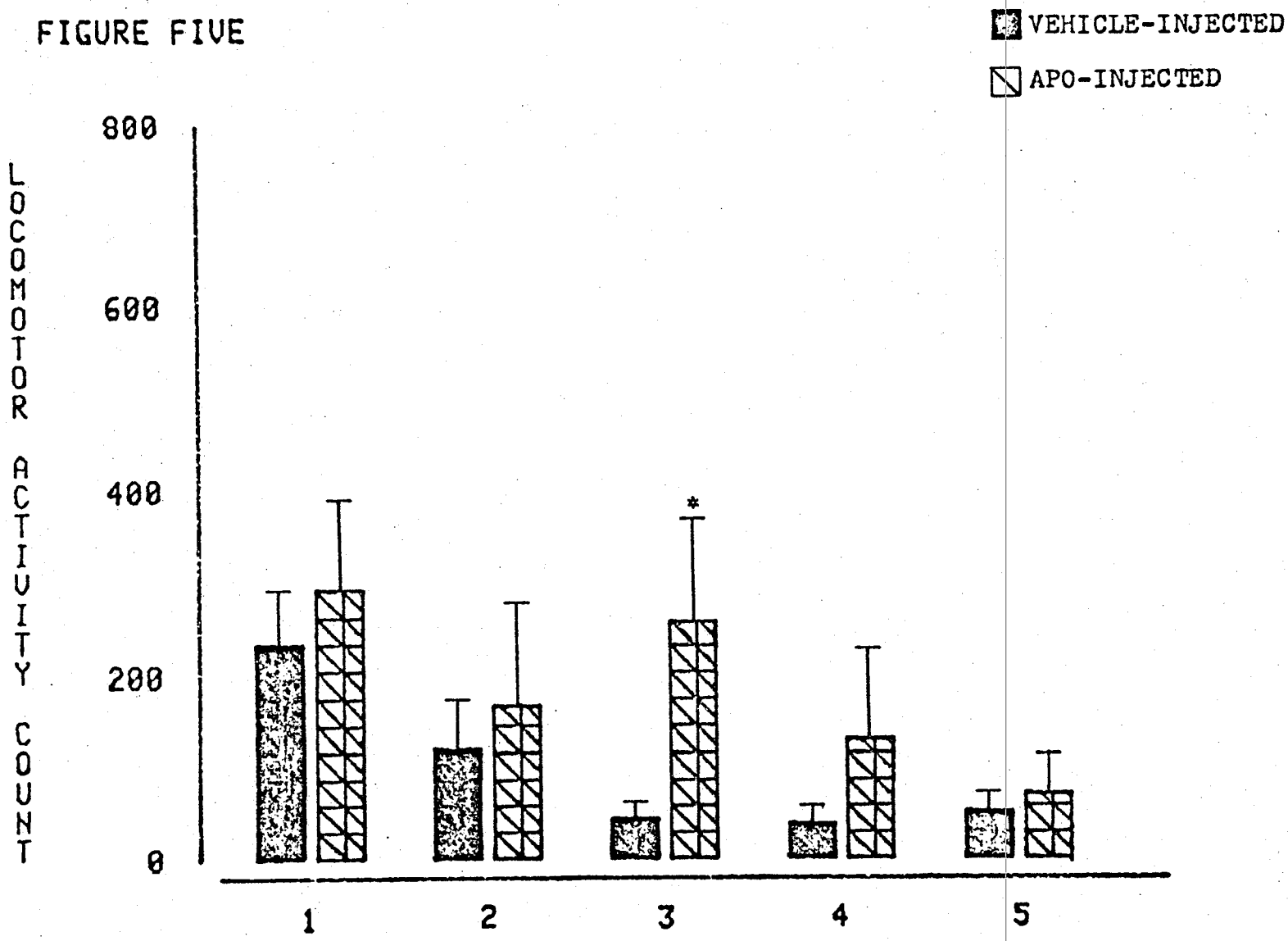

VEHICLE-INJECTED
APO-INJECTED
LOCOMOTOR ACTIVITY COUNT
800
600
400
200
0
*
1
2
3
4
5
TIME INTERVAL

Figure 6--Locomotor activity counts obtained after vehicle-injection compared to locomotor activity counts obtained after APO-injection: WKY (n = 6 groups of 3 rats).

Key: Time Interval 1 = 0-18 minutes after injection with vehicle or APO; 2 = 18-36 min; 3 = 36-54 min; 4 = 54-72 min; 5 = 72-90 min.

* = Significantly different from vehaicle-injected animals ($p<0.05$).

FIGURE SIX

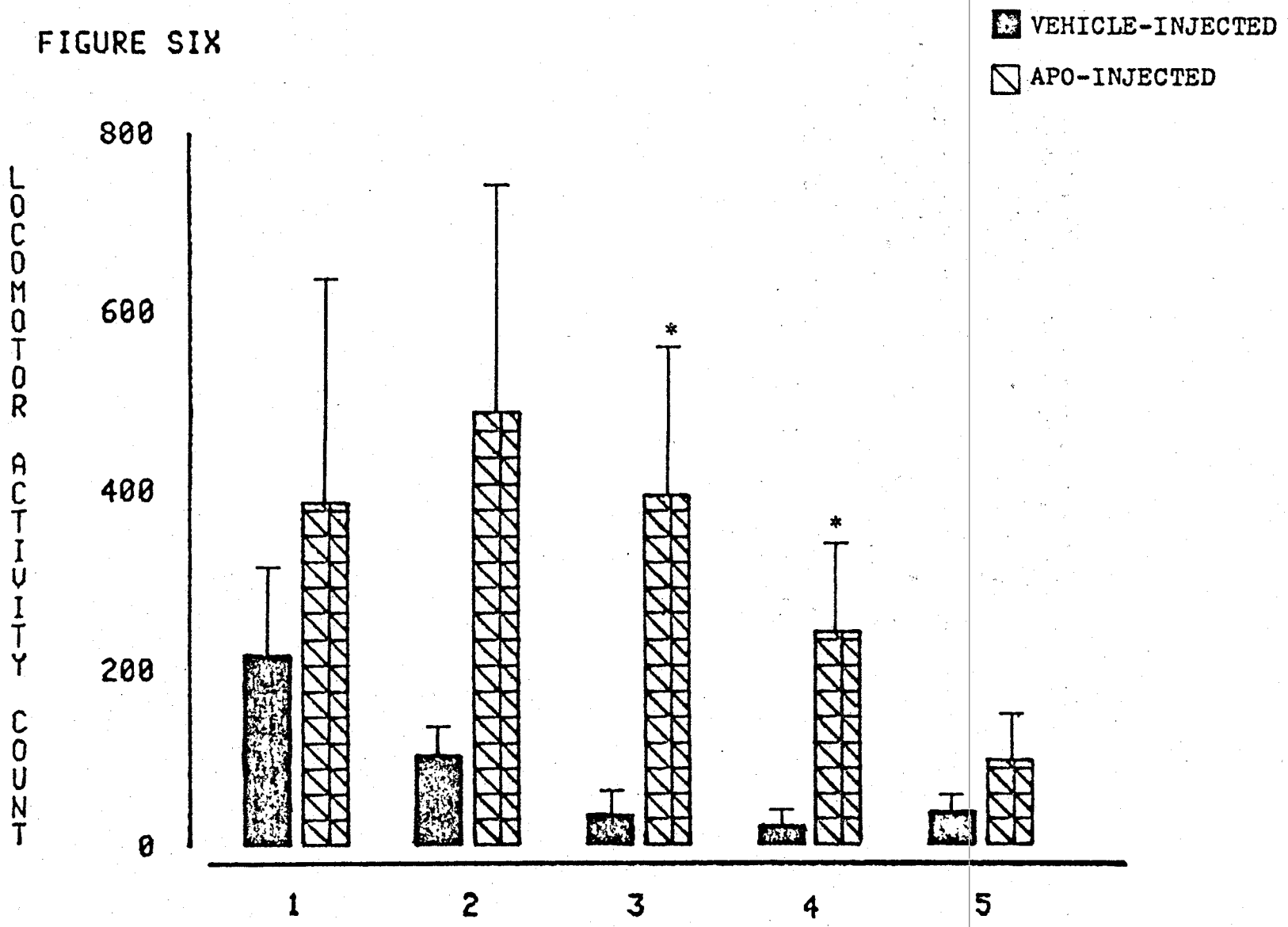

VEHICLE-INJECTED
APO-INJECTED
LOCOMOTOR ACTIVITY COUNT
800
600
400
200
0
*
*
1
2
3
4
5
TIME INTERVAL

Figure 7--Locomotor activity counts obtained after vehicle-injection compared to locomotor activity counts obtained after APO-injection: NWR (n = 8 groups of 3 rats).

Key: Time Interval 1 = 0-18 minutes after injection with vehicle or APO; 2 = 18-36 min; 3 = 36-54 min; 4 = 54-72 min; 5 = 72-90 min.

* = Significantly different from vehicle-injected animals ($p<0.05$).

FIGURE SEVEN

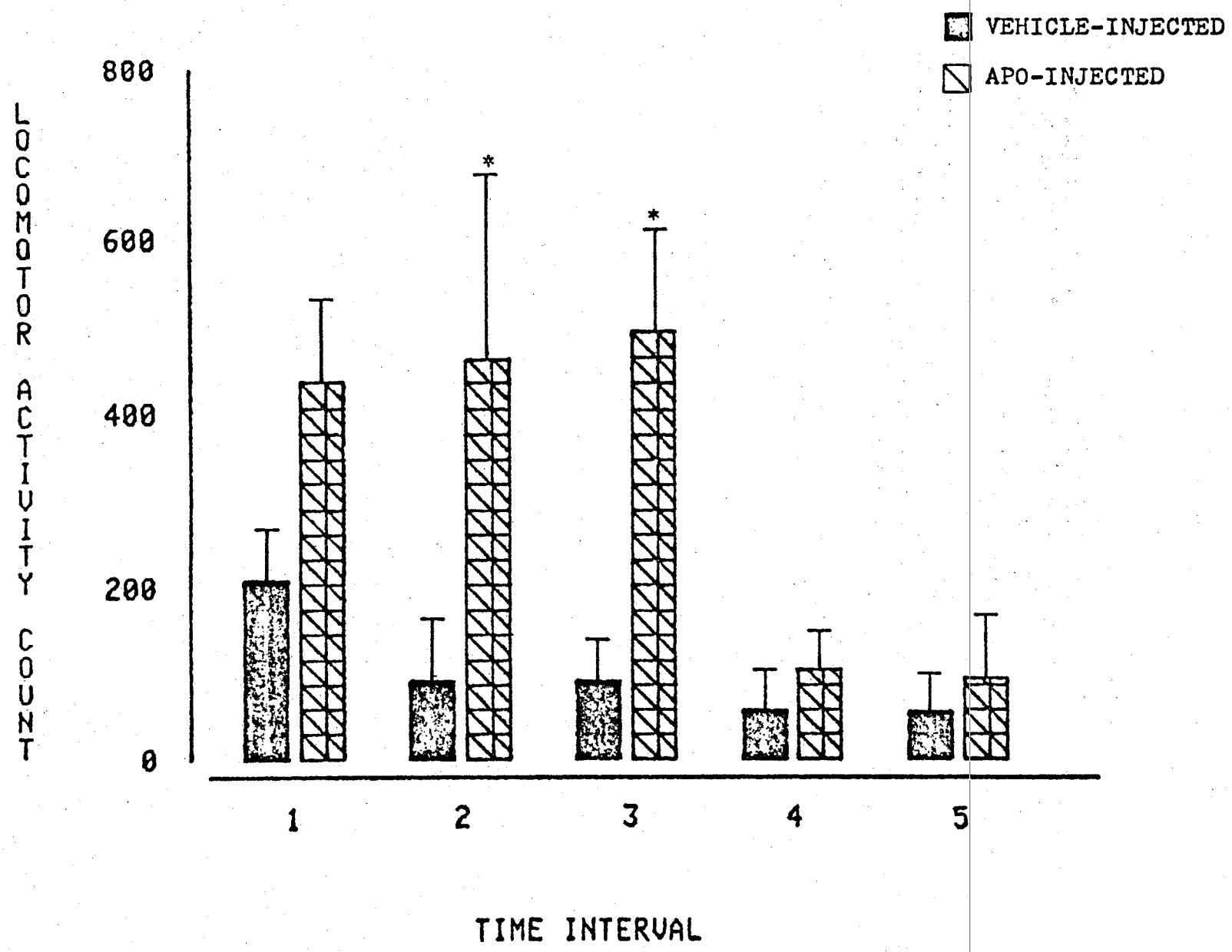


Figure 8--Locomotor activity induced by 2.0 mg/kg APO. (For n values see Figures 5, 6, and 7.)

Key: Time Interval 1 = 0-18 minutes after injection with APO; 2 = 18-36 min; 3 = 36-54 min; 4 = 54-72 min; 5 = 72-90 min.

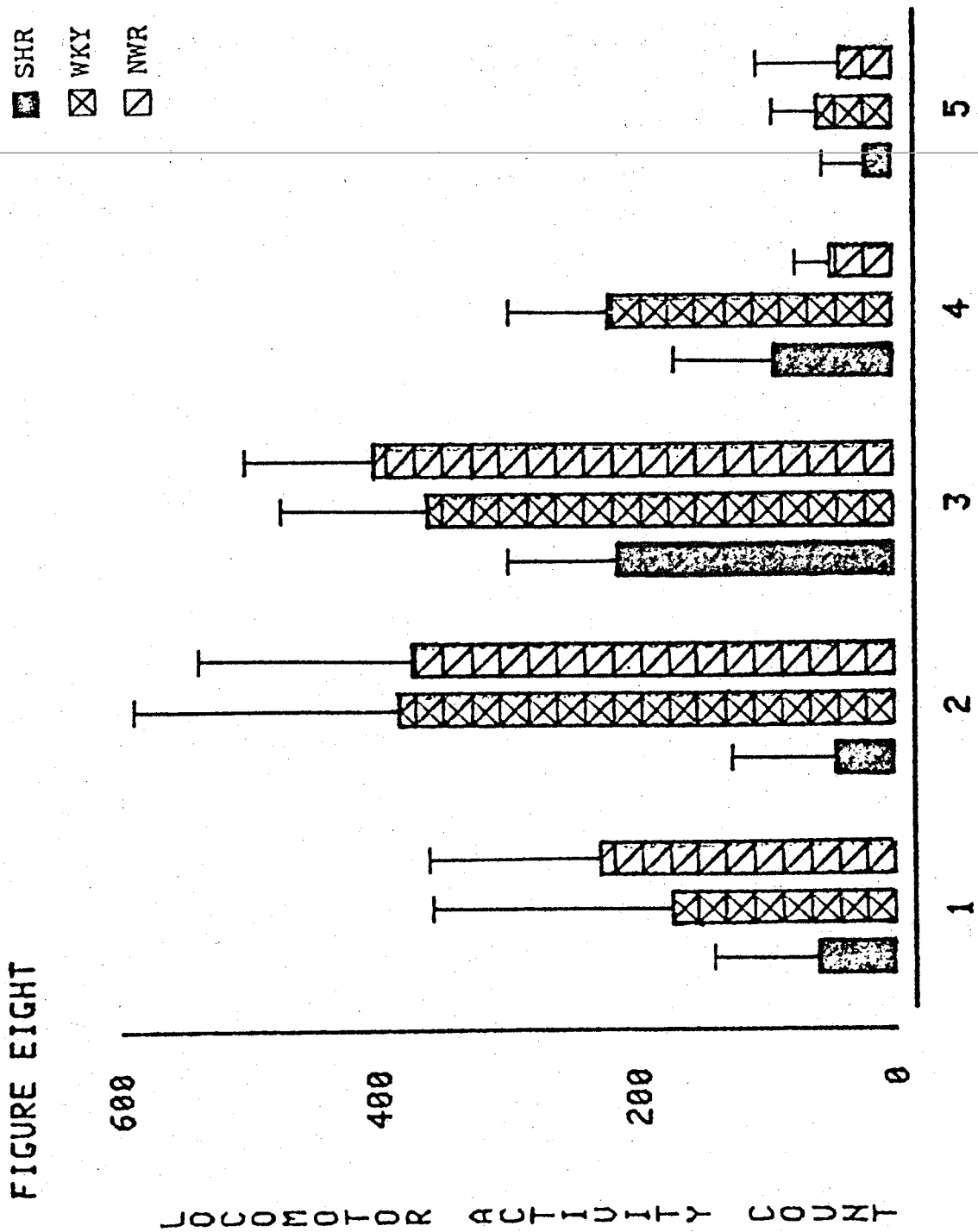


FIGURE EIGHT

TABLE III

Total activity induced by 2.0 mg/kg APO for 90 Min

Rat Strain (n)	Total Counts
NWR (8)	1090.9 ± 351.0
WKY (6)	1194.7 ± 516.8
SHR (11)	435.0 ± 292.4

ence in the change in body temperature from 75 min to the end of the hypothermic response when compared to SHR.

To determine whether this hypothermic effect was dependent on the stimulation of DA receptors, HAL at an intraperitoneal dose of 0.3 mg/kg was administered 30 min prior to the APO challenge. As illustrated in Figures 10 and 11, this dose of HAL significantly attenuated the hypothermic response in NWR and WKY. This same dose of HAL also significantly antagonized the hypothermia in SHR as illustrated in Figure 12. However, the drop in body temperature produced in SHR was still significantly greater than that produced in either NWR or WKY (Figure 13). A dose of 0.5 mg/kg HAL was required in SHR to attain the same degree of attenuation provided by 0.3 mg/kg HAL in NWR and WKY (Figures 12 and 13).

Graphic representations similar to the one obtained from the hypothermic response to 2.0 mg/kg APO were also obtained from the hypothermic responses to 0.25 mg/kg, 0.35 mg/kg, and 0.50 mg/kg doses of APO. These curves are shown in Figures 14, 15, and 16, respectively. At each of these three doses the magnitude and the duration of the hypothermic effect was greater in SHR than NWR. Only at the 0.5 mg/kg dose of APO was the hypothermic response more prolonged in SHR than WKY.

Since these time-course curves of APO-induced hypothermia employed log-doses of APO, construction of log-dose response curves from the results of these three doses was possible. These log-dose response curves were constructed by plotting the averages of the maximum decreases in body temperature displayed by each rat. Figure 17 shows the log-dose response

curves obtained for each rat strain. By use of linear regression analysis it was determined that the slopes of the curves equalled: 1) 2.54 for SHR; 2) 2.22 for WKY; and 3) 1.70 for NWR. These slopes were not significantly different as determined by linear regression analysis. Since the magnitude of the decrease in body temperature was significantly greater in SHR in comparison to NWR for each of the three doses of APO, it was calculated that APO appears to be approximately 1.78 times (95 % confidence limits: 1.37 to 2.30) more potent in SHR than NWR. Or, in other words, that SHR appears to be approximately 1.78 times more sensitive to the hypothermic effect of APO than NWR. Because all three of the average maximum drops in body temperature for WKY did not differ significantly from either SHR or NWR, apparent potency of APO in this strain was not calculated in comparison to either NWR or SHR.

During these studies, it became apparent that different doses of APO were required to elicit the same time-course of hypothermia in the three rat strains. Figure 18 shows that a dose of 4.0 mg/kg was necessary to elicit the same hypothermic response in NWR as 2.0 mg/kg APO in WKY, and 0.5 mg/kg in SHR. These doses also appear to be threshold doses as 4.0 mg/kg caused the same drop in body temperature as 2.0 mg/kg in WKY (Figure 19), and 0.5 mg/kg APO induced the same decrease as 2.0 mg/kg APO in SHR (Figure 20). Whereas the higher dose was capable of prolonging the duration of the hypothermic response in SHR, the hypothermic response to 4.0 mg/kg in WKY was not significantly different from that elicited by a

2.0 mg/kg APO.

To ensure that this difference in temperature response was not a phenomenon specific to APO, a second dopaminergic agonist, L-DOPA (coupled with pretreatment of the DOPA decarboxylase inhibitor, carbidopa) was used. It was assumed that L-DOPA is metabolized to the neurotransmitter DA in the brain. Figures 21 to 23 illustrate the time-course of the hypothermic response to three log-doses of L-DOPA. These curves are strikingly similar to those obtained after administration of APO in that the duration of the hypothermic effect was greater in SHR than NWR. In contrast to APO, the hypothermic response was more prolonged in SHR than WKY at 25 mg/kg and 50 mg/kg L-DOPA. A significantly greater decrease in body temperature occurred at 25 mg/kg and 50 mg/kg doses of L-DOPA in SHR than in WKY or NWR. At a dose of 100 mg/kg L-DOPA the drop in body temperature was significantly greater in SHR than NWR. WKY at this dose of L-DOPA displayed a response somewhat intermediate to the other two rat strains and thereby did not differ significantly from SHR.

Since log-doses of L-DOPA were used, it was again possible to construct log-dose response curves of the average maximum drop in body temperature per rat. The L-DOPA log-dose response curves, as shown in Figure 24, are very similar to those obtained from APO. Slopes calculated for these curves equalled: 1) 1.83 for SHR; 2) 2.18 for WKY; and 3) 1.99 for NWR. These slopes were not significantly different from one another. The average maximum decreases in body temperature induced by L-DOPA were significantly greater in SHR than in

NWR at the high and low dose. Since the points at the ends of the curves differed significantly, the potency of L-DOPA in SHR in comparison to NWR was calculated. L-DOPA was found to be approximately 1.78 times (95 % confidence limits: 1.34 to 2.35) more potent in producing hypothermia in SHR than NWR. Or, in other words, SHR appear to be approximately 1.78 times more sensitive than NWR to the hypothermic effect of L-DOPA. As with the APO data, potency of L-DOPA in WKY in comparison to SHR or NWR were not calculated as the hypothermic responses were not significantly different from either SHR or NWR.

Chronic lithium treatment did not significantly affect the hypothermia caused by 0.5 mg/kg APO in either NWR or WKY as illustrated in Figures 25 and 26, respectively. Both lithium- or sodium-treated NWR and WKY displayed a maximum drop in body temperature of approximately 0.7 to 0.8 ^{o}C at 30 min. Sodium-treated SHR, on the other hand, displayed a maximum drop of about 1.3 ^{o}C at 30-45 min as illustrated by Figure 27. This figure also shows that lithium treatment significantly attenuated the APO-induced hypothermic response in SHR. Instead of displaying a body temperature drop of 1.3 ^{o}C as in sodium-treated SHR, the lithium-treated animals displayed a drop of 0.7 ^{o}C at 30 min in much the same manner as sodium- or lithium-treated NWR and WKY. Therefore, as Figure 28 shows, lithium altered the hypothermic response in SHR so that it was similar to those displayed by NWR and WKY. Lithium-treated NWR differed significantly from lithium-treated SHR from 75-150 min. However, this

difference occurred after the hypothermic response had ended.

The effect that lithium may have had on body temperature was roughly analyzed to ensure that it did not affect the response to APO by altering baseline body temperature, particularly in SHR. It was found that the baseline body temperatures of sodium-treated rats averaged: 1) 36.5 ± 0.1 °C for SHR; 2) 36.7 ± 0.2 °C for WKY; and 3) 36.4 ± 0.1 °C for NWR. Lithium-treated rats demonstrated a lower baseline rectal temperature which was 36.1 ± 0.2 °C for all three of the rat strains.

Serum lithium levels were 0.0 mEq/l in sodium-treated rats, whereas in lithium-treated rats they were found to be: 1) 1.05 ± 0.11 mEq/l in SHR; 2) 1.24 ± 0.08 mEq/l in WKY; and 3) 1.01 ± 0.05 mEq/l in NWR. Blood pressures were measured on the 15th day (the day following cessation of chronic treatment) and were found not to be significantly changed in either NWR or SHR (Table IV). However, the blood pressures in SHR were lower, though not significantly, after lithium treatment. WKY blood pressures were not measured because of the lack of a significant change in the other normotensive strain.

Chronic treatment of SHR with hydralazine was found to normalize their blood pressure. The systolic blood pressure of these animals averaged 137 ± 3 mmHg. Despite this normalization of blood pressure, the hypothermia induced by 0.5 mg/kg APO was not significantly different from the hypothermic response induced in untreated SHR (Figure 29).

Figure 9--Hypothermia induced by 2.0 mg/kg APO. (SHR = 15 rats; WKY = 25 rats; NWR = 24 rats.)

Key: TEMP = Temperature

* = Significantly different from SHR ($p<0.05$).

** = Highly significantly different from SHR ($p<0.01$).

*** = Very highly significantly different from ($p<0.001$).

FIGURE NINE

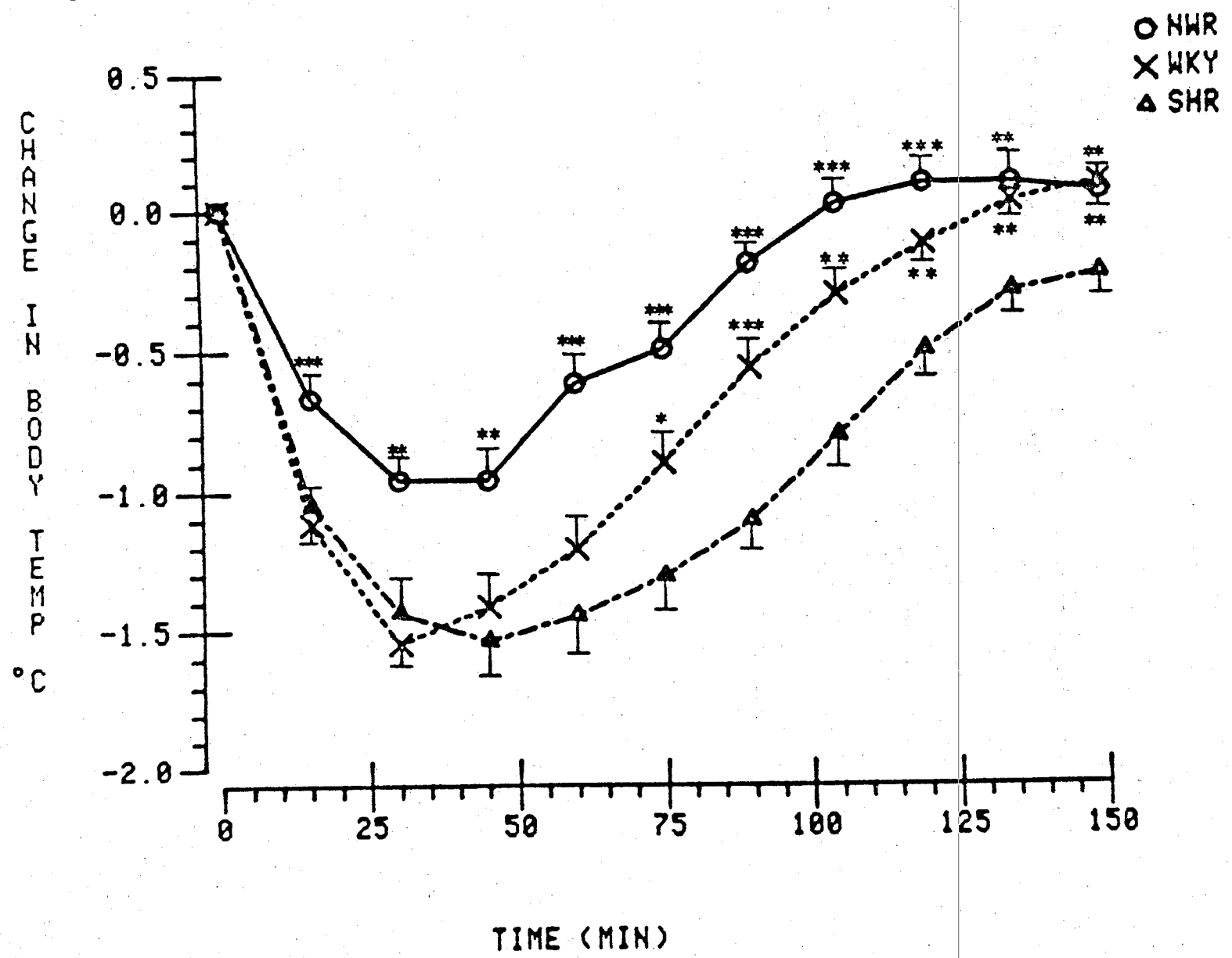

NWR
WKY
SHR
0.5
0.0
-0.5
-1.0
-1.5
-2.0
CHANGE IN BODY TEMP °C
0
25
50
75
100
125
150
TIME (MIN)

Figure 10--Antagonism of 2.0 mg/kg APO-induced hypothermia (n = 24 by 0.3 mg/kg HAL (n = 9) in NWR.

Key: TEMP = Temperature

* = Significantly different from rats injected only with APO ($p<0.05$).

*** = Very highly significantly different from rats injected only with APO ($p<0.001$).

FIGURE TEN

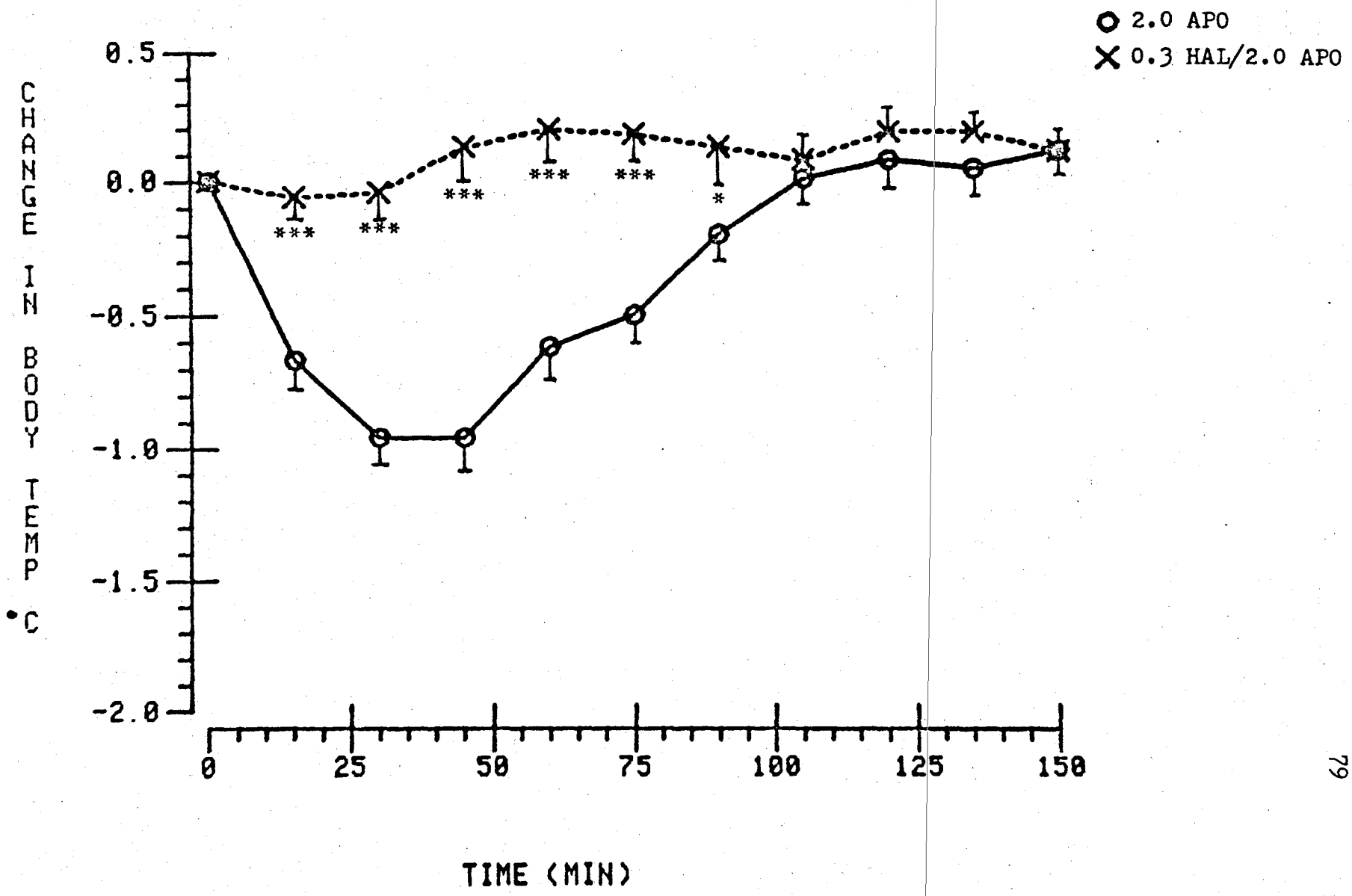

2.0 APO
0.3 HAL/2.0 APO
CHANGE IN BODY TEMP °C
0.5
0.0
-0.5
-1.0
-1.5
-2.0
***
***
***
***
***
*
0
25
50
75
100
125
150
TIME (MIN)

Figure 11--Antagonism of 2.0 mg/kg APO-induced hypothermia (n = 25) by 0.3 mg/kg HAL (n = 9) in WKY.

Key: TEMP = Temperature

** = Highly significantly different from rats injected only with APO ($p<0.01$).

*** = Very highly significantly different from rats injected only with APO ($p<0.001$).

FIGURE ELEVEN

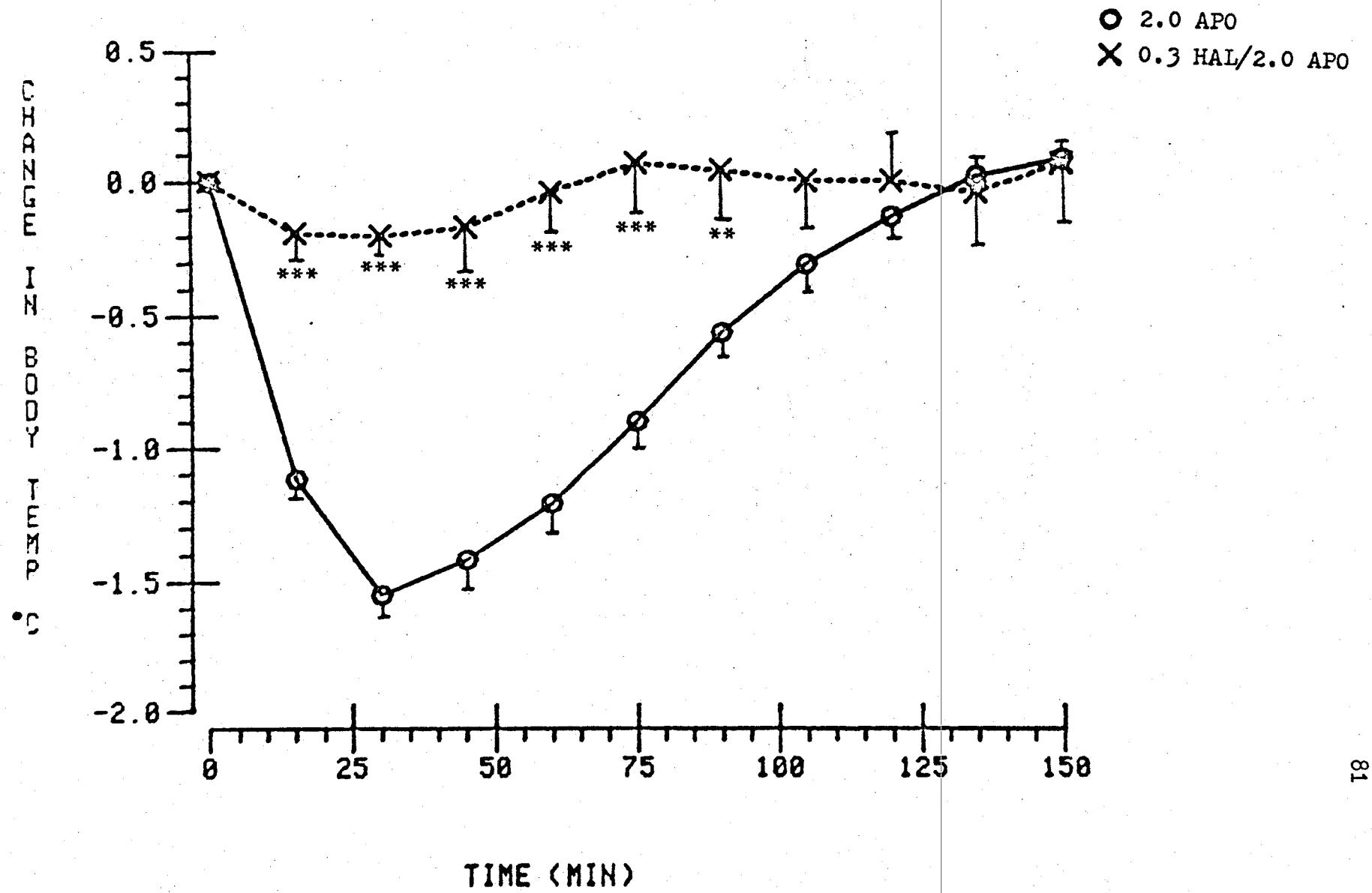


Figure 12--Antagonism of 2.0 mg/kg APO-induced hypothermia (n = 15) by 0.3 mg/kg (n = 9) and 0.5 mg/kg (n = 9) HAL in SHR.

Key: TEMP = Temperature

* = Significantly different from rats injected only with APO ($p<0.05$).

** = Highly significantly different from rats injected only with APO ($p<0.01$).

*** = Very highly significantly different from rats injected only with APO ($p<0.001$).

FIGURE TWELVE

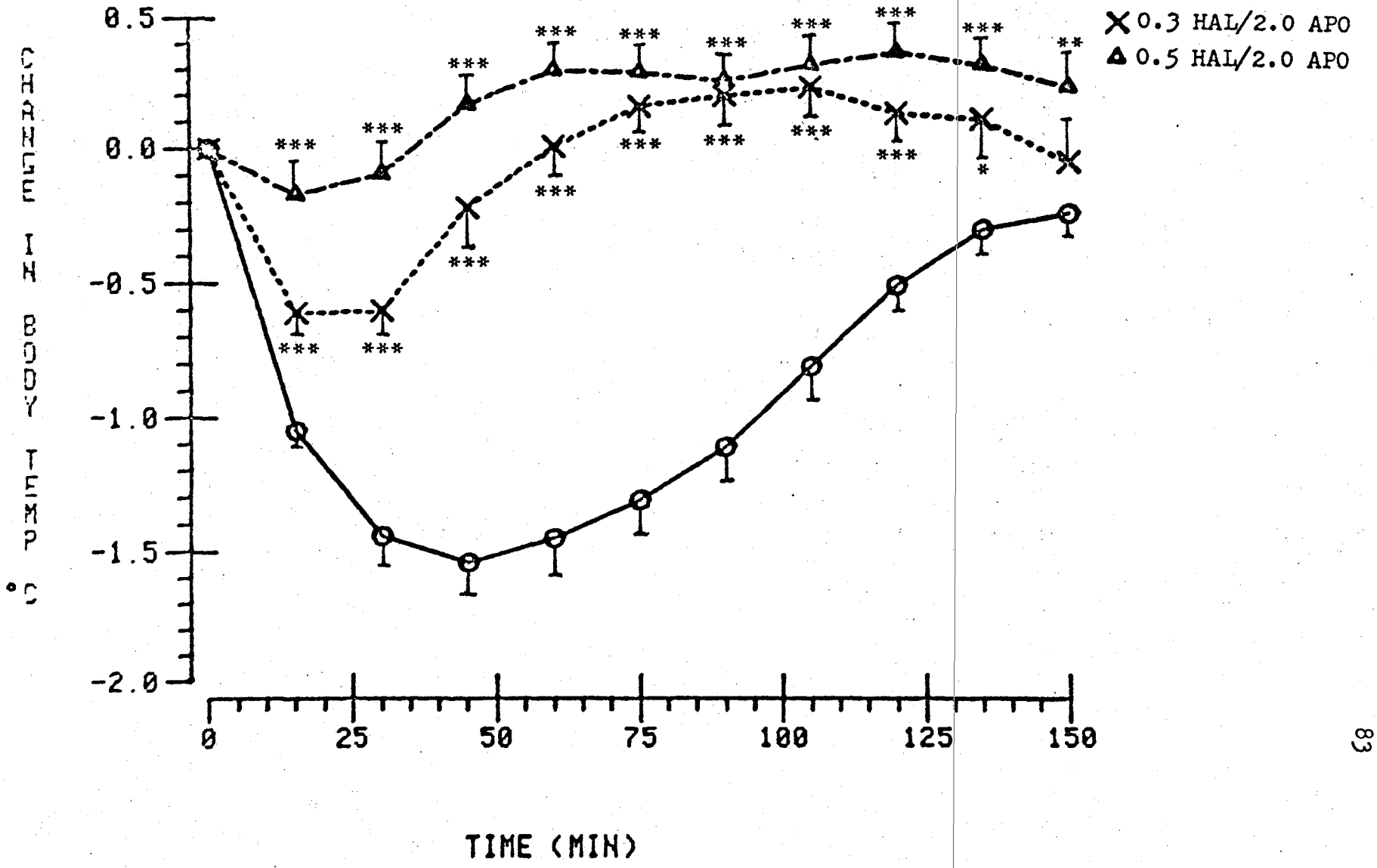


Figure 13--Change in body temperature induced by 2.0 mg/kg APO after pretreatment with 0.3 mg/kg HAL in SHR, WKY, and NWR, and after pretreatment with 0.5 mg/kg HAL in SHR. (For n values see Figures 10, 11, and 12.)

Key: TEMP = Temperature

** = Highly significantly different from SHR treated with 0.3 mg/kg HAL + 2.0 mg/kg APO ($p<0.01$).

*** = Very highly significantly different from SHR treated with 0.3 mg/kg HAL + 2.0 mg/kg APO ($p<0.001$).

FIGURE THIRTEEN

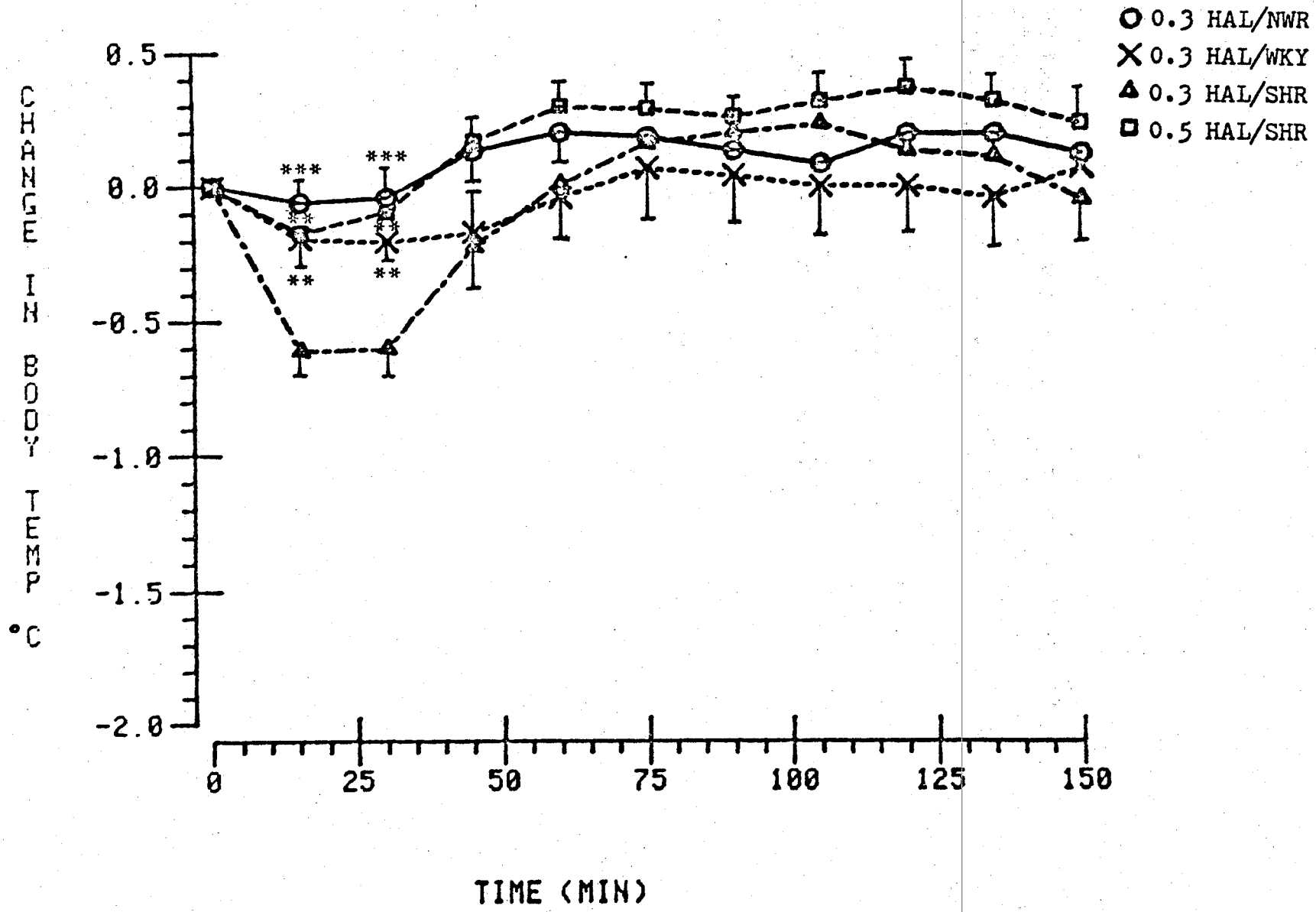


Figure 14--Hypothermia induced by 0.25 mg/kg APO. (SHR = 13 rats; WKY = 8 rats; NWR = 9 rats.)

* = Significantly different from SHR ($p<0.05$).

** = Highly significantly different from SHR ($p<0.01$).

FIGURE FOURTEEN

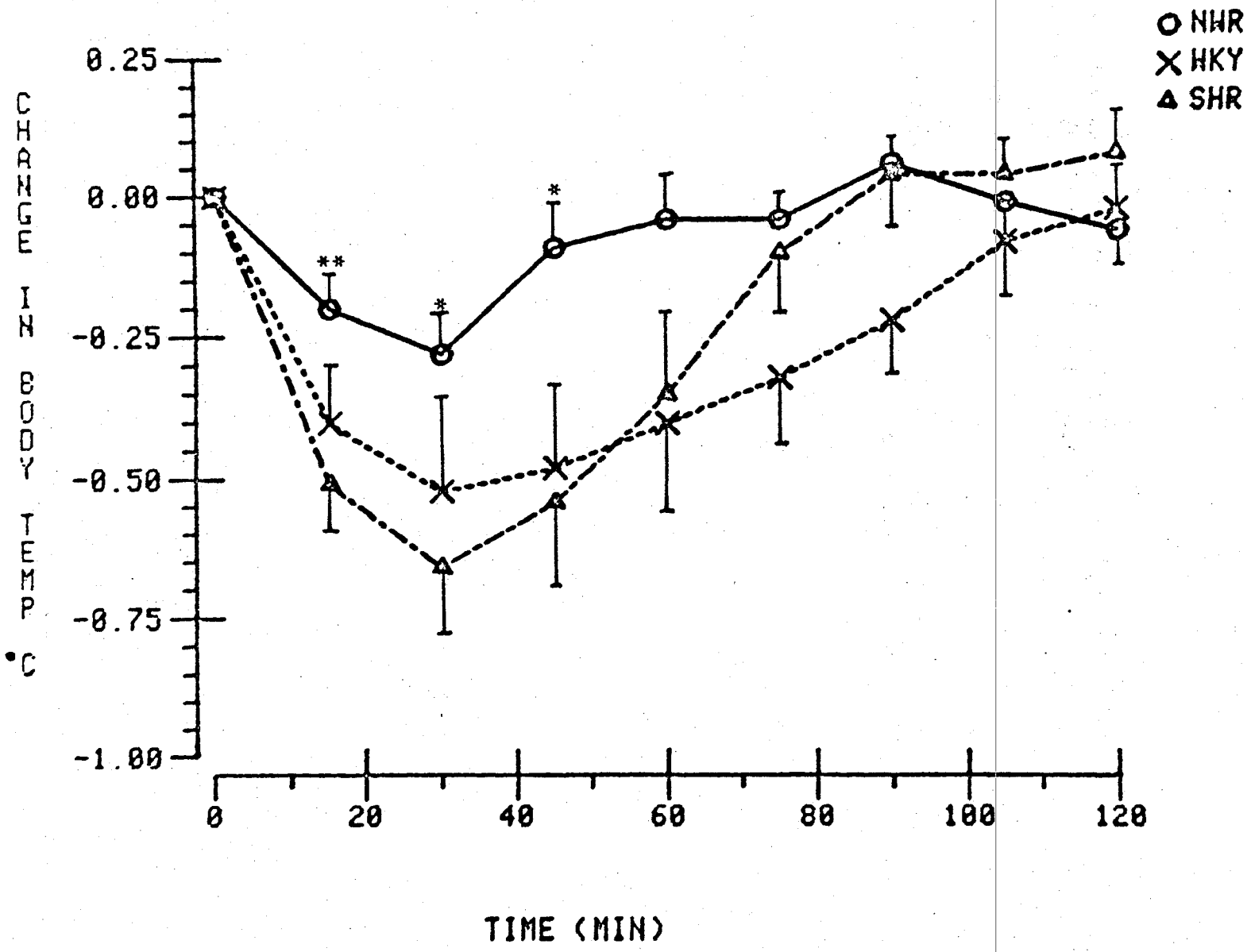


Figure 15--Hypothermia induced by 0.35 mg/kg APO. (SHR = 8 rats; WKY = 9 rats; NWR = 8 rats.)

Key: TEMP = Temperature

** = Highly significantly different from SHR ($p<0.01$).

*** = Very highly significantly different from SHR ($p<0.001$).

FIGURE FIFTEEN

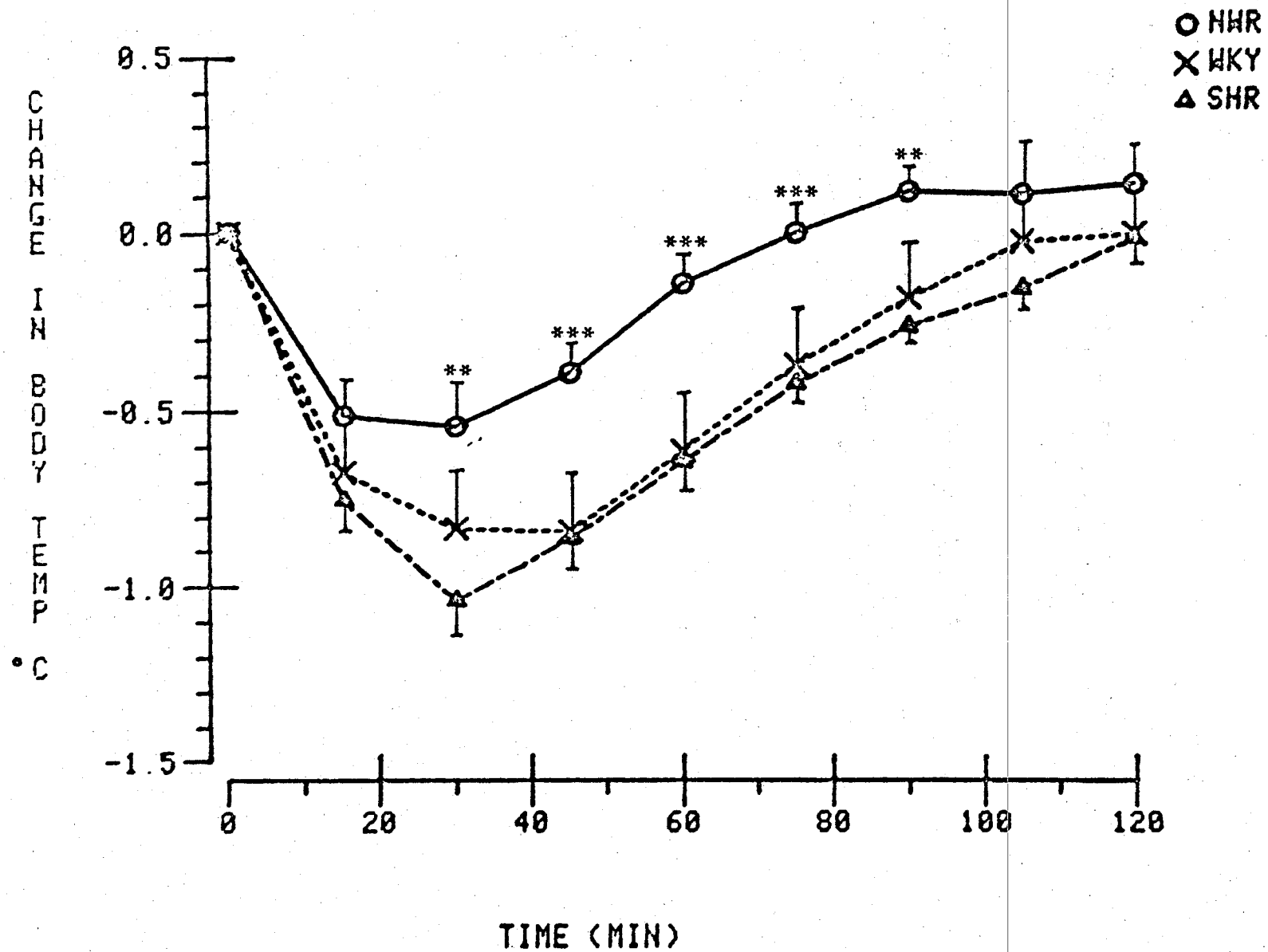


Figure 16--Hypothermia induced by 0.5 mg/kg APO. (n = 9 for each rat strain.)

Key: TEMP = Temperature

* = Significantly different from SHR ($p<0.05$).

** = Highly significantly different from SHR ($p<0.01$).

*** = Very highly significantly different from SHR ($p<0.001$).

FIGURE SIXTEEN

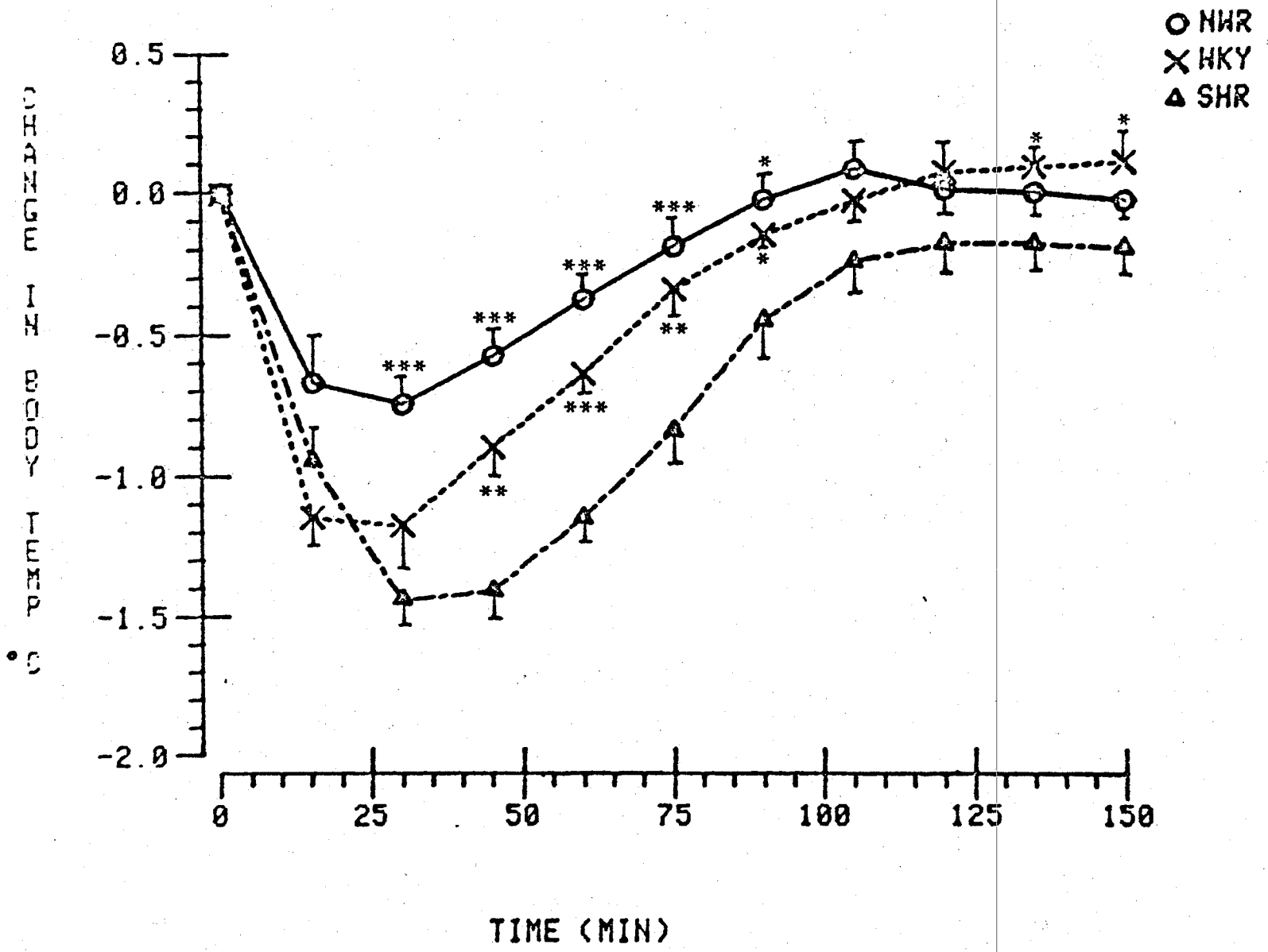


Figure 17--Log-dose response curves of maximum decreases in body temperature produced by APO. (For n values see Figures 14, 15, and 16.)

Key: MAX DROP IN B. T. = Maximum Drop in Body Temperature

** = Highly significantly different from SHR ($p<0.01$).

*** = Very highly significantly different from SHR ($p<0.001$).

FIGURE SEVENTEEN

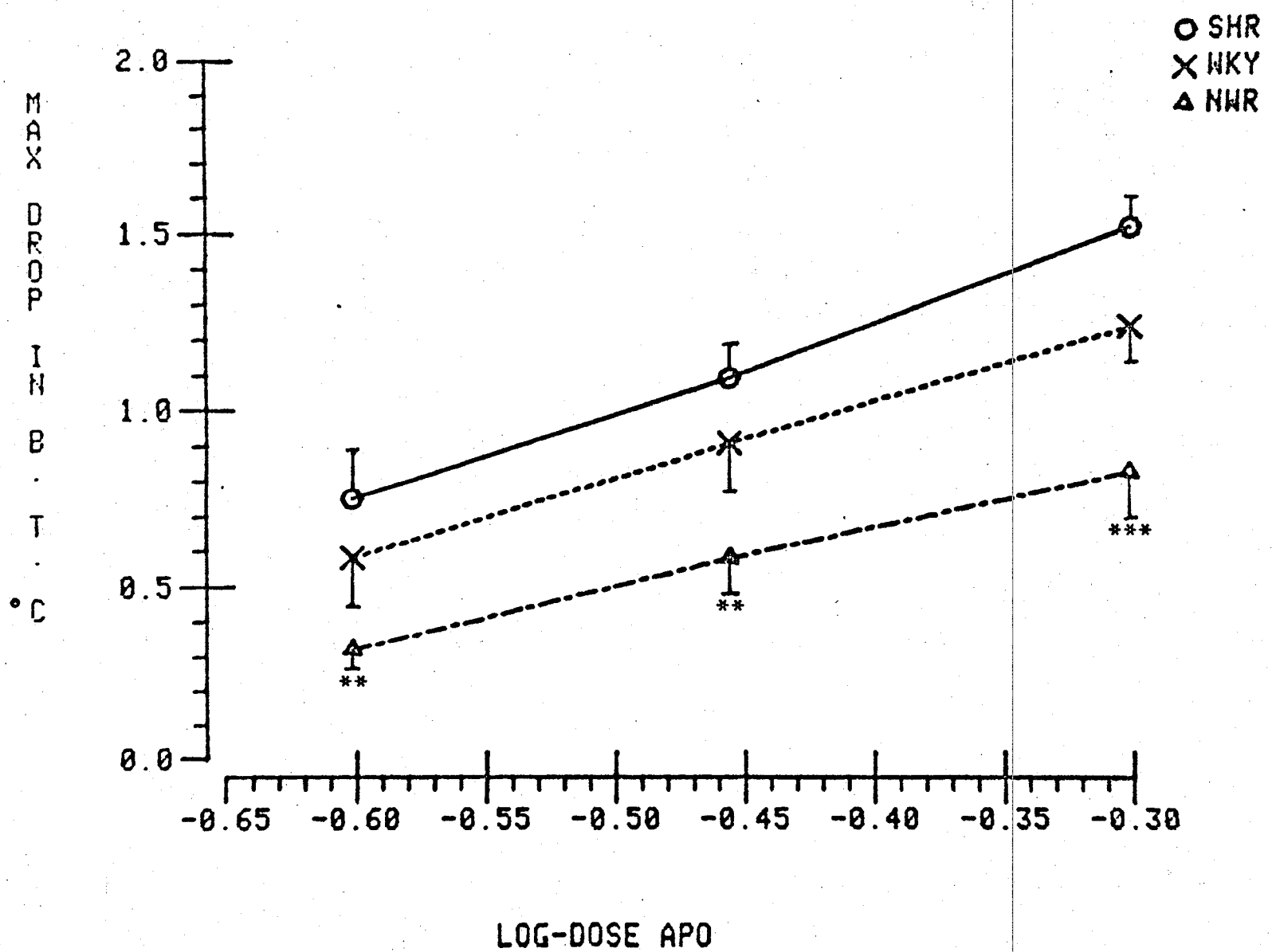


Figure 18--Doses of APO required to elicit the same hypothermic response:
0.5 mg/kg in SHR (n = 9);
2.0 mg/kg in WKY (n = 25);
4.0 mg/kg in NWR (n = 8).

Key: TEMP = Temperature

FIGURE EIGHTEEN

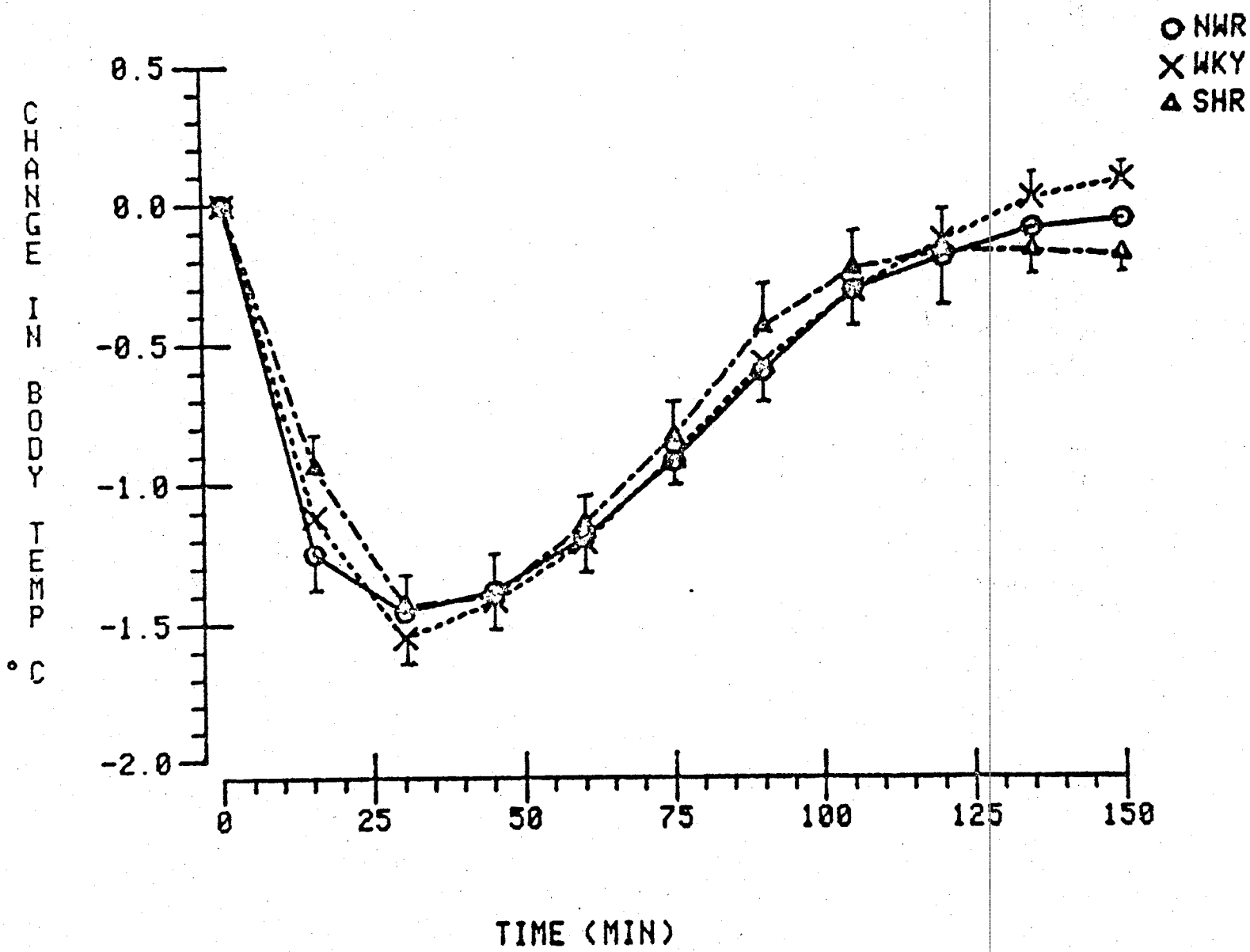


Figure 19--Hypothermia produced by threshold and suprathreshold doses of APO in WKY.

Key: 2.0 APO = 2.0 mg/kg dose of APO (n = 25 rats); 4.0 APO = 4.0 mg/kg dose of APO (n = 6 rats); TEMP = Temperature.

FIGURE NINETEEN

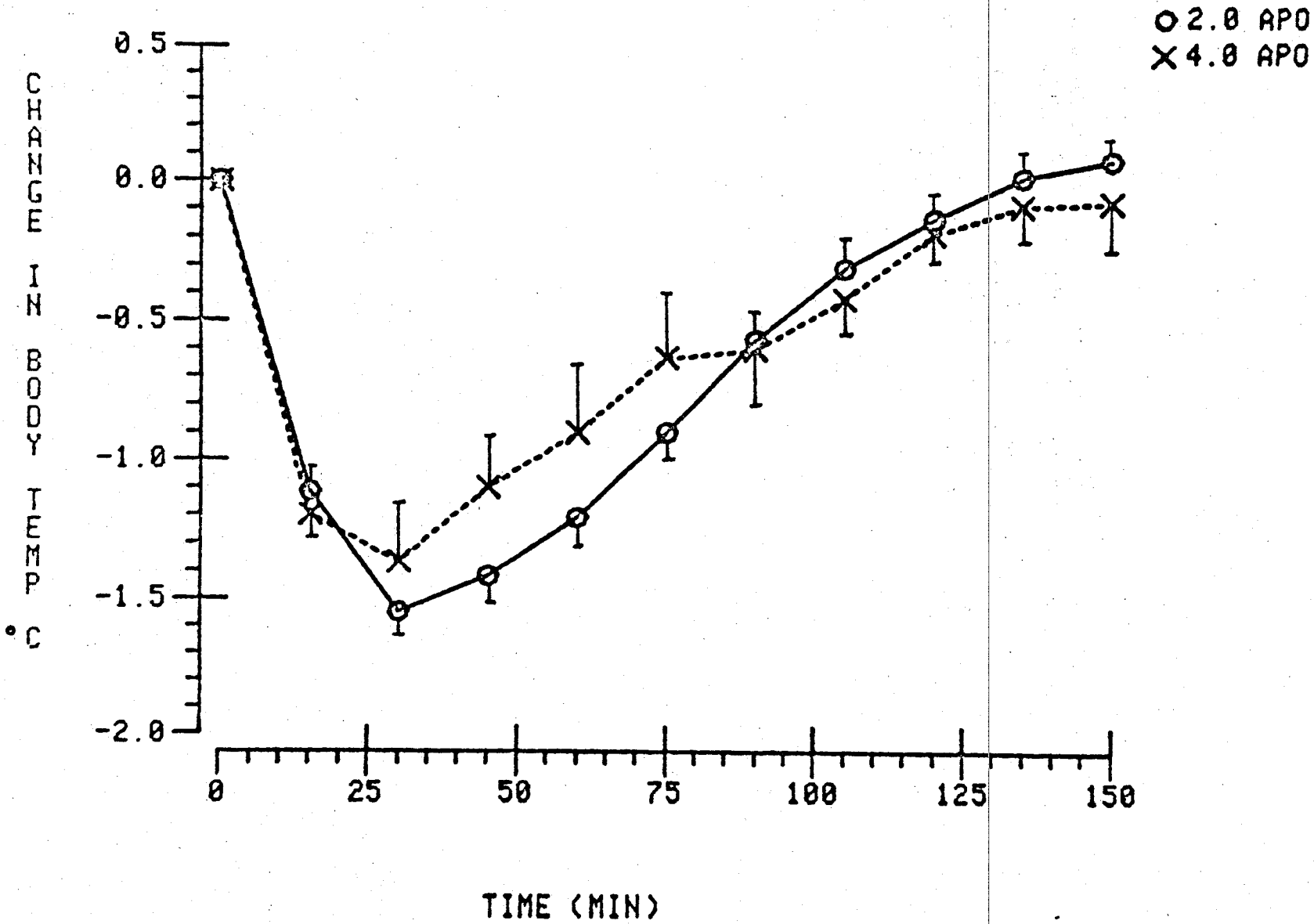


Figure 20--Hypothermia produced by threshold and suprathreshold doses of APO in SHR.

Key: 0.5 APO = 0.5 mg/kg dose of APO (n = 9 rats); 2.0 APO = 2.0 mg/kg dose of APO (n = 15 rats); TEMP = Temperature.

* = Significantly different from 2.0 mg/kg APO ($p<0.05$).

** = Highly significantly different from 2.0 mg/kg APO ($p<0.01$).

FIGURE TWENTY

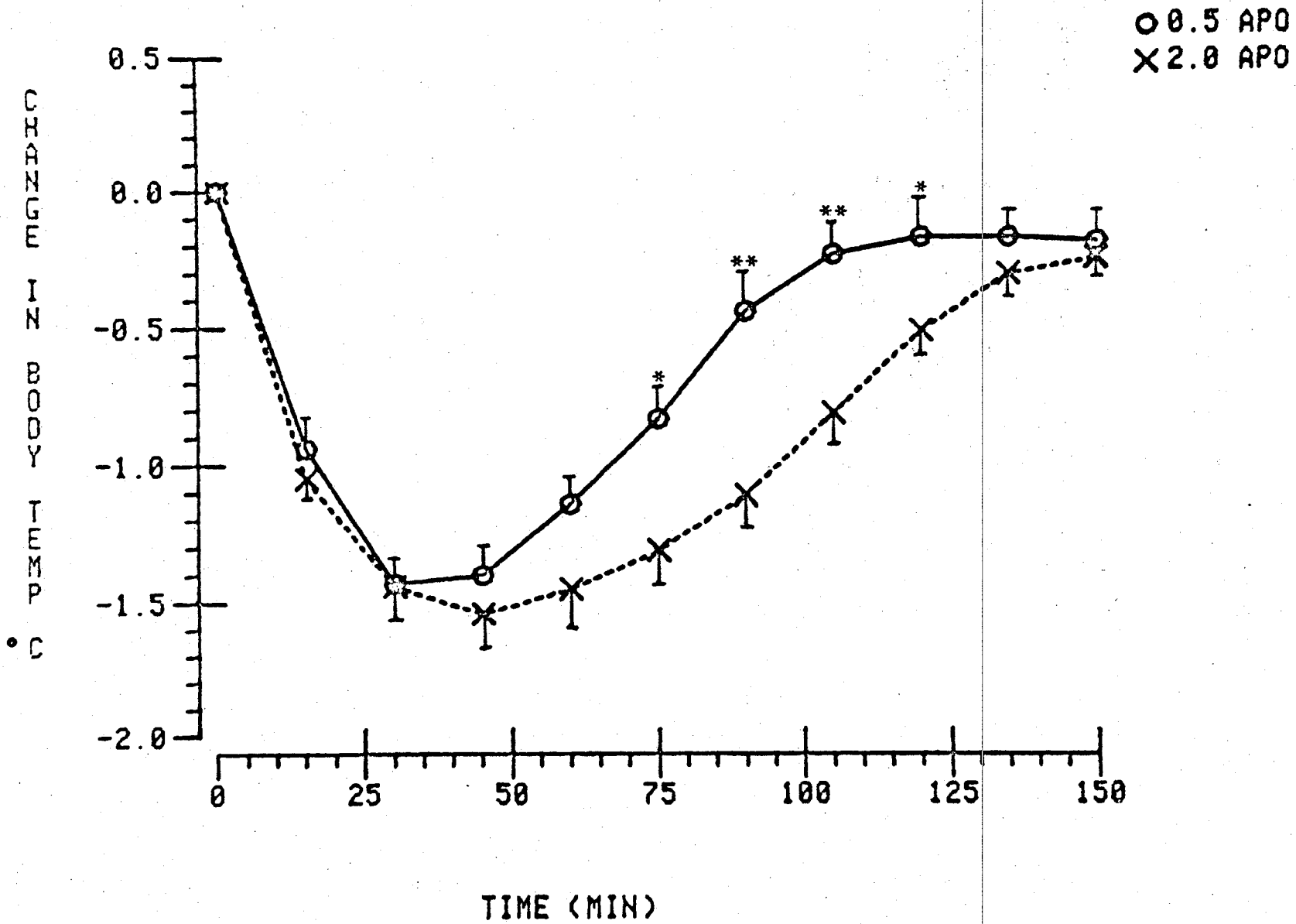


Figure 21--Hypothermia induced by 50 mg/kg carbidopa + 25 mg/kg L-DOPA. (SHR = 9 rats; WKY = 8 rats; NWR = 7 rats.)

Key: TEMP = Temperature.

* = Significantly different from SHR ($p<0.05$).

** = Highly significantly different from SHR ($p<0.01$).

*** = Very highly significantly different from SHR ($p<0.001$).

FIGURE TWENTY-ONE

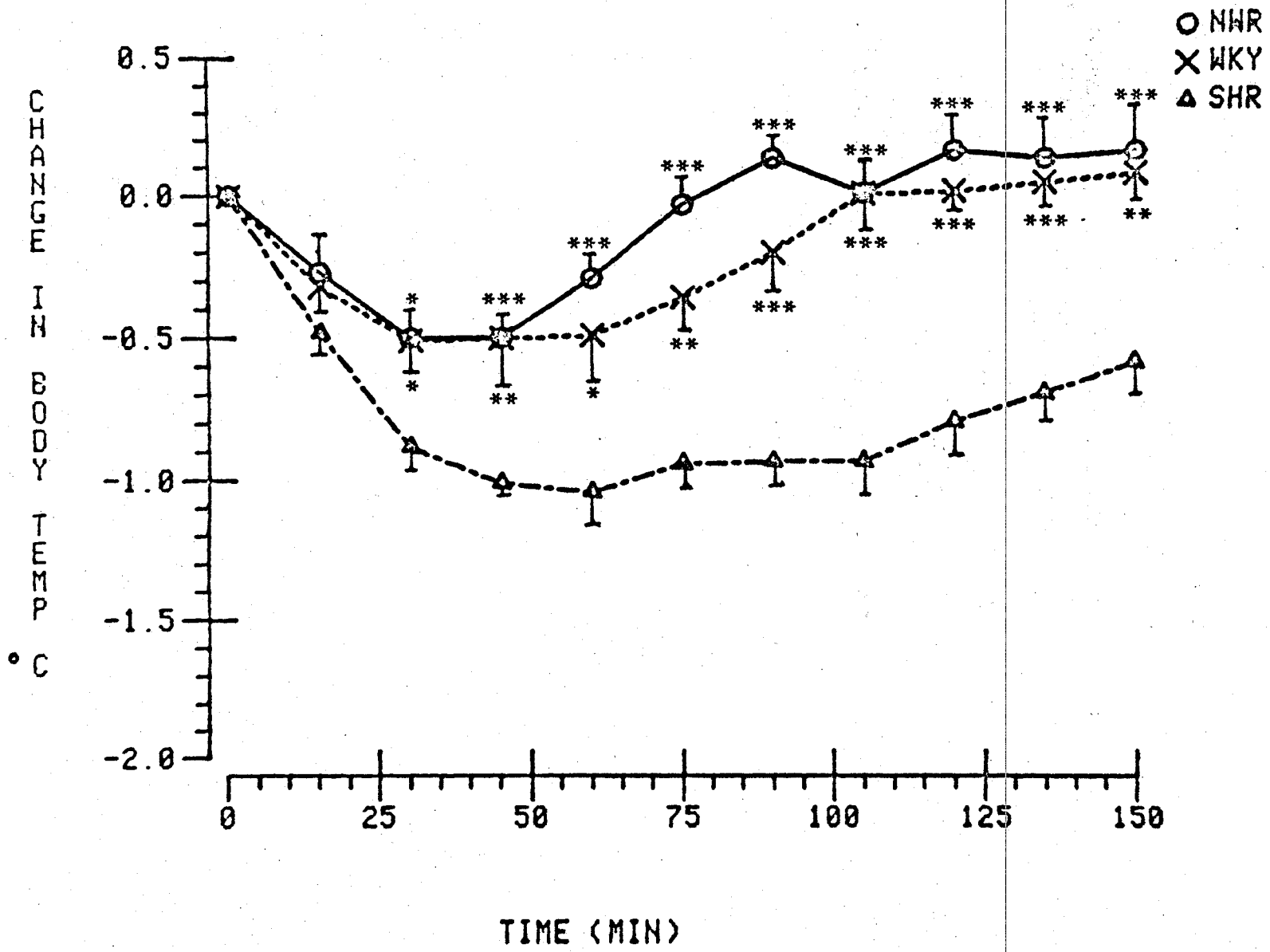


Figure 22--Hypothermia induced by 50 mg/kg carbidopa + 50 mg/kg L-DOPA. (SHR = 8 rats; WKY = 7 rats; NWR = 9 rats.)

Key: TEMP = Temperature.

* = Significantly different from SHR ($p<0.05$).

** = Highly significantly different from SHR ($p<0.01$).

*** = Very highly significantly different from SHR ($p<0.001$).

FIGURE TWENTY-TWO

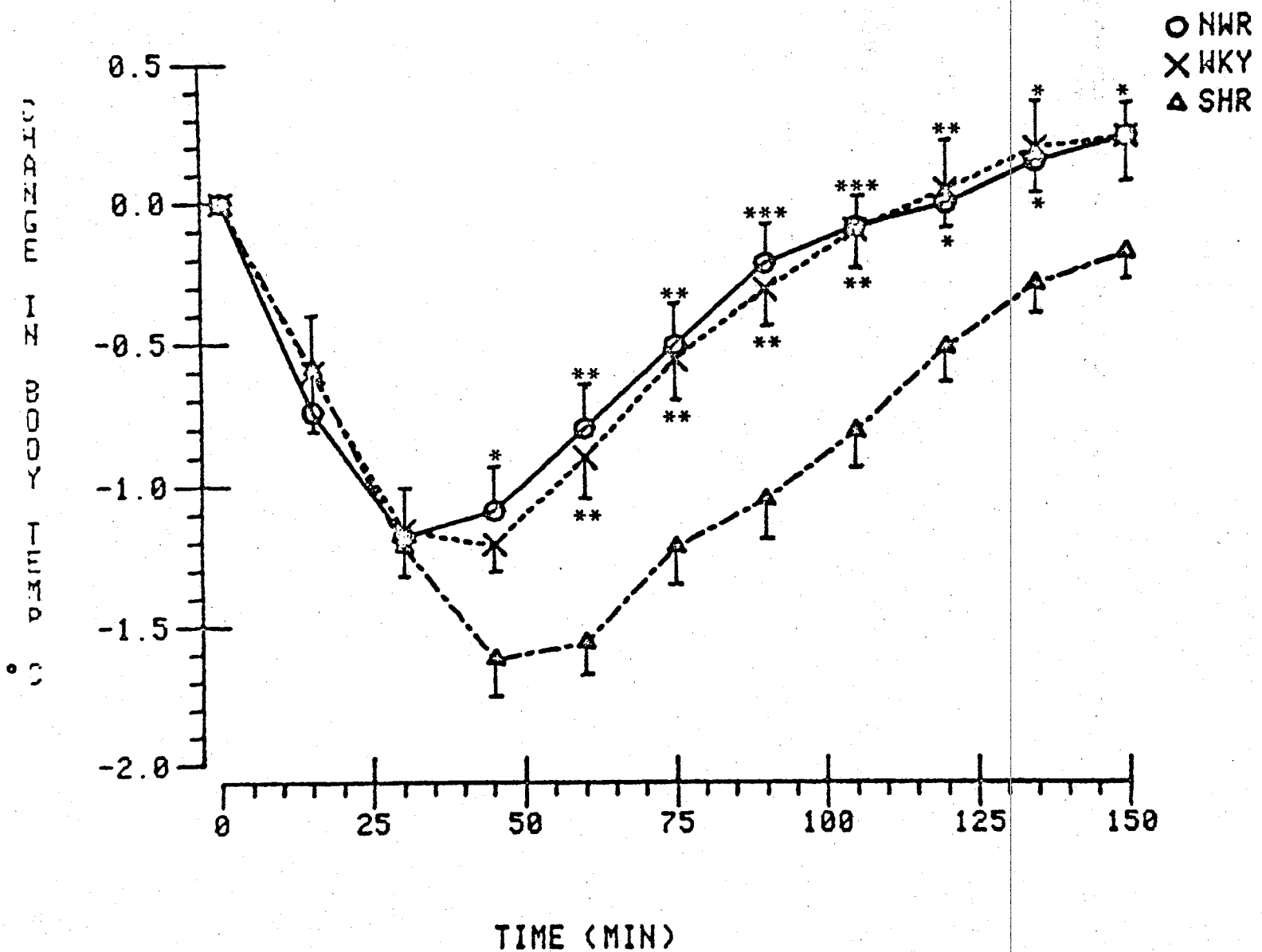


Figure 23--Hypothermia induced by 50 mg/kg carbidopa + 100 mg/kg L-DOPA. (n = 8 rats for each rat strain.)

Key: TEMP = Temperature.

* = Significantly different from SHR ($p<0.05$).

** = Highly significantly different from SHR ($p<0.01$).

*** = Very highly significantly different from SHR ($p<0.001$).

FIGURE TWENTY-THREE

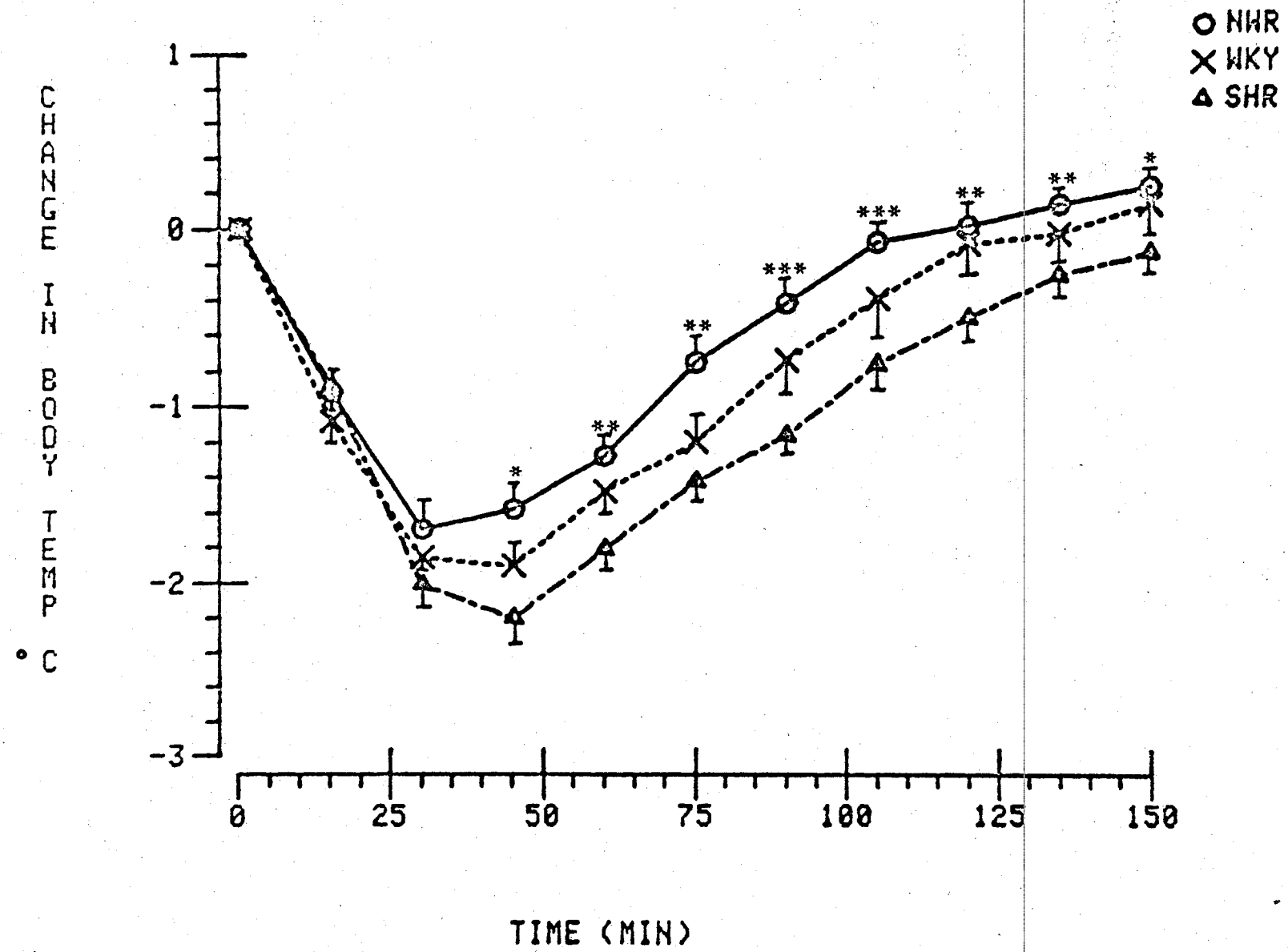


Figure 24--Log-dose response curves to carbidopa + L-DOPA-induced hypothermia. (For n values see Figures 21, 22, and 23.)

Key: MAX DROP IN B. T. = Maximum Drop in Body Temperature.

* = Significantly different from SHR ($p<0.05$).

*** = Very highly significantly different from SHR ($p<0.001$).

FIGURE TWENTY-FOUR

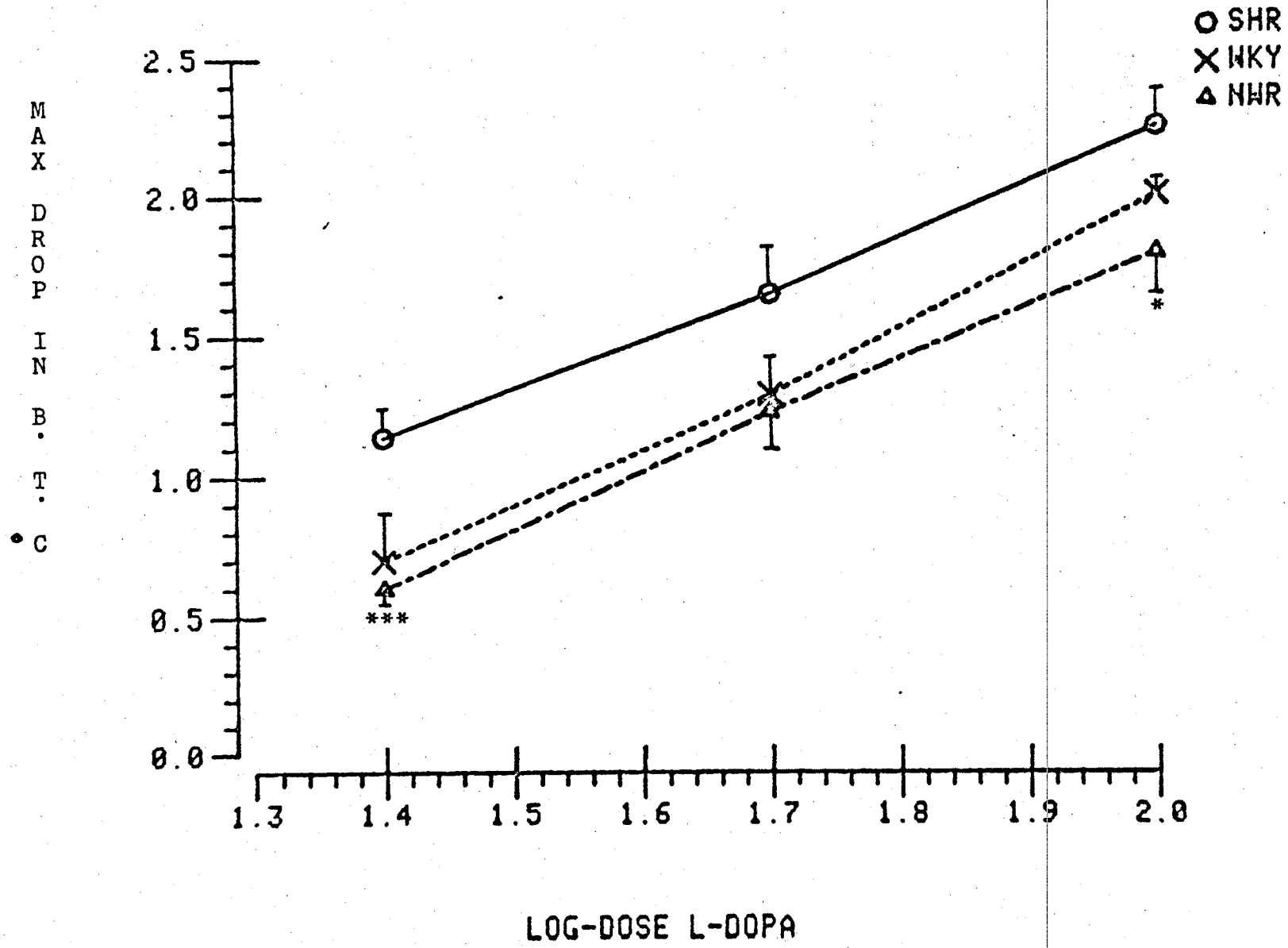


Figure 25--Hypothermia induced by 0.5 mg/kg APO after chronic treatment with sodium or lithium in NWR.

Key: NA TX = Sodium-Treated (n = 8);
LI TX = Lithium-Treated (n = 8);
TEMP = Temperature.

* = Significantly different from lithium-treated rats ($p<0.05$).

FIGURE TWENTY-FIVE

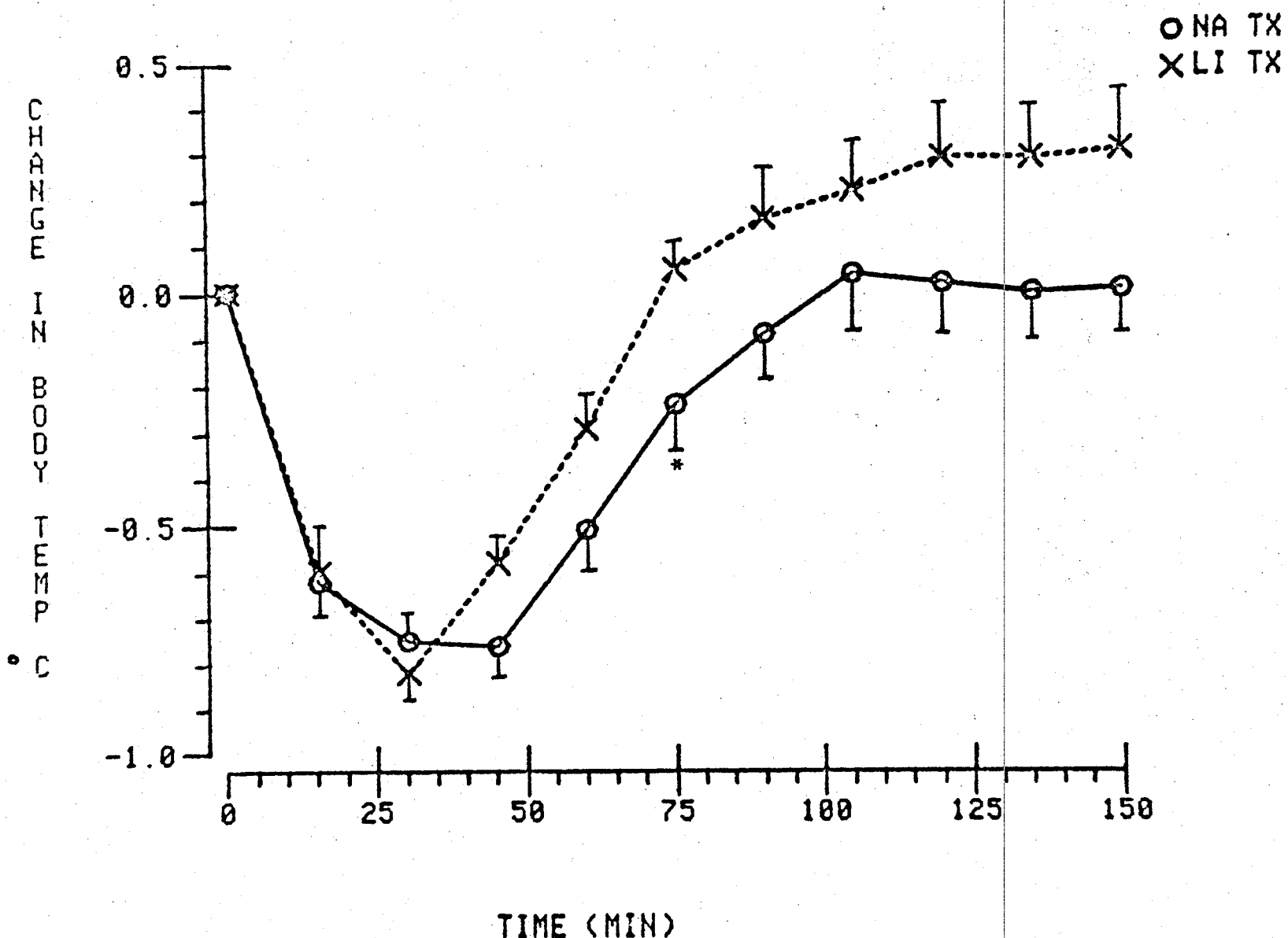


Figure 26--Hypothermia induced by 0.5 mg/kg APO after chronic treatment with sodium or lithium in WKY.

Key: NA TX = Sodium-Treated (n = 11);
LI TX = Lithium-Treated (n = 10);
TEMP = Temperature.

FIGURE TWENTY-SIX

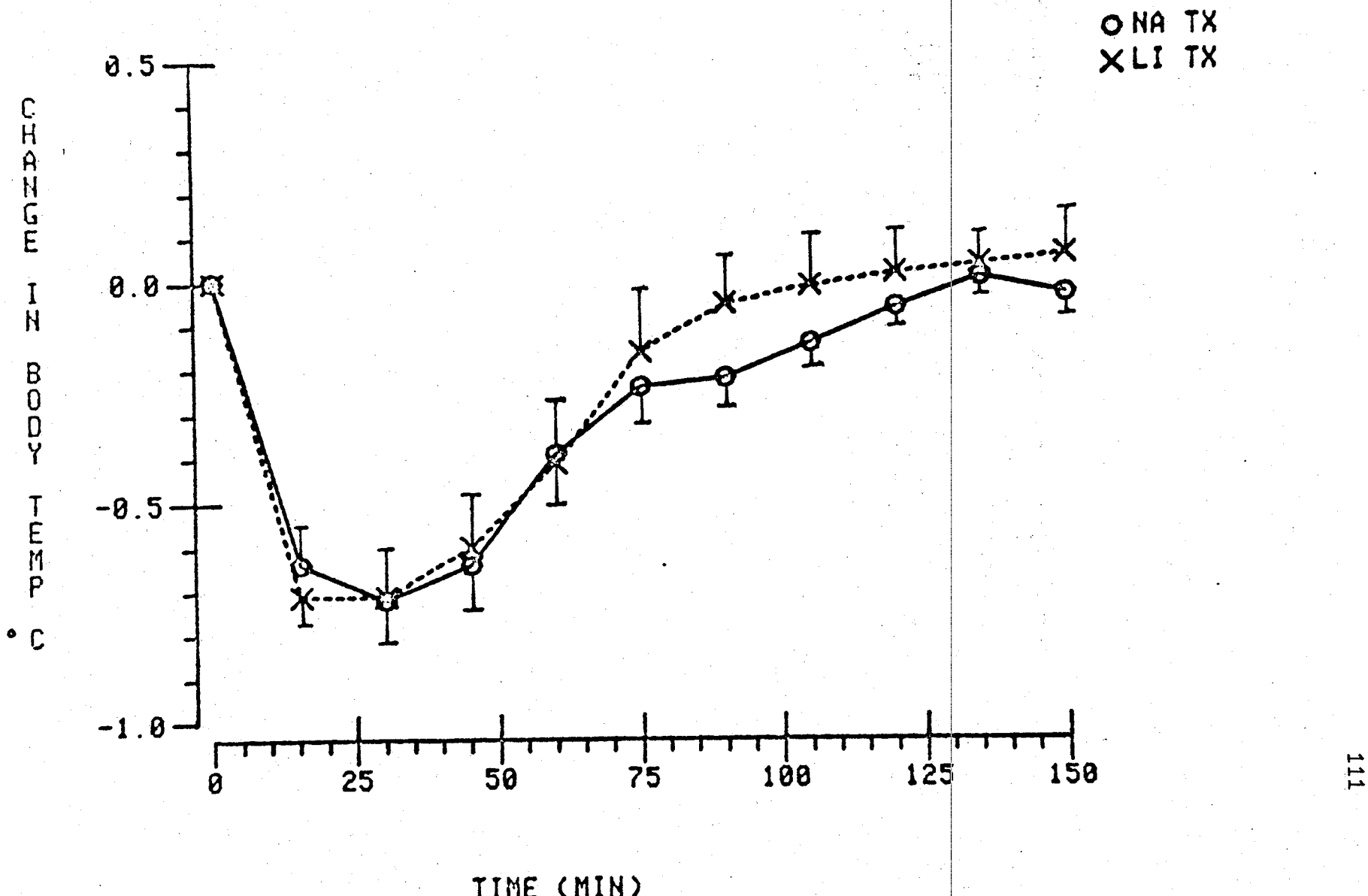


Figure 27--Hypothermia induced by 0.5 mg/kg APO after chronic treatment with sodium or lithium in SHR.

Key: NA TX = Sodium-Treated (n = 9);
LI TX = Lithium-Treated (n = 9);
TEMP = Temperature.

* = Significantly different from lithium-treated rats ($p<0.05$).

** = Highly significantly different from lithium-treated rats ($p<0.01$).

*** = Very highly significantly different from lithium-treated rats ($p<0.001$).

FIGURE TWENTY-SEVEN

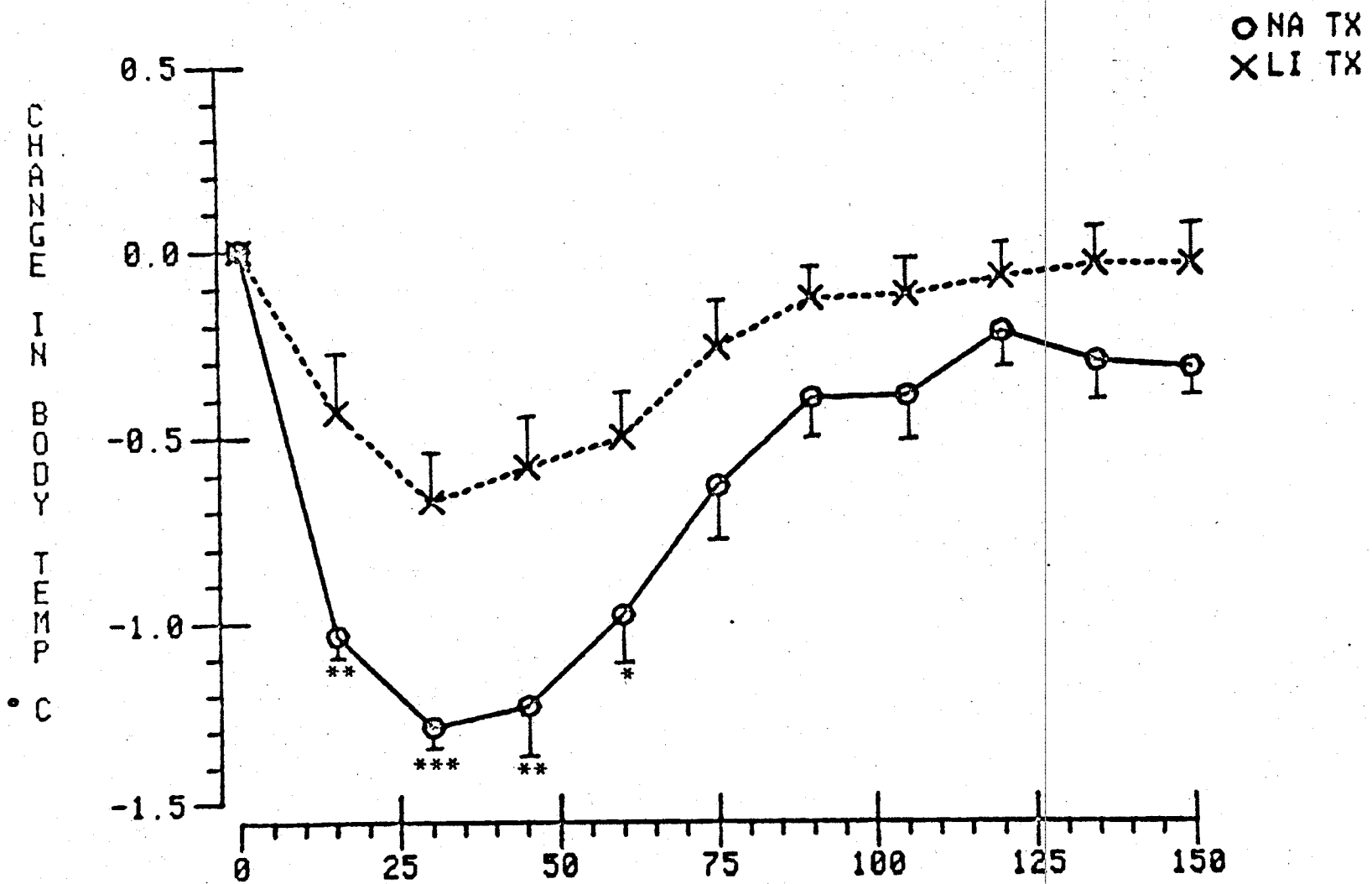


Figure 28--Hypothermia induced by 0.5 mg/kg APO after chronic treatment with lithium. (For n values see Figures 25, 26, and 27.)

Key: TEMP = Temperature.

* = Significantly different from SHR ($p<0.05$).

FIGURE TWENTY-EIGHT

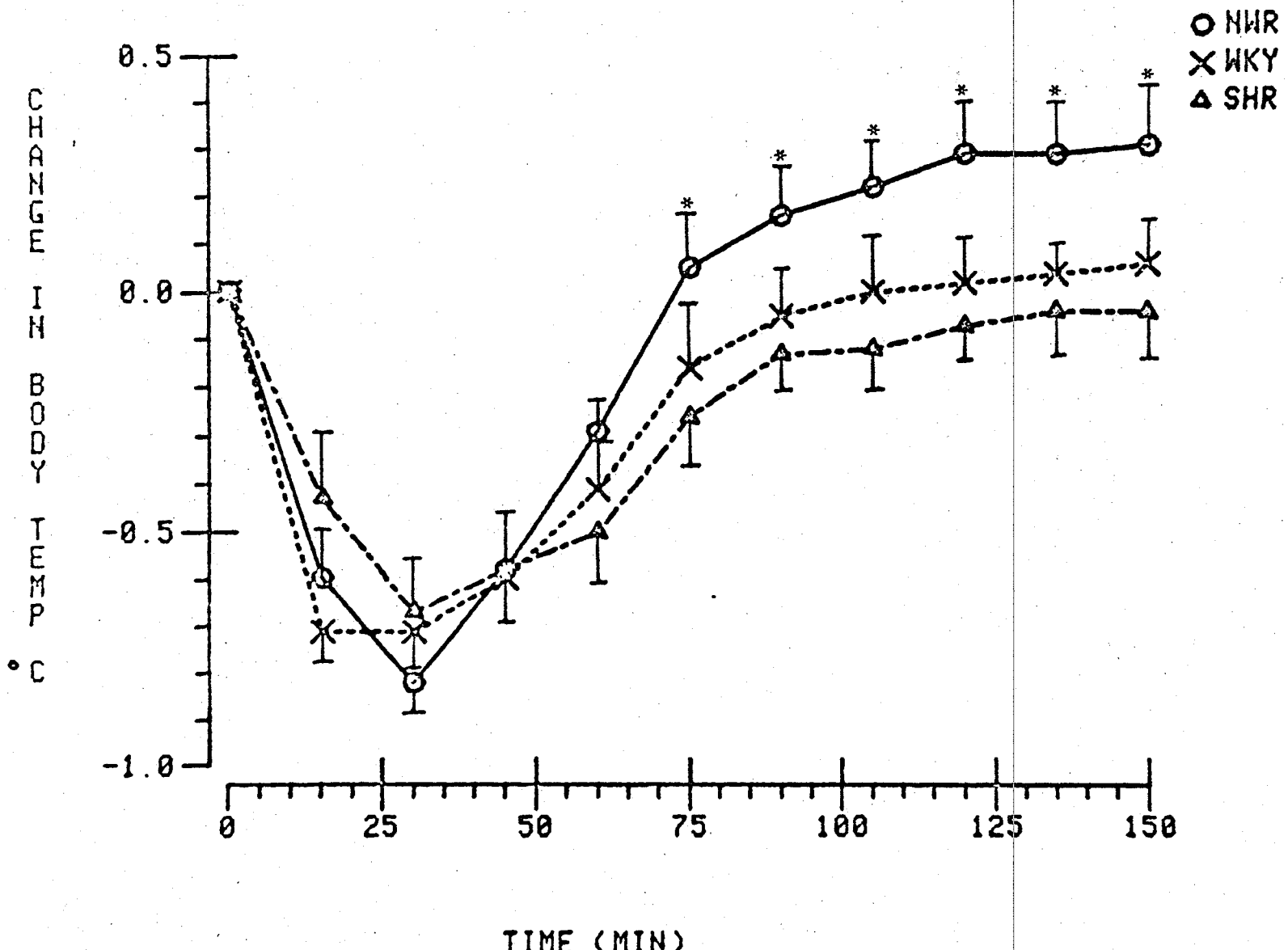


TABLE IV

Blood Pressures Before and After Sodium or Lithium Treatment

Rat Strain (n)	Treat-ment	Blood Pressures Before (mmHg)	After (mmHg)
NWR (8)	Na	134 ± 2	144 ± 6
	Li	134 ± 3	135 ± 4
SHR (9)	Na	198 ± 1	214 ± 8
	Li	203 ± 2	190 ± 10

Figure 29--Effect of chronic hydralazine treatment on hypothermia induced by 0.5 mg/kg APO in SHR.

Key: HYD TX = Hydralazine-Treated (n = 8);
NON TX = Non-Treated (n = 9);
TEMP = Temperature.

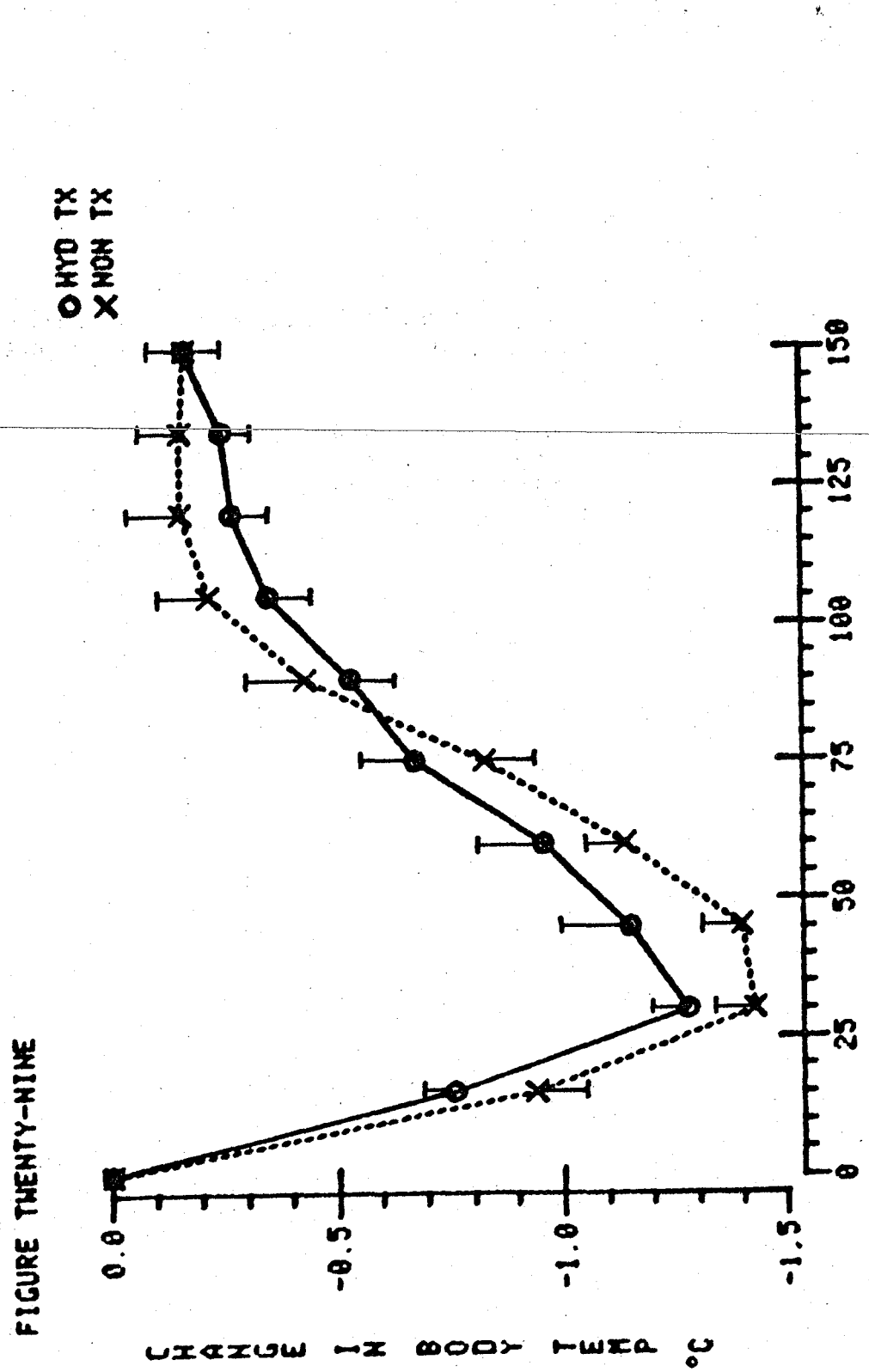


FIGURE TWENTY-NINE

DISCUSSION

Results of the present study agree and disagree with several of those reported for earlier studies. The stereotyped behavior results, in particular, agree with those reported by Shimamoto and Nagaoka (1972) thereby confirming the lack of difference in APO-induced stereotyped behavior between SHR and normotensive rat strains. SHR showed a spontaneous locomotor activity immediately after handling and placement into the test cage which was not significantly different from that of either WKY or NWR. These results conflict with the results obtained by other investigators (Shimamoto and Nagaoka, 1972; Pappas et al., 1974; Rosecrans and Adams, 1976; Myers et al., 1977). However, the present study appears to be the first on the effect that a direct-acting DA agonist has on locomotor activity of SHR. Additionally, this is also the first study on the effect that APO has on the body temperature of SHR. These latter experiments have given further insight into the possible alterations of hypothalamic function of SHR. Therefore the objectives set down for this study have been met as to determining the possible alterations in DA receptor sensitivity in SHR in the: 1) corpus striatum; 2) mesolimbic forebrain; and 3) hypothlalmus.

No significant difference in APO-induced stereotyped behavior was found to exist between the three rat strains either in onset, intensity, or duration. If SHR do possess supersensitive striatal DA receptors, then the gnawing behavior would have been more prolonged at 2.0 mg/kg APO than in the two normotensive rat strains. Furthermore, the intensity of behavior displayed at 0.5 mg/kg APO would probably have been greater in SHR. Instead, the duration of stereotyped behavior appeared to be slightly shorter in SHR which could possibly be suggestive of subsensitive striatal DA receptors. However, the intensity of stereotypy observed in each of the three strains was not significantly different from one another at either dose of APO. Therefore there appears to be no alteration in striatal DA receptor sensitivity in SHR.

The low intensity component of APO-induced stereotyped behavior is present upon return to normal behavior and this lower intensity of behavior is apparently mediated via the mesolimbic forebrain (Costall et al., 1975). This brain region is also apparently responsible for mediation of locomotor activity (Jackson et al., 1975; Costall and Naylor, 1976a). Serotonergic neuronal systems also apparently have an inhibitory effect on these mesolimbic dopaminergic neurons which mediate locomotor activity (Costall et al., 1976b) and may possibly have an inhibitory effect on the low intensity component of APO-induced stereotyped behavior since it has locomotor activity incorporated into it. As APO has been suggested to activate central serotonin neurons and thereby

attenuate its own stimulatory effect on locomotor activity (Grabowska et al., 1973a), it may be possible that the slightly faster return to normal behavior demonstrated by SHR is the result of stimulation by APO of the hypothesized (Rosecrans and Adams, 1976) hyperfunctional central serotonergic neuronal systems present in SHR.

APO was able to significantly increase locomotor activity in WKY and NWR for at least 36 min of the total 90 min, whereas in SHR APO increased locomotor activity for only 18 min of the entire 90 min. In addition, APO-induced locomotor activity was much less, although not significantly, in SHR than either WKY or NWR. A possible explanation for such results is similar to the one suggested for the faster return to normal behavior from stereotyped behavior. Since central serotonergic neuronal systems apparently inhibit the mesolimbic dopaminergic neuronal systems responsible for induction of locomotor activity (Costall et al., 1976b), and APO appears to stimulate these inhibitory neuronal systems (Grabowska et al., 1973a), then the possible stimulation of the hypothesized (Rosecrans and Adams, 1976) hyperfunctional serotonergic systems in SHR may attenuate the locomotor stimulatory effects of APO.

Both the stereotyped behavior and locomotor activity results obtained in the present study need further confirmation. This could probably be best determined by direct application of direct-acting dopaminergic agonists, including DA itself, to the corpus striatum and mesolimbic forebrain. This would, of course, require the implantation of bilateral

cannulae into the caudate nucleus and nucleus accumbens. Comparison of the responses elicited in SHR to those elicited in WKY and NWR, as in the present study, would supply further information as to the sensitivity of the DA receptors in these areas of the brain in SHR.

Spontaneous locomotor activity did not differ significantly between the three rat strains. These results disagree with those of earlier studies which have indicated a greater spontaneous locomotor activity in SHR (Shimamoto and Nagaoka, 1972; Pappas et al., 1974; Myers et al., 1977), and a lesser spontaneous locomotor activity in SHR (Rosecrans and Adams, 1976). These differences in results may be due to differences in experimental design. Whereas the present study was interested in locomotor activity, several of the earlier studies were interested in open field behavior as well as locomotor activity. Therefore in these earlier studies the animals had not been previously acclimated to their test cage or enclosure, whereas in the present study the animals had been acclimated to their test cage prior to commencement of the experiment. It may be possible that SHR will show greater exploratory activity and thus locomotor activity upon placement into strange surroundings than normotensive control animals. This increase in exploratory behavior and thus activity may be due to the animal's reaction to a new environment. Measuring an animal's reaction to new surroundings is the purpose of the open field test. Therefore these investigations may have measured "reactivity" whereas the present study, since the animals had already undergone their reaction to the environment,

In contrast, Rosecrans and Adams (1976) found activity to be less in SHR than normotensive rats upon placement into a strange environment. Upon repeated exposure to the test environment the activity of the normotensive control strain decreased dramatically while that of SHR changed very little. As a result, the locomotor activity of SHR subsequently became greater than that of the normotensive animals. This investigation apparently also measured reactivity rather than activity. It appears that the results obtained in the present study cannot be reconciled with those of the previously reported studies since the locomotor activity of SHR did not differ from either of the normotensive control rat strains utilized in this study. However, it seems that the earlier studies measured reactivity whereas the present study measured locomotor activity.

Furthermore, overnight spontaneous locomotor activity did not differ between SHR and the normotensive control strains. This would seem to provide further evidence that spontaneous activity does not differ between SHR and normotensive animals, especially when it is taken into consideration that rats are nocturnal creatures. In addition, if mesolimbic DA receptors of SHR were supersensitive or subsensitive, then compensatory mechanisms have occurred so that spontaneous locomotor activity remains similar to that of normotensive animals. Supersensitive mesolimbic DA receptors in SHR, however, are probably unlikely due to the smaller increase in locomotor activity induced by APO in SHR than WKY or NWR. The possibility of subsensitive mesolimbic DA receptors is at present diffi-

cult to eliminate and must await further investigations.

APO elicited a significantly greater fall in body temperature in SHR than NWR while the response elicited from WKY was intermediate to the other two rat strains at all doses used. As a result, the log-dose response curves obtained from the maximum drop in body temperature for the three rat strains were parallel to one another. Because of the significant difference between SHR and NWR, it was determined that APO appears to be approximately 1.78 times more potent in SHR than NWR. This apparent greater potency of APO could be due to one of several reasons. These reasons could also be used to explain why a larger dose of HAL was required in order to attenuate the hypothermic response in SHR to the same degree as in WKY and NWR.

The first explanation is that SHR may metabolize APO at a slower rate than WKY or NWR which would thus allow APO to act for a longer period of time on the DA receptors. A slower rate of metabolic degradation of APO would not be inconsistent with the finding that ethanol is metabolized at a slower rate in naive SHR than naive NWR (Israel et al., 1977). It is known that approximately 80 percent of APO is conjugated with glucuronide in rat liver and then excreted in the urine (Kaul et al., 1961a, 1961b). The other 20 percent is apparently metabolized by both liver and brain catechol-O-methyl transferase (COMT) (McKenzie and White, 1973). Since the liver is the major site for the metabolic degradation of APO, alterations of liver metabolic enzymes in SHR could increase the sensitivity of this rat strain to APO. However, several

earlier studies have found that this is not the case. Pentobarbital sleeping time was found to be significantly shorter in SHR than WKY which was suggested to be the result of a faster rate of metabolic degradation of pentobarbital in SHR (Willis and Queener, 1977). In a separate study it was found that the hexobarbital sleeping time, in vitro activities of microsomal enzymes, and the concentration of cytochrome P-450 in SHR did not differ significantly from those of NWR (Vainionpaa et al., 1974). Neither of these results are consistent with the concept of a slower rate of metabolic degradation of APO in SHR. In addition, if the rate of metabolic degradation of APO were slower in SHR, then the intensity and duration of APO-induced stereotyped behavior, and the duration of APO-induced locomotor activity should have been greater in SHR than in WKY and NWR rather than smaller as observed in the present study. It may be possible, however, that the greater amount of APO present due to its slower rate of breakdown and removal would allow stimulation of the hyperfunctional serotonergic system in SHR (Rosecrans and Adams, 1976) thereby causing these APO-induced responses to inhibit and attenuate themselves. However, the alteration of the hypothermic response in SHR by chronic lithium treatment would be difficult to account for by this hypothesis (as will be discussed later).

It could also be suggested that there may be an alteration in COMT activity in SHR which would have to be localized to the hypothalamus. However, it again would be difficult to explain the results obtained from the SHR chronically treated

with lithium (as will be discussed later).

A second explanation for the observed difference in the hypothermic response is that there may be an enhanced distribution of APO to the brain of SHR. If this were the case, however, then APO-induced stereotyped behavior and locomotor activity would also have been significantly greater in SHR. This lack of difference is not surprising considering that APO is highly lipid-soluble and easily permeates the blood-brain-barrier (B-B-B). Therefore no significant difference would be expected between the normotensive strains and SHR. This would be so even if there were a more permeable B-B-B present in SHR as suggested by Tanaka et al. (1972). As a result, any enhanced distribution of APO in SHR would have to be to the site of the hypothermic response which has been shown to reside within the preoptic anterior hypothalamus (Cox and Lee, 1977a, 1977b).

APO has been shown to have a distribution to the hypothalamus which is less than to the rest of the brain (Butterworth and Barbeau, 1974). An alteration within the brain of SHR which would allow a greater amount of APO to reach the hypothalamus could account for the increased sensitivity of this unique rat strain to the hypothermic effect of APO. This alteration would also have to allow enhanced distribution to the hypothalamus of very lipid-insoluble compounds. SHR was found to be more sensitive than normotensive strains to the hypothermic effect of L-DOPA, a highly lipid-insoluble molecule, in a manner similar to the way this strain was more sensitive to APO. Additionally, chronic lithium treatment would

have to be able to correct this alteration as lithium treatment changed the APO-induced hypothermic response to one similar to that shown by the normotensive strains. It seems, by this hypothesis, that lithium would also have to alter the distribution of APO to various regions in order to correct the original dysfunction in distribution. Such an alteration in SHR which can increase the distribution of both lipid-soluble and lipid-insoluble molecules to the hypothalamus, and which can be corrected by a positively-charged ion does not seem plausible. Therefore the possibility that a dysfunction exists within SHR brain that increases the distribution of APO to the hypothalamus can be eliminated.

L-DOPA, as previously mentioned, was also found to cause hypothermia in much the same manner as APO. This seems to be clearly illustrated by the similarities in the log-dose response curves. Similarly to APO, L-DOPA also appeared to be approximately 1.78 times more potent in producing hypothermia in SHR than NWR. These results would seem to suggest that the difference in the hypothermic response between SHR and NWR is not a phenomenon strictly reserved to APO. This would also argue against the first two explanations for the observed results; that is, that there may be an alteration of metabolic degradation of APO or that there may be an enhanced distribution of APO to the hypothalamus in SHR. On the other hand, these results would seem to indicate that in SHR there is some sort of postsynaptic alteration of the DA receptors responsible for mediating the hypothermic response. However, the mechanism of action of L-DOPA depends on pre-

synaptic events as well as on postsynaptic events whereas the responses observed from APO, at the doses used, should have been the result of postsynaptic actions. Since the L-DOPA log-dose response curve of SHR was parallel to the log-dose response curve of NWR it may be assumed that the mechanism of hypothermia was identical in the two strains. Also, the APO log-dose response curves were parallel which would again suggest the same mechanism of action. Since the APO-induced hypothermic response depended on a postsynaptic site of action, it could be assumed that the presynaptic events in response to L-DOPA were the same in the two rat strains. Therefore it can be argued that the difference in the hypothermic response to L-DOPA between the two strains was also the result of postsynaptic alterations in SHR.

It might also be argued that L-DOPA displaced serotonin from serotonergic neurons thereby leading to the hypothermic response (Maj and Pawlowski, 1973; Reichenburg and Vetulani, 1973) and that APO indirectly stimulated the release of serotonin from neurons which participate in the hypothermic response (Grabowska, 1974; Przewlocki, 1976). As SHR have been hypothesized to possess hyperfunctional serotonergic systems (Rosecrans and Adams, 1976), stimulation of the serotonergic neurons involved in the mediation of hypothermia by either L-DOPA or APO may have led to the significantly greater fall in rectal temperature. However, the dose of L-DOPA used in the present study (25 mg/kg to 100 mg/kg) was smaller than those used in an earlier study (100 mg/kg and above (Maj and Pawlowski, 1973)). Another study used intraventricularly-

administered L-DOPA (Reichenburg and Vetulani, 1973). Both of these studies reported that the DA antagonists, pimozide and spiroperidol, were unable to attenuate the L-DOPA-induced hypothermia. In contradiction to these earlier investigations, it was observed during the course of the present study that HAL could significantly attenuate L-DOPA-induced hypothermia in SHR and NWR (personal observation). It may be that large amounts of L-DOPA are able to displace serotonin from serotonergic neurons. The resulting response may be able to mask the responses resulting from DA receptor stimulation. Responses from lower amounts of L-DOPA may be the result of the conversion of L-DOPA to DA and the subsequent stimulation of DA receptors. HAL also significantly attenuated APO-induced hypothermia. Therefore the data obtained from the present study appear to indicate that the stimulation of DA receptors was responsible for the hypothermic response to L-DOPA and APO. As a result, it seems that the L-DOPA- and APO-induced hypothermia observed in the present study was not due, either directly or indirectly, to the release of serotonin.

Additionally, the results obtained from lithium-treated animals would not support the hypothesis that a serotonergic mechansim is the major contribution to the greater fall in body temperature induced by APO in SHR. Lithium has been found to interact with some of the serotonergic neuronal systems. Thus lithium has been found to decrease the amount of serotonin synthesized from L-tryptophan (Collard and Roberts, 1975). These results may be related to the observation that chronic lithium treatment can reduce brainstem sero-

tonin levels by approximately 26 percent (Ho et al., 1970). This decrease in brainstem serotonin could also be used as an explanation for the attenuation by lithium of the APO-induced hypothermia in SHR as it has been shown that it is brainstem serotonergic systems that participate in APO-induced hypothermia (Grabowska, 1974; Przewlocki, 1976). However, it seems reasonable that such a decrease in brainstem serotonin levels would also have occurred in the normotensive rat strains. Therefore it seems plausible that chronic lithium treatment would also have attenuated APO-induced hypothermia in WKY and NWR.

A third explanation is that in SHR there are postsynaptic alterations of the DA receptors in the hypothalamus which are responsible for mediating the drop in body temperature. This alteration in the hypothalamic DA receptor results in the greater sensitivity of SHR to the hypothermic effect of APO and L-DOPA. It was recently discovered that lithium has the ability to "stabilize" DA receptor sensitivity. This was accomplished by showing that concurrent lithium treatment could attenuate the increased response to APO-induced stereotyped behavior and locomotor activity observed when DA receptors are made supersensitive by chronic neuroleptic treatment (Gallager et al., 1978; Pert et al., 1978). "Stabilize", as used by these authors, means that the lithium was able to return the sensitivity of the DA receptor to normal. Similar results were observed in the present study, except that the DA receptors are apparently already supersensitive in SHR. As a result, lithium was found to alter the APO-in-

duced hypothermic response of SHR to that shown by WKY and NWR, just as lithium was able to alter the APO-induced behavioral responses of chronically neuroleptic-treated rats to that of untreated rats. Therefore it is suggested that the DA receptors of the hypothalamus responsible for mediating hypothermia are supersensitive in SHR.

This supersensitivity may be due, according to Pert et al. (1978), to: 1) an increase in the number of DA receptors; or 2) an increase in the sensitivity of the existing DA receptors. Pert and coworkers (1978) found that lithium could prevent the increase in DA receptor binding sites induced by haloperidol. These results support their suggestion that lithium prevents the development of supersensitive DA receptors by inhibiting the increase in the number of DA receptors. On the other hand, Stefanini and coworkers (1978) found that lithium could inhibit the stimulatory effects of DA on DA-sensitive adenylate cyclase. They also found that lithium at brain concentrations above those required for therapeutic effect could inhibit basal activity of DA-sensitive adenylate cyclase. From these results it could be suggested that lithium prevents the development of supersensitive DA receptors by inhibiting the sensitivity of the existing DA receptors. Therefore, with the data available at the present time, it cannot be determined whether the proposed hypothalamic DA receptor supersensitivity in SHR is due to: 1) an increase in the number of DA receptors; or 2) an increase in the sensitivity of existing DA receptors.

Chronic hydralazine treatment was found to have no effect

on APO-induced hypothermia in SHR despite being able to normalize systolic blood pressure. These results would seem to eliminate any possibility that the difference in the hypothermic response between SHR and NWR was the result of peripheral vasodilating effects. That is, it would seem unlikely that greater vasodilation in SHR, and therefore dissipation of heat from SHR, was the cause of the greater fall in rectal temperature produced by APO. This is because hydralazine is a smooth muscle relaxant and had already dilated the vasculature of SHR as evidenced by the normal systolic blood pressure. As lithium was observed to slightly decrease blood pressure in SHR, it could have been suggested that the alteration of the hypothermic response to APO in SHR by lithium was due to this decrease in blood pressure. Therefore, if the greater hypothermia induced by APO in SHR was the result of greater vasodilation, then hydralazine would have been able to alter the APO-induced hypothermia in the same manner as lithium was able to alter the hypothermic response. Since this response was not the same after chronic treatment with hydralazine or lithium, and hydralazine exerts its effect on the periphery, this suggests that lithium affected APO-induced hypothermia via a central mechanism of action. Therefore it appears that the lack of significant effect on APO-induced hypothermia in SHR by hydralazine supports the suggestion that the hypothalamic DA receptors responsible for mediating hypothermia are supersensitive in SHR.

The hypothermic responses obtained from WKY are more difficult to explain in that their responses were intermediate

to those of SHR and NWR. These results are similar to biochemical studies in which significant differences between SHR and NWR are lost when SHR are compared to WKY (Sjoerdsma, 1972; Yamabe et al., 1973; Osumi et al., 1974). Such observations have led to the controversy as to whether WKY or NWR is the proper control strain for comparison to SHR. It has been suggested that WKY may possess hypertensive traits (Sjoerdsma, 1972), and therefore the similarity in response of WKY to SHR may not be critical. However, the hypothermic response to APO of lithium-treated WKY was not significantly different from the hypothermic response produced by APO in sodium-treated animals. These results suggest that no difference exists between WKY and NWR as the APO-induced hypothermia was not affected by chronic lithium treatment in either rat strain, but that there is a difference between WKY and SHR as the hypothermic response elicited in SHR was altered by chronic lithium treatment.

It appears that the most plausible explanation for the results of the present study is that the hypothalamic DA receptors responsible for mediating hypothermia are supersensitive in SHR. This proposed alteration in hypothalamic function is consistent with previous studies which have also reported alterations in various hypothalamic functions in SHR. Thus Buñag and coworkers (1975) hypothesized that the posterior hypothalamic pressor areas of SHR are hypersensitive. These pressor areas have recently been related to hyperactivity of the sympathetic nervous system (Juskevich et al., 1978; Takeda and Buñag, 1978). Schmid et al. (1978) discovered

that cAMP levels are lower in SHR than WKY which was suggested by these authors to indicate a possible alteration of the adenylate cyclase-cAMP-phosphodiesterase system in hypothalamic structures of SHR. By observing elevated basal prolactin release and normal suppression of prolactin release in SHR, Sowers and coworkers (1979) hypothesized that hypothalamic DA metabolism is defective in SHR. Thus the results of the present study appear to be in agreement with other studies that have postulated alterations of normal hypothalamic functions in SHR.

The conclusions of the present study obviously requires further experimentation and verification. There are a number of procedures which might be employed in an effort to confirm this hypothesis of SHR hypothalamic DA receptor supersensitivity. Regional activity of DA-dependent adenylate cyclase could give an indication as to the sensitivity of the hypothalamic DA receptor in SHR. Additionally, regional DA receptor binding studies may also be helpful. Furthermore, it would be interesting to determine if there is a common denominator between the greater sensitivity of SHR to the hypothermic effect of APO and L-DOPA, and their greater sensitivity to the inhibitory effect of L-DOPA on sympathetic nerve activity (Judy et al., 1978). This may give a clue as to whether or not the postulated supersensitive DA receptors are related to the pathogenesis of hypertension in SHR. Studies such as these will be required to confirm the suggestion that the hypothalamic DA receptors responsible for mediating hypothermia are supersensitive in SHR.

CONCLUSIONS

1. There was no significant difference in stereotyped behavior induced by the direct-acting DA agonist APO between SHR and two normotensive control strains, WKY and NWR, suggesting that there is no alteration in the sensitivity of DA receptors in the corpus striatum of SHR.
2. There was no significant difference in locomotor activity, either spontaneous or induced by APO, between SHR, WKY, and NWR, suggesting that there is no alteration in the sensitivity of DA receptors in the mesolimbic forebrain of SHR.

3a. SHR demonstrated a significantly pronounced and prolonged hypothermic response to APO and L-DOPA than NWR.

b. WKY displayed a response intermediate to SHR and NWR; this may be due to the possession of hypertensive traits by WKY.

c. Lithium significantly attenuated APO-induced hypothermia in SHR, and altered it to a response similar to WKY and NWR.

d. Since lithium has the ability to "stabilize" supersensitive DA receptors, and APO-induced hypothermia in SHR, it is suggested that DA receptors in the hypothalamus

responsible for mediating hypothermia are supersensitive in SHR. This supersensitivity may be the result of: 1) an increase in the number of DA receptors; or 2) an increase in the sensitivity of the existing DA receptors.

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