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Mechanisms of Amitriptyline Induced Hypothermia in the Rat[1,2]

H. K. LEE, C. Y. CHAI, M. J. WAYNER,[3] P. M. CHUNG AND J. T. CHENG

Department of Biophysics, National Defense Medical Center, Taipei, Taiwan, Republic of China

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LEE, H. K., C. Y. CHAI, M. J. WAYNER, P. M. CHUNG AND J. T. CHENG. *Mechanisms of amitriptyline induced hypothermia in the rat.* PHARMAC. BIOCHEM. BEHAV. 7(2) 159–165, 1977. – Effects of amitriptyline on rectal temperature of male rats were studied at the ambient temperature of 25°C. Drugs were administered intraperitoneally. Amitryptyline elicited a dose related hypothermia. The hypothermia was attenuated by phenoxybenzamine 10 mg/kg, haloperidol 2 mg/kg, diphenhydramine 5 mg/kg, atropine 20 mg/kg, and cyproheptadine 5 mg/kg. Propranolol, at a dose of 5 mg/kg, had no effect on the hypothermia. Theophylline 50 mg/kg and dibutyryl cyclic AMP 20 mg/kg inhibited the hypothermia produced by amitriptyline. Pretreatment with parachloroamphetamine (PCA), 2 or 5 mg/kg daily for 3 days, strongly antagonized the hypothermia. In addition, pretreatment with parachlorophenylalanine (PCPA), 100 mg/kg daily for three days, reduced the brain 5-hydroxytryptamine (5-HT) concentration to 20% of the control level and completely blocked the hypothermia response. When brain 5-HT concentration recovered to 50% of the control level in PCPA treated rats following the administration of 10 mg/kg 5-hydroxytryptophan (5-HTP) the hypothermia induced by amitriptyline was restored. However, the administration of 5-HT, 5 mg/kg, to PCPA treated rats did not increase brain 5-HT concentration or restore the amitriptyline induced hypothermia (AIH). Results suggest that amitriptyline interacts with several transmitter substances to produce hypothermia. Since the ability of amitriptyline to produce hypothermia was correlated with brain 5-HT content, 5-HT might play an important role in the mediation of AIH.

Amitriptyline Hypothermia Neurotransmitter blocking agents Theophylline
Dibutyryl cyclic AMP Parachloroamphetamine Parachlorophenylalanine
5-Hydroxytryptamine 5-Hydroxytryptophan Brain 5-HT content Temperature regulation

AMITRIPTYLINE is a widely used drug in the treatment of depression [2]. Although the mechanisms of its antidepressant action has not been elucidated, it blocks the reuptake of norepinephrine (NE) and 5-hydroxytryptamine (5-HT) by nerve terminals [2,12]. Enhanced and prolonged adrenergic and serotoninergic activity due to blocked reuptake might account for the antidepressant activity [2]. In addition, amitryptyline and other tricyclic antidepressants are known to potentiate apomorphine induced gnawing behavior in mice [3,9]. This effect is due not only to adrenergic and serotoninergic mechanisms [3] but also the enhancement of dopaminergic and the inhibition of cholinergic systems in the central nervous system [9]. Apparently, the interactions between amitriptyline and neurotransmitters are complex. Results of preliminary experiments indicate that amitriptyline produces hypothermia in the rat. Therefore, the present study was designed to evaluate the possible importance of several transmitter substances in amitriptyline induced hypothermia (AIH). Results indicate that although acetylcholine (ACh), NE, dopamine, and histamine might play a role in AIH, 5-HT seems to be the most important. At least 50% of the normal brain 5-HT must be present for amitriptyline to exert its hypothermic effect.

METHOD

Animals

Male Sprague-Dawley rats, 200–300 g in weight, were used. At least one day prior to the experiment, the animals were transferred from the breeding quarters to the air conditioned laboratory (25 ± 1°C). Rectal temperature was obtained by a YSI 423 probe inserted 5 cm into the rectum and displayed on a YSI 47 telethermometer. In a total of 146 untreated rats the average temperature was 38.5 ± 0.5°C (SD). After the initial rectal temperature was obtained saline or drug pretreatments were administered intraperitoneally. The control rectal temperature was then

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[2] Send reprint requests to Dr. H. K. Lee, Department of Biophysics, National Defense Medical Center, P. O. Box 8244, Taipei, Taiwan, Republic of China.

[3] Visiting Professor at the National Defense Medical Center, Taipei, sponsored by the National Science Foundation, USA and the National Science Council, ROC Joint Research Program.

obtained twice with an interval of 15 min. The second reading was used as a reference value for each of the respective experiments. After the second control reading, amitriptyline was injected intraperitoneally and additional readings were obtained 15, 30, 60, 90, 120, 150, 180, 240 and 300 min later. There were six rats in each group. Drug solutions were administered at a volume of 0.1 ml/100 g body weight.

Drug Treatment

The hypothermic effect of amitriptyline HCl (Hoffman-La Roche) was studied after doses of 10, 20, and 50 mg/kg were administered intraperitoneally. In the experiments in which the hypothermia was to be antagonized, amitriptyline was used in a dose of 20 mg/kg.

Pretreatments

All drugs were administered intraperitoneally: phenoxybenzamine HCl (Smith Kline and French) 10 mg/kg, 30 min before the amitriptyline administration; propranolol HCl (Sigma) 5 mg/kg, 30 min before; haloperidol (Janssen) 2 mg/kg as a suspension (two drops of surfactant polysorbate in 10 ml saline), 30 min before; diphenhydramine HCl (Sigma) 5 mg/kg, 30 min before; atropine sulfate (Sigma) 20 mg/kg, 30 min before; cyproheptadine HCl (Merck Sharp and Dohme) 5 mg/kg, 30 min before; theophylline (Sigma) 50 mg/kg, 30 min before; monosodium dibutyryl cyclic AMP (Sigma) 20 mg/kg, 30 min before. Both parachloroamphetamine (PCA, Sigma) 2 or 5 mg/kg and parachlorophenylalanine (PCPA, Sigma) 100 mg/kg were administered daily for three days and the last dose was injected 24 hr before the amitriptyline treatment. Two groups of PCPA pretreated rats were further treated with 5-hydroxytryptophan (5-HTP, Sigma) or 5-HT oxalate (Sigma) before the amitriptyline injection. 5-HTP (5 mg/kg) was administered 12 and 24 hr after the last dose of PCPA and the amitriptyline administration then followed the last treatment of 5-HTP by 16 hr. 5-HT was administered in a dose of 5 mg/kg 30 min before the amitriptyline injection. All doses of premedications selected did not affect rectal temperature significantly by themselves.

Brain 5-HT Concentration

The 5-HT concentration in the whole brain was determined according to a spectroflurophotometric method [1] in four groups of five rats. They were the normal control group, PCPA pretreated group, PCPA and 5-HTP pretreated group, and the PCPA and 5-HT pretreated group.

RESULTS

Dose Dependent Hypothermia Induced by Amitriptyline

The hypothermic effects produced by 10 (△—△), 20 (□- - -□), and 50 (○—○) mg/kg of amitriptyline are illustrated in Fig. 1. Data are presented in terms of the mean ± SE rectal temperature for each group at the times indicated. Data were analyzed by means of a 3 × 11 ANOVA having repeated measures on one factor [13]. The three doses of 10, 20 and 50 mg/kg of amitriptyline

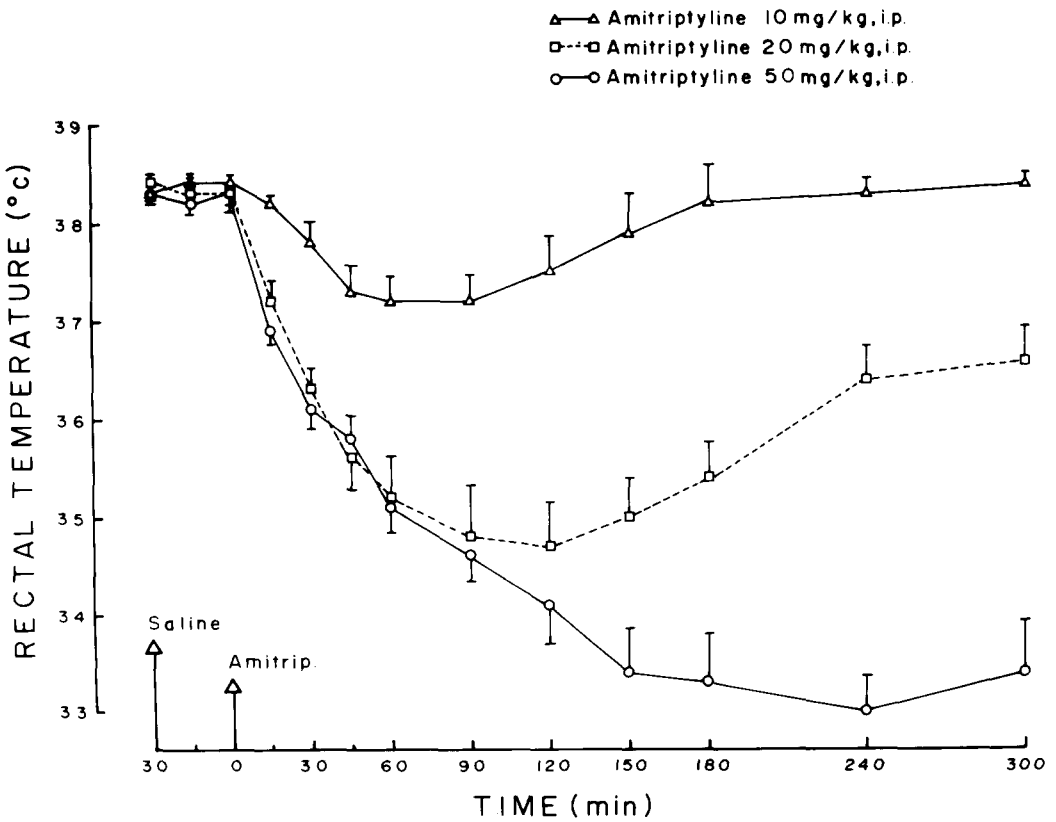

FIG. 1. Dose response relation of amitriptyline induced hypothermia (AIH). Each point represents a mean ± SE (N = 6). Amitriptyline elicited a dose dependent hypothermic effect with doses of 10–50 mg/kg, IP.

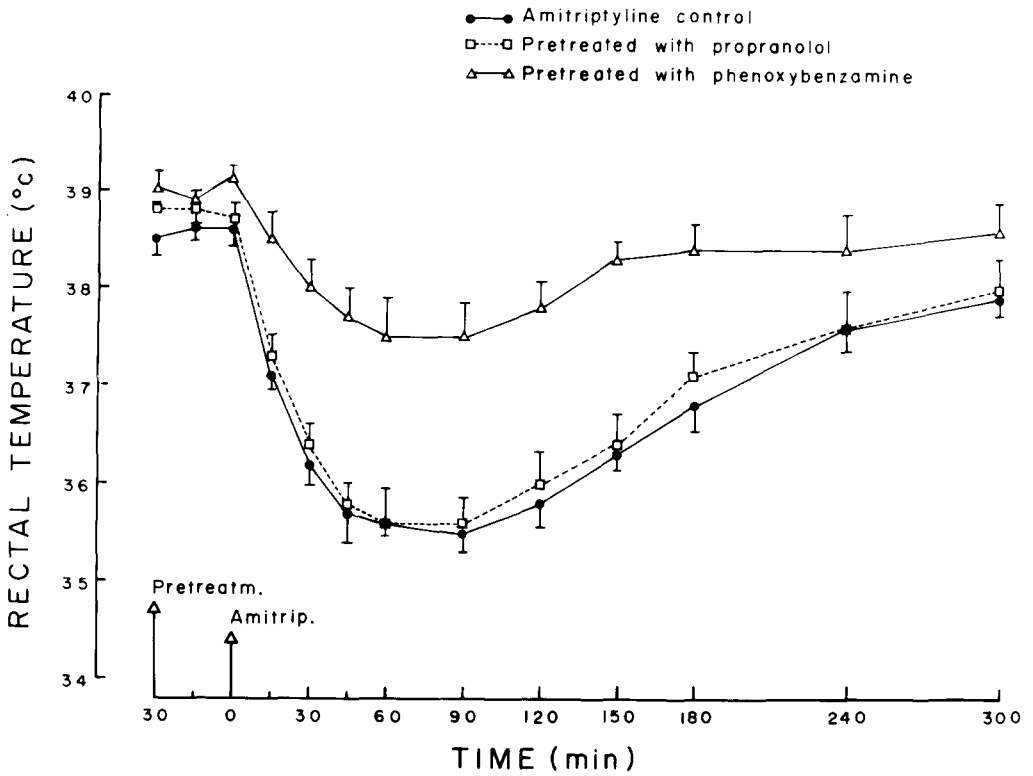

FIG. 2. Effects of propranolol and phenoxybenzamine on AIH. Each point represents a mean ± SE (N = 6). Propranolol pretreatment did not interfere with the hypothermic effect induced by amitriptyline. Phenoxybenzamine pretreatment attenuated the hypothermia.

represented one factor (dose). Temperature at zero (control) just before amitriptyline was administered and at 15, 30, 45, 60, 90, 120, 150, 180, 240 and 300 min after the amitriptyline treatment constituted the other factor (time). The main effects were significant: dose, $F(2,15) = 28.38$, $p<0.01$; time, $F(10,150) = 76.52$, $p<0.01$. The interaction dose by time was significant, $F(20,150) = 28.74$, $p<0.01$. Single main effects analyses revealed significant differences for 20 mg/kg, $F(10,150) = 6.23$, $p<0.01$ and 50 mg/kg amitriptyline, $F(10,150) = 14.70$, $p<0.01$ but not for the 10 mg/kg dose. Temperature at each time was then compared to the control temperature prior to amitriptyline treatment by means of post hoc Dunnett tests [13]. The post hoc analysis revealed that both groups, 20 and 50 mg/kg, produced significant decreases in rectal temperature during the period from 30–300 min after the amitriptyline treatment ($p<0.01$). Therefore, the AIH endured for at least 5 hr. Single main effects across three doses at times 15, 30, 45, 60 and 90 min after the amitriptyline treatment were not significant, but were significant at times 120, 150, 180, 240 and 300 min, $F(2,165) = 5.08$, 8.02, 9.30, 11.11 and 9.88, respectively, $p<0.01$. Temperatures produced by three doses at a given time were compared to each other by means of post hoc Tukey A tests. These tests revealed that at 120 and 150 min, 20 and 50 mg/kg were significantly different from 10 mg/kg ($p<0.01$) but did not differ significantly from each other. At 180 min, each dose was significantly different from each other ($p<0.01$). At 240 and 300 min, 10 and 20 mg/kg were significantly different from 50 mg/kg ($p<0.01$) but no significant difference was found between 10 and 20 mg/kg. These results indicate that AIH is a dose related effect.

Effects of Adrenergic Blocking Agents on AIH

Effects of an alpha adrenergic blocking agent, phenoxybenzamine (△——△), and a beta adrenergic blocking agent, propranolol (□– – –□), on AIH are shown in Fig. 2. Data are presented as mean ± SE. A 3 × 11 ANOVA as described previously was applied to the data of the present experiment. Three groups of amitriptyline control, phenoxybenzamine pretreatment and propranolol pretreatment represented one factor (drug). The 11 temperature measurements obtained at different times constituted the other factor (time). The main effects were significant: drug, $F(2,15) = 36.96$, $p<0.01$; time, $F(10,150) = 52.70$, $p<0.01$. The interaction drug by time was significant, $F(20,150) = 2.96$, $p<0.01$. Significant single main effects were found for the amitriptyline control group (●——●), $F(10,150) = 4.47$, $p<0.01$, and for the propranolol pretreated group, $F(10,250) = 4.30$, $p<0.01$. However, there was no significant single main effect for the phenoxybenzamine pretreated group. Post hoc Dunnett tests indicated that amitriptyline produced a significant decrease of rectal temperature in the control group at 30–180 min after the drug treatment ($p<0.01$) and in the propranolol pretreated group at 30 to 150 min ($p<0.01$). The results suggest that phenoxybenzamine, but not propranolol, attenuates the hypothermic response induced by amitriptyline.

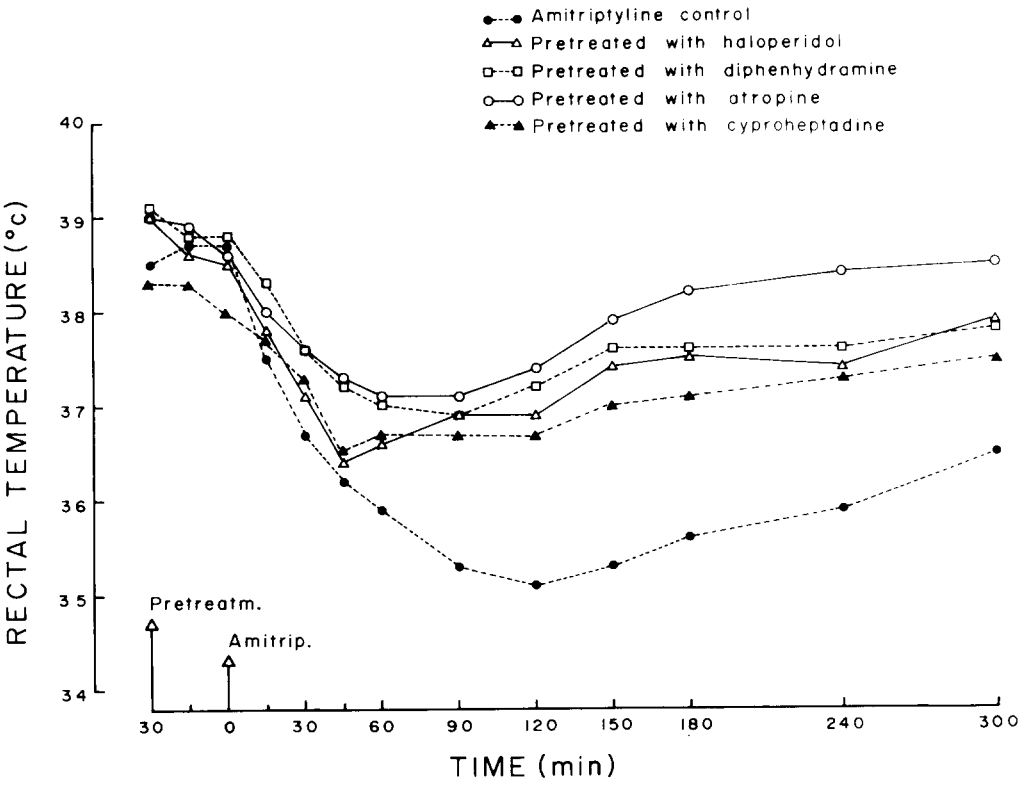

FIG. 3. Effects of haloperidol, diphenhydramine, atropine, and cyproheptadine on AIH. Each point represents a mean value (N = 6). All drug pretreated groups showed less response to amitriptyline treatment.

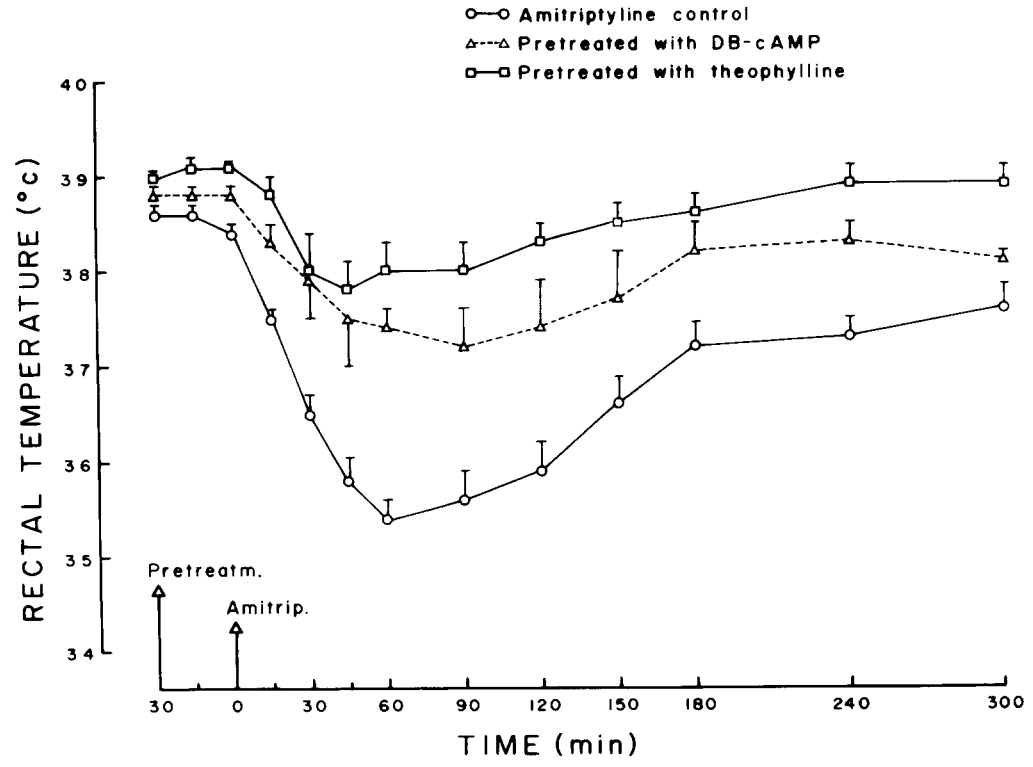

FIG. 4. Effects of dibutyryl cyclic AMP (DB-cAMP) and theophylline on AIH. Each point represents a mean ± SE (N = 6). Pretreatment with either DB-cAMP or theophylline inhibited the AIH.

Effects of Haloperidol, Diphenhydramine, Atropine and Cyproheptadine on AIH

Effects of a dopaminergic receptor blocker, haloperidol (△——△), a histamine antagonist, diphenhydramine (□– – –□), a cholinergic blocking agent, atropine (○——○), and a 5-HT antagonist, cyproheptadine (▲– – –▲) on AIH are shown in Fig. 3. A 5 × 11 ANOVA as previous described was applied to the data of the present experiment. The amitriptyline control group and the other four groups pretreated with either haloperidol, diphenhydramine, atropine or cyproheptadine represented one factor (drug). The 11 temperature measurements obtained at different times constituted the other factor (time). Main effects were significant: drug, $F(4,25) = 6.27$, $p<0.01$; time, $F(10,150) = 37.60$, $p<0.01$. The interaction drug by time was significant, $F(4,250) = 3.16$, $p<0.01$. A significant single main effect was found for the amitriptyline control group (●–––●), $F(10,250) = 4.09$, $p<0.01$, but not for the other four drug pretreated groups. Post hoc Dunnett tests indicated that amitriptyline caused a significant decrease of rectal temperature in control groups at 45–240 min after the drug treatment ($p<0.01$). The results suggest that haloperidol, diphenhydramine, atropine and cyproheptadine significantly reduce amitriptyline induced hypothermia.

Effects of Theophylline and Dibutyryl Cyclic AMP on AIH

The effects of dibutyryl cyclic AMP (△– – –△) and an inhibitor of cyclic AMP phosphodiesterase, theophylline (□——□) [10], are illustrated in Fig. 4. Data are presented as mean ± SE. A 3 × 11 ANOVA as described previously was carried out. Amitriptyline control treatment, dibutyryl cyclic AMP pretreatment and theophylline pretreatment represented one factor (drug). The 11 time measurements constituted the other factor (time). Main effects were significant: drug, $F(2,15) = 17.18$, $p<0.01$; time, $F(10,150) = 26.61$, $p<0.01$. The interaction drug by time was significant, $F(20,150) = 2.49$, $p<0.01$. A significant single main effect was only found for the amitriptyline control group (○——○), $F(10,150) = 3.51$, $p<0.01$, but not for the groups pretreated with dibutyryl cyclic AMP, or theophylline. Post hoc Dunnett tests indicated that amitriptyline produced a significant decrease in rectal temperature of the control group at 30–150 min after the drug treatment ($p<0.01$). Results indicate that both dibutyryl cyclic AMP and theophylline significantly reduce the hypothermic effect induced by amitriptyline.

Effects of PCA on AIH

Effects of PCA, a selective depletor of brain 5-HT [11], are illustrated in Fig. 5. Data are presented as mean ± SE. When PCA, 2 or 5 mg/kg, was initially administered, it produced hyperthermia, sweating, increase of motor activity and hyperreactive to auditory stimuli. PCA induced hyperthermia has been reported previously [5]. However, these reactions were not observed after the second or the third administration of PCA. A 3 × 11 ANOVA as described previously was used to analyze the data. The amitriptyline control treatment (○——○), PCA 2 mg/kg pretreatment (□– – –□), and PCA 5 mg/kg pretreatment (△——△) represented one factor (drug). The 11 time measurements constituted the other factor (time). The main effects were significant: drug, $F(2,15) = 28.85$, $p<0.01$; time, $F(10,150) = 25.16$, $p<0.01$. The interaction drug by time was significant, $F(20,150) = 9.02$, $p<0.01$. A significant main effect was found for the amitriptyline control group, $F(10,150) = 5.22$, $p<0.01$; but not for the PCA 2 mg/kg pretreated group, or the PCA 5 mg/kg pretreated group. Post hoc Dunnett tests indicated amitriptyline produced a significant decrease in rectal temperature at 30–240 min after the drug treatment ($p<0.01$). These results indicate that PCA dramatically attenuates the AIH.

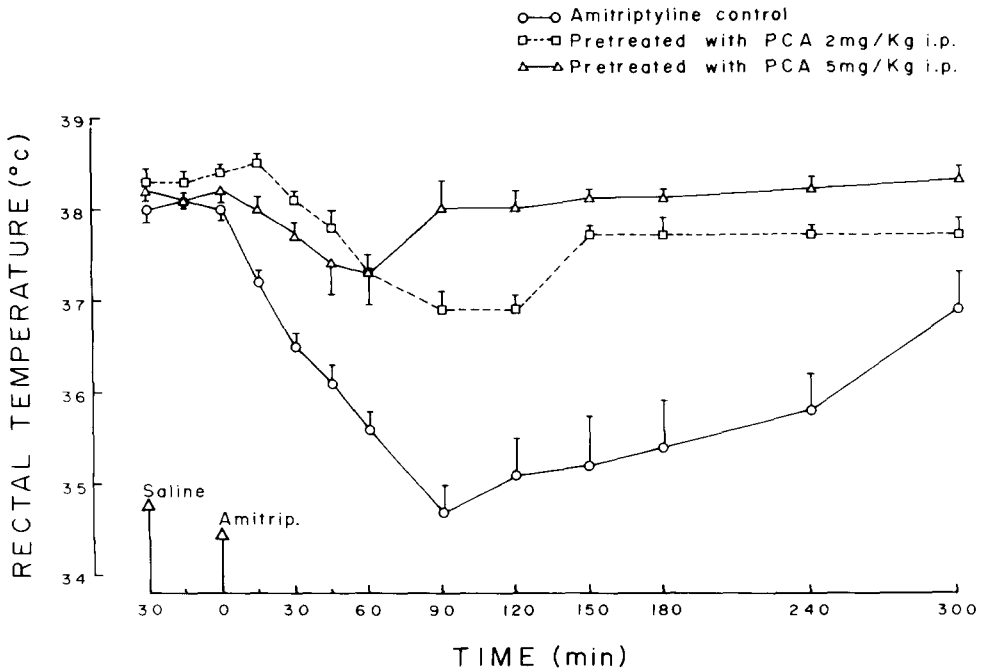

FIG. 5. Effects of parachloroamphetamine (PCA) on AIH. Each point represents a mean ± SE (N = 6). PCA with doses of 2 or 5 mg/kg significantly attenuated the AIH.

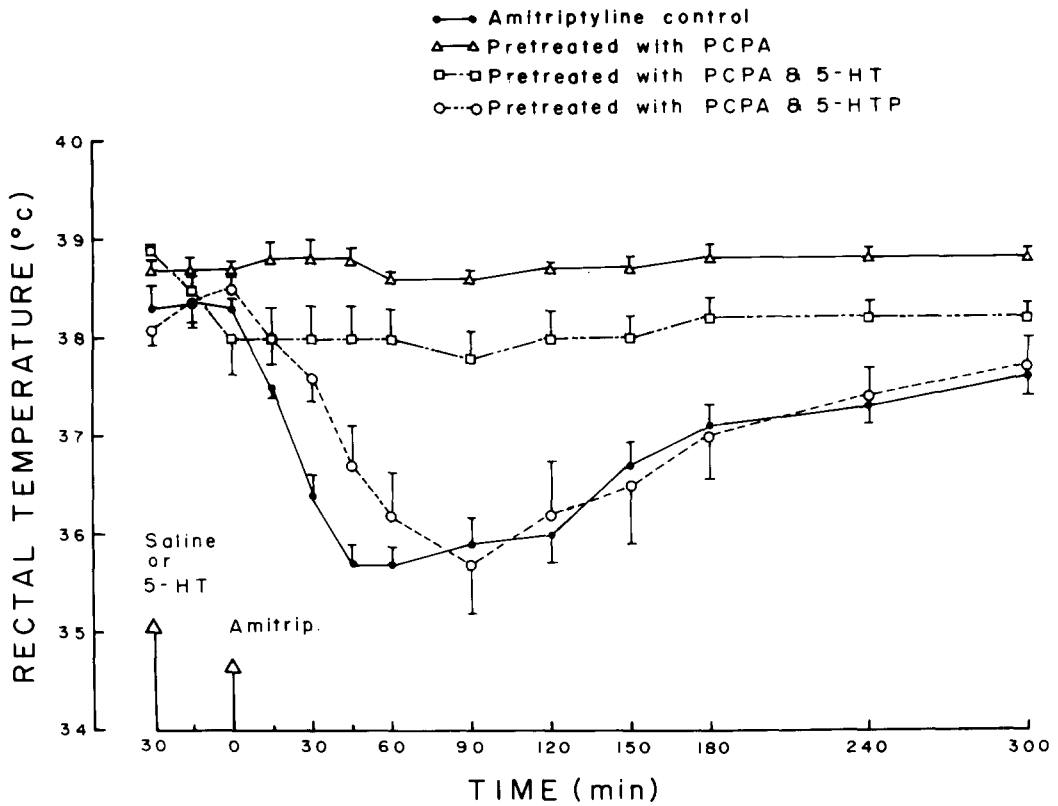

FIG. 6. Effects of parachlorophenylalanine (PCPA) on AIH. Each point represents a mean ± SE (N = 6). Pretreatment with PCPA abolished the hypothermic action induced by amitriptyline. Further administration of 5-hydroxytryptophan (5-HTP), but not of 5-hydroxytryptamine (5-HT), to the PCPA pretreated group restored the AIH.

Brain 5-HT Concentration and AIH

PCPA, a potent and selective depletor of 5-HT [7], was used to study the relation between brain 5-HT concentrations and AIH. The effects of drug treatment on rectal temperature are illustrated in Fig. 6. The data were analyzed by means of a 4 × 11 ANOVA as described previously. The amitriptyline control treatment (•——•), PCPA pretreatment (△——△), PCPA and 5-HT pretreatment (□——□), and PCPA and 5-HTP pretreatment (○——○) represented one factor (drug). The 11 time measurements constituted the other factor (time). Main effects were significant: drug, $F(3,20) = 18.01$, $p<0.01$; time, $F(10,200) = 19.38$, $p<0.01$. The interaction drug by time was significant, $F(30,200) = 6.04$, $p<0.01$. Significant single main effects were found in the amitriptyline control group, $F(10,200) = 3.12$, $p<0.01$, and in the PCPA and 5-HTP pretreated group, $F(10,200) = 3.04$, $p<0.01$, but not in groups pretreated with either PCPA or PCPA and 5-HT. Post hoc Dunnett tests indicated that significant decreases in rectal temperature were produced by amitryptyline at 30–120 min after the drug treatment and by PCPA and 5-HTP at 60–120 min ($p<0.01$). These results suggest that PCPA pretreatment can abolish AIH. Treatment with 5-HTP but not 5-HT can reverse this effect.

Table 1 summarizes the effects of drug treatment on brain 5-HT concentration. Mean 5-HT brain content of the control group was 0.40 ± 0.02 (SE) μg/g wet weight. PCPA treatment decreased the brain 5-HT concentration to 20% of the control level (0.08 ± 0.01 μg/g). When the PCPA pretreated rats were further treated with 5-HT, which does not penetrate the blood brain barrier readily [4], the brain 5-HT concentration only slightly increased to 25% of the control level (0.10 ± 0.03 μg/g). On the other hand, if the PCPA pretreated rats were treated with 5-HTP, which easily penetrates the blood brain barrier [4], brain 5-HT concentration increased to 50% of the control level (0.20 ± 0.01 μg/g). When the data in Table 1 were analyzed by means of a one-way ANOVA, a significant main effect was found, $F(3,16) = 45.73$, $p<0.01$. Individual comparisons were then performed by means of post hoc Tukey A tests. These tests indicated that the brain 5-HT concentrations of both the PCPA or PCPA and 5-HT pretreated groups were significantly lower than those of controls ($p<0.01$). No

TABLE 1

EFFECT OF 5-HYDROXYTRYPTAMINE (5-HT) AND 5-HYDROXYTRYPTOPHAN (5-HTP) ON THE LEVELS OF 5-HT IN THE RAT BRAIN FOLLOWING PRETREATMENT WITH PARACHLOROPHENYLALANINE (PCPA)

	Drug	Brain 5-HT content μg/g wet weight
I	Control	0.40 ± 0.02*
II	PCPA	0.08±0.01
III	PCPA & 5-HT	0.10 ± 0.03
IV	PCPA & 5-HTP	0.20±0.01

*Mean ± SE, N = 5.

significant difference was found between the control group and the group treated with PCPA and 5-HTP, or between any other groups. The data suggest that relatively intact levels of brain 5-HT are required for amitriptyline to exert its hypothermic action.

DISCUSSION

A dose related hypothermic action induced by amitriptyline in rats was demonstrated. Results of the present study also indicate that AIH can be attenuated by an alpha adrenergic blocking agent (phenoxybenzamine), a dopamine receptor blocker (haloperidol), a histamine antagonist (diphenhydramine), a cholinergic blocking agent (atropine), and a 5-HT antagonist (cyproheptadine). Therefore, AIH might be related to the actions of NE, dopamine, histamine, ACh, or 5-HT. These results agree with several observations [2, 3, 9, 12] which indicate that the antidepressive and some other behavioral effects of amitriptyline can be attributed to an interaction with many neurotransmitters, such as NE, 5-HT, dopamine, and ACh.

Both theophylline and dibutyryl cyclic AMP markedly inhibited AIH. At the cellular level theophylline is known to inhibit phosphodiesterase and thus increase cyclic AMP concentration [10]. Dibutyryl cyclic AMP is similar to cyclic AMP but it penetrates cell membranes more easily and is resistant to the catabolic effect of cyclic AMP phosphodiesterase [10]. Therefore, the attenuation of AIH by theophylline or dibutyryl cyclic AMP indicates that the pharmacological effect of amitriptyline might be mediated indirectly by cyclic AMP. Since many physiological actions of neurotransmitters are known to be mediated by cyclic AMP [10], amitriptyline might increase the amount of NE, 5-HT, or dopamine in the synaptic cleft due to an inhibition of reuptake [2,12] which in turn would activate adenylate cyclase activity [6,10] and raise the cyclic AMP content in the brain.

The ability of amitriptyline to produce hypothermia correlated closely with the brain 5-HT levels. PCA, in doses which produce a marked reduction in the brain level of 5-HT [11], greatly attenuated the AIH. In addition, when brain 5-HT concentration was significantly decreased to 20% of the control level by treatment with PCPA, amitriptyline did not produce a decrease in rectal temperature. An increase of brain 5-HT concentration to 50% of the control level in PCPA treated rats by administration of 5-HTP, restored the hypothermia. On the other hand, administration of 5-HT to PCPA treated rats neither increased the brain 5-HT concentration significantly nor restored the AIH. Therefore, it appears that the amount of brain 5-HT is critical for the hypothermic effect of amitriptyline. The ability of amitriptyline to block the reuptake of 5-HT by nerve terminals [2,12] alone, which increases the bioavailability of 5-HT to postsynaptic receptors, could explain its hypothermic effect. Results of the present study indicate that amitriptyline might interact with several neurotransmitters, such as 5-HT, NE, dopamine or ACh. However, among all these transmitter substances only 5-HT has been shown to decrease rectal temperature when it is administered intracerebroventricularly in the vicinity of the preoptic anterior hypothalamic region in the rat [8]. Therefore, 5-HT might play an important role in the mediation of amytryptyline induced hypothermia.

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