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5-HT_{1A} and 5-HT_2 Receptors Mediate Hypo- and Hyperthermic Effects of Tryptophan in Pargyline-Pretreated Rats

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ABDEL-FATTAH, A.-F. M., K. MATSUMOTO, K. A.-W. EL-HADY AND H. WATANABE. *5-HT_{1A} and 5-HT_2 receptors mediate hypo- and hyperthermic effects of tryptophan in pargyline-pretreated rats.* PHARMACOL BIOCHEM BEHAV **52**(2) 379-384, 1995.—Mechanisms of tryptophan (a 5-HT precursor)-induced changes in body temperature were investigated in rats pretreated with pargyline, a monoamine oxidase inhibitor (MAO-I). Tryptophan (100 mg/kg, IP) did not affect the body temperature in rats, but it produced significant hypothermia followed by marked hyperthermia and higher mortality in the pargyline-pretreated rats. 5-HT depletion with p-chlorophenylalanine (p-CPA, 100 mg/kg/day for 3 days) significantly suppressed not only the body temperature change but also the mortality and 5-HT syndrome following tryptophan plus pargyline administration. Although propranolol (10 mg/kg, IP), a β-adrenoceptor antagonist, did not alter the hypothermia caused by tryptophan in the pargyline-pretreated rats, pindolol (2 mg/kg, SC), a 5-HT_{1A} receptor and β-adrenoceptor antagonist, suppressed the hypothermia but not the hyperthermia or mortality caused by the same treatment. On the other hand, spiperone and ketanserin, 5-HT_2 receptor antagonists, at doses of 3 mg/kg, potentiated the hypothermia and completely suppressed the hyperthermia and mortality caused by tryptophan in the pargyline-pretreated rats. These results suggest that tryptophan-induced hypo- and hyperthermia are mediated by 5-HT_{1A} and 5-HT_2 receptors, respectively, in the pargyline-pretreated rats.

Tryptophan Pargyline 5-HT 5-HT_{1A} receptor 5-HT_2 receptor Hypothermia Hyperthermia Rat

SEROTONIN has been shown to play an important role in regulation of body temperature since Feldberg and Myers (6) implicated monoamines in thermoregulation. Administration of L-tryptophan, a serotonin precursor, to the rat pretreated with monoamine oxidase inhibitor (MAO-I) also affects body temperature, accompanied by behavioral effects such as tremor, forepaw treading, hind limb abduction, and head weaving (8). The behavioral changes caused by tryptophan plus MAO-I have been extensively studied, but the thermoregulatory effects of this combination are controversial. Tryptophan administration to pargyline-pretreated rats has been reported to produce hyperthermia associated with hyperactivity (8). In contrast, Francesconi and Mager (7) and Pawlowski (14) observed hypothermia following administration of tryptophan or 5-hydroxytryptophan with a peripheral decarboxylase inhibitor Ro 4-4602 in rats.

In the last decade, it has been demonstrated that 5-HT_{1A} and 5-HT_2 receptors, respectively, are involved in hypo- and hyperthermic effects of 5-HT agonists in rats (9,12). Thus, in the present study we investigated effects of tryptophan on rectal temperature in pargyline-pretreated rats to clarify the role of 5-HT receptor subtypes in endogenous 5-HT regulation of body temperature.

METHOD

Male Wistar rats weighing 250-350 g (Japan SLC Inc., Hamamatsu, Japan) were used in the experiments. The ani-

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mals were housed in groups of four to five per cage (35 × 30 × 16 cm) for at least 1 week before starting the experiment, in an animal room under a 12 L : 12 D cycle (lights on: 0730 h) in an ambient condition of 24 ± 2°C and 55 ± 5% R.H. Animals were given food and water ad lib.

Drugs

The test drugs used were as follows: pargyline hydrochloride and propranolol (Nacalai Tesque, Inc., Kyoto, Japan), DL-p-chlorophenylalanine methylester hydrochloride (p-CPA), pindolol, and spiperone (Sigma Chem. Co., St. Louis, MO), DL-tryptophan (Aldrich Chem. Co., Inc., Milwaukee, WI), and ketanserin tartrate (Research Biochemicals Inc., Natick, MA). Drugs were dissolved in saline except for specially stated cases. Tryptophan and spiperone were suspended in saline containing 0.5% carboxymethyl-cellulose Na (CMC), and pindolol was dissolved in saline by adding a few drops of 1 N HCl and then neutralized with 1 N NaOH. Ketanserin was dissolved in 5% glucose solution. Drug solutions were prepared immediately before experiments and were injected intraperitoneally in a constant volume of 1 ml/kg body weight except for pindolol, which was administered subcutaneously.

Measurement of Body Temperature

Rectal temperature was measured using a digital thermometer (Delta SK-1250 Mc, Sato Keiryoki Mfg. Co. Ltd., Japan). Briefly, a probe (2 mm in diameter, 10 cm long, Mc-100, Sato Keiryoki Mfg. Co. Ltd., Japan) was inserted 3 cm into the rectum until the recorded temperature reached plateau. Rectal temperature of each animal was recorded just before and every 30 min over 6 h after drug administration. Animals were pretreated with pargyline (50 mg/kg, IP) 60 min before tryptophan administration (25, 50, and 100 mg/kg, IP) except for p-CPA experiment where tryptophan and pargyline were injected simultaneously at time 0. Control animals received 0.5% CMC in saline.

Pretreatment With 5-HT Related Agents, β-Adrenoceptor Antagonists, and a Dopamine Antagonist

p-CPA (100 mg/kg/day, IP), a 5-HT synthesis inhibitor, was administered daily for 3 days before the experiments. Propranolol (10 mg/kg), a β-adrenoceptor antagonist, and spiperone (3 mg/kg), a 5-HT_2- and D_2 receptor antagonist, were injected IP 2 and 1 h before the experiments, respectively. Pindolol (0.05 and 2 mg/kg, SC), a 5-HT_{1A} receptor and β-adrenoceptor antagonist, and ketanserin (3 mg/kg, IP), a selective 5-HT_2 antagonist, were injected 30 min before the experiments.

Behavioral Assessment

Immediately before temperature measurement, behavior was assessed according to the criteria described by Jacobs (10). Briefly, a behavioral effect was scored as present if at least four of the following six component signs were observed: resting tremor (especially of the head and forelimbs) that occurs when the rat was undisturbed or at rest, rigidity or hypertonic-

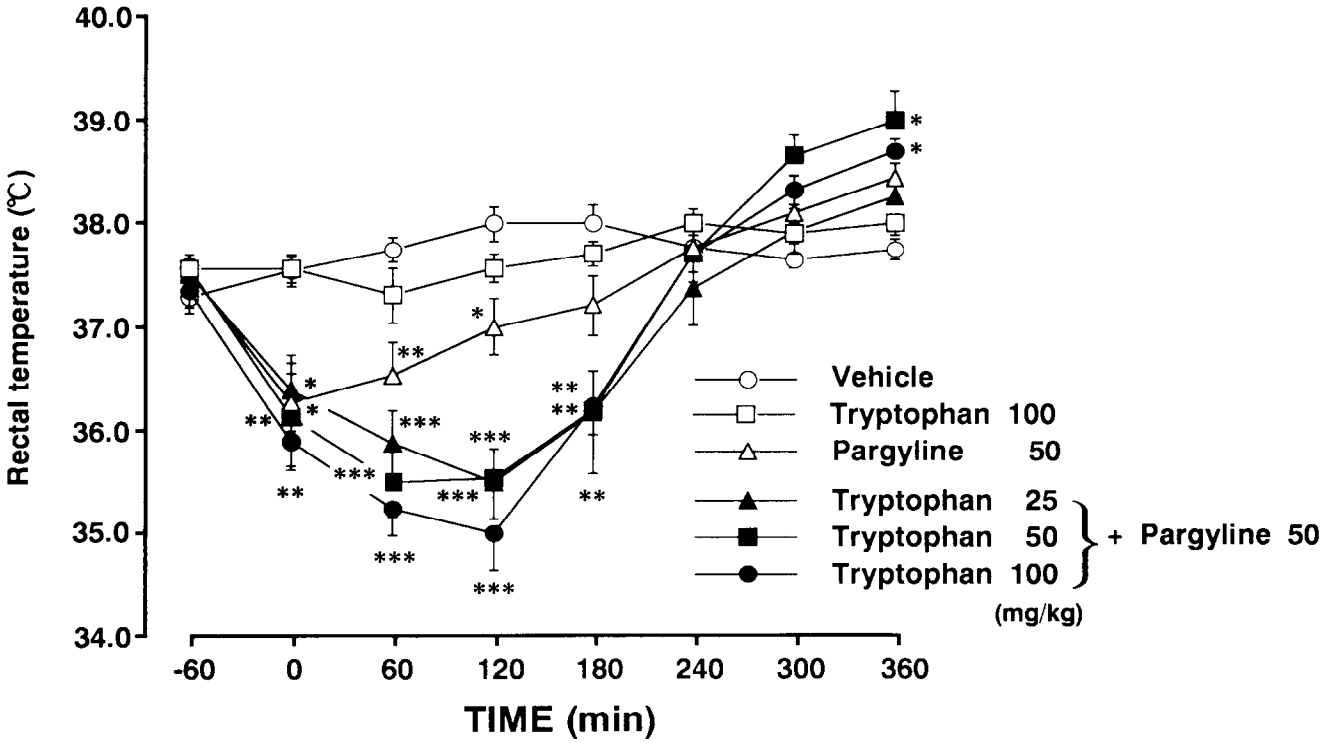

FIG. 1. Effect of tryptophan on the rectal temperature in pargyline-pretreated rats. Pargyline (50 mg/kg) or saline was intraperitoneally injected 60 min before tryptophan administration. At time 0, either 0.5% CMC or tryptophan (25–100 mg/kg, IP) was injected. The rectal temperature was measured every 30 min over 6 h. Each point represents the mean ± SEM from five to seven rats. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ compared with the respective vehicle control values.

ity (assessed both by grasping the rat around the torso and by passively extending and flexing the hindlimbs), reciprocal forepaw treading (rhythmic dorso-ventral movements of the forelimbs), hindlimb abduction (a dramatic splaying out of the hindlimbs), straub tail, and lateral head weaving (slow side-to-side head movements).

Statistics

The data are expressed as the mean (±SEM) change in rectal temperature (°C) and analyzed with one-way analysis of variance (ANOVA) followed by Dunnett's test. Comparison between two groups was made by the Student's *t*-test. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

Effect of Tryptophan on Body Temperature of Pargyline-Treated Rats

As shown in Fig. 1, tryptophan (25, 50, and 100 mg/kg, IP) produced dose-dependent hypothermia followed by hyperthermia in the rats pretreated with pargyline (50 mg/kg, IP). Hypothermia, which was observed 30 min after pargyline injection, persisted over 3 h after tryptophan administration, whereas the hyperthermic effect was recorded 4 to 6 h after tryptophan injection. The 5-HT syndrome such as tremor, head weaving, and hindlimb abduction appeared 3–4 h after tryptophan administration. Some animals died within 24 h after tryptophan administration, and the mortality rates of rats receiving 25, 50, and 100 mg/kg tryptophan (IP) were 0, 25, and 40%, respectively. Pargyline alone produced significant hypothermia that was not followed by hyperthermia.

Effect of p-CPA Pretreatment on the Body Temperature Changes Induced by Tryptophan Plus Pargyline in Rats

Pretreatment with p-CPA significantly blocked both the hypo- and hyperthermic effects produced by tryptophan plus pargyline (Fig. 2). Moreover, p-CPA completely attenuated the tryptophan-induced 5-HT syndrome and mortality in the pargyline-pretreated rats.

Effect of Pindolol on the Tryptophan-Induced Body Temperature Changes in the Pargyline-Pretreated Rats

As shown in Fig. 3A, pindolol, at 0.05 mg/kg (SC), did not affect the hypothermic effect of tryptophan in the pargyline-pretreated rats, whereas it at 2 mg/kg (SC) significantly attenuated the hypothermia induced by tryptophan plus pargyline and decreased the latency to induce hyperthermia. This treatment was not able to depress the behavioral syndrome or mortality (66.6%).

Effect of Propranolol on the Tryptophan-Induced Body Temperature Changes in the Pargyline-Pretreated Rats

Propranolol, a β-adrenoceptor antagonist, prolonged the hypothermic effect and delayed the appearance of the hyperthermic action of tryptophan in the pargyline-pretreated rats (Fig. 3B).

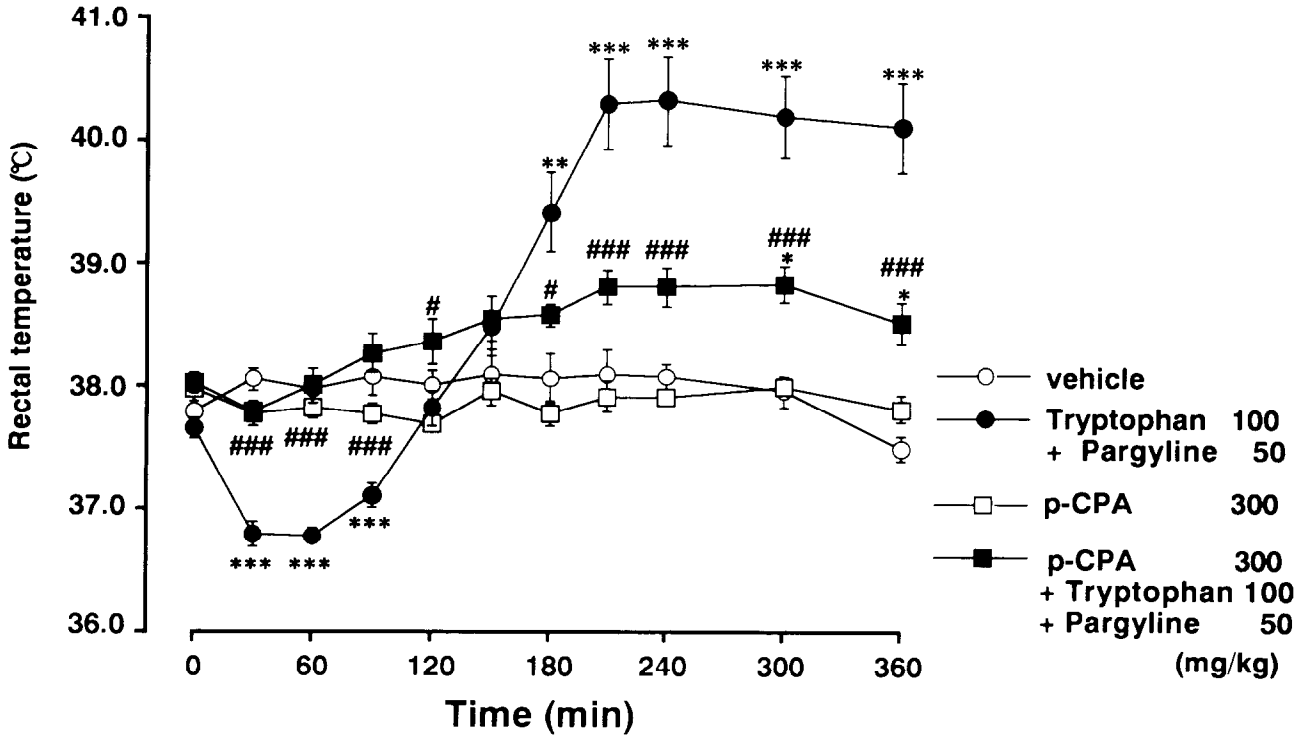

FIG. 2. Effect of p-CPA treatment on the tryptophan plus pargyline-induced changes in the rectal temperature. p-CPA (100 mg/kg, IP) was daily administered for 3 days before the experiments. Tryptophan (100 mg/kg) and pargyline (50 mg/kg) were intraperitoneally injected at time 0 immediately after measuring the basal temperature. The rectal temperature was recorded every 30 min over 6 h. Each point represents the mean ± SEM from nine rats. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ compared with the respective vehicle control values. $\#p < 0.05$ and $\#\#\#p < 0.001$ compared with tryptophan plus pargyline.

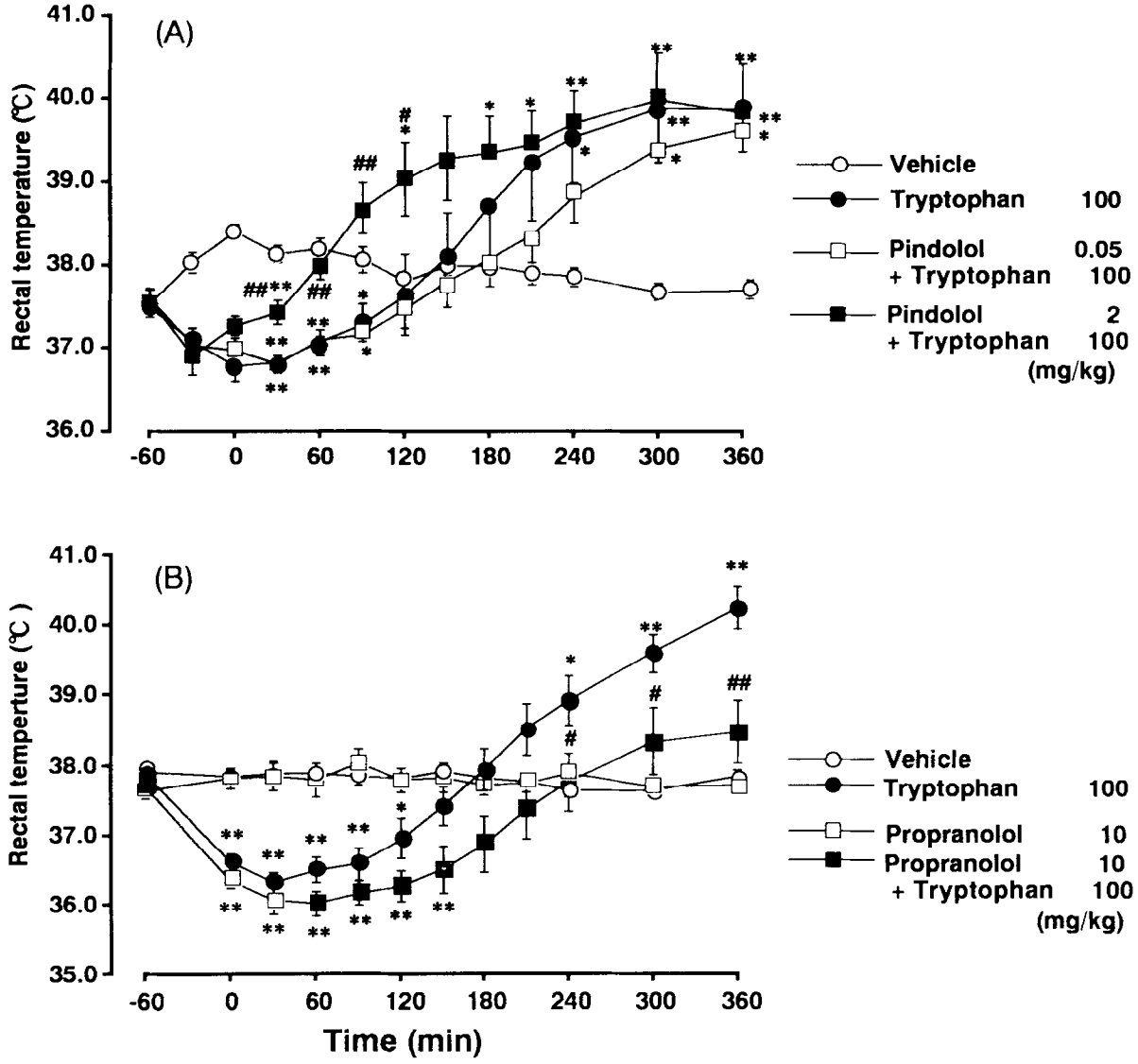

FIG. 3. Effects of pindolol (A) and propranolol (B) on the tryptophan-induced body temperature change in the pargyline-pretreated rats. The animals were pretreated with pargyline (50 mg/kg, IP) 60 min before tryptophan (100 mg/kg, IP) administration. Pindolol (0.05 and 2 mg/kg, SC) and propranolol (10 mg/kg, IP) were administered 30 min and 2 h before tryptophan, respectively. The rectal temperature was recorded every 30 min over 6 h. Each point represents the mean ± SEM from seven rats. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ compared with the respective vehicle control values. $\#p < 0.05$ and $\#\#p < 0.01$ compared with tryptophan plus pargyline.

Effect of Spiperone and Ketanserin on the Body Temperature Changes Induced by Tryptophan in the Pargyline-Pretreated Rats

The hypothermic effect of tryptophan in the pargyline-pretreated rats was significantly prolonged by spiperone (3 mg/kg, IP) and ketanserin (3 mg/kg, IP), whereas its hyperthermic effect was significantly blocked by these drugs (Fig. 4). No mortality was recorded in these spiperone- and ketanserin-treated animals. Spiperone-treated rats showed a cataleptic posture but not the 5-HT syndrome, while 50% of the rats treated with ketanserin showed a moderate 5-HT syndrome consisting of muscle rigidity, flat body posture, hindlimb abduction, and head tremor.

DISCUSSION

The present results showed that administration of tryptophan to the pargyline-pretreated rats produced short-lasting hypothermia followed by hyperthermia and the 5-HT syndrome. Administration of tryptophan, a precursor of 5-HT, to MAO-I-pretreated rats elevates the 5-HT levels in the brain (8). The hypothermic effect of tryptophan plus pargyline agrees with the finding by others (4,5,14) that serotonin and

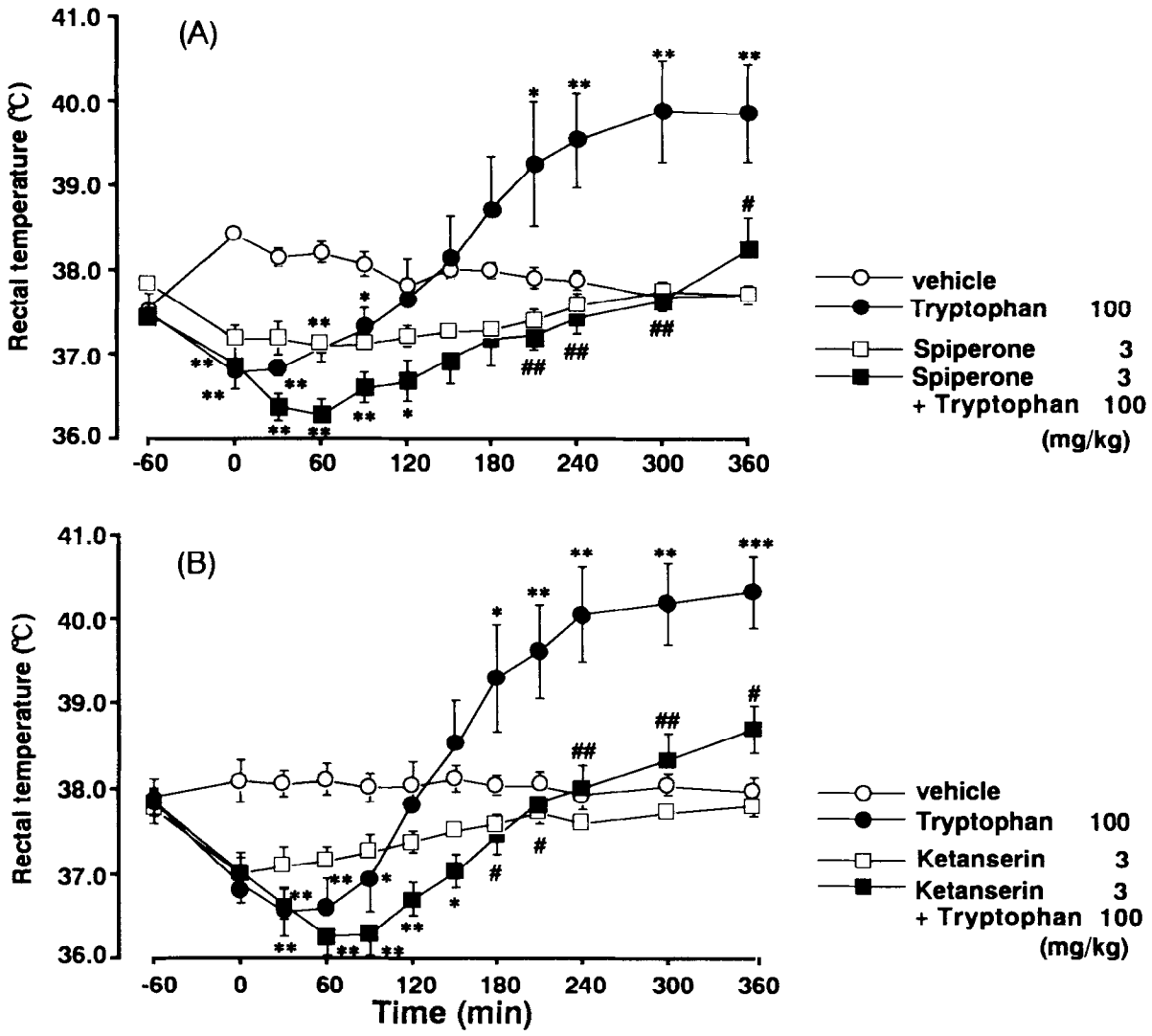

FIG. 4. Effect of 5-HT_2 receptor antagonists, spiperone (A) and ketanserin (B), on the tryptophan-induced body temperature change in the pargyline-pretreated rats. The animals were pretreated with pargyline (50 mg/kg, IP) 60 min before tryptophan (100 mg/kg, IP) administration. Spiperone (3 mg/kg, IP) and ketanserin (3 mg/kg, IP) were administered 60 and 30 min before tryptophan injection, respectively. The rectal temperature was recorded every 30 min over 6 h. Each point represents the mean ± SEM from seven rats. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ compared with the respective vehicle control values. $\#p < 0.05$ and $\#\#p < 0.01$ compared with tryptophan plus pargyline.

tryptophan produce hypothermia when injected intraventricularly, intraperitoneally, or directly into the lateral septum in rat. However, the role of serotonin in thermoregulation evokes controversy, because the thermoregulatory response to serotonin depends on the location of injection (2,4,5,13), the species of animals tested (13,17), sex (1), and the ambient temperature examined (7).

The present data that p-CPA, a 5-HT synthesis inhibitor, prevented the tryptophan plus pargyline-induced hypo- and hyperthermia indicate that the changes in body temperature following administration of tryptophan plus pargyline are, indeed, related to the 5-HT synthesized from exogenously administered tryptophan. Moreover, these results seem to exclude the possible involvement of tryptamine in the body temperature change, because p-CPA reportedly produces its action through the inhibition of tryptophan hydroxylase but not of aromatic amino acid decarboxylase (8).

Pindolol, a 5-HT_{1A} and β-adrenoceptor antagonist, but not propranolol, a β-adrenoceptor antagonist, significantly attenuated the hypothermia caused by tryptophan plus pargyline, suggesting that the 5-HT_{1A} receptor is important for the hypothermic effect of tryptophan. The involvement of the 5-HT_{1A} receptor subtype in the hypothermia agrees with the data reported by others [(9,12), Abdel-Fattah et al., unpublished data] that hypothermia is a highly specific and sensitive response to the activation of the postsynaptic 5-HT_{1A} receptor in rats. The appearance of the hyperthermic effect of tryptophan in the pargyline-pretreated rats was markedly accelerated by pindo' ,1 but not by propranolol in this study. These results further support the hypothesis that the 5-HT_{1A} receptor sub-

type is involved in hypothermia but not hyperthermia, and that blockade of this receptor subtype stimulates the appearance of the hyperthermic activity. On the other hand, the failure of (±)pindolol (0.05 and 2 mg/kg) to block the 5-HT syndrome, especially head weaving and ambulation produced by tryptophan plus pargyline, is consistent with the data reported by Tricklebank (15) and Tricklebank et al. (16) that (+)- and (−)-pindolol (0.5–4 mg/kg) was unable to attenuate head weaving and ambulation induced by 8-hydroxy-2-(di-n-propylamino) tetralin, a selective 5-HT_{1A} receptor agonist, in rats.

The failure of spiperone and ketanserin, 5-HT_2 receptor antagonists, to antagonize the hypothermic effect of tryptophan in the pargyline-pretreated rats excludes the possible involvement of the 5-HT_2 receptor subtype in this effect. On the other hand, both antagonists significantly blocked the hyperthermia induced by the same treatment, indicating involvement of the 5-HT_2 receptor subtype in the hyperthermic response to tryptophan in the pargyline-pretreated rats. These results are consistent with the data of Gudelsky (9) that a selective 5-HT_2 receptor agonist MK-212 produced hyperthermia in rats. Moreover, in the present study, although in the pargyline-pretreated rats tryptophan caused muscle rigidity and a moderate 5-HT syndrome in about 50% of the rats pretreated with ketanserin, it produced long-lasting hypothermia but not significant hyperthermia in these animals. These findings suggest that the hyperthermia caused by tryptophan in the pargyline-pretreated rats is not a secondary action due to the prolonged tremor or motor activity (1,8).

In this study, pargyline itself slightly but significantly induced hypothermia. Pargyline has been reported to act as a preferential inhibitor of the MAO-B type, which deaminates dopamine and elevates the dopamine level in the brain (18,19). In fact, injection of dopamine into the preoptic anterior hypothalamus is known to produce hypothermia in rats (3). Moreover, stimulation of the nigrostriatal dopaminergic system also produces hypothermia in rats (11). Thus, the present results do not exclude the possible involvement of dopaminergic system in the hypothermic effect of tryptophan in the pargyline-pretreated rats.

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