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Hypothermic Effect of Harmala Alkaloid in Rats: Involvement of Serotonergic Mechanism

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ABDEL-FATTAH, A.-F. M., K. MATSUMOTO, H. A.-K. GAMMAZ AND H. WATANABE. *Hypothermic effect of harmala alkaloid in rats: Involvement of serotonergic mechanism.* PHARMACOL BIOCHEM BEHAV **52**(2) 421–426, 1995.—The effect of total alkaloid extracted from *Peganum harmala* seeds collected in Egypt on body temperature was studied in rats. Intraperitoneal administration of the *Peganum harmala* extract produced significant and dose-dependent hypothermia. Similarly, harmine and harmaline, major constituents of the harmala alkaloid, lowered the body temperature. Pretreatment with p-chlorophenylalanine (100 mg/kg/day for 3 days), a 5-HT synthesis inhibitor, significantly attenuated the hypothermic effect of the total alkaloid and harmine, while it tended to block the hypothermic action of harmaline. Methysergide (2 mg/kg), a 5-HT antagonist, significantly attenuated the hypothermia induced by harmala alkaloids. Pindolol (0.05–2 mg/kg), a 5-HT_{1A} receptor and β-adrenoceptor antagonist, partly blocked the hypothermic effect of the harmala alkaloids in a dose-dependent manner, whereas propranolol (10 mg/kg), a β-adrenoceptor antagonist, failed to alter it, suggesting that β-adrenoceptor is not involved in the hypothermia caused by the alkaloids. Pretreatment with a dopamine receptor antagonist haloperidol (5 mg/kg, SC and 2 mg/kg, IP 24 and 2 h before the experiment, respectively) significantly attenuated the hypothermic effect of harmala alkaloids. Moreover, in haloperidol pretreated rats, methysergide (2 mg/kg, IP) and pindolol (0.05 and 2 mg/kg) completely attenuated the hypothermic effect of the alkaloids. These data suggest that harmala alkaloids produce hypothermic effect mainly through endogenous 5-HT stimulation of 5-HT_{1A} receptor.

Harmala alkaloids Harmine Harmaline Hypothermia 5-HT_{1A} receptor Dopamine receptor Rats

PEGANUM HARMALA L. is a member of the family Zygophyllaceae (13). This plant is commonly distributed in North Africa and the Middle East (8). Several alkaloids are isolated from the seeds and roots of the plant, and identified as chemicals with β-carboline structure such as harmine, harmaline, harmalol, and harman (2,22) or with quinazoline structure such as vasicine and vasicinon (21,22,25). β-Carboline compounds are known to affect several neurotransmitter systems in the brain (5,23). For example, harmine, harmaline, and related compounds are potent and reversible inhibitors of monoamine oxidase and produce various pharmacological actions such as hypothermia, tremor, and hypotensive activity (6,9,12). Moreover, it has been hypothesized that the decrease of body temperature by harmaline is due to its modulation of monoaminergic function at thermoreceptor sites in the brain (3,4), but the exact mechanism of thermoregulatory action of the harmala alkaloids still remains to be clarified.

A number of reports indicate that the 5-HT receptor in the brain, especially in the hypothalamus, plays important roles in thermoregulation, and that drugs acting on such receptors change body temperature [for review, see (14,19)]. Recently, Millan et al. (18) demonstrated that hypothermia is a highly specific and sensitive response to activation of the postsynaptic 5-HT_{1A} receptor in rats. Besides, the dopaminergic system in the brain also appears to play a role in thermoregulation mechanisms, i.e., the presynaptic dopamine neurons are involved in hypothermia, whereas both postsynaptic dopamine D_1 and D_2 receptors mediate hyperthermia in mice (28). These findings prompted us to investigate the possible involvement of 5-HT and dopamine systems in the hypothermic effect of harmala alkaloids.

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METHOD

Animals

Male Wistar rats weighing 250–350 g (Japan SLC Inc., Hamamatsu, Japan) were used in the experiments. The animals were housed in groups of four to five per cage (35 × 30 × 16 cm), for at least 1 week before starting the experiment. Housing conditions were thermostatically maintained at 24 ± 2°C with constant humidity (55 ± 5%) and a light/dark cycle (lights on: 0730–1930). Animals were given food and water ad lib.

Drugs

The test drugs used were as follows: harmaline HCl and propranolol HCl (Nacalai Tesque, Inc., Kyoto, Japan), haloperidol (Serenace®, Dai Nippon Pharmaceutical Co. Ltd., Osaka, Japan), harmine HCl, DL-p-chlorophenylalanine methylester HCl (p-CPA) and pindolol (Sigma Chemical Co., St. Louis, MO), and methysergide hydrogen maleate (Sandoz, Switzerland). In addition, total alkaloid was extracted from *Peganum harmala* seeds collected in Egypt as described previously (1). All drugs were dissolved in saline except for total alkaloid and pindolol. Total alkaloid fraction was suspended in saline containing 0.5% carboxymethyl cellulose Na (CMC). Pindolol was dissolved in saline by adding a few drops of 1 N HCl and then neutralized with 1 N NaOH. Drug solutions were prepared immediately before injection and were administered in a volume of 0.5 ml/kg body weight.

Measurement of Body Temperature

Rectal temperature was measured using Digital Thermometer (Delta SK-1250 Mc, Sato Keiryoki Mfg. Co., Ltd., Japan). Briefly, a probe (2 mm in diameter, 10 cm long, MC-T100) was inserted into the rectum 3 cm deep until the recorded temperature reach plateau. Rectal temperature of each animal was recorded just before and 30, 60, 90, and 120 min after drug administration. Animals were injected IP with 2.5, 5, 10, and 20 mg/kg of either total alkaloid, its major components, harmine and harmaline, or vehicle.

Pretreatment of Animals With 5-HT-Related Agents, β-Adrenoceptor Antagonists, or a Dopamine Antagonist

p-CPA (100 mg/kg/day for 3 days), a 5-HT synthesis inhibitor, and propranolol (10 mg/kg), a β-adrenoceptor antagonist, were IP injected 72 and 2 h before the experiments, respectively. Methysergide (2 mg/kg, IP), a 5-HT receptor antagonist, and pindolol (0.05 and 2 mg/kg, SC), a 5-HT_{1A} receptor and β-adrenoceptor antagonist, were injected 30 min before the experiments. Haloperidol was injected at doses of 5 mg/kg (SC) and 2 mg/kg (IP) 24 and 2 h, before the experiment, respectively. Haloperidol produced a hypothermic effect, but the effect disappeared 90 min after the last administration of this drug.

Statistics

The data were expressed as the mean (°C ± SEM) change in rectal temperature and analyzed with one-way analysis of variance (ANOVA) followed by Dunnett's test. Comparisons between two groups were made by the Student's *t*-test. Data from agonist-antagonist interaction experiments were analyzed with two-way ANOVA followed by Tukey's test. Difference with a $p < 0.05$ was considered statistically significant.

RESULTS

Effect of Total Alkaloid, Harmine, and Harmaline on Body Temperature of Rats

As shown in Fig. 1, total alkaloid, harmine, and harmaline (2.5–20 mg/kg) induced a dose-dependent hypothermia in rats. Maximal changes in body temperature were observed at 30–60 min. after alkaloid injection. Potency of hypothermic effects of these agents was following rank of order; harmaline > total alkaloid ≥ harmine.

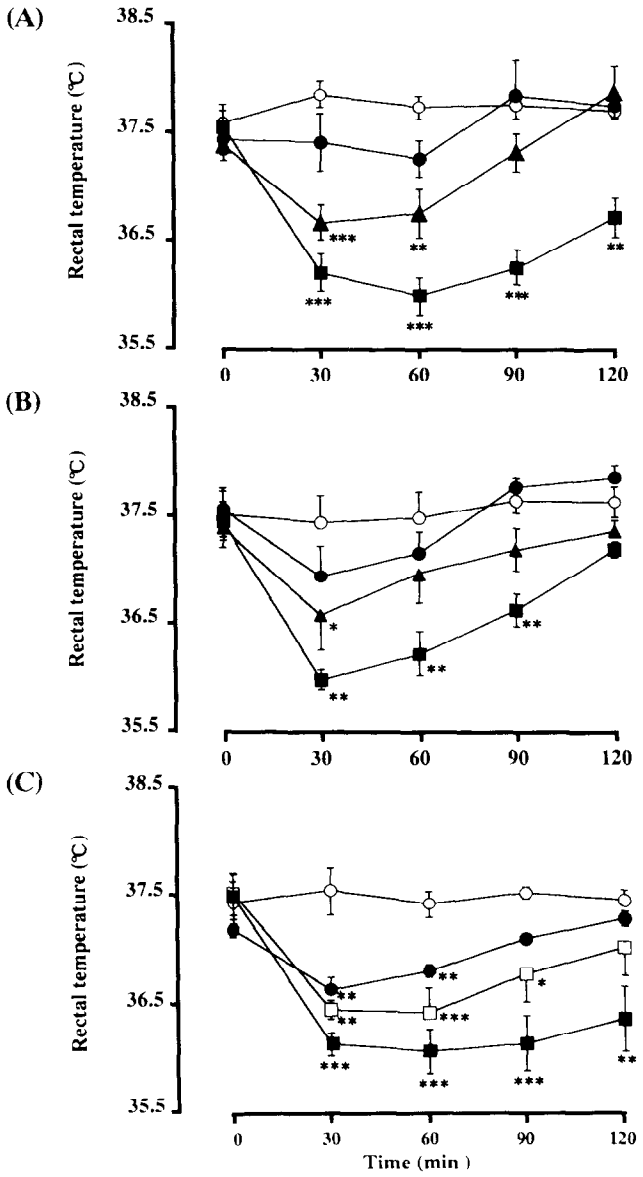

FIG. 1. Effects of total alkaloid (A), harmine (B) and harmaline (C) on rectal temperature in rats. Vehicle (○: saline or 0.5% CMC) or test agents (●: 2.5; ▲: 5; □: 10; ■: 20 mg/kg, IP) were administered at time 0. The temperature was measured every 30 min over 120 min. Each point represents the mean ± SEM from five to seven rats. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared with the respective vehicle control values.

Effect of p-CPA on Total Alkaloid-, Harmine- and Harmaline-Induced Hypothermia

Pretreatment with p-CPA significantly blocked the hypothermic effects of total alkaloid and harmine, while it slightly

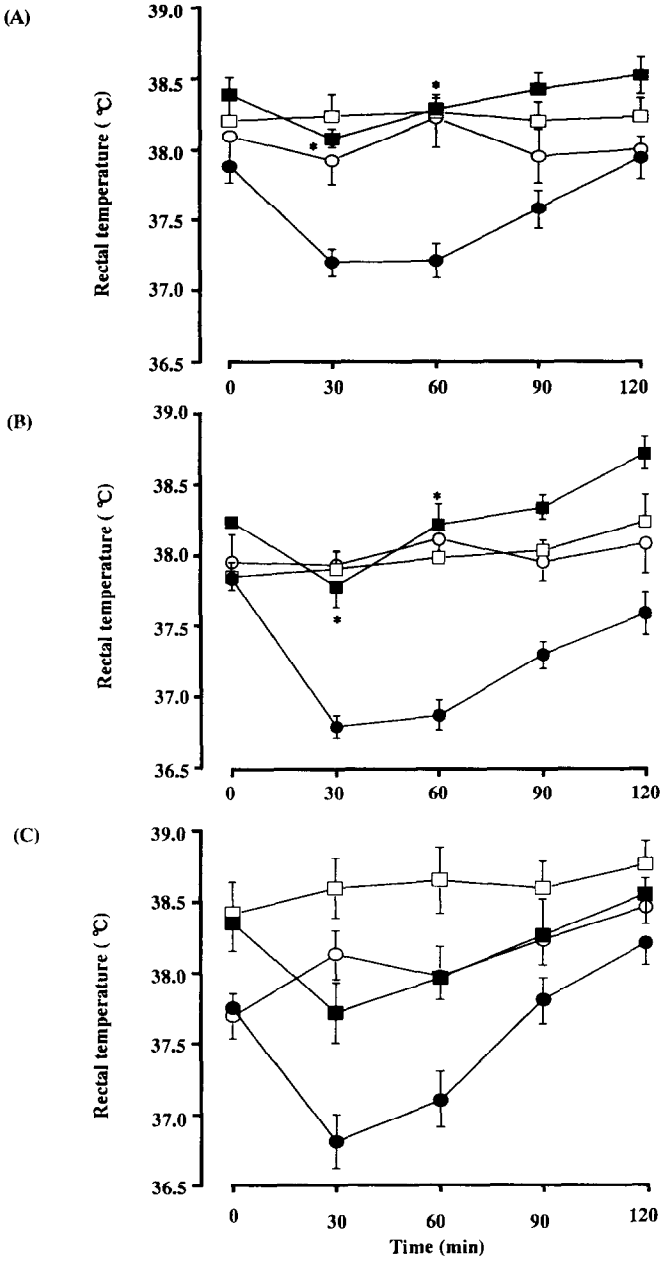

FIG. 2. Effect of p-CPA pretreatment on total alkaloid, harmine, and harmaline-induced changes in the rectal temperature. p-CPA (100 mg/kg, IP) was daily injected for 3 days before testing. Total alkaloid (5 mg/kg, A), harmine (10 mg/kg, B), or harmaline (5 mg/kg, C) was intraperitoneally injected at time 0 immediately after measuring the basal temperature. Rectal temperature was recorded every 30 min over 120 min. Each point represents the mean ± SEM from nine rats. *$p < 0.05$ compared with the total alkaloid alone. (○) vehicle; (●) total alkaloid (A), harmine (B) or harmaline (C); (□) p-CPA alone; (■) p-CPA plus total alkaloid, harmine, or harmaline.

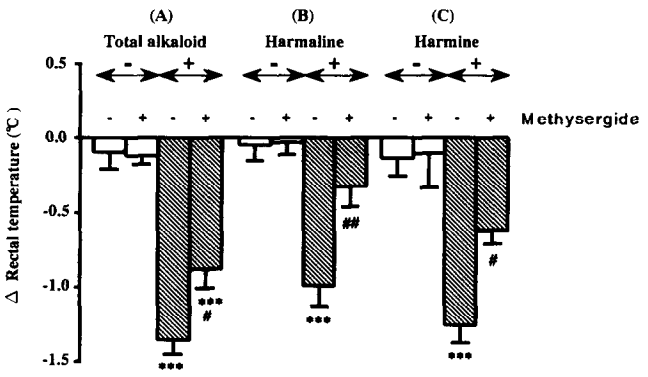

FIG. 3. Effect of methysergide on total alkaloid, harmine, and harmaline-induced hypothermia. Methysergide (2 mg/kg, IP) was injected 30 min before test drugs administration. The differences in the rectal temperature measured before and 30 min after vehicle, total alkaloid (5 mg/kg), harmine (10 mg/kg), or harmaline (5 mg/kg) administration were compared. Each datum represents the mean ± SEM from six rats. ***$p < 0.001$ vs. vehicle control. #$p < 0.05$ and ##$p < 0.01$ vs. total alkaloid, harmine, or harmaline alone. Hatched and open columns represent the treatment with and without total alkaloid (A), harmaline (B), or harmine (C), respectively.

but not significantly attenuated the hypothermic response following harmaline injection (Fig. 2). Effect of p-CPA alone on the body temperature was variable and not statistically significant.

Effect of Methysergide on Total Alkaloid, Harmine, and Harmaline-Induced Hypothermia

The effect of pretreatment with methysergide on the hypothermia induced by harmala alkaloids is shown in Fig. 3. Methysergide (2 mg/kg, IP) significantly attenuated the hypothermic effect of total alkaloid measured at 30 min after total alkaloid injection, but the effect of total alkaloid in the methysergide-treated animals was still significant compared with vehicle treated groups ($p < 0.001$). Methysergide also significantly blocked the hypothermic effects of harmine ($p < 0.01$) and harmaline ($p < 0.05$) recorded at 30 min after injection.

Effect of Pindolol and Propranolol on the Total Alkaloid-Induced Hypothermia

Pindolol (0.05 and 2 mg/kg) significantly antagonized the hypothermia produced by the total alkaloid in a dose-dependent manner (Fig. 4). On the other hand, a nonselective β-adrenoceptor antagonist propranolol (10 mg/kg) did not alter the hypothermic effect induced by the total alkaloid.

Effect of Haloperidol Alone and in Combination With 5-HT_{1A} Antagonists on Total Alkaloid-Induced Hypothermia in Rats

Pretreatment with haloperidol significantly attenuated the hypothermic effect of total alkaloid in rats without inducing hypothermia by itself. Combination of methysergide (2 mg/kg, IP) or pindolol (2 mg/kg, SC) with haloperidol pretreatment completely antagonized the hypothermic effect of the total alkaloid in an additive manner (Fig. 5).

DISCUSSION

The present results demonstrated that total alkaloid extracted from *Peganum harmala* seeds and its major compo-

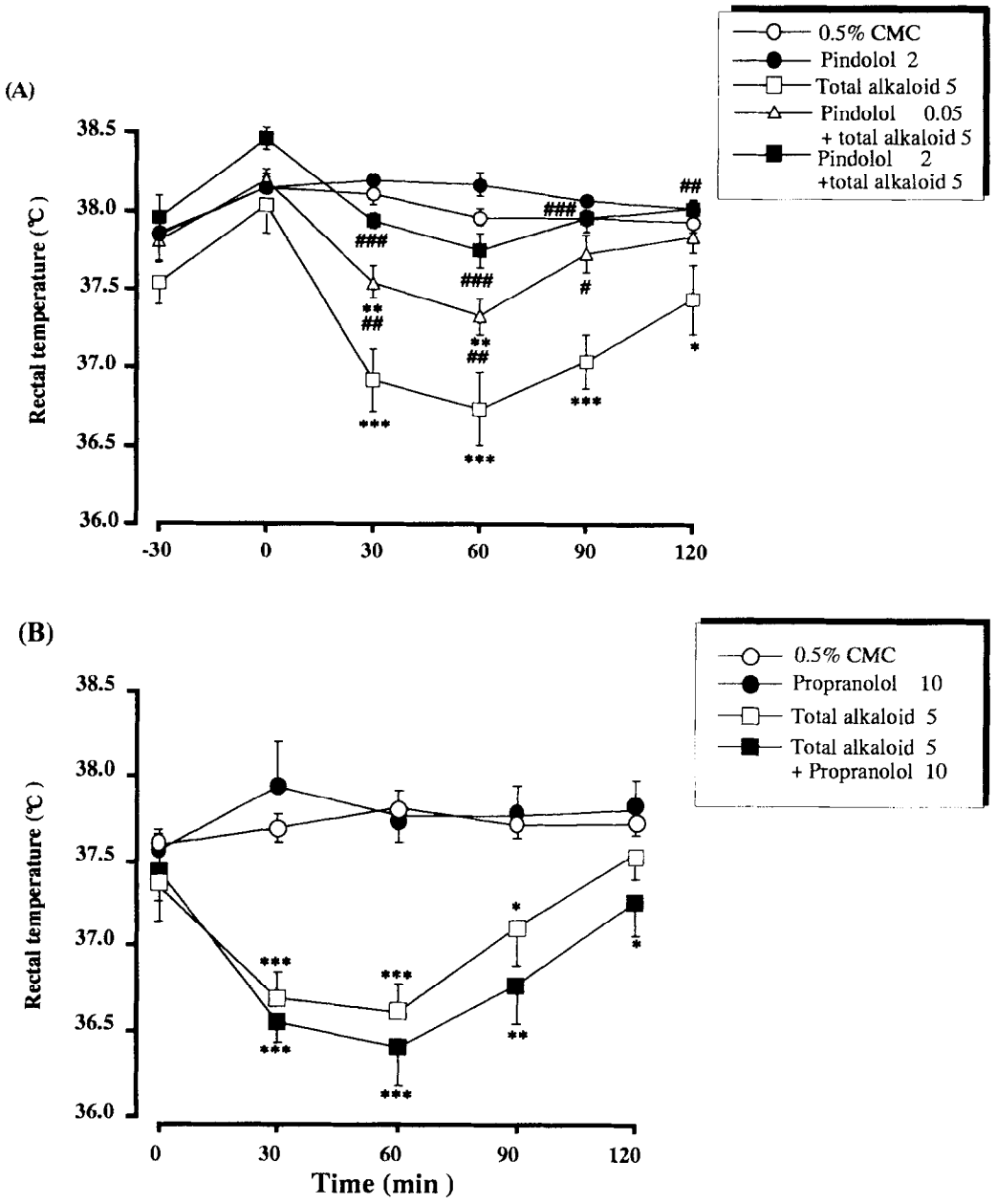

FIG. 4. Effects of pindolol (A) and propranolol (B) on total alkaloid-induced hypothermia. Pindolol (0.05 and 2 mg/kg, SC) and propranolol (10 mg/kg, IP) were administered 30 min and 2 h, respectively, before total alkaloid injection (5 mg/kg, IP). Rectal temperature was recorded every 30 min over 120 min. Each point represents the mean ± SEM from seven rats. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ compared with vehicle control. $\#p < 0.05$, $\#\#p < 0.01$, and $\#\#\#p < 0.001$ compared with the total alkaloid alone.

nents, harmine and harmaline, produced a dose-dependent hypothermia in rats mainly through endogenous 5-HT stimulation of the 5-HT_{1A} receptor. p-CPA, a 5-HT synthesis inhibitor, completely abolished the hypothermic effect of total alkaloid, indicating that harmala alkaloid requires endogenous serotonin to exert its hypothermic effect. On the other hand, p-CPA failed to significantly block the hypothermic effect induced by harmaline. The latter finding agrees with the data reported by Bruinvels et al. (4) that treatment with p-CPA, as well as with α-methyldopa and α-methyl-*p*-tyrosine, did not affect the decrease in temperature induced by harmaline. Several factors may explain the difference in the endogenous 5-HT dependency between the extract and harmaline. First, the remaining portion of endogenous 5-HT after p-CPA treatment may be enough for harmaline to produce hypothermia in rats, because pretreatment with the dosage of p-CPA re-

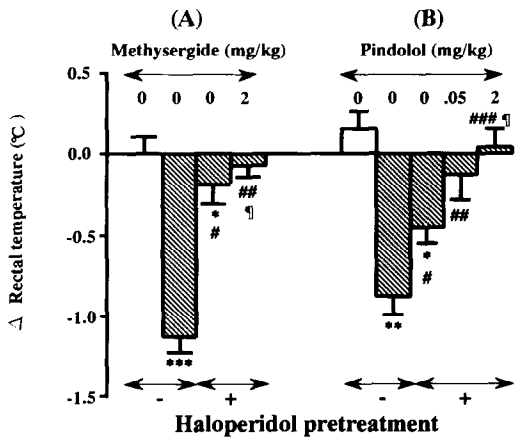

FIG. 5. Effect of haloperidol alone and its combination with methysergide (A) and pindolol (B) on the total alkaloid-induced hypothermia. Haloperidol was given at the doses of 5 (SC) and 2 mg/kg (IP) 24 and 2 h, respectively, before the experiments. Methysergide (2 mg/kg, IP) and pindolol (0.05 and 2 mg/kg, SC) were given 30 min before the total alkaloid. Difference in the rectal temperature recorded immediately before and 30 min after administration of the alkaloid (5 mg/kg, IP). Each datum represents the mean ± SEM from seven rats. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ compared with vehicle control. $\#\#p < 0.01$ and $\#\#\#p < 0.001$ compared with the total alkaloid. $¶p < 0.05$ compared with haloperidol + the total alkaloid.

portedly decreases endogenous 5-HT in the brain by 80–90% (16). Thus, component(s) of the harmala alkaloid extract different from harmaline may play important roles in the hypothermic effect in an endogenous 5-HT-dependent manner. Secondly, harmaline itself directly stimulates the 5-HT receptor, resulting in a hypothermic effect. Third, other neurotransmitter mechanism(s) differing from the serotonergic system may be involved in harmaline-induced hypothermia, as described below.

The antagonism by the 5-HT antagonist methysergide indicates that the hypothermic actions of the total alkaloid and its major components are, in part, mediated by 5-HT receptor stimulation. Moreover, in this study, pindolol, a 5-HT_{1A}- and β-receptor antagonist, but not propranolol, a β-receptor antagonist, significantly suppressed the hypothermic action of the total alkaloid. Methysergide reportedly attenuates the responses caused by 8-hydroxy-2-(di-n-propylamino)tetraline, a selective 5-HT_{1A} receptor agonist, in vivo and in vitro (10,15). Thus, taken together with the endogenous 5-HT dependency of the total alkaloid-induced hypothermia, these findings suggest that harmala alkaloids indirectly stimulate the 5-HT_{1A} receptor subtype by elevating the 5-HT level in the synaptic cleft, resulting in hypothermia. This idea seems to be supported by the data reported by Cox and Lee (7), that an increase of the endogenous 5-HT level in the brain decreases heat production and/or increases heat loss, leading to hypothermia in rats.

In this study, pretreatment with a dopamine antagonist haloperidol not only blocked the hypothermic response to the total alkaloid but also potentiated the suppressing actions of methysergide and pindolol on the total alkaloid-induced hypothermia in an additive manner. These results indicate that dopamine receptors also partly participate in the harmala alkaloids-induced hypothermia. Harmala alkaloids are known to be capable of inhibiting reversibly MAO subtype A (17,20). Dopamine is exclusively metabolized by MAO-A in rat brain in vivo, even though it is capable of serving as a substrate for both MAO-A and MAO-B in vitro (11,24,26,27). Thus, the inhibitory action of harmala alkaloids on MAO-A may contribute to the hypothermia through enhancing the dopamine mechanism. In fact, harmaline administration has been reported to increase the endogenous 5-HT level without changing the noradrenaline level in the brain (4). Harmaline-induced hypothermia may be partly mediated by the dopaminergic system, because the hypothermic effect of this drug was insensitive to the treatment with the 5-HT synthesis inhibitor p-CPA.

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