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Different Effects of Methylxanthines on Central Serotonergic Postsynaptic Neurons in a Mouse Behavioral Model

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KITATANI, T., Y. WATANABE AND T. SHIBUYA. *Different effects of methylxanthines on central serotonergic postsynaptic neurons in a mouse behavioral model.* PHARMACOL BIOCHEM BEHAV **44**(2) 457–461, 1993.—Effects of the four methylxanthines (100 mg/kg, IP)—caffeine, theophylline, theobromine, and pentoxifylline—on the central serotonergic neuron were studied in mice using a behavioral model, the head-twitch response. The four methylxanthines potentiated the head twitches induced by 5-hydroxytryptophan (5-HTP) in pargyline-pretreated mice; pentoxifylline was the most potent. The potentiating effect of pentoxifylline was increased by paroxetine, the selective inhibitor of uptake of 5-hydroxytryptamine (5-HT), but those of the other drugs were not. In nontreated animals, caffeine directly induced head-twitch responses, which were not affected by pargyline pretreatment but were increased by prior treatment with 5,7-dihydroxytryptamine (5,7-DHT). The number of head twitches produced by caffeine in 5,7-DHT-treated mice was increased twofold by *p*-chlorophenylalanine (*p*-CPA), the tryptophan hydroxylase inhibitor. In mice treated with both 5,7-DHT and *p*-CPA, theophylline induced the responses, although much less potently than caffeine. Theobromine and pentoxifylline produced even fewer responses. From the results of the present study, it may be concluded that the methylxanthines possess qualitatively different actions on the central serotonergic neuron; caffeine and theophylline appear to have direct effects on the postsynaptic neuron, but theobromine and pentoxifylline do not.

Methylxanthines Caffeine Theophylline Theobromine Pentoxifylline Paroxetine 5,7-DHT
p-CPA 5-HTP 5-HT Postsynaptic neuron 5-HT_2 receptors Head-twitch response Mice

IT has been documented that methylxanthines have effects not only on adenosinergic (6,25,29) but also on serotonergic neurons in the brain. Most reports on the effects of methylxanthines on serotonergic neurons have shown that caffeine, theophylline, and/or pentoxifylline facilitate the synthesis (1, 2,4,11,16,22,23) and release (3,21) of serotonin [5-hydroxytryptamine (5-HT)] and inhibit its reuptake (3,24,28). These findings suggest that such methylxanthines enhance the activity of the serotonergic presynaptic neuron in the brain. Our previous study revealed that caffeine, theophylline, theobromine, and pentoxifylline increased the number of 5-hydroxytryptophan (5-HTP)-induced head twitches in pargyline-pretreated mice (23). However, little is known about the mechanisms of the stimulating actions of the methylxanthines on the serotonin system, especially at the postsynaptic site. The few articles that have been published report that a) caffeine had no influence on the number of 5-HT_2 receptors 24 h after the last dose of a 3-week course of treatment (28) and b) pentoxifylline possessed no direct effect in producing head twitches in 5,7-dihydroxytryptamine (5,7-DHT)-pretreated mice (24). Although Reith et al. (28) reported that caffeine exerted no effect on postsynaptic serotonin functions, this interaction needs to be reexamined because the drug may (not?) have been washed out even as late as 24 h after the last treatment. Therefore, the present study was undertaken to examine further the mechanism of action of methylxanthines on the serotonergic postsynaptic neuron.

The head-twitch response in animals has been well investigated pharmacologically, and it has already been established that the response in mice is closely related to the function of 5-HT_2 receptors on postsynaptic neurons (12–14,19). Intrathecal administration of 5,7-DHT has been reported to induce supersensitivity of central postsynaptic 5-HT_2 receptor function (8,9,12,19,24,26,27) by depleting 5-HT levels via its neurotoxic actions (9,10,12,15,19,20,27,30). This indicates that 5,7-DHT-treated animals can be used to test for direct effects of drugs acting at postsynaptic 5-HT receptors. In the present study, therefore, we examined the effects of caffeine, theophylline, theobromine, and pentoxifylline in 5,7-DHT-treated mice using the head-twitch response as the behavioral model.

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The following effects were determined: a) the presynaptic neuronal function using paroxetine, the specific 5-HT uptake inhibitor; and b) the postsynaptic neuronal responses in mice made supersensitive to the postsynaptic effects of serotonin by pretreating with 5,7-DHT and *p*-chlorophenylalanine (*p*-CPA), which depletes the brain stores of 5-HT.

METHOD

Animals

Male ICR mice were obtained from CLEA Japan and used at 8–12 weeks of age after at least 1 week of acclimatization to the laboratory environment. Animals were kept in individual polycarbonate cages with wooden chips (White Flake®, Charles River, Japan) in a room at a temperature of 22 ± 1°C and relative humidity of 55 ± 5% with lighting from 8:00 a.m.–8:00 p.m. They were allowed free access to tapwater and lab chow (CE-2, CLEA Japan) ad lib except on the day of determination of head-twitch responses.

Drugs

The following substances were used: caffeine, theophylline, theobromine, and ascorbic acid (Wako Chemical); pentoxifylline (Hoechst AG, Frankfurt, Germany); 5,7-DHT creatinine phosphate salt, *p*-CPA methylester, 5-HTP, and desipramine HCl (Sigma Chemical Co., St. Louis, MO); pargyline HCl (Aldrich Chemical Co., Milwaukee, WI); pentobarbital-Na (Nembutal®, Abbott Laboratories, North Chicago, IL); and paroxetine HCl (a gift from SmithKline Beecham). These substances were dissolved or suspended in physiological saline for parenteral administration or suspended in a 0.5% solution of carboxymethylcellulose (CMC) for oral treatment. They were given in a volume of 0.1 ml/10 g body weight unless otherwise stated.

Administration of Drugs

The methylxanthines were administered IP at 100 mg/kg unless otherwise indicated. This dose was chosen because 30 mg/kg or lower did not enhance 5-HTP-induced head-twitch responses in mice (23). As previously described (20,24,30), we prepared the 5,7-DHT solution by dissolving 80 μg of the base in 5 μl physiological saline containing 0.2% ascorbic acid. This solution was injected ICV (1 mm dorsolaterally, 3 mm in depth) at the rate of 1 μl/10 s in mice anesthetized with pentobarbital. Animals were pretreated with desipramine (30 mg/kg, IP) 30 min prior to the 5,7-DHT treatment. Pargyline was given IP at 100 mg/kg and *p*-CPA was injected IP at 200 mg/kg twice at an interval of 24 h. Paroxetine was administered PO at 1 mg/kg. 5-HTP was injected IP at 25 mg/kg.

Dosage Schedule

To confirm presynaptic effects of the methylxanthines, we administered paroxetine (or 0.5% CMC as a control) concomitantly with pargyline. The methylxanthines were injected 30 min and 5-HTP 60 min later. The number of head twitches was counted for 10 min at 10 min after 5-HTP injection. Caffeine was examined in more detail than the other three drugs because it is known to have more potent central action than any other methylxanthine and is contained in many drug preparations and used as a reference drug in various animal experiments. Caffeine (100 mg/kg) was administered 30 min after pargyline or 7 days after 5,7-DHT when the maximum

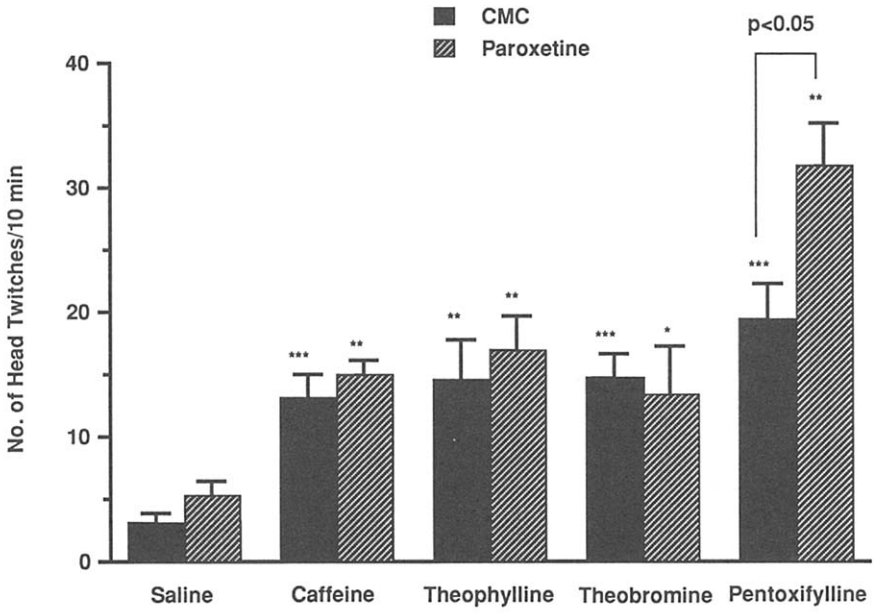

FIG. 1. Effects of paroxetine plus methylxanthines on 5-hydroxytryptophan (5-HTP)-induced head-twitch responses in pargyline-pretreated mice (n = 7 or 8). Paroxetine (1 mg/kg, PO) or carboxymehtylcellulose (CMC) was given concomitantly with pargyline (100 mg/kg, IP); the methylxanthines were injected IP 30 min later. 5-HTP was given (25 mg/kg, IP) 30 min following methylxanthine injection. Head-twitch responses were counted for a period of 10 min at 10 min after 5-HTP injection. Mean values ± SEM are shown. *, **, ***Significantly different from the saline-treated group at $p < 0.05$, 0.01, and 0.001, respectively.

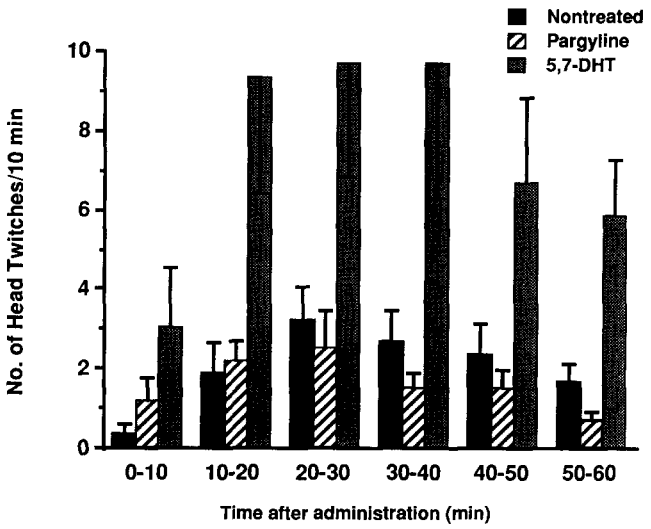

FIG. 2. Caffeine-induced head-twitch responses in 5,7-dihydroxytryptamine (5,7-DHT)- or pargyline-pretreated mice (n = 6). Caffeine (100 mg/kg, IP) was administered 30 min after pargyline (100 mg, IP) or 7 days after 5,7-DHT and head twitches were counted for 60 min immediately thereafter. Mean values ± SEM are shown.

supersensitivity of 5-HT_2 receptors was obtained (24). Head twitches were counted for 60 min immediately thereafter. An additional dose of 50 mg/kg was also injected 7 days after 5,7-DHT and head-twitch responses measured for 60 min thereafter. To determine direct effects of the methylxanthines on the postsynaptic neuron, we performed the following two experiments: a) The methylxanthines were given 7 days after 5,7-DHT and head twitches were counted for 10 min from 30–40 min after drug administration; and b) p-CPA was given 5 and 6 days after 5,7-DHT, the methylxanthines were administered 24 h after the second dose of p-CPA, and head-twitch responses were measured for 60 min.

Statistics

The Mann–Whitney U-test was used to determine differences between groups at the 5, 1, and 0.1% levels of significance.

RESULTS

Presynaptic Effects of the Methylxanthines

Caffeine, theophylline, theobromine, and pentoxifylline increased the 5-HTP-induced head-twitch responses in pargyline-pretreated mice; pentoxifylline was the most potent (Fig. 1). The potentiating effect of pentoxifylline was increased by paroxetine but those of the other three drugs were not.

Direct Effects of Caffeine

As shown in Fig. 2, caffeine at 100 mg/kg induced head twitches, which peaked at 3.2 ± 0.8 (mean ± SEM) during the observation period of 20–30 min after administration. Caffeine also exhibited a similar effect on head twitches in pargyline-pretreated mice. In 5,7-DHT-lesioned animals, the head twitches elicited by caffeine alone further increased to 9.7 ± 2.9 and 9.7 ± 3.4 during the observation periods of 20–30 and 30–40 min, respectively; the increase caused by 50 mg/kg was almost half that caused by 100 mg/kg, as shown in Table 1.

Direct Effects of the Methylxanthines on the Postsynaptic Neuron

As shown in Table 2, in both nontreated and 5,7-DHT-lesioned animals caffeine was more potent in inducing head-twitch responses than any other drugs tested. Theophylline, theobromine, and pentoxifylline had slight or almost no effect, and saline elicited no head twitches in 5,7-DHT-lesioned mice. The number of head twitches produced by caffeine in 5,7-DHT-treated mice (9.7 ± 2.9, Fig. 2) was increased twofold in 5,7-DHT + p-CPA-pretreated mice (17.7 ± 2.5, Fig. 3) when observed during the period of 20–30 min after caffeine administration. As illustrated in Fig. 3, theophylline induced 2.8 ± 0.8 head twitches during the period of 10–20 min. However, theobromine and pentoxifylline produced fewer responses (0–0.8 and 0.2–0.5, respectively) throughout the 60-min observation period.

DISCUSSION

There have been many reports indicating that methylxanthines have effects on the central serotonergic system, especially on the presynaptic neuron, such as synthesis, uptake, and/or turnover of 5-HT (1–4,11,16,21–24,28). In the present study, caffeine, theophylline, theobromine, and pentoxifylline at 100 mg/kg increased the number of 5-HTP-induced head-twitch responses in pargyline-pretreated mice, but none of the effects, except that of pentoxifylline, were further potentiated by paroxetine, the specific inhibitor of 5-HT reuptake, the binding site of which is known to be associated with the neuronal 5-HT transport complex (7,15). Our results confirmed earlier reports that caffeine, theophylline, and theobromine have some stimulating effects on central serotonergic neurons but also revealed that their effects on 5-HT reuptake were weak. Our findings with caffeine are in good agreement with

TABLE 1

ELICITING EFFECT OF CAFFEINE ON HEAD-TWITCH RESPONSES IN MICE TREATED WITH 5,7-DHT (n = 6 or 7)

	Time (min) After Drug Administration					
Dose (mg/kg)	0–10	10–20	20–30	30–40	40–50	50–60
50	1.7 ± 0.64	4.0 ± 0.53	5.1 ± 0.67	4.6 ± 0.61	4.6 ± 0.78	3.7 ± 0.49
100	3.0 ± 1.51	9.3 ± 2.87	9.7 ± 2.85	9.7 ±3.37	6.7 ± 2.12	5.8 ± 1.38

Caffeine was injected IP 7 days after 5,7-DHT (80 μg as the base, ICV), and head-twitch responses were measured immediately for 60 min thereafter. Values represent the mean ± SEM.

TABLE 2

ELICITING EFFECTS OF METHYLXANTHINES IN MICE TREATED WITH 5,7-DHT (n = 6)

Treatment	Without 5,7-DHT	With 5,7-DHT
Saline (control)	0.2 ± 0.17	0.0 ± 0.0
Caffeine	2.7 ± 0.76*	9.7 ± 3.37*
Theophylline	0.3 ± 0.21	1.5 ± 0.43*
Theobromine	0.9 ± 0.46	1.0 ± 0.52†
Pentoxifylline	0.1 ± 0.17	0.3 ± 0.21

The methylxanthines (100 mg/kg, IP) were administered to different groups of mice 7 days following 5,7-DHT treatment. The induced head twitches were counted for 10 min from 30–40 min after drug administration. Values represent the mean ± SEM.

*†Significantly different from control at $p < 0.01$ and 0.05, respectively.

the in vitro study of Reith et al. (28), who reported that caffeine inhibited 5-HT uptake only at concentrations above 10^{-4} M, indeed high. In nontreated mice, caffeine alone induced head-twitch responses, which were further increased by 5,7-DHT pretreatment, but not by pargyline, the monoamine oxidase inhibitor, which presumably promoted the accumulation of 5-HT released from presynaptic neurons. Berkowitz et al. (2) reported that the toxicity of caffeine, theophylline, and theobromine was increased by the monoamine oxidase inhibitors in rats. The discrepancy between our and their results might be dependent upon animals used, but further investigation should be necessary for the precise explanation.

Our study results suggest that caffeine exerted its stimulating effect on serotonergic neuronal function by acting directly on the postsynaptic more than on the presynaptic neuron. This conclusion is based upon the facts that: a) Intrathecal administration of 5,7-DHT specifically decreases brain levels of 5-HT (9,10,12,15,19,20,27,30); b) 5,7-DHT promotes the development of supersensitivity of postsynaptic 5-HT_2 receptors (8,9,12,19,24,26,27); and c) the function of postsynaptic 5-HT_2 receptors is closely related to the head-twitch response in mice (12–14,19).

When the effects of the four methylxanthines were compared in nontreated and 5,7-DHT-lesioned mice during the time period of 30–40 min after drug administration, caffeine was found to be the most potent in eliciting head-twitch responses in both groups of animals. Theophylline, theobromine, and pentoxifylline showed weak or almost no effects. The supersensitivity of the serotonergic postsynaptic receptors is reportedly dependent upon the decreased contents of brain 5-HT (19). Therefore, p-CPA was concomitantly administered with 5,7-DHT to produce greater supersensitivity of the postsynaptic receptors and eliminate the presynaptic effect as much as possible. The number of head twitches produced by caffeine and theophylline in 5,7-DHT-lesioned mice was increased twofold by p-CPA. In animals given the combination of the two depletors, the effects of theobromine and pentoxifylline were not potentiated. These results suggest that: a) The methylxanthines had qualitatively different actions on the central serotonergic neuron; b) pentoxifylline inhibited 5-HT reuptake but the other three drugs did not; and c) caffeine and theophylline had direct effects on the postsynaptic neuron but theobromine and pentoxifylline did not.

There have been suggestions that the head-twitch response is caused by an imbalance between cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) (5) and is induced by treatments with many sub-

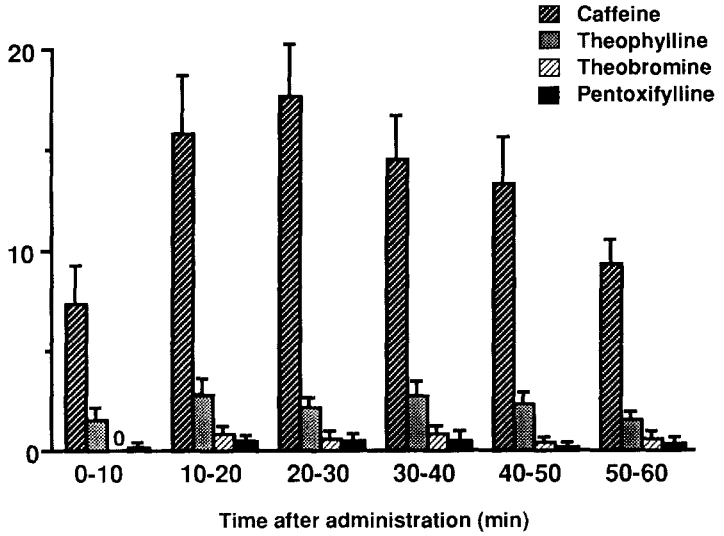

FIG. 3. Methylxanthine-induced head-twitch responses in mice treated with 5,7-dihydroxytryptamine (5,7-DHT) and p-chlorophenylalanine (p-CPA) (n = 5 or 6). p-CPA (200 mg, IP) was given 5 and 6 days after 5,7-DHT; the methylxanthines (100 mg/kg, IP) were administered 24 h after the second doses of p-CPA, and head-twitches responses were measured immediately for 60 min. Mean values ± SEM are shown.

stances such as β-adrenoceptor agonists and opioids (17). Methylxanthines have been reported to possess effects on adenosinergic neurons (6,25,29) and produce the inhibition of phosphodiesterase (PDE), resulting in accumulation of cAMP in the brain (18). Such data suggest that the head-twitch responses caused by caffeine and theophylline may be associated with the PDE-related mechanism in the brain. However, further studies are required to elucidate the mechanisms of the actions of the methylxanthines on the central serotonergic postsynaptic neuron because pentoxifylline, which has the same inhibitory effect on PDE as caffeine and theophylline, did not induce head twitches.

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