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BRIEF COMMUNICATION

Potentiation by Serotonergic Inhibition of Yawning Induced by Dopamine Receptor Agonists in Rats

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MATSUMOTO, S., K. YAMADA, M. NAGASHIMA, N. MATSUO, K. SHIRAKAWA AND T. FURUKAWA. *Potentiation by serotonergic inhibition of yawning induced by dopamine receptor agonists in rats.* PHARMACOL BIOCHEM BEHAV **32**(3) 815–818, 1989.—Low doses of the dopamine D_2-receptor agonist, B-HT 920 (25 μg/kg, SC), and the dopamine D_1/D_2-receptor agonists, apomorphine (50 μg/kg, SC) and piribedil (1 mg/kg, SC), evoked yawning. However, the dopamine D_1-receptor agonist, SK&F 38393 (2 mg/kg, SC), failed to induce yawning. The yawning responses induced by B-HT 920, apomorphine or piribedil were markedly increased without eliciting stereotypy by pretreatment with reserpine (5 mg/kg, IP, 24 hr). These yawning responses were also enhanced by p-chlorophenylalanine (PCPA) (300 mg/kg, IP, 72 hr), but not by α-methyl-p-tyrosine (300 mg/kg, IP, 6 hr). The yawning induced by B-HT 920 given alone and in combination with reserpine or PCPA was inhibited by spiperone (0.5 mg/kg, IP) or scopolamine (0.5 mg/kg, IP), but not by SCH 23390 (0.5 mg/kg, IP). The present results suggest that yawning is evoked by stimulation of dopamine D_2-receptors having a high affinity and consequent muscarinic activation, and that the yawning induced by dopamine receptor agonists is potentiated by decreases in serotonergic neuron activity.

Yawning Dopamine D_2-receptors B-HT 920 Apomorphine Piribedil SK&F 38393 Reserpine p-Chlorophenylalanine Serotonergic neuron

THERE is accumulated evidence that systemic administrations of low doses of dopamine receptor agonists, such as apomorphine, bromocriptine, piribedil and 3-PPP, elicit yawning in rats. The yawning behavior induced by dopamine receptor agonists is blocked by dopamine D_2-receptor antagonists (9, 17, 20, 22–24). On the basis of such findings, it has been proposed that dopamine D_2-receptor stimulation participates in the occurrence of yawning.

B-HT 920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo [4,5-d] azepine) has recently been characterized as a selective agonist at brain dopamine autoreceptors (1, 4, 11, 16). B-HT 920 also induced yawning behavior without eliciting stereotyped behavior in rats (8,12). The present authors (22) and Serra *et al.* (18) have previously reported that apomorphine-induced yawning behavior is enhanced by treatment with reserpine which reduces levels of catecholamine and serotonin in the brain.

The present study was therefore performed to investigate whether catecholaminergic and/or serotonergic neuron activities are related to the enhancement by reserpine of yawning evoked by dopamine receptor agonists.

METHOD

Animals

Male Wistar rats (200–230 g) were obtained from Kyudo Animal Laboratory (Kumamoto, Japan) and maintained in an animal room with a 12 hr light-dark cycle (lights on at 7:00 a.m.). Commercial food (CE-2, Clea Japan Ltd.) and tap water were freely available except during the time of the experiments. All experiments were carried out at an environmental temperature of 23 ± 1°C.

Behavioral Observation

Pairs of rats were placed in a transparent plastic box (33 ×

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30 × 17 cm) containing wood shavings and were allowed to habituate to the observation box for 15 min before injection of drugs. Yawning was counted for 60 min after injection as total number of mouth openings.

Administration of Drugs

Rats received injections of B-HT 920 (25 μg/kg, SC), apomorphine (50 μg/kg, SC), piribedil (1 mg/kg, SC) or SK&F 38393 (2 mg/kg, SC). To study drug effects on yawning, drugs were injected at the following times before yawning inducers: 30 min for saline, 6 hr for α-methyl-p-tyrosine (α-MT, 300 mg/kg, IP), 24 hr for reserpine (5 mg/kg, IP) and 72 hr for p-chlorophenylalanine (PCPA, 300 mg/kg, IP). For pretreatment with antagonists, spiperone (0.5 mg/kg, IP), SCH 23390 (0.5 mg/kg, IP) and scopolamine (0.5 mg/kg, IP) were injected 30 min before B-HT 920 (25 μg/kg, SC).

Drugs

The drugs used were apomorphine hydrochloride (Sandoz, Basel, Switzerland), piribedil monomethylsulfonate (ET-495, Servier Laboratories, Paris, France), 6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo [4,5-d] azepine (B-HT920) dihydrochloride (Boehringer Ingelheim, Kawanishi, Japan), 1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol (SK&F 38393) hydrochloride (R.B. Inc., Natick, USA), reserpine (Apoplon Injection, Daiichi, Tokyo, Japan), dl-p-chlorophenylalanine (Nakarai, Kyoto, Japan), dl-α-methyl-p-tyrosine (Nakarai, Kyoto, Japan), spiperone (Spiropitan Injection, Eisai, Tokyo, Japan), R(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol (SCH 23390) hydrochloride (R.B. Inc., Natick, USA) and scopolamine hydrobromide (Nakarai, Kyoto, Japan). PCPA and α-MT were administered as a suspension in 0.5% carboxymethylcellulose. The other drugs were dissolved or diluted in saline. These drugs were injected intraperitoneally (IP) or subcutaneously (SC) to experimental animals as mentioned above. Doses are expressed in terms of the salts except for PCPA, α-MT and spiperone.

Statistical Analysis

Yawning responses were expressed as the mean values. Statistical analysis was done using Kruskal-Wallis one-way analysis of variance and the two-tailed Mann-Whitney U-test (19).

RESULTS

As shown in Table 1, control rats treated subcutaneously with saline yawned only occasionally. Injections of B-HT 920 (25 μg/kg, SC), apomorphine (50 μg/kg, SC) or piribedil (1 mg/kg, SC) induced yawning in the saline-pretreated rats. However, stereotyped behaviors, such as licking and biting, were not evoked. The yawning elicited by the dopamine receptor agonists was markedly increased by 24 hr pretreatment with reserpine (5 mg/kg, IP), but stereotyped behaviors were not yet induced. The yawning was also enhanced after treatment with PCPA (300 mg/kg, IP, 72 hr), but was not affected after α-MT (300 mg/kg, IP, 6 hr). SK&F 38393 (2 mg/kg, SC), alone or even in combination with reserpine or PCPA, was not able to evoke yawning. Reserpine, PCPA or α-MT given alone did not elicit yawning.

As demonstrated in Table 2, the yawning induced by B-HT 920 was markedly inhibited by treatment with spiperone (0.5 mg/kg, IP) or scopolamine (0.5 mg/kg, IP), but not by SCH 23390 (0.5 mg/kg, IP). Moreover, the potentiation of yawning produced by B-HT 920 in combination with reserpine or PCPA was inhibited by spiperone or scopolamine. On the other hand, the yawning was not significantly affected by SCH 23390. These drugs when given alone at these doses did not elicit general motor debilitation.

TABLE 1

EFFECTS OF RESERPINE, p-CHLOROPHENYLALANINE AND α-METHYL-p-TYROSINE ON THE YAWNING INDUCED BY DOPAMINE RECEPTOR AGONISTS

Pretreatment (mg/kg)	Yawns in 60 Min				
	Saline	B-HT 920	Apomorphine	Piribedil	SK&F 38393
Saline	0.3 ± 0.2	10.6 ± 1.8*	5.2 ± 1.2*	8.0 ± 1.6*	0.3 ± 0.2
Reserpine (5)	1.1 ± 0.5	31.2 ± 2.8‡	14.3 ± 1.5‡	17.9 ± 3.3†	0.8 ± 0.3
PCPA (300)	0.1 ± 0.1	21.1 ± 2.1‡	12.8 ± 2.1‡	17.1 ± 2.3†	1.3 ± 0.7
α-MT (300)	0.3 ± 0.3	12.1 ± 1.8	4.3 ± 1.2	4.0 ± 1.8	0.3 ± 0.2

The number of yawns was counted immediately after the injection of B-HT 920 (25 μg/kg, SC), apomorphine (50 μg/kg, SC), piribedil (1 mg/kg, SC) or SK&F 38393 (2 mg/kg, SC).

Intraperitoneal pretreatment with drugs was done at respective times before yawning inducers: 30 min for saline, 24 hr for reserpine (5 mg/kg), 72 hr for PCPA (300 mg/kg) and 6 hr for α-MT (300 mg/kg).

Each value represents the mean ± S.E. of numbers of yawns from 8–10 rats.

*$p<0.01$; Significant difference from the saline control group.

†$p<0.05$, ‡$p<0.01$; Significant difference from the respective saline-pretreated group.

DISCUSSION

The present experiment has confirmed that the mixed dopamine D_1/D_2-receptor agonists, apomorphine and piribedil, induce yawning behavior in rats but the selective dopamine D_1-receptor agonist, SK&F 38393, is inactive. It is also observed that the dopamine autoreceptor agonist, B-HT 920, induced yawning behavior. The yawning produced by B-HT 920 was inhibited by the selective dopamine D_2-receptor antagonist, spiperone at a dose of 0.5 mg/kg, or the muscarinic receptor antagonist, scopolamine at a dose of 0.5 mg/kg, but was unaffected by the selective dopamine

TABLE 2

EFFECTS OF ANTAGONISTS ON THE YAWNING INDUCED BY B-HT 920 ALONE AND IN COMBINATION WITH RESERPINE OR PCPA

Antagonists (mg/kg)	Yawns in 60 Min		
	Saline + B-HT	Reserpine + B-HT	PCPA + B-HT
Saline	10.5 ± 1.7	19.9 ± 2.0†	19.2 ± 2.6†
Spiperone (0.5)	0.1 ± 0.1*	2.8 ± 1.0*	1.4 ± 0.7*
SCH 23390 (0.5)	9.1 ± 1.0	13.7 ± 2.2	15.9 ± 2.4
Scopolamine (0.5)	0.9 ± 0.3*	7.5 ± 1.3*	2.9 ± 1.0*

Antagonists were treated intraperitoneally 30 min before B-HT 920 (25 μg/kg, SC).

The number of yawns was counted immediately after the injection of B-HT 920 (25 μg/kg, SC).

Each value represents the mean ± S.E. of numbers of yawns from 8–10 rats.

†$p<0.05$; Significant difference from the saline plus B-HT group.

*$p<0.01$; Significant difference from the respective saline-treated group.

D_1-receptor antagonist, SCH 23390 at a dose of 0.5 mg/kg. The doses of these receptor antagonists were selected according to the other experiment (23) in which the yawning elicited by apomorphine or piribedil was inhibited by spiperone and scopolamine, but not by SCH 23390, while that induced by physostigmine was inhibited by scopolamine, but not by spiperone and SCH 23390, at above-mentioned doses. At these doses, the antagonists did not induce motor debilitation. Therefore, these receptor antagonists at the doses used are producing their inhibitory effects on B-HT 920-induced yawning behavior in a pharmacologically relevant manner, and are not exerting their antagonistic actions through general motor debilitation. Thus, the antagonisms by the receptor antagonists for B-HT 920-induced yawning coincide with those for apomorphine-elicited yawning (9, 20–24). Accordingly, it is now well acceptable that yawning involves dopamine D_2-receptor stimulation and consequent muscarinic activation.

Serra *et al.* (18) reported that a significant increase in the number of yawns was observed in rats 24 hr, but not 1, 6 and 12 hr, after treatment with reserpine (5 mg/kg, IP). This reserpine-induced yawning was antagonized by the dopamine D_2-receptor antagonist, sulpiride, and by the catecholamine synthesis inhibitor, α-MT (16), suggesting that this behavior may be induced by endogenously released dopamine. The treatment with reserpine for 24 hr also potentiated the yawning induced by the dopamine D_2-receptor agonist, (+)-3-PPP. From these results, they (18) have proposed that yawning behavior is due to the stimulation of a population of dopamine receptors having a high affinity for dopamine receptor agonists similar to that of dopamine autoreceptors but located postsynaptically. On the other hand, Longoni *et al.* (12) observed that the yawning responses to dopamine D_2-receptor agonists, such as B-HT 920 and (+)-3-PPP, were reduced by 6 hr treatment with reserpine. The yawning induced by these dopamine receptor agonists was completely abolished by sulpiride and was also slightly but significantly reduced by SCH 23390 (12). Thus, it was proposed that stimulation of D_1-receptors by endogenously released dopamine plays a permissive-facilitatory role for the behavioral expression of dopamine D_2-receptor activation. In the present experiments, the yawning responses to B-HT 920, apomorphine and piribedil were enhanced by 24 hr treatment with reserpine (5 mg/kg, IP). However, SK&F 38393 failed to induce yawning even after reserpine. Furthermore, the yawning elicited by B-HT 920 in combination with reserpine was markedly inhibited by spiperone or scopolamine, but was not significantly reduced by SCH 23390. Accordingly, although dopamine released endogenously is proposed to play at least in part a facilitatory role, the stimulation of dopamine D_1-receptors may not be an essential factor in the occurrence of yawning.

Interestingly, the dopamine receptor agonist-induced yawning was also increased by the serotonin synthesis inhibitor, PCPA, but was not influenced by α-MT, implying that depletion of serotonin plays a more important role than that of catecholamines in potentiation of yawning. The yawning evoked by combined administration of B-HT 920 and PCPA was completely inhibited following spiperone or scopolamine. Most recently, it was reported that apomorphine-elicited yawning was enhanced by pretreatment with PCPA or the serotonergic neurotoxin, 5,7-dihydroxytryptamine, and was contrarily reduced by pretreatment with the serotonin precursor, 5-hydroxytryptophan (14). In fact, serotonin is present in relatively high concentrations in the rat striatum (2,3), where is proposed to be one of the sites of action of dopamine receptor agonists in the occurrence of yawning (6, 14, 24). Various lines of evidence have shown that the origin of serotonergic neurons in the striatum is the dorsal raphe (13), and that there are inhibitory serotonin receptors located on terminals of dopaminergic neurons in the striatum (5, 7, 15). Lesioning of the raphe nucleus which reduces serotonin levels in the forebrain has been reported to cause an increase of dopamine release (10). Therefore, treatment with PCPA may increase the release of dopamine, which is proposed to play a facilitatory role in the occurrence of yawning, though more work is clearly warranted to clarify the nature of the observed serotonergic-dopaminergic neuron interaction.

From the results, it is assumed that the occurrence of yawning following dopamine receptor agonists involves stimulation of dopamine D_2-receptors having a high affinity and consequent muscarinic activation, and that the potentiation by reserpine or PCPA of yawning induced by dopamine receptor agonists is due to decreases in serotonergic neuron activity.

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