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Behavioral Effects of Dilazep on Cholinergic, Dopaminergic, and Purinergic Systems in the Rat

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USHIJIMA, I., Y. MIZUKI, T. UKITA, H. KANEYUKI, S. INANO AND M. YAMADA. *Behavioral effects of dilazep on cholinergic, dopaminergic, and purinergic systems in the rat.* PHARMACOL BIOCHEM BEHAV 43(3) 673–676, 1992.—This study examined the effects of 1,4-*bis*[3-(3,4,5-trimethoxy benzoyloxy)-propyl] perhydro-1,4-diazepine (dilazep; Comelian) on central dopaminergic, cholinergic, and purinergic neuronal systems in rats. Intraperitoneal injections of dilazep (1–5 mg/kg) produced yawning responses, the most effective dose being 2 mg/kg. Dilazep potentiated physostigmine-induced yawning but not pilocarpine- and bromocriptine-induced yawning. Dilazep-induced yawning was not affected by low doses of haloperidol or sulpiride, but was completely inhibited by atropine, a muscarinic M_1 receptor antagonist. Dilazep-induced yawning, as well as physostigmine-induced yawning, were markedly inhibited by pretreatment with SK & F 38393, a dopamine D_1 receptor agonist, and were potentiated by SCH23390, a dopamine D_1 receptor antagonist that alone does not elicit yawning. Caffeine, an adenosine receptor antagonist, inhibited dilazep- and physostigmine-induced yawning responses but N^6-cyclohexyl adenosine (CHA) and N^6-(L-phenylisopropyl, adenosine (L-PIA), adenosine A_1 receptor agonists, were inactive. These results suggest that because the effects of dilazep on central cholinergic neurons are similar to those of physostigmine dilazep may potentiate indirectly the action of endogenous acetylcholine. Cholinergic neurons activated by dilazep may be modulated by postsynaptic dopamine D_1 receptor activity but may not be affected by dopamine D_2 receptor activity. Furthermore, the stimulatory effects of dilazep on cholinergic neuron may not be due to an inhibition of dopamine D_1 receptors via purinergic (adenosine A_1 receptor) stimulation by dilazep.

Dilazep Yawning Cholinergic system Dopaminergic system Purinergic system

DILAZEP (Comelian; 1,4-*bis*[3-(3,4,5-trimethoxy benzoyloxy)-propyl]perhydro-1,4-diazepine) has been proposed to increase coronary blood flow and potentiate the dilating effect of adenosine on coronary vessels (6,8,9). Because dilazep also has a dilating effect on central vessels, probably via inhibiting adenosine uptake (2), the drug has been proposed as a valuable therapeutic agent in cerebrovascular dementia. Dilazep and N^6-(L-phenylisopropyl) adenosine (L-PIA: adenosine A_1 receptor agonist) inhibit aggressive behaviors induced by high doses of clonidine, which antagonizes adenosine A_1 receptors (15). On the other hand, the memory dysfunction associated with senile dementia of the Alzheimer type has been associated with a deficiency and loss of cholinergic neurons (3). An adenosine receptor antagonist, theophylline, inhibits physostigmine-induced yawning behaviors, which result in activation of central cholinergic neurons (23).

Yawning behaviors induced by physostigmine, a cholinesterase inhibitor, and pilocarpine, a direct acetylcholine receptor agonist, in rats are essentially mediated through the stimulation of central muscarinic receptors (14,20) but not nicotinic receptors (18). On the other hand, yawning can also be elicited by apomorphine, a D_1/D_2 agonist, and bromocriptine, a D_2 agonist, which have been reported to be mediated by cholinergic activation secondary to the inhibition of dopamine transmission (16,17,20,21). This behavior may be mediated by septal-striatal and hypothalamic D_2 receptor activation (1,4,22). In the present study, we found that yawning was induced by IP injection of dilazep to rats. These results were examined more closely to clarify whether the behavioral effects of dilazep involve central cholinergic and dopaminergic or purinergic systems.

METHOD

Animals

Male Wistar rats weighing 280–320 g were housed at constant room temperature (23 ± 1°C) and humidity (50 ± 10%) and allowed free access to standard chow (solid diet,

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MF, Oriental, Japan) and water. The animal colony was maintained on a 12 L : 12 D cycle with light on at 0700 h.

Behavioral Measurement

Yawning behavior is characterized by a slow, wide opening of the mouth. Pairs of rats were placed in transparent plastic boxes (35 × 30 × 17 cm) containing wood shavings. Yawning responses were counted every 10 min for 60 min after injection of dilazep or saline as control. Further details regarding the expression of drug effects are given in tables.

Administration of Drugs

To examine the effects of dilazep on physostigmine-, pilocarpine-, and bromocriptine-induced yawning, we administered dilazep (1.0–5.0 mg/kg, IP) 15 min before saline, physostigmine (0.2 mg/kg, IP), pilocarpine (4.0 mg/kg, IP), and bromocriptine (5.0 mg/kg, IP).

To examine the effects of dopaminergic and cholinergic antagonists on dilazep- and bromocriptine-induced yawning, we administered dilazep (2.0 mg/kg, IP) and bromocriptine (5.0 mg/kg, IP) 15 min after haloperidol (0.02 mg/kg, IP) and sulpiride (20 mg/kg, IP) and 30 min after atropine (5.0 mg/kg, IP). To examine the dopaminergic and purinergic effects on dilazep-, physostigmine-, and pilocarpine-induced yawning, we injected SK&F38393 (1.0 mg/kg, IP), SCH23390 (0.05 mg/kg, IP), caffeine (1.0 mg/kg, IP), L-PIA (0.1 and 0.2 mg/kg, IP), and CHA (0.2 mg/kg, IP) 15 min before dilazep (2.0 mg/kg, IP), physostigmine (0.2 mg/kg, IP), and pilocarpine (4.0 mg/kg, IP).

Drugs

Drugs used were dilazep (Kowa, Tokyo), physostigmine HCl (Wakoh, Tokyo), pilocarpine sulfate (Wakoh, Tokyo), atropine sulfate (E. Merck, Germany), haloperidol HCl (Dainippon, Tokyo), sulpiride (Sigma Chemical Co., St. Louis, MO), bromocriptine mesylate (Sandoz, A.G.), SK&F38393 HCl [Research Biochemicals, Inc. (RBI), Natick, MA], SCH23390 HCl (RBI), N^6-cyclohexyl adenosine (CHA; Boehringer-Mannheim, Mannheim), L-PIA (RBI), and caffeine (Nakarai, Kyoto). SK&F38393 HCl, SCH23390 HCl, and CHA were dissolved in ethanol (0.05 ml) and subsequently diluted by saline (10 ml). Bromocriptine and sulpiride were suspended in 3% Tween-80 solution, and other drugs were dissolved in saline. Drugs were injected IP in a volume of 1 ml/kg. Doses are expressed in terms of the salt.

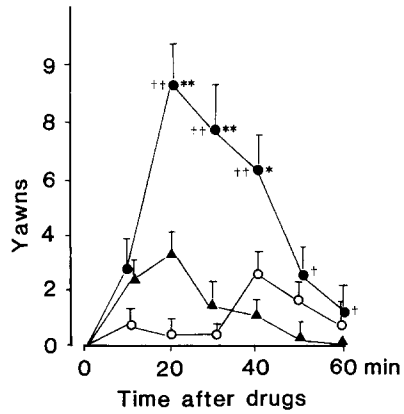

FIG. 1. Time course of yawning responses induced by dilazep, physostigmine, and dilazep plus physostigmine. Dilazep 2.0 mg/kg IP (○), physostigmine 0.2 mg/kg IP (▲), dilazep 2.0 mg/kg IP + physostigmine 0.2 mg/kg IP (●). Each point indicates mean value ± SE of yawns observed every 10 min after drug injection for 60 min in six to nine rats. *†$p < 0.05$, **††, $p < 0.02$, significant difference from dilazep-injected group (*,**) and physostigmine-injected group (†,††), determined by the Mann-Whitney *U*-test.

Statistical Analysis

Yawning responses are expressed as mean values. Statistical analysis was carried out using the two-tailed Mann-Whitney *U*-test.

RESULTS

Yawning Responses Induced by Dilazep

Dilazep at doses ranging from 1.0–5.0 mg/kg elicited yawning, accompanied by hypomotility, in rats. The maximal effect was observed at 2.0 mg/kg 30–40 min after injection of the drug (Table 1, Fig. 1).

Effects of Different Doses of Dilazep on Physostigmine-, Pilocarpine-, and Bromocriptine-Induced Yawning

Dilazep potentiated physostigmine (0.2 mg/kg, IP)-induced yawning (physostigmine yawning), additively increased bromocriptine (5.0 mg/kg, IP)-induced yawning (bromocriptine yawning), and did not affect pilocarpine (4.0

TABLE 1

EFFECTS OF DILAZEP ON YAWNING RESPONSES INDUCED BY PHYSOSTIGMINE, PILOCARPINE, OR BROMOCRIPTINE

		Yawns in 60 min		
Drugs (mg/kg)	Saline (1 ml/kg)	Physostigmine (0.2 mg/kg)	Pilocarpine (4.0 mg/kg)	Bromocriptine (5.0 mg/kg)
Control	0.0	8.6	9.7	13.0
Dilazep (1.0)	1.8*	18.8*	11.3	15.0
Dilazep (2.0)	6.0†	26.6†	8.5	20.3
Dilazep (5.0)	2.0†	20.7†	10.3	16.2

Physostigmine, pilocarpine, bromocriptine, and saline were administered immediately after dilazep (1.0–5.0 mg/kg, IP).

*$p < 0.05$, †$p < 0.02$, significant difference from the control group, determined by analysis of variance and subsequent Mann-Whitney *U*-tests (n = 6–9).

TABLE 2

EFFECTS OF VARIOUS DRUGS ON DILAZEP-INDUCED YAWNING

Drugs (mg/kg)	Yawns in 60 min		
	Saline (1 ml/kg)	Dilazep (2.0 mg/kg)	Bromocriptine (5 mg/kg)
Control	0.0	6.0	18.0
Haloperidol (0.02)	0.0	4.8	0.0*
(-)-Sulpiride (20.0)	0.0	5.2	0.0*
Atropine (5.0)	0.0	0.8*	0.5*

Dilazep (2.0 mg/kg, IP) was injected 15 min after haloperidol, (-)-sulpiride, and apomorphine and 30 min after atropine.

*$p < 0.02$, significant difference from the control group (n = 6-9).

mg/kg, IP)-induced yawning (pilocarpine yawning) (Table 1, Fig. 1).

Effects of Dopaminergic and Cholinergic Drugs on Dilazep Yawning and Bromocriptine Yawning

Dilazep (2.0 mg/kg, IP)-induced yawning (dilazep yawning) was not affected by pretreatment with a low dose of haloperidol (0.02 mg/kg, IP) and (-)-sulpiride (20 mg/kg, IP), but was completely blocked by atropine (5.0 mg/kg, IP). Bromocriptine yawning was completely blocked by either haloperidol (0.02 mg/kg IP), (-)-sulpiride (20 mg/kg, IP), or atropine (5 mg/kg, IP) (Table 2).

Effects of Dopaminergic and Purinergic Systems on Dilazep-, Physostigmine-, or Pilocarpine-Induced Yawning

Pretreatment with SK&F38393 (1.0 mg/kg, IP) significantly inhibited dilazep and physostigmine yawning but did not affect pilocarpine yawning, whereas pretreatment with SCH23390 (0.05 mg/kg, IP) potentiated dilazep and physostigmine yawning but did not affect pilocarpine yawning. Caffeine inhibited dilazep and physostigmine yawning, but CHA (0.2 mg/kg, IP) and L-PIA (0.1 and 0.2 mg/kg, IP) were without effect. SCH23390, CHA, or L-PIA alone did not induce yawning responses (Table 3).

DISCUSSION

In this study, dilazep alone induced yawning and potentiated physostigmine yawning but did not affect pilocarpine and bromocriptine yawning. There was a bell-shaped dose-response curve for the effects of dilazep on yawning. This suggests that dilazep yawning may involve an indirect activation of cholinergic neurons, probably by releasing acetylcholine and/or inhibiting acetylcholinesterase. Because dilazep yawning was not affected by (-)-sulpiride, a D_2 antagonist, and by a low dose of haloperidol that preferentially inhibits presynaptic dopamine receptors (D_2 receptors) but was completely blocked by atropine, a muscarinic receptor antagonist, dilazep yawning appears unlikely to be due to cholinergic neuronal activation secondary to the activation of presynaptic dopamine D_2 receptors.

There is some evidence that physostigmine yawning is inhibited by SK&F38393, a D_1 agonist (17) but is potentiated by SCH23390, a D_1 antagonist (23), whereas pilocarpine yawning is not affected by SK&F38393 (17), suggesting that activation of D_1 receptors may inhibit cholinergic neurons activated by cholinesterase inhibition, that is, endogenous acetylcholine, but may not affect the stimulation of cholinergic neurons to a direct acetylcholine agonist (muscarinic M_1 receptor agonist). In this study, the effects of SK&F38393 and SCH23390 on dilazep yawning were compatible with those observed on physostigmine yawning (17,23). Pretreatment with SK&F38393 markedly inhibited dilazep yawning and physostigmine yawning whereas SCH23390 potentiated them. Pilocarpine yawning was unaffected by either SK&F38393 or SCH23390. From these results, it is suggested that dilazep as well as physostigmine yawning may be mediated by dopamine D_1 receptor activity. The inhibitory effects of high doses of apomorphine on dilazep and physostigmine yawning appear to be due to the activation of dopamine D_1 receptors. D_1 and D_2 dopamine receptors that exist in the striatum stimulate and inhibit, respectively, the formation of striatal cyclic adenosine monophosphate (7,12,13). The striatum contains the highest concentration of acetylcholine in the brain (10) and dopamine receptors have a regulatory role on striatal acetylcholine (11).

TABLE 3

EFFECTS OF DOPAMINERGIC AND PURINERGIC DRUGS ON YAWNING RESPONSES INDUCED BY DILAZEP, PHYSOSTIGMINE, AND PILOCARPINE

Drugs (mg/kg)	Yawns in 60 min			
	Saline (1 ml/kg)	Dilazep (2.0 mg/kg)	Physostigmine (0.2 mg/kg)	Pilocarpine (4.0 mg/kg)
Control	0.0	6.0	8.6	9.7
SK&F38393 (1.0)	0.1	1.3*	3.5*	8.3
SCH23390 (0.05)	0.2	13.5*	18.3*	7.5
L-PIA (0.1)	0.0	7.2	8.8	8.2
L-PIA (0.2)	0.0	8.5	9.4	9.5
CHA (0.2)	0.0	8.8	9.2	10.1
Caffeine (1.0)	0.0	0.3†	0.5†	8.9

Dilazep, physostigmine pilocarpine, and saline were administered 15 min after SK&F38393, SCH23390, CHA, caffeine, or saline. L-PIA, N^6-(L-phenylisopropyl) adenosine; CHA, N^6-cyclohexyladenosine.

*$p < 0.05$, †$p < 0.02$, significant difference from the control group (n = 6-9).

The opposing effects of D_1 and D_2 receptors on striatal cholinergic neurons have also been shown in biochemical studies (5).

On the other hand, dilazep has an adenosine action as mentioned in the introductory section. Adenosine receptors have also been classified into two different types, called A_1 and A_2 receptors on the basis of inhibitory and stimulatory effects, respectively, of adenosine on rat brain adenylate cyclase activity (19). An adenosine A_1/A_2 receptor antagonist, theophylline, inhibits physostigmine yawning (23). In this study, the adenosine A_1/A_2 receptor antagonist, caffeine, also inhibited dilazep as well as physostigmine yawning. However, only dilazep and not L-PIA or CHA (adenosine A_1 receptor agonists) potentiated physostigmine yawning. These results suggest that the stimulatory effect of dilazep on physostigmine yawning may not be due to an inhibition of dopamine D_1 receptors via stimulation of adenosine A_1 receptors but rather may be due to an activation of cholinergic neuron by endogenous acetylcholine. The mode of action of dilazep is similar to that of physostigmine, which indirectly stimulates cholinergic neurons.

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