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Muscarinic and Nicotinic Effects on Yawning and Tongue Protruding in the Rat

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USHIJIMA, I., K. YAMADA, T. INOUE, T. TOKUNAGA, T. FURUKAWA AND Y. NODA. *Muscarinic and nicotinic effects on yawning and tongue protruding in the rat.* PHARMACOL BIOCHEM BEHAV 21(2) 297–300, 1984.—Physostigmine, an anticholinesterase agent, elicited yawning with a marked protrusion of the tongue and teeth chattering. Yawning and chattering were also observed after pilocarpine, a cholinergic agonist predominantly acting upon muscarinic receptors. Apomorphine at low doses (0.1–0.5 mg/kg), which preferentially activates presynaptic dopamine autoreceptors, elicited yawning, whereas at high doses (1–2 mg/kg) it produced stereotypy. Yawning induced by both cholinergic agonists and apomorphine was inhibited by scopolamine, a muscarinic receptor blocking agent, but not by methylscopolamine, a peripheral anticholinergic agent and mecamylamine, a nicotinic receptor blocking agent. Low dose (0.02 mg/kg) of haloperidol, which has been reported to block presynaptic dopamine autoreceptors, inhibited apomorphine-induced yawning but did not affect cholinergic agonist-induced yawning. Physostigmine-elicited tongue protruding was inhibited by mecamylamine. The results imply that yawning behavior is essentially associated with the stimulation of central muscarinic receptors, and that physostigmine also induces tongue protruding by activating the central nicotinic receptors.

Yawning Tongue protruding Physostigmine Pilocarpine Apomorphine Muscarinic receptors Nicotinic receptors Dopamine receptors

IT is known that a characteristic stretching-yawning syndrome appears after intracerebral injection of α-melanocyte-stimulating hormone (α-MSH) or a chemically related peptide, adrenocorticotrophic hormone (ACTH) in many animals [4, 5, 12, 15]. The stretching-yawning syndrome elicited by α-MSH or ACTH is inhibited by the treatment with cholinergic and dopaminergic antagonists [4,15], suggesting a possible involvement of cholinergic and dopaminergic neurons. Moreover, Wood *et al.* [12] have proposed that the septal-hippocampal cholinergic neurons are necessary to elicit a specific stretching-yawning syndrome following α-MSH or ACTH since intraventricular injection of α-MSH or ACTH increases the turnover rate of acetylcholine in the hippocampus of rats. On the other hand, cholinergic agonists have also been reported to induce yawning in infant and adult rats [7, 11, 13, 15]. Our previous results [13–15] show that intraperitoneal injections of apomorphine at low doses, which inhibit dopamine release from presynaptic sites, induced yawning in adult rats and a dopaminergic-cholinergic neuron link was involved in yawning behavior.

In the present experiments, we noticed yawning accompanied by a marked protrusion of the tongue or teeth chattering following physostigmine and pilocarpine, and investigated influences of muscarinic and nicotinic cholinergic agents on these symptoms. Furthermore, we studied effects of haloperidol at a low dose on yawning behavior.

METHOD

Animals

Male Wistar rats (250–300 g) were obtained from Kyudo Animal Laboratory (Kumamoto, Japan) and maintained in an animal room with a 12 hr light-dark cycle (7:00 a.m.–7:00 p.m.). Commercial food (MF, Oriental Yeast Ltd.) and tap water were available ad lib 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 (33×30×17 cm) containing wood shavings. Yawns were counted for 90 min after injection of drugs as total number of mouth openings. Tongue protrudings were counted as numbers of tongue protrusions when rats yawn. Stereotypy was rated according to the scale of Costall and Naylor [2]: (0) normal behavior; (1) exploratory activity, discontinuous

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FIG. 1. The yawning with tongue protruding induced by physostigmine, 0.2 mg/kg, IP, in a male rat.

sniffing; (2) continuous sniffing; (3) continuous sniffing, discontinuous licking, biting or gnawing; (4) continuous licking, biting or gnawing. Chatters were calculated as percent incidence of rats which continuously chattered for over one min.

Administration of Drugs

For dose-related analyses of behaviors, apomorphine (0.1–2 mg/kg), physostigmine (0.1–0.8 mg/kg) and pilocarpine (1–8 mg/kg) were injected intraperitoneally. For the time course study, apomorphine (0.25 and 2 mg/kg) physostigmine (0.2 and 0.8 mg/kg) and pilocarpine (4 and 8 mg/kg) were injected. To examine influences of drugs on yawning, scopolamine (0.5 mg/kg), methylscopolamine (0.5 mg/kg), mecamylamine (0.05–0.2 mg/kg) and haloperidol (0.02 and 0.2 mg/kg) were injected intraperitoneally 30 min before apomorphine (0.25 mg/kg), physostigmine (0.2 mg/kg) or pilocarpine (4 mg/kg). To study drug effects on chattering, scopolamine (0.5 mg/kg) was injected 30 min before physostigmine (0.8 mg/kg) or pilocarpine (8 mg/kg). Animals receiving injection of saline served as controls. Individual animals received only a single drug treatment except for drug-pretreatment groups.

Drugs

The drugs used were apomorphine hydrochloride (Sandoz AG, Basel), physostigmine sulfate (Wakoh Chemicals), pilocarpine hydrochloride (Wakoh Chemicals), haloperidol (Serenace Injection, Dainippon Pharmaceutical), scopolamine hydrobromide (Wakoh Chemicals), scopolamine methylbromide and mecamylamine hydrochloride (Meiji Chemicals). These drugs were dissolved or diluted in saline, and injected intraperitoneally (IP) into experimental animals. Doses are expressed in terms of the salts except for haloperidol.

Statistical Analysis

Yawning and tongue protruding responses were expressed as the mean values. Statistical analysis was done using the two-tailed Mann-Whitney U-test. The incidence of chattering was statistically evaluated by means of 2×2 contingency table and the Fisher exact probability test [10].

RESULTS

Dose-Responses of Yawning, Chattering and Stereotypy to Physostigmine, Pilocarpine and Apomorphine

As shown in Figs. 1 and 2, physostigmine at doses ranging from 0.1–0.8 mg/kg elicited yawning with a marked protrusion of the tongue in the rat. Frequency of yawning was most pronounced at a dose of 0.2 mg/kg. Pilocarpine (1–8 mg/kg) also induced yawning with the highest frequency at 4 mg/kg. At high doses, both drugs elicited teeth chattering in a dose-dependent manner. The chattering was most marked at 0.8 mg/kg for physostigmine and at 8 mg/kg for pilocarpine.

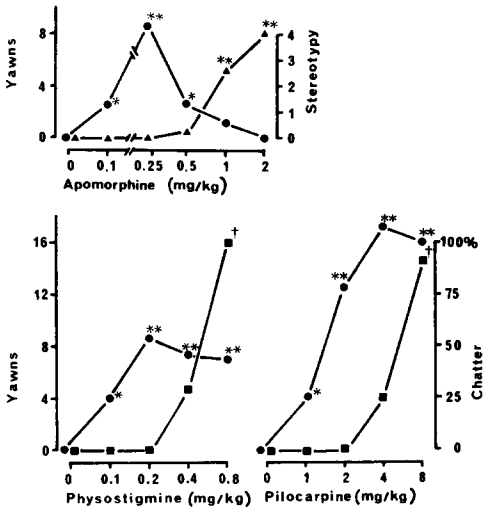

FIG. 2. Dose-responses of yawning, chattering and stereotypy to physostigmine, pilocarpine and apomorphine. Each point represents mean number of yawns, percent incidence of chattering during 90 min, or stereotypy scores at 20 min after respective drugs. ●—● Yawning, ■—■ Chattering, ▲—▲ Stereotypy. $*p<0.05$, $**p<0.02$; significant difference from the saline group, determined by the Mann-Whitney U-test, $\dagger p<0.01$ by the Fisher exact probability test (N=8).

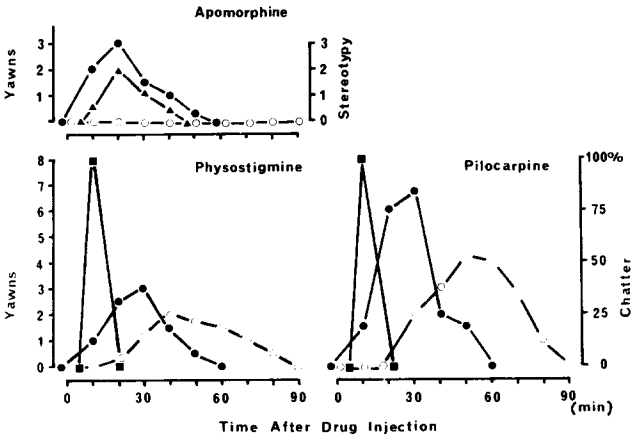

FIG. 3. Time course of yawning, chattering and stereotypy induced by different doses of physostigmine, pilocarpine and apomorphine. ●—● Yawning induced by lower doses of physostigmine (0.2 mg/kg), pilocarpine (4 mg/kg) and apomorphine (0.25 mg/kg). ○—○ Yawning induced by higher doses of physostigmine (0.8 mg/kg), pilocarpine (8 mg/kg) and apomorphine (2 mg/kg). ■—■ Chattering induced by physostigmine (0.8 mg/kg) and pilocarpine (8 mg/kg), and ▲—▲ Stereotypy induced by apomorphine (2 mg/kg). Each point indicates mean value of yawns, percent incidence of chattering or stereotypy scores observed every 10 min after drug injection for 90 min in 8 rats.

Yawning and chattering did not occur simultaneously in a rat.

Apomorphine-evoked yawning (Fig. 2) exhibited a bell-shape dose response curve with the most pronounced effect occurring at 0.25 mg/kg. At high doses apomorphine induced dose-dependent stereotypy which is characterized by slight sniffing at 0.5 mg/kg and continuous licking and biting without yawning at doses over 2.0 mg/kg. The yawning and stereotypy did not appear simultaneously in a rat.

Time-Course of Yawning, Chattering and Stereotypy

The results are depicted in Fig. 3. After lower doses of physostigmine (0.2 mg/kg) and pilocarpine (4 mg/kg), yawning began at about 10 min, was marked after 20–30 min and almost terminated within 60 min, while chattering did not occur. At higher doses of physostigmine (0.8 mg/kg) and pilocarpine (8 mg/kg), chattering appeared immediately after administration, reached maximum 10 min later and lasted for 10 min, while yawning occurred at about 20 min, reached maximum 40–60 min later and ceased within 90 min after administration.

After apomorphine (0.25 mg/kg) yawning began within 5 min, reached a maximum after 10–20 min and usually ceased within 60 min, while stereotypy did not appear. After a dose of 2 mg/kg, stereotypy began within 10 min, was most marked after 20 min and usually disappeared within 60 min, whereas yawning did not occur. However, the onset and time-course of yawning and stereotypy were about the same at different doses of apomorphine.

Effects of Various Drugs on Yawning, Tongue Protruding and Chattering

As demonstrated in Table 1, the yawning produced by three inducers was inhibited by pretreatment with scopolamine (0.5 mg/kg), but not by methylscopolamine (0.5 mg/kg). After treatment with haloperidol (0.02 and 0.2 mg/kg), the yawning produced by apomorphine was inhibited but that by physostigmine and pilocarpine was unaffected. The tongue protruding induced by physostigmine was inhibited by pretreatment with scopolamine (0.5 mg/kg) and mecamylamine (0.05–0.2 mg/kg) (Table 1). On the other hand, the chattering induced by physostigmine (0.8 mg/kg) and pilocarpine (8 mg/kg) was eliminated by pretreatment with scopolamine (0.5 mg/kg) (data not shown).

DISCUSSION

Physostigmine, an anticholinerase agent, and pilocarpine, a cholinergic agonist predominantly acting upon muscarinic receptors, elicited yawning with a marked tongue protruding or teeth chattering. The yawning was blocked by scopolamine, which blocks muscarinic cholinergic receptors, but not by methylscopolamine, a peripheral anticholinergic agent [11, 13, 15]. Moreover, the yawning induced by the cholinergic agonists was not inhibited by mecamylamine, a nicotinic receptor blocking agent, supporting a previous report [11] that nicotine did not induce yawning. Considered together, these results confirm that yawning behavior is essentially mediated through the stimulation of central muscarinic receptors but not nicotinic receptors.

Tongue protruding was also observed after the treatment with physostigmine which increases the concentrations of endogenous acetylcholine in the synaptic clefts. Mecamylamine did not inhibit yawning but selectively blocked the tongue protruding. The tongue protruding, as well as yawning, was also inhibited by scopolamine but this inhibition may be resulting from the decrease of yawning. Moreover, a muscarinic receptor agonist, pilocarpine, failed to evoke tongue protruding. Accordingly, it is likely that

TABLE 1

EFFECTS OF VARIOUS DRUGS ON YAWNING OR TONGUE PROTRUDING INDUCED BY PHYSOSTIGMINE, PILOCARPINE AND APOMORPHINE

Drugs	(mg/kg)	Physostigmine Yawns	Physostigmine Tongue Protrusions	Pilocarpine Yawns	Apomorphine Yawns
Saline		8.1	5.3	18.5	7.8
Scopolamine	0.5	1.0*	0.6*	0.6*	1.5*
Methylscopolamine	0.5	7.0	5.0	16.3	6.4
Mecamylamine	0.05	7.8	1.3*	18.6	7.0
	0.1	8.0	1.5*	17.5	6.3
	0.2	7.1	1.0*	17.0	6.5
Haloperidol	0.02	7.6	6.2	16.3	1.3*
	0.2	8.0	5.8	17.9	0.5*

Each drug was injected 30 min before an injection of physostigmine (0.2 mg/kg), pilocarpine (4 mg/kg) or apomorphine (0.25 mg/kg).

Yawns and tongue protrusions were counted for 90 min after the inducers.

Each value is the mean number of 8 rats.

*$p<0.02$; significant difference from the saline group, determined by the Mann-Whitney U-test.

physostigmine-induced tongue protruding may involve, in part, an activation of nicotinic receptors.

Recently, Dubuc *et al.* [3] have reported that physostigmine-induced yawning was unaffected by neuroleptics such as haloperidol, mezilamine and sulpiride but inhibited by clozapine and thioridazine, which have antimuscarinic actions in addition to a dopamine receptor blocking action [8]. In the present experiments, low doses of haloperidol, which have been reported to block presynaptic dopamine receptors [1, 6, 9], failed to affect the yawning elicited by physostigmine and pilocarpine. On the other hand, yawning was also elicited by low doses of apomorphine, which have been reported to stimulate presynaptic dopamine autoreceptors, and consequently inhibit dopamine release [3, 7, 13, 14]. This apomorphine-induced yawning was blocked by scopolamine and a low dose (0.02 mg/kg) of haloperidol, these results being compatible with reported data [9] that apomorphine-induced yawning was blocked by doses of haloperidol well below those inhibiting the stereotypy evoked by apomorphine. Considered together, these experimental findings lend additional support to the hypothesis [13–15] that an activation of cholinergic neurons resulting from an inhibition of dopamine release from presynaptic sites in the dopaminergic-cholinergic neuron link is involved in apomorphine-induced yawning.

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