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Influence of Different Adrenoceptor Agonists and Antagonists on Physostigmine-Induced Yawning in Rats

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ZARRINDAST, M.-R., S. FAZLI-TABAI, S. SEMNANIAN AND Y. FATHOLLAHI. *Influence of different adrenoceptor agonists and antagonists on physostigmine-induced yawning in rats.* PHARMACOL BIOCHEM BEHAV **62**(1) 1–5, 1999.—In the present study, effects of adrenoceptor agonists and antagonists on physostigmine-induced yawning was investigated. Intraperitoneal (IP) injection of different doses of physostigmine (0.03, 0.05, 0.1, and 0.2 mg/kg) induced yawning in rats. The maximum response was obtained by 0.2 mg/kg of the drug. The α_1-adrenoceptor agonist, phenylephrine, and the α_2-adrenoceptor agonist, clonidine, decreased yawning induced by physostigmine. Prazosin and higher doses of phenoxybenzamine reduced the inhibitory effect of phenylephrine. Higher doses of yohimbine also reduced the clonidine response. The adrenoceptor antagonists, prazosin, phenoxybenzamine, and propranolol, did not significantly alter the physostigmine response. However, yohimbine, or lower doses of prazosin, decreased the physostigmine response. It may be concluded that α_1- and α_2-adrenoceptor stimulation decreases the physostigmine-induced yawning behavior in rats. © 1998 Elsevier Science Inc.

Adrenergic agents Physostigmine Yawning Rats

YAWNING behavior is a curious and still little-understood phenomenon that is displayed in many vertebrate species.

Despite the great sociobiological importance and extraordinarily widespread incidence of yawning, this stereotyped behavior has been the subject of very little empirical research. The behavior, if practiced in excess, may be a sign of diseases such as chorea, brain tumors, or encephalitis (2).

Most rats normally show one to two episodes of yawning per hour. They are usually sleepy during the time of yawning, but yawning can be elicited in experimental animals by the cholinergic agents physostigmine and pilocarpine, which is inhibited by muscarinic blockade (11,14).

Considerable interest has developed in a yawning syndrome that is produced by dopamine agonists in rats (4,7,8). The paraventricular nucleus (1), incerto-hypothalamic dopamine system (7), and septal and striatal dopamine receptors have all been suggested to mediate yawning in rats (12). The behavior seems to be mediated through D_2 dopamine receptors (4,10,14). Central cholinergic mechanisms mediate the yawning induced by dopamine receptor agonists, and the response is inhibited by muscarinic antagonists such as scopolamine and atropine (8,14).

The influences of GABAergic agents (15), adenosine receptor agents (16) and opioidergic agents (13) on cholinergic-induced yawning have been investigated in our previous work. There is evidence that the adrenceptor system may influence some behaviors in rats (3). In the present study, effects of adrenoceptor mechanism(s) on physostigmine-induced yawning were studied.

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METHOD

Animals

Male albino Wistar rats weighing 150–250 g were used in these experiments. They were kept five per cage in a room on a 12 L:12 D cycle at 22 ± 2°C. Food and water were freely available except during experimentation. The experimental protocol was approved by the Research and Ethics Committee of Azad University of Kazeron [3040;21,05,96].

Behavioral Observation

Rats were placed individually in a glass cylinder (25 cm wide, 25 cm high) at 22 ± 2°C during experimentation and were allowed to adapt for 30 min before the first drug injection. No more than two rats were observed simultaneously. Yawning was counted by direct observation immediately after physostigmine administration for a period of 60 min. Physostigmine was injected 15 min after administration of adrenoceptor agonists and antagonists.

Drugs

The following drugs were used: phenoxybenzamine HCl (SmithKline & French Labs, Philadelphia), propranolol (ICI, UK), physostigmine salicylate, phenylephrine hydrochloride, prazosin hydrochloride, clonidine hydrochloride, and yohimbine (Sigma, Poole, UK). The drugs were given intraperitoneally in a volume of 5 ml/kg and were prepared immediately before use. The control groups received saline.

Statistical Analysis

One-way analysis of variance (ANOVA) followed by the Newman–Keuls test was used for statistical analysis. A difference of $p < 0.05$ was considered statistically significant.

RESULTS

Yawning Behavior induced by Physostigmine

Intraperitoneal (IP) administration of different doses of physostigmine (0.03, 0.05, 0.1, and 0.2 mg/kg) induced yawning in rats, $F(4, 40) = 7.6$, $p < 0.0001$. The response was dose related, and reached a maximum level with 0.2 mg/kg of the drug (Fig. 1).

Effects of Adrenoceptor Agonists on Physostigmine-Induced Yawning

The α_1-adrenoceptor agonist, phenylephrine (0.25, 0.5, 2.5, and 10 mg/kg, IP), or the α_2-adrenoceptor agonist, clonidine (0.025, 0.05, and 0.1 mg/kg, IP), reduced yawning induced by physostigmine (0.05 mg/kg, IP), $F(7, 64) = 6.0$, $p < 0.0001$ (Fig. 2).

Effects of Adrenoceptor Agonists in the Presence or Absence of Antagonists on Physostigmine-Induced Yawning

ANOVA shows a significant difference between animals which received physostigmine (0.05 mg/kg) plus saline and those that received physostigmine plus phenylephrine (2.5 mg/kg) in the presence or absence of prazosin (0.5 and 1 mg/kg) or phenoxybenzamine (5 and 10 mg/kg), $F(5, 48) = 4.14$, $p < 0.01$ (Fig. 3A). Further analyses indicate that phenylephrine was able to reduce yawning induced by physostigmine and prazosin, and higher doses of phenoxybenzamine decreased the phenylephrine response.

ANOVA also indicates a significant difference between animals that were administered physostigmine (0.05 mg/kg) plus saline or those that received physostigmine plus clonidine (0.025 mg/kg) in the presence or absence of yohimbine, $F(4, 40) = 5.7$, $p < 0.001$ (Fig. 3B). Further analyses indicate that

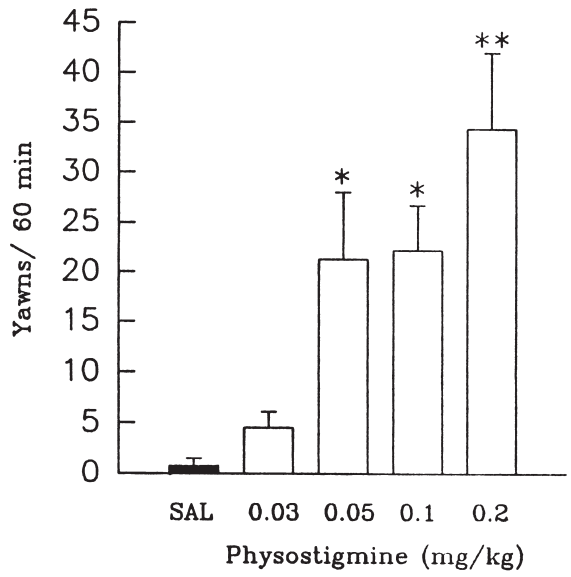

FIG. 1. Yawning induced by administration of physostigmine in rats. Animals were injected intraperitoneally (IP) with saline (SAL; 5 ml/kg) or with different doses of physostigmine (0.03–0.2 mg/kg). The number of yawns were recorded for 60 min. Each point is the mean ± SEM of nine experiments. *$p < 0.05$, **$p < 0.001$ significantly different from saline-treated group.

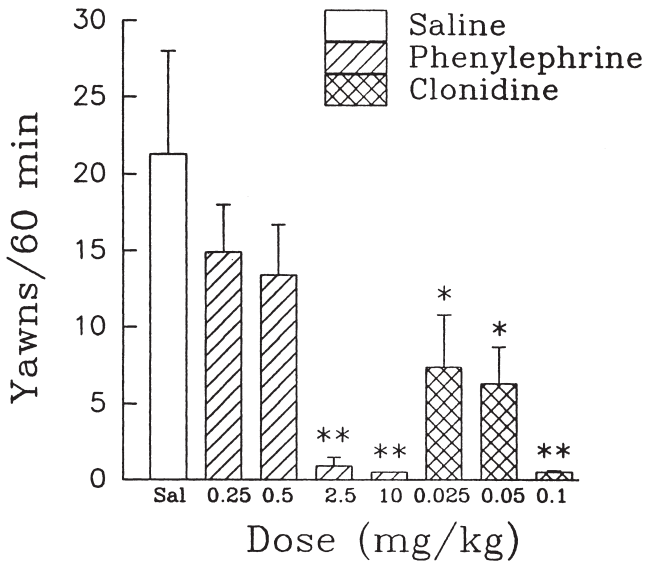

FIG. 2. Effects of phenylephrine or clonidine on yawning induced by physostigmine. Rats were treated intraperitoneally (IP) either with saline (5 ml/kg), phenylephrine (0.25, 0.5, and 2.5 mg/kg), or clonidine (0.025, 0.05, and 0.1 mg/kg) 15 min prior to physostigmine (0.05 mg/kg). Each point is the mean ± SEM of nine experiments. *$p < 0.01$, **$p < 0.001$ significantly different from saline-treated group.

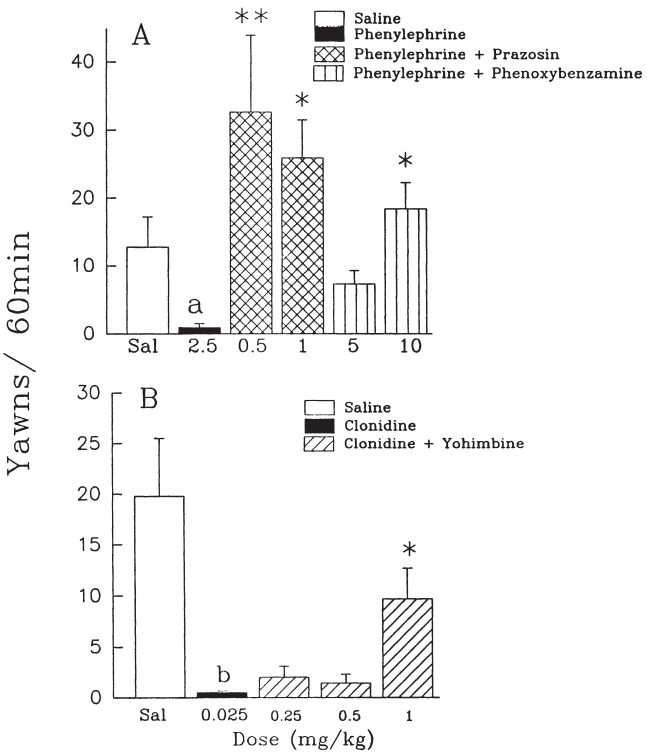

FIG. 3. Effects of phenyephrine or clonidine on yawning induced by physostigmine in the presence or the absence of adrenoceptor antagonists. Animals were treated intraperitoneally (IP) with saline (5 ml/kg), phenylephrine (2.5 mg/kg), phenylephrine plus prazosin (0.5 and 1 mg/kg), or phenylephrine plus phenoxybenzamine (5 and 10 mg/kg) 15 min before physostigmine (0.05 mg/kg) (A). Another group of animals were treated with clonidine (0.025 mg/kg, IP) or clonidine plus Yohimbine (0.25, 0.5, and 1 mg/kg) 15 min prior to physostigmine (0.05 mg/kg) (B). Each point is the mean ± SEM of nine experiments. [a]$p < 0.05$, [b]$p < 0.01$ different from respective saline-treated animals. *$p < 0.01$, **$p < 0.001$ different from respective phenylephrine-treated control groups.

clonidine reduced the physostigmine effect, and only higher doses of yohimbine (1 mg/kg) were able to decrease clonidine's inhibitory response.

Effects of Adrenoceptor Antagonists on Physostigmine-Induced Yawning in Rats

Effects of adrenoceptor antagonists are shown in Table 1. Pretreatment of animals with the adrenoceptor antagonists prazosin (0.25, 0.5, and 1 mg/kg), $F(3, 32) = 0.5$, $p > 0.05$, phenoxybenzamine (2.5, 5, and 10 mg/kg), $F(3, 32) = 3.7$, $p > 0.05$, or propranolol (1.25, 2.5, and 5 mg/kg), $F(3, 32) = 1.5$, $p > 0.05$, did not alter the physostigmine response. However, the α_2-adrenoceptor antagonist, yohimbine (0.25 and 2 mg/kg), decreased yawning induced by physostigmine (0.05 mg/kg), $F(4, 40) = 4.1$, $p < 0.01$.

When yohimbine (0.25 or 1 mg/kg) plus prazosin (0.5 or 1 mg/kg) was administered 15 min before physostigmine (0.5 mg/kg) and compared to the saline group (saline + physostigmine), yawning was significantly decreased, $F(4, 37) = 3.1$, $p < 0.05$. However, the results were not significantly different from those of prazosin or yohimbine alone, $F(5, 45) = 2.76$, $p > 0.05$.

TABLE 1

EFFECTS OF α-ADRENOCEPTOR ANTAGONISTS ON YAWNING INDUCED BY PHYSOSTIGMINE (0.05 mg/kg)

Drug Treatment (mg/kg)	Yawns/60 min	P < vs. Saline
Saline 5 ml/kg	14.0 ± 2.6	NS
Prazosin 0.25	12.4 ± 2.4	NS
Prazosin 0.5	18.0 ± 1.3	NS
Prazosin 1	18.2 ± 6.9	NS
Yohimbine 0.25	2.3 ± 0.5	0.01
Yohimbine 0.5	7.7 ± 2.0	NS
Yohimbine 1	10.2 ± 2.9	NS
Yohimbine 2	5.6 ± 2.1	0.05
Phenoxybenzamine 2.5	7.8 ± 1.9	NS
Phenoxybenzamine 5	24.9 ± 5.4	NS
Phenoxybenzamine 10	11.7 ± 2.5	NS
Propranolol 1.25	20.4 ± 6.5	NS
Propranolol 2.5	12.0 ± 1.9	NS
Propranolol 5	28.2 ± 9.6	NS
Yohimbine 0.25 + Prazosin 0.5	7.0 ± 1.6	0.05
Yohimbine 0.25 + Prazosin 1	6.0 ± 2.0	0.05
Yohimbine 1 + Prazosin 0.5	7.3 ± 1.8	0.05
Yohimbine 1 + Prazosin 1	5.0 ± 2.0	0.05

Animals were treated intraperitoneally (IP) either saline (5 ml/kg), prazosin (0.25, 0.5, and 1 mg/kg), or yohimbine (0.25, 0.5, 1, and 2 mg/kg) 15 min prior to physostigmine (0.05 mg/kg). Each point is the mean ± SEM of nine experiments.

DISCUSSION

In this work, the influence of adrenoceptor agonists and antagonists on physostigmine-induced yawning were investigated. Previous studies have shown that cholinergic or dopamine D_2 receptor activation induce yawning. The behavior induced by cholinoceptor agents is antagonized by muscarinic receptor antagonists but not by dopaminergic receptor antagonists (11,14), and behavior induced by dopamine D_2 receptor agonists is inhibited by both dopaminergic and muscarinic receptor antagonists (12,14). These data imply that the yawning induced by cholinoceptor agents involves activation of cholinergic neuronal mechanism(s), and the yawning induced by dopaminergic receptor agonists appears to be mediated via activation of dopaminergic and cholinergic mechanisms. The yawning can also be influenced by different systems (e.g., dopamine D_1 receptor stimulation; opioidergic, GABAergic and adenosine mechanisms) [(13–16); See also Table 2]. Together with evidence for the role of dopaminergic, cholinergic, and other mechanisms in mediating yawning, evidence also indicates an important modulatory role for the noradrenergic system (6).

The present results show that intraperitoneal (IP) injection of the cholinesterase inhibitor, physostigmine, induced dose-dependent yawning. The response was maximal with 0.2 mg/kg of the drug. This is in agreement with previous work that found that activation of the cholinergic mechanism may induce yawning (11,13–16). The present data indicate that administration of the α_1-adrenoceptor agonist, phenylephrine, and the α_2-adrenoceptor agonist, clonidine, decreased physostigmine's effect. Therefore, it can be suggested that adrenergic system(s) is involved in the present response. This may be in agreement with other reports that indicate that adrenocep-

TABLE 2

DRUG AND SYSTEM INFLUENCES ON YAWNING BEHAVIOR IN RATS

Drugs	Response	Mechanism
Physostigmine	↑	Muscarinic receptor activation
Pilocarpine	↑	Muscarinic receptor activaton
Apomorphine	↑	Dopamine D_2 receptor stimulation
Bromocriptine	↑	Dopamine D_2 receptor stimulation
Atropine	↔C, ↔D	Muscarinic receptor blockade
Sulpiride	↔D	Dopamine D_2 receptor blockade
SKF38393	↓C, ↓D	Dopamine D_1 receptor activation
High doses of apomorphine	↓D	Dopamine D_1 receptor activation
Morphine	↓C, ↓D	Opioid receptor stimulation
Naloxone	↑, ↔M	Opioid receptor blockade
$GABA_A$ or $GABA_B$	↓C	GABA receptor stimulation
GABA antagonist	↔G	GABA receptor blockade
A_1 adenosine	↓C, ↓D	A_1 receptor activation

↑ = induces yawning; ↔C = prevents yawning induced by cholinergic agents; ↔D = prevents yawning induced by dopaminergic agents; ↓C = decreases yawning induced by cholinergic agents; ↓D = decreases yawning induced by dopaminergic agents; ↔M = inhibits morphine inhibitory response; ↔G = inhibits GABA agonists' effect; Data for Table 2 derived from (13–16).

tor mechanisms may influence some of the behaviors in rats (3). The effect induced by phenylephrine can be decreased by the α_1-adrenoceptor antagonist, prazosin, which may show that inhibition of the yawning induced by phenylephrine is mediated through an α_1-adrenoceptor mechanism. This is not consistent with data obtained by some authors who showed that physostigmine-induced yawning could not be affected by α_1-adrenoceptors (6). The higher dose of the α_2-adrenoceptor antagonist, yohimbine, also reduced the inhibition induced by clonidine. The possibility may exist that clonidine produces its effect through α_2-receptor activity. This is in agreement with results obtained by some investigators who showed clonidine could reduce cholinergic-induced yawning (5). Because low doses of yohimbine by itself reduced physostigmine-induced yawning, receptor blockade by the α_2-receptor antagonist cannot be shown. Thus, low doses of yohimbine may block α_2-presynaptic adrenoceptors and, in turn, may release noradrenaline. The released neurotransmitter may cause inhibition of physostigmine's response through activation of adrenoceptors. However, prazosin administration could not reduce the yohimbine influence on physostigmine-induced yawning.

Evidence also exists that α_2-adrenoceptor activation may decrease dopamine release (9). Therefore, there may be a possibility that blockade of α_2-adrenoceptor by yohimbine releases dopamine. Stimulation of D_1 dopamine receptors has also been shown to reduce yawning [(14); Table 2]. Thus, the released dopamine may inhibit yawning through D_1 receptor activation.

Overall, whether the activation of adrenergic system inhibited the release of acetylcholine or directly interacted with cholinergic mechanisms involved in yawning it is not presently clear, and should be examined further. Prazosin, phenoxybenzamine, and propranolol by themselves did not change physostigmine-induced yawning. Therefore, the negative influence of the brain adrenergic mechanism(s) on the yawning behavior seems unlikely. However, it has been proposed that β-adrenoceptor antagonists are able to increase yawning induced by cholinergic and dopaminergic drugs (6).

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