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Role of Adrenergic Neuronal Activity in the Yawning Induced by Tacrine and NIK-247 in Rats

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KIMURA, H., K. YAMADA, M. NAGASHIMA, S.-I. MATSUMOTO, Y. ISHII, S. YOSHIDA, K. FUJII AND T. FURUKAWA. *Role of adrenergic neuronal activity in the yawning induced by tacrine and NIK-247 in rats.* PHARMACOL BIOCHEM BEHAV **43**(4) 985-991, 1992.—The present experiments were performed to investigate the potential role of central adrenergic neurons in regulating occurrence of yawning in rats. Intraperitoneal injection of tacrine (THA) or 9-amino-2,3,5,6,7,8-hexahydro-1*H*-cyclopenta(*b*)-quinoline monohydrate HCl (NIK-247), cholinesterase inhibitors, induced yawning, which was markedly increased by pretreatment with the β-adrenoceptor antagonist, pindolol. The yawning evoked by tacrine or NIK-247 given alone or in combination with pindolol was inhibited by pretreatment with scopolamine but not by mecamylamine or spiperone. Treatment with tacrine or NIK-247 increased acetylcholine content of the striatum, but this effect was not enhanced by pindolol, which per se did not affect basal acetylcholine content. Moreover, pretreatment with the central adrenaline synthesis inhibitors, (±)-2,3-dichloro-α-methylbenzylamine HCl (LY-78335) and 2-cyclooctyl-2-hydroxyethylamine HCl (UK-1187A), increased tacrine-induced yawning. Subcutaneous injection of talipexole (B-HT 920), a dopamine D_2 receptor agonist, evoked yawning, which was also increased by pindolol, LY-78335, and UK-1187A. These receptors antagonists and synthesis inhibitors per se did not cause yawning responses. The results suggest that the β-adrenoceptor blockade and the inhibition of adrenaline synthesis facilitate the occurrence of yawning induced by cholinergic and dopaminergic agonists, and thus the central adrenergic neuronal systems may be implicated in the regulation of yawning responses.

Yawning Cholinesterase inhibitor β-Adrenoceptor antagonists Adrenaline synthesis inhibitors Central adrenergic neurons

BEHAVIORAL studies have shown that physostigmine, an anticholinesterase agent, and pilocarpine, a muscarinic receptor agonist, induce yawning behavior that is abolished by scopolamine, a muscarinic receptor antagonist (11,34,39,45). The yawning induced by dopamine receptor agonists such as apomorphine and talipexole (B-HT 920) is also completely inhibited by scopolamine (21,39,41,43,44). On the basis of these results, it has been proposed that cholinergic neuronal activity is indispensably involved in eliciting yawning behavior.

9-Amino-1,2,3,4-tetrahydroacridine (tacrine (THA)) is proposed to be a potent, centrally acting cholinesterase inhibitor (13,25). 9-Amino-2,3,5,6,7,8-hexahydro-1*H*-cyclopenta-(*b*)-quinoline monohydrate HCl (NIK-247) has also been reported to exert inhibitory effects on cholinesterase (17,18,33). These cholinesterase inhibitors are found to improve cognitive functions at different phases of the learning and memory processes in animals (24,25,30,33) and have been clinically developed as potential cognitive improvers (3,31). However, there is little documentation on yawning and acetylcholine content in animals treated with tacrine or NIK-247.

The potential functional role of adrenaline-containing neuronal systems was suggested in some of the earliest research on brain catecholamines (12). The earlier hypothesis (4,26) that adrenaline is a neurotransmitter candidate in the brain were validated by detailed studies of the distribution of brain neurons containing phenylethanolamine *N*-methyltransferase (PNMT) (15) and their close correlation with the regional distribution of enzymatic activity (28) and adrenaline content (19). As inhibitors of PNMT were reported to decrease adrenaline formation without affecting dopamine and noradrena-

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line levels (8,9,22), several lines of investigation with the inhibitors subsequently suggested that brain adrenaline may be involved in cerebral cardiovascular (38), neuroendocrine (1,32), and behavioral regulation (7). Recently, we reported that administration of various β-adrenoceptor antagonists such as pindolol, propranolol, and indenolol increased the yawning responses induced by treatment with apomorphine, talipexole, physostigmine, or pilocarpine (41,42), suggesting that β-adrenoceptor blockade facilitates the occurrence of yawning induced by dopaminergic and cholinergic agonists. Thus, central β-adrenoceptors relating to central adrenergic neuronal systems might be involved in the regulation of yawning responses.

The present experiments were therefore performed to investigate whether or not tacrine and NIK-247 elicit yawning that may possibly be regulated by central adrenergic neurons via β-adrenoceptor activities in rats.

METHOD

Animals

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

Behavioral Observations

Pairs of rats were placed in a transparent plastic box (33 × 30 × 17 cm) containing wood shavings. They were allowed to habituate to the observation box for 15 min before drug injection. Yawning is a fixed innate motor pattern, characterized by slow, wide opening of the mouth (34,35). The total number of yawns was counted for 60 min following injection.

Drugs

Drugs used were THA HCl (Sigma Chemical Co., St. Louis, MO), NIK-247 HCl (Nikken Chemicals, Tokyo, Japan), talipexole (B-HT 920) HCl (Boehringer Ingelheim, Kawanishi, Japan), pindolol (Sigma), (±)-2,3-dichloro-α-methylbenzylamine HCl (LY-78335) (RBI, Natick, MA), 2-cyclooctyl-2-hydroxyethylamine HCl (UK-1187A) (RBI), spiperone (Spiropitan Injection, Eisai, Tokyo, Japan), mecamylamine HCl (Merck Sharp and Dohme, Rahway, NJ), and scopolamine HBr (Nakarai, Kyoto, Japan). Pindolol was dissolved in an excess of equimolar tartaric acid solution, with subsequent dilution in saline. The other drugs were dissolved or diluted in saline. Doses are expressed according to the salt with the exceptions of pindolol and spiperone.

Administration of Drugs

Rats received IP injections of tacrine (0.5–10 mg/kg) or NIK-247 (1–20 mg/kg) and SC injections of talipexole (0.02 mg/kg). The β-adrenoceptor antagonist, pindolol (5–40 mg/kg, IP) was injected 1 h and the PNMT inhibitors, LY-78335 (2.5–20 mg/kg, IP) and UK-1187A (1–20 mg/kg, IP), 4–24 h prior to injection of anticholinesterase agents or a dopamine receptor agonist. For pretreatment with antagonists, spiperone (0.5 mg/kg, IP), scopolamine (0.5 mg/kg, IP), or mecamylamine (0.2 mg/kg, IP) was injected 30 min before the yawning inducers.

Biochemical Analysis

Rats were killed by head-focused microwave irradiation (5 kW, 1.3 s) 30 min after tacrine (5 mg/kg, IP) or NIK-247 (10 mg/kg, IP). Pindolol (20 mg/kg, IP) was given 60 min before anticholinesterase agents. After brains were rapidly removed from the skull, the striata were dissected as described previously (10). The tissue was then weighed and stored at −70° C until use. The tissue was homogenized in 0.025 N HCl containing ethylhomocholine as an internal standard. The homogenates were centrifuged at 3,000 rpm for 20 min and the supernatants were filtered through membrane filter with 0.45-μm pores.

Acetylcholine was measured using a modification of the high-performance liquid chromatography (HPLC) method of Potter et al. (27), in which acetylcholine is converted to betaine and H_2O_2 in a postcolumn reactor containing choline oxidase and acetylcholinesterase. These enzymes were immobilized on an AC-enzympak column (Eicom, Kyoto, Japan). AC-Gel column (Eicom) was used to separate acetylcholine and choline. The mobile phase consisted of 0.1 M K_2HPO_4, pH 8.0, containing decanesulfonic acid (300 mg/l) and tetramethylammonium (65 mg/l). The flow rate was 1.0 ml/min. The HPLC system was composed of a 510 pump (Waters Assoc., Milford, MA), an ECD-100 electrochemical detector (Eicom) with a platinum electrode at a potential of + 0.45 V, and a 741 data module (Waters).

Statistical Analysis

Behavioral and biochemical results were expressed as mean values ± SEM. Statistical analysis was done using either two-tailed Student's *t*-test (differences between two groups) or a

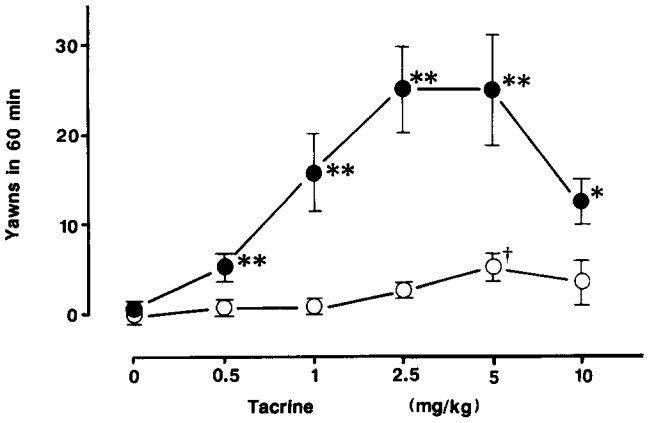

FIG. 1. Dose-response curve for yawning induced by tacrine given alone or in combination with pindolol in rats. The number of yawns was counted immediately following the intraperitoneal injection of saline (1 ml/kg, IP) or tacrine (0.5–10 mg/kg, IP). Pindolol (●) (20 mg/kg, IP) or saline (○) (1 ml/kg, IP) was given 60 min before tacrine. Points represent mean ± SEM (vertical lines) of the number of yawns from 8–10 rats during a 60-min observation period. †$p < 0.01$: Significant difference from saline plus saline-injected group (0 mg/kg), determined by a one-way ANOVA followed by Dunnett's *t*-test. *$p < 0.05$, **$p < 0.01$; Significant difference from respective control groups, determined by Student's *t*-test.

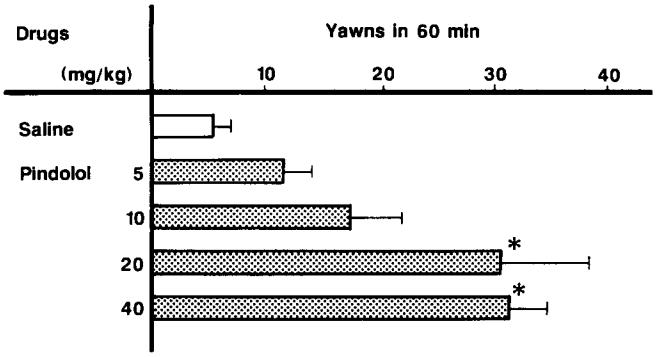

FIG. 2. Dose-dependent facilitatory effects of pindolol on tacrine-induced yawning. Pindolol (5–40 mg/kg, IP) was given 60 min before tacrine (5 mg/kg, IP). Columns represent mean ± SEM (horizontal bars) of the number of yawns from 8–10 rats. *$p < 0.01$; Significant difference from saline-injected group, determined by a one-way ANOVA followed by Dunnett's t-test.

one-way analysis of variance (ANOVA) followed by two-tailed Dunnett's t-test (differences between the control and all groups) or Tukey's test (differences between all groups).

RESULTS

Yawning Induced by the Anticholinesterase Agents

Control rats treated with saline (1 ml/kg, IP) yawned only occasionally. Tacrine (0.5–10 mg/kg, IP) induced a small number of yawning response in saline-pretreated rats, the maximal effect being observed at a dose of 5 mg/kg (Fig. 1).

Tacrine-elicited yawning was markedly increased by pretreatment with pindolol, forming a bell-shaped curve (Fig. 1).

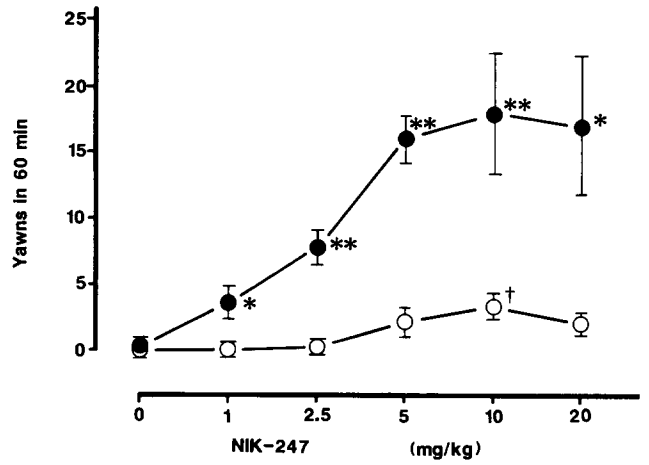

FIG. 3. Dose-response curve for yawning induced by NIK-247 given alone or in combination with pindolol in rats. The number of yawns was counted immediately after saline (1 ml/kg, IP) or NIK-247 (1–20 mg/kg, IP). Pindolol (●) (20 mg/kg, IP) or saline (○) (1 ml/kg, IP) was given 60 min before NIK-247. Points represent mean ± SEM (vertical bars) of the number of yawns from 8–10 rats. †$p < 0.01$: Significant difference from saline plus saline-injected group (0 mg/kg), determined by a one-way ANOVA followed by Dunnett's t-test. *$p < 0.05$, **$p < 0.01$: Significant difference from respective control groups, determined by Student's t-test.

As for dose effect of pindolol ranging from 5–40 mg/kg, the incidence of behavior increased dose dependently and reached a maximum at 20 mg/kg (Fig. 2). Pindolol given alone did not evoke yawning.

NIK-247 (1–20 mg/kg, IP) also induced a modest yawning response. The maximal effect of the drug was obtained at a dose of 10 mg/kg. The NIK-247-elicited yawning was also markedly increased by pretreatment with pindolol (20 mg/kg, IP) (Fig. 3).

Effects of Various Receptor Antagonists on Yawning Induced by the Anticholinesterase Agents

As demonstrated in Table 1, the yawning induced by tacrine (5 mg/kg, IP) or NIK-247 (10 mg/kg, IP) given alone or in combination with pindolol was inhibited by pretreatment with scopolamine (0.5 mg/kg, IP) but not by mecamylamine (0.2 mg/kg, IP) and spiperone (0.5 mg/kg, IP). These receptor antagonists given alone did not elicit yawning.

Acetylcholine Content in the Striatum After Treatment with Tacrine or NIK-247

As shown in Fig. 4, the mean acetylcholine content in the striatum of the saline-treated group was 80.0 ± 3.1 nM/g wet tissue. This content was significantly increased by tacrine (5 mg/kg, IP) or NIK-247 (10 mg/kg, IP). Although pindolol (20 mg/kg, IP) increased yawning responses, this β-adrenoceptor antagonist did not alter basal acetylcholine content. Moreover, pindolol failed to affect the increase of acetylcholine content induced by tacrine or NIK-247.

Effects of PNMT Inhibitors on Tacrine-induced Yawning

As shown in Fig. 5, the yawning induced by tacrine (5 mg/kg, IP) was markedly increased by LY-78335 (2.5–20 mg/kg, IP) or UK-1187A (1–20 mg/kg, IP), which was injected 8 h before the yawning inducer. These PNMT inhibitors were shown to have bell-shaped responses, the maximal effect being observed at 10 mg/kg in the LY-78335-treated group and 5 mg/kg in the UK-1187A-treated group. The PNMT inhibitors given alone did not induce yawning.

Figure 6 shows time course of effects of the PNMT inhibitors. When LY-78335 (10 mg/kg, IP) or UK-1187A (5 mg/kg,IP) was given 4–24 h before tacrine (5 mg/kg, IP), the maximal facilitative effect of both inhibitors was observed at 8 h.

Effects of Pindolol, LY-78335, and UK-1187A on Talipexole-Induced Yawning

Subcutaneous injection of talipexole (0.02 mg/kg) elicited yawning, the mean number of yawns from eight rats in 60 min being 9.3 ± 1.5. Talipexole-induced yawning was increased significantly by pretreatment with pindolol (20 mg/kg, IP, 1 h), LY-78335 (10 mg/kg, IP, 8 h), or UK-1187A (5 mg/kg, IP, 8 h), as demonstrated by the respective mean number of yawns from eight rats in 60 min, 19.9 ± 2.0, 15.5 ± 1.1, and 16.9 ± 1.8.

DISCUSSION

Previous experiments have shown that physostigmine and pilocarpine elicit yawning and this behavior is blocked by muscarinic but not dopamine receptor antagonists (11,34, 35,39,45). On the other hand, the yawning induced by dopa-

TABLE 1

EFFECTS OF VARIOUS DRUGS ON THE YAWNING INDUCED BY TACRINE OR NIK-247 GIVEN ALONE OR IN COMBINATION WITH PINDOLOL

Drugs (mg/kg)		Yawns in 60 min				
		Saline	Tacrine	NIK-247	Pindolol + Tacrine	Pindolol + NIK-247
Saline		0.3 ± 0.3	5.3 ± 1.4	3.3 ± 0.9	25.6 ± 6.3	17.9 ± 4.6
Scopolamine	0.5	0.0 ± 0.0	0.0 ± 0.0*	0.0 ± 0.0*	0.1 ± 0.1†	0.0 ± 0.0†
Mecamylamine	0.2	0.4 ± 1.8	4.6 ± 1.8	1.4 ± 0.4	22.5 ± 6.3	20.4 ± 3.2
Spiperone	0.5	0.5 ± 0.4	2.0 ± 1.1	1.7 ± 1.3	19.4 ± 5.3	19.4 ± 3.3

Scopolamine (0.5 mg/kg, IP), mecamylamine (0.2 mg/kg, IP), and spiperone (0.5 mg/kg, IP) were given 30 min before tacrine (5 mg/kg, IP) or NIK-247 (10 mg/kg, IP) alone or in combination with pindolol (20 mg/kg, IP). Values represent mean ± SEM of the number of yawns from 8–12 rats.

*$p < 0.05$, †$p < 0.01$: Significant difference from respective control groups, determined by a one-way ANOVA followed by Dunnett's t-test.

mine D_2 receptor agonists is antagonized by both dopamine and muscarinic receptor antagonists (21,40,41), implying that dopamine receptor agonist-induced yawning can result from a consequent activation of cholinergic neurons. From such findings, it has been proposed that a dopaminergic-cholinergic-linked neuronal system may participate in inducing yawning (16,39–41).

In the present experiment, treatment with tacrine or NIK-247 evoked a yawning response to a small extent. This yawning response was markedly increased by a β-adrenoceptor antagonist, pindolol, which did not evoke yawning when given alone. Moreover, the yawning elicited by tacrine or NIK-247 given alone or in combination with pindolol was inhibited by scopolamine, a muscarinic receptor antagonist, but not by mecamylamine, a nicotinic receptor antagonist, and spiperone, a dopamine receptor antagonist. The present results suggest that tacrine and NIK-247 were able to elicit yawning via muscarinic receptor activation, as our previous experiments with physostigmine-induced yawning (41). On the other hand, the dopamine D_2 receptor agonist, talipexole, also elicited yawning, which was increased by pindolol, as previously reported (42). Accordingly, the stimulation of muscarinic receptors is obligatory for the induction of yawning and central β-adrenoceptor activity may regulate the occurrence of yawning induced by anticholinesterase agents and dopamine D_2 receptor agonists.

A previous experiment (41) showed that the dopamine D_1/D_2 receptor agonist, apomorphine, induced yawning that was enhanced by pindolol, propranolol, indenolol, and alprenolol, which block central β-adrenoceptors, but not by the peripheral

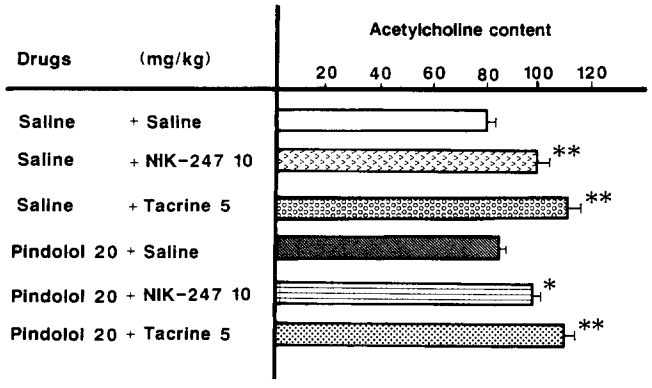

FIG. 4. Effects of tacrine or NIK-247 given alone or in combination with pindolol on acetylcholine content (nM/g wet tissue) in the striatum. Rats were killed by microwave irradiation 30 min after saline (1 ml/kg, IP), tacrine (5 mg/kg, IP), or NIK-247 (10 mg/kg, IP). Pindolol (20 mg/kg, IP) was given 60 min before cholinesterase inhibitors. Columns represent mean ± SEM (horizontal bars) of the contents of acetylcholine from 16–18 rats. *$p < 0.05$, **$p < 0.01$: Significant difference from saline-injected group, determined by a one-way ANOVA followed by Tukey's test.

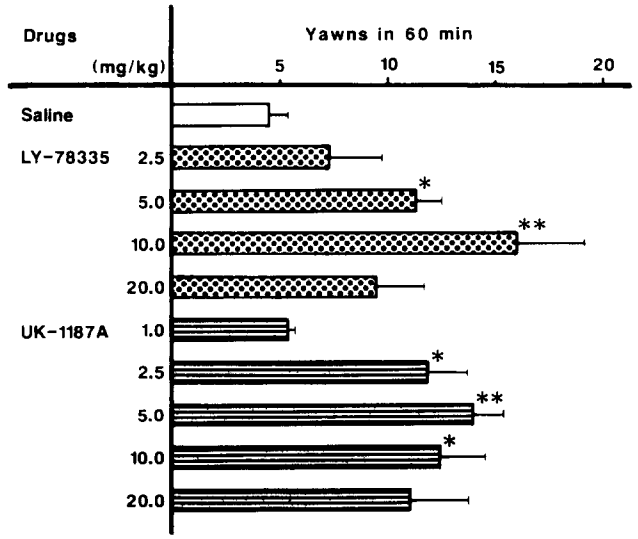

FIG. 5. Dose-dependent facilitatory effects of PNMT inhibitors on tacrine-induced yawning. LY-78335 (2.5–20 mg/kg, IP) and UK-1187A (1–20 mg/kg, IP) were given 8 h before tacrine (5 mg/kg, IP). Columns represent mean ± SEM (horizontal bars) of the number of yawns from eight rats. *$p < 0.05$, **$p < 0.01$: Significant difference from saline-injected group, determined by a one-way ANOVA followed by Dunnett's t-test.

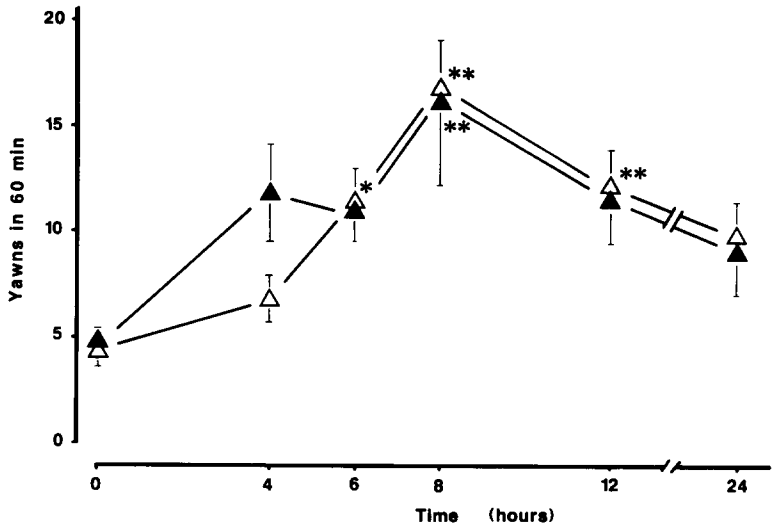

FIG. 6. Time course of effects of PNMT inhibitors on tacrine-induced yawning. LY-78335 (△) (10 mg/kg) and UK-1187A (▲) (5 mg/kg) were given intraperitoneally 4–24 h before tacrine (5 mg/kg, IP). Points represent mean ± SEM (vertical lines) of the number of yawns from eight rats. $*p < 0.05$, $**p < 0.01$: Significant difference from tacrine-alone injected group (zero time), determined by a one-way ANOVA followed by Dunnett's *t*-test.

β-adrenoceptor antagonists, carteolol and atenolol, suggesting that yawning is enhanced by central β-adrenoceptor blockade but not by peripheral blockade. Pindolol is also known to have serotonin (5-HT) receptor blocking actions in addition to β-adrenoceptor antagonistic properties (14). However, the yawning induced by talipexole was increased by pretreatment with pindolol (42) but was unaffected by either metergoline, a potent antagonist of 5-HT_1, and 5-HT_2 receptors, or ketanserin, a specific antagonist of 5-HT_2 receptors (20). Moreover, we found that various β-adrenoceptor antagonists, which have no 5-HT receptor antagonistic properties, also increased the yawning induced by dopamine receptor agonists (41). Accordingly, the 5-HT receptor antagonistic property of pindolol may not be related to the observed enhancement of yawning responses by the drug.

Dopamine receptor agonists, locally applied into the striatum, elicited yawning behavior, and those administered systemically were ineffective in evoking yawning after bilateral lesions of the striatum by 6-hydroxydopamine. Therefore, the striatum is proposed to be one of the important sites in the rat brain areas involved in evoking yawning behavior (5,6,44). In contrast with these reports, microinjection of dopamine receptor agonists into the paraventricular nucleus (PVN) of the hypothalamus induced yawning behavior in rats and electrolytic lesion of the hypothalamic PVN prevented the drug-induced yawning responses (2,23), suggesting that the PVN is also the brain area where dopamine receptor agonists act for inducing yawning. However, a role of cholinergic neuron activity in the PVN for evoking yawning is still unknown. On the other hand, high β-adrenoceptor density was found in the rat striatum (36). Accordingly, in this experiment acetylcholine content of the striatum was measured after treatment with cholinesterase inhibitors. The systemic administration of tacrine or NIK-247 increased acetylcholine content of the striatum, and this was accompanied by a modest yawning response. These results seem to be compatible with recent findings (25) that intraperitoneal injection of tacrine increased acetylcholine content in the striatum, cortex, and hippocampus in rats. However, although pindolol increased the yawning responses induced by tacrine or NIK-247 and the β-adrenoceptor antagonist did not alter basal acetylcholine content and failed to enhance the increase of acetylcholine content in the striatum induced by the two cholinesterase inhibitors. Our previous experiments (41) have also shown that pretreatment with pindolol or propranolol increased the yawning responses induced by the direct muscarinic receptor agonist, pilocarpine, suggesting that β-adrenoceptor antagonists may not affect the synthesis and/or release of acetylcholine in the brain areas involved in eliciting yawning behavior. Furthermore, various β-adrenoceptor antagonists, including pindolol, did not change the activity of acetylcholinesterase in the brain (29). Taken together, it may be assumed that central β-adrenoceptors do not regulate the activities of striatal cholinergic neurons.

According to expectation, in the present experiment, the tacrine-induced yawning responses were increased notably by LY-78335 and UK-1187A. These drugs are reported to block adrenaline synthesis without change of dopamine and noradrenaline levels in the brain via the inhibition of noradrenaline *N*-methyltransferase (9,22,37). However, the dose-response curve of yawning to PNMT inhibitors in combination with tacrine was bell shaped. Similarly, the combined treatment with pindolol and tacrine was shown to have bell-shaped responses of yawning. Although the real reason for showing a bell-shaped dose-response curve is obscure at present, it is a possibility that a high dose of PNMT inhibitors or the β-adrenoceptor antagonist with the anticholinesterase agent produces changes of the other neuronal activities that inhibit the

occurrence of yawning. Furthermore, pretreatment with LY-78335 or UK-1187A facilitated talipexole-induced yawning. Therefore, PNMT inhibitors as well as β-adrenoceptor blockers seem to enhance yawning responses via the inhibition of central adrenergic neuronal activity.

The results suggest that the β-adrenoceptor blockade and inhibition of central adrenaline synthesis facilitate the occurrence of yawning induced by tacrine, NIK-247, or talipexole and that the central adrenergic neuronal systems may participate in the regulation of yawning evoked by cholinergic and dopaminergic agonists. However, further work is warranted to clarify a neuronal circuit between a dopaminergic-cholinergic-linked neuronal system and an adrenergic neuronal system involved in yawning behavior.

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REFERENCES

1. Adler, B. A.; Johnson, M. D.; Lynch, C. O.; Crowley, W. R. Evidence that norepinephrine and epinephrine systems mediate the stimulatory effects of ovarian hormones on luteinizing hormone and luteinizing hormone-releasing hormone. Endocrinology 113:1431–1438; 1983.
2. Argiolas, A.; Melis, M. R.; Mauri, A.; Gessa, G. L. Paraventricular nucleus lesion prevents yawning and penile erection induced by apomorphine and oxytocin but not by ACTH in rats. Brain Res. 421:349–352; 1987.
3. Becker, R.; Giacobini, E. Advances in the therapy of Alzheimer's disease: Issues in the study and development of cholinomimetic therapies. In: Giacobini, E.; Becker, R., eds. Current research in Alzheimer therapy. New York: Taylor & Francis; 1988:113–122.
4. Ciaranello, R. D.; Barchas, R. E.; Byers, G. S.; Stemmle, D. W.; Barchas, J. D. Enzymatic synthesis of adrenaline in mammalian brain. Nature 221:368–369; 1969.
5. Dourish, C. T.; Cooper, S. J.; Philips, S. R. Yawning elicited by systemic and intrastriatal injection of piribedil and apomorphine in the rat. Psychopharmacology (Berl.) 86:175–181; 1985.
6. Dourish, C. T.; Hutson, P. H. Bilateral lesions of the striatum induced with 6-hydroxydopamine abolish apomorphine-induced yawning in rats. Neuropharmacology 24:1051–1055; 1985.
7. Durcan, M. J.; Lister, R. G.; Linnoila, M. Behavioral effects of the inhibitors of phenylethanolamine-N-methyltransferase, LY 78335 and LY 134046, and their interactions with ethanol. Psychopharmacology (Berl.) 101:196–202; 1990.
8. Fuller, R. W. Pharmacology of brain epinephrine neurons. Annu. Rev. Pharmacol. Toxicol. 22:31–55; 1982.
9. Fuller, R. W.; Perry, K. W. Lowering of epinephrine concentration in rat brain by 2,3-dichloro-α-methylbenzylamine, an inhibitor of norepinephrine *N*-methyltransferase. Biochem. Pharmacol. 26:2087–2090; 1977.
10. Glowinski, J.; Iversen, L. L. Regional studies of catecholamines in the brain—I. The disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]DOPA in various regions of the brain. J. Neurochem. 13:655–699; 1966.
11. Gower, A. J. Effects of acetylcholine agonists and antagonists on yawning and analgesia in the rat. Eur. J. Pharmacol. 139:79–89; 1987.
12. Gunne, L. M. Relative adrenaline content in brain tissue. Acta Physiol. Scand. 56:324–333; 1962.
13. Hallak, M.; Giacobini, E. Physostigmine, tacrine and metrifonate: The effect of multiple doses on acetylcholine metabolism in rat brain. Neuropharmacology 28:199–206; 1989.
14. Hjorth, S.; Carlsson, A. Is pindolol a mixed agonist-antagonist at central serotonin (5-HT) receptors? Eur. J. Pharmacol. 129: 131–138; 1986.
15. Hökfelt, T.; Fuxe, K.; Goldstein, M.; Johansson, O. Immunohistochemical evidence for the existence of adrenaline neurons in the rat brain. Brain Res. 66:235–251; 1974.
16. Holmgren, B.; Urbá-Holmgren, R. Interaction of cholinergic and dopaminergic influences on yawning behavior. Acta Neurobiol. Exp. 40:633–642; 1980.
17. Ishii, Y.; Sumi, T. Evaluation of a cholinomimetic drug, 9-amino-2,3,5,6,7,8-hexahydro-1*H*-cyclopenta[*b*]-quinoline (NIK-247), as an enhancer of endogenous efflux of acetylcholine from brain slices. Neuropharmacology 31:61–66; 1992.
18. Kojima, J.; Yoshida, S.; Ishii, Y.; Fujii, K.; Okumura, M. Effects of NIK-247 and 9-amino-1,2,3,4-tetrahydroacridine on scopolamine-induced amnesia, long-term potentiation and acetylcholinesterase. Eur. J. Pharmacol. 183:929; 1990.
19. Koslow, S. H.; Schlumpf, M. Quantitation of adrenaline in rat brain nuclei and areas by mass fragmentography. Nature 251: 530–531; 1974.
20. Longoni, R.; Spina, L.; Di Chiara, G. Permissive role of D-1 receptor stimulation by endogenous dopamine for the expression of postsynaptic D-2-mediated behavioural responses. Yawning in rats. Eur. J. Pharmacol. 134:163–173; 1987.
21. Matsumoto, S.; Yamada, K.; Nagashima, M.; Domae, M.; Shirakawa, K.; Furukawa, T. Occurrence of yawning and decrease of prolactin levels via stimulation of dopamine D_2-receptors after administration of SND 919 in rats. Naunyn Schmiedeberg's Arch. Pharmacol. 340:21–25; 1989.
22. Mefford, I. N. Epinephrine in mammalian brain. Prog. Neuropsychopharmacol. Biol. Psychiatry 12:365–388; 1988.
23. Melis, M. R.; Argiolas, A.; Gessa, G. L. Apomorphine-induced penile erection and yawning: Site of action in brain. Brain Res. 415:98–104; 1987.
24. Nabeshima, T.; Yoshida, S.; Kameyama, T. Effects of the novel compound NIK-247 on impairment of passive avoidance response in mice. Eur. J. Pharmacol. 154:263–269; 1988.
25. Nielsen, J. A.; Mena, E. E.; Williams, I. H. Correlation of brain levels of 9-amino-1,2,3,4-tetrahydroacridine (THA) with neurochemical and behavioral changes. Eur. J. Pharmacol. 173:53–64; 1989.
26. Pohorecky, L. A.; Zigmond, M.; Karten, H.; Wurtman, R. J. Enzymatic conversion of norepinephrine to epinephrine by the brain. J. Pharmacol. Exp. Ther. 165:190–195; 1969.
27. Potter, P. E.; Meek, J. L.; Neff, N. H. Acetylcholine and choline in neuronal tissue measures by HPLC with electrochemical detection. J. Neurochem. 41:188–194; 1983.
28. Saavedra, J. M.; Palkovits, M.; Brownstein, M. J.; Axelrod, J. Localization of phenylethanolamine *N*-methyl transferase in the rat brain. Nature 248:695–696; 1974.
29. Simon, G.; Winter, M. The effects of sympatholytic and sympathomimetic agents on acetylcholinesterase and cholinesterase activity, in vitro. Biochem. Pharmacol. 25:881–882; 1976.
30. Speiser, Z.; Reicher, S.; Gitter, S.; Cohen, S. Tacrine or arecoline mediates reversal of anoxia- or AF64A-induced behavioural disorders in the developing rat. Neuropharmacology 28:1325–1332; 1989.
31. Summers, W. K.; Majovski, L. V.; Marsh, G. M.; Tachiki, K.; Kling, A. Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. N. Engl. J. Med. 315:1241–1245; 1986.
32. Terry, L. C. Regulation of thyrotropin secretion by the central epinephrine system. Neuroendocrinology 42:102–108; 1986.
33. Ueki, A.; Miyoshi, K. Effects of cholinergic drugs on learning impairment in ventral globus pallidus-lesioned rats. J. Neurol. Sci. 90:1–21; 1989.
34. Urbá-Holmgren, R.; González, R. M.; Holmgren, B. Is yawning a cholinergic response? Nature 267:261–262; 1977.

35. Ushijima, I.; Yamada, K.; Inoue, T.; Tokunaga, T.; Furukawa, T.; Noda, Y. Muscarinic and nicotinic effects on yawning and tongue protruding in the rat. Pharmacol. Biochem. Behav. 21: 297–300; 1984.
36. Vantini, G.; Perry, B. D.; Guchhait, R. B.; U'Prichard, D. C.; Stolk, J. M. Brain epinephrine systems: Detailed comparison of adrenergic and noradrenergic metabolism, receptor number and in vitro regulation, in two inbred rat strains. Brain Res. 296:49–65; 1984.
37. Vincek, W. C.; Aldrich, C. S.; Borchardt, R. T.; Grunewald, G. L. Importance of the aromatic ring in adrenergic amines. 5. Nonaromatic analogues of phenylethanolamine as inhibitors of phenylethanolamine *N*-methyltransferase: Role of hydrophrobic and steric interactions. J. Med. Chem. 24:7–12; 1981.
38. Ward-Routledge, C.; Marsden, C. A. Adrenaline in the CNS and the action of antihypertensive drugs. Trends Pharmacol. Sci. 9: 209–214; 1988.
39. Yamada, K.; Furukawa, T. Direct evidence for involvement of dopaminergic inhibition and cholinergic activation in yawning. Psychopharmacology (Berl.) 67:39–43; 1980.
40. Yamada, K.; Furukawa, T. The yawning elicited by α-melanocyte-stimulating hormone involves serotonergic–dopaminergic-cholinergic neuron link in rats. Naunyn Schmiedeberg's Arch. Pharmacol. 316:155–160; 1981.
41. Yamada, K.; Matsumoto, S.; Nagashima, M.; Kumagai, M.; Kimura, H.; Furukawa, T. Involvement of central β-adrenoceptors in the regulation of yawning responses. Naunyn Schmiedeberg's Arch. Pharmacol. 340:26–30; 1989.
42. Yamada, K.; Matsumoto, S.; Nagashima, M.; Shirakawa, K.; Furukawa, T. Potentiation of yawning responses to the dopamine receptor agonists B-HT 920 and SND 919 by pindolol in the rat. J. Neural Trans. 79:19–24; 1990.
43. Yamada, K.; Nagashima, M.; Kimura, H.; Matsumoto, S.; Furukawa, T. Possible involvement of differing classes of dopamine D-2 receptors in yawning and stereotypy in rats. Psychopharmacology (Berl.) 100:141–144; 1990.
44. Yamada, K.; Tanaka, M.; Shibata, K.; Furukawa, T. Involvement of septal and striatal dopamine D-2 receptors in yawning behavior in rats. Psychopharmacology (Berl.) 90:9–13; 1986.
45. Zarrindast, M. R.; Poursoltan, M. Interactions of drugs acting on central dopamine receptors and cholinoceptors on yawning responses in the rat induced by apomorphine, bromocriptine or physostigmine. Br. J. Pharmacol. 96:843–848; 1989.