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Pharmacology Biochemistry & Behavior, Vol. 55, No. 1, pp. 55–60, 1996

0091-3057/96 $15.00 + .00

SSDI 0091-3057(95)02216-3

The New Muscarinic M_1-Receptor Agonist YM796 Evokes Yawning and Increases Oxytocin Secretion from the Posterior Pituitary Gland in Rats

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Received 25 March 1995; Revised 7 September 1995; Accepted 7 October 1995

FUJIKAWA, M., K. YAMADA, M. NAGASHIMA, M. DOMAE AND T. FURUKAWA. *The new muscarinic M_1-receptor agonist YM796 evokes yawning and increases oxytocin secretion from the posterior pituitary gland in rats.* PHARMACOL BIOCHEM BEHAV **55**(1) 55–60, 1996.—The present experiments were performed to examine the effects of a new muscarinic M_1-receptor agonist, (−)-YM796 ((−)-S-2,8-dimethyl-3-methylene-1-oxa-8-azaspiro[4.5]decane L-tartrate monohydrate), on yawning and oxytocin secretion from the posterior pituitary gland in rats. YM796, at doses of 2.5–50 mg/kg (SC), elicited yawning. The yawning response was markedly increased by pretreatment with a β-adrenoceptor antagonist, pindolol (20 mg/kg, IP), which per se did not elicit yawning. The yawning induced by YM796 (10 mg/kg, SC) in combination with pindolol (20 mg/kg, IP) was inhibited by scopolamine (0.5 mg/kg, SC), a muscarinic receptor antagonist, and pirenzepine (300 μg/rat, ICV) and EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) (5 mg/kg, IP), muscarinic M_1-receptor antagonists, but not by spiperone (0.5 mg/kg, SC), a dopamine D_2-receptor antagonist, 4-DAMP (4-diphenylacetoxy-N-methylpiperidine methiodide) (100 μg/rat, ICV), a muscarinic M_3-receptor antagonist, and [d$(CH_2)_5$, Tyr(Me)^2, Orn^8]-vasotocin (100 ng/rat, ICV), an oxytocin receptor antagonist. YM796 at 2.5–50 mg/kg (SC) did not exert an action on prolactin levels but increased oxytocin secretion from the posterior pituitary gland in rats. This augmentation of oxytocin secretion by YM796 was inhibited by scopolamine (0.5 mg/kg, SC) and pirenzepine (3 mg/kg, SC), but not by mecamylamine (1 mg/kg, IP), a nicotinic receptor antagonist. The present findings obtained with YM796 suggest that the muscarinic M_1-receptor stimulation participates in causing yawning behavior and oxytocin secretion in rats.

Yawning YM796 Muscarinic M_1-receptors β-Adrenoceptor antagonists Oxytocin secretion
Posterior pituitary gland Radioimmunoassay

PREVIOUS behavioral studies, including our experimental findings, have shown that the yawning behavior was induced by dopamine D_2-receptor agonists and was blocked by both dopamine D_2-receptor and muscarinic receptor antagonists, thereby indicating that the behavior involves dopaminergic and cholinergic activation (19,27,28). Cholinesterase inhibitors and muscarinic receptor agonists also induce yawning behavior, which is blocked by muscarinic receptor antagonists, but not by dopamine receptor antagonists, suggesting that the dopaminergic activation precedes cholinergic one (10,11,16,24,27,32). Thus, the dopaminergic-cholinergic activation seems to participate essentially in eliciting yawning behavior (6,10,16, 17,28). Besides, since administration of oxytocin has been reported to cause yawning (1,10,20), oxytocinergic neuronal activities seems to be participated in producing the yawning behavior.

On the other hand, intraventricular administration of bethanechol or carbachol produces the sustained release of oxytocin, which is abolished by atropine, but not by mecamyl-

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amine or hexamethonium (7). In cultured bovine granulosa, continuous exposure to acetylcholine resulted in a dose-dependent increase in oxytocin secretion, which was completely abolished by the specific muscarinic receptor antagonists, atropine and scopolamine, but not by nicotinic receptor antagonists, implying that cholinergic neurotransmitters play a direct role in the regulation of oxytocin release through muscarinic receptors (18).

The muscarinic receptors have been designated as M_1- or M_2-receptors, depending on whether they have high or low affinity for pirenzepine (12). Recently, molecular cloning studies have revealed the existence of five distinct muscarinic receptor proteins (M_1–M_5) (5,14), but their functions are as yet tentative.

(±)-YM796 was reported to exert muscarinic M_1-receptor agonistic activity in the central nervous system, with relatively weak muscarinic M_2-receptor and/or muscarinic M_3-receptor agonistic activity for behavioral changes at a high dose (26). Moreover, the (−)-isomer of YM796 having higher affinity for muscarinic M_1-receptors than (±)-YM796 was identified from the ratio of the IC_{50} value (26,31).

To determine the possible involvement of the M_1 subtype of muscarinic receptor in yawning and oxytocin release, we examined whether a new muscarinic M_1-receptor agonist, (−)-YM796, elicits yawning responses and affects oxytocin release from the posterior pituitary gland in rats.

METHOD

Animals

Male Wistar rats (200–230g) were obtained from Kyudo Animal Laboratory (Kumamoto, Japan). They were kept in an animal room with a 12 L:12 D cycle (lights on at 0700 h). Commercial food (CE-2, Clea Japan Ltd.) and tap water were freely available except during the experiments. All experiments were carried out at an environmental temperature of 23 ± 1°C.

Behavioral Observation

Pairs of rats were placed in transparent plastic boxes (33 × 30 × 17 cm) containing wood shavings. They were allowed to habituate to the observation boxes for 30 min prior to drug injection. Yawns were counted for 60 min immediately after subcutaneous (SC) injection of YM796.

Determination of Serum Oxytocin and Prolactin Levels

Blood was taken from the trunks of decapitated rats and centrifuged at 3000 × *g* for 30 min. Separated serum was stored at −40°C until the hormone assay. Oxytocin levels (13) and prolactin levels (8,19,30) in serum were measured by radioimmunoassay as previously reported.

Administration of Drugs

To count the yawning behavior, (−)-YM796 (2.5 − 50 mg/kg) was SC injected to the neck area in rats. Time intervals between treatment with respective receptor antagonists and YM796 (SC) were 30 min for pindolol (20 mg/kg, IP), spiperone (0.5 mg/kg, SC), scopolamine (0.5 mg/kg, SC) and EEDQ (20 mg/kg, IP) and 15 min for pirenzepine (300 μg/rat, ICV), 4-DAMP (100 μg/rat, ICV) and [d(CH_2)$_5$, Tyr(Me)^2, Orn^8]-vasotocin (100 ng/rat, ICV).

To evaluate the effects on serum oxytocin levels, saline (1 ml/kg) or YM796 (2.5–50 mg/kg) was injected SC 30 min and nicotine (2.5 mg/kg) IP 10 min before sacrifice. Pirenzepine (3 mg/kg, SC), scopolamine (0.5 mg/kg, SC), and mecamylamine (20 mg/kg, IP) were injected 30 min before saline, YM796, or nicotine. To determine the effects on serum prolactin levels, saline (1 ml/kg) or YM796 (2.5–50 mg/kg) was injected SC 30 min before sacrifice.

For ICV injection of pirenzepine (300 μg/rat), 4-DAMP (100 μg/rat), and [d(CH_2)$_5$, Tyr(Me)^2, Orn^8]-vasotocin (100 ng/rat), rats were anesthetized with pentobarbital sodium and placed on a stereotaxic apparatus (Narishige). Stainless steel guide cannulas were implanted into the lateral ventricle of rats using stereotaxic coordinates according to the atlas of Pellegrino et al. (22) (0.2 mm posterior to bregma, 1.5 mm lateral to midline, and 2.5 mm ventral to dura). The cannulas were fixed to the skull with dental cement. At least 2 weeks after surgery, pirenzepine (300 μg in 20 μl of 0.9% NaCl), 4-DAMP (100 μg in 20 μl of 0.9% NaCl) or [d(CH_2)$_5$, Tyr(Me)^2, Orn^8]-vasotocin (100 ng in 20 μl of 0.9% NaCl) was administered for 60 s using a Hamilton syringe (100 μl) connected through polyethylene tubing to an internal cannula that extended 1.0 mm beyond the tip of the guide cannula. After injection, the tip of the cannula was left in the injection site for 60 s to allow the spread of the injected solution. The dosage of oxytocin antagonist was selected according to the previous report by Argiolas et al. (2), and those of other drugs, pindolol, spiperone, scopolamine (16,19,28), nicotine (23,25), and mecamylamine (23) were chosen according to the above previous reports.

Drugs

The following drugs were used: (−)-YM796 ((−)-S-2,8-dimethyl-3-methylene-1-oxa-8-azaspiro[4.5]decane L-tartrate monohydrate) (Yamanouchi Pharmaceutical Co., Ltd., Osaka, Japan), oxytocin (Peptide Institute, Osaka, Japan), pindolol (Sigma, St. Louis, MO), spiperone (Spiropitan Injection, Eisai, Tokyo, Japan), nicotine tartrate (Wakoh Pure Chemical Industries Ltd., Osaka, Japan), mecamylamine hydrochloride (Merck, Darmstadt, Germany), [d(CH_2)$_5$, Tyr(Me)^2, Orn^8]-vasotocin (Peninsula Laboratories, Inc., Belmont, CA), pirenzepine dihydrochloride (Nippon C.H. Boehringer Sohn, Osaka, Japan), EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) (Sigma, St. Louis, MO), 4-DAMP (4-diphenylacetoxy-N-methylpiperidine methiodide) (Research Biochemicals Inc., Natick, MA), and scopolamine hydrobromide (Nacalai tesque, Kyoto, Japan). Pindolol was dissolved in an excess of equimolar tartaric acid solution, with subsequent dilution in saline and EEDQ was dissolved in an ethanol:water (1:1, v/v) solution. Both the agents were administered IP into experimental animals. The other drugs dissolved in saline were injected SC, IP, or ICV into experimental animals as mentioned above. Doses were expressed in terms of respective salts, with the exception of oxytocin, pindolol, spiperone, [d(CH_2)$_5$, Tyr(Me)^2, Orn^8]-vasotocin and EEDQ.

Statistical Analysis

Yawning responses were expressed as mean values ± SEM. Statistical analysis was done using either two-tailed Student's *t*-test for differences between two groups or a one-way analysis of variance (ANOVA) followed by two-tailed Dunnett's test for differences between the control and all groups. Oxytocin and prolactin levels were expressed as mean values ± SEM. Statistical analysis of these levels was done using a one-way ANOVA followed by two-tailed Dunnett's test for differences

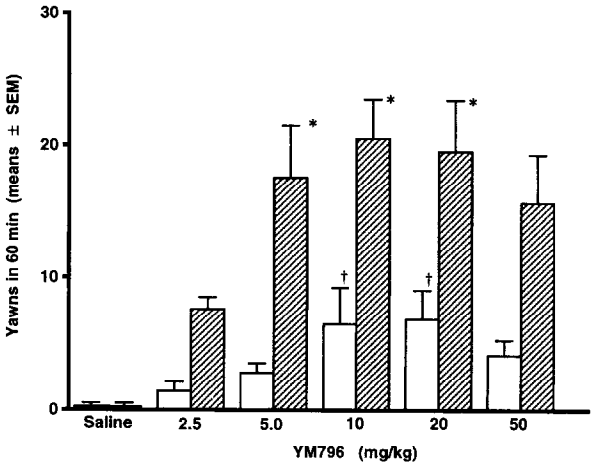

FIG. 1. Dose–response of yawning to YM796 in saline- or pindolol-pretreated rats. The number of yawns was counted for 60 min following injection of YM796 (2.5–50 mg/kg, SC). Pindolol (20 mg/kg, IP) or saline (1 ml/kg, IP) was administered 30 min before YM796. Open columns indicate saline-pretreated groups and hatched columns pindolol-pretreated groups. Columns represent means ± SEM (vertical lines) of the number of yawns from eight rats during a 60-min observation period. †$p < 0.01$; significant difference from saline plus saline-injected group, determined by a one-way ANOVA followed by Dunnett's test. *$p < 0.01$; Significant difference between saline- and pindolol-pretreated groups, determined by Student's t-test.

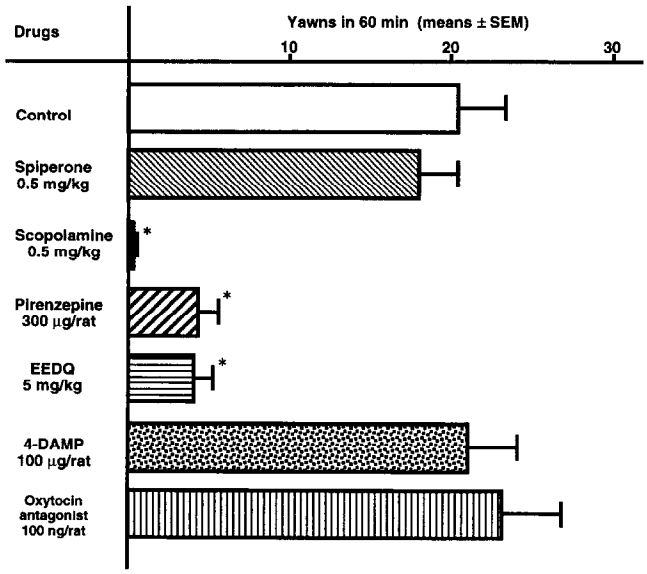

FIG. 2. Effects of various receptor antagonists on the yawning induced by YM796 in combination with pindolol in rats. Pindolol (20 mg/kg, IP) was administered 30 min before YM796 (10 mg/kg, SC). Pirenzepine (300 μg/rat), 4-DAMP (100 μg/rat) and oxytocin receptor antagonist (100 ng/rat) were administered intracerebroventricularly (ICV) 15 min and spiperone (0.5 mg/kg, SC), EEDQ (5 mg/kg, IP), and scopolamine (0.5 mg/kg, SC) 30 min before YM796. Values represent means ± SEM (horizontal lines) of the number of yawns from 8–12 rats. *$p < 0.01$: Significant difference from the control group, determined by Dunnett's test.

between the control and all means and Tukey's test for differences between all means.

RESULTS

Yawning Induced by YM796 Administered Alone or in Combination with Pindolol

Control rats treated with saline (1 ml/kg, SC) yawned only occasionally. Similarly, pindolol (20 mg/kg, IP) administered alone evoked no behavioral changes including yawning. YM796 (2.5–50 mg/kg, SC) induced yawning in saline-pretreated rats, the maximal effect (6.8 ± 2.2 yawns in 60 min) being observed at a dose of 20 mg/kg (Fig. 1). In addition, YM796-elicited yawning was markedly increased by pretreatment with pindolol, forming a bell-shape with the maximal effect at 10 mg/kg (20.4 ± 3.0 yawns in 60 min) (Fig. 1).

Effects of Various Antagonists on the Yawning Induced by YM796 Administered in Combination with Pindolol

As shown in Fig. 2, control rats were treated with YM796 (10 mg/kg, SC) after administration of pindolol (20 mg/kg, IP). The yawning induced by YM796 after pindolol was strongly inhibited by pretreatment with scopolamine (0.5 mg/kg, SC), a muscarinic receptor antagonist, pirenzepine (300 μg/rat, ICV), or EEDQ (5 mg/kg, IP), muscarinic M_1-receptor antagonists, but was not influenced by spiperone (0.5 mg/kg, SC), a dopamine D_2-receptor antagonist, 4-DAMP (100 μg/rat, ICV), a muscarinic M_3-receptor antagonist, and [d$(CH_2)_5$, Tyr(Me)^2, Orn^8]-vasotocin (100 ng/rat, ICV), an oxytocin receptor antagonist (Fig. 2). None of these six receptor antagonists administered alone elicited yawning.

Effects of YM796 on Serum Oxytocin Levels

The mean serum prolactin level in the saline-treated group was 16.3 ± 2.3 ng/ml. Prolactin levels were not changed in YM796-treated rats at respective doses of 2.5, 5, 10, 20, and 50 mg/kg, as compared with the saline-treated group (data not shown). At the same dose range of YM796 (2.5–50 mg/kg), oxytocin levels were dose dependently increased as compared with the saline-treated group in which the mean serum oxytocin level was 22.9 ± 1.9 pg/ml (Fig. 3).

Effects of Muscarinic and Nicotinic Receptor Antagonists on YM796-Induced Increases in Serum Oxytocin Levels

As shown in Fig. 4, YM796 (50 mg/kg, SC) and nicotine (2.5 mg/kg, IP) increased the oxytocin levels, the increased level being 84.6 ± 5.9 and 87.8 ± 7.1 pg/ml, respectively. Scopolamine (0.5 mg/kg, SC), a muscarinic receptor antagonist, pirenzepine (3 mg/kg, SC), a muscarinic M_1-receptor antagonist, or mecamylamine (1 mg/kg, IP), a nicotinic receptor blocking agent, did not affect the static levels of oxytocin. The oxytocin level elevated by YM796 (50 mg/kg, SC) was markedly inhibited by pretreatment with scopolamine. The level was also inhibited by pirenzepine, but was unaffected by mecamylamine, which reduced the nicotine-produced high level of oxytocin.

DISCUSSION

(−)-YM796, a novel muscarinic M_1-receptor agonist, was capable of inducing yawning in this study. Thus, the present result confirms the previous reports describing that RS-86

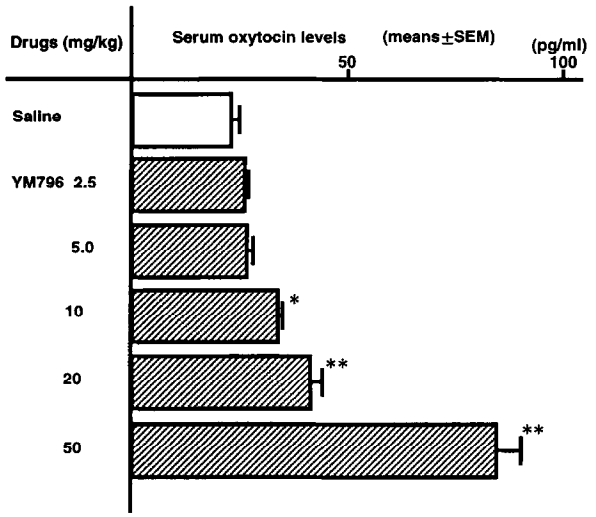

FIG. 3. Effects of YM796 on serum oxytocin levels in rats. Saline (1 ml/kg) and YM796 (2.5–50 mg/kg) were injected subcutaneously 30 min before sacrifice. Oxytocin levels are shown as means ± SEM (horizontal lines) from 10 rats. $*p < 0.05$, $**p < 0.01$: Significant difference from saline-injected group, determined by Dunnett's test.

(10,26) and AF102B (9,21,26), putative muscarinic M_1-receptor agonists, induce yawning. As for the potency of producing yawning, the frequencies were 6.8 ± 2.2 yawns in 60 min by YM796, a muscarinic M_1-receptor agonist, alone at 10 mg/kg SC and 20.4 ± 3.0 yawns by YM796 in combination with pindolol in this study. The frequency produced by pilocarpine, a muscarinic M_1- and M_2-receptor agonist, was 3.1 ± 0.6 yawns in 60 min at 4 mg/kg, IP alone and 20.9 ± 3.1 yawns in combination with pindolol under similar experimental conditions in our previous reports (28). Studies on the agents that act selectively on muscarinic M_3-receptors have not been reported. Accordingly, the muscarinic M_1-receptors are definitely involved in causing yawning, but the possible involvement of muscarinic M_2- and M_3-receptors may not be dismissed at present.

In our previous reports (28,29), blockade of β-adrenoceptors caused by central β-adrenoceptor antagonists, pindolol, and others, which reach the brain through the blood–brain barrier, but not by peripheral β-adrenoceptor antagonists, facilitates the occurrence of yawning induced by dopaminergic agonists, such as apomorphine and talipexole (B-HT 920). Administration of LY-78335 and UK-1187A, phenylethanolamine-*N*-methyltransferase inhibitors, which decrease adrenaline formation without affecting dopamine and noradrenaline levels in the brain, similarly potentiates the incidence of yawning as does β-adrenoceptor blockade (16). Therefore, the central adrenergic neuronal system was proposed to participate in the regulation of yawning for suppressive direction via β-adrenoceptors (16). The yawning induced by YM796 was also potentiated by treatment with the β-adrenoceptor antagonist, pindolol, in the present study.

The yawning induced by muscarinic receptor agonists, AF102B and RS-86, has been reported to be blocked by muscarinic receptor antagonists but to be unaffected by dopamine receptor antagonists (10,11). In the present study, the yawning behavior elicited by YM796 administered after pindolol was inhibited by scopolamine, a muscarinic receptor antagonist, and was also antagonized by pirenzepine and EEDQ,

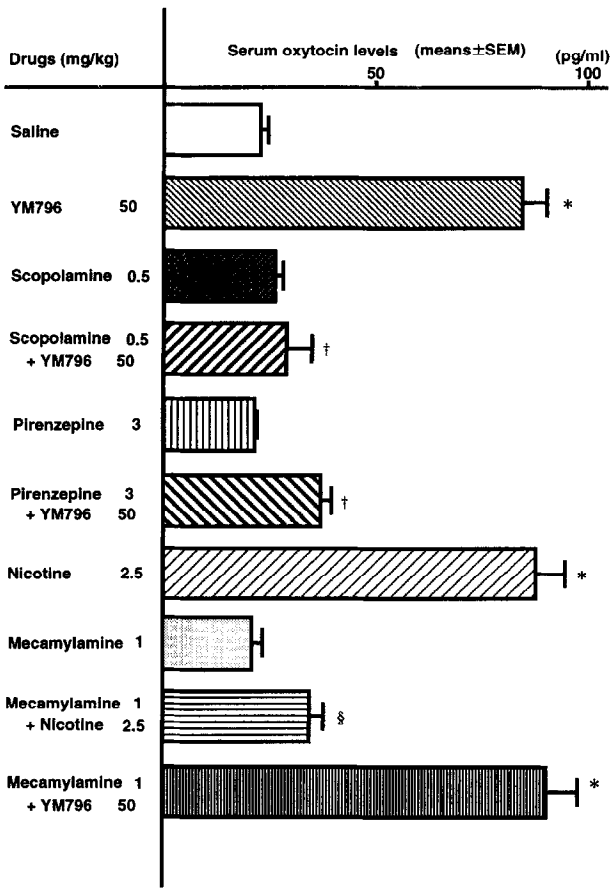

FIG. 4. Effects of various receptor antagonists on YM796-induced increases in serum oxytocin levels. Saline (1 ml/kg, SC) or YM796 (50 mg/kg, SC) was injected 30 min and nicotine (2.5 mg/kg, IP) 10 min before sacrifice. Scopolamine (0.5 mg/kg, SC) or pirenzepine (3 mg/kg, SC) was injected 30 min before saline or YM796. Mecamylamine (1 mg/kg, IP) was injected 30 min before saline, YM796 or nicotine. Oxytocin levels are demonstrated as means ± SEM (horizontal lines) from 10 rats. $*p < 0.01$: Significant difference from saline-injected group, $\dagger p < 0.01$: that from YM796-injected group, and $\S p < 0.01$: that from nicotine-injected group, respectively, determined by Tukey's test.

muscarinic M_1-receptor antagonists, without being affected by 4-DAMP, a muscarinic M_3-receptor antagonist, and spiperone, a dopamine D_2-receptor antagonist. Thus, muscarinic M_1-receptors are involved in the induction of the yawning behavior.

Nicotinic receptor stimulation causes an increase of oxytocin release (3,4,15), and the muscarinic pathway also seems to be involved in regulating oxytocin release (7). In the present study, serum oxytocin levels were increased in a dose-dependent manner not only by nicotine but also by YM796, indicating the participation of both nicotinic and muscarinic receptor mechanisms. The elevation of oxytocin levels produced by YM796 was inhibited by scopolamine, a muscarinic receptor antagonist, but not by mecamylamine, a nicotinic receptor antagonist, thus implying that the muscarinic receptor stimulation participates in the acceleration of oxytocin release from the posterior pituitary gland. In further experiments, pirenzepine, a muscarinic M_1-receptor antagonist, did not affect the static oxytocin levels but antagonized the elevation of the

oxytocin level by YM796. These findings indicate that muscarinic M_1-receptors play a role in increasing oxytocin release.

As for possible involvement of oxytocinergic neuronal activities in the yawning, central administration of oxytocin has been reported to elicit yawning behavior (1,10,20). Therefore, it might be a problem whether or not the release of oxytocin found in the plasma is related to the yawning response. In this study, the yawning induced by YM796 after pindolol was not prevented by the oxytocin receptor blockade. Accordingly, the release of oxytocin from the neurohypophysis after YM796 may not be related to the yawning response. This would also suggest that central and peripheral oxytocin release is differentially regulated and YM796 acts at another level in the central nervous structures involved in the regulation and/or expression of this behavior. Moreover, as described in the introduction, the dopaminergic-cholinergic neuronal linkage seems to be essentially involved in eliciting yawning behavior. In fact, YM796-induced yawning was inhibited by muscarinic antagonists, but not by a dopamine D_2-receptor antagonist in the present study. There has been also the proposal that the expression of yawning induced by dopaminergic agonists involves dopamine–oxytocin, but not oxytocin–dopamine, neuronal linkage (10,20). Besides, the oxytocin-elicited yawning behavior is inhibited by oxytocin receptor antagonists and muscarinic receptor antagonists, but not by dopamine receptor antagonists (1,10). In this study, the YM796-induced yawning was antagonized by muscarinic M_1-receptor antagonists, but not by the oxytocin receptor antagonist. Therefore, the findings suggest that the oxytocinergic mechanism precedes the muscarinic one and is in position between dopaminergic and muscarinic mechanism in producing yawning behavior.

The present findings suggest that the muscarinic M_1-receptor stimulation is involved in the occurrence of yawning behavior and secretion of oxytocin from the posterior pituitary gland in rats.

ACKNOWLEDGEMENTS

The authors are grateful to Yamanouchi Pharmaceutical Company (Osaka, Japan) for the gift of YM796 and Prof. Higuchi for its generous provision of the anti-rat oxytocin serum.

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