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Possible Involvement of β_1 Receptors in Various Emetogen-Induced Increases in Salivary Amylase Activity in Rats

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Abstract. We investigated the inhibitory effects of β_1- or β_2-adrenoceptor (AR) antagonists on salivary amylase secretion produced by various emetic agents, such as cisplatin, apomorphine, and lithium chloride (LiCl), or the non-emetic agent $\beta_{1/2}$-AR agonist isoprenaline in rats. We also determined the inhibitory effect of metoclopramide, a dopamine D_2–receptor antagonist, on increases in the salivary amylase activity induced by apomorphine or granisetron, a 5-HT_3–receptor antagonist, on LiCl-induced increased salivary amylase activity. Isoprenaline (0.01 mg/kg, s.c.) produced an increase in salivary amylase and the increase was inhibited by the $\beta_{1/2}$-AR antagonist propranolol (5 mg/kg, s.c.) and β_1-AR antagonist atenolol (2 mg/kg, s.c.) but not by the β_2-AR antagonist butoxamine (8 mg/kg, s.c.). The increased amylase activity induced by cisplatin (15 mg/kg, i.v.), apomorphine (3 mg/kg, s.c.), or LiCl (120 mg/kg, i.p.) was inhibited significantly by atenolol (2 mg/kg, s.c.) but not by butoxamine (8 mg/kg, s.c.). In addition, increases in amylase activities induced by apomorphine and LiCl were inhibited significantly by metoclopramide (10 mg/kg, i.v.) and granisetron (3 mg/kg, i.v.), respectively. These results suggest that salivary amylase secretion induced by various emetogens is involved in β_1-adrenoceptor activity and that salivary amylase activity is useful to detect emetogens with no direct β_1-AR activation in rats, a species that does not exhibit vomiting.

Keywords: rat salivary amylase activity, vomiting, emesis, β_1 adrenoceptor

Introduction

Nausea and vomiting are still recognized as significant adverse effects of cancer chemotherapy. However, studies of the mechanisms of vomiting and the development of new anti-emetic agents have been limited because emesis has not been observed in common laboratory rats and animal models available for studies on emesis such as ferrets, dogs, and monkeys are expensive, difficult to handle, and their use is sometimes resisted by animal rights advocates in many countries. Thus, emesis research using small animals would be valuable for the development of this field.

In the research on emesis in rats, pica, the eating of non-nutritive substances such as kaolin, has been suggested as an adverse response behavior analogous to vomiting in species that have a developed emetic reflex (1, 2). It has been reported that various compounds such as cisplatin, copper sulfate, apomorphine, and lithium chloride (LiCl), which can cause emesis, induce increased kaolin consumption (2 – 5) and some anti-emetics can suppress pica induced by emetic agents (1, 6, 7). This suggests that pica could be used to evaluate emesis in rats and could be a good index of emesis. Our recent study in rats has shown that various stimuli such as cisplatin, apomorphine, LiCl, rolipram, or sibutramine induced a significant increase in salivary amylase secretion and granisetron, a 5-HT_3–receptor antagonist, or bilateral abdominal vagotomy inhibited or tended to inhibit cisplatin-induced acute increases in salivary amylase (8). This study might shed some light on the emesis research using rats. Salivary amylase is thought to reflect changes in noradrenaline levels due to increased activation of the sympathetic-adrenal-medullary system (9, 10). It is conceivable that salivary amylase is released due to activation of β-adrenoceptors (ARs) in the acinar cells of the

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parotid glands (11) and/or the sympathetic nerves in the central nervous systems. However, the involvement of the sympathetic nerves or β_1- and β_2-ARs on the secretion of salivary amylase induced by various stimuli such as cisplatin, apomorphine, and LiCl has not yet been clarified.

In the present study, the effects of the β_1-AR antagonist atenolol or the β_2-AR antagonist butoxamine on increased salivary amylase activity induced by various emetogens such as cisplatin, apomorphine, and LiCl and a non-emetic agent such as $\beta_{1/2}$-AR agonist isoprenaline were investigated. In addition, the antiemetic effect of a dopamine D_2–receptor antagonist on the apomorphine-induced secretion of salivary amylase and that of a 5-HT_3–receptor antagonist on LiCl-induced secretion of salivary amylase were also investigated.

Materials and Methods

Animals

Male Wistar (Crlj:WI) rats aged 5 weeks were obtained from Charles River Japan (Yokohama) and were acclimatized to the environmental conditions for 1 week after receipt. The rats were weighed and assigned randomly to each of the treated and control groups. At dosing, the animals were 6-week-old and their body weights were 226 ± 1 g (mean ± S.E.M.). These animals were placed in an animal room with a temperature of 20°C – 26°C, relative humidity of 40% – 70%, and a 12-h light/dark cycle. The animals were allowed free access to a commercial pelleted diet and tap water. All animals were used only once. Procedures involving the animals and their care were conducted in conformity with the institutional guidelines.

Salivary amylase activity induced by isoprenaline or noradrenaline and the effects of $\beta_{1/2}$-AR antagonist propranolol, β_1-AR antagonist atenolol, or β_2-AR antagonist butoxamine on increased salivary amylase activity induced by isoprenaline

The amylase activity induced after dosing with isoprenaline (0.01 mg/kg, s.c.) or noradrenaline (0.1 mg/kg, s.c.) was examined. The amount of saliva following administration of each compound was measured at each sampling point. The control animals for isoprenaline and noradrenaline received the same volume of physiological saline in a similar way. The effect of subcutaneous (s.c.) administration of the $\beta_{1/2}$-AR antagonist propranolol (5 mg/kg), β_1-AR antagonist atenolol (2 mg/kg), or β_2-AR antagonist butoxamine (8 mg/kg) on the increased salivary amylase levels induced by isoprenaline was examined and each of them was administered 15 min before isoprenaline. Control animals received the same volume of physiological saline in a similar way.

Assessment of the effects of atenolol or butoxamine on increased salivary amylase activity induced by cisplatin, apomorphine, and LiCl

The effects of s.c. administration of atenolol (2 mg/kg) or butoxamine (8 mg/kg) on the enhanced salivary amylase levels induced by cisplatin (15 mg/kg, i.v.), apomorphine (3 mg/kg, s.c.), or LiCl (120 mg/kg, i.p.) were examined. Each of them was administered 15 min before and 1.25 h after cisplatin or 15 min before apomorphine or LiCl. Control animals received the same volume of physiological saline in a similar way.

Assessment of the effects of metoclopramide or granisetron on increased salivary amylase activity induced by apomorphine or LiCl

The effects of metoclopramide (10 mg/kg, i.v.) or granisetron (3 mg/kg, i.v.) on the enhanced salivary amylase levels induced by apomorphine (3 mg/kg, s.c.) or LiCl (120 mg/kg, i.p.) were examined. Each of them was administered 15 min before apomorphine or LiCl. Control animals received the same volume of physiological saline in a similar way.

Measurement of amylase activity in rat saliva

The weights of roller cotton balls (3 mm in diameter; Richmond Dental, Charlotte, NC, USA) and the disposal tubes were measured together beforehand. The rats were fasted and water-deprived for at least 30 min before measurement. The cotton ball was inserted under their tongues with forceps. The cotton ball was taken out of their mouths about 1 min later and was weighed. The quantity of saliva was calculated from the difference in weight. Saliva was diluted 1:30 or 1:50 with physiological saline by assuming 1 mg as 1 μl of saliva. The activity of the salivary amylase in the solution was determined by an automated blood chemistry analyzer (Hitachi 7600; Hitachi, Tokyo) using an amylase determination kit (L-Type Amylase; Wako Pure Chemical Industry, Ltd., Osaka) containing *p*-nitrophenylbenzyl-α-maltopentaoside as the substrate. The salivary amylase activity was also expressed by the area under the concentration–time curve (AUC) values. The AUC values for each animal after dosing with each drug were calculated from the measured activities at the following limited 1 to 3 sampling points by the trapezoidal rule. These points included before dosing, one or a few points showing the maximum amylase activity, and/or one point when the activity had returned or tended to return to control levels. The mean and S.E.M. of AUC values were calculated for each dose group. Samples of saliva were collected before and at 1.5 h after the administration of cisplatin in combination

with each antagonist. The saliva was collected before and at 0.25 and 1 h after apomorphine and each antagonist or before and at 1 and 3 h after LiCl and each antagonist. Sampling points in the cisplatin, apomorphine, or LiCl experiment were selected based on the results of the previous study (8).

Statistics

The data in the figures are expressed as the means ± S.E.M. Data on the AUC values for amylase activity induced by isoprenaline, noradrenaline, cisplatin, apomorphine, or LiCl and the amount of saliva induced by isoprenaline or noradrenaline were analyzed for differences from the control. An F-test followed by Student's or Welch's *t*-test was performed to compare the means with that for the control group.

Drugs

Isoprenaline hydrochloride (Tokyo Chemical Industry Co., Ltd., Tokyo) and noradrenaline (Daiichi Sankyo Co., Ltd., Tokyo) were dissolved in physiological saline and were administered s.c. at dosages of 0.01 and 0.1 mg/kg. Propranolol hydrochloride (Wako Pure Chemical Industries, Ltd.), atenolol (Wako Pure Chemical Industries, Ltd.), and butoxamine hydrochloride (Sigma-Aldrich Japan K.K., Tokyo) dissolved in physiological saline were administered s.c. at 5, 2, and 8 mg/kg, respectively. Cisplatin (Sigma-Aldrich Japan K.K.) was purchased and injected intravenously (i.v.) at 15 mg/kg. Granisetron hydrochloride (Nichi-Iko Pharmaceutical Co., Ltd., Toyama) was administered i.v. at 3 mg/kg. Apomorphine hydrochloride hemihydrate (Sigma-Aldrich Japan K.K.) dissolved in physiological saline was administered s.c. at the dosage level of 3 mg/kg. Metoclopramide hydrochloride (Sigma-Aldrich Japan K.K.) dissolved in physiological saline was administered i.v. at 10 mg/kg. Lithium chloride monohydrate (LiCl; Wako Pure Chemical Industries, Ltd.) for intraperitoneal (i.p.) injection was dissolved in physiological saline and was administered i.p. at a dosage of 120 mg/kg. The injection volumes were 1 ml/kg for cisplatin, apomorphine, propranolol, atenolol, butoxamine, or metoclopramide; 1.5 ml/kg for isoprenaline or noradrenaline; 2 ml/kg for LiCl; and 3 ml/kg for granisetron. Each drug was administered immediately after preparation.

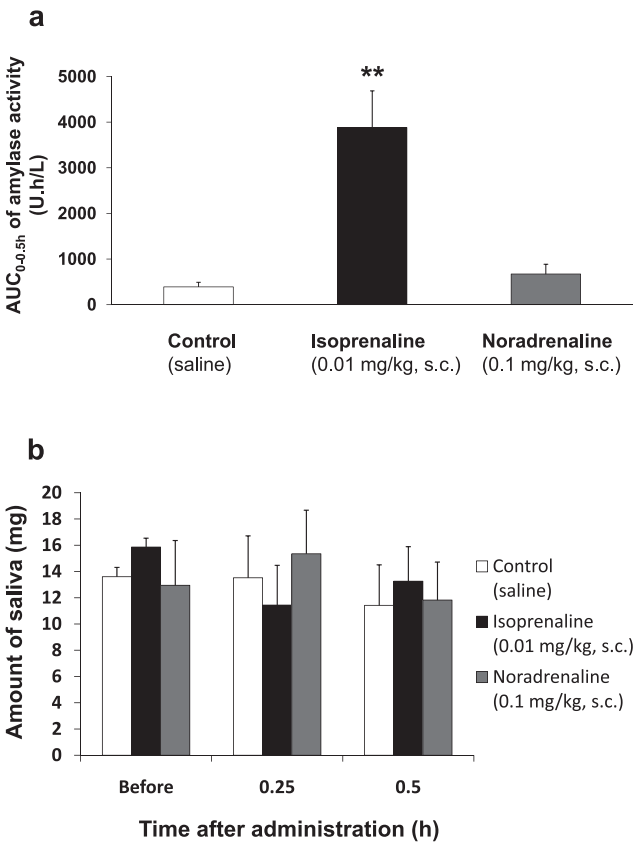

Fig. 1. The $AUC_{0-0.5h}$ values of amylase activity (a) and the amount of saliva (b) in rats following isoprenaline (0.01 mg/kg, s.c.) or noradrenaline (0.1 mg/kg, s.c.) administration. Vertical bars represent the mean degree of the amylase activity or the amount of saliva with S.E.M. (n = 5). Control animals were given saline solution s.c. Compared with the control group at each respective time point; $^{**}P < 0.01$ (Student's or Welch's *t*-test).

Results

Salivary amylase activity induced by isoprenaline or noradrenaline and the effects of the $\beta_{1/2}$-AR antagonist propranolol, β_1-AR antagonist atenolol, or β_2-AR antagonist butoxamine on increased salivary amylase activity induced by isoprenaline

The patterns of the salivary amylase activity and the amounts of saliva with isoprenaline or noradrenaline are summarized in Figs. 1 and 2. Statistically significant increases in the $AUC_{0-0.5h}$ values for salivary amylase were produced by s.c. administration of 0.01 mg/kg isoprenaline, but not by 0.1 mg/kg noradrenaline (Fig. 1a). No significant differences were observed in the volume of saliva at almost all points (Fig. 1b). The increase in the $AUC_{0-0.5h}$ values of amylase activity induced by isoprenaline was inhibited by s.c. administration of 5 mg/kg propranolol and 2 mg/kg atenolol, but not by 8 mg/kg butoxamine (Fig. 2).

The effects of atenolol or butoxamine on increased salivary amylase activity induced by cisplatin

The results are shown in Fig. 3. Cisplatin at 15 mg/kg caused a significant increase in the $AUC_{0-1.5h}$ values of salivary amylase activity. Atenolol (2 mg/kg, s.c.) significantly inhibited an increase in the $AUC_{0-1.5h}$, but bu-

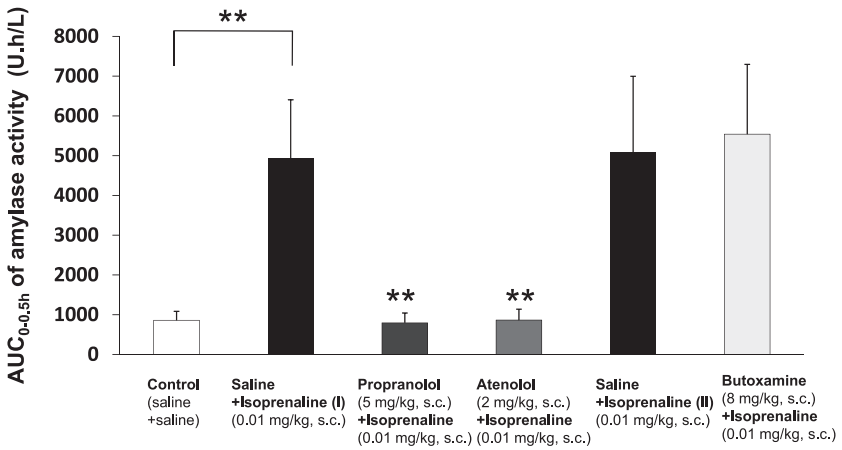

Fig. 2. Effects of propranolol (5 mg/kg, s.c.), atenolol (2 mg/kg, s.c.), or butoxamine (8 mg/kg, s.c.) on increased salivary amylase activity induced by isoprenaline (0.01 mg/kg, s.c.). Vertical bars represent the mean $AUC_{0–0.5h}$ values of the amylase activity with S.E.M. (n = 6 – 11). Control animals were given saline solution s.c. Compared with the control group: $^{**}P < 0.01$ (Student's or Welch's *t*-test). The effects of propranolol and atenolol were compared to the data of isoprenaline (I), and those of butoxamine were compared to the data of isoprenaline (II) because these studies were conducted separately.

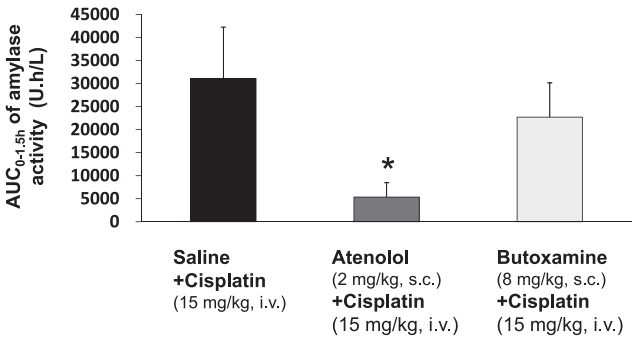

Fig. 3. Effects of atenolol (2 mg/kg, s.c.) or butoxamine (8 mg/kg, s.c.) on increased salivary amylase activity in rats following cisplatin administration (15 mg/kg, i.v.). Vertical bars represent the mean $AUC_{0–1.5h}$ values of the amylase activity with S.E.M. (n = 10). Control animals were given saline solution s.c. Compared with the control group: $^{*}P < 0.05$ (Student's or Welch's *t*-test).

toxamine (8 mg/kg, s.c.) had no significant effects.

The effects of metoclopramide, atenolol, or butoxamine on increased salivary amylase activity induced by apomorphine

The results are shown in Fig. 4, a and b. Apomorphine induced increases in the $AUC_{0–1h}$ values for salivary amylase at 3 mg/kg, and the $AUC_{0–1h}$ values were suppressed by metoclopramide (10 mg/kg, i.v.) and atenolol (2 mg/kg, s.c.), but not by butoxamine (8 mg/kg, s.c.).

The effects of granisetron, atenolol, or butoxamine on increased salivary amylase activity induced by LiCl

The results are shown in Fig. 5. The $AUC_{0–3h}$ values for salivary amylase were increased at 120 mg/kg of LiCl. The increased $AUC_{0–3h}$ values were significantly inhibited by granisetron (3 mg/kg, i.v.) and atenolol (2 mg/kg, s.c.), but not by butoxamine (8 mg/kg, s.c.).

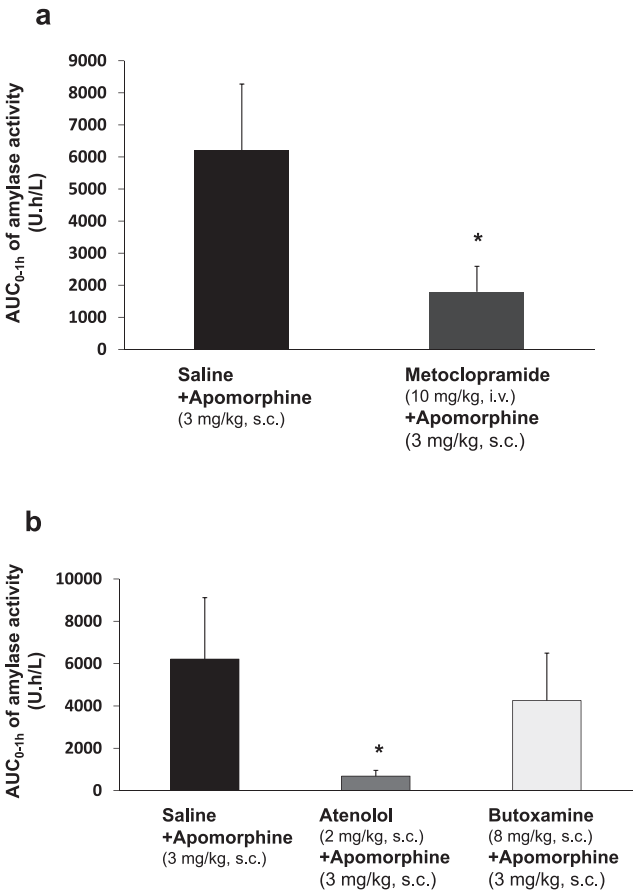

Fig. 4. Effects of metoclopramide (10 mg/kg, i.v.; panel a), atenolol (2 mg/kg, s.c.; panel b), or butoxamine (8 mg/kg, s.c.; panel b) on increased salivary amylase activity in rats following apomorphine administration (3 mg/kg, s.c.). Vertical bars represent the mean $AUC_{0–1h}$ values of the amylase activity with S.E.M. (n = 9 – 12). Control animals were given saline solution s.c. Compared with the control group; $^{*}P < 0.05$ (Student's or Welch's *t*-test).

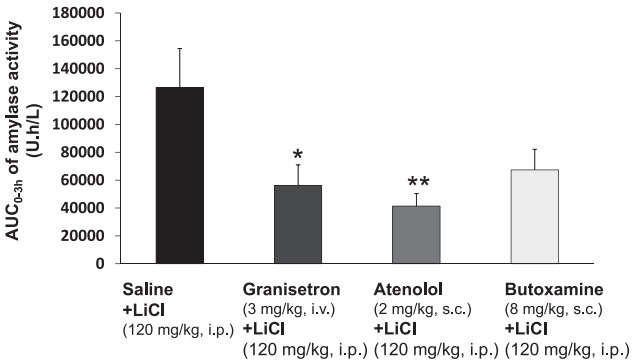

Fig. 5. Effects of granisetron (3 mg/kg, i.v.), atenolol (2 mg/kg, s.c.), or butoxamine (8 mg/kg, s.c.) on increased salivary amylase activity in rats following LiCl administration (120 mg/kg, i.p.). Vertical bars represent the mean AUC_{0-3h} values of the amylase activity with S.E.M. (n = 10 – 11). Control animals were given saline solution s.c. Compared with the control group: $*P < 0.05$, $**P < 0.01$ (Student's or Welch's *t*-test).

Discussion

Salivary amylase is thought to reflect changes in noradrenaline levels due to increased activation of the sympathetic–adrenal-medullary system (9, 10). It is considered that salivary amylase is released due to direct activation of the β_1-ARs in the acinar cells of the parotid glands (11). On the other hand, salivary amylase is induced by activation of the sympathetic nerves in the central nervous systems. It is well known that the β-AR agonist isoprenaline is the most potent secretagogue in amylase release from the rat parotid gland (12). The β-ARs in the rat parotid gland play the major role in the regulation of the secretion of amylase, whereas the α-ARs play a minor role in the regulation of this parameter (13). It is also described in an in vitro study that the parotid salivary amylase activity responds with an increase to the administration of isoprenaline, whereas submandibular salivary amylase activity does not, and this effect was inhibited by the selective β_1-AR antagonist atenolol, but not by the β_2-AR antagonist butoxamine (14). The present study demonstrated that isoprenaline increased amylase secretion in rat saliva and the increased amylase was inhibited by both the $\beta_{1/2}$-AR antagonist propranolol and atenolol but not by butoxamine. These results suggest that the secretion of salivary amylase induced by isoprenaline is attributable to direct action on the β_1-ARs in the acinar cells of the parotid glands. On the contrary, s.c. bolus administration of 0.1 mg/kg noradrenaline failed to increase salivary amylase levels, although a 1.5 mg/kg-h i.v.–infusion of noradrenaline is reported to increase salivary amylase in rats (15), suggesting that a certain level of noradrenaline concentrations in plasma might be needed to elicit the secretion of salivary amylase.

When an emetic is given to rats, this may automatically excite the sympathetic nervous system and/or suppress the parasympathetic nervous system and decrease the motility of the gastrointestinal tract to prevent the toxin from being absorbed. This phenomenon might represent one of the protective responses. This excitation of the sympathetic nervous system may result in the secretion of salivary amylase. Although the reason why the sympathetic nervous system is excited by emetics is not clear, this may be secondary to stress from the vomiting response to emetic stimuli. On the other hand, it is reported that the activation of the parasympathetic nerves causes salivary secretion containing a significant quantity of amylase, which is even increased in the absence of sympathetic innervation (16). However, saliva evoked from the parotid gland of the rat from parasympathetic activity contains very low levels of amylase activity (17), whereas saliva after sympathetic activity contains amylase in a concentration that is much higher (about 20 – 25 times) than that in saliva after parasympathetic activity (18). In addition, the stress caused by the vomiting response to emetic stimuli may not be able to activate both the sympathetic and parasympathetic nerves simultaneously. In the present study, increased amylase secretion in rat saliva was observed following dosing with all the emetic agents used and the increased amylase was inhibited by atenolol but not by butoxamine. These results suggest that all emetogens may also induce an increase in amylase activity by activating mainly the β_1-ARs in the parotid gland resulting from activation of the sympathetic nerves, even though involvement of the parasympathetic nerves cannot be completely ruled out.

The present study demonstrated that a dopamine D_2–receptor antagonist inhibited the increased activity of salivary amylase produced by apomorphine and the 5-HT_3 antagonist granisetron inhibited the LiCl-induced amylase secretion. These results are also in good agreement with those in rats (6), dogs (19), and suncus (20). In previous studies, abdominal vagotomy does not inhibit the increased activity of salivary amylase produced by LiCl in rats (8) or vomiting induced by apomorphine in dogs (19), indicating that central D_2 and 5-HT_3 receptors are mainly involved in the salivary amylase secretion due to apomorphine and LiCl, respectively. In addition, peripheral 5-HT_3 receptors mainly mediate the increase in amylase in rat saliva induced by cisplatin (8). From the results of the previous and present studies, the secretory pathway of the salivary glands could be accounted for as follows: The stimuli from peripheral 5-HT_3 receptors due to cisplatin, central dopamine D_2 receptors due to apomorphine, and central 5-HT_3 receptors due to LiCl acti-

vate the sympathetic nerves in the central nervous systems. The excitation of the sympathetic nerves may activate the sympathetic-adrenal-medullary system (9, 10). The increased activation of this system may result in the release of noradrenaline from the adrenal gland and then in increased noradrenaline levels in plasma. This increase in plasma noradrenaline may activate the β_1-ARs in the acinar cells of the parotid glands. β_1-AR activation causes elevation of intracellular cAMP, which is linked to the secretion of salivary proteins that are stored in membrane-bound secretory granules (21, 22). As a result, salivary amylase activity may be increased following the administration of various emetogens to rats. In fact, it is reported that noradrenaline levels in plasma are increased when LiCl is injected to rats (23), indicating that the increased amylase activity might be dependent on hormone levels. On the other hand, there are no reports on the emetic effects of isoprenaline in the species showing emesis, suggesting that direct action of the sympathetic nerves through β_1-ARs on the rat parotid gland fails to produce emesis. Therefore, it may be possible to detect emetogens except for compounds modulating the autonomic nervous systems directly.

In conclusion, salivary amylase secretion induced by various emetogens such as cisplatin, apomorphine, and LiCl and a non-emetic agent such as the $\beta_{1/2}$-AR agonist isoprenaline is inhibited by atenolol, a β_1-AR antagonist, but not by butoxamine, a β_2-AR antagonist. These results suggest that salivary amylase activity is useful for detecting emetogens with no direct β_1-AR activation in rats a species that does not exhibit vomiting.

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