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# Various Emetogens Increase the Secretion of Salivary Amylase in Rats: a Potential Model in Emesis Research

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**Abstract.** We investigated the effects of various emetic agents: cisplatin, apomorphine, lithium chloride (LiCl), rolipram, sibutramine, and the $\beta_3$-adrenoceptor (AR) agonist CL316243 on salivary amylase secretion in rats. We also determined the inhibitory effect of granisetron, a 5-$HT_3$–receptor antagonist, on cisplatin-induced increased salivary amylase activity and the inhibitory effect of bilateral abdominal vagotomy on increases in salivary amylase activity induced by cisplatin and LiCl. Granisetron was administered 15 min before and 1 h after cisplatin administration. Cisplatin (10 – 15 mg/kg, i.v.) increased salivary amylase activity dose-dependently and induced an acute increase from 1.5 h post-treatment with 15 mg/kg. Apomorphine (1 – 3 mg/kg, s.c.), LiCl (120 mg/kg, i.p.), rolipram (3 – 10 mg/kg, p.o.), and sibutramine (10 mg/kg, p.o.) induced significant increases in salivary amylase secretion. On the other hand, CL316243 did not stimulate salivary amylase secretion. The increased amylase activity induced by cisplatin (15 mg/kg, i.v.) was inhibited significantly by granisetron (1 or 3 mg/kg × 2, i.v.) or tended to be inhibited by bilateral abdominal vagotomy, whereas an increase in amylase activity produced by LiCl was not inhibited by abdominal visceral nerve section. These results suggest that salivary amylase activity is useful as a marker for emesis in rats, a species that does not exhibit vomiting.

***Keywords***: rat salivary amylase activity, vomiting, emesis, cisplatin

## Introduction

Nausea and vomiting are some of the most common side effects of medicines, especially in 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 study for vomiting using 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. However, there are some disadvantages in using pica, for example, it is difficult to follow kaolin intake with time, and it is necessary to train animals in the pretreatment period because control animals eat kaolin (8).

When cisplatin is given to rats, gastric emptying is delayed, producing gastric stasis and stomach distention, and the transit of the food in the small and large intestines is influenced by the delayed gastric emptying (9, 10). These may be indicative of a defensive response in the rats because this action probably delays transport of any toxic compounds into the small intestine where they may produce more systemic toxicity, especially if absorbed (11). A delay in motility of the upper gastrointestinal tract including the stomach and small intestine may be due to the increased activity of the sympathetic nervous system and/or the reduced activity of the parasympathetic

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nervous system. If excitation of the sympathetic nerves occurs with some emetics, it is considered that amylase activity in the saliva may be increased together with increases in noradrenaline in the brain and noradrenaline and adrenaline in the adrenal glands.

In the present study, the response of salivary amylase activity to various emetic agents such as cisplatin, apomorphine, LiCl, and rolipram, which selectively inhibit phosphodiesterase 4, and sibutramine, which is an anorexiant drug that inhibits the reuptake of noradrenaline and serotonin, was investigated. The response of the salivary amylase activity to $\beta_3$-adrenoceptor (AR) agonist CL316243, which inhibits gut motility via the $\beta_3$-AR, was also studied. In addition, the antiemetic effect of a 5-$HT_3$–receptor antagonist on the cisplatin-induced secretion of salivary amylase and the inhibitory effects of abdominal visceral nerve section on cisplatin- and LiCl-induced secretion of salivary amylase were investigated.

## Materials and Methods

*Animals*

Male Wistar (Crlj:WI) rats aged 5 weeks were obtained from Charles River Japan (Yokohama) and then acclimatized to the environmental conditions for 1 week before the experiments. The rats were weighed, weight-ranked, and assigned randomly to each of the treated and control groups. At dosing, the animals were 6-week-old and their body weights were 223 ± 4 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 animals and their care were conducted in conformity with the institutional guidelines.

*Bilateral abdominal vagotomy and its verification*

Vagotomized and sham-operated animals were purchased from the Institute for Animal Reproduction (Ibaraki). Experiments were performed on rats 14 – 21 days after undergoing a chronic sub-diaphragmatic vagotomy under aseptic conditions. The animals were anaesthetized with a single intraperitoneal injection of pentobarbital (Schering-Plough Animal Health K.K., Osaka) at 40 mg/kg. A midline laparotomy was performed. The stomach and liver were carefully maneuvered to expose the oesophagus as it emerged from the diaphragm. The two vagal branches running anterior and posterior to the oesophagus were carefully separated from the oesophageal wall, ligated, and sectioned as close to the diaphragm as possible by using a bipolar coagulator (MICRO-3E; Mizuho, Co., Ltd., Tokyo). After cutting the bilateral vagal nerves, the pylorus was dilated since sudden death can occur from pyloric stenosis. A 0.5 – 0.7-cm-long incision was made in the musculus sphincter pylori longitudinally, and the incised part was sutured using sterilized surgical catgut in a vertical direction against the direction of the incision. Sham-operated rats underwent laparotomy and gentle lifting of the stomach, but no tissue was cauterized. The peritoneum and abdominal muscles were sealed with surgical catgut, and the abdominal skin incision was closed with a surgical ligature, which was removed 7 days after surgery. The rats were allowed a minimum of 7 days to recover before experimentation. To confirm the sub-diaphragmatic vagotomy, the operated sites were inspected macroscopically immediately after the end of the experiment; and the oesophagus and surrounding tissue were removed, fixed with 10% neutral formalin, and the operated sites were observed microscopically.

*Salivary amylase activity induced by cisplatin, apomorphine, LiCl, rolipram, sibutramine, and the $\beta_3$-AR agonist CL316243*

The amylase activity induced after dosing with cisplatin (10 – 15 mg/kg, i.v.), apomorphine (1 – 3 mg/kg, s.c.), LiCl (120 mg/kg, i.p.), rolipram (1 – 10 mg/kg, p.o.), sibutramine (1 – 10 mg/kg, p.o.), or CL316243 (0.1 – 1 mg/kg, p.o.) was examined. The amount of saliva following administration with each compound was measured at each sampling point. The control animals for cisplatin, apomorphine, and LiCl received the same volume of physiological saline and those for rolipram, sibutramine, and CL316243 received the same volume of 0.5 w/v% methylcellulose solution in a similar way.

*Assessment of the effects of granisetron on increased salivary amylase activity induced by cisplatin*

The effect of intravenous administration of granisetron (1 or 3 mg/kg) on the enhanced salivary amylase levels induced by cisplatin (15 mg/kg, i.v.) was examined. Granisetron was administered 15 min before and 1 h after cisplatin. Control animals for granisetron received the same volume of physiological saline in a similar way.

*Assessment of bilateral abdominal vagotomy on increased salivary amylase activity induced by cisplatin or LiCl*

The effects of bilateral abdominal vagotomy on the enhanced salivary amylase levels induced by cisplatin (15 mg/kg, i.v.) or LiCl (120 mg/kg, i.p.) were examined. Nine to eleven animals were allocated to each of 4 groups (normal-saline, normal-drug, sham-drug, and vagotomized-drug). Body weights (mean ± S.E.M.) of the vago-

tomized animals (160 ± 3 g) were lighter than those of the normal (223 ± 2 g) and sham-operated (213 ± 2 g) animals before the administration of each drug.

*Measurement of amylase activity in rat saliva*

The weights of the roller cotton ball (3 mm in diameter; Richmond Dental, Charlotte, NC, USA) and the disposal tube were measured together beforehand. Rats were fasted and water-deprived for at least 30 min before measurement. The cotton ball was inserted under the tongue of each rat with forceps. The cotton ball was taken out of their mouths about 1 min later and then weighed. The quantity of saliva was calculated from the weight difference. Saliva was diluted 1:30 or 1:50 with physiological saline by assuming 1 mg as 1 $\mu$l 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-$\alpha$-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 3 – 5 sampling points by the trapezoidal rule. These points included before dosing, one or a few points showing the maximum amylase activity, and 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. The saliva was collected before and at 1.5, 3, and 6 h after cisplatin; before and at 0.25 and 1 h after apomorphine; before and at 1 and 3 h after LiCl; before and at 0.5 and 1 h after rolipram; before and at 0.5, 1, 3, and 7 h after sibutramine; and before and at 1 and 3 h after CL316243. Samples of saliva were collected before and at 1 and 1.5 h after the administration of cisplatin in combination with granisetron. The salivary samples were collected from normal, vagotomized, or sham-operated animals before and at 3 and 6 h after cisplatin administration or before and 1 and 3 h post-dose for LiCl. Sampling points in the cisplatin or apomorphine experiment were selected based on the duration of the emetic episodes in dogs (12) and/or monkeys (13). Sampling points of LiCl, rolipram, and sibutramine were selected based on the onset of increased amylase activity through the time to return to the control level. Sampling points of CL316243 were selected based on the time when the motility of the gastrointestinal tract is inhibited (14).

*Statistics*

The data in the figures are expressed as the means ± S.E.M. Data on the salivary amylase activity and the AUC values for amylase activity induced by cisplatin, apomorphine, LiCl, rolipram, sibutramine, or CL316243 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. Bartlett's test followed by Williams' test or Shirley-Williams' test was conducted to compare the mean for the control group with those for the multiple dosage groups.

*Drugs*

Cisplatin (Sigma-Aldrich Japan K.K., Tokyo) was purchased and injected intravenously at 10 and 15 mg/kg. Granisetron hydrochloride (Nichi-Iko Pharmaceutical Co., Ltd., Toyama) was administered intravenously at 1 and 3 mg/kg. Apomorphine hydrochloride (Sigma-Aldrich Japan K.K.) dissolved in physiological saline was administered subcutaneously at the dosage levels of 1, 3, and 10 mg/kg. Lithium chloride (LiCl, Sigma-Aldrich Japan K.K.) for intraperitoneal (i.p.) injection was dissolved in physiological saline and was administered i.p. at a dosage of 120 mg/kg. Rolipram (Sigma-Aldrich Japan K.K.), sibutramine hydrochloride (Tocris Bioscience, Bristol, UK), and CL316243 (Tocris Bioscience) were suspended in a 0.5 w/v% methylcellulose solution for oral administration; and rolipram and sibutramine at 1, 3, and 10 mg/kg or CL316243 at 0.1 and 1 mg/kg were administered orally. The injection volumes were 10 ml/kg for cisplatin, apomorphine, rolipram, sibutramine, or CL316243; 1 or 3 ml/kg for granisetron; and 2 ml/kg for LiCl. Each drug was administered immediately after preparation.

## Results

*Salivary amylase activity induced by cisplatin, apomorphine, LiCl, rolipram, sibutramine, and CL316243 in rats*

The patterns of the salivary amylase activity and the amounts of saliva with cisplatin, apomorphine, LiCl, rolipram, sibutramine, and CL316243 are summarized in Figs. 1 – 6, respectively.

Cisplatin (15 mg/kg, i.v.) produced a statistically significant increase in the secretion of salivary amylase from 1.5 h after dosing and maximal values were observed from 1.5 to 3 h after dosing (Fig. 1a), and statistically significant increases in the $AUC_{0-6h}$ values were observed at 10 and 15 mg/kg (Fig. 1c). Apomorphine induced statistically significant increases in the $AUC_{0-1h}$ values for salivary amylase at 1 and 3 mg/kg (Fig. 2c), and 3 mg/kg apomorphine tended to increase the activity at 0.25 h after dosing (Fig. 2a). Statistically significant

increases in amylase activity were observed when the control and treated animals were compared at 1 h after dosing with LiCl (Fig. 3a). This increase returned to normal by 3 h after administration (Fig. 3a). The $AUC_{0-3h}$ values for salivary amylase were increased at 120 mg/kg of LiCl (Fig. 3c). In the animals receiving 1 – 10 mg/kg

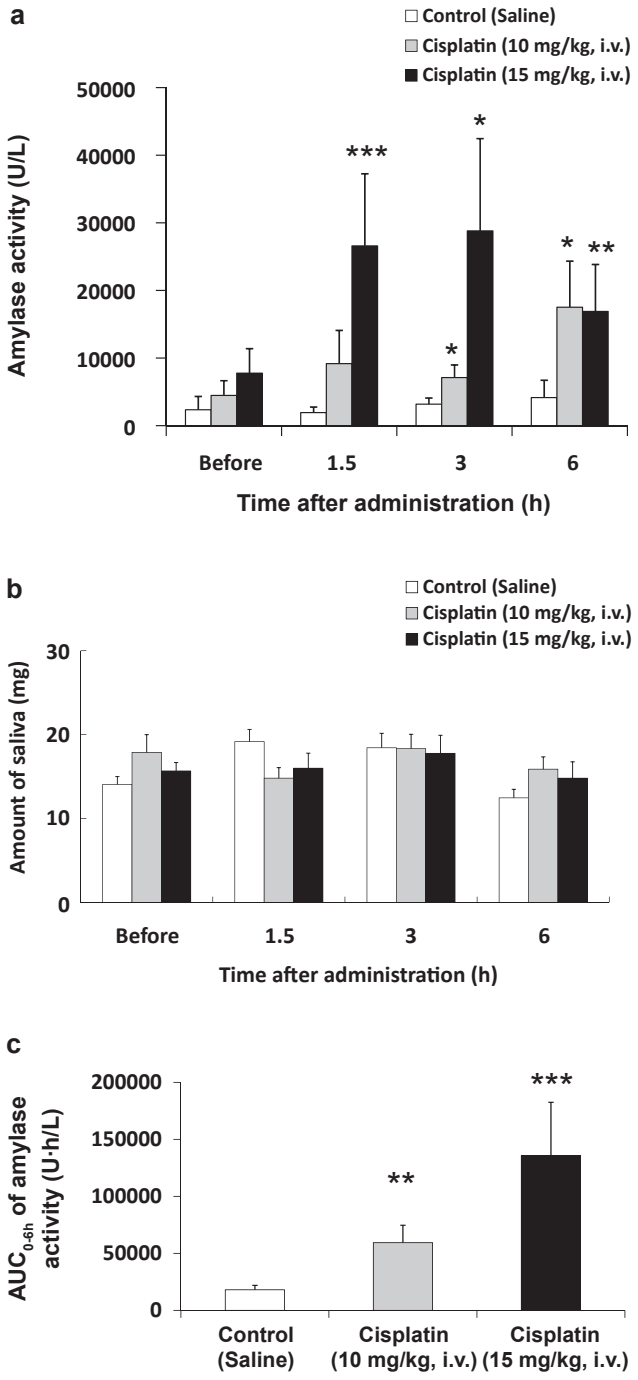


**Fig. 1.** The pattern of salivary amylase activity (a), amount of saliva (b), and $AUC_{0-6h}$ values of amylase activity (c) in rats following cisplatin administration (10 and 15 mg/kg, i.v.). Vertical bars represent the mean activity of amylase with S.E.M. (n = 10) for each time and dose. Control animals were given saline solution i.v. Compared with the control group at each respective time point: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (Shirley-Williams).

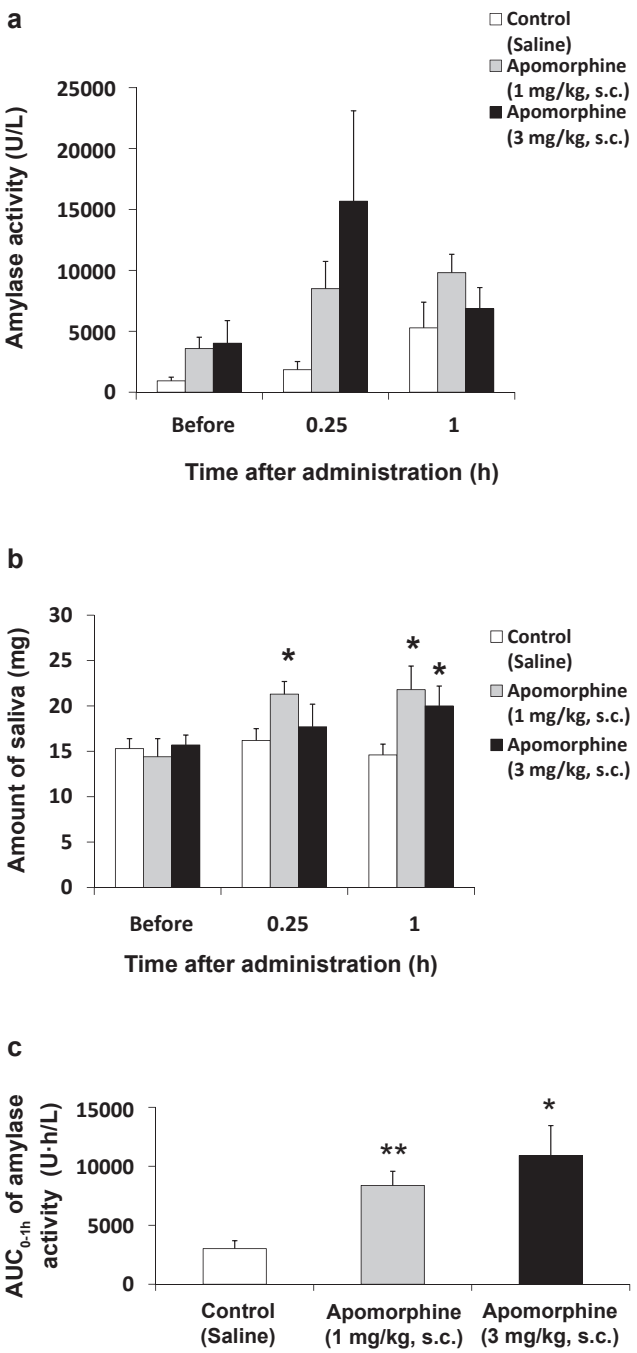


**Fig. 2.** The pattern of salivary amylase activity (a), amount of saliva (b), and $AUC_{0-1h}$ values of amylase activity (c) in rats following apomorphine administration (1 and 3 mg/kg, s.c.). Vertical bars represent the mean activity of amylase with S.E.M. (n = 6) for each time and dose. Control animals were given saline solution s.c. Compared with the control group at each respective time point: $*P < 0.05$, $**P < 0.01$ (Shirley-Williams).

of rolipram, statistically significant increases in amylase secretion were observed at 0.5 h after dosing (Fig. 4a). Thereafter, the secretion decreased gradually (Fig. 4a). Statistically significant increases in the $AUC_{0–1h}$ values were observed at 3 and 10 mg/kg (Fig. 4c). Oral admin-

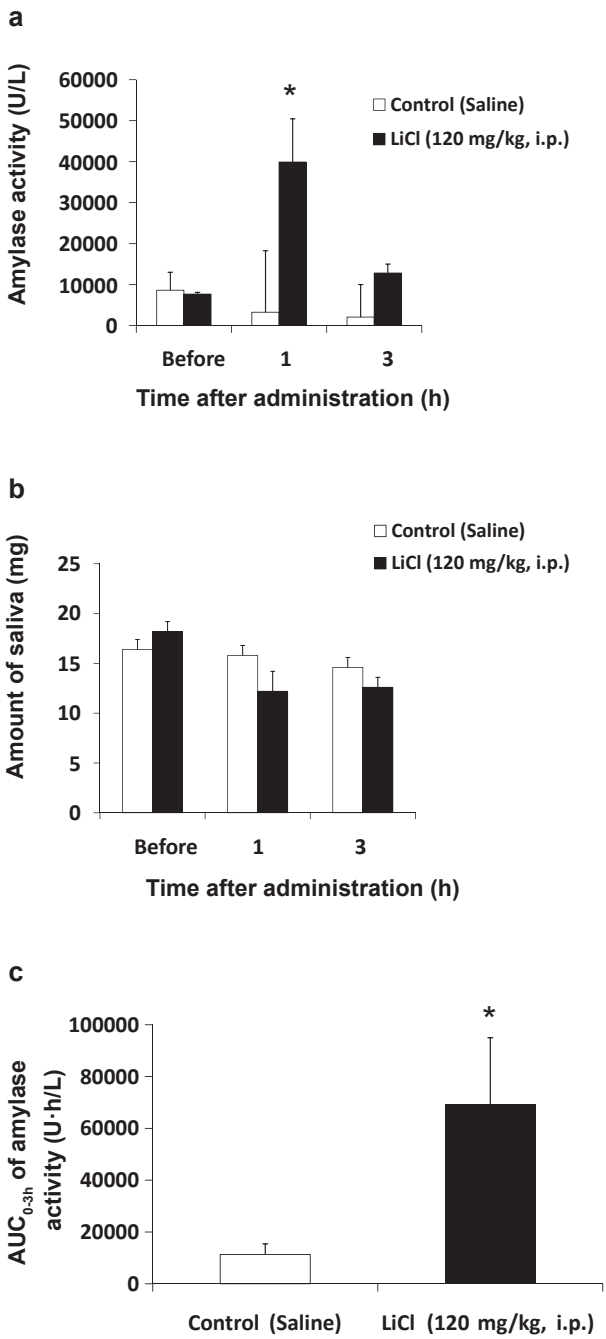


**Fig. 3.** The pattern of salivary amylase activity (a), amount of saliva (b), and $AUC_{0–3h}$ values of amylase activity (c) in rats following LiCl administration (120 mg/kg, i.p.).Vertical bars represent the mean activity of amylase with S.E.M. (n = 10) for each time. Control animals were given saline solution i.p. Compared with the control group at each respective time point: $*P < 0.05$ (Student's or Welch's *t*-test).

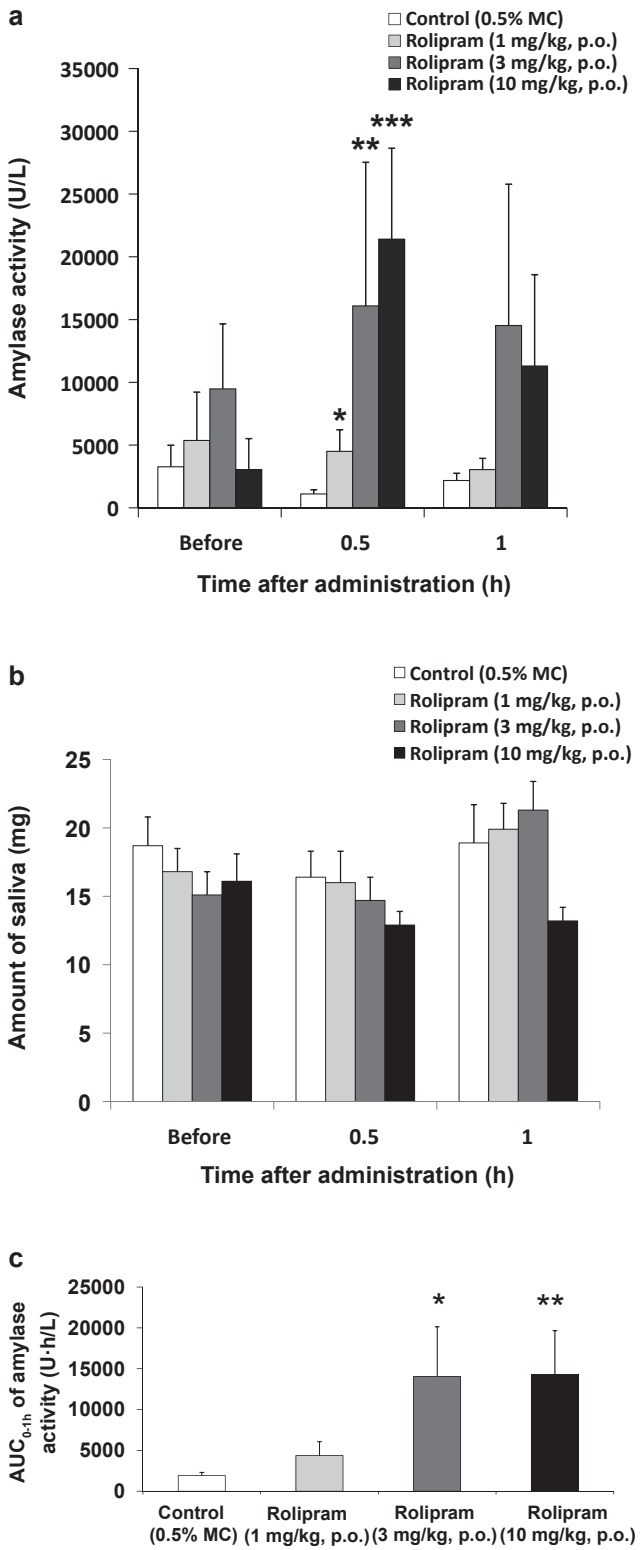


**Fig. 4.** The pattern of salivary amylase activity (a), amount of saliva (b), and $AUC_{0–1h}$ values of amylase activity (c) in rats following rolipram administration (1 – 10 mg/kg, p.o.). Vertical bars represent the mean activity of amylase with S.E.M. (n = 5) for each time and dose. Control animals were given 0.5 w/v% MC orally. Compared with the control group at each respective time point: $*P < 0.05$, $**P < 0.01$, $*** P < 0.001$ (Shirley-Williams).

istration of sibutramine at the high dose (10 mg/kg), but not at the low and middle doses (1 and 3 mg/kg), significantly induced amylase secretion from 0.5 to 3 h after dosing, and the enhanced levels returned to basal levels at 7 h after dosing (Fig. 5a). The $AUC_{0–7h}$ values were increased significantly at 10 mg/kg of sibutramine (Fig. 5c). CL316243 caused no significant increase in amylase activity (Fig. 6a) or the $AUC_{0–3h}$ values (Fig. 6c) at 0.1 and 1 mg/kg. No significant differences were observed in the volume of saliva elicited by cisplatin (Fig. 1b), LiCl (Fig. 3b), rolipram (Fig. 4b), sibutramine (Fig. 5b), or CL316243 (Fig. 6b) at almost all points, although the volume of saliva after dosing with apomorphine was slightly increased at 0.25 and 1 h with 1 mg/kg and at 1 h with 3 mg/kg (Fig. 2b). Amylase activity tended to increase in the control animals [0.5% methylcellulose solution (MC), p.o.] over a 1-h period in the sibutramine and CL316243 experiments (Figs. 5a and 6a).

*Effects of granisetron on increased salivary amylase activity induced by cisplatin in rats*

The results are shown in Fig. 7. Cisplatin at 15 mg/kg caused a significant increase in the $AUC_{0–1.5h}$ values of salivary amylase activity. Granisetron was administered twice since the pharmacologically effective period of granisetron in rats was within 1 – 1.5 h after intravenous administration (data not shown). Intravenous administration of granisetron (1 mg/kg × 2) significantly reduced the increase in the $AUC_{0–1.5h}$ values of amylase activity induced by cisplatin by 65%. Furthermore, granisetron (3 mg/kg × 2, i.v.) significantly inhibited the $AUC_{0–1.5h}$ values of amylase secretion produced by cisplatin by 73%.

*Effects of bilateral abdominal vagotomy on increased salivary amylase activity induced by cisplatin or LiCl*

The results are shown in Figs. 8 and 9. Cisplatin (15 mg/kg, i.v.) produced a statistically significant increase in the $AUC_{0–6h}$ values in both normal rats and sham-operated rats but did not induce a statistically significant increase in bilateral abdominal vagotomized rats (Fig. 8), although there was no statistical significance between the sham and vagotomy groups. On the other hand, the increased $AUC_{0–1h}$ values for amylase activity induced by LiCl were unaffected by bilateral abdominal vagotomy (Fig. 9).

## Discussion

In the present study, increased amylase secretion in rat saliva was observed following dosing with all the emetic agents used. These results are in agreement with those indicating an increase in kaolin consumption caused by almost the same dosage levels of cisplatin (6) or LiCl (15) administration to rats. In general, cisplatin is injected intravenously to patients with various kinds of cancer at

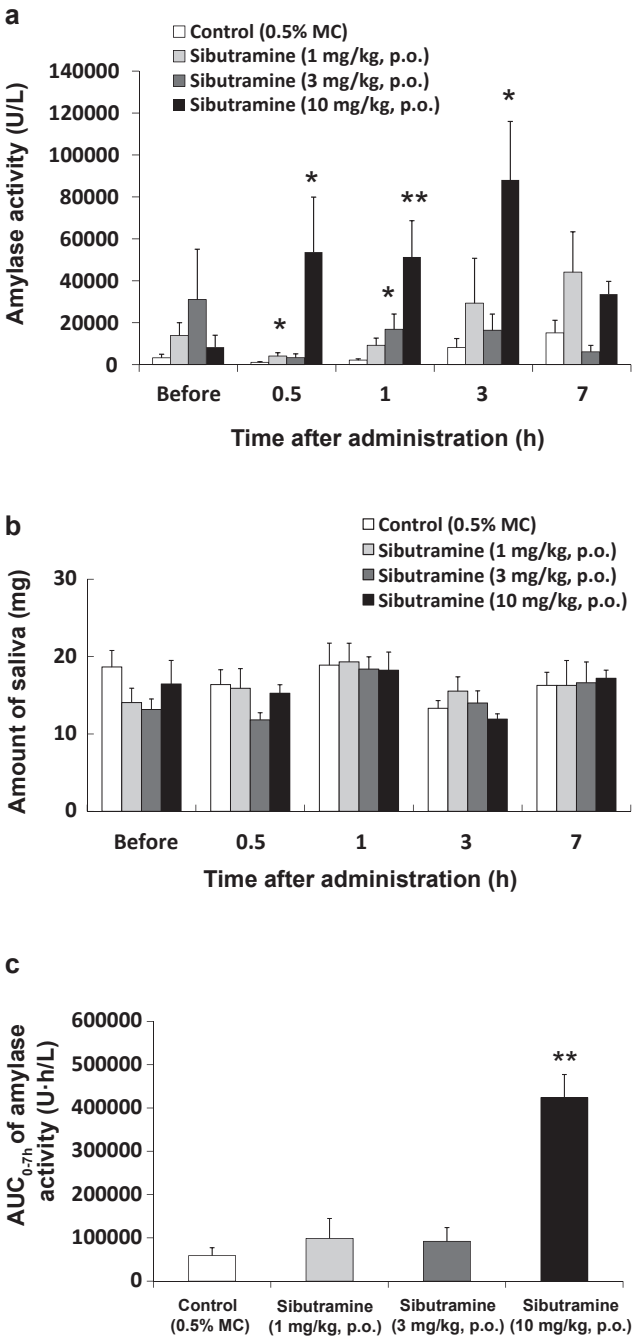


**Fig. 5.** The pattern of salivary amylase activity (a), amount of saliva (b), and $AUC_{0–7h}$ values of amylase activity (c) in rats following sibutramine hydrochloride administration (1 – 10 mg/kg, p.o.). Vertical bars represent the mean activity of amylase with S.E.M. (n = 5) for each time and dose. Control animals were given 0.5 w/v% MC orally. Compared with the control group at each respective time point: $*P < 0.05$, $**P < 0.01$ (Shirley-Williams).

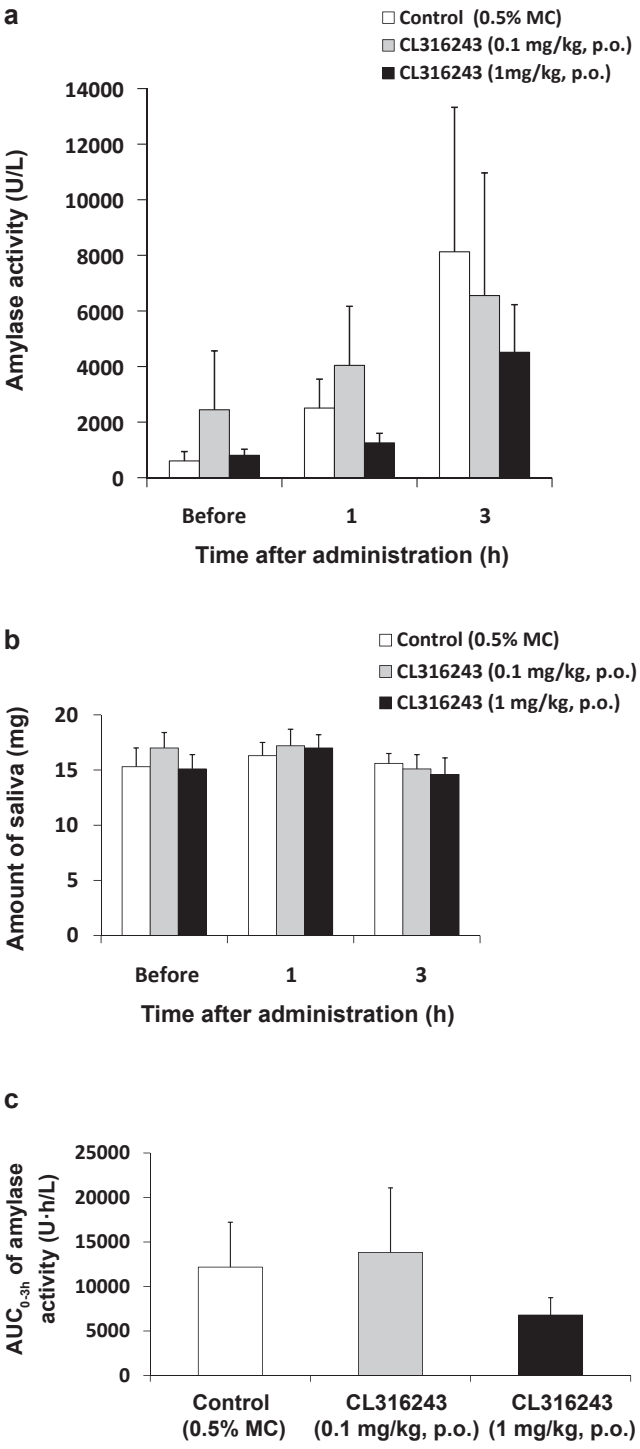


**Fig. 6.** The pattern of salivary amylase activity (a), amount of saliva (b), and $AUC_{0-3h}$ values of amylase activity (c) in rats following CL316243 administration (0.1 or 1 mg/kg, p.o.). Vertical bars represent the mean activity of amylase with S.E.M. (n = 10) for each time. Control animals were given 0.5 w/v% MC orally.

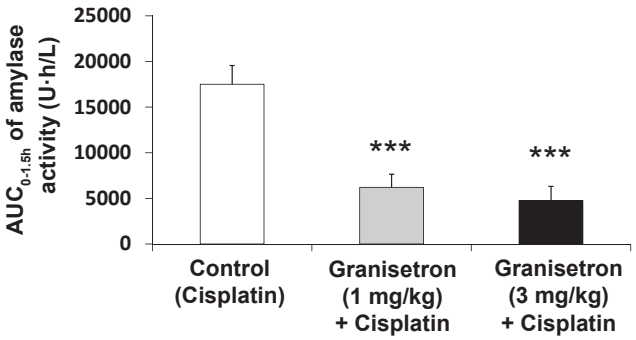


**Fig. 7.** Effect of granisetron (1 or 3 mg/kg, i.v.) on increased salivary amylase activity in rats following cisplatin administration (15 mg/kg, i.v.). Vertical bars represent the mean $AUC_{0-1.5h}$ values of amylase activities with S.E.M. (n = 10) for each time. Control animals were given saline solution i.v. Compared with the control group: ***$P < 0.001$ (Shirley-Williams).

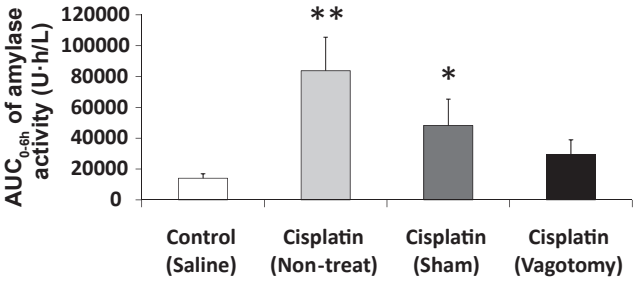


**Fig. 8.** Effect of bilateral abdominal vagotomy on increased salivary amylase activity in rats following cisplatin administration (15 mg/kg, i.v.). Vertical bars represent the mean $AUC_{0-6h}$ values of amylase activities with S.E.M. [Control (saline): n = 9, cisplatin (non-treated): n = 10, cisplatin (sham-operated): n = 10, cisplatin (vagotomy): n = 11]. Compared with the control group: *$P < 0.05$, **$P < 0.01$ (Student's *t*-test).

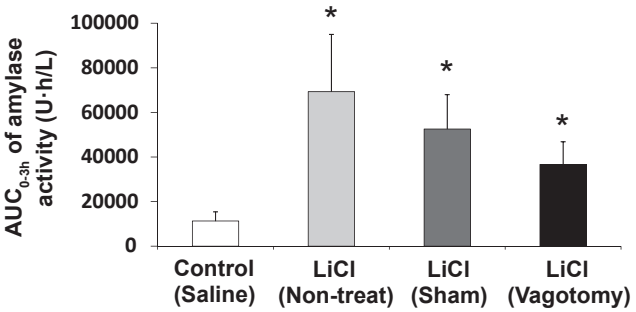


**Fig. 9.** Effect of bilateral abdominal vagotomy on increased salivary amylase activity in rats following LiCl administration (120 mg/kg, i.p.). Vertical bars represent the mean $AUC_{0-3h}$ values of amylase activities with S.E.M. [Control (saline): n = 10, LiCl (non-treated): n = 9, LiCl (sham-operated): n = 10, LiCl (vagotomy): n = 11]. Compared with the control group: *$P < 0.05$ (Welch's *t*-test).

dosage levels of 50 – 120 mg/m$^2$ (16). In the previous study using dogs, the dose of intravenous cisplatin that produced 100% emesis was 3 mg/kg (12). In this study, intravenous administration of cisplatin significantly induced salivary amylase secretion in rats at 10 and 15 mg/kg. These dosage levels in dogs and rats are equivalent to 60 and 60 – 90 mg/m$^2$, respectively. This result demonstrates that clinical dosage levels of cisplatin elicit increases in salivary amylase in rats. It is well known that cisplatin (12, 17, 18) and rolipram (19 – 24) are strong emetic agents in humans, dogs, and ferrets; and the latency to the first vomiting is 1 – 1.5 h after cisplatin administration to humans, dogs, monkeys and ferrets (12, 13, 25 – 27) and within 0.5 h after rolipram administration to humans, ferrets, and dogs (21, 22, 28). In this study, significant increases in amylase activity were observed at 1.5 h after the administration of cisplatin and at 0.5 h after the administration of rolipram. The results demonstrated that the timing of the increased amylase secretion is consistent with the onset of vomiting induced by cisplatin and rolipram and the increased amylase levels reflect the response to emetics in the species showing emesis. In addition, a 5-HT$_3$ antagonist inhibited the cisplatin-induced amylase secretion in rats, indicating that this result is good agreement with that in humans and other species showing emesis. Emesis induced by cisplatin is prevented by abdominal vagotomy in dogs (12), monkeys (13), and ferrets (17). In rats, cisplatin-induced pica is suppressed by 61% by common hepatic branch vagotomy (29). In contrast, bilateral subdiaphragmatic vagotomy in rats does not prevent subsequent acquisition of a conditioned taste aversion induced by LiCl (30). The present study demonstrated that abdominal vagotomy inhibited or tended to inhibit the increased activity of salivary amylase produced by cisplatin but not that by LiCl. These results are in good agreement with the results of previous studies, suggesting that cisplatin induces an increase in amylase activity by activating mainly the abdominal vagal afferent fibers, while LiCl induces increased activity by an effect on the central nervous system. Therefore, it is considered that it is possible to study the mechanisms for vomiting produced by drugs.

In the present study, LiCl (120 mg/kg, i.p.) and a high dose (10 mg/kg, p.o.) of sibutramine also stimulated an increase in amylase activity. It is known that LiCl induces conditioned taste aversion at ca 120 mg/kg in rats (31 – 33) and produces vomiting with a low incidence in ferrets (34). Sibutramine is administered orally to patients at a dosage level of 15 mg (35) and induces vomiting with a low incidence in humans (36). A high dose (10 mg/kg, p.o.) of sibutramine, which is equivalent to 80 – 112 mg/person, is around 5 – 7-times higher than the clinical dose (15 mg). These results suggest that it is possible to predict the emetogenic potential of drugs, especially new candidates or compounds with a low emetogenic potential, since humans are more sensitive to some kinds of emetogens than are some animal species that show vomiting (37), and the development of new medicines is sometimes discontinued because of the vomiting observed in clinical practice but which was not noted in the non-clinical studies.

In this study, apomorphine (3 mg/kg, s.c.) tended to increase the salivary amylase at 15 min after dosing without an increase in the volume of saliva, and 1 and 3 mg/kg apomorphine increased the saliva volume at 0.25 and/or 1 h after dosing without an increase in the amylase activity. Our results are inconsistent with those reported by Koga et al. (38) who found that apomorphine causes an increase in salivary secretion in rats within 5 min after dosing with 3 – 10 mg/kg, i.v. The reason for this might be due to the differences in the sampling time or administration route. In addition, there was no clear hypersalivation in rats receiving emetogens such as cisplatin, LiCl, rolipram, sibutramine, and CL316243 and no apparent relationship between amylase activity and the amount of saliva in this study. Therefore, not all emetogens necessarily cause hypersalivation.

A delay in gastric emptying has been discussed as a component of the defensive response in rodents and in species with emesis because this action would delay delivery of any toxic compound from the stomach into the small intestine where it could produce more damage, especially if absorbed (39). In fact, cisplatin is reported to induce a delay in motility of the gastrointestinal tract in rats (10). It is well-known that CL316243 is a potent $\beta_3$-AR agonist that inhibits cholinergic-induced motility of the gastrointestinal tract and chemically induced diarrhea following oral administration at 1 mg/kg (14). Intravenous administration of CL316243 at 3 mg/kg fails to produce significant changes in heart rate in rats (40). In addition, there are no reports on vomiting induced by CL316243 in species showing emesis. It has been reported that $\beta_3$-AR agonists other than CL316243 do not produce vomiting in dogs (41). In this study, CL316243 did not produce an increase in salivary amylase activity at 1 mg/kg. It is, therefore, conceivable that the inhibition of gastrointestinal motility without stimulation of the sympathetic nerves or this non-emetogenic compound does not induce any increase in salivary amylase activity.

It has been reported that pica is useful as an emetic model in rats (2 – 5). However, there are some disadvantages with pica as follows: i) it is necessary to investigate the emetic potential of cisplatin for at least a 24-h observation period, since kaolin intake is increased during the 24 – 48 h after dosing when cisplatin is injected to rats

(42); ii) administration of an optimum dosage level for cisplatin is needed to induce pica because a low dose of cisplatin does not induce pica, whereas a high dose also fails to produce pica due to deterioration in the animals' physical condition (43); iii) it is difficult to judge the effects of drugs since non-treated rats sometimes also eat kaolin (8); iv) it is not easy to judge the effects of drugs since it is not feasible to measure the kaolin weight correctly as kaolin is mixed with food, water, urine, feces, and so on beneath the cage (8). On the other hand, salivary amylase has some advantages as an index of emesis as follows: i) Rat salivary amylase increases dose-dependently. ii) It is possible to collect saliva simply, easily, quickly, and repeatedly, so that amylase activity can be measured over time. As a result, statistically significant increases in amylase activity can be seen from 1.5 h after dosing with cisplatin. On the contrary, cisplatin-induced pica was seen 24 h after dosing (6). These results suggest that the emetic potential of cisplatin could be detected in a shorter period using salivary amylase rather than pica. iii) The latency period (1.5 h) of salivary amylase activity following cisplatin administration in rats is almost comparable to the latencies (1 – 2 h) to the first vomiting induced by cisplatin in humans, dogs, monkeys, and ferrets (12, 13, 26, 27). However, there may be still some disadvantages of this model as follows: i) Individual variability: the manipulation of the rat is more important. The cursory handling of the rats may affect amylase activity in saliva as well as the amount of saliva. ii) Circadian variability of salivary amylase activity: salivary amylase activity in the control animals seems to increase in the afternoon compared to the values in the morning.

It also remains an important question as to whether the increase in salivary amylase is induced solely by emetic agents, generally toxic compounds, or by the toxic dose of a drug that does not induce vomiting. However, it is should be possible to evaluate the emesis by measuring the amylase activity in rat saliva as well as by pica, which is also recognized as an animal model in emesis research, since the change in amylase activity correlates with the actual emetic reaction to some emetics in humans and species showing emesis.

In conclusion, 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. These results suggest that salivary amylase activity is useful as a marker for emesis in rats, a species that does not exhibit vomiting, and that this animal model could be useful for revealing the mechanisms of emesis and for investigating new antiemetic agents for humans.

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