Involvement of Hypothalamic Glutamate in Cisplatin-Induced Emesis in *Suncus murinus* (House Musk Shrew)

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**Abstract.** We investigated the effect of cisplatin on glutamate release in the hypothalamus of *Suncus murinus* measured by brain microdialysis. Dialysis samples were collected every 20 min for 1 h before and 3 h after the cisplatin (30 mg/kg, i.p.) administration with the animals also being observed for the development of emesis. Cisplatin increased glutamate levels within 1 h and this was closely associated with the occurrence of emesis. Pretreatment with the 5-HT₃–receptor antagonist ondansetron (2 mg/kg, i.p.) inhibited both the emesis and the increased glutamate levels. These results suggest that hypothalamic glutamate is involved in cisplatin-induced emesis in *Suncus murinus*.

**Keywords:** glutamate, microdialysis, *Suncus murinus*

Emesis is frequently observed in patients receiving cancer chemotherapy. Serotonin 5-HT₃–receptor antagonists such as ondansetron and granisetron, combined with glucocorticoids such as dexamethasone and the tachykinin NK₁–receptor antagonist aprepitant, have been used extensively for the prophylaxis and treatment of nausea and emesis for over a decade (1, 2). Unfortunately, whilst the combination of these drugs can prevent emesis in a majority of the patients, the symptom of nausea may still persist (3).

It is well known that the brainstem dorsal vagal complex is a critical site for emesis control (4). However, nausea and emesis may also be associated with an alteration of output from the autonomic system that may result in anorexia, hypotension, tachycardia, and salivation and changes of hypothalamic–pituitary–adrenal (HPA) axis function such as secretion of vasopressin that has been argued to be a biomarker of nausea (5, 6). Since the glutamatergic system is known to be involved in the development of these emesis-accompanied autonomic symptoms (7, 8), we hypothesized that it is involved in the development of chemotherapy-induced emesis. To investigate the role of the glutamatergic system for the development of emesis, we examined the changes of glutamate release in the hypothalamus after administration of cisplatin with or without treatment of ondansetron, a serotonin 5-HT₃–receptor antagonist, by using in vivo microdialysis in *Suncus murinus* (house musk shrew) (9).

Female *Suncus murinus* (Jic:SUN-Her; 28 – 35 g) were obtained from CLEA Japan (Tokyo) and housed in cages (35 cm × 10 cm × 25 cm) in a room at a constant temperature (25 ± 1°C) and humidity (50 ± 5%). Animals were allowed free access to water and pelleted chow (CIEA-311; CLEA Japan). All studies were approved and conducted in accordance with the Animal Care Committee of the Graduate School of Medicine, Osaka University.

For surgery, animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.; Dainippon Sumitomo Pharma, Osaka) and lidocaine (8%, 0.05 ml, s.c.; Astrazeneca K.K., Osaka). One end of an intraperitoneal catheter made of polyethylene tubing (INTRAMEDIC PE50; Becton Dickinson, Sparks, MD, USA) was inserted into the abdominal cavity and the other end was threaded subcutaneously to the top of the skull. Then the animals were placed in a stereotaxic apparatus (Kopf Instrument, Tujunga, CA, USA). A guide cannula (MAB 2/6/9.14. IC.; Microbiotech/se AB, Stockholm, Sweden) was implanted into the hypothalamus using
the following coordinates: anterior +4.7 mm, lateral +0.7 mm, and ventral −3.7 mm; stereotaxic zero was lambda, according to Veney and Rissman’s previous report (10). The guide cannula was fixed with dental cement and incisions closed using interrupted suture. The animals were allowed at least 7 days to recover.

On the day of the experiment, a microdialysis probe (MAB 6.14, Microbiotech/se AB) with 1 mm effective length was inserted into the hypothalamus through the guide cannula. The probe was perfused with artificial cerebrospinal fluid (CSF: 140 mM NaCl, 3 mM KCl, 2.5 mM CaCl$_2$, and 1 mM MgCl$_2$, pH 7.4) at a rate of 1.0 µl/min. Dietary amino acids contained in the pelleted chow could potentially have an effect on glutamate measurements, so food was removed from the cages during the experimental period. The first 6 h of perfusate was discarded before collecting 20-µl samples every 20 min for 1 h to establish baseline measurements. Then the animals were administered four sets of drug combinations: A) saline and saline, B) saline and cisplatin (30 mg/kg; Sigma-Aldrich, St. Louis, MO, USA), C) ondansetron (2 mg/kg; GlaxoSmithKline, Tokyo) and saline, and D) ondansetron (2 mg/kg) and cisplatin via the previously implanted intraperitoneal catheter in a total volume of 30 ml/kg (n = 3, each group). Twenty-microliter samples were collected at 20-min intervals for a further 3 h. The glutamate contents were analyzed by a high performance liquid chromatography (HPLC)–fluorimetric method using a pre-column derivatization method (11). The behavior of *Suncus murinus* were recorded for the sampling period using a video recording system and an episode of emesis was counted. After the experiment, the animals were deeply anesthetized with pentobarbital (100 mg/kg, i.p.) and then perfused with 0.9% saline through the left ventricle, followed by 100 ml of 10% formalin. Brains were removed and histological verification of the dialysis probe placement was performed. Because the absolute basal release of glutamate in *Suncus murinus* varied between subjects, the mean of the first three fractions was defined as the mean basal release, and subsequent fractions were expressed as the percent of the mean basal release.

Latency data are indicated as medians and were analyzed by a Kruskal-Wallis test followed by Dunn’s multiple comparison tests; other data indicate the mean ± S.E.M. Significant differences between episode data or glutamate basal levels and the effect of treatment were assessed by a one or two-way repeated-measures ANOVA, as appropriate, followed by Bonferroni tests. A value of $P<0.05$ was considered statistically significant.

Histological examination of brain sections obtained from the experimental animals indicated that the probe was mainly sited in the ventromedial nucleus of the hypothalamus. The basal level of glutamate was $0.22 ± 0.07 \mu M$ (n = 12, pooled data from all animals). The administration of saline or ondansetron alone at $t = 0$ min did not modify significantly glutamate levels nor did they induce emesis ($P>0.05$; Fig. 1, Table 1). However, in the saline-treated animals, cisplatin at 30 mg/kg induced emesis following a latency of $32 ± 3.2$ min, with the highest frequency of episodes occurring during the 60–80 min period (Fig. 2). There was also a gradual increase in glutamate levels following cisplatin administration that peaked during the 80–100 min period ($P<0.01$, Fig. 1). There were some episodes of emesis in 1 out of 3 animals at around 120 min, but glutamate levels had returned to baseline during this period. Cisplatin induced $45 ± 0.83$ episodes

![Fig. 1. The effect of cisplatin (30 mg /kg, i.p.) on extracellular glutamate levels in the hypothalamus of *Suncus murinus*. Basal glutamate levels were $0.22 ± 0.07 \mu M$. The average mean value of the first three samples was taken as 100%. Significant differences relative to saline-saline controls are indicated as **$P<0.01$; significant differences relative to the cisplatin-ondansetron-treated group are indicated as ††$P<0.01$.](image)
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during the entire 180-min observation period in all animals (Table 1). Ondansetron prevented emesis in all animals (Table 1, \(P<0.01\)) and also antagonized significantly the cisplatin-induced increase in extracellular glutamate (\(P<0.05\), Fig. 1).

There have been several previous studies attempting to link the hypothalamus to mechanisms involved in nausea and emesis (4, 12). This is partly because the hypothalamus is known to be involved in homeostatic processes altered during nausea and emesis (see introduction) and also because it is a controlling site regulating the release of vasopressin from the posterior pituitary (6, 13). There are also several studies showing increases in c-fos immunoreactivity in the hypothalamus following emetic treatments (4). However, these latter observations are largely indirect or have been made several hours after the start of the emetic treatment, in animals incapable of emesis.

The importance of our studies using microdialysis, therefore, was to show for the first time that cisplatin treatment induces emesis with close temporal mirroring of elevations in extracellular glutamate in the hypothalamus of a species capable of emesis. In our studies, ondansetron predictably antagonized emesis and also reduced the cisplatin-induced increases in extracellular glutamate levels. Glutamate is considered to be one of the neurotransmitters coordinating the gastrointestinal, cardiovascular, and endocrinological functions. Moreover, Lehmann and Karrberg (14) and Fink-Jensen et al. (15) reported that the cisplatin-induced emesis in ferrets was prevented by pretreatment with glutamate-receptor antagonists, and Zhang and Fogel (7) reported that hypothalamic glutamate regulated gastrointestinal function via the dorsal vagal complex of rats. Darlington et al. (8) reported that activation of the hypothalamic glutamatergic system induced autonomic nervous symptoms such as an increase of plasma vasopressin level, bradycardia, and hypotension. From these findings, we hypothesized that the central glutamatergic system is involved in the emesis and related autonomic symptoms.

In summary, our studies revealed that administration of an emetic dose of cisplatin induced a significant increase of extracellular glutamate release in the hypothalamus. Importantly, we also found that emesis and increases of extracellular glutamate in the hypothalamus were significantly inhibited by ondansetron (Fig. 2). The time required to increase glutamate levels appeared similar to the latency of cisplatin-induced emesis and the duration of the increased level of glutamate was also similar to the timing of the emetic episodes. These results indicate that the central glutamatergic system may contribute to the development of emesis in *Suncus murinus*. Further experiments are required to elucidate the precise etiology of cisplatin-induced emesis and in particular the role of the hypothalamus. It is possible that our approach to studying transmitter function in the hypothalamus could be used to investigate if novel anti-emetics can prevent not just emesis but also the associated activation of the hypothalamic system that may be relevant to mechanisms involved in nausea.

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**Table 1.** The effect of ondansetron (2 mg/kg) on cisplatin (30 mg/kg)-induced emesis in *Suncus murinus*

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<thead>
<tr>
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<th>Number of animals</th>
<th>Number of vomiting</th>
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<tr>
<td>Saline – Saline</td>
<td>3</td>
<td>0</td>
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<tr>
<td>Saline – Ondansetron</td>
<td>3</td>
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<td>Cisplatin – Saline</td>
<td>3</td>
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<tr>
<td>Cisplatin – Ondansetron</td>
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**Fig. 2.** The emetic profile of cisplatin (30 mg/kg) in *Suncus murinus*. Results are shown as the mean cumulative numbers of retches and/or vomiting occurring at the 20-min time interval after cisplatin administration.
References

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