Involvement of a Peripheral Mechanism in the Emesis Induced by Cardiac Glycosides in *Suncus murinus*

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The ability of three cardiac glycosides, ouabain, digitonin and digitoxin, to induce emesis and their mechanism(s) of action were investigated in *Suncus murinus*. The intraperitoneal injection of ouabain but not digitonin nor digitoxin caused vomiting in a dose-dependent manner. However, the administration of ouabain into the cerebroventricle did not cause emesis. Ouabain-induced emesis was partly prevented by surgical abdominal vagotomy. Pretreatment with tropisetron, a selective 5-HT₃ (5-hydroxytriptamine) receptor antagonist, did not affect the emetic response evoked by ouabain. These results suggest that ouabain exerts emetic effects via peripheral mechanism(s), but 5-HT₃ receptors are not involved in the pathway.

Key words  emesis; cardiac glycoside; ouabain; *Suncus*

Emesis is considered to be a defensive reflex for excluding toxic substances ingested accidentally. However, there are a number of emetogenic stimuli which apparently do not fulfill this defensive purpose. Emesis caused by cardiac glycosides as a side effect produces serious discomfort in patients with heart disease. Considering the quality of life of patients, it is very important to overcome such side effects.

Pharmacological investigation of the mechanism of digitalis-induced emesis has been extensively performed using laboratory animals such as dogs¹⁻² and cats.⁴⁻⁶ It is suggested that the chemoreceptor trigger zone (CTZ), a chemosensitive region in the area postrema outside the blood brain barrier, mainly constitutes the central site of the emetic action of cardiac glycosides. However, the difficulty of lesion of area postrema without damaging the subarea has recently been indicated.⁷,⁸ It is possible that the operation had damaged neuronal inputs or blood supply to the nucleus tractus solitarius (NTS). Furthermore, it is considered that digitalis-induced emesis is partly mediated by peripheral mechanism(s).²,⁵,⁶,⁹ The emesis induced by cardiac glycosides was not abolished completely by ablation of the area postrema.⁴,⁶ Gut denervation, combined with the ablation of the area postrema, did not prevent the emetic action of digitals.⁶ These observations suggest that digitalis-induced emesis is initiated largely by peripheral stimulation. Therefore, the precise mechanism(s) of the emesis caused by digitals glycosides remain to be resolved.

Remarkable species variations in the response to emetics are recognized in several animal models.¹⁰ Cardiac glycosides elicit a notable emetic response in dogs and cats.⁹ The monkey, however, is clearly not sensitive to cardiac glycosides.¹¹ Only few attempts have so far been made to elucidate the effect of cardiac glycosides in *Suncus murinus*. *Suncus murinus* (a house musk shrew) is a species of insectivore that is considered to be closer to primates than rodents, lagomorphs and carnivores in the phylogenetic system.¹² Because its body is small in size compared to other experimental animals used for research on emesis, the use of suncus is of great advantage with regard to pharmacological studies.¹³ We have shown previously that *Suncus murinus* vomits in response to various emetic drugs, motion stimulus or cancer chemotherapeutic agents and that the animal is suitable for research on emesis.¹³⁻¹⁵

In the present study, we determined whether cardiac glycosides (ouabain, digitonin, digitoxin) have emetogenic potency, and if so, we tried to clarify the mechanism(s) of the emesis induced by these drugs in *Suncus murinus*.

MATERIALS AND METHODS

Animals Three to six-month-old *Suncus murinus* of either sex weighing 50–70 g (male) and 30–50 g (female) were used. The animals, originally introduced from the Central Institute for Experimental Animals (Kanagawa), were bred and housed in a temperature-controlled room at 24 ± 1 °C under a 12 h light/dark cycle. All experiments were performed between 13:00–18:00.

Experimental Procedures Drug Treatment: The following three cardiac glycosides were studied: ouabain, digitonin, digitoxin. These drugs have different lipophilic properties. The lipophilicity of digitoxin is highest, followed by digitonin and ouabain. Cardiac glycosides were administered intraperitoneally or intracerebroventricularly, and behavioral changes including vomiting were observed for 120 min. Control animals received the vehicle. The number of vomiting episodes and the latency until the first vomit were recorded. Initial vomiting is always accompanied by an expulsion of gastric contents, and our recent study revealed that several retches always precede the vomiting but they are too rapid to be counted accurately.¹⁶ Therefore, we recorded the vomiting episode which includes several retches and one vomiting.

The antiemetic effects of tropisetron (0.2 mg/kg, s.c.), a 5-HT₃ receptor antagonist, on ouabain (0.1 mg/kg, i.p.)-induced emesis were also investigated. The agent was administered 30 min prior to the injection of ouabain.

Chronic Cannulation into the Cerebral Ventricle: For injection of substances into the cerebral ventricles, a stainless-steel guide cannula was inserted stereotaxically into the left lateral ventricle (AP: 9.5, LM: 0.8, DV: 3.4) under anesthesia with a combination of ketamin hydrochloride (47.2 mg/kg, i.m.) and xylazine hydrochloride.
(4.1 mg/kg, i.m.). Animals were allowed to recover for at least 1 week following the operation. A needle connected to a microinjection syringe via a polyethylene tube was inserted through the guide cannula, and under unanesthetized conditions, a drug solution was slowly injected over one min. After the experiment, the success of intracerebroventricular (i.c.v.) administration was confirmed in each animal by post-mortem injection of trypan blue solution. The dye should diffuse immediately throughout the ventricles.

Surgical Vagotomy: Abdominal surgical vagotomy was performed according to Torii et al. The animals operated upon were allowed to recover for at least 1 week before the experiment. We have reported that surgical vagotomy eliminated the emetic effect of copper sulfate (p.o.) but not nicotine (s.c.) or veratrine (s.c.). Thus, the success of surgical vagotomy was confirmed by the absence of the emetogenic potency of copper sulfate (40 mg/kg, p.o.).

**Drugs** The drugs used in this experiment were as follows: ouabain, digitoxin, digitoxin (Wako Pure Chemical Industries), and tropisetron (Sandoz). Ouabain, digitoxin and tropisetron were dissolved in sterile saline. Digitoxin was suspended in saline with a small amount of carboxymethyl cellulose sodium salt (CMC). The volume of cardiac glycosides solution for intraperitoneal injection was adjusted to 2 ml/kg body weight. Tropisetron was administered subcutaneously in a volume of 10 ml/kg. The injection volume for the i.c.v. administration was 3 µl per animal.

**RESULTS**

**Effect of Cardiac Glycosides on Emesis and General Behavior** Table 1 presents a comparison of the emetogenic potency of ouabain, digitoxin and digitoxin given by intraperitoneal administration in *Suncus murinus*. Ouabain (0.03—0.3 mg/kg) caused emesis dose-dependently. One out of five animals died within 30 min when the dose was increased to 0.3 mg/kg. The same dose of ouabain also caused severe convulsions. Digitoxin was emetogenic at a dose of 1 mg/kg. The treated animals died after the observation period of 120 min. Only one out of five animals vomited during the terminal convulsion 3 min after receiving 100 mg/kg digitoxin. However, this high dose of digitoxin was fatal and all animals died after the completion of the experiment. Non-fatal doses of digitoxin did not cause emesis. Besides emesis, other noted behavioral changes after injection of maximum doses of the cardiac glycosides were ataxia, tremor, convulsions and panting.

**Intracerebroventricular Administration of Ouabain** Since ouabain (i.p.) showed a marked emetic effect, we investigated its mechanism in more detail. First, we tested the possibility that ouabain stimulates the central nervous system directly. The injection of ouabain (0.3—6 µg/body) into the lateral ventricle of the brain did not induce emesis (Table 2). Although i.c.v. administration of ouabain was not emetogenic, other neurotoxic effects such as ataxia, tremor, vocalization, convulsions and panting were observed. A central injection of higher doses of ouabain was also fatal. To exclude the possibility that the operation itself affects vomiting responses, we used nicotine. Nicotine (50 µg/body, i.c.v.) elicited vomiting in all animals tested. In no case did vomiting occur after the injection of saline.

**Influence of Surgical Vagotomy on Ouabain-Induced Emesis** The effect of surgical vagotomy on ouabain-induced emesis was investigated (Table 3). Ouabain (0.1 mg/kg, i.p.) caused vomiting in all animals of the sham operated group with a mean latency of 12.0±1.7 min (mean±S.E.M.) and the mean number of vomiting episodes was 14.0±1.3 times. Surgical vagotomy did not prevent ouabain-induced emesis. However, the latency was significantly prolonged in vagotomized animals.

**Effect of Selective 5-HT3 Receptor Antagonist on Ouabain-Induced Emesis** In order to investigate the participation of peripheral 5-HT3 (5-hydroxytryptamine) receptors in ouabain-induced emesis, we tested whether or not the emetic effect of ouabain (0.1 mg/kg, i.p.) was blocked by pre-treatment with tropisetron, a selective 5-HT3 receptor antagonist. Subcutaneous administration of 0.2 mg/kg
tropisetron 30 min prior to ouabain did not affect emetic responses (Table 4). However, this treatment is shown to block cisplatin-induced emesis completely.\cite{19} No obvious behavioral change was noted after the injection of tropisetron alone.

**DISCUSSION**

Cardiac glycosides have been shown to have various effects on the cardiovascular system, as well as on the central and peripheral nervous systems. Nausea and vomiting are the apparent toxic reactions to cardiac glycosides. Numerous attempts have been made to clarify the mechanism of emesis induced by cardiac glycosides. Glycoside-induced emesis was not abolished by chronic denervation of the abdominal viscera and heart.\cite{11} Ablation of the area postrema prevented an emetic response of intravenously injected cardiac glycosides in dogs\cite{22} and cats.\cite{5} Therefore, it has been proposed that cardiac glycosides induce emesis by stimulating CTZ, or the area postrema. However, it is impossible not to damage the subarea postrema region by conventional ablation of the area postrema. We must be cautious to interpret experiments in which the area postrema has been lesioned.\cite{7,8} The area postrema may be a part of a “vomiting center” rather than a single organ having a specialized purpose such as being a sensor for emetogenic substance. Furthermore, ablation of the area postrema did not completely eliminate the emetic activity of oral cardiac glycosides. In addition, the delayed phase emesis of i.v. administered glycoside was not attenuated by lesions of the area postrema.\cite{60} Cardiac glycosides failed to evoke emesis when applied intracerebroventricularly in cats.\cite{4,20,21} These results suggest that cardiac glycosides can elicit emesis by stimulating more than one receptive site and that a peripheral mechanism(s) is involved.

In the present study, we showed that intraperitoneal administration of ouabain induced emesis in *Suncus murinus*. It is suggested that cardiac glycosides have a general emetogenic effect in *Suncus murinus*. However, both digitonin and digitoxin were unable to cause an emetic response at non-fatal doses. One possible explanation for the lack of emetic effect of digitonin and digitoxin is pharmacokinetic differences among the three cardiac glycosides due to their different lipophilic properties. Another possible explanation is that there is some variation in the effect of different cardiac glycosides.\cite{5,6,22}

In order to explore the mechanism(s) of ouabain-induced emesis, ouabain was administered intracerebroventricularly. The central administration of ouabain did not cause emesis. However, this does not reflect a lack of effect of ouabain, because it induced prominent and dose-related neurotoxicity, such as ataxia, tremor, convulsions and pating, within a minute. This neurotoxicity was less significant when ouabain was injected intraperitoneally. These results also suggest that the emetic effect is separable from the neurotoxic effects.

Gaitonde *et al.*\cite{20} showed similar results in cats, and suggested that cardiac glycosides stimulate the emetic receptors from the vascular but not from the ependymal side of the CTZ. This interpretation is not convincing because i.c.v. injected cardiac glycosides diffuse rapidly throughout the ventricles. On the other hand, application of ouabain into the lateral ventricles in cat specifically induces a large dose-dependent release of 5-HT (serotonin), which is considered to play a major role in the genesis of cardiac glycoside-related neurotoxicity.\cite{23} Serotonin is one of the neurotransmitters which may be involved in the mechanism of the emetic reflex. Recently, it has been shown that selective agonists for the 5-HT*1A* or 5-HT*2* receptors completely prevent emetic responses caused by various stimuli in animal models, including *Suncus murinus*.\cite{24,25,26} Therefore, a lack of emetogenic potency when ouabain was applied into the cerebral ventricles may be due to a stimulation of these 5-HT receptor subtypes by endogenous 5-HT, which is released by the excitatory effects of ouabain on nerve cells.

In order to elucidate the mechanism(s) of ouabain-induced emesis further, we studied the effect of surgical vagotomy on the emetic response. Vagotomy did not block but delayed the onset of ouabain-induced emesis. Therefore, stimulation of the vagus afferent sensory nerves is probably involved, at least in part.

Cisplatin, a potent cancer chemotherapeutic agent, is one of the most emetogenic drugs.\cite{27} The emetic effect of cisplatin is considered to be mediated via peripheral 5-HT*3* receptors because it was inhibited completely by both 5-HT*3* receptor antagonists and vagotomy.\cite{18,19,28} Since a partial involvement of the vagus afferents had been suggested in the present study, we investigated the participation of peripheral 5-HT*3* receptors in ouabain-induced emesis. Tropisetron failed to affect ouabain-induced emesis, eliminating the possible involvement of peripheral 5-HT*3* receptors. It is possible that ouabain may stimulate the vagal afferents or gastrointestinal smooth muscle directly or via certain types of receptors other than the 5-HT*3* receptor.

Because ouabain-induced emesis was not abolished by surgical vagotomy, the participation of other peripheral or central sites for the emetogenic effect is suggested. Cardiac glycosides exert significant effects on both the central and peripheral nervous systems.\cite{22} They are also considered to enhance α-adrenergic tone by stimulating the area postrema of the medulla oblongata\cite{29} and to increase the discharge rate of chemoreceptor and baroreceptor afferent nerve fibers located in the carotid sinus nerve.\cite{30,31} These regions are considered to be responsible, in part, for the emesis. Further investigations are necessary to clarify the exact mechanism of the emetic effect of ouabain.
REFERENCES