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5-HT3 receptor antagonists injected into the area postrema inhibit cisplatin-induced emesis in the ferret

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1 The purpose of the present study was to identify and investigate the role of 5-hydroxytryptamine$_3$ (5-HT$_3$) receptors in the area postrema in the control of cisplatin-induced emesis in the ferret.

2 Homogenate binding and autoradiography experiments using the high affinity 5-HT$_3$ receptor ligand, [^{3}H]-GR65630, identified the presence of a high concentration of 5-HT$_3$ receptors in the area postrema of the ferret.

3 Intraperitoneal injection of the 5-HT$_3$ receptor antagonists, GR38032F, GR65630A and MDL72222, at doses of 1, 0.1 and 1 mg kg^{-1} respectively, inhibited emesis induced by cisplatin, 9 mg kg^{-1} i.p.

4 Discrete injection of low doses of the 5-HT$_3$ receptor antagonists directly into the area postrema region also inhibited cisplatin-induced (9 mg kg^{-1} i.p.) emesis. The dose ranges used were: GR38032F, 0.01–1 μg; GR65630A, 0.001–0.1 μg; MDL72222, 0.1–10 μg.

5 Cisplatin-induced emesis was not inhibited by discrete injection of ketanserin (30 μg) or methiothepin (30 μg) into the area postrema. Injection of the 5-HT$_3$ receptor agonist, 2-methyl-5-HT, directly into the area postrema produced an incomplete emetic response.

6 These results confirm a role of 5-HT, and in particular 5-HT$_3$ receptors, in the control of cisplatin-induced emesis, and show that at least one functional site for these receptors in modulating the emetic response is the area postrema, the locus of the chemoreceptor trigger zone.

Introduction

It is well documented that the selective 5-hydroxytryptamine$_3$ (5-HT$_3$)-receptor antagonist, GR38032F (Butler *et al.*, 1988), inhibits emesis induced by cytotoxic drugs in man (Cunningham *et al.*, 1987) and in experimental animals (Stables *et al.*, 1987). Experiments in ferrets have revealed that cisplatin-induced emesis is associated with intestinal damage and changes in the levels of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) present in the intestinal mucosa (Gunning *et al.*, 1987), and that abdominal vagotomy ameliorates such emesis (Hawthorn *et al.*, 1988). These latter studies have led to the proposition that cytotoxic drugs cause 5-HT release from the enterochromaffin cells of the gastrointestinal mucosa to induce stimulation of 5-HT$_3$ receptors on the afferent vagal fibres (Ireland & Tyers, 1987). Stimulation of 5-HT$_3$ receptors then induces abnormal discharge of the vagal fibres which evokes the vomiting reflex (Hawthorn *et al.*, 1988).

However, emesis induced by cytotoxic drugs appears to involve centrally, as well as peripherally, located mechanisms. For example, it is known that ablation of the area postrema, the site of the chemoreceptor trigger zone (CTZ) causes inhibition of cisplatin-induced emesis (McCarthy & Borison, 1984). In addition, the involvement of 5-HT mechanisms in the area postrema in the control of emesis is implicated by the observations that the area postrema contains a large number of 5-HT-containing neurones (Pickel & Armstrong, 1984), and that depletion of 5-HT from the area postrema totally inhibits cisplatin-induced emesis (Barnes *et al.*, 1988).

The latter observations on 5-HT in the central nervous system, together with the proposed central role of the area postrema in controlling emesis (Borison *et al.*, 1984), prompted us to undertake studies to investigate directly the role of 5-HT$_3$ receptors in this medullary structure in the emetic response to cisplatin. To this end, we have carried out experiments in the ferret to (i) identify 5-HT$_3$ receptor binding sites in the area postrema, using the

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techniques of homogenate receptor binding and receptor autoradiography, and (ii) determine the effects of discrete injections of GR38032F and other 5-HT_3 receptor antagonists directly into the area postrema on cisplatin-induced emesis.

A preliminary account of these findings has been presented to the British Pharmacological Society (Higgins *et al.*, 1988).

Methods

Animals

Fitch and albino ferrets of either sex weighing 0.5–2 kg were used. The ferrets were allowed free access to food and water before the experiment. Unless stated otherwise each animal was used for one experiment only.

Surgery

Ferrets were anaesthetized with pentobarbitone (30–40 mg kg^{-1} i.p.) and a guide cannula (23 gauge stainless steel tube, 13 mm length) was lowered vertically so that the tip was 9 mm below the skull surface (midline) and fixed to the skull with dental cement and two holding screws. The coronal co-ordinate used was a cross-suture located approximately 2 mm from the posterior edge of the skull. These co-ordinates sited the guide cannula tip 3–5 mm directly above the area postrema. Following treatment with antibiotic (50 mg Streptopen injection) the ferrets were returned to holding pens for a period of 24–96 h before experimentation.

Experimental protocol in conscious ferrets

Emesis was induced in all ferrets by intraperitoneal injection of cisplatin, 9 mg kg^{-1} (dose volume 4.5 ml kg^{-1}). For intraperitoneal administration the 5-HT_3 receptor antagonists were injected immediately after the dose of cisplatin. When injected directly into the area postrema the 5-HT_3 receptor antagonists were administered immediately after the first vomiting episode induced by cisplatin. Following intraperitoneal injection of compounds the ferrets were observed for 3 h, and following central injection they were observed for 1 h and the total number of vomits and retches were recorded. In some experiments the ferrets received two central injections into the area postrema (2-methyl-5-HT followed by apomorphine) which were separated by at least 5 h. All central drug injections were made at a point approximately 1 mm below the area postrema into the hindbrain using a 30 gauge injection cannula. During injection the needle passed between the two rostral projections of the area postrema and thus did not damage the structure. The injection volume of 2 μl was slowly infused over 2 min with the ferret fully conscious.

Histological verification of central injection site

At the end of each experiment, the ferrets were anaesthetized with pentobarbitone (30–40 mg kg^{-1} i.p.) and 2 μl of Indian ink injected into the area postrema via the indwelling cannula. Ten minutes later the ferrets were decapitated, the hindbrain including the cerebellum removed, and sectioned on a freezing microtome. Any ferrets showing no ink penetration into the area postrema were excluded from the study.

Homogenate binding of [^{3}H]-GR65630

Ferrets were decapitated under pentobarbitone anaesthesia, their brains removed and dissected. Tissue samples were homogenised (ultra turrax, 15 s) in 10 volumes of HEPES buffer (50 mM, pH 7.4, 4°C) and centrifuged at 48,000 *g* and 4°C (Sorvall RC5C). The pellet was retained, the homogenisation and centrifugation repeated and the final pellet resuspended in 10 volumes of HEPES buffer.

For the binding assay, tubes (in triplicate) contained 100 μl [^{3}H]-GR65630 (81 Ci $mmol^{-1}$; final concentration 0.2 nM) in HEPES buffer, 50 μl of metoclopramide (30 μM, to define non-specific binding) or vehicle (HEPES buffer) and 100 μl of tissue homogenate (0.2–0.4 mg protein). Tubes were incubated for 30 min at 37°C in a shaking water bath. The reaction was terminated by rapid vacuum filtration through Whatman GF/B filters and extensive washing (5 × 2.5 ml; HEPES buffer) using a Brandel cell harvester. Filters were placed with 10 ml of Picofluor 30 and left overnight before radioactivity counting in a Packard CA2000 scintillation spectrometer. Tissue protein was assayed by the method of Bradford (1976).

Autoradiography

Ferret brains were frozen in liquid nitrogen and mounted in Tissue-Tek O.C.T. embedding medium. Thin sections (20 μm) were cut using an Anglia AS600 cryotome (−15°C). Sections were thaw-mounted on to gelatin-coated glass slides.

Slide-mounted brain sections were incubated with [^{3}H]-GR65630 (0.2 nM) in HEPES buffer (50 mM, pH 7.4, 37°C) in the presence or absence of metoclopramide (30 μM, to define non-specific binding) for 30 min. Sections were washed twice in an excess of HEPES buffer (pH 7.4, 20°C) for 30 s followed by a 2 s wash in distilled water. Sections were dried in a stream of cool air. The slide-mounted brain sections

Table 1 The effect of intraperitoneal injection of 5-HT$_3$ receptor antagonists on cisplatin-induced emesis in the ferret

Compound and dose (mg kg^{-1} i.p.)	n	*No. of vomits*	*No. of retches*	*Latency to emesis* (min)
Control	9	14.3 ± 1.5	123.7 ± 11.9	75.4 ± 2.9
GR38032F (1)	4	0*	0*	313.3 ± 18.2*
GR65630A (0.1)	4	0*	1.0 ± 1.0*	256.7 ± 25.7*
MDL72222 (1)	4	0.25 ± 0.25*	4.3 ± 3.6*	227.5 ± 69.7

Results expressed as means ± s.e.mean.
Dose of cisplatin = 9 mg kg^{-1} i.p. Test compounds were injected immediately after the injection of cisplatin. Vomiting and retching were measured for 3 h following injection of cisplatin. Latencies were measured up to 6 h following injection of cisplatin. *$P < 0.05$ vs controls (Mann-Whitney 'U'-test).

were then opposed to photographic film (Amersham Hyperfilm) for 3 months. Film was developed using Kodak D-19 developer.

Compounds

The 5-HT$_3$ receptor antagonists used were GR38032F (1,2,3,9-tetrahydro-3-[(methyl-imidazol-1-yl)methyl]-9-methyl-4H-carbazol-4-one hydrochloride, $2H_2O$), GR65630A (3-(5-methyl-1H-imidazol-4-yl)-1-(1-methyl-1H-indol-3-yl)-1-propanone maleate) and MDL72222 (1αH,3α,5αH-tropan-3yl-3,5-dichlorobenzoate), and the selective 5-HT$_3$ receptor agonist used was 2-methyl-5-HT; these compounds were synthesized in our own laboratories. The compounds described above and cisplatin (Lederle) were dissolved in saline immediately before use. Methiothepin maleate (Roche) and ketanserin (Janssen) were dissolved in minimal quantities of 1 M HCl and 0.1% tartaric acid respectively, made up to volume with distilled water and adjusted to pH 5 with sodium bicarbonate. Apomorphine hydrochloride (Sigma) was dissolved in saline containing

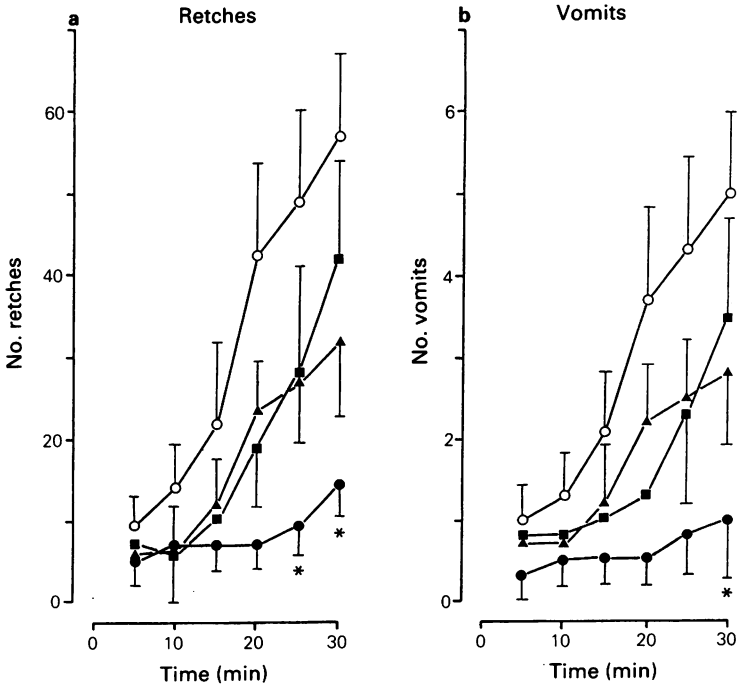

Figure 1 The effect of an injection of GR38032F into the area postrema on cisplatin-induced emesis in the ferret. GR38032F was injected at doses of 0.01 (▲, $n = 6$), 0.1 (■, $n = 4$) and 1 (●, $n = 4$) μg. (○) Shows vehicle control with cisplatin, 9 mg kg^{-1} i.p. ($n = 7$). Results are expressed as cumulative (a) retches/(b) vomits over the appropriate time period. Each point is the mean, and vertical lines s.e.mean, for n observations. *$P < 0.05$.

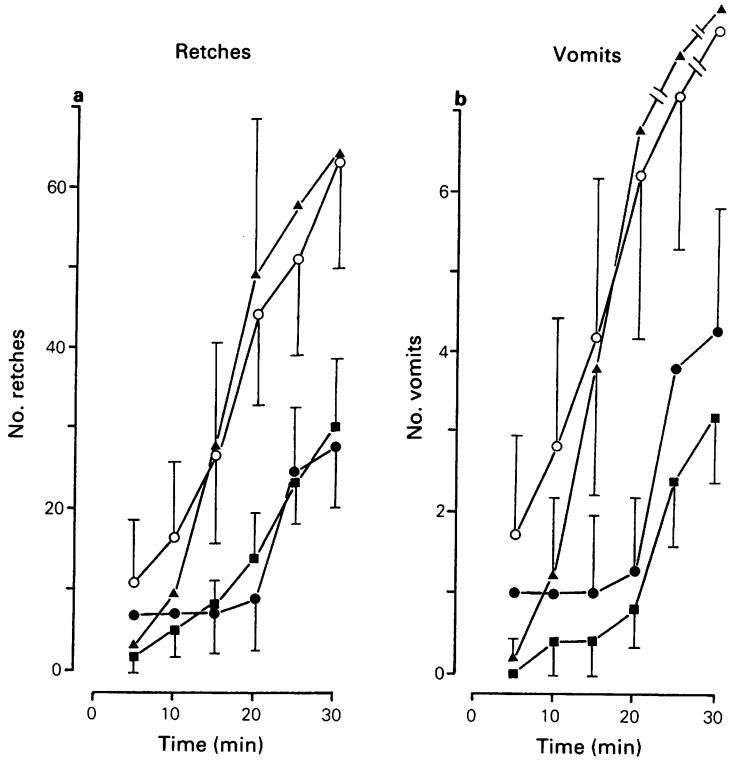

Figure 2 The effect of injection of GR65630A into the area postrema on cisplatin-induced emesis in the ferret. GR65630A was injected at doses of 0.001 (▲, $n = 5$), 0.01 (■, $n = 5$) and 0.1 (●, $n = 4$) μg. (○) Shows vehicle control with cisplatin, 9 mg kg^{-1} i.p. ($n = 6$). Results are expressed as cumulative (a) retches/(b) vomits over the appropriate time period. Each point is the mean and vertical lines s.e.mean for n observations.

200 mg l^{-1} ascorbate. Control solutions for each drug were prepared with the appropriate vehicle.

Doses of drugs are expressed as that of the free base.

Expression of data

Retches and vomits are expressed as cumulative means ± s.e.mean over the specified time period following drug injection. Differences between drug treatments and vehicle pretreated controls were analysed by use of the Mann-Whitney 'U' test.

Results

The effect of intraperitoneal injection of 5-HT$_3$ antagonists on cisplatin-induced emesis

Before commencing the studies on the central injection of compounds, some preliminary experiments were carried out using intraperitoneal injections to establish the anti-emetic actions of the compounds; the results are shown in Table 1. All three 5-HT$_3$ receptor antagonists, given intraperitoneally, were anti-emetic in the ferret.

The effect of 5-HT$_3$ receptor antagonists injected into the area postrema on cisplatin-induced emesis

In these experiments the 5-HT$_3$ receptor antagonists were injected into the area postrema immediately after the first emetic episode, the latency of which did not vary between groups. Within 5 min of microinjection into the area postrema, both GR38032F (0.01–1 μg, Figure 1) and GR65630A (0.001–0.1 μg, Figure 2) produced some inhibition of the number of cisplatin-induced retches and vomits by comparison with controls. This antagonism was particularly marked between 5–20 min after dosing with almost complete inhibition in all animals at 1 μg GR38032F (vomits 5–20 min post dosing: vehicle 2.7 ± 0.8, GR38032F 1 μg 0.3 ± 0.3; $P < 0.05$) and 0.01–0.1 μg

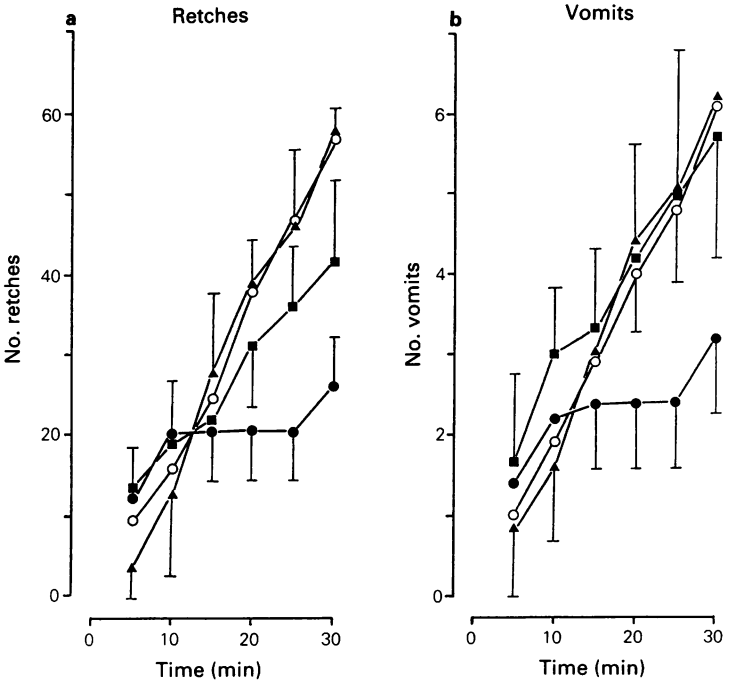

Figure 3 The effect of injection of MDL72222 into the area postrema on cisplatin-induced emesis in the ferret. MDL72222 was injected at doses of 0.1 (▲, $n = 5$), 1 (■, $n = 5$) and 10 (●, $n = 4$) μg. (○) Shows vehicle control with cisplatin, 9 mg kg^{-1} i.p. ($n = 6$). Results are expressed as cumulative (a) retches/(b) vomits over the appropriate time period. Each point is the mean and vertical lines s.e.mean for n observations.

GR65630A (vomits 5–20 min post dosing: vehicle 4.5 ± 0.8, GR65630A 0.1 μg 0.3 ± 0.3; $P < 0.05$). After this time, particularly with GR65630A, the mean emetic response began to return towards control levels. MDL72222 (10 μg) also produced a marked inhibition of cisplatin-induced emesis (Figure 3). However, this compound had a slower rate of onset compared with GR38032F and GR65630A, blockade of emesis being particularly marked 10–25 min after dosing (vomits 10–25 min post dosing: vehicle 2.9 ± 0.8, MDL 72222 10 μg 0.2 ± 0.2; $P < 0.05$).

Following 5-HT$_3$ antagonist treatment, the ferrets also displayed fewer signs of nausea, i.e. searching behaviour, backwards walking, licking of lips and yawning were reduced. Instead, the ferrets tended to lie quietly on the cage floor; reactivity and co-ordination were not impaired as assessed by visual observation.

Effect of methiothepin and ketanserin injected into the area postrema on cisplatin-induced emesis

Neither methiothepin (30 μg) nor ketanserin (30 μg) injected into the area postrema, significantly modified the emetic response induced by cisplatin (Figure 4). The ferrets treated with methiothepin, but not ketanserin, showed some sedation and a flattened posture approximately 10 min after administration.

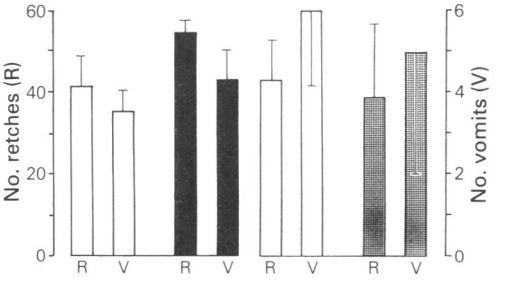

Figure 4 Effects of ketanserin or methiothepin injected into the area postrema against cisplatin-induced emesis in the ferret. Cisplatin was administered at a dose of 9 mg kg^{-1} i.p. Open columns, vehicle control ($n = 3$ to 4). Solid columns, methiothepin, 30 μg ($n = 3$). Stippled columns, ketanserin, 30 μg ($n = 3$). Both retches (R) and vomits (V) are the cumulative total obtained 0–30 min following injection of the test compound. Results are expressed as means and vertical bars show s.e.mean.

Table 2 Effect of 2-methyl-5-HT following injection into the area postrema of the ferret

	Dose (μg)	n	*Vomit latency*	*Behavioural effects*	*Emesis*	*Other changes* (>10 min)
2-Me-5-HT	1	2	>30 min	NSE	0	NSE
	3	2	>30 min	Search, back walk, licking lips, sl. salivation	0	NSE
	10	2	>30 min	As above 1/2 retching	0	Flat posture LMA markedly reduced, hypotension?
	30	5	>30 min	As above 1/5 retching	0	As above
Apomorphine	10	4	0.3 ± 0.1 min[a]	Search, back walk, licking lips	23.3 ± 7.8 ret.[a] 2.8 ± 0.9 vom.[a] 4/4 responder	Effects gone >15 min

Ferrets had no prior treatment with cisplatin. Dose volume = 2 μl.
[a] Data are means ± s.e.mean.
NSE = no significant effect.

Effect of 2-methyl-5-HT and apomorphine injected into the area postrema

The 5-HT_3 receptor agonist 2-methyl-5-HT (1–30 μg) injected into the area postrema induced signs of 'nausea' (salivation, searching, licking of lips, backwards walking, some retching) following doses of 3–30 μg, although at 10 μg and above marked sedation was apparent approximately 10 min after injection (Table 2). However, at no dose was any emesis observed within 30 min of treatment.

Apomorphine (10 μg) produced a full emetic response within 0.5 min of dosing in ferrets that failed to respond to 2-methyl-5-HT (Table 2). Both retching and vomiting were observed in all animals. These effects had a duration of approximately 15 min, after which time the behaviour of the ferrets returned to normal.

Homogenate binding and autoradiography

Specific [^{3}H]-GR65630 binding was evident in homogenates of ferret brain. This binding was discretely localised (Table 3). The highest level of specific binding was in homogenates of the area postrema. Other brain areas showing high levels of specific binding were the striatum, nucleus accumbens/olfactory tubercle, septum and amygdala.

Autoradiographic analysis of [^{3}H]-GR65630 binding to sections of ferret brain confirmed the observation of high specific binding in the area postrema (Figure 5). No specific binding was evident in areas surrounding the area postrema.

Discussion

Intraperitoneal injection of the 5-HT_3 receptor antagonists, GR38032F, GR65630A and MDL72222, inhibited cisplatin-induced emesis in the

Table 3 The specific binding of [^{3}H]-GR65630 to homogenates of discrete areas of ferret brain

Brain region	*Bound* (fmol mg^{-1} protein)
Entorhinal cortex	0.4 ± 0.1
Retrosplenic cortex	0.1 ± 0.04
Frontal cortex	0.4 ± 0.1
Cingulate cortex	0.3 ± 0.1
Temporal cortex	0.3 ± 0.1
Occipital cortex	0.2 ± 0.05
Parietal cortex	0.3 ± 0.1
Amygdala	1.0 ± 0.4
Hippocampus	0.5 ± 0.2
Nucleus accumbens/ olfactory tubercle	2.8 ± 0.7
Septum	1.4 ± 0.3
Thalamus	0.4 ± 0.02
Hypothalamus	0.8 ± 0.2
Striatum	2.9 ± 0.11
Cerebellum	0.1 ± 0.03
Area postrema	37.1 ± 12.3

Results are the means ± s.e.mean of three separate experiments. Non-specific binding was determined by the inclusion of metoclopramide (30 μM). The specific proportion of total binding varied between 15% and 90% depending on brain area. Protein was assayed by the method of Bradford (1976).

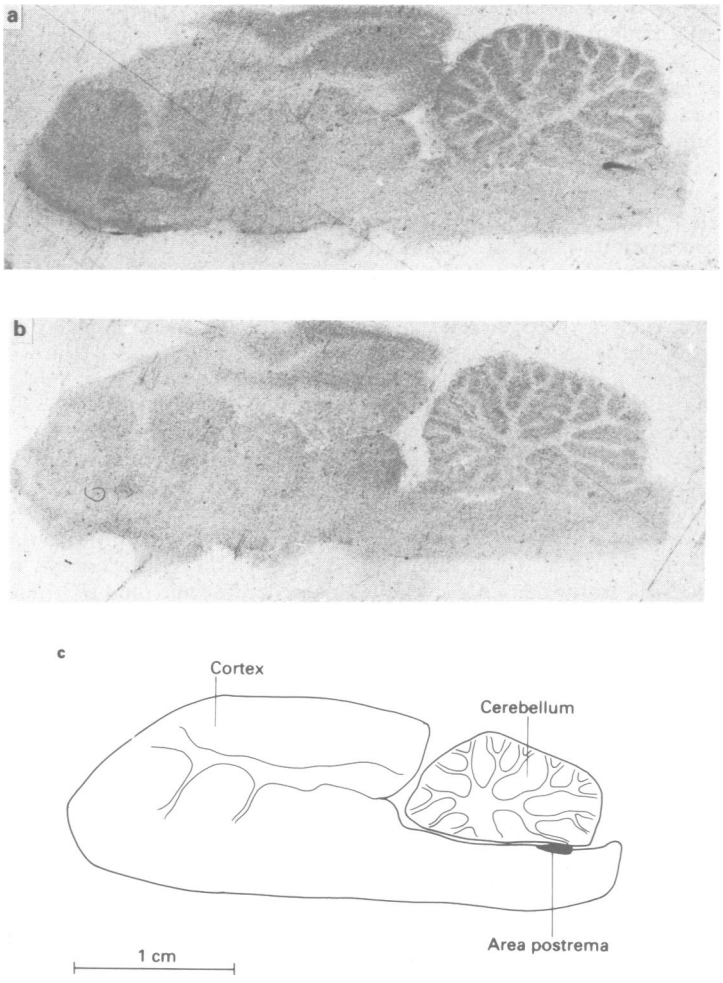

Figure 5 The autoradiography of (a) total and (b) non-specific [^{3}H]-GR65630 binding to longitudinal sections (20 μm) of ferret brain. Darker regions represent areas with high receptor density. Sections shown are approximately on the midline. (c) Line drawing of ferret brain corresponding to the autoradiographs (a) and (b). The area postrema is a flat medullary structure lying just beneath the cerebellum.

ferret. Similar doses of GR38032F and MDL72222 to those used intraperitoneally in the present study have previously been shown to be anti-emetic against cisplatin in the ferret when administered by the intravenous route (Miner & Sanger, 1986; Costall *et al.*, 1987). Like GR38032F, GR65630A at the dose used produced an almost complete inhibition of cisplatin-induced emesis over the 3 h observation period.

Although GR65630A has been shown previously to bind specifically to several discrete areas of rat brain (Kilpatrick *et al.*, 1987), the area postrema was not included in that study. However, as pointed out above, a number of observations led us to investigate the specific 5-HT$_3$ receptor binding in the area postrema, the most important being that this medullary structure is the site of the chemoreceptor trigger zone (CTZ). Also, it was necessary to carry out these experiments on ferret tissue since this species was used to investigate emesis. The results obtained with experiments on both receptor binding and autoradiography showed that the area postrema contains a high concentration of 5-HT$_3$ receptors.

Having established the presence of 5-HT$_3$ receptors in the area postrema it was important to investigate their role, if any, in the control of emesis. This was done by examining the effect of discrete injections of 5-HT$_3$ receptor antagonists into the area

postrema on cisplatin-induced emesis. All three of the compounds tested inhibited emesis with a rank order of potency of GR65630A > GR38032F > MDL72222, which agrees well with their affinities for 5-HT_3 receptors in rat vagus nerve (Kilpatrick *et al.*, 1987). Also, when administered directly into the area postrema the anti-emetic dose of each compound was considerably smaller than when given by intraperitoneal or intravenous injection, for instance, the minimal effective intravenous doses of GR38032F and MDL72222 against cisplatin-induced emesis in the ferret are 0.01 mg kg^{-1} and 2 × 0.05 mg kg^{-1}, respectively (Miner & Sanger, 1986; Stables *et al.*, 1987). Thus it is unlikely that the anti-emetic response following injection into the area postrema was due to diffusion of the compound from the injection site into the general circulation. Administration of methiothepin or ketanserin into the area postrema had no effect on emesis, indicating that other 5-HT receptor types, 5-HT_1-like and 5-HT_2, are unlikely to be involved. In the light of the results obtained with the 5-HT_3 receptor antagonists, the failure of the agonist 2-methyl-5-HT to evoke emesis readily was surprising and contrasted sharply with the powerful emetic effect of apomorphine. A possible explanation for this is that 5-HT_3 receptors in the area postrema have a permissive role in emesis so that their activation does not evoke emesis, but is necessary for emesis to occur. Alternatively, since 2-methyl-5-HT is a basic hydrophilic compound insufficient penetration into the area postrema may account for the absence of a full emetic response. A third explanation is that the sedative effect of 2-methyl-5-HT may have overcome any underlying emetic response. This, however, is unlikely because methiothepin also caused sedation yet it was not able to inhibit cisplatin-induced emesis. Finally, at present we have no knowledge as to the intrinsic activity of 2-methyl-5-HT at area postrema 5-HT_3 receptors. Thus, 2-methyl-5-HT may function as a partial agonist at this site with insufficient efficacy to evoke a full emetic response. Clearly further studies are necessary to clarify this interesting result.

The data in Figures 1, 2 and 3 reveal two important points. First, the 5-HT_3 receptor antagonists did not produce an immediate effect since some emesis occurred up to 5 min after their central injection. Second, following intravenous injection even the minimally effective doses of GR38032F (0.01 mg kg^{-1}) and MDL72222 (2 × 0.5 mg kg^{-1}) must exhibit a duration of approximately 60–90 min since this is the latency to emesis following administration of cisplatin (Miner & Sanger, 1986; Stables *et al.*, 1987). However, when the 5-HT_3 receptor antagonists were injected into the area postrema emesis started to recur only 20–30 min after injection. The area postrema is a 'V' shaped structure and the injections were made between the two rostral projections so as to avoid tissue damage. The short delay in the onset of the anti-emetic effect may be attributable to the time required for the compounds to diffuse to all regions of the CTZ within the area postrema. In addition, the area postrema has a rich blood supply and is continuously being flushed with cerebrospinal fluid, and these factors would increase the rate of elimination of compounds from the site of injection and so limit their duration of action.

It has previously been shown that injection of the 5-HT_3 receptor antagonist, zacopride, into the fourth ventricle inhibits cisplatin-induced emesis (Smith *et al.*, 1988). However, the present study is believed to be the first to demonstrate inhibition of cisplatin-induced emesis by discrete injections of a 5-HT_3 receptor antagonist into the area postrema, the region containing the CTZ. The precise mechanisms affected by the antagonists are unclear at present, although some clues may be derived from previous work with cisplatin. Intravenous injection of cisplatin induces emesis with a delay of approximately 60 min and 100 min in the ferret and cat respectively (Stables *et al.*, 1987; Smith *et al.*, 1988), whereas injection of cisplatin directly into the fourth ventricle of the cat caused emesis within 4 min (Smith *et al.*, 1988). Since the area postrema is outside the blood–brain barrier, intravenously injected cisplatin would be expected to make rapid contact with this structure. Nevertheless, the emetic response to intravenous cisplatin is delayed (see above), and it therefore seems improbable that cisplatin exerts its emetic effect principally through a mechanism located solely in the area postrema. It is more likely that cisplatin activates a peripheral mechanism, probably involving the gastro-intestinal (GI) tract. Indeed, such a contention is supported by the observation that cisplatin accumulates (Rosenberg, 1985), causes degenerative changes (Vermorken & Pinedo, 1982) and affects the levels of 5-HT and 5-HIAA (Gunning *et al.*, 1987) in the GI tract. Also, sectioning of the visceral afferent nerves in the ferret completely blocks cisplatin-induced emesis (Hawthorn *et al.*, 1988). Why then, if cisplatin induces emesis mainly through an action on the GI tract, do 5-HT_3 receptor antagonists inhibit such emesis when injected into the area postrema? Three observations are pertinent to this discussion: first, the area postrema contains large numbers of 5-HT-containing neurones (Pickel & Armstrong, 1984); second, it contains a high concentration of 5-HT_3 receptors (this study); and third, it receives visceral afferents from the GI tract (Andrews & Hawthorn, 1988). Thus, is it possible that the area postrema acts as a relay between the visceral afferents and the vomiting centre identified in the reticular formation, and that disruption of

5-hydroxytryptaminergic pathways, by injection of 5-HT$_3$ receptor antagonists into the area postrema, inhibits emesis.

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