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European Journal of Pharmacology 461 (2003) 197–206
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Antiemetic effect of a tachykinin NK_1 receptor antagonist GR205171 on cisplatin-induced early and delayed emesis in the pigeon

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Received 29 August 2002; received in revised form 31 December 2002; accepted 8 January 2003

Abstract

Cisplatin (4 mg/kg, i.v.) induced both early emesis, which appears within the first 8-h period, and delayed emesis, which appears between 8 and 48 h after its administration to pigeons. GR205171 ([(2*S*-*cis*)-*N*-((2-methoxy-5(5-(trifluoromethyl)-1*H*-tetrazol-1-yl)-phenyl) methyl)-2-phenyl-3-piperidinamine dihydrochloride]) administered intramuscularly (1–10 mg/kg) reduced significantly the number of emetic response to cisplatin: this reduction was 60–81% ($P<0.05$) for early emesis and 48–64% ($P<0.05$) for the delayed response. Intracerebroventricularly administered GR205171 (30 μg/kg) also reduced the number of emetic responses: 53% ($P<0.05$) in early emesis and 88% ($P<0.05$) in the delayed response. However, the latency time to the first emesis was not affected by GR205171. Direct injection of cisplatin (10 μg/kg) into the fourth ventricle produced emesis, which was reduced by GR205171 administered via the peripheral or central route. Substance P-immunoreactive fibres were distributed throughout the dorsal vagal complex. These results suggest that the antiemetic effect of GR205171 on both emetic responses to cisplatin acts on a central site, and that the onset of the emetic response may be mediated partly via GR205171-insensitive mechanisms.

Keywords: Emesis; Cisplatin; Tachykinin NK_1 receptor antagonist; GR205171; (Pigeon)

1. Introduction

Nausea and emesis are the main factors of reduced drug compliance in patients receiving anticancer drugs such as cisplatin (Kris et al., 1985; Martin, 1996). In humans, cisplatin induces both early and delayed emesis, and both types have also been observed in ferrets (Rudd et al., 1994, 1996a,b; Rudd and Naylor, 1994) and piglets (Milano et al., 1995; Grélot et al., 1996).

Recently, tachykinin NK_1 receptor antagonists have been shown to possess broad-spectrum antiemetic activity (Watson et al., 1995; Gardner et al., 1995; Gonsalves et al., 1996). It was confirmed that the antiemetic action of tachykinin NK_1 receptor antagonists is centrally mediated (Gardner et al., 1994; Hargreaves et al., 1994; Tattersall et al., 1996), and it was proposed that the antiemetic site of tachykinin NK_1 receptor antagonists is located in the nucleus of the tractus solitarius (Watson et al., 1995). The antiemetic effect of selective tachykinin NK_1 receptor antagonists, CP 99,994 ((+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine, Rudd et al., 1996b) and PD 154075 ([(2-benzofuran)-CH_2OCO]-(*R*)-α-MeTrp-(*S*)-$NHCH(CH_3)$Ph, Singh et al., 1997) against the cisplatin-induced early and delayed emesis was demonstrated in ferrets. Furthermore, GR205171 ([(2*S*-*cis*)-*N*-((2-methoxy-5(5-(trifluoromethyl)-1*H*-tetrazol-1-yl)-phenyl) methyl)-2-phenyl-3-piperidinamine dihydrochloride]) as a tachykinin NK_1 receptor antagonist produced a potent and long-lasting inhibition of both early and delayed emesis induced by cisplatin in piglets (Grélot et al., 1998).

Experimental animals known to be used in studies on emesis include dogs, cats, *Suncus murinus*, piglets and ferrets. Sensitivity to various emetics or antiemetic drugs differs among animal species (Borison et al., 1981; King, 1988). Early and delayed emesis induced by cisplatin in humans was also observed in pigeons (Uchiyama and Suzuki, 1992). In our previous study, we showed that intravenously

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doi:10.1016/S0014-2999(03)01311-6

injected cisplatin dose-dependently induces emesis in the pigeon, and at a dose of 4 mg/kg induces both types of emesis consisting of early and delayed phases which appear within the first 8-h period and between 8 and 48 h after cisplatin administration, respectively (Tanihata et al., 2000).

The present study was aimed at confirming the antiemetic effect of GR205171, a tachykinin NK_1 receptor antagonist, on the cisplatin-induced early and delayed emesis in the pigeon, and an immunohistochemical study was undertaken to determine the presence of substance P-immunoreactive fibres in regions of the dorsal vagal complex including the area postrema, the nucleus of the tractus solitarius and the dorsal motor nucleus of the vagus nerve in the pigeon. In preliminarily experiments we observed that cisplatin injected into the fourth ventricle induced emesis, suggesting a central site for the cisplatin-induced emesis. Therefore, the emetogenic activity of centrally administered cisplatin and the antiemetic effect of GR205171 against this emesis were also examined.

2. Materials and methods

2.1. Animals

Adult domestic pigeons of either sex weighing between 400 and 550 g (Saitama Experimental Animal Supply, Saitama, Japan) were used. All pigeons were housed under a 12-h light/dark cycle, and standard pigeon chow (Daiki, Saitama, Japan) and water were available ad libitum. All animal experimental procedures were carried out under the Guidelines for Animal Experiments, Faculty of Medicine, Toho University.

2.2. Cisplatin-induced emesis

The pigeons were placed in individual cages, and cisplatin was administered intravenously (i.v.) via a brachial wing vein or intracerebroventricularly (i.c.v.) through an implanted cannula. The behaviour of the pigeons was observed with a

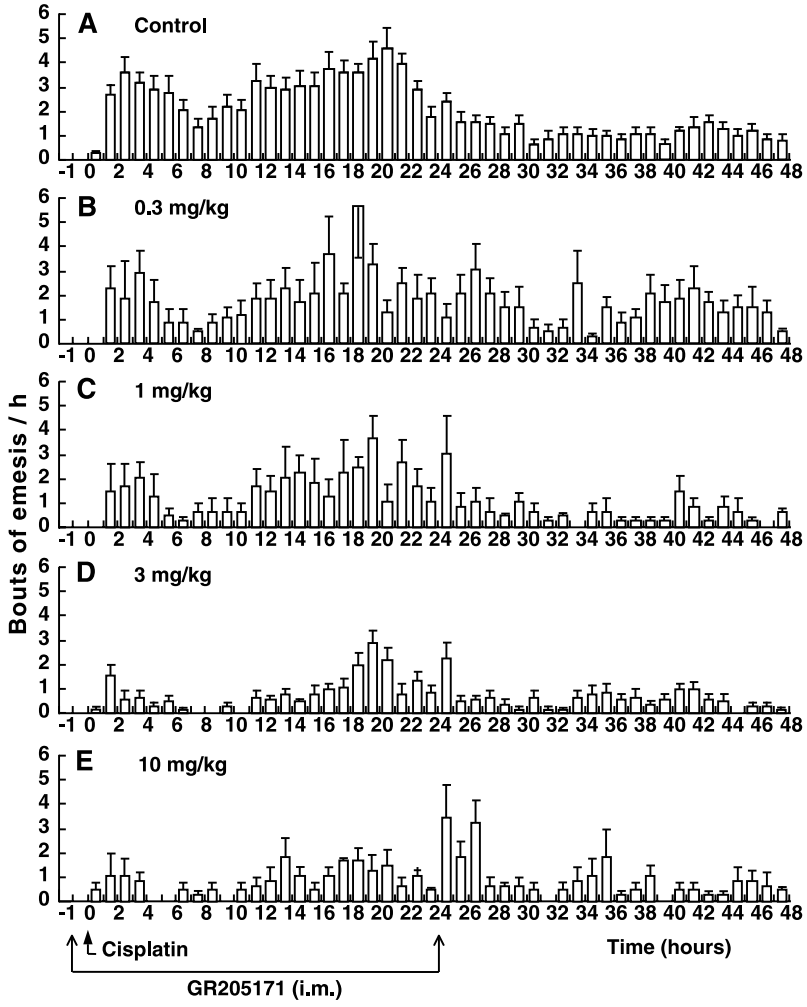

Fig. 1. The profile of the emetic response induced by intravenously injected cisplatin (4 mg/kg) in pigeons during a 48-h observation period ($n = 22$), and the effect of GR205171 (0.3–10 mg/kg, i.m., administered twice, 1 h before and 24 h after cisplatin administration) on the cisplatin-induced emetic response ($n = 5–10$). Each hourly bin with a vertical bar represents the mean ± S.E.M. of the number of bouts of emesis occurring in 1-h time intervals after cisplatin administration at time zero.

video-monitoring system for up to 24 or 48 h under unrestricted conditions. During the observation period, food and water were available ad libitum. Each pigeon was used once. Vomiting and retching associated with and without oral expulsion, respectively, were considered as the emetic response (Preziosi et al., 1992). The emetic responses were characterized by a bout of emesis and more than one emetic behaviour occurred within one bout. The latency time to first emesis, the number of bouts of emesis and the total number of emetic behaviours were recorded.

The antiemetic effect of GR205171 was studied by intramuscular injections (0.3–10 mg/kg, i.m.) into the greater pectoral muscle or by intracerebroventricular administration (3 and 30 μg/kg, i.c.v.) through an implanted cannula. GR205171 is reported to have long-lasting antiemetic activity against cisplatin-induced emesis in piglets (Grélot et al., 1998). In the present study, GR205171 was administered twice: 1 h before and 24 h after cisplatin administration.

Experiments were usually performed with 10 pigeons at a time, and several control pigeons were always placed in each experiment. The present study primarily focused on the antiemetic activity of intramuscularly administered GR205171 at the most effective dose of 3 mg/kg, and we attempted to use a minimum number of animals to provide meaningful results. Therefore, the sizes were unequal for the control and the experimental (intramuscular GR205171 treatment) groups.

2.3. Surgical preparation for i.c.v. administration

Pigeons were anaesthetized with pentobarbital sodium (35 mg/kg, i.m., Sigma, St. Louis, MO, USA), and a stainless-steel guide cannula (G-14, Eicom, Kyoto, Japan) was surgically implanted into the fourth ventricle using a stereotaxic apparatus (SR-6N, Narishige Scientific Instrument Lab., Tokyo, Japan) with a Revzin pigeon adaptor (Karten and Hodos, 1967). The stereotaxic coordinates were 0.50 mm posterior from the interaural line in the midline, and 9.30 mm below the surface of the calvaria according to our adjustment from the atlas of Karten and Hodos (1967). The cannula was fixed with dental cement and screws to the skull, and a dummy cannula (D-14, Eicom) was inserted into the guide cannula. The animals were administered benzylpenicillin (20,000 U/kg, i.m., crystalline penicillin G potassium, Meiji Seika Kaisha, Tokyo, Japan) and gentamicin sulfate ointment (Gentacin® ointment, Schering-Plough, Osaka, Japan) was applied to the sutural sites. The animals were allowed 5 days to recover from surgery.

2.4. Immunohistochemistry for substance P

Pigeons were deeply anaesthetized with pentobarbital sodium (50 mg/kg, i.m.), and perfused through the ascending aorta with 100 ml of 0.2% heparinized 0.1 M phosphate buffer (pH 7.4) followed by 1000 ml of a cold fixative containing 4% paraformaldehyde in phosphate buffer. The brainstem was removed and postfixed overnight in fixative at 4 °C, immersed in 20% sucrose in phosphate buffer for 8 h at 4 °C. Fifty-micrometer horizontal sections were cut on a freezing microtome and collected in cold phosphate buffer (4 °C).

The sections were washed in phosphate buffer, and one series of free-floating sections was used for immunocytochemistry for substance P, and a second series was stained with 0.25% thionine to correlate immunocytochemical data with cytoarchitectonic subdivisions of the dorsal vagal complex including the area postrema, nucleus of the tractus solitarius and dorsal motor nucleus of the vagus nerve described by Katz and Karten (1983a,b).

The sections for immunocytochemistry were incubated for 2 days at 4 °C with rabbit primary antibody against substance P (UCB-Bioproducts, Braine-l'Alleud, Belgium) diluted 1:5,000 in phosphate buffer containing 4% normal goat serum and 0.3% Triton X-100. After being washed in

Table 1
Effect of intramuscularly injected GR205171 on the cisplatin (4 mg/kg, i.v.)-induced emetic response during a 48-h observation period in pigeons

GR205171 (mg/kg)	No. of pigeons tested	% Emetic pigeons	Onset (min)	Bouts of emesis	Total emetic behaviours
0–8 h after cisplatin administration					
Control	22	100	90.5 ± 6.0	18.3 ± 2.4	143.8 ± 16.4
0.3	5	100	168.8 ± 66.9	10.4 ± 4.8	142.2 ± 75.9
1	5	80	135.5 ± 21.8	7.4 ± 3.0[a]	62.2 ± 21.2
3	10	80	107.3 ± 22.4	3.4 ± 0.7[b]	28.3 ± 6.8[b]
10	5	60	144.3 ± 47.8	3.8 ± 3.1[b]	25.6 ± 19.3[b]
8–24 h after cisplatin administration					
Control	22	100	–	48.2 ± 4.4	413.3 ± 41.6
0.3	5	100	–	32.8 ± 11.3	382.6 ± 155.1
1	5	100	–	26.4 ± 8.2[a]	259.8 ± 64.7
3	10	100	–	14.6 ± 2.3[b]	134.1 ± 22.0[b]
10	5	100	–	14.2 ± 2.9[b]	128.2 ± 32.9[b]
24–48 h after cisplatin administration					
Control	22	100	–	26.2 ± 4.0	128.1 ± 27.4
0.3	5	100	–	33.0 ± 9.3	210.6 ± 69.5
1	5	100	–	14.6 ± 5.8	115.0 ± 46.5
3	10	100	–	12.1 ± 4.3	73.8 ± 22.7
10	5	100	–	20.0 ± 6.3	134.0 ± 40.0
0–48 h after cisplatin administration					
Control	22	100	90.5 ± 6.0	92.8 ± 8.2	685.3 ± 68.6
0.3	5	100	168.8 ± 66.9	76.2 ± 19.8	735.4 ± 257.6
1	5	100	267.6 ± 133.2	48.4 ± 13.2[a]	437.0 ± 82.1
3	10	100	295.0 ± 129.7	30.1 ± 6.6[b]	236.2 ± 41.9[b]
10	5	100	438.6 ± 184.2	38.0 ± 8.8[b]	287.8 ± 64.9[a]

GR205171 was administered twice, 1 h before and 24 h after cisplatin administration. The values (mean ± S.E.M.) for the bouts of emesis and the total emetic behaviours are the numbers of bouts of emesis and the total number of emetic behaviours, respectively. The values (mean ± S.E.M.) for onset are the latency times to first emesis in the pigeons which vomited after cisplatin administration.

[a] $P < 0.05$: statistically different from controls with cisplatin alone.

[b] $P < 0.01$: statistically different from controls with cisplatin alone.

phosphate buffer, the sections were incubated in biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) in phosphate buffer containing 2% normal goat serum and 0.3% Triton X-100 for 3 h at 20 °C, and incubated in the avidin–biotin–peroxidase complex solution (ABC kit, Vector) for 90 min at 20 °C. Following washes in phosphate buffer, the sections were incubated for 5–10 min at room temperature in 0.02% 3,3′-diaminobenzidine (Wako, Osaka, Japan) in phosphate buffer with 0.002% hydrogen peroxide for visualization. Then the sections were washed in phosphate buffer, mounted on gelatinized slides, dehydrated and coverslipped.

2.5. Drugs

Cisplatin [*cis*-platinum (II) diamine dichloride, Sigma] was dissolved in 0.9% saline solution at 65–70 °C, followed by cooling to 45–50 °C and administered immediately. GR205171 [(2*S*-*cis*)-*N*-((2-methoxy-5(5-(trifluoromethyl)-1*H*-tetrazol-1-yl)-phenyl) methyl)-2-phenyl-3-piperidinamine dihydrochloride] was generously supplied by Glaxo Wellcome Research and Development (UK), and dissolved in 0.9% saline solution just before use.

2.6. Data analysis

The values presented are the mean ± S.E.M. The differences among means were evaluated for statistical significance using the one-way analysis of variance followed by either Dunnett's test or Tukey's multiple comparison test. Fisher's exact test was used for the statistical evaluation of the incidence of emesis. $P<0.05$ was considered statistically significant.

3. Results

3.1. Intravenously injected cisplatin-induced emesis

Cisplatin at a dose of 4 mg/kg induced emesis in all of the injected pigeons with a latency of 90.5 ± 6.0 min ($n=22$). The emetic response reached a peak at 2–3 h, and decreased gradually within 8 h after injection. Then the second-phase emetic response, whose peak was found at 19–22 h, lasted up to 48 h (Fig. 1).

The emetic responses within the first 8-h period and the period between 8 and 48 h were called early and delayed emesis, respectively (Tanihata et al., 2000).

3.2. Effect of GR205171 on intravenously injected cisplatin-induced emesis

GR205171 was administered twice by an intramuscular or intracerebroventricular route, 1 h before and 24 h after cisplatin administration. Intramuscularly injected GR205171 at doses of 0.3, 1, 3 and 10 mg/kg reduced the

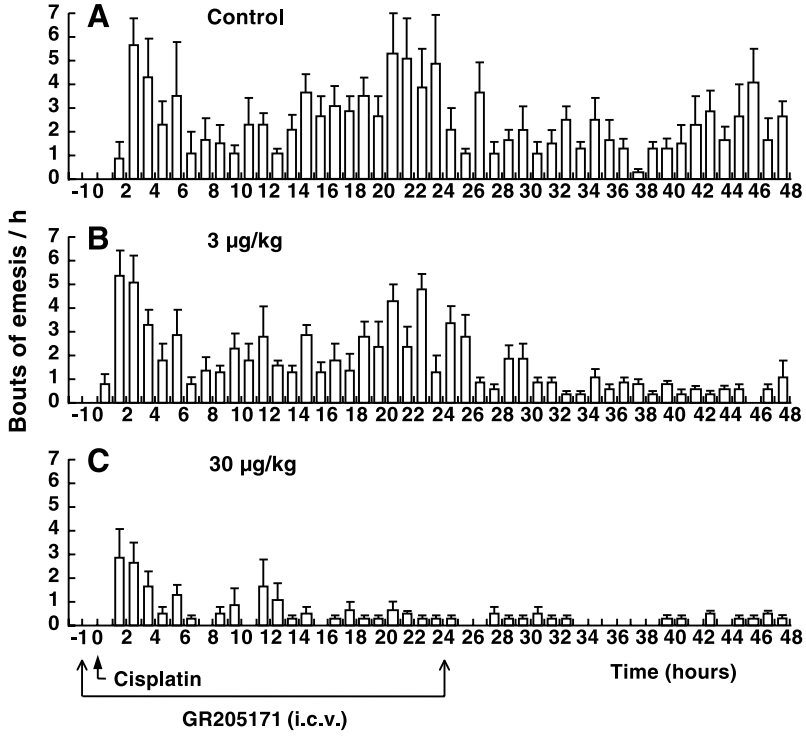

Fig. 2. The effect of intracerebroventricularly injected GR205171 (3 or 30 μg/kg, administered twice, 1 h before and 24 h after cisplatin administration) on the cisplatin (4 mg/kg, i.v.)-induced emetic response during a 48-h observation period in pigeons ($n=5–6$). Each hourly bin with a vertical bar represents the mean ± S.E.M. of the number of bouts of emesis occurring in 1-h time intervals after cisplatin administration at time zero.

number of bouts of cisplatin-induced emesis by 43.2% ($P>0.05$), 59.6% ($P<0.05$), 81.4% ($P<0.05$) and 79.2% ($P<0.05$), respectively, in the early phase and by 23.8% ($P>0.05$), 47.7% ($P<0.05$), 64.2% ($P<0.05$) and 54.1% ($P<0.05$), respectively, in the delayed phase. GR205171 significantly reduced the early emetic response to cisplatin within the first 8-h period, but the latency time to first emesis in the pigeons which vomited within 8 h after cisplatin administration was not significantly affected by GR205171 (Fig. 1; Table 1).

Intracerebroventricularly injected GR205171 at the dose of 3 μg/kg did not affect the early emetic response but reduced the number of bouts of emesis in the delayed phase by 38.4% ($P<0.05$). GR205171 (30 μg/kg) administered intracerebroventricularly reduced the number of bouts of emesis induced by cisplatin by 53.2% ($P<0.05$) in early emesis and 88.3% ($P<0.05$) in delayed emesis, without affecting the latency time to the first emesis (Fig. 2; Table 2).

3.3. Emesis induced by intracerebroventricularly injected cisplatin

Cisplatin at doses of 5 μg/kg or less did not induce emesis within 8 h after injection, while 10 μg/kg induced emesis in all of the injected pigeons with a latency of 225.3 ± 22.8 min ($n=6$). The emetic response declined within 24 h. The dual response (early and delayed emesis) observed with the intravenous administration of cisplatin was obscured with the intracerebroventricular administration. Four of six pigeons died between 24 and 48 h after cisplatin injection (10 μg/kg) (Table 3).

Table 2
Effect of intracerebroventricularly injected GR205171 on the cisplatin (4 mg/kg, i.v.)-induced emetic response during a 48-h observation period in pigeons

GR205171 (μg/kg)	No. of pigeons tested	% Emetic pigeons	Onset (min)	Bouts of emesis	Total emetic behaviours
0–8 h after cisplatin administration					
Control	5	100	129.4 ± 14.1	18.8 ± 5.1	180.2 ± 77.0
3	6	100	70.8 ± 7.9	20.7 ± 4.6	188.0 ± 56.6
30	5	100	122.6 ± 27.7	8.8 ± 2.0[a]	73.0 ± 22.6
8–24 h after cisplatin administration					
Control	5	100	–	46.6 ± 5.9	326.8 ± 53.4
3	6	100	–	34.7 ± 4.6	249.8 ± 54.1
30	5	100	–	7.0 ± 2.8[b]	23.0 ± 10.6[b]
24–48 h after cisplatin administration					
Control	5	100	–	43.8 ± 10.1	216.8 ± 71.9
3	6	100	–	21.0 ± 3.9[a]	160.2 ± 54.4
30	5	40	–	3.6 ± 2.4[b]	11.0 ± 7.1[a]
0–48 h after cisplatin administration					
Control	5	100	129.4 ± 14.1	109.2 ± 15.0	723.8 ± 130.4
3	6	100	70.8 ± 7.9	76.3 ± 8.4[a]	598.0 ± 134.7
30	5	100	122.6 ± 27.7	19.4 ± 5.0[b]	107.0 ± 16.2[b]

GR205171 was administered twice, 1 h before and 24 h after cisplatin administration. The values (mean ± S.E.M.) for the bouts of emesis and the total emetic behaviours are the numbers of bouts of emesis and the total number of emetic behaviours, respectively. The values (mean ± S.E.M.) for onset are the latency times to first emesis after cisplatin administration.

[a] $P<0.05$: statistically different from controls with cisplatin alone.

[b] $P<0.01$: statistically different from controls with cisplatin alone.

Table 3
Intracerebroventricularly injected cisplatin-induced emetic response during a 48-h observation period in pigeons

Cisplatin (μg/kg)	No. of pigeons	Latency to first emesis (min)[a]	% Pigeons showing emesis		
			0–8 h	8–24 h	24–48 h
3	5	–	0	0	0
5	5	925.6 ± 98.4	0	100	100
7	7	629.3 ± 155.7	29	100	71
10	6	225.3 ± 22.8	100	100	–[b]

[a] The values (mean ± S.E.M.) are the latency times to first emesis after cisplatin injection.

[b] Four of six pigeons died between 24 and 48 h after cisplatin (10 μg/kg) injection.

3.4. Effect of GR205171 on emesis induced by intracerebroventricularly injected cisplatin

GR205171 administered by the intramuscular (3 mg/kg) or intracerebroventricular (30 μg/kg) route 1 h before cisplatin did not affect the incidence of emesis due to cisplatin (10 μg/kg, i.c.v.), but significantly reduced the number of emetic responses during a 24-h observation period. The latency time to first emesis was not significantly

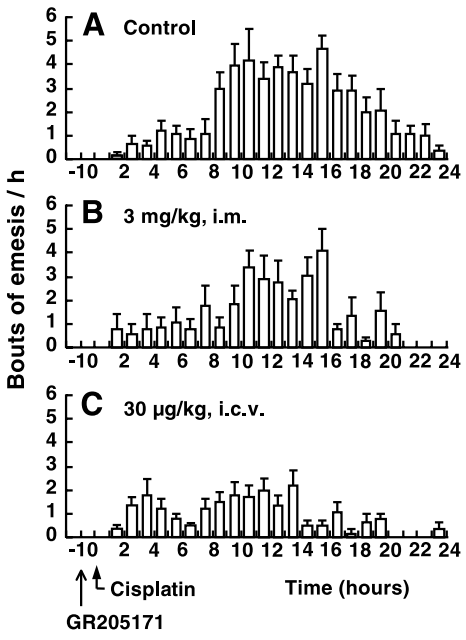

Fig. 3. The profile of the emetic response induced by intracerebroventricularly injected cisplatin (10 μg/kg) in pigeons during a 24-h observation period, and the effect of GR205171 (3 mg/kg, i.m. or 30 μg/kg, i.c.v., 1 h before cisplatin administration) on the cisplatin-induced emetic response ($n=6–8$). Each hourly bin with a vertical bar represents the mean ± S.E.M. of the number of bouts of emesis occurring in 1-h time intervals after cisplatin administration at time zero.

Table 4
Effect of GR205171 on the emetic response induced by intracerebroventricularly injected cisplatin (10 μg/kg) during a 24-h observation period in pigeons

GR205171	No. of pigeons tested	% Emetic pigeons	Onset (min)	Bouts of emesis	Total emetic behaviours
Control	8	100	298.9 ± 58.2	46.9 ± 4.3	483.8 ± 57.9
3 mg/kg, i.m.	6	100	416.0 ± 92.6	30.7 ± 6.2[a]	257.8 ± 48.4[a]
30 μg/kg, i.c.v.	7	100	487.0 ± 210.3	20.3 ± 5.7[a]	145.7 ± 49.0[a]

GR205171 was administered intramuscularly (3 mg/kg, i.m.) or intracerebroventricularly (30 μg/kg, i.c.v.) 1 h before cisplatin administration. The values (mean ± S.E.M.) for the bouts of emesis and total emetic behaviours are the numbers of bouts of emesis and the total number of emetic behaviours, respectively. The values (mean ± S.E.M.) for onset are the latency times to first emesis after cisplatin administration.

[a] $P < 0.05$: statistically different from controls with cisplatin alone.

affected by GR205171, possibly due to the large intergroup variability (Fig. 3; Table 4).

3.5. Immunohistochemistry for substance P

Between the level of the obex and approximately 1.00 mm rostral to the obex, the area postrema is located dorsal to the nucleus of the tractus solitarius and dorsal motor nucleus of the vagus nerve. The area postrema is separated from the nucleus of the tractus solitarius by a band of glial limitation. At these levels, subnuclei of the nucleus of the tractus solitarius and dorsal motor nucleus of the vagus nerve were identified cytoarchitectonically according to the nomenclature described in the white Carneaux pigeon by Katz and Karten (1983a,b), and the subnuclear organizations of the nucleus of the tractus solitarius and dorsal motor nucleus of the vagus nerve in the domestic pigeon used in the present study corresponded closely to those in the white Carneaux pigeon. The nucleus of the tractus solitarius is divided into the medial and lateral zones, which are further subdivided into the dorsoventral subdivisions: medialis superficialis pars posterior, medialis dorsalis pars posterior, medialis intermedius pars posterior, medialis ventralis pars posterior, lateralis dorsalis pars posterior and lateralis parasolitarius. The dorsal motor nucleus of the vagus nerve is also divided into the dorsoventral divisions: posterior dorsalis magnocellularis, posterior intermedius mediocellularis and posterior ventralis parvicellularis (Fig. 4A).

Substance P-immunoreactive fibres and terminals were distributed throughout the nucleus of the tractus solitarius and dorsal motor nucleus of the vagus nerve. Intense

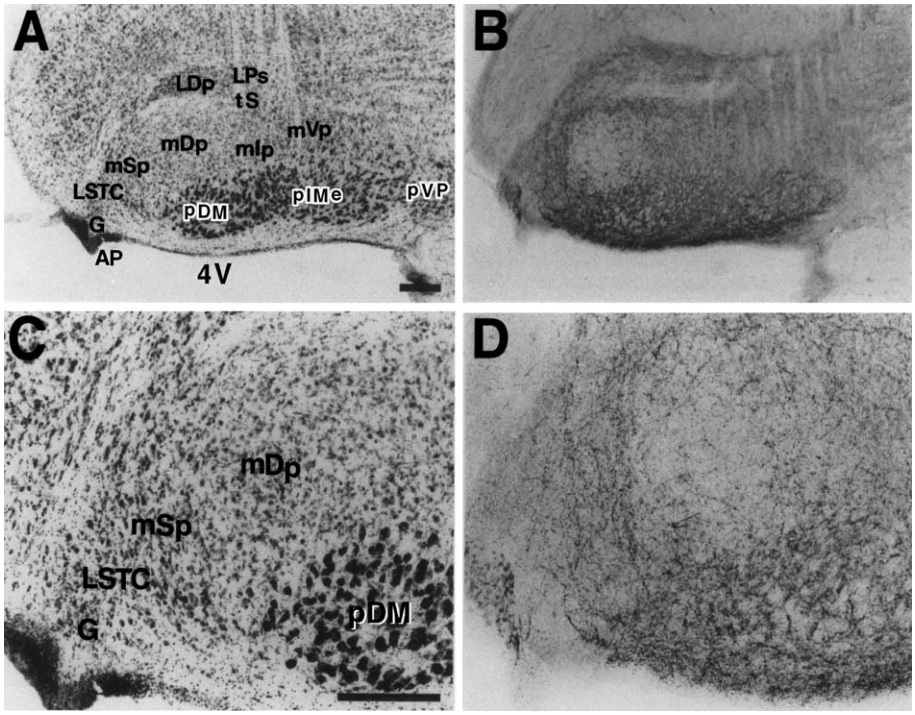

Fig. 4. Photomicrographs showing representative transverse sections of the dorsal brainstem including the area postrema, the nucleus of the tractus solitarius and the dorsal motor nucleus of the vagus nerve of a pigeon. (A) Nissl-stained section. (B) Immunohistochemical staining of substance P corresponding to the sections in A. (C, D) Enlargements of selected fields from A and B, respectively. The scale bar represents 200 μm. The abbreviations are as follows: AP, area postrema; G, glial limitation; LDp, lateralis dorsalis pars posterior; LPs, lateralis parasolitarius; LSTC, lateralis superficialis taenia choroidea; mDp, medialis dorsalis pars posterior; mIp, medialis intermedius pars posterior; mSp, medialis superficialis pars posterior; mVp, medialis ventralis pars posterior; pDM, posterior dorsalis magnocellularis; pIMe, posterior intermedius mediocellularis; pVP, posterior ventralis parvicellularis; tS, tractus solitarius; 4 V, fourth ventricle.

immunoreactive elements were observed within the subnuclei of the dorsal motor nucleus of the vagus nerve. In the nucleus of the tractus solitarius, immunoreactivity varied among the subnuclei. Immunoreactivity for substance P in the area postrema was relatively low (Fig. 4B and D).

4. Discussion

4.1. Cisplatin-induced emesis

Cisplatin administered by either the intravenous or intracerebroventricular route induced emesis in the pigeon.

Intravenously injected cisplatin at a dose of 4 mg/kg induced both early and delayed emesis in all of the injected pigeons. We classified the emetic response within the first 8-h period as early, and the emetic response between 8 and 48 h as delayed emesis. Although individual variation appeared in the time course and severity of cisplatin-induced emesis, the cisplatin-induced emetic response markedly decreased after 48 h in all of the control pigeons. No emesis was observed between 7 and 9 h after cisplatin in 10 of 22 control pigeons and a less intense crisis of emesis was observed in the other 12 control pigeons. Therefore, we determined that the transition from the early to delayed phases occurred between 7 and 9 h. In humans (Kris et al., 1985) and ferrets (Rudd et al., 1994), the early phase is presumed to last for one day, and in piglets for the first 16 h (Milano et al., 1995). Cisplatin-induced early emesis should be prevented with 5-HT_3 receptor antagonists as observed in humans (Cubeddu et al., 1990; Ruff et al., 1994), ferrets (Costall et al., 1987), cats (Lucot, 1989), *S. murinus* (Torii et al., 1991) and piglets (Milano et al., 1995). However, in pigeons, we could not observe the antiemetic activity of 5-HT_3 receptor antagonists such as granisetron, since indolic 5-HT_3 receptor antagonists have intrinsic emetic activity in pigeons (Preziosi et al., 1992). Although there may be species differences in the time course of the early and delayed phases of cisplatin-induced emesis, the cisplatin-induced early emesis in pigeons may be mediated partially via serotonergic mechanisms, since the inhibition of 5-HT synthesis by *p*-chlorophenylalanine significantly reduced the early emetic response, but did not affect delayed emesis (Tanihata et al., 2000).

In the present study, direct injection of a low dose of cisplatin (10 μg/kg) into the fourth ventricle produced emesis, suggesting a central site of the emetic response to cisplatin in pigeons. The emetic response declined within 24 h, and observations carried out for longer than 24 h cannot give meaningful results in this study. The dual response (early and delayed emesis) observed with the peripheral (i.v.) administration of cisplatin was obscured with the central (i.c.v.) administration.

Intravenously injected cisplatin (4 mg/kg) produced emesis at relative long latencies (90.5 ± 6.0 min, $n = 22$) in pigeons as has been observed in other animals, including ferrets, cats and piglets (Stables et al., 1987; Smith et al., 1988; Milano et al., 1995). The latency time to the first emesis induced by the centrally (i.c.v.) administered cisplatin in pigeons was longer (298.9 ± 58.2 min, $n = 8$) than that induced by peripherally (i.v.) administered cisplatin. In cats, it was conversely observed that the latency times were 4.0 and 100.6 min when cisplatin was administered intracerebroventricularly (0.3 mg/0.1 ml/animal) and intravenously (7.5 mg/kg), respectively (Smith et al., 1988). The long latency time to the onset of emesis after intracerebroventricular injection in pigeons might reflect an indirect action of cisplatin and/or the time required for cisplatin to diffuse to central sites at which cisplatin can act to induce emesis. Further studies are required to elucidate the central mechanisms of cisplatin-induced emesis.

4.2. Antiemetic profile of GR205171 against cisplatin-induced emesis

GR205171 is reported to have long-lasting antiemetic activity against cisplatin-induced emesis in ferrets (Gardner et al., 1996) and piglets (Grélot et al., 1998). In the present study, GR205171 was administered twice 1 h before and 24 h after cisplatin administration. Intramuscular injection of GR205171 (3 mg/kg), which was administered twice, 1 h before and 24 h after cisplatin administration, significantly reduced the number of emetic responses to cisplatin (4 mg/kg, i.v.) in both the early and delayed phases. In preliminary experiments, we observed that the antiemetic activity of GR205171 (3 mg/kg, i.m.) against cisplatin (4 mg/kg, i.v.)-induced emesis could not be augmented even when GR205171 was administered intramuscularly four times, 1 h before and 12, 24 and 36 h after cisplatin administration. GR205171 (3 mg/kg, i.m.), which was administered four times, reduced the number of bouts of emesis induced by cisplatin by 54.2% ($P < 0.05$) in early emesis and 35.5% ($P > 0.05$) in the delayed response ($n = 5$); however, the reductions were not significantly different ($P > 0.05$) from the reductions observed with GR205171 (3 mg/kg, i.m.) that were administered twice, 1 h before and 24 h after cisplatin administration.

The cisplatin-induced emesis was also reduced by injection of a low dose of GR205171 (30 μg/kg) directly into the fourth ventricle of pigeons. In a preliminary study, GR205171 at a dose of 30 μg/kg when administered intramuscularly did not show any antiemetic effects on the cisplatin-induced emesis. The antiemetic effect of GR205171 when administered intracerebroventricularly was approximately 100 times more potent than when administered intramuscularly, suggesting a central site of antiemetic action of GR205171 in pigeons, as has been demonstrated for other tachykinin NK_1 antagonists in other species (Gardner et al., 1994; Hargreaves et al., 1994; Tattersall et al., 1996). Additionally, the antiemetic effect of GR205171 was still apparent 24 h after its administration by either the intramuscular or

intracerebroventricular route, indicating a long duration of antiemetic action of GR205171 in pigeons.

GR205171 failed to show a statistically significant effect on the latency of the emetic response to cisplatin in pigeons. Although 2 of 10 pigeons treated with GR205171 (3 mg/kg, i.m.) did not exhibit any emetic response to cisplatin within the first 8-h period, the latency of the emetic response to cisplatin in the remaining 8 GR205171-treated pigeons was similar to that in untreated pigeons. Consequently, there was no significant difference in the latency of the emetic response to cisplatin between GR205171 intramuscularly treated and untreated pigeons. Furthermore, administration of GR205171 into the fourth ventricle could not at all affect the latency of the emetic response to cisplatin. These results suggest that the onset of the emetic response to cisplatin in pigeons may be mediated partly via GR205171-insensitive mechanisms. A similar lack of significant effect for another tachykinin NK_1 receptor antagonist, CP 99,994, on latency to cisplatin-induced emesis was reported in the ferret (Watson et al., 1995). A lack of effect of CP 99,994 on latency was also observed in the cat to reduce motion-induced emesis (Lucot et al., 1997).

4.3. Immunohistochemistry for substance P

It has been suggested that the site of the antiemetic action of tachykinin NK_1 receptor antagonists is at the tachykinin NK_1 receptors located in the dorsal vagal complex including the area postrema, nucleus of the tractus solitarius and dorsal motor nucleus of the vagus nerve, which are known to be associated with the emetic pathway (Gardner et al., 1994; Watson et al., 1995; Tattersall et al., 1996). In the ferret, a direct injection of a tachykinin NK_1 receptor antagonist, GR82334 (D-Pro^9-[spiro-γ-lactam]-Leu^{10}-Trp^{11}-physalaemin(1–11)), into the region of the nucleus of the tractus solitarius antagonized the early emesis induced by cisplatin (Gardner et al., 1994), and the immunohistochemical study revealed the presence of substance P-immunoreactive nerve fibres and terminals in regions of the nucleus of the tractus solitarius and dorsal motor nucleus of the vagus nerve (Gardner et al., 1995).

In the present study, cisplatin-induced early and delayed emesis was reduced by intracerebroventricular GR205171, suggesting that substance P, an endogenous ligand for tachykinin NK_1 receptors, plays a role in the central mechanism of cisplatin-induced emesis in the pigeon. Additionally, our immunohistochemical study demonstrated that substance P-immunoreactive fibres and terminals are present in regions of the dorsal vagal complex including the area postrema, nucleus of the tractus solitarius and dorsal motor nucleus of the vagus nerve in the domestic pigeon used in this study. The presence of substance P-immunoreactive nerve fibres and terminals in regions of the dorsal vagal complex including the area postrema, nucleus of the tractus solitarius and dorsal motor nucleus of the vagus nerve of pigeon was also demonstrated by Berk et al. (1993) using the white Carneaux pigeon. The pigeon has many strains and strain differences have been indicated in the shape and structure of the brain (Ebinger and Löhmer, 1984; Dubbeldam, 1998). As we have been using the domestic pigeon as an emetogenic animal model, we determined the localization of substance P-immunoreactive nerve components by immunohistochemical staining, and observed a dense distribution of substance P-immunoreactive fibres and terminals throughout the nucleus of the tractus solitarius and dorsal motor nucleus of the vagus nerve in the domestic pigeon used in the present study.

4.4. Species differences in antiemetic activity of GR205171 against cisplatin-induced emesis

Grélot et al. (1998) reported that an intravenous administration of GR205171 at a dose of 1 mg/kg almost abolished the early emesis and, furthermore, had a potent and long-lasting antiemetic activity against both the early and delayed emesis to cisplatin (5.5 mg/kg, i.v.) in the piglet.

In the pigeon, intramuscularly injected GR205171 (3 mg/kg) significantly reduced the number of emetic responses to cisplatin (4 mg/kg, i.v.) in both the early and delayed phases, but could not significantly affect the latency time to the first emesis. When the dose was increased to 10 mg/kg, GR205171 failed not only to completely inhibit the cisplatin-induced emesis, but also to significantly prolong the latency time to first emesis, suggesting that the antiemetic profile of GR205171 against cisplatin-induced emesis in the pigeon differs from that in the piglet.

Gardner et al. (1996) observed that the doses of GR205171 required to inhibit the cisplatin-induced early emesis in the *S. murinus* were at least 10 times higher than those required in the ferret. Furthermore, GR205171 could not affect the latency time to the first emesis induced by cisplatin in the *S. murinus*, which differed from the observation in the ferret. Consequently, Gardner et al. (1996) suggested that the relative lower potency of GR205171 in inhibiting the cisplatin-induced emesis in the *S. murinus* when compared with that in the ferret might be related to differences in the affinities of their tachykinin NK_1 receptors for tachykinin NK_1 receptor antagonists. Although we have not studied the affinity of GR205171 for tachykinin NK_1 receptors in the pigeon, the relative lower antiemetic potency of GR205171 may reflect a similar species difference. Moreover, the species differences in the variation in the antiemetic potency may be based on those in the mechanisms of cisplatin-induced emesis (Miller and Nonaka, 1992). Therefore, further studies are required to substantiate the mechanism involved in the cisplatin induction of emesis in the pigeon.

5. Conclusion

Cisplatin (4 mg/kg, i.v.) induced both early and delayed emesis in the pigeon, and GR205171 administered via

either an intramuscular or intracerebroventricular route reduced both types of emesis. Direct injection of cisplatin (10 μg/kg) into the fourth ventricle produced emesis, and GR205171 significantly reduced the emetic response to centrally administered cisplatin. The antiemetic effect of centrally administered GR205171 was more potent than when it was peripherally administered. However, the latency time to the first emesis induced by cisplatin administered via the peripheral or central route was not significantly affected by GR205171. Substance P-immunoreactive fibres were distributed throughout the dorsal vagal complex in the pigeon. In pigeons, these results suggest that the emetic response to cisplatin occurs via its central cite of action, the antiemetic action of GR205171 against both the early and delayed emetic responses to cisplatin involves a central site, and the tachykinin NK_1 receptor could not play a role in the onset of the emetic response to cisplatin.

Acknowledgements

We would like to thank Prof. K. Kishi (Department of Anatomy, Faculty of Medicine, Toho University) for his helpful advice and discussion of the cytoarchitectonic and immunohistochemical studies. We are also grateful to Glaxo Wellcome Research and Development (UK) for generously supplying GR205171.

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