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Influence of 5-HT$_1$ receptor agonists on feline stomach relaxation

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Abstract

Sumatriptan is known for its stomach relaxing properties in both humans and cats, but the receptor involved has not been characterized. In a barostat study the intragastric volume was monitored in sedated cats at constant pressure. The maximum intragastric volume increase after subcutaneous or intravenous administration of saline or agonists was registered [mean (n=4–5)]. Sumatriptan (0.01–1 mg kg^{-1}) induced a dose-dependent intragastric volume increase vs. saline (4–15 vs. 5 ml, respectively) that was sometimes accompanied by retching after 8–10 min. Pre-treatment with nitric oxide-synthase inhibitors and different 5-HT$_1$ receptor antagonists *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide(WAY-100635), 2-methyl-4-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic-acid[4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide(GR-127935), *N*-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan-amide(5-HTP-DP) and 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine-HCl(NAN-190) did not affect the sumatriptan-induced effect. Alniditan (5-HT$_{1A/1D}$ receptor agonist) and flesinoxan (5-HT$_{1A}$ receptor agonist) did not induce significant intragastric volume changes. The 5-HT$_{1F}$ receptor agonists 5-hydroxy-3-(1-methylpiperidin-4-yl)-1H-indole(BRL-54443) and (*R*)-(+)-*N*-(3-dimethylamino-1,2,3,4-tetrahydro-9H-carbazol-6-yl)-4-fluorobenzamide(LY-344864; 0.003–3 mg kg^{-1}) however induced a dose-dependent intragastric volume increase (6–36 and 5–26 ml, respectively), no retching was seen. Our results suggest that stimulation of 5-HT$_{1F}$ receptors induces feline stomach relaxation. Whether the sumatriptan-induced gastric relaxation in cats is due to interaction with 5-HT$_{1F}$ receptors could not be proven absolutely in view of the lack of selective 5-HT$_{1F}$ receptor antagonists.

Keywords: 5-HT$_{1F}$; Barostat; Proximal stomach; Sumatriptan; Retching

1. Introduction

Physiological modulation of gastric tone is based upon a constant vagal cholinergic excitatory and nitrergic inhibitory drive. Since in basal conditions the cholinergic drive is predominant, interruption of the cholinergic drive by vagal cooling (Azpiroz and Malagelada, 1987) or vagotomy (Abrahamsson and Thoren, 1973) decreases gastric tone. Administration of the nitric oxide (NO)-synthase inhibitor N^G-nitro-L-arginine (L-NNA) on the other hand increases gastric tone in the dog (Paterson et al., 2000). Serotonin (5-hydroxytryptamine; 5-HT) has proven to be an important mediator of gastro-intestinal motility (Read and Gwee, 1994; Hoyer et al., 2002). With regard to gastric smooth muscle activity, 5-HT is generally linked with a stimulatory influence; stimulation of 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_3$ and 5-HT$_4$ receptors was shown to induce gastric contraction or to enhance contractility in different species (Janssen et al., 2002; Baxter et al., 1994; Buchheit and Buhl, 1994; Prins et al., 2001). Stimulation of 5-HT$_1$ receptors on the other hand was shown to have a relaxatory influence on gastric tone in different species. The selective 5-HT$_{1A}$ receptor agonists buspirone and flesinoxan were shown to increase human gastric accommodation and canine gastric relaxation, respectively (Tack et al., 1999; Janssen et al., 2003). Sumatriptan, an antimigraine drug with affinity at different 5-HT$_1$ receptor subtypes (Dahlof, 2001) induced gastric fundus relaxation and enabled accommodation of considerably larger volumes before thresholds for perception or discomfort were reached in humans (Tack et al.,

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2000). In dogs, sumatriptan shifted the barostat-assessed pressure–volume curves towards higher volumes and enhanced gastric accommodation, an effect mediated by 5-HT_{1B} receptors (De Ponti et al., 2003). In the same study it was shown that sumatriptan induced an immediate gastric relaxation in dogs, but this response was not dose-dependent. In cats on the other hand, a consistent sumatriptan-induced dose-dependent relaxation of the gastric fundus was reported (Coulie et al., 1999). In this study Coulie et al. could partially block the sumatriptan-induced stomach relaxation by N^G-nitro-L-arginine methyl ester (L-NAME), indicating involvement of nitrergic nerves. The sumatriptan-induced feline stomach relaxation was not antagonized by the 5-HT_{1A} receptor antagonist 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine HCl (NAN-190), indicating that this receptor subtype is not involved.

The aim of the present study was to pharmacologically characterize the receptor that mediates the sumatriptan-induced feline gastric relaxation by means of selective agonists and antagonists.

2. Materials and methods

2.1. Experimental set-up

Seven adult male cats (weight between 2 and 4 kg) were used. During the course of the experiments, two cats dropped out but each type of experiment was performed in at least four cats. The cats were housed individually in a temperature-controlled (19 ± 1 °C) and light-controlled (lights on from 0700 to 2100 h) room. The animals were fasted for 24 h prior to the beginning of the experiment (water was available ad libitum). All procedures were reviewed and approved by the local ethics committee.

A polyethylene bag (70 ml maximal capacity) was adhered to a double lumen tube (Salem sump tube, 3.3 mm diameter, Sherwood Medical, Petit Rechain, Belgium). The two lumens of the tube could be connected to a pressure recording port and an air inflation port respectively at a computer driven barostat (Distender series II; G and J electronics, Ontario, Canada) to record volume changes while the pressure was kept constant. Before the beginning of the experiment, the bag was connected to the barostat and the intrabag pressure was raised to 10 mm Hg. By monitoring a constant intrabag volume it was ensured that there was no leak.

At the beginning of the experiment, the animals were sedated intramuscularly (i.m.) with a single dose of ketamine (35 mg kg^{-1}). Thereafter, a dose of 10 mg kg^{-1} was administered i.m. every hour to maintain sedation. During the experiment the animals were always positioned lying down on their right side. The ketamine sedation allowed the cats to tolerate swallowing of the finely folded polyethylene bag adherent to the double lumen tube and intubation of the tube during the experiment while spontaneous breathing was preserved. The bag was positioned in the stomach via a guide-wire.

In preliminary experiments we noticed large basal intragastric volume variability by applying a constant intragastric pressure of 6 mm Hg. Administration of sumatriptan in this condition did not result in a reproducible gastric volume increase. Therefore, the pressure was increased until the intragastric volume was above 10 ml; this pressure (4–20 mm Hg) was then kept constant throughout the whole experiment, while volume was recorded. The recording of the volume was terminated 30 min after administration of the agonist. If the agonist induced retching the recording was stopped.

2.2. Drug administration

Drugs were administered after the intragastric volume maintained a stable baseline value during at least 10 min at the constant pressure used. Per experiment, a single dose of a single agonist [sumatriptan, 5-hydroxy-3-(1-methylpiperidin-4-yl)-1H-indole (BRL-54443), alniditan, flesinoxan or (*R*)-(+)-*N*-(3-dimethylamino-1,2,3,4-tetrahydro-9H-carbazol-6-yl)-4-fluorobenzamide (LY-344864)] was administered. Different doses of agonists were randomly divided over different experiments. All agonists were injected subcutaneously (s.c.) except LY-344864 that was also injected intravenously (i.v.). Saline, L-NNA, L-NAME and different 5-HT receptor antagonists [*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide (WAY-100635), NAN-190, [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide HCl (GR-127935) and *N*-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide (5-HTP-DP)] were tested vs. 1 mg kg^{-1} sumatriptan and administered i.v. (bolus in an antecubital vein with a maximal volume of 2 ml) 10 min prior to sumatriptan. A washout period of 1 week was allowed between two consecutive experiments in the same animal. After a session with sumatriptan preceded by an antagonist, a session with sumatriptan alone (control) always followed.

2.3. Data analysis

A regression curve (Lowess regression) was calculated from the original gastric volume data to correct for extreme outliers. The Lowess regression method obtains a smoothed *Y* value at a given *X* by fitting a linear regression to the data in the neighbourhood of the *X* value (Cleveland, 1979; Neter et al., 1996). SigmaPlot 2000 for Windows Version 6 was used for the calculation of the Lowess regression curve. All intragastric volume calculations were determined from the Lowess regression curve. The maximal intragastric volume increase induced by a given treatment was calculated as the difference between the highest volume after treatment and the mean intragastric volume in the last 5 min before treatment (=mean baseline volume). In Table 1 the mean baseline volume before and after pre-treatment were deter-

Table 1
Mean baseline volume before and after addition of different pre-treatments

Pre-treatment (mg kg^{-1})	Mean baseline volume before pre-treatment (ml)	Mean baseline volume after pre-treatment (ml)
L-NAME (50)	15 ± 1	14 ± 2
L-NNA (20)	13 ± 2	12 ± 2
WAY-100635 (0.1)	17 ± 1	18 ± 1
NAN-190 (0.05)	12 ± 1	10 ± 2
GR-127935 (0.1)	19 ± 4	17 ± 5
5-HTP-DP (1)	13 ± 3	11 ± 4

Mean ± S.E.M. (n = 4); all pre-treatments were injected intraveneously.

mined as the mean baseline volume in the 5 min before addition of pre-treatment and the mean baseline volume between addition of pre-treatment and agonist, respectively. If the treatment induced retching, the maximal intragastric volume increase was calculated before the onset of the retching event.

Results were expressed as mean ± S.E.M.; the number of cats used is denoted by n. All comparisons of two or more treatment sessions were analysed using PROC MIXED (SAS System for Windows V8; SAS code available on request) except for comparisons between the time to retch after drug administration (a non-parametric Mann–Whitney test was used). The Page-test was applied to determine a dose-dependent relationship of the effects induced by different doses of the same drug. A Page-test compares K populations, in which N samples are drawn independently from each population. The Page test determines if each of the N samples is correlated across the K populations, and furthermore, if there is a natural ordering of the K populations (SAS System for Windows V8; the one-sided asymptotic p-value was used). A level of $p < 0.05$ was taken as significant.

2.4. Membrane preparation and binding assay

The binding affinity of a set of 5-HT receptor agonists and antagonists (samples) at 5-HT$_{1F}$ receptors was tested. To do this h5-HT$_{1F}$-HEK 293 cells were cultured in Petri dishes till 90% confluency was reached. The cells were scraped from the plates and suspended in 50 mM Tris–HCl buffer (pH 7.4). After centrifugation (23,500 × g) the pellet was suspended in 5 mM Tris–HCl buffer (pH 7.4) and homogenized with an Ultra Turrax homogeniser. The membranes were collected by centrifugation at 18,000 × g for 20 min, resuspended in 50 mM Tris–HCl (pH 7.4) and stored at − 80 °C.

At the day of the binding experiment, the membranes were defrosted and homogenized. The protein concentration was determined using the Bradford protein assay and the membranes were diluted in the assay buffer down to 65 μg protein ml^{-1} (50 mM Tris–HCl, 10 mM $MgCl_2$, 1 mM EGTA and 10 μM pargyline, pH 7.4). Assay mixtures (0.5 ml) contained 0.4 ml membrane preparation and 2 nM of tritiated 5-HT (118 Ci mmol^{-1}); to determine the non-specific binding 1 μM sumatriptan was added. Per sample studied 15 mixtures were created containing different concentrations (10 μM–1 pM) of the sample. The mixtures were then incubated for 60 min at 25 °C (protected from light), incubation was stopped by rapid filtration on a Filtermate 96 (Packard).

Bound radioactivity was determined by liquid scintillation counting. Data were analysed graphically with inhibition curves and IC_{50} values were derived. K_i values were calculated according to the equation $K_i = IC_{50}/(1+(C/K_d))$, with C as the concentration of [^{3}H]5-HT (2 nM) and K_d as the equilibrium dissociation constant of [^{3}H]5-HT (2.61 nM).

2.5. Drugs

The following drugs were used (respective suppliers within parentheses): alniditan, buspirone, 5-HTP-DP, NAN-190, sumatriptan (Johnson & Johnson Pharmaceutical Research and Development, Beerse, Belgium), BRL-54443 (Tocris Cookson, Bristol, UK), flesinoxan (Solvay Pharma, Brussels, Belgium), LY-344864 (kindly donated by Eli Lilly, Indianapolis, IN, USA), GR-127935 (kindly donated by Glaxo Group Research, Ware, UK), L-NNA (Acros Organics, Geel, Belgium), L-NAME, WAY-100635 (Sigma-Aldrich, Bornem, Belgium), Ketamin (Ketalar® (50 mg/ml); Pfizer, Brussels, Belgium).

BRL-54443 was dissolved in distilled water containing 1 equivalent 2,3-dihydroxybutanedioic acid + 0.9% NaCl, GR-127935 in distilled water containing 10% cyclodextrin + 0.9% NaCl, 5-HTP-DP in distilled water containing 1 equivalent NaOH + 0.9% NaCl, L-NNA in distilled water containing 1 equivalent NaOH + 20% cyclodextrin, LY-344864 in distilled water, sumatriptan in distilled water containing 2 equivalents 2,3-dihydroxybutanedioic acid + 0.9% NaCl and WAY-100635 in distilled water containing three equivalents 2,3-dihydroxybutanedioic acid + 0.9% NaCl. The other drugs were dissolved in saline. All drugs used were dissolved isotonically and pH was between 3.5 and 9 except for L-NNA (pH was 10).

3. Results

3.1. Influence of different pre-treatments on the sumatriptan-induced effects

In a first series of experiments we tested the effect of 1 mg kg^{-1} sumatriptan vs. saline on the intragastric volume in four cats. The mean baseline volume before addition of saline was 14 ± 2 ml; this did not significantly differ from the mean baseline volume before addition of 1 mg kg^{-1} sumatriptan (16 ± 1 ml). In comparison to saline sumatriptan induced a significant maximal intragastric volume increase (5 ± 2 vs. 10 ± 1 ml, respectively; $p < 0.05$; Fig. 1). In three out of four cats sumatriptan

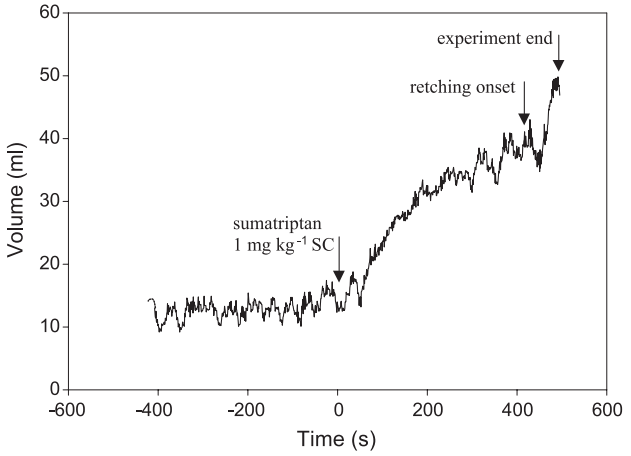

Fig. 1. Representative example of a feline intragastric volume recording as determined with a barostat (15 mm Hg intrabag pressure). Influence of sumatriptan (1 mg kg^{-1}; s.c.) on the intragastric volume.

induced retching after 8 ± 3 min whereas saline never induced retching.

The influence of L-NAME, L-NNA and different 5-HT receptor antagonists (WAY-100635, NAN-190, GR-127935 and 5-HTP-DP) on the 1 mg kg^{-1} sumatriptan-induced effects was examined in comparison to the effects of 1 mg kg^{-1} sumatriptan in control sessions. Although L-NAME and L-NNA induced a small but sustained contraction in three out of four cats (data not shown), the average volume before administration of L-NNA and L-NAME was not significantly different from the average volume after administration of both drugs (Table 1). The different 5-HT_1 receptor antagonists did not show intrinsic effects on the intragastric volume. The mean baseline volume before administration of sumatriptan in the control sessions did not significantly differ from the mean baseline volume in the sessions with pre-treatment. Addition of the different pre-treatments did not significantly influence the sumatriptan-induced maximal intragastric volume and time to retch (Table 2).

3.2. Influence of different 5-HT receptor agonists on the feline intragastric volume

A dose–response curve for sumatriptan was established in four to five cats. The mean baseline volume before treatment with saline was 17 ± 3 ml; this did not significantly differ from the mean baseline volume before addition of 0.01, 0.1 and 1 mg kg^{-1} sumatriptan (13 ± 2, 15 ± 4 and 19 ± 3 ml, respectively). Although sumatriptan induced a dose-dependent (as determined by the Page test: $p < 0.05$) maximal intragastric volume increase compared to saline, no significant difference in mean baseline volume was found between the sessions with administration of sumatriptan versus that with administration of saline (Table 3). Saline and 0.01 mg kg^{-1} sumatriptan did not induce retching. Administration of 0.1 and 1 mg kg^{-1} sumatriptan on the other hand induced retching after 10 ± 2 and 8 ± 3 min, respectively, both in three out of five cats. As confirmed with the Page-test the sumatriptan-induced retching was dose-dependent ($p < 0.01$).

A dose–response curve of the 5-ht_{1e} and 5-HT_{1F} receptor agonist BRL-54443 was established on four to five cats (Fig. 2). The mean baseline volume in the saline treatment session was 10 ± 2 ml. This was not significantly different from the mean baseline volume in the 0.003, 0.03, 0.3 and 3 mg kg^{-1} BRL-54443 treated sessions (16 ± 2, 12 ± 2,

Table 2
Mean baseline volume before addition of sumatriptan, maximal (max.) intragastric volume increase, time to retch and number of cats that retch after administration of sumatriptan (1 mg kg^{-1}; s.c.) in control condition or after pre-treatment with different antagonists

Pre treatment (mg kg^{-1})	Mean baseline volume (ml)	Max. intragastric volume increase (ml)	Number of cats that retch	Time to retch (min)
Control	15 ± 2	11 ± 1	2	6
L-NAME (50)	14 ± 2	12 ± 3	2	8
Control	12 ± 2	12 ± 7	4	4 ± 1
L-NNA (20)	9 ± 3	10 ± 4	2	15
Control	14 ± 1	17 ± 2	4	4 ± 1
WAY-100635 (0.1)	19 ± 4	16 ± 3	4	2 ± 0.3
Control	11 ± 3	7 ± 1	2	4
NAN-190 (0.05)	11 ± 1	11 ± 4	3	5 ± 1
Control	17 ± 3	14 ± 3	4	6 ± 2
GR-127935 (0.1)	19 ± 3	18 ± 7	3	8 ± 4
Control	14 ± 1	8 ± 2	4	3 ± 0.3
5-HTP-DP (1)	11 ± 3	7 ± 2	4	6 ± 2

Results shown as mean ± S.E.M. ($n = 4$); all pre-treatments were injected i.v. Time to retch after sumatriptan administration was calculated only from cats that retch, no S.E.M. was estimated in those groups where $n < 3$.

Table 3
Maximal (max.) intragastric volume increase after addition of different agonists

Agonist	Dose (route)	Max. intragastric volume increase (ml)
Sumatriptan ($n = 4–5$)	0 (s.c.)	5 ± 1
	0.01 (s.c.)	4 ± 2
	0.1 (s.c.)	16 ± 5
	1 (s.c.)	15 ± 5
BRL-54443 ($n = 4–5$)	0 (s.c.)	5 ± 3
	0.003 (s.c.)	6 ± 2
	0.03 (s.c.)	15 ± 3^{a}
	0.3 (s.c.)	23 ± 5^{b}
	3 (s.c.)	36 ± 4^{c}
LY-344864 ($n = 4$)	0 (i.v.)	3 ± 1
	0.003 (i.v.)	5 ± 2
	0.03 (i.v.)	19 ± 2^{c}
	0.3 (i.v.)	26 ± 2^{c}
Flesinoxan ($n = 4$)	0.1 (s.c.)	3 ± 1
Alniditan ($n = 4$)	0.5 (s.c.)	8 ± 3

Results presented as mean ± S.E.M.; dose expressed as mg kg^{-1}.
Sumatriptan, BRL-54443 and LY-344864 induced a dose-dependent volume increase as determined by the Page test.
[a] $p < 0.05$ vs. saline addition in the same condition.
[b] $p < 0.005$ vs. saline addition in the same condition.
[c] $p < 0.0005$ vs. saline addition in the same condition.

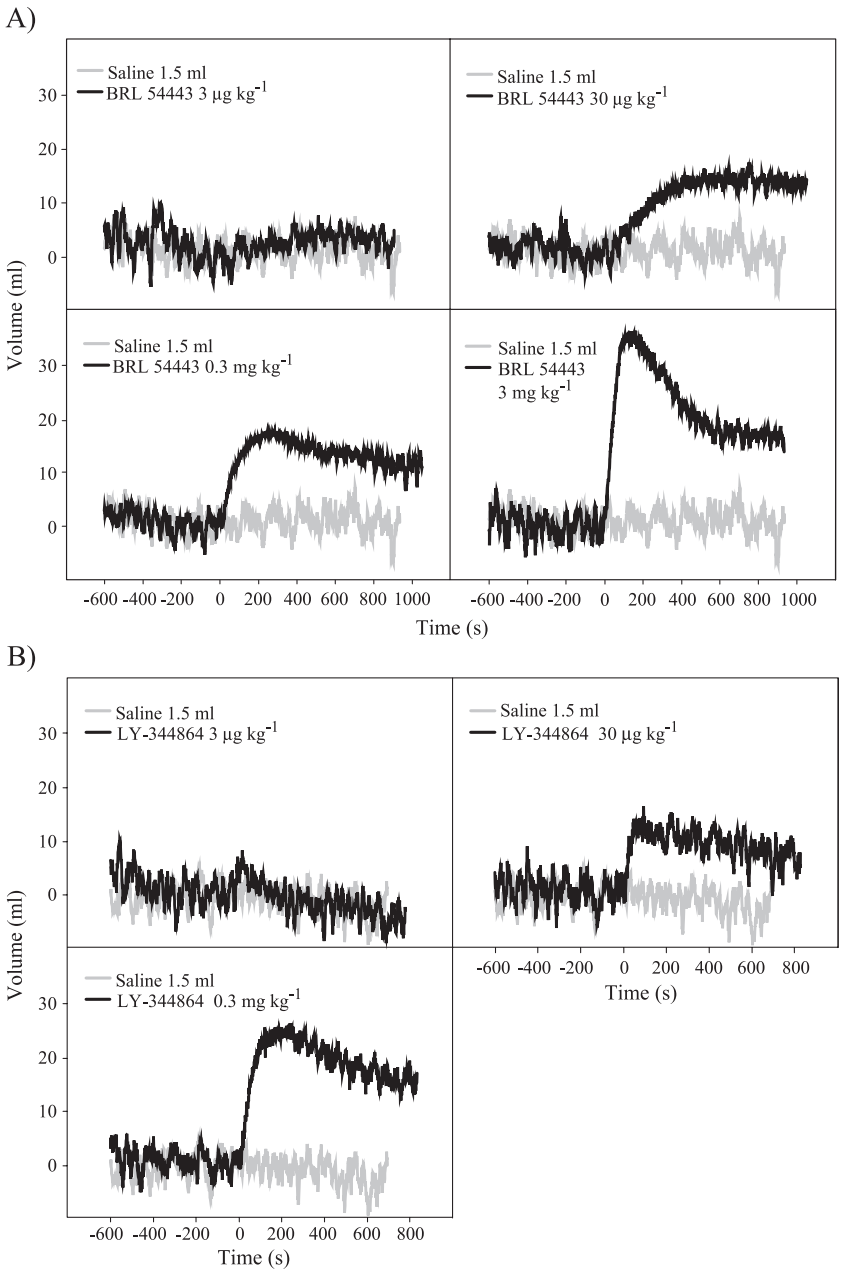

Fig. 2. Mean barostat recordings ($n = 4–5$) of feline intragastric volume before and after administration of different doses of BRL-54443 (A) and LY-344864 (B) at time 0. All curves are shown after subtraction of the mean baseline volume. BRL-54443 was administered subcutaneously, while LY-344864 was administered intravenously.

13 ± 2 and 14 ± 5 ml, respectively). BRL-54443 induced an immediate dose-dependent maximal intragastric volume increase vs. saline (as determined by the Page test: $p < 0.001$; Table 3). In preliminary experiments the selective 5-HT_{1F} receptor agonist LY-344864 (0.003 and 0.03 mg kg^{-1}) did not induce an effect on the intragastric volume when injected s.c. (results not shown). When injected at high dose (0.3 mg kg^{-1}) LY-344864 induced a non-significant maximal intragastric volume increase of 15 ± 6 ml. It was therefore decided to administer this drug i.v. A dose–response curve of LY-344864 (0.003, 0.03 and 0.3 mg kg^{-1}; i.v.) was established in four cats (Fig. 2). The mean baseline volume in the saline treatment session (17 ± 4 ml) did not significantly differ from the mean baseline volume in LY-344864 treatment sessions (16 ± 4, 16 ± 2 and 14 ± 2 ml, respectively). LY-344864 induced a dose-dependent increase in the maximal intragastric volume increase (as determined by the Page test: $p < 0.001$; Table 3). Saline, BRL-54443 and LY-344864 did not induce retching.

The selective 5-HT_{1A} receptor agonist flesinoxan (0.1 mg kg^{-1}; s.c.; $n = 6$) and the 5-$HT_{1A/1D}$ receptor agonist alniditan (0.5 mg kg^{-1}; s.c.; $n = 5$) were also tested for their possible influence on the intragastric volume. The mean baseline volume before addition of both agonists (15 ± 3

and 15 ± 4 ml, respectively) was not significantly different from the mean baseline volume in the three saline treatment sessions as described above. Both agonists did not induce a maximal intragastric volume increase that reached significance versus the maximal intragastric volume increase induced by the 3 saline treatment sessions as described above (Table 3). Neither flesinoxan nor alniditan induced retching.

4. Discussion

Subcutaneous administration of sumatriptan in humans resulted in an immediate and profound relaxation of the gastric fundus which enabled accommodation of considerably larger volumes before thresholds for perception or discomfort were reached (Tack et al., 2000). Coulie et al. (1999) studied sumatriptan-induced gastric relaxation in cats and showed involvement of nitrergic pathways. Neither one of these studies elucidated the receptor involved. In the present study, we used different 5-HT_1 receptor agonists and antagonists in order to characterize the receptor involved in the sumatriptan-induced feline stomach relaxation.

In our experiments sumatriptan induced a dose-dependent intragastric volume increase similar to that described by Coulie et al. (1999) In the latter study however, the maximum volume increase to administration of a similar dose of sumatriptan was somewhat greater compared to our observations (26 vs. 16 ml, respectively). Differences in the weight of the cats used and/or the gastric volume assessment might account for this difference. Shortly after administration of 0.1 and 1 mg kg^{-1} sumatriptan, retching occurred in most of our experiments. The sumatriptan-induced retching was dose-dependent; retching did not occur after administration of the 5-HT_{1A} receptor agonist flesinoxan, the 5-$HT_{1A/1D}$ receptor agonist alniditan, the 5-$HT_{1e/1F}$ receptor agonist BRL-54443 and the 5-HT_{1F} receptor agonist LY-344864. Pre-treatment with the 5-HT_{1A} receptor antagonists WAY-100635 and NAN-190, the 5-$HT_{1B/1D}$ receptor antagonist GR-127935 and with 5-HTP-DP, thought to interfere with the effect of 5-HT at 5-HT_{1P} receptors, did not block or delay the sumatriptan-induced retching suggesting that the sumatriptan-induced retching is not mediated by a 5-HT_1 receptor. As sumatriptan has very low binding affinities towards other 5-HT receptors (Leysen et al., 1996) it is unlikely that sumatriptan would mediate retching by activation of 5-HT receptors. It is also not likely that the sumatriptan-induced retching is related to the intragastric bag or to the rapid gastric volume increase as BRL-54443 and LY-344964 can induce a similar intragastric volume increase without retching. Neither retching, vomiting nor nausea are known side effects of sumatriptan in humans (Vingerhagen et al., 2000). Although the cat is known for the emetic responses to several drugs (King, 1990) and is used as a model to study vomiting and motion sickness (Lucot and Crampton, 1989), there has been no description of sumatriptan-induced retching or vomiting. We therefore conclude that the sumatriptan-induced retching is a specific side effect seen in the cats we used sedated with ketamine.

The influence of the NO synthase inhibitors L-NAME and L-NNA was tested in order to determine the possible involvement of nitrergic pathways. Although L-NAME and L-NNA induced no overall significant volume decrease, a small but sustained contraction in some cats was noticed. These findings are in concordance with the findings of Coulie et al. suggesting that the resting gastric tone is under influence of a continuous inhibitory nitrergic drive. In contrast to the findings of Coulie et al. however, we were not able to block the sumatriptan-induced intragastric volume increase with L-NAME. We confirmed this finding by pre-treatment of sumatriptan with L-NNA. Our findings are in concordance with a report on sumatriptan-induced relaxation in the isolated guinea-pig stomach; this relaxation was also not blocked by L-NNA (Meulemans et al., 1996). We did thus not find evidence for nitrergic pathways in the sumatriptan-induced stomach relaxation. Although Coulie et al. only reported a partial inhibition of the relaxation after administration of L-NAME, an inconsistency remains and no clear explanation could be found.

Sumatriptan displays affinity towards different 5-HT_1 receptors (Table 4) but has little affinity towards non-5HT_1 receptors (Leysen et al., 1996). By means of different 5-HT_1 receptor agonists and antagonists we tried to characterize the receptor involved in the gastric relaxant effect of sumatriptan in the cat. In dogs, activation of 5-HT_{1A} receptors by the selective 5-HT_{1A} receptor agonist flesinoxan was shown to induce gastric relaxation by a mechanism not involving NO (Janssen et al., 2003). In our experiments, flesinoxan was not able to induce feline stomach relaxation, although the dose used was previously shown to induce canine stomach relaxation (Janssen et al.,

Table 4
Binding affinities (pKi) for different drugs at different 5-HT receptors

Drug	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{1e}	5-HT_{1F}
Sumatriptan	6.43[a]	7.60[a]	7.92[a]	5.80[a]	8.21[b]
Alniditan	8.42[a]	7.31[a]	9.10[a]	6.62[a]	6.35[b]
Flesinoxan	8.91[c]	7.19[c]	7.86[c]	Nd.	6.11[b]
BRL-54443	7.2[d]	6.9[d]	7.2[d]	8.7[d]	9.25[b]
LY-344864	6.28[e]	6.26[e]	6.24[e]	5.85[e]	8.90[b]
Buspirone	7.50[c]	< 5[c]	5.82[c]	Nd.	5.18[b]
GR-127935	7.58[f]	9.18[f]	8.41[f]	5.88[f]	6.96[b]
WAY-100635	9.05[f]	5.88[f]	6.48[f]	< 5[f]	6.19[b]
NAN-190	8.9[g]	6.2[g]	6.7[g]	Nd.	6.93[b]
5-HTP-DP	Nd.	Nd.	Nd.	Nd.	< 5[b]
Ketamine	Nd.	Nd.	Nd.	Nd.	< 5[b]

[a] (Leysen et al., 1996).
[b] See methods.
[c] (Koek et al., 1998).
[d] (Brown et al., 1997).
[e] (Phebus et al., 1997).
[f] (Gommeren et al., 1998).
[g] (Briejer et al., 1995).

2003) and anti-emetic effects in cats (Lucot, 1994). Furthermore, the 5-HT_{1A} receptor antagonists NAN-190 and WAY-100635 did not influence the feline gastric relaxation by sumatriptan again confirming that it is not mediated via 5-HT_{1A} receptors. These observations indicate that 5-HT_{1A} receptor activation does not induce feline stomach relaxation, in contrast to dogs. Recently it was shown that 5-HT_{1B} receptors play a role in modulation of canine gastric accommodation and relaxation (De Ponti et al., 2003). As sumatriptan is regarded as a 5-$HT_{1B/1D}$ receptor agonist, the influence of the 5-$HT_{1B/1D}$ receptor antagonist GR-127935 was tested on the sumatriptan-induced stomach relaxation. GR-127935, administered in a ten times higher dose than one that was previously shown to inhibit sumatriptan-induced vasoconstriction in dogs (Villalon et al., 1997), was not able to block the sumatriptan-induced feline stomach relaxation. 5-$HT_{1B/1D}$ receptors are thus most likely not involved and this was corroborated by the observation that alniditan, a 5-$HT_{1A/1D}$ receptor agonist with moderate affinity for 5-HT_{1B} receptors, was not able to mimic the sumatriptan-induced effects. In 1986, Mawe et al. described 5-HT_{1P} receptors that mediated slow depolarisation in response to 5-HT in the myenteric neurons of the guinea-pig jejunum (Mawe et al., 1986). This effect could be blocked by 5-HTP-DP. Although 5-HT_{1P} receptors have been described in other studies as well (Mawe et al., 1989; Kuemmerle et al., 1995) these receptors were never officially recognized by the International Union of Pharmacology. Nevertheless is has been suggested by Tack et al. (2000) that the sumatriptan-induced gastric relaxation in humans might be mediated via 5-HT_{1P} receptors. Therefore the influence of the 5-HT_{1P} receptor antagonist 5-HTP-DP on the sumatriptan-induced feline stomach relaxation was tested. No effect was noticed, indicating that this receptor is not involved.

Sumatriptan displays a high affinity towards 5-HT_{1F} receptors. To date, no selective 5-HT_{1F} receptor antagonist has been described in literature and only few 5-HT_{1F} receptor agonists are available. Both the selective 5-HT_{1F} receptor agonist LY-344864 and the selective 5-$HT_{1e/1F}$ receptor agonist BRL-54443 were tested for their effects on feline gastric tone. LY-344864 is a selective, high affinity agonist at 5-HT_{1F} receptors (Phebus et al., 1997) that has not been administered in cats to date. In anaesthetized mice however, intraperitoneally administered LY-344864 (0.1 and 1 mg kg^{-1}) decreased c-fos expression after capsaicin administration, a response that was shown to be mediated by 5-HT_{1F} receptors (Mitsikostas et al., 2002). BRL-54443 is a selective, high affinity 5-$HT_{1e/1F}$ receptor agonist (Brown et al., 1997) and has been administered to conscious rats (0.01 and 0.1 mg kg^{-1}) where the drug increases the maximal electroshock seizure threshold (Lightowler et al., 1997). In our experiments, both agonists, in doses comparable to those cited above, dose-dependently induced feline gastric volume increase suggestive of 5-HT_{1F} receptor involvement. However, no real characterization of the receptor can be performed, as no antagonists are available.

In 1999, Coulie et al. found that buspirone was not able to mimic the sumatriptan-induced gastric relaxation in cats (Coulie et al., 1999). As buspirone displays low binding affinity towards 5-HT_{1F} receptors (Table 4) this may confirm our hypothesis. No clear explanation was found for the non-effect of subcutaneously administered LY-344864, moreover we have no clear explanation for the rapid intragastric volume decrease after administration of 3 mg kg^{-1} BRL-54443.

Since sumatriptan was shown to be effective in the treatment of migraine, it has been suggested that 5-HT_{1F} receptors may in addition to 5-$HT_{1B/1D}$ receptors also be involved in the pathophysiology of migraine. Research on 5-HT_{1F} receptors in this regard has predominantly been conducted towards the presence of 5-HT_{1F} receptors in brain and vascular system (Mitsikostas et al., 2002; Cohen and Schenck, 2000). Sumatriptan and LY-344864 are known to poorly penetrate the blood–brain barrier (Phebus et al., 1997; Dechant and Clissold, 1992) but as this barrier is not present throughout the whole brain (Bloom, 1996) a central site of action cannot fully be excluded. If the site of action would be located peripherally it would not be at the level of the smooth muscle cells as activation of 5-HT_{1F} receptors leads to inhibition of adenylate cyclase (Adham et al., 1993); activation of muscular 5-HT_{1F} receptors would therefore lead to contraction. A possible mechanism of action for 5-HT_{1F} receptors to mediate gastric relaxation is to inhibit the constant cholinergic drive (Azpiroz and Malagelada, 1987; Paterson et al., 2000). Decreasing the acetylcholine release to the stomach can be mediated centrally by inhibiting the cholinergic excitatory vagal pathway to the stomach (Chang et al., 2003). Inhibition of acetylcholine release could also take place at a local level as receptors negatively coupled to adenylate cyclase are frequently located presynaptically where they inhibit neurotransmitter release (e.g. M_2 receptors; Caulfield, 1993). Our experiments showed no involvement of nitrergic nerves in the sumatriptan-induced gastric relaxation, but this does not exclude that activation of 5-HT_{1F} receptors may induce release of other smooth muscle relaxing neurotransmitters such as purinergic or peptidergic substances (Azpiroz and Malagelada, 1985).

Inducing gastric relaxation with compounds similar to sumatriptan might be of therapeutic relevance in the treatment of an important subgroup of patients with functional dyspepsia where gastric accommodation is impaired (Tack et al., 2000). Our results suggest that stimulation of 5-HT_{1F} receptors induces feline stomach relaxation. Whether the sumatriptan-induced relaxation in cats is due to interaction with 5-HT_{1F} receptors could not be proven absolutely because of the lack of selective 5-HT_{1F} receptor antagonists. Still our study indicates that further investigation of the gastric relaxant effect of 5-HT_{1F} receptor agonists for possible therapeutic application in functional dyspepsia is war-

ranted. The sumatriptan-induced retching on the other hand has never been described before and is believed to be specific for ketamine-sedated cats.

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