J. Pharm. Pharmacol. 1995, 47: 219–222
Received January 14, 1994
Accepted September 23, 1994

Antagonism by SB 204070 of 5-HT-evoked Contractions in the Dog Stomach: an In-vivo Model of 5-HT_4 Receptor Function

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Abstract

The ability of 5-hydroxytryptamine (5-HT) to evoke contractile activity in the gastric Heidenhain pouch was measured in conscious dogs using a method in which 5-HT_4 receptor-antagonist activity can be measured in-vivo.

At doses of 5-HT which evoked short-lived measurable responses (5 or 10 $\mu g\,kg^{-1}$, i.v.), it was found that this activity was greatly reduced by atropine (100 $\mu g\,kg^{-1}$, i.v.), but was unaffected by methysergide, methiothepin, ketanserin (each at 100 $\mu g\,kg^{-1}$, i.v.) or granisetron (10 or 100 $\mu g\,kg^{-1}$, i.v.). At best SDZ 205-557 2-diethylaminoethyl-[2-methoxy-4-amino-5-chloro] benzoate; 100 $\mu g\,kg^{-1}$, i.v.) reduced the action of 5-HT in 4/5 animals and increased it in the other but its effects were variable in magnitude and not consistently maintained. However, the more potent and selective 5-HT_4-receptor antagonist SB 204070 (1-butyl-4-piperidinylmethyl 8-amino-7-chloro-1,4-benzodioxan-5-carboxylate hydrochloride) dose-dependently antagonized the 5-HT-evoked contractions in all dogs tested. This action was reversible, but long-lasting with an effective half-life of 18·0 h when administered at 1 $\mu g\,kg^{-1}$. The estimated ID50 value was 0·55 $\mu g\,kg^{-1}$.

Many 5-HT_4-receptor antagonists have now been reported in the literature, characterized mostly by their activity in-vitro (e.g. ICS 205-930 (3α-tropanyl)-1H-indole-3-carboxylic acid ester (Dumuis et al 1988), DAU 6285 (endo-8-methoxy-8-azabicyclo[3.2.1]oct-3-yl-2,3-dihydro-3-oxo-1H-benzimidazole-1-carboxylate hydrochloride) (Schiavone et al 1992), SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester) (Bucheit et al 1992), RS-23597-190 (Eglen et al 1993), GR 113808 ((1-(2-methylsulphonyl) amino)ethyl)-4-piperidinyl)methyl 1-methyl-1H-indole-3-carboxylate (Gale et al 1994). However, a full investigation of their function in-vivo is limited by the paucity of animal models of 5-HT_4-receptor function. Extension of the range of such in-vivo models is useful as it provides a background to possible species variation/subtype determination of effects which may have relevance to man. The most commonly used in-vivo system relies on the measurement of the tachycardia that is evoked by 5-hydroxytryptamine (5-HT) in the anaesthetized pig (Villalon et al 1990). However, whilst this preparation may be useful for determining the acute effects of 5-HT_4-receptor antagonists, it is not ideal for determining pharmacodynamic measurements such as duration of action. Responses may also be modified by anaesthetic agents.

Bermudez et al (1990) reported the use of the conscious dog Heidenhain pouch preparation to assess the efficacy of the motility-stimulating agent and 5-HT_4-receptor agonist, renzapride. This preparation allows the measurement of 5-HT_4-receptor function over extended periods of time without the complication of anaesthesia and also the potential to monitor possible side-effects in an awake animal. In the present study we have used the dog Heidenhain pouch to investigate and characterize the contractile activity evoked by 5-HT and developed a method for evaluating the efficacy of putative 5-HT_4-receptor antagonists in conscious animals. Some of the results in this paper have previously been reported to the British Pharmacology Society (Bingham et al 1993).

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Materials and Methods

5-HT and antagonists

Eight adult male beagle dogs, with previously prepared Heidenhain pouches (Bermudez et al 1990), were fasted overnight and restrained in Pavlov slings. The cephalic vein was cannulated for administration of 5-HT. Fifteen minutes after cannulation the first dose of 5-HT was given. This was repeated at 30-min intervals until two consecutive, consistent contractile responses to 5-HT were obtained as controls. Fifteen minutes after the second control response the antagonist or saline was given intravenously followed, after a further 15 min, by 5-HT. Thereafter 5-HT was administered at 30-min intervals until the end of the observation period (75 min from the administration of antagonist). At least one week was left between each experiment for each dog. Although gastric motility only was monitored during these experiments 5-HT had no other overt physiological or behavioural effects. All husbandry and procedures were in accordance with the UK Animal (Scientific Procedures) Act 1986 and were approved and monitored by trained veterinary staff.

During experiments, Heidenhain pouch motility was measured by monitoring pouch pressure via a pressure transducer (LEC) and displayed on a chart recorder

(LEC). The pressure signal was stored on video tape and at the end of the experiment the stored record was played back through an integrator (BPIES), which measured the area under the pressure signal at 1-min intervals. In some experiments the motility record was integrated on line every minute and the two signals displayed simultaneously on a chart recorder.

Duration of action

In these experiments the ability of the 5-HT_4-receptor antagonist SB 204070 (1 and 10 $\mu g\,kg^{-1}$, i.v.) (Wardle et al 1993) to inhibit the contractile response to 5-HT was measured at various time points after administration. The cephalic vein was acutely cannulated and SB 204070 given intravenously 3, 6, 9, 18 and 24 h before restraining the dogs in slings and cannulation of the other cephalic vein. Fifteen minutes later 5-HT was administered twice, 30 min apart.

Analysis and statistics

5-HT and antagonists. For the two control responses to 5-HT, the total height of the integrated record was measured over a 3-min period before and after administration of 5-HT, and a mean control difference obtained for each dog. Fifteen, 45 and 75 min after administration of antagonist or saline the response to 5-HT was calculated in the same way and expressed as a percentage of the mean control response, which was taken as 100%. For the dose-response curve to SB 204070 a single time point (75 min) was chosen when the effect of the antagonist was maximal and compared with control using Student's *t*-test for paired samples.

The total integrated height was measured 3-min before and 3-min after the injection of an antagonist and compared using Student's *t*-test for paired samples. These data were used to ascertain whether the antagonist possessed any intrinsic activity.

Duration of action. For each dog the response to the two injections of 5-HT after administration of antagonist was calculated as above from the integrated record and the mean difference taken. This was expressed as a percentage of the control response. In these experiments the control response was an historical control and was the mean value, for each dog, of the integrated response to 5-HT alone, obtained over the course of several experiments ($n = 2$–14). The percentage responses obtained for each time point were curve-fitted and the half-life for the return to control levels calculated using a logistic model and an iterative least-squares curve fitting procedure in RS1.

Materials

The following were dissolved in 154 mM NaCl (saline): 5-hydroxytryptamine creatinine sulphate (5-HT), atropine sulphate (Sigma), methysergide hydrogen maleate (Sandoz), methiothepin hydrochloride (Roche), ketanserin (Janssen), granisetron, and SB 204070 (1-butyl-4-piperidinylmethyl 8-amino-7-chloro-1,4-benzodioxan-5-carboxylate hydrochloride; SmithKline Beecham). SDZ 205-557 (SmithKline Beecham) was dissolved in 154 mM NaCl and tartaric acid.

Results

Responses to 5-HT

All dogs received intravenous randomized doses of 5-HT over the dose range 2·5–15 $\mu g\,kg^{-1}$. The contractile response to 5-HT was largely dose-dependent, but different dogs displayed different levels of sensitivity and experimental doses were chosen on the basis of their ability to evoke a measureable and consistent contractile response without adverse side-effects. This was either 5 or 10 $\mu g\,kg^{-1}$. Both these doses produced contractile responses that were near maximal within the above criteria (Fig. 1). In the absence of antagonist, repeated injection of these doses of 5-HT at 30-min intervals tended to evoke consistent responses over the duration of the experiment and also between experiments for each dog.

Effect of antagonists on the response to 5-HT

Atropine. At 30 $\mu g\,kg^{-1}$ intravenously, atropine had little or

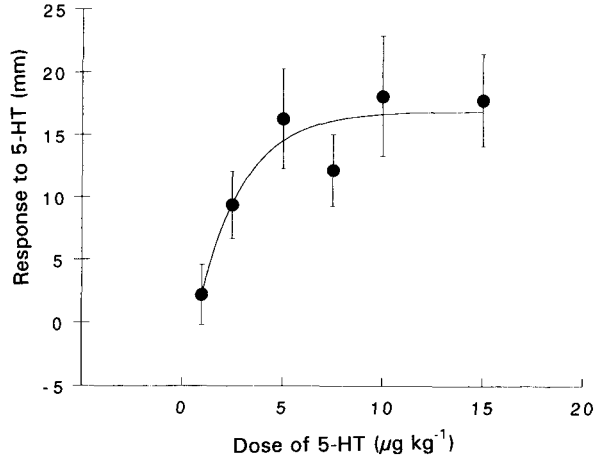

FIG 1. The mean (± s.e.m.) contractile response to 5-HT (2·5–15 $\mu g\,kg^{-1}$) in the dog Heidenhain pouch expressed in mm of trace. Doses of 5-HT were administered in a randomized manner ($n = 8$).

Table 1. The effect of antagonists on the contractile response to 5-HT in the dog Heidenhain pouch expressed as a percentage of the control response before administration of antagonist. Data are mean ± s.e.m. and numbers in parentheses are the number of animals in each group.

Antagonist	Time after injection of antagonist (min) 15	45	75
Saline (7)	126 ± 16	139 ± 26	106 ± 13
Methysergide (6) 100 $\mu g\,kg^{-1}$	128 ± 32	145 ± 19	127 ± 24
Methiothepin (2) 100 $\mu g\,kg^{-1}$	93 ± 10	94 ± 9	113 ± 2
Ketanserin (6) 100 $\mu g\,kg^{-1}$	166 ± 66	150 ± 45	155 ± 55
Granisetron (3) 10 $\mu g\,kg^{-1}$	144 ± 23	109 ± 17	133 ± 24
Granisetron (4) 100 $\mu g\,kg^{-1}$	84 ± 31	86 ± 29	99 ± 17
SDZ 205-557 (5) 100 $\mu g\,kg^{-1}$	65 ± 54	69 ± 60	164 ± 76
Atropine (3) 30 $\mu g\,kg^{-1}$	83 ± 14	87 ± 11	88 ± 23
Atropine (4) 100 $\mu g\,kg^{-1}$	67 ± 33	28 ± 13	25 ± 11

no effect on the contractile response to 5-HT at any time point after administration (Table 1). At 100 μg kg^{-1} atropine, the response to 5-HT was reduced within 45 min to 28 ± 13% of control and remained at this level for the duration of the experiment (105 min) (Table 1). There was no effect of atropine at either dose on spontaneous motility in the Heidenhain pouch.

5-HT-receptor antagonists. The 5-HT$_1$- and 5-HT$_2$-receptor antagonist, methiothepin (100 μg kg^{-1}, i.v.) had no effect on the response to 5-HT in either of the dogs tested (Table 1), but was profoundly sedative; therefore, no further experiments were carried out with this agent. The 5-HT$_1$-receptor and 5-HT$_2$-receptor antagonist, methysergide (100 μg kg^{-1}, i.v.) had variable effects on the response to 5-HT ranging from a sustained large increase in the percentage response (220–240%) in 1/6 dogs, a smaller increase (110–157%) lasting at most 45 min in 4/6 dogs, to a decrease (47%) in the response that returned to control levels within 30 min in 1/6 dogs. The mean data were not significantly different from those obtained in the saline control group (Table 1). The 5-HT$_2$-receptor antagonist ketanserin (100 μg kg^{-1}, i.v.) also had very variable and non-significant effects on the contractile response to 5-HT (Table 1). In 2/6 animals there was no effect, in 2/6 there was a sustained increase in the response to 5-HT (139–455%), in 1/6 the response was briefly increased (168%) before decreasing (67%) and in 1/6 dogs the response was decreased (48–86%). The 5-HT$_3$-receptor antagonist granisetron (10 μg kg^{-1}) had no effect in 2/3 dogs and gave a sustained increase of the 5-HT response (133–181%) in the third. At 100 μg kg^{-1}, granisetron transiently increased the response to 5-HT in 1/4 dogs, transiently decreased it in another, gave a sustained decrease in the third dog (14–88%) and had no effect in a fourth. Overall, none of these effects were statistically significant compared with the saline control (Table 1). None of these antagonists had consistent effects on spontaneous motility.

The 5-HT$_4$- and 5-HT$_3$-receptor antagonist SDZ 205-557 (100 μg kg^{-1}, i.v.) also had no consistent effect on the contractile response to 5-HT (Table 1). In 4/5 animals SDZ 205-557 reduced the response to 5-HT (−67–83%) for between 15 and 45 min after administration. In the fifth dog it increased (252%) and then decreased this response (32–52%). In contrast, the selective 5-HT$_4$-receptor antagonist, SB 204070 (Wardle et al 1993) reduced the response to 5-HT in a dose-dependent manner with complete abolition at 3 μg kg^{-1} (Fig. 2). Following the higher doses (1–100 μg kg^{-1}, i.v.) the maximal effects of SB 204070 occurred within 15 min of administration and lasted for the duration of the experiment (75 min). Over this dose range, SB 204070 decreased the response to 5-HT in all dogs at all time points measured. At 0·1–0·5 μg kg^{-1} the maximum effect tended to occur within 45–75 min. Statistically significant ($P < 0.05$) effects of SB 204070 were seen 75 min after administration at all doses except 0·1 μg kg^{-1} and an ID50 value of 0·55 μg kg^{-1} with 95% confidence limits of 0·20 to 1·49 μg kg^{-1} was calculated for this time point. Neither of the 5-HT$_4$-receptor antagonists had consistent effects on spontaneous motility at any dose.

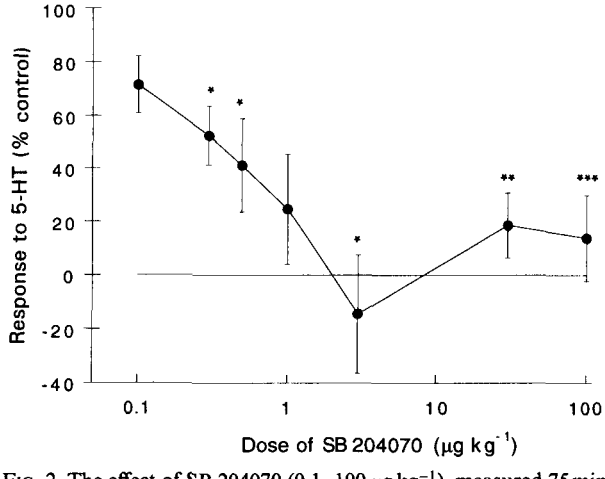

FIG. 2. The effect of SB 204070 (0·1–100 μg kg^{-1}), measured 75 min after administration, on the mean (± s.e.m.) contractile response to 5-HT (5 or 10 μg kg^{-1}) in the dog Heidenhain pouch expressed as a percentage of control. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ compared with control (n = 4 for each point).

Duration of action of SB 204070

The duration of action of SB 204070 was examined at two doses, both of which abolished the contractile response to 5-HT. At 10 μg kg^{-1} given intravenously 18 and 24 h before challenge with 5-HT, the response to 5-HT was still considerably reduced to 20 ± 12 and 36 ± 12% of control, respectively. However, at 1 μg kg^{-1} the response to 5-HT returned to control levels within 24 h (Table 2). The effective half-life was estimated as 18·0 h, with confidence limits of 1·9 and 176·8 h. In each group the responses to 5-HT at each time point after administration of SB 204070 were usually similar in two out of three dogs but often varied in the third. This may explain the variation in the confidence limits for this parameter.

Discussion

The search for selective antagonists at the 5-HT$_4$ receptor have relied on models of receptor function in isolated tissues (Sanger & Gaster 1994) which do not allow determination of pharmacodynamic activity and apart from the assessment of cardiac function in anaesthetized pigs (Villalon et al 1990), suitable models have not yet been developed in conscious animals. For this reason, we further characterized the dog Heidenhain gastric pouch preparation (Bermudez et al 1990).

It was previously reported that the contractile activity of the dog Heidenhain gastric pouch can be increased by renzapride, a compound which acts as an agonist at 5-HT$_4$ receptors but which is also an antagonist at 5-HT$_3$

Table 2. The mean ± s.e.m. contractile response to 5-HT (5 or 10 μg kg^{-1}) in the dog Heidenhain pouch expressed as a percentage of control, 3, 6, 9, 18 or 24 h after dosing with SB 204070.

SB 204070 (pre-dose)	Contractile response to 5-HT (% control) Time after pre-dose with SB 204070 (h)				
	3	6	9	18	24
1 μg kg^{-1}	−8 ± 17	21 ± 12	12 ± 4	51 ± 9	87 ± 14
10 μg kg^{-1}				20 ± 12	36 ± 12

n = 3 for each dose.

receptors. Since this response was neither mimicked nor blocked by the selective 5-HT_3-receptor antagonist, granisetron and could be prevented by atropine, it was concluded that renzapride evoked a cholinergically-mediated response predominantly by activation of the 5-HT_4 receptor (Bermudez et al 1990). In the present study, a similar contractile response was evoked by a single, bolus intravenous injection of 5-HT. This response was clearly measurable only when using doses of 5-HT which evoked a near-maximal response. However, since 5-HT can activate 5-HT receptors which exert both excitatory and inhibitory actions on gastrointestinal cholinergic function (Sanger & Wardle 1990), and since high doses of 5-HT can act on the vagus nerve, suppressing gastric motility and affecting cardio-respiratory function (Sanger 1993), we cannot be certain that the observed dose-response relationship for 5-HT represented the degree of receptor occupation or, more likely, a combination of a number of different, confounding factors.

Although the contractile responses evoked by 5-HT were not clearly affected by methiothepin, methysergide, ketanserin or granistron, at doses which antagonize 5-HT_1-like, 5-HT_2 and 5-HT_3 receptors in other in-vivo preparations (Connor et al 1986; Bermudez et al 1988) there was a certain amount of variability in these data particularly with the mixed 5-HT_1/5-HT_2-receptor antagonist, methysergide and the 5-HT_2-receptor antagonist, ketanserin. The predominant effect was a trend towards an increase in the response to 5-HT in the presence of these agents. This might suggest a possible activation of an inhibitory 5-HT_2 receptor mechanism by 5-HT under these conditions, although it should be noted that the 5-HT_1/5-HT_2-receptor antagonist, methiothepin had little effect on the contractile response to 5-HT. Nevertheless, it is clear that the selective 5-HT_4-receptor antagonist SB 204070 (Wardle et al 1993) potently and dose-dependently reduced the response to 5-HT, without consistently affecting the spontaneous contractility of the stomach. This action was long-lasting, but reversible. Further testing for selectivity of action was not undertaken in the dog, because the selectivity of action for SB 204070 has already been established in an isolated gastrointestinal preparation and by radioligand binding techniques (Wardle et al 1993). These results would suggest that the contractile response to 5-HT involves activation of 5-HT_4 receptors with a possible inhibitory, modulatory role for 5-HT_2 receptors. The existence of the latter might explain some of the variability observed with methysergide and ketanserin.

In contrast to the clear activity of SB 204070, the mixed 5-HT_4 and 5-HT_3-receptor antagonist SDZ 205-557 was without consistent effect on the contractile response to 5-HT. Whilst we have no conclusive explanation for this lack of consistency of action, one possibility is that the short biological half-life of this compound prevents significant activity from being detected in our model. Thus, in anaesthetized pigs, the effective half-life of SDZ 205-557 was only 23 min, when tested at 6 $mg\,kg^{-1}$ against the tachycardia evoked by 5-HT (Eglen et al 1993). Another possibility is that submaximally effective doses of SDZ 205-557 were used in this experiment, although a dose of 100 $\mu g\,kg^{-1}$ had some utility in conscious mice in a model of 5-hydroxytryptophan-induced defecation (Banner et al 1993).

In conclusion, this study has demonstrated that in the conscious dog, contractile responses elicited by 5-HT in the Heidenhain pouch are abolished by low doses of the selective 5-HT_4-receptor antagonist SB 204070 and are substantially reduced by atropine. These data indicate that this 5-HT-evoked response is mediated via 5-HT_4 receptors and involves the activation of cholinergic neurons. As such, the dog Heidenhain gastric pouch preparation provides a novel and reproducible means of investigating 5-HT_4-receptor antagonist and agonist activity in conscious animals.

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