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Interactions of GR127935, a 5-$HT_{1B/D}$ receptor ligand, with functional 5-HT receptors

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Abstract GR127935 (N-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl) [1, 1-biphenyl]-4-carboxamide hydrochloride) has been recently introduced as an experimental tool to antagonize 5-$HT_{1B/D}$ receptor-mediated functional responses. The compound indeed exhibits a very high affinity and selectivity for 5-$HT_{1B/D}$ binding sites and it antagonizes a number of 5-$HT_{1B/D}$ receptor-mediated responses. The present experiments were performed to investigate the selectivity of GR127935 against functional responses mediated by 5-HT_1-like, 'orphan' 5-HT_1-like (5-ht_7?), 5-HT_2, 5-HT_3 or 5-HT_4 receptors in several in vivo preparations. Intravenous (i.v.) treatment with GR127935 (300 $\mu g \times kg^{-1}$) potently antagonized decreases in total carotid blood flow as well as hypotensive responses induced by the 5-HT_1-like receptor agonist sumatriptan in rabbits. I.v. bolus injections of GR127935 (up to 500 and/or 1500 $\mu g \times kg^{-1}$) did not significantly modify 5-HT-induced: (i) tachycardia in the pig (5-HT_4 receptor-mediated) and cat ('orphan' 5-HT_1-like or, perhaps, 5-ht_7 receptor-mediated); (ii) depressor effects in the rat and cat ('orphan' 5-HT_1-like or 5-ht_7 receptor-mediated); (iii) von Bezold-Jarisch reflex in the rat or the early phase of the urinary bladder contraction in the cat (both 5-HT_3 receptor-mediated). In contrast, high doses (500-1500 $\mu g \times kg^{-1}$) of GR127935 suppressed 5-HT-induced pressor responses in the rat and cat and urinary bladder contractions (secondary phase) in the cat as well as the DOI ((±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride)-induced pressor responses in the rat, which are all mediated by 5-HT_{2A} receptors. In conclusion, the present study demonstrates that GR127935 is a selective 5-$HT_{1B/D}$ receptor antagonist devoid of interactions at 'orphan' 5-HT_1-like (5-ht_7?), 5-HT_3 and 5-HT_4 receptors. However, GR127935 possesses a moderate 5-HT_{2A} receptor blocking property, which is consistent with its binding profile (pK_i: 7.4). Lastly, in view of the potent antagonist action of GR127935, the sumatriptan-induced hypotension in rabbits seems to be mediated by 5-$HT_{1B/D}$ receptors.

Key words DOI · GR127935 · 5-HT · 5-HT receptors · 5-HT_{1B} receptor · 5-$HT_{1B/D}$ receptor antagonist · 5-HT_1-like receptor · Sumatriptan

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Introduction

The present decade has indisputably witnessed a remarkable progress in the classification of serotonin (5-hydroxytryptamine; 5-HT) receptors; this achievement is due not only to the adoption of structural and transductional criteria, but also to the discovery of compounds acting selectively at 5-HT receptors (Hoyer et al. 1994). For example, it is nowadays clear that the cardiovascular effects of 5-HT are mediated by several different receptor types, including 5-HT_1-like, 5-HT_2, 5-HT_3 and 5-HT_4 receptors (Saxena and Villalón 1990, 1991). Notwithstanding, the exact identity of 5-HT_1-like receptor mediating vascular contraction is in debate, although several lines of pharmacological evidence (see Hoyer et al. 1994) suggest a close resemblance with the 5-$HT_{1B/D}$ (formerly designated as 5-$HT_{1D\beta}$ and 5-$HT_{1D\alpha}$, respectively; see Hartig et al. 1996) receptor subtypes. This notion has been hampered because of the lack of compounds acting selectively at 5-$HT_{1B/D}$ receptor subtypes, but the advent of GR127935, a piperazinylbenzanilide derivative with high affinity for and selective antagonist

activity at 5-$HT_{1B/D}$ receptors (Clitherow et al. 1994), will hopefully overcome this obstacle. Indeed, some studies already show that GR127935 potently antagonizes: (i) contralateral turning in guinea-pigs induced by the 5-$HT_{1B/D}$ receptor agonist GR56764 (3-(2-methylaminoethyl)-1H-indole-5-yl-1H-[1, 2, 4]triazol-3-yl)-methyanol; Skingle et al. 1996); (ii) sumatriptan-evoked inhibition of 5-HT release in the guinea-pig dorsal raphe nucleus (Clitherow et al. 1994; Starkey and Skingle 1994); and (iii) sumatriptan-induced contraction of porcine carotid arteriovenous anastomoses (De Vries et al. 1996), canine external carotid vessels (Villalón et al. 1996), rabbit isolated saphenous vein (Valentin et al. 1996) and canine basilar artery (Skingle et al. 1996); several of these responses have been previously described as being mediated by 5-HT_1-like receptors (see Saxena and Villalón 1990, 1991).

In view of the pharmacological relevance that GR127935 has been acquiring as an experimental tool to identify functional 5-$HT_{1B/D}$ receptors, we have considered it most crucial to ascertain the selectivity of the drug. For this purpose, the effect of GR127935 has been studied on several functional responses mediated by different 5-HT receptors: (i) sumatriptan-induced decrease in carotid blood flow in the rabbit (5-HT_1-like); (ii) 5-HT-induced hypotension in the rat and tachycardia in the cat ('orphan' 5-HT_1-like); (iii) 5-HT and/or DOI-induced pressor responses in the rat and cat as well as the secondary phase of urinary bladder contraction in the cat (5-HT_{2A}); (iv) 5-HT-induced bradycardia (von Bezold-Jarisch reflex) in the rat and the early phase of urinary bladder contraction in the cat (5-HT_3); and (v) 5-HT-induced tachycardia in pigs (5-HT_4) (for references, see Saxena and Villalón 1990, 1991; Hoyer et al. 1994; Choppin and O'Connor 1996).

Material and methods

Experiments in rabbits. Experiments were carried out in 7 male New Zealand White rabbits (2.5-3.0 kg). After initial anaesthesia with sodium pentobarbital (45 mg×kg^{-1}, i.v.) and tracheal cannulation, the rabbits were artificially ventilated with a mixture of oxygen and room air using a respiratory pump (Infant ventilator MK3, HoekLoos, The Netherlands) at a rate of 30 strokes×min^{-1} (15 ml×kg^{-1}). At this point a continuous infusion of pentobarbital sodium (18 mg×kg^{-1}×h^{-1}, i.v.) was started. The left femoral artery and vein were dissected free and cannulated for blood pressure measurement with a pressure transducer (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and the administration of drugs, respectively. Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by ECG signals. After cutting both vagosympathetic trunks, the right common carotid artery was dissected free. Blood flow was measured in the right common carotid artery with a flow probe (internal diameter: 1.4 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). Blood pressure and heart rate were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). After a stable haemodynamic condition for about 30 min, all animals received consecutive bolus injections of sumatriptan (1, 3, 10, 30, 100 μg×kg^{-1}, i.v. every 5-15 min). These responses were elicited again 15 min after a bolus injection of physiological saline (0.5 ml, i.v.) and, subsequently, 15 min after an i.v. infusion of GR127935 (300 μg×kg^{-1}) over a period of 1 min.

Experiments in rats. Experiments were carried out in 39 male Wistar rats (300-350 g). After initial anaesthesia with ether, the trachea was cannulated and a catheter was placed in the left external jugular vein. At this point, ether anaesthesia was stopped and, subsequently, the animals received i.v. bolus injections of sodium pentobarbital (30-40 mg×kg^{-1}). Hereafter, the left carotid artery was cannulated for the recording of blood pressure, using a pressure transducer (Combitrans disposable pressure transducer, Braun, Melsungen, Germany) and the rats were artificially ventilated with a mixture of oxygen and room air using a respiratory pump (Infant ventilator MK3, HoekLoos, The Netherlands) at a rate of 40 strokes×min^{-1} (volume: 20 ml×kg^{-1}). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered from electrocardiogram signals. Both blood pressure and heart rate were recorded simultaneously on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). It should be noted that three experiments were carried out simultaneously; because of limited channels for heart rate, this variable was determined in only one out of three rats. At this point, the animals were then divided into three groups; in the first group both vagus nerves were left intact (n = 12), whilst the second and third group underwent sectioning of vagosympathetic trunk bilaterally (n = 12 and 15, respectively) to avoid the bradycardia and hypotension caused by 5-HT via the von Bezold Jarisch reflex (Paintal 1973). After a stable haemodynamic condition for at least 30 min, baseline values of mean blood pressure and heart rate were determined. Then, the first and second group of animals received intravenous (i.v.) bolus injections of 5-HT (3, 10 and 30 μg×kg^{-1}) every 5-10 min, while the third group received i.v. bolus injections of DOI (1, 3, 10 and 30 μg×kg^{-1}) and the changes produced in mean blood pressure and heart rate were noted. At this point, all groups of animals were subdivided again into two treatment groups, which received i.v. bolus injections of either GR127935 (20, 100 and 500 μg×kg^{-1}) or the corresponding volumes of saline (control animals); at the end, the control animals received an i.v. infusion of ritanserin (50 μg×kg^{-1}) over a period of about 5 min to establish that the hypertensive response was amenable to blockade by this 5-HT_2 receptor antagonist. Before each antagonist or saline treatment, the animals received small i.v. bolus injections of pentobarbital (20-30 mg×kg^{-1}), necessary to establish equal baseline values of blood pressure. Fifteen to twenty min after each antagonist or saline treatment, the responses to 5-HT and DOI were elicited again.

Experiments in cats. Two cats (4 and 6 kg) were fasted overnight and anaesthetized with ketamine (50 mg×kg^{-1}, i.m.) and sodium pentobarbital (30 mg×kg^{-1}, i.v.). Anaesthesia was maintained with a continuous infusion of sodium pentobarbital (15-20 mg×kg^{-1}×h^{-1}, i.v.). Subsequently, the animals were artificially ventilated with a mixture of oxygen and room air using a respiratory pump (Infant ventilator MK3, HoekLoos, The Netherlands). The left femoral vessels were catheterized for the i.v. injection of drugs and measurement of arterial blood pressure with a pressure transducer (Combitrans disposable pressure transducer, Braun, Melsungen, Germany). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered from electrocardiogram signals. Subsequently, the spinal cord (at the level of C_1-C_2) and both vagosympathetic trunks were sectioned, as previously reported (Saxena et al. 1985a). Hereafter, the urinary bladder was cannulated with a suitable polyethylene tube inserted into its cavity via a cut in the urethra. Intravesical pressure was monitored by connecting the bladder cannula to another pressure transducer. Blood pressure, heart rate and intravesical pressure were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands).

After the animals had been in a stable haemodynamic condition for at least 30 min, bolus injections of 5-HT (3, 10 and 30 μg×kg^{-1}, i.v.) were given at intervals of 10-15 min and the changes pro-

duced in mean blood pressure, heart rate and urinary bladder pressure were noted. Subsequently, GR127935 (500 μg×kg^{-1}, i.v.) was slowly administered over a period of 5 min and the responses to 5-HT were elicited again after 15 min. The responses induced by 5-HT (30 μg×kg^{-1}) on the urinary bladder pressure were also analysed after subsequent administrations of ketanserin and MDL 72222 (both 500 μg×kg^{-1}, i.v.) at 15 min interval.

Experiments in pigs. After an overnight fast, 5 domestic pigs (Yorkshire × Landrace; 10-15 kg) were anaesthetized with azaperone (160 mg, i.m.), midazolan hydrochloride (5 mg, i.m.) and metomidate (200 mg, i.v.), intubated and connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO2: 35-48 mmHg; pO2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of pentobarbitone sodium at 20 mg×kg^{-1}×h^{-1} and both vagi and the accompanying cervical sympathetic nerves were cut to avoid possible reflex changes. Catheters were placed in the inferior vena cava via the left femoral vein for the administration of drugs and in the aortic arch via the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered from electrocardiogram signals. Blood pressure and heart rate were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). During the experiment, body temperature was kept at about 37°C and the animals were continuously infused with saline to compensate for fluid losses.

After the animals had been in a stable haemodynamic condition for at least 30 min, they were given methiothepin (500 μg×kg^{-1}, i.v.) in order to block 5-HT-induced changes in arterial blood pressure without affecting heart rate responses (Bom et al. 1988). After 10 min, i.v. bolus injections of 5-HT (10 and 30 μg×kg^{-1}) were administered at intervals of 10-15 min and the changes produced in heart rate were noted (for further details, see Villalón et al. 1990, 1991). Subsequently, GR127935 (500 μg×kg^{-1}, i.v.) was slowly administered over a 5 min period and the responses to 5-HT were elicited again.

Ethical approval. The protocols of the investigation were approved by the joint Ethical Committee of the Erasmus University Rotterdam and the University Hospital Rotterdam 'Dijkzigt' dealing with the use of animals in scientific experiments.

Data presentation and statistical evaluation. All data in the text and the illustrations are presented as means±SEM The changes and percent changes from baseline in blood pressure, heart rate and carotid blood flow by the different doses of the agonists were calculated in each experiment. The mean±SEM of the changes in blood pressure, heart rate and carotid blood flow by the agonists before and after GR127935 or saline treatment were compared by the use of Duncan's new multiple range test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel and Torrie 1980). In case of the rat experiments, the changes from baseline caused by 5-HT in the animals treated with the different doses of GR127935 were compared with those in the control group at the same dose of 5-HT by Student's *t*-test, while the sumatriptan-induced changes after GR127935 in rabbits were compared with those after saline by a paired *t*-test. A *P* value of 0.05 or less (two-tailed) was considered statistically significant.

Compounds. Apart from the anaesthetics, azaperone, metomidate (both from Janssen Pharmaceutica, Beerse, Belgium), midazolan hydrochloride (Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and pentobarbitone sodium (Apharmo, Arnhem, The Netherlands), the drugs used in this study (obtained from the sources indicated) were: GR127935 (N-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl) [1, 1-biphenyl]-4-carboxamide hydrochloride) and sumatriptan succinate (both from Glaxo Group Research, Ware, UK; courtesy Dr. Helen Connor); 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Company, St. Louis, MO, USA); methiothepin maleate (Hoffmann La Roche b.v., Mijdrecht, The Netherlands); DOI ((±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride) and ritanserin (both from RBI, Natick, USA); ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium); MDL 72222 (1α-H,3α,5αH-tropan-3-yl-3,5-dichlorobezoate; Merrell Dow Research Institute, Strasbourg, France) and heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) to prevent clotting of the catheters. GR127935 was solubilized according to the instructions of the supplier by heating the dispersion in distilled water to about 70°C for 10 s and then allowing to cool down to room temperature. Ritanserin was dissolved in distilled water (30% methanol). The other drugs were dissolved in physiological saline. All doses refer to the respective salts, whereas those of 5-HT and ritanserin refer to the free base.

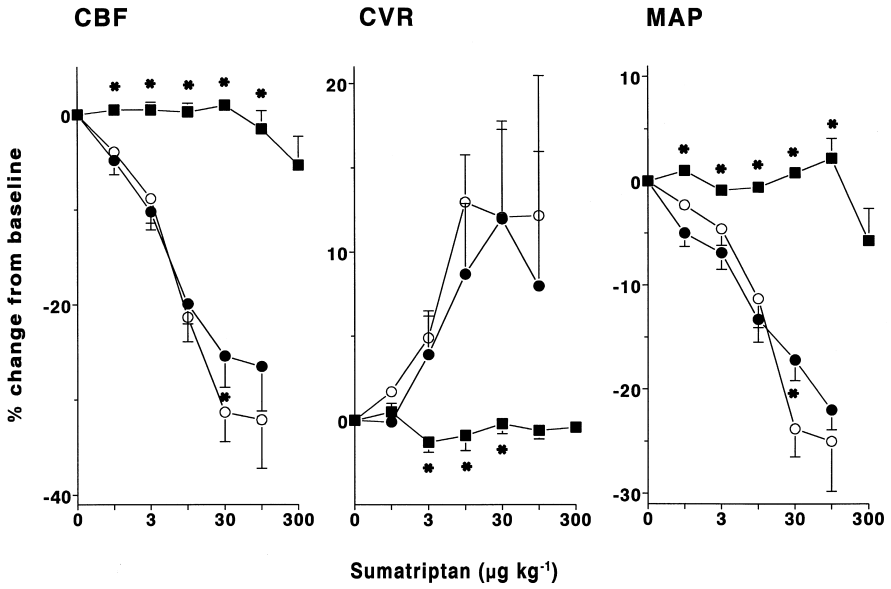

Fig. 1 Percentage changes from baseline values by sumatriptan (1, 3, 10, 30, 100 and 300 μg×kg^{-1}, i.v.) in carotid blood flow (*CBF*), vascular resistance (*CVR*) and mean arterial blood pressure (*MAP*) in rabbits (n = 7), obtained before (❍) and after treatment with saline (●) and GR127935 (300 μg×kg^{-1}, i.v., ■).
* P<0.05 vs. the corresponding dose of sumatriptan before treatments

Results

Effect of sumatriptan on total carotid blood flow, heart rate and mean arterial blood pressure in rabbits before and after GR127935

As depicted in Fig. 1, sumatriptan (1-100 μg×kg^{-1}, i.v.) elicited dose-dependent decreases in the total carotid blood flow (maximum change 32±5%; baseline 53.9±7.8 ml×min^{-1}) and concomitant increases in carotid vascular resistance (maximum change 13±3%, baseline 1.6±0.2 mmHg×ml^{-1}×min^{-1}). Moreover, sumatriptan produced dose-dependent decreases in mean arterial blood pressure (maximum change 25±5%; baseline 74.7±2.4 mmHg); baseline heart rate (275±16 beats×min^{-1}) was not altered by sumatriptan. The percent changes in these variables (pre-saline values of carotid blood flow, carotid vascular resistance and blood pressure were 46.0±9.3 ml×min^{-1}, 1.9±0.3 mmHg×ml^{-1}×min^{-1} and 76.7±3.9 mmHg, respectively) induced by the same doses of sumatriptan about 15 min after the administration of physiological saline were not significantly different, except for a small change in the response to 30 μg×kg^{-1} sumatriptan on carotid blood flow and arterial blood pressure. Subsequent treatment with GR127935 (300 μg×kg^{-1}, i.v.) potently antagonized the sumatriptan-induced changes in common carotid blood flow (maximum change -5±3%), vascular resistance (maximum change -1±1%) and mean arterial blood pressure (maximum change -6±3%) (Fig. 1). Post-GR127935 values of carotid blood flow, carotid vascular resistance and blood pressure were 45.8±8.9 ml×min^{-1}, 1.9±0.3 mmHg×ml^{-1}×min^{-1} and 72.3±3.3 mmHg, respectively and, thus, contrary to some earlier reports (Pauwels and Colpaert 1995; Watson et al. 1995; De Vries et al. 1996), GR127935 did not show any agonist activity.

Cardiovascular effects of 5-HT in intact and vagosympathectomized rats and of DOI in vagosympathectomized rats before and after GR127935

The baseline values of mean arterial blood pressure and heart rate in the three groups of rats (see Methods section) were, respectively, 111±2, 101±2 and 86±2 mmHg and 265±13, 298±18 and 216±29 beats×min^{-1}. These variables were not significantly modified by the subsequent injection of GR127935 (20, 100 and 500 μg×kg^{-1}) or the corresponding volumes of physiological saline (data not shown).

Animals with intact vagus. As depicted in Fig. 2, 5-HT produced a triphasic effect on arterial blood pressure in rats with intact vagus, comprising of an initial hypotension associated with a brief, but intense, bradycardia (not shown in the Fig. 2) via the von Bezold-Jarisch reflex, followed by a pressor effect and, finally, a longer-lasting hypotension. These responses were reproducible and remained essentially unchanged in control animals receiving 3 doses of saline (Fig. 2 *upper panel*). Treatment with ritanserin (50 μg×kg^{-1}, i.v.) completely antagonized the pressor responses, while the early and late hypotensive responses were significantly potentiated (data not shown).

GR127935 (100 and 500 μg×kg^{-1}), which showed no agonist effect, significantly attenuated the pressor effects induced by the highest dose of 5-HT. When compared to the respective responses in the saline-treated control animals, only at 500 μg×kg^{-1} of GR127935 a significant attenuation of the response to the highest dose of 5-HT was observed. Additionally, the pressor effects induced by 3 μg×kg^{-1} of 5-HT were slightly, but significantly potentiated by GR127935 (20 and 100 μg×kg^{-1}). Similarly, GR127935 (100 and 500 μg×kg^{-1}) slightly enhanced the late depressor responses induced by the lowest dose of 5-HT (Fig. 2, *lower panel*).

Vagotomized animals. After section of both vagosympathetic trunks, 5-HT no longer produced bradycardia or

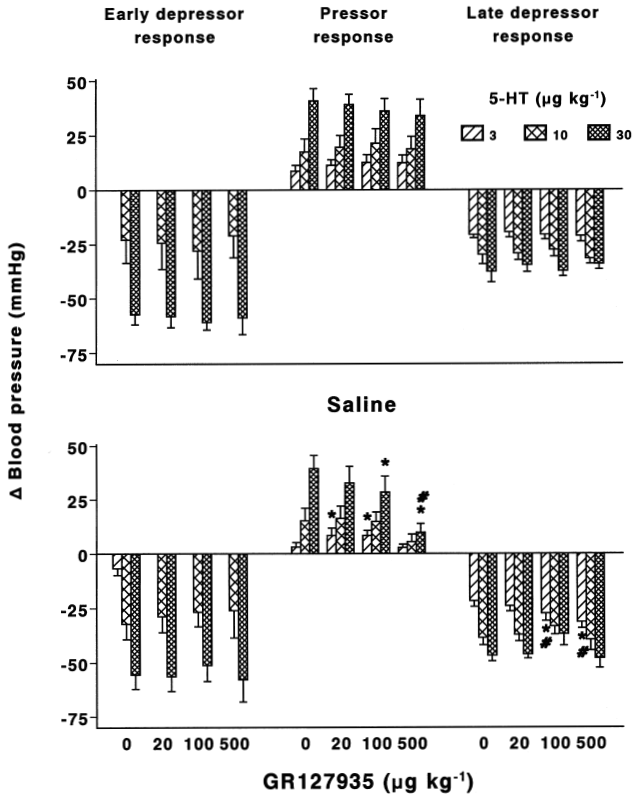

Fig. 2 Effect of either saline (three consecutive infusions; *upper panels*; n = 6) or GR127935 (20, 100 and 500 μg×kg^{-1}, i.v.; *lower panels*; n = 6) on mean arterial blood pressure responses to 5-HT (3, 10 and 30 μg×kg^{-1}, i.v.) in rats with intact vagus. * P<0.05 vs. the respective changes by 5-HT at baseline. # P<0.05 vs. the respective changes by 5-HT in the saline-treated (control) animals

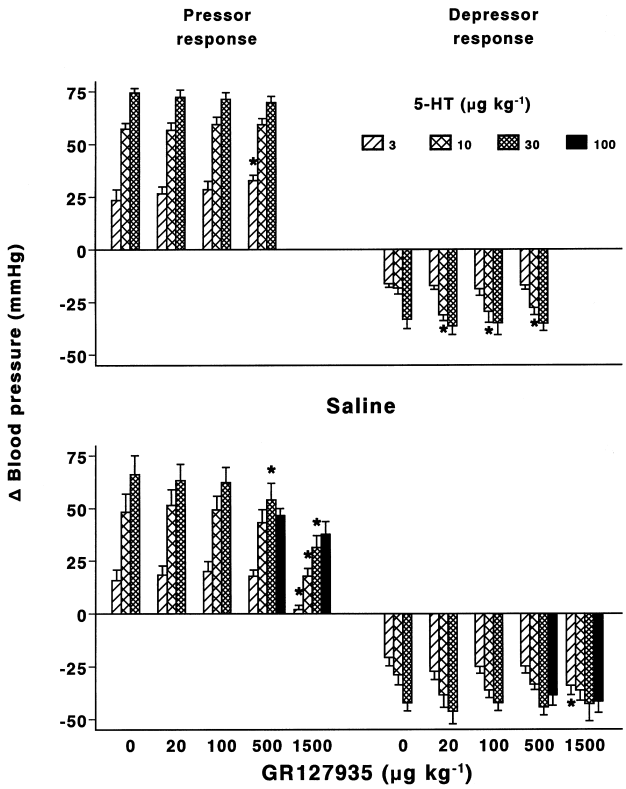

Fig. 3 Effect of either saline (three consecutive infusions; *upper panels*; $n = 6$) or GR127935 (20, 100, 500 and 1500 µg×kg^{-1}, i.v.; $n = 6$) on mean arterial blood pressure responses to 5-HT (3, 10, 30 and 100 µg×kg^{-1}, i.v.) in vagosympathectomized rats. * $P<0.05$ vs. the respective changes by 5-HT at baseline. # $P<0.05$ vs. the respective changes by 5-HT in the saline-treated (control) animals

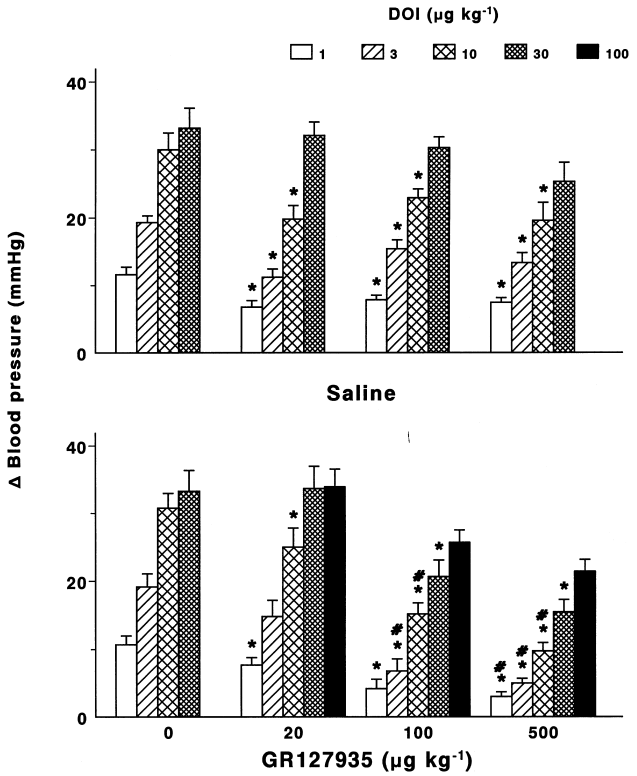

Fig. 4 Effect of either saline (three consecutive infusions; *upper panel*; $n = 9$) or GR127935 (20, 100 and 500 µg×kg^{-1}, i.v.; $n = 6$) on mean arterial blood pressure responses to DOI (1, 3, 10, 30 and 100 µg×kg^{-1}, i.v.) in vagosympathectomized rats. * $P<0.05$ vs. the respective changes by 5-HT at baseline. # $P<0.05$ vs. the respective changes by 5-HT in the saline-treated (control) animals

the associated initial hypotensive response, but the pressor responses, which became more pronounced, and the late hypotensive responses were present. These responses were reproducible and remained essentially unchanged in control animals receiving 3 subsequent doses of saline (Fig. 3, *upper panels*); after additional treatment with ritanserin (50 µg×kg^{-1}, i.v.) the pressor response was completely abolished, while the late depressor response was significantly potentiated (data not shown). As shown in the *lower panel* of Fig. 3, under these experimental conditions, GR127935 (500 and, primarily, 1500 µg×kg^{-1}) produced a significant blockade of the pressor responses to 5-HT.

As depicted in Fig. 4, the selective 5-HT$_{2A}$ receptor agonist DOI exclusively produced dose-dependent pressor responses in vagosympathectomized rats. These responses were reproducible, but slightly (though significantly) decreased in control animals receiving 3 subsequent doses of saline (Fig. 4, *upper panel*). The responses to DOI, particularly to 3 and 10 µg×kg^{-1}, i.v., were significantly more attenuated by treatment with GR127935 (100 and 500 µg×kg^{-1}, i.v.) than was the case in the saline-treated animals (Fig. 4, *lower panel*).

Effects of 5-HT on heart rate, mean arterial blood pressure and intravesical pressure in spinal cats before and after GR127935

Intravenous administration of 5-HT (3, 10 and 30 µg×kg^{-1}) produced dose-dependent increases in heart rate, mean arterial blood pressure and intravesical pressure. After GR127935 (500 µg×kg^{-1}) the increases in heart rate (26, 55 and 72 beats×min^{-1} for cat 1 and 7, 31, 49 beats×min^{-1} for cat 2) remained unaltered (23, 49 and 66 beats×min^{-1} for cat 1 and 4, 30 and 47 beats×min^{-1} for cat 2), but the increases in mean arterial blood pressure (10, 21 and 42 mmHg for cat 1 and 1, 15 and 40 mmHg for cat 2) seemed to be attenuated (9, 11 and 5 mmHg for cat 1 and 5, 4 and 12 mmHg for cat 2).

As has been previously reported (Saxena et al. 1985b), the contractile responses of the cat urinary bladder induced by 5-HT (3, 10 and 30 µg×kg^{-1}) were biphasic, consisting of an initial spike followed by a longer-lasting (secondary) increase in intravesical pressure (Fig. 5). GR127935 (500 µg×kg^{-1}) did not modify the first phase of the increase in intravesical pressure, but seemed to attenuate the second contractile phase. This remaining re-

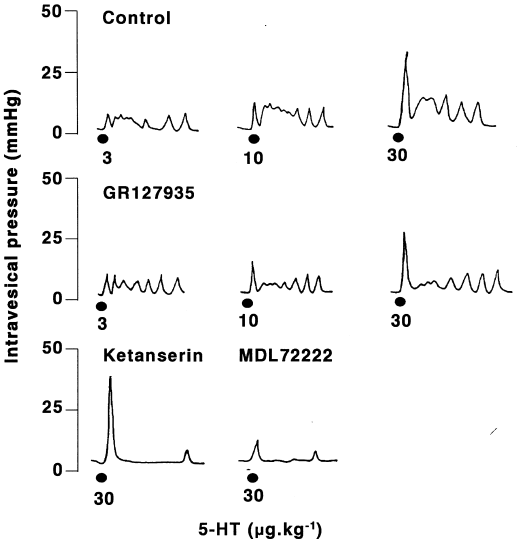

Fig. 5 Tracings illustrating the effects produced by bolus injections of 5-HT (3, 10 and 30 μg×kg^{-1}, i.v.) on intravesical pressure in a cat before (control) and after GR127935 (500 μg×kg^{-1}, i.v.). Note that 5-HT produced a biphasic response and that only the secondary phase was attenuated by GR127935. Subsequent administrations of ketanserin (500 μg×kg^{-1}, i.v.) and MDL 72222 (500 μg×kg^{-1}, i.v.) blocked, respectively, the second and the first phase responses produced by 30 μg×kg^{-1} of 5-HT

sponse to 5-HT (30 μg×kg^{-1}) was changed after the administration of ketanserin (500 μg×kg^{-1}, i.v.) into a monophasic (spike) response, which was then markedly antagonized by the subsequent administration of MDL 72222 (Fig. 5).

Tachycardiac responses to 5-HT in pigs

Consistent with previous findings (Villalón et al. 1990, 1991), i.v. bolus injections of 5-HT (10 and 30 μg×kg^{-1}) resulted in dose-dependent increases in heart rate of, respectively, 45±8 and 70±9 beats min^{-1}. These tachycardiac responses to 5-HT were not blocked after administration of 500 μg×kg^{-1} of GR127935 (59±7 and 73±9 beats min^{-1}, respectively).

Discussion

General

The lack of availability of selective 5-HT$_{1B/D}$ receptor antagonists has been felt as an impediment in investigating the relationship between the 5-HT$_1$-like and 5-HT$_{1B/D}$ receptors (see Saxena and Villalón 1990; Den Boer et al. 1992). Thanks to the advent of GR127935, it is becoming increasingly evident that sumatriptan-sensitive 5-HT$_1$-like receptors do indeed resemble the 5-HT$_{1B/D}$ subtype, with the remaining 5-HT$_1$-like receptors resembling the pharmacological profile of the 5-ht$_6$ or 5-ht$_7$ receptors or even conforming the operational characteristics of 'orphan' 5-HT receptors (Hoyer et al. 1994).

Binding studies show that GR127935 displays high affinities for human 5-HT$_{1D}$, human 5-HT$_{1B}$ and rat 5-HT$_{1B}$ receptors (pKi: 8.9, 9.9 and 8.5, respectively), moderate affinity for 5-HT$_{2A}$ receptors (pK$_i$: 7.4) and very low affinity for 5-HT$_3$, 5-HT$_4$ and 5-ht$_5$ receptors (pK$_i$ < 5.2) (see Skingle et al. 1996). There is only limited information available concerning the selectivity of GR127935 in functional studies and, therefore, the present investigation deals with this issue. However, when interpreting the results, one should keep in mind that the activity of GR127935 at different 5-HT receptors is based only on binding data for human and rat 5-HT receptors; binding data of GR127935 for the cat, pig and rabbit 5-HT receptors are, at this moment, not available.

Effect of sumatriptan on total carotid blood flow, heart rate and mean arterial blood pressure in rabbits before and after GR127935

As described by Choppin and O'Connor (1996), sumatriptan dose-dependently decreased total carotid blood flow in anaesthetized rabbits. This effect was antagonized by methiothepin, suggestive for the involvement of 5-HT$_1$-like receptors. Taking into account the high affinity of sumatriptan, but also of methiothepin for 5-HT$_{1B/D}$ receptors (Beattie et al. 1994), it is argued that these 5-HT$_1$-like receptors are identical to 5-HT$_{1B/D}$ receptors (De Vries et al. 1996). In agreement with this, the present results demonstrate that sumatriptan-induced reduction in the rabbit carotid blood flow, which was amenable to blockade by GR127935, involves 5-HT$_1$-like receptors resembling 5-HT$_{1B/D}$ receptors. Interestingly, sumatriptan produced dose-dependent hypotensive responses and, in view of the potent antagonist action of GR127935, the involvement of 5-HT$_{1B/D}$ receptors is likely.

Effects of GR127935 on 5-HT-induced cardiovascular responses in the rat

As previously described by Saxena and Lawang (1985), i.v. administration of 5-HT produced a triphasic response on the arterial blood pressure of rats with intact vagus; this response consists of an initial hypotension associated with a brief, but intense, bradycardia via the von Bezold-Jarisch reflex (mediated by 5-HT$_3$ receptors), followed by a vasopressor effect (mediated by 5-HT$_{2A}$ receptors) and, finally, a longer-lasting hypotension (mediated by 5-HT$_1$-like, probably 'orphan' and/or 5-ht$_7$ receptors) (Saxena and Villalón 1990, 1991; unpublished observations). After bilateral resection of vagosympathetic trunks, the initial hypotension due to reflex bradycardia was abolished, consistent with the concept that the von Bezold-Jarisch reflex is elicited by stimulation of 5-HT$_3$ receptors located on sensory vagal nerve endings

in the heart (Saxena and Villalón 1990, 1991). Our finding that only the vasopressor response to 5-HT elicited in intact as well as vagosympathectomized rats was significantly antagonized by 500 and 1500 μg×kg^{-1} of GR127935 suggests that the drug produces a moderate blockade of vascular 5-HT$_{2A}$ receptors. Moreover, the use of DOI, a compound acting selectively at 5-HT$_{2A}$ receptors, enabled us to study the pressor response without the influence of the initial and late hypotensive responses. Indeed, GR127935 dose-dependently antagonized DOI-induced pressor responses. The latter blockade is in keeping with the affinity of GR127935 for 5-HT$_{2A}$ binding sites (see above). However, it may be remarked that Skingle et al. (1996) have reported that GR127935 (1-10 mg×kg^{-1}, s.c.) did not affect wet dog shakes induced by DOI (3 mg×kg^{-1}, s.c.) in the guinea-pig, a response mediated by 5-HT$_2$ receptors (Skingle et al. 1991).

The fact that GR127935 failed to antagonize both the initial transient hypotensive (in intact animals) and the late hypotensive responses to 5-HT in intact and vagosympathectomized rats implies that the drug does not interact with, respectively, 5-HT$_3$ and 5-HT$_1$-like (orphan; 5-ht$_7$) receptors. In this connection, preliminary data from our laboratory suggest that the receptors mediating the 5-HT-induced late hypotensive response in rats, which are insensitive to sumatriptan, closely resemble the pharmacological profile of the 5-ht$_7$ receptor (unpublished observations at our laboratory).

Effects of 5-HT on the urinary bladder of spinal cats before and after GR127935

Saxena et al. (1985b) have previously shown in anaesthetized cats that i.a. administration of 5-HT elicits a biphasic contractile response of the urinary bladder, consisting of an initial spike followed by a longer-lasting increase in the intravesical pressure. These early and late phases of the cat urinary bladder contraction elicited by 5-HT are mediated, respectively, by 5-HT$_3$ receptors (amenable to blockade by MDL 72222) located on parasympathetic ganglia and 5-HT$_2$ receptors (amenable to blockade by ketanserin, cyproheptadine or methysergide) located on smooth muscle (Saxena et al. 1985b). The results of the present study confirm that the contractile response of the cat urinary bladder to 5-HT is biphasic in nature (Fig. 5). Since GR127935 did not significantly antagonize the early contractile phase, it is concluded that the drug does not interact with 5-HT$_3$ receptors located on parasympathetic ganglia. In contrast, GR127935 moderately attenuated the 5-HT-induced secondary phase of urinary bladder contraction. In conformity with its moderate affinity for 5-HT$_{2A}$ binding sites (Starkey and Skingle 1994) as well as the attenuation of pressor responses to 5-HT and DOI (see above), these results show that high doses of GR127935 can antagonize functional 5-HT$_{2A}$ receptors located on the cat urinary bladder smooth muscle. Indeed, for this reason we decided to confirm the involvement of both 5-HT$_2$ and 5-HT$_3$ receptors by using their respective antagonists ketanserin and MDL 72222 (Fig. 5) after treatment with GR127935. Thus, the fact that a monophasic response was obtained after ketanserin, and that this latter response was markedly blocked after MDL 72222 unequivocally demonstrates the involvement of both 5-HT$_2$ and 5-HT$_3$ receptors.

Effects of 5-HT on heart rate and mean blood pressure of spinal cats before and after GR127935

It has previously been shown that the increases in heart rate produced by 5-HT in the cat, being mimicked by 5-carboxamidotryptamine, are mediated by 5-HT$_1$-like receptors (Saxena et al. 1985a). Since these receptors are amenable to blockade by some ergot derivatives, including mesulergine (Saxena 1988), but are not stimulated by sumatriptan (Saxena et al. unpublished), the operational characteristics of these receptors rather resemble that of the 5-ht$_7$ type (Hoyer et al. 1994). Although there are no binding data available thus far showing the affinity of GR127935 for 5-ht$_7$ receptors, our finding demonstrating that the drug failed to antagonize 5-HT-induced tachycardia in cats implies that GR127935 does not interact with cardiac 5-ht$_7$ (or orphan 5-HT$_1$-like) receptors in the cat.

The antagonism of the pressor responses by GR127935 is in keeping with the findings described above, that GR127935 displays moderate affinity for 5-HT$_{2A}$ receptors.

Effects of GR127935 on 5-HT-induced porcine tachycardia

It has been well characterized that the 5-HT-induced tachycardia in the pig is mediated by 5-HT$_4$ receptors (Duncker et al., 1985; Bom et al., 1988; Villalón et al. 1990, 1991), which are positively coupled to adenylate cyclase (Hoyer et al. 1994). In the present investigation, the animals were deliberately vagosympathectomized (to avoid reflex bradycardia) and pretreated with methiothepin (500 μg×kg^{-1}) to block 5-HT$_2$ receptor-mediated vasopressor and 5-HT$_1$-like receptor-mediated late depressor responses. Under these experimental conditions, i.v. bolus injections of 5-HT produced dose-dependent increases in heart rate which were resistant to antagonism by GR127935. Therefore, we conclude that GR127935 does not interact with cardiac 5-HT$_4$ receptors in the pig.

In conclusion, the present study demonstrates that the piperazinyl-benzanilide derivative, GR127935, is a selective 5-HT$_{1B/D}$ receptor antagonist devoid of interactions at 5-HT$_1$-like ('orphan' and/or 5-ht$_7$), 5-HT$_3$ and 5-HT$_4$ receptors. However, GR127935 possesses moderate 5-HT$_{2A}$ blocking properties which are consistent with its binding profile.

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