



Characterization of putative 5-HT₇ receptors mediating tachycardia in the cat

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1 It has been suggested that the tachycardic response to 5-hydroxytryptamine (5-HT) in the spinal-transected cat is mediated by '5-HT₁-like' receptors since this effect, being mimicked by 5-carboxamidotryptamine (5-CT), is not modified by ketanserin or MDL 72222, but it is blocked by methiothepin, methysergide or mesulergine. The present study was set out to reanalyse this suggestion in terms of the IUPHAR 5-HT receptor classification schemes proposed in 1994 and 1996.

2 Intravenous (i.v.) bolus injections of the tryptamine derivatives, 5-CT (0.01, 0.03, 0.1, 0.3, 1, 3, 10 and 30 µg kg⁻¹), 5-HT (3, 10 and 30 µg kg⁻¹) and 5-methoxytryptamine (3, 10 and 30 µg kg⁻¹) as well as the atypical antipsychotic drug, clozapine (1000 and 3000 µg kg⁻¹) resulted in dose-dependent increases in heart rate, with a rank order of agonist potency of 5-CT >> 5-HT > 5-methoxytryptamine >> clozapine.

3 The tachycardic effects of 5-HT and 5-methoxytryptamine were dose-dependently antagonized by i.v. administration of lisuride (30 and 100 µg kg⁻¹), ergotamine (100 and 300 µg kg⁻¹) or mesulergine (100, 300 and 1000 µg kg⁻¹); the highest doses of these antagonists used also blocked the tachycardic effects of 5-CT. Clozapine (1000 and 3000 µg kg⁻¹) did not affect the 5-HT-induced tachycardia, but attenuated, with its highest dose, the responses to 5-methoxytryptamine and 5-CT. However, these doses of clozapine as well as the high doses of ergotamine (300 µg kg⁻¹) and mesulergine (300 and 1000 µg kg⁻¹) also attenuated the tachycardic effects of isoprenaline. In contrast, 5-HT-, 5-methoxytryptamine- and 5-CT-induced tachycardia were not significantly modified after i.v. administration of physiological saline (0.1 and 0.3 ml kg⁻¹), the 5-HT_{1B/1D} receptor antagonist, GR127935 (500 µg kg⁻¹) or the 5-HT_{3/4} receptor antagonist, tropisetron (3000 µg kg⁻¹).

4 Intravenous injections of the 5-HT₁ receptor agonists, sumatriptan (30, 100 and 300 µg kg⁻¹) and indorenate (300 and 1000 µg kg⁻¹) or the 5-HT₄ receptor (partial) agonist cisapride (300 and 1000 µg kg⁻¹) were devoid of effects on feline heart rate *per se* and failed to modify significantly 5-HT-induced tachycardic responses.

5 Based upon the above rank order of agonist potency, the failure of sumatriptan, indorenate or cisapride to produce cardioacceleration and the blockade by a series of drugs showing high affinity for the cloned 5-HT₇ receptor, the present results indicate that the 5-HT receptor mediating tachycardia in the cat is operationally similar to other putative 5-HT₇ receptors mediating vascular and non-vascular responses (e.g. relaxation of the rabbit femoral vein, canine external carotid and coronary arteries, rat systemic vasculature and guinea-pig ileum). Since these responses represent functional correlates of the 5-HT₇ gene product, the 5-HT₇ receptor appellation is reinforced. Therefore, the present experimental model, which is not complicated by the presence of other 5-HT receptors, can be utilized to characterize and develop new drugs with potential agonist and antagonist properties at functional 5-HT₇ receptors.

Keywords: Heart rate; 5-HT (5-hydroxytryptamine); 5-hydroxytryptamine receptors; 5-HT₇ receptors; tachycardia

Introduction

5-Hydroxytryptamine (5-HT) elicits complex changes in the cardiovascular system comprising bradycardia or tachycardia, hypotension or hypertension and vasodilatation or vasoconstriction (for reviews, see Saxena & Villalón, 1990; 1991). In most species, bradycardia induced by 5-HT is mediated by 5-HT₃ receptors, via the activation of the von Bezold Jarisch reflex; in marked contrast, 5-HT-induced tachycardia is notoriously species-dependent and is mediated, directly or indirectly, either by '5-HT₁-like' (cat), 5-HT₂ (rat, dog), 5-HT₃ (rabbit, dog) and 5-HT₄ (pig, human) receptors or by tyramine-like (guinea-pig) or unidentified mechanisms (Saxena, 1986; Saxena & Villalón, 1990; 1991).

The so called '5-HT₁-like' receptors mediating tachycardia in the cat are potentially stimulated by 5-carboxamidotryptamine (5-CT) and blocked by methiothepin and methysergide (Saxena *et al.*, 1985; Saxena, 1988). However, it is noteworthy that these receptors do not satisfactorily fulfill some classification requirements for the 5-HT₁ type, including insensitivity to

stimulating doses of RU 24969 and 8-OH-DPAT at typical 5-HT₁-like receptor-mediated responses (e.g. constriction of porcine carotid arteriovenous anastomoses; Saxena & Villalón, 1990), blockade by mesulergine (Saxena, 1988), an ergoline devoid of interactions with the 5-HT₁ receptor family (Hoyer *et al.*, 1994), and inconsistency with a negative coupling to adenylyl cyclase (Hoyer *et al.*, 1994). These cardiac receptors, consequently, are different from the typical 5-HT₁-like receptors mediating vasoconstriction, a response usually associated with a decrease in cyclic AMP (Sumner *et al.*, 1992), but are similar to those mediating direct relaxation of vascular and non-vascular smooth muscle, a response that involves an increase in cyclic AMP (for references see Saxena & Villalón, 1990; Martin, 1994).

While evidence is emerging that sumatriptan-sensitive 5-HT₁-like receptors mediating vasoconstriction resemble 5-HT_{1D/1D₂} receptors (De Vries *et al.*, 1996; Villalón *et al.*, 1996), presently known as a 5-HT_{1B/1D} receptors (Hartig *et al.*, 1996), the recently cloned 5-HT₇ receptor (e.g. Bard *et al.*, 1993; Ruat *et al.*, 1993) seems to be a suitable candidate for responses

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mediated by the atypical, sumatriptan-insensitive 5-HT₁-like receptors. Indeed, the cloned 5-HT₇ receptor is positively coupled to adenylyl cyclase and binding studies show that the cloned 5-HT₇ receptor displays high affinities for 5-CT, 5-HT, methiothepin, mesulergine and methysergide, but relatively low affinities for RU 24969, 8-OH-DPAT and sumatriptan (Hoyer *et al.*, 1994).

In the light of these findings, the present study was set out to investigate the operational characteristics of the 5-HT receptors mediating tachycardia in the spinal cat, with particular emphasis on verifying if these receptors display the pharmacological profile of the cloned 5-HT₇ receptor. Hence, the drugs employed included agonists and/or antagonists at 5-HT₁, 5-HT₂, 5-HT₃ and 5-HT₄ receptors, as well as some compounds with high affinity for the cloned 5-HT₅, 5-HT₆ and/or 5-HT₇ receptors (see Hoyer *et al.*, 1994).

Methods

General

Forty one cats of either sex, not selected for breed (2.5–4.0 kg) were fasted overnight and initially anaesthetized with sodium pentobarbitone (30 mg kg⁻¹, i.v.). Left femoral vein and artery were cannulated for, respectively, drug injections and the measurement of aortic blood pressure by a Statham pressure transducer (P23 ID). Heart rate was obtained by triggering a tachograph (Grass Instruments Co., Quincy, MA, USA; model 7P4) with the ECG signals. Blood pressure and heart rate were recorded simultaneously by a model 7D Grass polygraph (Grass Instrument Co., Quincy, MA, U.S.A.). The animals were then given an additional dose of pentobarbitone (10–15 mg kg⁻¹, i.v.) and intubated with an endotracheal tube for artificial ventilation with room air, by a Palmer ventilation pump (rate: 20 strokes min⁻¹; stroke volume: 13–16 ml kg⁻¹). Subsequently, both vagosympathetic trunks and the spinal cord (at the level of C₁–C₂) were sectioned in all animals, as previously described (Saxena *et al.*, 1985; Saxena, 1988).

Experimental protocols

After the cats had been in a stable haemodynamic condition for at least 60 min, baseline values of blood pressure and heart rate were determined. Then, the animals were divided into two groups.

The first group ($n=34$) received consecutive i.v. bolus injections, every 5 to 10 min, of 5-HT (3, 10 and 30 $\mu\text{g kg}^{-1}$), 5-methoxytryptamine (3, 10 and 30 $\mu\text{g kg}^{-1}$) and isoprenaline (0.01, 0.03 and 0.1 $\mu\text{g kg}^{-1}$) and the changes produced in blood pressure and heart rate were noted. At this point, the animals were divided into six subgroups. Four subgroups were treated, by use of a cumulative dose schedule, with either physiological saline (0.1 and 0.3 ml kg⁻¹; $n=5$), lisuride (30 and 100 $\mu\text{g kg}^{-1}$; $n=6$), ergotamine (100 and 300 $\mu\text{g kg}^{-1}$; $n=6$) or mesulergine (100, 300 and 1000 $\mu\text{g kg}^{-1}$; $n=6$). The fifth subgroup ($n=6$) was given clozapine (1000 and 3000 $\mu\text{g kg}^{-1}$) following a sequential (not cumulative) dosing because it produced an immediate short-lasting tachycardic response by itself. The sixth subgroup ($n=5$) was treated with GR127935 (500 $\mu\text{g kg}^{-1}$) and, subsequently, with tropisetron (3000 $\mu\text{g kg}^{-1}$). The responses to i.v. injections of 5-HT, 5-methoxytryptamine and isoprenaline, at the doses and sequence listed above, were elicited again 10 min after each dose of physiological saline or the above compounds.

The second group ($n=7$) received consecutive i.v. injections of 5-HT (3, 10 and 30 $\mu\text{g kg}^{-1}$) and isoprenaline (0.01, 0.03 and 0.1 $\mu\text{g kg}^{-1}$) and the changes in blood pressure and heart rate were noted. The animals were then divided into two subgroups. The first subgroup ($n=4$) was given, sequentially, i.v. injections of the 5-HT₁ receptor agonist, indorenate (300 and 1000 $\mu\text{g kg}^{-1}$ spaced by 5 min) followed by the 5-HT₄ receptor (partial) agonist, cisapride (300 and 1000 $\mu\text{g kg}^{-1}$

spaced by 5 min) and the second subgroup ($n=3$) was treated with sequential i.v. injections of the 5-HT₁ receptor agonist, sumatriptan (30, 100 and 300 $\mu\text{g kg}^{-1}$ spaced by 5 min). After each treatment and dose, the responses to 5-HT and isoprenaline were reanalysed.

Subsequently, each of the above 8 subgroups received, at the end of their corresponding protocol, i.v. injections of 5-CT at cumulative total dose levels of 0.03, 0.1, 0.3, 1, 3, 10 and 30 $\mu\text{g kg}^{-1}$ every 5–7 min. The changes in heart rate and blood pressure, produced after each dose of 5-CT, were noted. Lastly, in at least three cats of each of the above eight subgroups (see Results section), the highest dose used of saline or the corresponding drug was administered once again in an attempt to investigate which compounds could reverse the tachycardia elicited by the highest dose of 5-CT (30 $\mu\text{g kg}^{-1}$), as previously described for methysergide (Saxena *et al.*, 1985).

With the exception of 5-CT (see above), the dose-intervals between the different doses of agonists ranged between 1 and 10 min, as in each case we waited until the heart rate had returned to baseline values. For the antagonists, as well as for indorenate, cisapride and sumatriptan, a period of 10 min was allowed to elapse before the dose-response curves for the agonists were elicited again. The dosing with 5-HT, 5-methoxytryptamine, isoprenaline, sumatriptan, indorenate, cisapride and clozapine was sequential, whilst that for 5-CT and the rest of the antagonists was cumulative.

Data presentation and statistical analysis

All data in the text and figures are presented as mean \pm s.e.-mean and these were analysed by a computer programme (Saxena, 1985). The agonist-induced increases in heart rate just before and after a particular dose of saline or antagonist drug within one group of animals were compared by Student Newman-Keuls' test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations. Furthermore, the increases in heart rate by 5-HT and isoprenaline before and after sumatriptan (430 $\mu\text{g kg}^{-1}$), indorenate (1300 $\mu\text{g kg}^{-1}$) or cisapride (1300 $\mu\text{g kg}^{-1}$), given cumulatively, were compared by using paired Student's *t* test. Finally, the tachycardic responses to 5-CT in the different groups of animals were compared by using unpaired Student's *t* test. A *P* value of 0.05 or less (two-tailed) was considered statistically significant.

Drugs

Apart from the anaesthetic (sodium pentobarbitone), the drugs used in the present study (obtained from the sources indicated) were the following: 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Company, St. Louis, MO, U.S.A.); lisuride hydrogen maleate, isoprenaline hydrochloride, 5-methoxytryptamine hydrochloride and 5-carboxamidotryptamine maleate (Research Biochemicals Int., Natick, MA, U.S.A.); sumatriptan succinate (gift: Prof. P.P.A. Humphrey, Glaxo Institute of Applied Pharmacology, Cambridge, U.K.); N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1,-biphenyl]-4-carboxamide hydrochloride monohydrate (GR127935; gift: Dr M. Skingle, Glaxo Group Research Limited, Ware, Herts, U.K.); tropisetron (ICS 205-930), clozapine base, mesulergine hydrochloride and ergotamine tartrate (gift: Sandoz A.G., Basel, Switzerland); indorenate (5-methoxytryptamine- β -methylcarboxylate hydrochloride or TR3369; gift: Prof. Dr. E. Hong, CINVESTAV-IPN, Mexico City, Mexico); and cisapride (gift: Janssen Pharmaceutica, Beerse, Belgium). All compounds were dissolved in distilled water. When needed, 4% (w/v) ascorbic acid (clozapine) or 5% (v/v) dimethylsulphoxide (DMSO) (lisuride) was added; these vehicles had no effect on the haemodynamic variables or the agonist-induced responses. The doses mentioned in the text refer to the salts of substances, except in the case of all agonists and clozapine, where they refer to the free base.

Results

Initial blood pressure and heart rate changes by 5-HT receptor agonists

Baseline values of mean arterial blood pressure and heart rate in the forty one cats were, respectively, 84 ± 3 mmHg and 184 ± 35 beats min^{-1} . The changes induced in mean arterial blood pressure by the agonist drugs were: 5-HT (-2 ± 1 , $+3 \pm 4$ and $+15 \pm 5$ mmHg after 3, 10 and $30 \mu\text{g kg}^{-1}$, respectively; $n=41$), 5-methoxytryptamine (-1 ± 2 , $+7 \pm 4$ and $+23 \pm 4$ mmHg after 3, 10 and $30 \mu\text{g kg}^{-1}$, respectively; $n=34$), 5-CT (-1 ± 17 , -13 ± 5 , -17 ± 6 and -20 ± 7 mmHg after 0.03, 0.1, 0.3 and $1 \mu\text{g kg}^{-1}$, with 3, 10 and $30 \mu\text{g kg}^{-1}$ producing no subsequent change; $n=5$, i.e. saline-treated animals), sumatriptan (-12 ± 12 , $+17 \pm 9$, $+3 \pm 2$ mmHg after 30, 100 and $300 \mu\text{g kg}^{-1}$, respectively; $n=3$), indorenate (-5 ± 7 and -3 ± 5 mmHg after 300 and $1000 \mu\text{g kg}^{-1}$, respectively; $n=4$), cisapride (-1 ± 2 and -7 ± 3 mmHg after 300 and $1000 \mu\text{g kg}^{-1}$, respectively; $n=4$) and clozapine (-9 ± 5 and -18 ± 5 mmHg after 1000 and $3000 \mu\text{g kg}^{-1}$, respectively; $n=6$). It should be pointed out that these effects were not evaluated further because in spinal cats the baseline blood pressure is low and, consequently, the hypotensive responses produced by the above agonists were smaller and not strictly dose-dependent (see above).

The onset of the increases in heart rate induced by the agonists under study was immediate. Figure 1 shows that isoprenaline, 5-CT, 5-HT, 5-methoxytryptamine and clozapine caused dose-dependent increases in heart rate; in contrast, sumatriptan, indorenate and cisapride, at the doses tested, failed to increase feline heart rate. Isoprenaline was about 1.5 log units more potent than 5-CT, which was itself distinctly more potent (1.5 log units) than 5-HT and 5-methoxytryptamine; clozapine was the least potent. Thus, the apparent rank order of agonist potency was: isoprenaline $>>$ 5-CT $>>$ 5-HT $>$ 5-methoxytryptamine $>>$ clozapine.

The duration of tachycardic effects of agonist drugs (except 5-CT) was relatively short: isoprenaline (3.9 ± 0.2 , 4.8 ± 0.2 and 6.2 ± 0.2 min after 0.01, 0.03 and $0.1 \mu\text{g kg}^{-1}$, respectively), 5-HT (2.6 ± 0.2 , 4.6 ± 0.2 and 7.0 ± 0.3 min after 3, 10 and $30 \mu\text{g kg}^{-1}$, respectively), 5-methoxytryptamine (2.3 ± 0.2 , 3.5 ± 0.2 and 5.2 ± 0.2 min after 3, 10 and

$30 \mu\text{g kg}^{-1}$, respectively) or clozapine (2.1 ± 1.0 and 3.0 ± 1.0 after 1000 and $3000 \mu\text{g kg}^{-1}$, respectively). Furthermore, the 5-CT-induced tachycardia has been shown to last longer than 1 h (Saxena *et al.*, 1985; Saxena, 1988) and, therefore, as expected, no recovery was observed with 5-CT during the dosing intervals (5–7 min) or for at least 10 min after the last dose.

Effect of physiological saline and 5-HT receptor antagonists on the tachycardic responses induced by 5-HT, 5-methoxytryptamine and isoprenaline

The effects of physiological saline or lisuride on the tachycardic responses induced by 5-HT, 5-methoxytryptamine and isoprenaline are depicted in Figure 2. No evidence of tachyphylaxis was observed since the responses to these agonists, at the doses and time intervals (330 min) used in the present study, were reproducible and remained essentially unchanged in control animals receiving two doses (0.1 and 0.3 ml kg^{-1} , i.v.) of physiological saline (Figure 2a). In contrast, the administration of low doses (30 and $100 \mu\text{g kg}^{-1}$, i.v.) of lisuride potently and dose-dependently antagonized the tachycardic responses induced by 5-HT and 5-methoxytryptamine; this blockade was specific as lisuride did not alter isoprenaline-induced tachycardia (Figure 2b). Ergotamine (100 and $300 \mu\text{g kg}^{-1}$) and mesulergine (100, 300 and $1000 \mu\text{g kg}^{-1}$) also produced a dose-dependent blockade of the tachycardic responses to 5-HT and 5-methoxytryptamine (Figure 3a and b, respectively). However, the blockade produced by the high doses of ergotamine ($300 \mu\text{g kg}^{-1}$) and mesulergine (300 and $1000 \mu\text{g kg}^{-1}$) was not specific as they also attenuated the tachycardic effects of isoprenaline (Figure 3a and b). Moreover, the atypical antipsychotic drug, clozapine (1000 and $3000 \mu\text{g kg}^{-1}$), did not affect 5-HT-induced tachycardia, but significantly blocked, at its highest dose, the responses to 5-methoxytryptamine. However, these doses of clozapine also attenuated the tachycardic effects of isoprenaline (Figure 4a).

The apparent order of potency for blockade of both 5-HT- and 5-methoxytryptamine-induced tachycardic responses was ergotamine \geq lisuride $>$ mesulergine $>$ clozapine. Finally, as shown in Figure 4b, the tachycardic responses to 5-HT, 5-methoxytryptamine and isoprenaline were not significantly modified after administration of the selective 5-HT_{1B/1D} receptor antagonist, GR127935 (Skingle *et al.*, 1996) or by the subsequent administration of tropisetron which, at $3000 \mu\text{g kg}^{-1}$, is a 5-HT₃ and 5-HT₄ receptor antagonist (Villalón *et al.*, 1990b; 1991).

Except for an immediate and sustained increase in mean blood pressure by ergotamine (from 103 ± 9 to 134 ± 13 mmHg, after its highest dose; $P < 0.05$), the values of mean blood pressure and heart rate before and 10 min after the administration of physiological saline or the above 5-HT receptor antagonists were not significantly different (data not shown).

Tachycardic effects of 5-HT before and after administration of indorenate, cisapride or sumatriptan

Since the 5-HT receptor agonists, indorenate, cisapride and sumatriptan, failed to mimic 5-HT in increasing heart rate (see Figure 1), we decided to investigate these compounds ($1300 \mu\text{g kg}^{-1}$ each of indorenate and cisapride in one subgroup and $430 \mu\text{g kg}^{-1}$ of sumatriptan in another subgroup) as potential antagonists of the tachycardic responses to 5-HT. 5-HT (3, 10 and $30 \mu\text{g kg}^{-1}$) elicited a dose-dependent tachycardia (first subgroup: 5 ± 2 , 26 ± 10 and 46 ± 11 beats min^{-1} , $n=4$, respectively; second subgroup: 7 ± 2 , 17 ± 6 and 43 ± 7 beats min^{-1} , $n=3$, respectively). These responses as well as the tachycardia elicited by isoprenaline (0.01, 0.03 and $0.1 \mu\text{g kg}^{-1}$) remained unaffected after administration of the above compounds (data not shown).

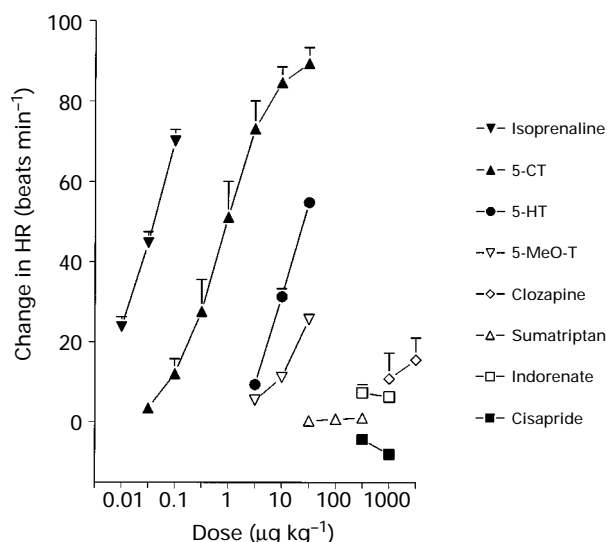


Figure 1 Comparative effects of i.v. bolus injections of isoprenaline ($n=41$), 5-carboxamidotryptamine (5-CT; $n=5$), 5-HT ($n=41$), 5-methoxytryptamine (5-MeO-T; $n=34$), clozapine ($n=6$), sumatriptan ($n=3$), indorenate ($n=4$) and cisapride ($n=4$) on heart rate (HR) in vagosympathectomized spinal cats. Vertical lines show s.e.mean.

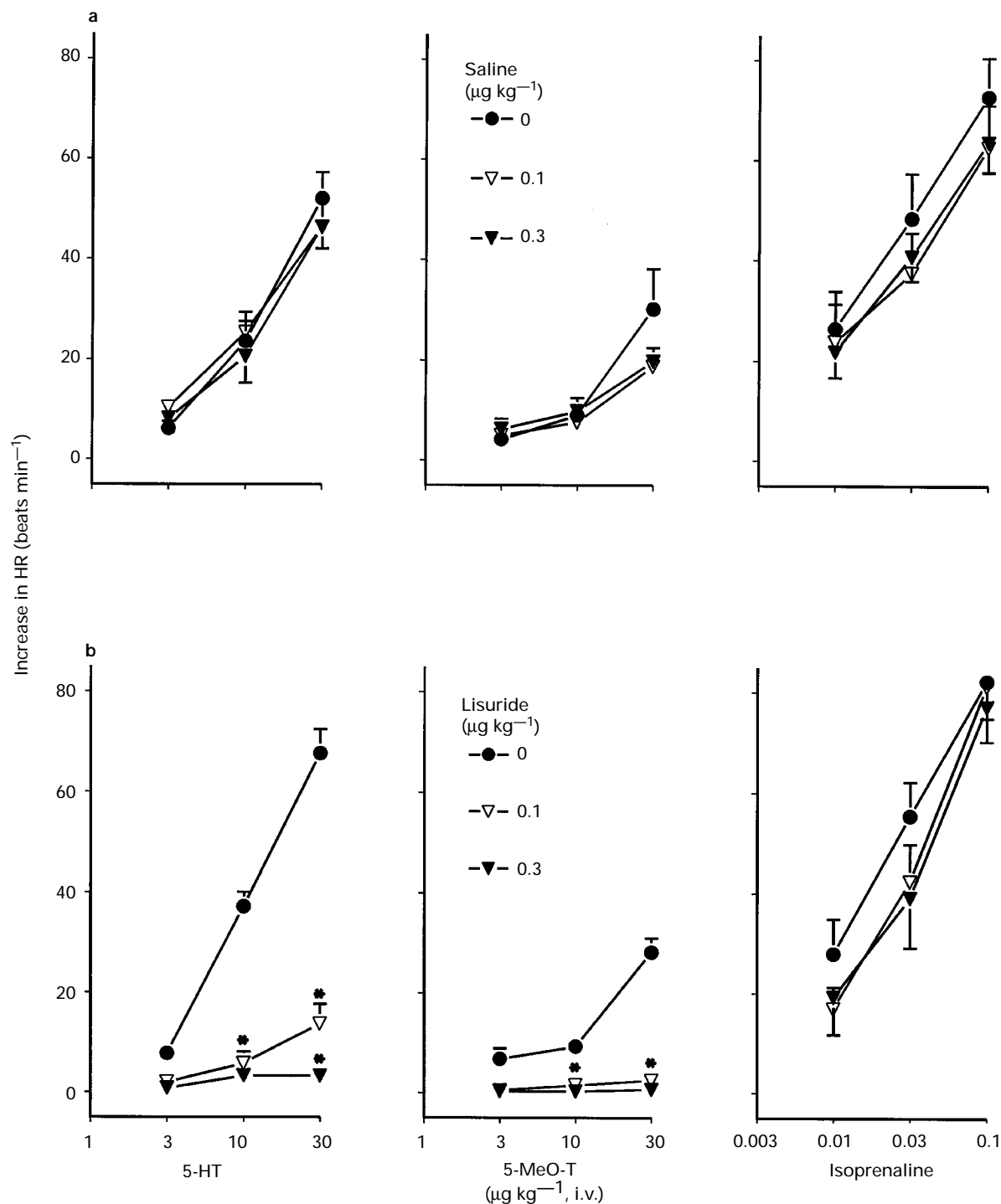


Figure 2 Effects of i.v. bolus injections of (a) physiological saline ($n=5$) or (b) lisuride ($n=6$) on tachycardic responses to 5-HT, 5-methoxytryptamine (5-MeO-T) and isoprenaline in vagosympathectomized spinal cats. HR, heart rate. * $P<0.05$ vs the corresponding control response. Vertical lines show s.e.mean.

Tachycardic effects of 5-CT after physiological saline or some compounds acting at 5-HT receptors

Figure 5 shows the comparative effects of different doses of 5-CT obtained in cats pretreated with cumulative doses of either saline (0.3 ml kg^{-1}), lisuride (100 µg kg^{-1}), ergotamine (300 µg kg^{-1}), clozapine (4000 µg kg^{-1}), mesulergine (1000 µg kg^{-1}), GR127935 (500 µg kg^{-1}) plus tropisetron (3000 µg kg^{-1}), indorenate (1300 µg kg^{-1}) plus cisapride (1300 µg kg^{-1}) or sumatriptan (430 µg kg^{-1}). Only lisuride, mesulergine, ergotamine and clozapine produced a significant blockade of the responses to 5-CT, with an apparent order of antagonist potency of ergotamine \geq lisuride $>$ mesulergine $>$

clozapine; in addition, indorenate + cisapride slightly, though significantly, attenuated the response to the highest dose of 5-CT (30 µg kg^{-1}). The remaining compounds produced no significant effect (Figure 5).

After the tachycardic response to 30 µg kg^{-1} 5-CT (given cumulatively) had been stable for at least 10 min, a subsequent administration of the same dose of saline, GR127935 + tropisetron, indorenate + cisapride or sumatriptan ($n=3$ each) in the corresponding subgroups produced no further change in heart rate (data not shown). In contrast, 5-CT-induced tachycardia was significantly ($P<0.05$) decreased from the prevailing values after i.v. administration of the same dose of lisuride ($-17 \pm 1\%$; $n=4$), ergotamine

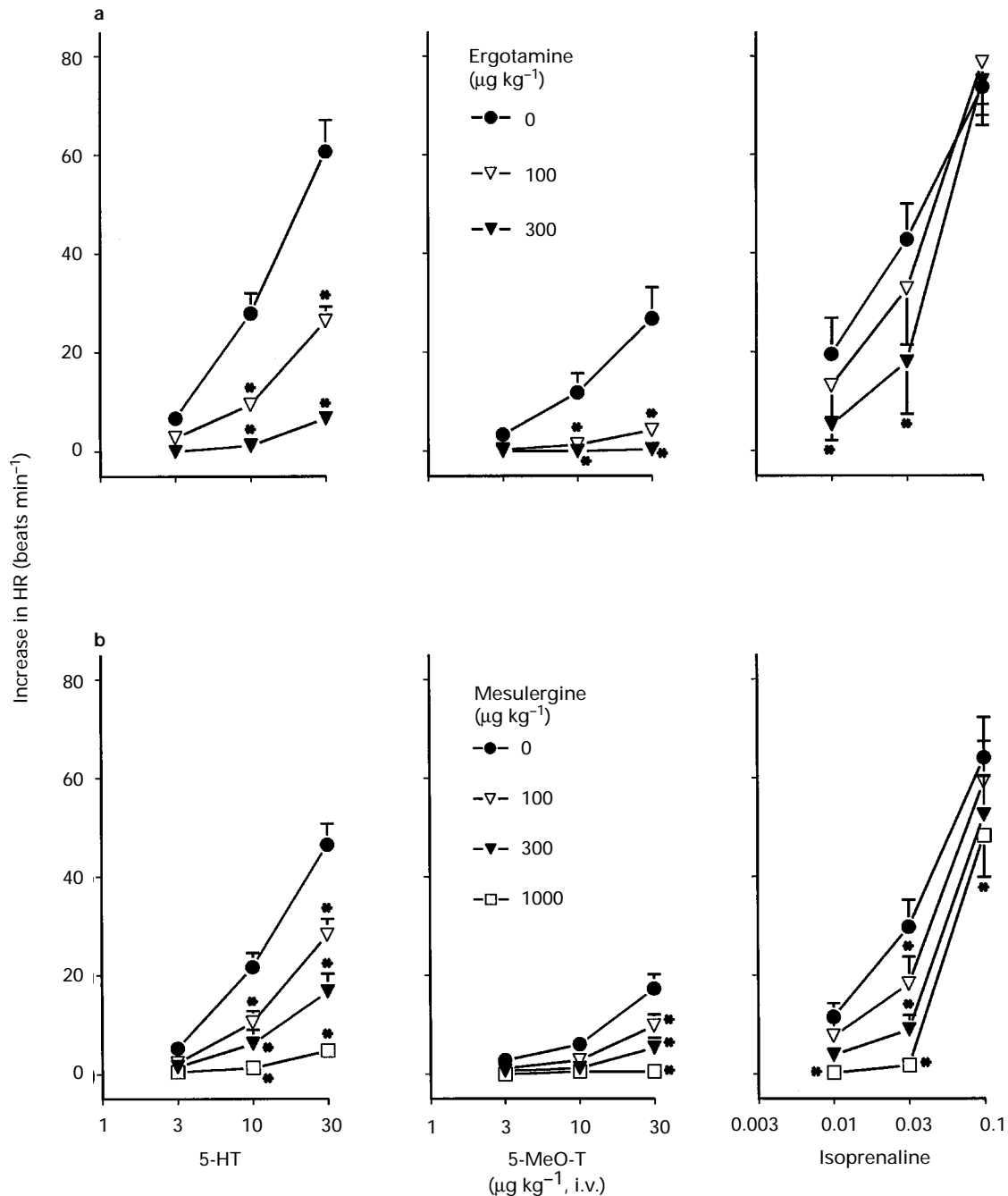


Figure 3 Effects of i.v. bolus injections of (a) ergotamine ($n=6$) or (b) mesulergine ($n=6$) on tachycardic responses to 5-HT, 5-methoxytryptamine (5-MeO-T) and isoprenaline in vagosympathectomized spinal cats. HR, heart rate. * $P<0.05$ vs the corresponding control response.

($-8 \pm 2\%$; $n=4$), clozapine ($-22 \pm 4\%$; $n=3$) or mesulergine ($-25 \pm 2\%$; $n=6$).

Discussion

In previous studies, Saxena *et al.* (1985) had proposed that the tachycardia induced by 5-HT in the cat, being potently mimicked by 5-CT and blocked by methysergide, is mediated by '5-HT₁-like' receptors on the basis of the resistance to blocking doses of antagonists at 5-HT₂ (ketanserin, ritanserin) and 5-HT₃ (MDL 72222) receptors. However, it is noteworthy that, in contrast to its constrictor effects via vascular 5-HT₁-like receptors (see Saxena & Villalón, 1990), methysergide did not show any agonist activity at the feline cardiac '5-HT₁-like'

receptors. Subsequently, Saxena (1988) showed that these atypical cardiac receptors were unrelated to the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} (now 5-HT_{2C}) and 5-HT_{1D} subtypes based on: (i) the blockade, in decreasing order of potency, by methiothepin, mesulergine and metergoline (an order which does not match with their corresponding affinities for these receptors; Hoyer, 1988); (ii) the low agonist potency of the 5-HT_{1A} receptor agonist, 8-OH-DPAT; and (iii) the lack of activity of 5-HT_{1A/1B} receptor agonist, RU 24969.

Certainly, the approach established by the IUPHAR 5-HT receptor classification scheme (Hoyer *et al.*, 1994) lead us to conclude that the appellation '5-HT₁-like' is no longer regarded as appropriate for the 5-HT receptors mediating tachycardia in the cat. The operational approach of the present study strengthens this view, as the feline cardiac 5-HT

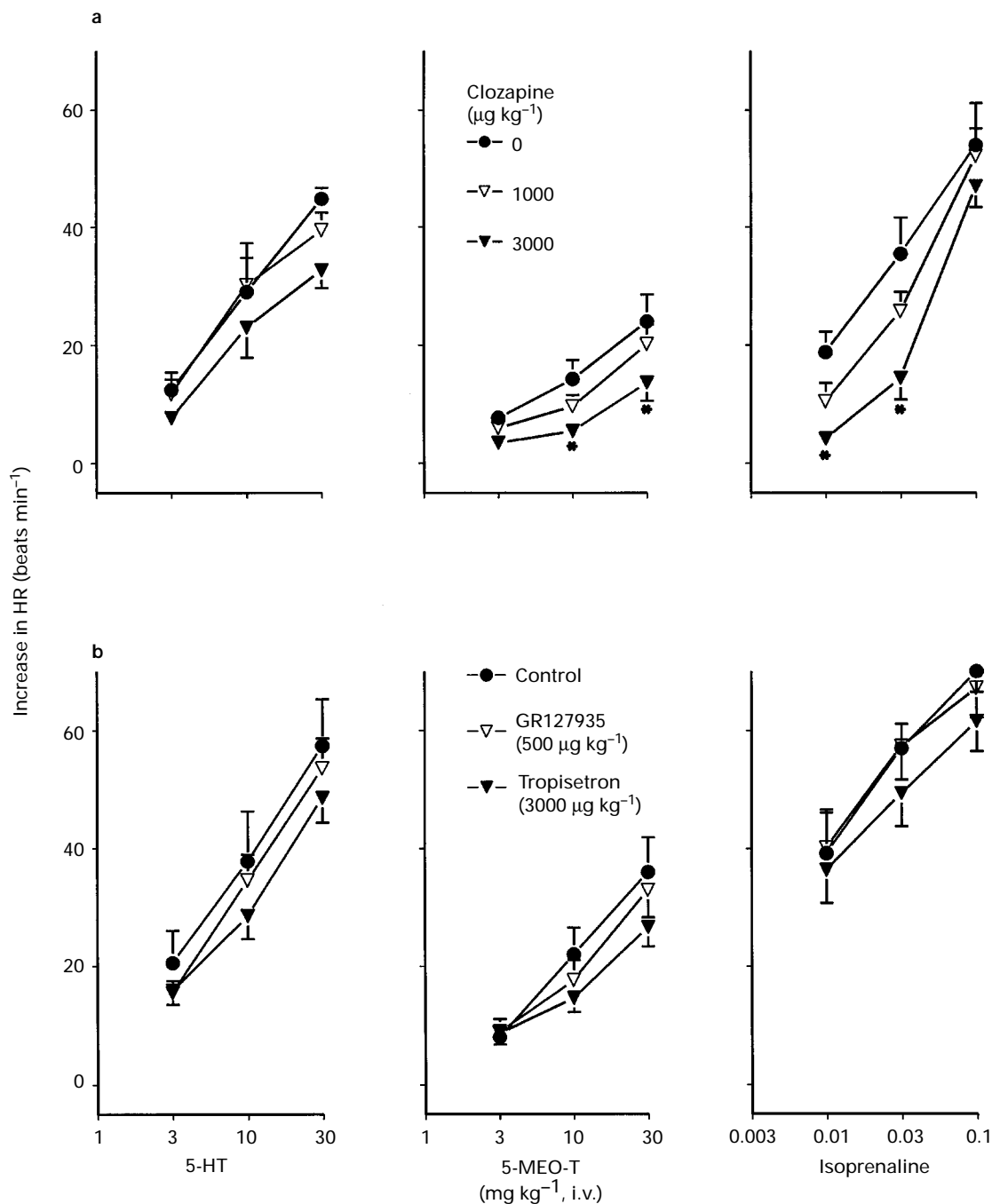


Figure 4 Effects of bolus injections of (a) clozapine ($n=6$) or (b) GR127935 ($n=5$) followed by tropisetron ($n=5$) on tachycardic responses to 5-HT, 5-methoxytryptamine (5-MeO-T) and isoprenaline in vagosympathectomized spinal cats. HR, heart rate. * $P<0.05$ vs the corresponding control response.

receptors were not stimulated by sumatriptan or indorenate (both agonists at typical 5-HT₁-like receptors mediating vasoconstrictor responses; Saxena & Villalón, 1990; Villalón *et al.*, 1990a; Hoyer *et al.*, 1994) and could be blocked by a series of drugs showing high affinity for cloned 5-HT₇ receptors. Thus, these cardiac receptors resemble those mediating smooth muscle relaxation and elevation of adenosine 3':5'-cyclic monophosphate (cyclic AMP) in neonatal porcine vena cava (Trevithick *et al.*, 1986; Sumner *et al.*, 1989), hypotension in anaesthetized rats (Saxena & Lawang, 1985; De Vries *et al.*, 1997) and cats (Connor *et al.*, 1986) and vasodilatation in the canine external carotid bed (Villalón *et al.*, 1997). Apart from the implications discussed below, these data suggest that the 5-HT receptors mediating tachycardia in the cat may represent a functional correlate of the recombinant 5-HT₇ receptor.

Agonist action of some tryptamine derivatives on the feline heart 5-HT receptor

Admittedly, the rank order of agonist potency (5-CT \gg 5-HT $>$ 5-methoxytryptamine) observed in our study is similar to that found for the prejunctional sympatho-inhibitory 5-HT_{1B/1D} receptors mediating canine external carotid vasodilatation (Villalón & Terrón, 1994). Nevertheless, at these prejunctional 5-HT_{1B/1D} receptors, indorenate and sumatriptan behaved as agonists (Villalón *et al.*, 1993; Villalón & Terrón, 1994), as previously shown for other 5-HT_{1B/1D} receptors (Saxena & Villalón, 1990; Villalón *et al.*, 1990a; 1996; De Vries *et al.*, 1996). Contrasting with this 5-HT_{1B/1D} receptor operational profile, our results in the cat heart clearly showed that sumatriptan and indorenate did not behave as agonists (or antagonists). The above rank order of agonist potency, therefore, closely resem-

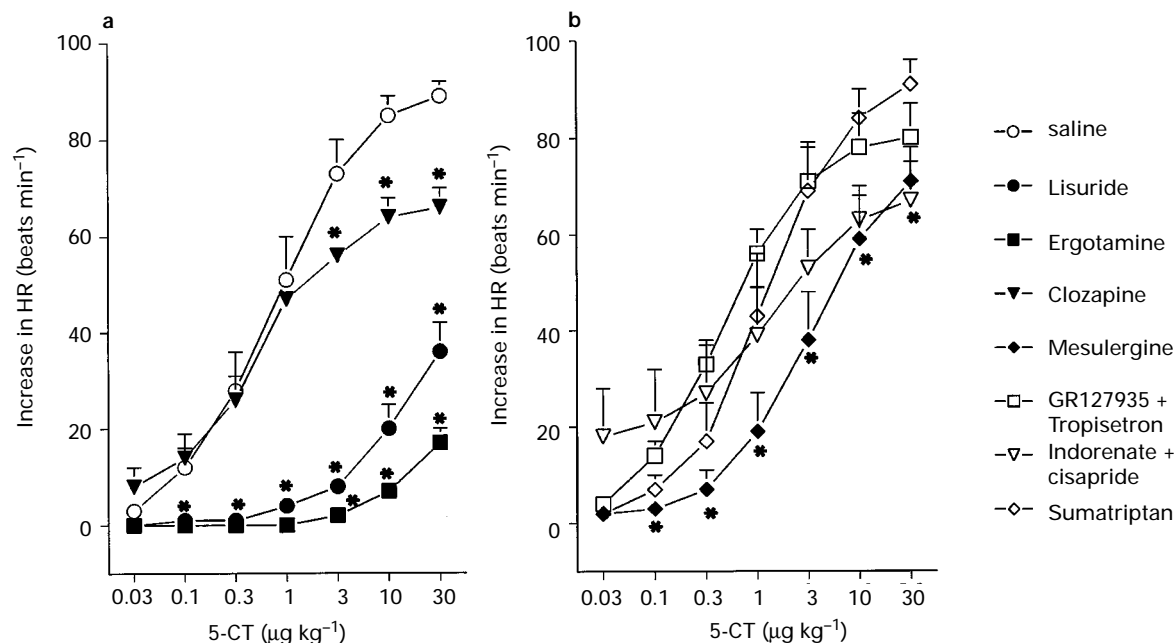


Figure 5 Effects of a number of drugs acting on 5-HT receptors on tachycardic responses to 5-carboxamidotryptamine (5-CT) in vagosympathectomized spinal cats. The cumulative doses of various drugs used were: (a) lisuride 100 $\mu\text{g kg}^{-1}$ ($n=6$), ergotamine 300 $\mu\text{g kg}^{-1}$ ($n=6$), clozapine 4000 $\mu\text{g kg}^{-1}$ ($n=6$); (b) mesulergine 1000 $\mu\text{g kg}^{-1}$ ($n=6$), GR127935 500 $\mu\text{g kg}^{-1}$ + tropisetron 3000 $\mu\text{g kg}^{-1}$ ($n=5$), indorenate 1300 $\mu\text{g kg}^{-1}$ + cisapride 1300 $\mu\text{g kg}^{-1}$ ($n=4$) and sumatriptan 430 $\mu\text{g kg}^{-1}$ ($n=3$). HR, heart rate. * $P < 0.05$ vs the corresponding control response in animals treated with saline (0.3 ml kg^{-1} , $n=5$), indicated for the sake of clarity only in (a). Vertical lines show s.e.mean.

bles the pharmacological properties of the cloned 5-HT₇ receptor subtype; pK_D values obtained for recombinant 5-HT₇ receptors for 5-CT, 5-HT, 5-methoxytryptamine and sumatriptan are 9.5, 8.7, 8.8 and 6.2, respectively (Hoyer *et al.*, 1994).

Do feline heart 5-HT receptors correlate with any subtype of the 5-HT₁ receptor family?

Although the above rank order of agonist potency with indorenate and sumatriptan already excludes the involvement of 5-HT_{1A}, 5-HT_{1B/1D} and sumatriptan-sensitive 5-HT₁-like receptors, it is well known that the 5-HT₁ receptor family includes two additional subtypes, namely, the cloned 5-HT_{1E} and 5-HT_{1F} receptors (Hoyer *et al.*, 1994). However, the involvement of these subtypes in the present study is unlikely because (i) 5-CT and 5-methoxytryptamine, which display very low affinity for the recombinant 5-HT_{1E} and 5-HT_{1F} receptors (Adham *et al.*, 1993; Hoyer *et al.*, 1994), potently mimicked 5-HT in eliciting tachycardia; (ii) the tachycardic responses to 5-CT, 5-HT and 5-methoxytryptamine were antagonized by mesulergine and clozapine, which do not interact with any subtype of the 5-HT₁ receptor family (Hoyer *et al.*, 1994); (iii) these tachycardic responses were not antagonized by GR127935 at doses that are high enough to block 5-HT_{1B/1D} receptors (De Vries *et al.*, 1996; Skingle *et al.*, 1996; Villalón *et al.*, 1996) and (iv) the 5-HT₁ receptor family is, by definition, negatively coupled to adenylyl cyclase (Hoyer *et al.*, 1994), a signal transduction system usually associated with vasoconstriction and bradycardia, not tachycardia (Rand *et al.*, 1987; Saxena & Villalón, 1990, 1991; Sumner *et al.*, 1992).

Lack of resemblance of the feline heart 5-HT receptor with either 5-HT₂, 5-HT₃, 5-HT₄, 5-HT₅ or 5-HT₆ receptors

The 5-HT receptors mediating tachycardia in the cat also seem to differ from the 5-HT₂, 5-HT₃, 5-HT₄ and 5-HT₆ receptor types on the basis of: (i) the high potency of 5-CT relative to 5-HT (present results), an order which is reversed for the aforementioned types (Hoyer *et al.*, 1994); (ii) insensitivity to antagonism by high doses of 5-HT₂ (ketanserin, ritanserin or

ciproheptadine) and 5-HT₃ (MDL 72222) receptor antagonists (Saxena *et al.*, 1985); (iii) the inactivity (as agonist or antagonist) of cisapride (present study), a benzamide with partial agonist properties at the 5-HT₄ receptors mediating tachycardia in the pig (Villalón *et al.*, 1991); (iv) resistance to antagonism by tropisetron (ICS 205-930) at doses (3000 $\mu\text{g kg}^{-1}$) that block 5-HT₃ and 5-HT₄ receptors (Villalón *et al.*, 1990b; 1991) and (v) the potent blockade by mesulergine, a compound showing an almost 300 fold selectivity for the cloned 5-HT₇ receptor ($pK_D = 8.2$) over the cloned 5-HT₆ receptor ($pK_D = 5.8$) (Hoyer *et al.*, 1994). Furthermore, based on the above findings, the ability of 5-methoxytryptamine to induce tachycardia in the cat, which is, in its own right, an additional criterion to exclude the participation of 5-HT₃ receptors (Hoyer *et al.*, 1994), cannot be attributed to its agonist properties at 5-HT₄ receptors (Villalón *et al.*, 1991).

Interestingly, 5-CT (pK_D : 9.5 and 7.4, respectively), methiothepin (pK_D : 7.0 and 7.8, respectively) and ergotamine (pK_D : 8.4 and 8.5, respectively) display a relatively high affinity for the recombinant 5-HT_{5A} and 5-HT_{5B} receptors (Hoyer *et al.*, 1994). This, therefore, raises the question whether the cloned 5-HT_{5A/5B} receptors are related to the 5-HT receptors mediating tachycardia in the cat. However, this does not seem very likely because: (i) ergotamine failed to increase heart rate in the cat (present results) and (ii) methiothepin, which has a ten fold lower affinity than ergotamine for 5-HT_{5A/5B} receptors (see above), was more potent (not less potent as may be expected from their affinities for 5-HT_{5A/5B} receptors) than ergotamine in blocking 5-HT-induced feline tachycardia (Saxena, 1988; present results). Moreover, the antagonism by mesulergine (present results) and metergoline (Saxena, 1988), both with pK_D values of < 6.0 at cloned 5-HT_{5A/5B} receptors (Hoyer *et al.*, 1994), also points against these receptors mediating the 5-HT-induced tachycardia in the cat.

Resemblance of the feline heart 5-HT receptor to putative 5-HT₇ receptors

Since the involvement of 5-HT₁-5-HT₆ receptors seems improbable, the possibility has finally to be discussed that the

5-HT receptors mediating tachycardia in the cat resemble the cloned 5-HT₇ receptor. Indeed, the rank order of agonist potency of 5-CT >> 5-HT > 5-methoxytryptamine >> clozapine to produce tachycardia in the cat, with sumatriptan and indorenate inactive (present results) parallels that obtained with the mouse (Lovenberg *et al.*, 1993; Plassat *et al.*, 1993), rat (Ruata *et al.*, 1993; Shen *et al.*, 1993) and human (Bard *et al.*, 1993) cloned 5-HT₇ receptors (5-CT > 5-methoxytryptamine ≥ 5-HT). Significantly, the above rank order of agonist potency is practically identical to that found in other preparations where the relaxant effects of 5-HT have been ascribed to stimulation of muscletropic 5-HT₇-like receptors; some of these preparations include the rabbit femoral vein (Martin & Wilson, 1995), the *Cynomolgus* monkey isolated jugular vein (Leung *et al.*, 1996), the guinea-pig ileum (Carter *et al.*, 1995) and the canine coronary (Terrón, 1996) and external carotid (Villalón *et al.*, 1997) arteries.

This suggestion is further strengthened when considering that the tachycardia induced by 5-HT in the cat was blocked in previous studies (Saxena, 1988) by methiothepin (pK_D: 9.0), methysergide (pK_D: 7.9) and metergoline (pK_D: 8.7), compounds that display high affinities for cloned 5-HT₇ receptors (Hoyer *et al.*, 1994). On this basis, in the present study, we deliberately selected ergotamine, lisuride, clozapine and mesulergine as potential antagonists because they show either high affinity (the former three) or relative selectivity (the latter) for the cloned 5-HT₇ receptor (see Hoyer *et al.*, 1994). Accordingly, all of these compounds blocked the 5-CT-, 5-HT- and 5-methoxytryptamine-induced tachycardic responses, though this blockade appeared to be highly specific only for lisuride. Indeed, ergotamine, lisuride, mesulergine or clozapine have been shown to antagonize smooth muscle relaxant responses mediated by other functional 5-HT₇ receptors (e.g.

Carter *et al.*, 1995; Martin & Wilson, 1995; Terrón, 1996; Villalón *et al.*, 1997).

Admittedly, there are no selective agonists and antagonists at cloned 5-HT₇ receptors available so far. Thus, although we recognize that the antagonists blocking 5-HT-induced feline tachycardia display varying degrees of affinity for receptors other than 5-HT, including α (α₁ and α₂)-adrenoceptors, H₂ histamine and muscarinic receptors (Leysen, 1985), the agonists used in the present study do not interact with these receptors; most significantly, these and other mechanisms in 5-HT-induced feline tachycardia (including stimulation of cardiac β-adrenoceptors and the indirect release of catecholamines) have already been excluded (Saxena *et al.*, 1985).

In conclusion, it is suggested that the tachycardic effect of 5-HT in the cat is mediated by a receptor similar to the cloned 5-HT₇ subtype. Since this cardiac 5-HT receptor represents another functional correlate of the 5-HT₇ gene product, the change of the appellation 5-HT₇ for 5-HT₇ is favoured. Although species variations should not be dismissed (Saxena & Villalón, 1991), the present findings in the cat seem to be the first to show a tachycardic effect being mediated by the 5-HT₇ receptor. This *in vivo* experimental model, which is not complicated by the presence of other 5-HT receptors, can be utilized to characterize and develop, including the possibility of studying oral absorption, new drugs with potential agonist and antagonist properties at functional 5-HT₇ receptors.

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References

- ADHAM, N., KAO, H.-T., SCHECHTER, L.E., BARD, J., OLSEN, M., URQUHART, D., DURKIN, M., HARTIG, P.R., WEINSHANK, R.L. & BRANCHEK, T.A. (1993). Cloning of another human serotonin receptor (5-HT_{1F}): a fifth 5-HT₁ receptor subtype coupled to the inhibition of adenylate cyclase. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 408–412.
- BARD, J.A., ZGOMBICK, J., ADHAM, N., VAYSSE, P., BRANCHEK, T.A. & WEINSHANK, R.L. (1993). Cloning of a novel human serotonin receptor (5-HT₇) positively linked to adenylate cyclase. *J. Biol. Chem.*, **268**, 23422–23426.
- CARTER, D., CHAMPNEY, M., HWANG, B. & EGLIN, R.M. (1995). Characterization of a postjunctional 5-HT receptor mediating relaxation of guinea-pig isolated ileum. *Eur. J. Pharmacol.*, **280**, 243–250.
- CONNOR, H.E., FENIUK, W., HUMPHREY, P.P.A. & PERREN, M.J. (1986). 5-Carboxamidotryptamine is a selective agonist at 5-hydroxytryptamine receptors mediating vasodilatation and tachycardia in anaesthetized cats. *Br. J. Pharmacol.*, **87**, 417–426.
- DE VRIES, P., HEILIGERS, J.P.C., VILLALÓN, C.M. & SAXENA, P.R. (1996). Blockade of porcine carotid vascular responses to sumatriptan by GR127935, a selective 5-HT_{1D} receptor antagonist. *Br. J. Pharmacol.*, **118**, 85–92.
- DE VRIES, P., VILLALÓN, C.M., HEILIGERS, J.P.C. & SAXENA, P.R. (1997). Nature of 5-HT₁-like receptors mediating depressor responses in vagosympathectomized rats; close resemblance to the cloned 5-HT₇ receptor. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, (in press).
- HARTIG, P.R., HOYER, D., HUMPHREY, P.P.A. & MARTIN, G.R. (1996). Alignment of receptor nomenclature with the human genome: classification of 5-HT_{1B} and 5-HT_{1D} receptor subtypes. *Trends Pharmacol. Sci.*, **17**, 103–105.
- HOYER, D. (1988). Functional correlates of serotonin 5-HT₁ recognition sites. *J. Receptor Res.*, **8**, 59–81.
- HOYER, D., CLARKE, D.E., FOZARD, J.R., HARTIG, P.R., MARTIN, G.R., MYLECHARANE, E.J., SAXENA, P.R. & HUMPHREY, P.P.A. (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.*, **46**, 157–203.
- LEUNG, E., WALSH, L.K.M., PULIDO-RIOS, M.T. & EGLIN, R.M. (1996). Characterization of putative 5-HT₇ receptors mediating direct relaxation in *Cynomolgus* monkey isolated jugular vein. *Br. J. Pharmacol.*, **117**, 926–930.
- LEYSSEN, J.E. (1985). Serotonin binding sites. In *Serotonin and the Cardiovascular System*. ed. Vanhoutte, P.M. pp. 43–62. New York: Raven Press.
- LOVENBERG, T.W., BARON, B.M., DE LECEA, L., MILLER, J.D., PROSSER, R.A., REA, M.A., FOYE, P.E., RACKE, M., SLONE, A.L., SIEGEL, B.W., DANIELSON, P.E., SUTCLIFFE, J.G. & ERLANDER, M.G. (1993). A novel adenylyl cyclase-activating serotonin receptor (5-HT₇) implicated in the regulation of mammalian circadian rhythms. *Neuron*, **11**, 449–458.
- MARTIN, G.R. (1994). Vascular receptors for 5-hydroxytryptamine: distribution, function and classification. *Pharmacol. Ther.*, **62**, 283–324.
- MARTIN, G.R. & WILSON, R.J. (1995). Operational characteristics of a 5-HT receptor mediating direct vascular relaxation: identity with the 5-HT₇ receptor? *Br. J. Pharmacol.*, **114**, 383P.
- PLASSAT, J.L., AMLAIKY, N. & HEN, R. (1993). Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase. *Mol. Pharmacol.*, **44**, 229–236.
- RAND, M.J., MAJEWSKI, H., WONG-DUSTING, H., STORY, D.F., LOIACONO, R.E. & ZIOGAS, J. (1987). Modulation of neuroeffector transmission. *J. Cardiovasc. Pharmacol.*, **10** (Suppl. 12), S33–S44.
- RUAT, M., TRAFFORT, E., LEURS, R., TARDIVEL-LACOMBE, J., DIAZ, J., ARRANG, J.-M. & SCHWARTZ, J.-C. (1993). Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT₇) activating cAMP formation. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 8547–8551.
- SAXENA, P.R. (1985). An interactive computer program for data management and parametric and non-parametric statistical analysis. *Br. J. Pharmacol.*, **86**, 818P.
- SAXENA, P.R. (1986). Nature of the 5-hydroxytryptamine receptors in mammalian heart. *Prog. Pharmacol.*, **6**, 173–185.

- SAXENA, P.R. (1988). Further characterization of 5-hydroxytryptamine₁-like receptors mediating tachycardia in the cat: no apparent relationship to known subtypes of the 5-hydroxytryptamine₁ binding site. *Drug Develop. Res.*, **13**, 245–258.
- SAXENA, P.R. & LAWANG, A. (1985). A comparison of cardiovascular and smooth muscle effects of 5-hydroxytryptamine and 5-carboxamidotryptamine, a selective agonist at 5-HT₁ receptors. *Arch. Int. Pharmacodyn.*, **277**, 235–252.
- SAXENA, P.R., MYLECHARANE, E.J. & HEILIGERS, J. (1985). Analysis of the heart rate effects of 5-hydroxytryptamine in the cat; mediation of tachycardia by 5-HT₁-like receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **330**, 121–129.
- SAXENA, P.R. & VILLALÓN, C.M. (1990). Cardiovascular effects of serotonin agonists and antagonists. *J. Cardiovasc. Pharmacol.*, **15** (Suppl. 7), S17–S34.
- SAXENA, P.R. & VILLALÓN, C.M. (1991). 5-Hydroxytryptamine, a chameleon in the heart. *Trends Pharmacol. Sci.*, **12**, 223–227.
- SHEN, Y., MONSMA, F.J., JR, METCALF, M.A., JOSE, P.A., HAMBLIN, M.W. & SIBLEY, D.R. (1993). Molecular cloning and expression of a 5-hydroxytryptamine₇ serotonin receptor subtype. *J. Biol. Chem.*, **268**, 18200–18204.
- SKINGLE, M., BEATTIE, D.T., SCOPES, D.I.C., STARKEY, S.J., CONNOR, H.E., FENIUK, W. & TYERS, M.B. (1996). GR 127935: a potent and selective 5-HT_{1D} receptor antagonist. *Behav. Brain Res.*, **73**, 157–161.
- SUMNER, M.J., FENIUK, W. & HUMPHREY, P.P.A. (1989). Further characterization of the 5-HT receptor mediating vascular relaxation and elevation of cyclic AMP in porcine isolated vena cava. *Br. J. Pharmacol.*, **97**, 292–300.
- SUMNER, M.J., FENIUK, W., McCORMICK, J.D. & HUMPHREY, P.P.A. (1992). Studies on the mechanism of 5-HT₁ receptor-induced smooth muscle contraction in dog saphenous vein. *Br. J. Pharmacol.*, **105**, 603–608.
- TERRÓN, J.A. (1996). The relaxant 5-HT receptor in the dog coronary artery smooth muscle: pharmacological resemblance to the cloned 5-HT₇ receptor subtype. *Br. J. Pharmacol.*, **118**, 1421–1428.
- TREVETHICK, M.A., FENIUK, W. & HUMPHREY, P.P.A. (1986). 5-Carboxamidotryptamine: a potent agonist mediating relaxation and elevation of cyclic AMP in isolated neonatal porcine vena cava. *Life Sci.*, **38**, 1521–1528.
- VILLALÓN, C.M., BOM, A.H., HEILIGERS, J.P.C., DEN BOER, M.O. & SAXENA, P.R. (1990a). Porcine arteriovenous anastomotic constriction by indorenate is unrelated to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} receptor subtype. *Eur. J. Pharmacol.*, **190**, 167–176.
- VILLALÓN, C.M., CENTURIÓN, D., LUJÁN-ESTRADA, M., TERRÓN, J.A. & SÁNCHEZ-LÓPEZ, A. (1997). Mediation of 5-HT-induced external carotid vasodilatation in GR 127935-pretreated vagosympathectomized dogs by the putative 5-HT₇ receptor. *Br. J. Pharmacol.*, **120**, 1319–1327.
- VILLALÓN, C.M., DEN BOER, M.O., HEILIGERS, J.P.C. & SAXENA, P.R. (1990b). Mediation of 5-hydroxytryptamine-induced tachycardia in the pig by the putative 5-HT₄ receptor. *Br. J. Pharmacol.*, **100**, 665–667.
- VILLALÓN, C.M., DEN BOER, M.O., HEILIGERS, J.P.C. & SAXENA, P.R. (1991). Further characterization, using tryptamine and benzamide derivatives, of the 5-HT₄ receptor mediating tachycardia in the pig. *Br. J. Pharmacol.*, **102**, 107–112.
- VILLALÓN, C.M., SÁNCHEZ-LÓPEZ, A. & CENTURIÓN, D. (1996). Operational characteristics of the 5-HT₁-like receptors mediating external carotid vasoconstriction in vagosympathectomized dogs: close resemblance to the 5-HT_{1D} receptor subtype. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **354**, 550–556.
- VILLALÓN, C.M. & TERRÓN, J.A. (1994). The 5-HT₁-like receptor mediating the increase in canine external carotid blood flow: close resemblance to the 5-HT_{1D} subtype. *Br. J. Pharmacol.*, **113**, 13–20.
- VILLALÓN, C.M., TERRÓN, J.A. & HONG, E. (1993). Further characterization of the 5-HT₁-like receptors mediating the increase in external carotid blood flow in the dog. *Drug Develop. Res.*, **29**, 271–281.

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