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Characterization of putative 5-HT_7 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-HT_1-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 $\mu g\ kg^{-1}$), 5-HT (3, 10 and 30 $\mu g\ kg^{-1}$) and 5-methoxytryptamine (3, 10 and 30 $\mu g\ kg^{-1}$) as well as the atypical antipsychotic drug, clozapine (1000 and 3000 $\mu g\ kg^{-1}$) 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 $\mu g\ kg^{-1}$), ergotamine (100 and 300 $\mu g\ kg^{-1}$) or mesulergine (100, 300 and 1000 $\mu g\ kg^{-1}$); the highest doses of these antagonists used also blocked the tachycardic effects of 5-CT. Clozapine (1000 and 3000 $\mu g\ kg^{-1}$) 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 $\mu g\ kg^{-1}$) and mesulergine (300 and 1000 $\mu g\ kg^{-1}$) 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 $\mu g\ kg^{-1}$) or the 5-$HT_{3/4}$ receptor antagonist, tropisetron (3000 $\mu g\ kg^{-1}$).

4 Intravenous injections of the 5-HT_1 receptor agonists, sumatriptan (30, 100 and 300 $\mu g\ kg^{-1}$) and indorenate (300 and 1000 $\mu g\ kg^{-1}$) or the 5-HT_4 receptor (partial) agonist cisapride (300 and 1000 $\mu g\ kg^{-1}$) 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-ht_7 receptor, the present results indicate that the 5-HT receptor mediating tachycardia in the cat is operationally similar to other putative 5-HT_7 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-ht_7 gene product, the 5-HT_7 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-HT_7 receptors.

Keywords: Heart rate; 5-HT (5-hydroxytryptamine); 5-hydroxytryptamine receptors; 5-HT_7 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-HT_3 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-HT_1-like' (cat), 5-HT_2 (rat, dog), 5-HT_3 (rabbit, dog) and 5-HT_4 (pig, human) receptors or by tyramine-like (guinea-pig) or unidentified mechanisms (Saxena, 1986; Saxena & Villalón, 1990; 1991).

The so called '5-HT_1-like' receptors mediating tachycardia in the cat are potently 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-HT_1 type, including insensitivity to stimulating doses of RU 24969 and 8-OH-DPAT at typical 5-HT_1-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-HT_1 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-HT_1-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-HT_1-like receptors mediating vasoconstriction resemble 5-$HT_{1D\beta/1D\alpha}$ 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-ht_7 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-HT$_1$-like receptors. Indeed, the cloned 5-ht$_7$ receptor is positively coupled to adenylyl cyclase and binding studies show that the cloned 5-ht$_7$ 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-ht$_7$ receptor. Hence, the drugs employed included agonists and/or antagonists at 5-HT$_1$, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptors, as well as some compounds with high affinity for the cloned 5-ht$_5$, 5-ht$_6$ and/or 5-ht$_7$ 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 C_1-C_2) 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 μg kg^{-1}), 5-methoxytryptamine (3, 10 and 30 μg kg^{-1}) and isoprenaline (0.01, 0.03 and 0.1 μg 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 μg kg^{-1}; $n=6$), ergotamine (100 and 300 μg kg^{-1}; $n=6$) or mesulergine (100, 300 and 1000 μg kg^{-1}; $n=6$). The fifth subgroup ($n=6$) was given clozapine (1000 and 3000 μg 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 μg kg^{-1}) and, subsequently, with tropisetron (3000 μg 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 μg kg^{-1}) and isoprenaline (0.01, 0.03 and 0.1 μg 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-HT$_1$ receptor agonist, indorenate (300 and 1000 μg kg^{-1} spaced by 5 min) followed by the 5-HT$_4$ receptor (partial) agonist, cisapride (300 and 1000 μg kg^{-1} spaced by 5 min) and the second subgroup ($n=3$) was treated with sequential i.v. injections of the 5-HT$_1$ receptor agonist, sumatriptan (30, 100 and 300 μg 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 μg 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 μg 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 ± 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 μg kg^{-1}), indorenate (1300 μg kg^{-1}) or cisapride (1300 μg 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±4 and +15±5 mmHg after 3, 10 and 30 μg kg^{-1}, respectively; n=41), 5-methoxytryptamine (−1±2, +7±4 and +23±4 mmHg after 3, 10 and 30 μg 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 μg kg^{-1}, with 3, 10 and 30 μg kg^{-1} producing no subsequent change; n=5, i.e. saline-treated animals), sumatriptan (−12±12, +17±9, +3±2 mmHg after 30, 100 and 300 μg kg^{-1}, respectively; n=3), indorenate (−5±7 and −3±5 mmHg after 300 and 1000 μg kg^{-1}, respectively; n=4), cisapride (−1±2 and −7±3 mmHg after 300 and 1000 μg kg^{-1}, respectively; n=4) and clozapine (−9±5 and −18±5 mmHg after 1000 and 3000 μg 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 μg kg^{-1}, respectively), 5-HT (2.6±0.2, 4.6±0.2 and 7.0±0.3 min after 3, 10 and 30 μg kg^{-1}, respectively), 5-methoxytryptamine (2.3±0.2, 3.5±0.2 and 5.2±0.2 min after 3, 10 and 30 μg kg^{-1}, respectively) or clozapine (2.1±1.0 and 3.0±1.0 after 1000 and 3000 μg 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.

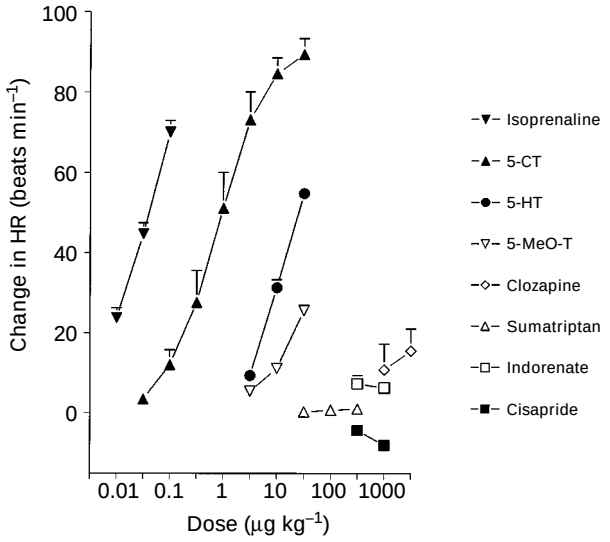

Figure 1 Comparative effects of i.v. bolus injections of isoprenaline (n=41), 5-carboxamidotryptamine (5-CT; n=5), 5-HT (n=41), 5-methoxytryptamine (5-MeO-T; n=34), clozapine (n=6), sumatriptan (n=3), indorenate (n=4) and cisapride (n=4) on heart rate (HR) in vagosympathectomized spinal cats. Vertical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

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 μg 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 μg kg^{-1}) and mesulergine (100, 300 and 1000 μg 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 μg kg^{-1}) and mesulergine (300 and 1000 μg 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 μg 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 ⩾ 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 μg kg^{-1}, is a 5-HT$_3$ and 5-HT$_4$ 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 μg kg^{-1} each of indorenate and cisapride in one subgroup and 430 μg kg^{-1} of sumatriptan in another subgroup) as potential antagonists of the tachycardic responses to 5-HT. 5-HT (3, 10 and 30 μg 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 μg kg^{-1}) remained unaffected after administration of the above compounds (data not shown).

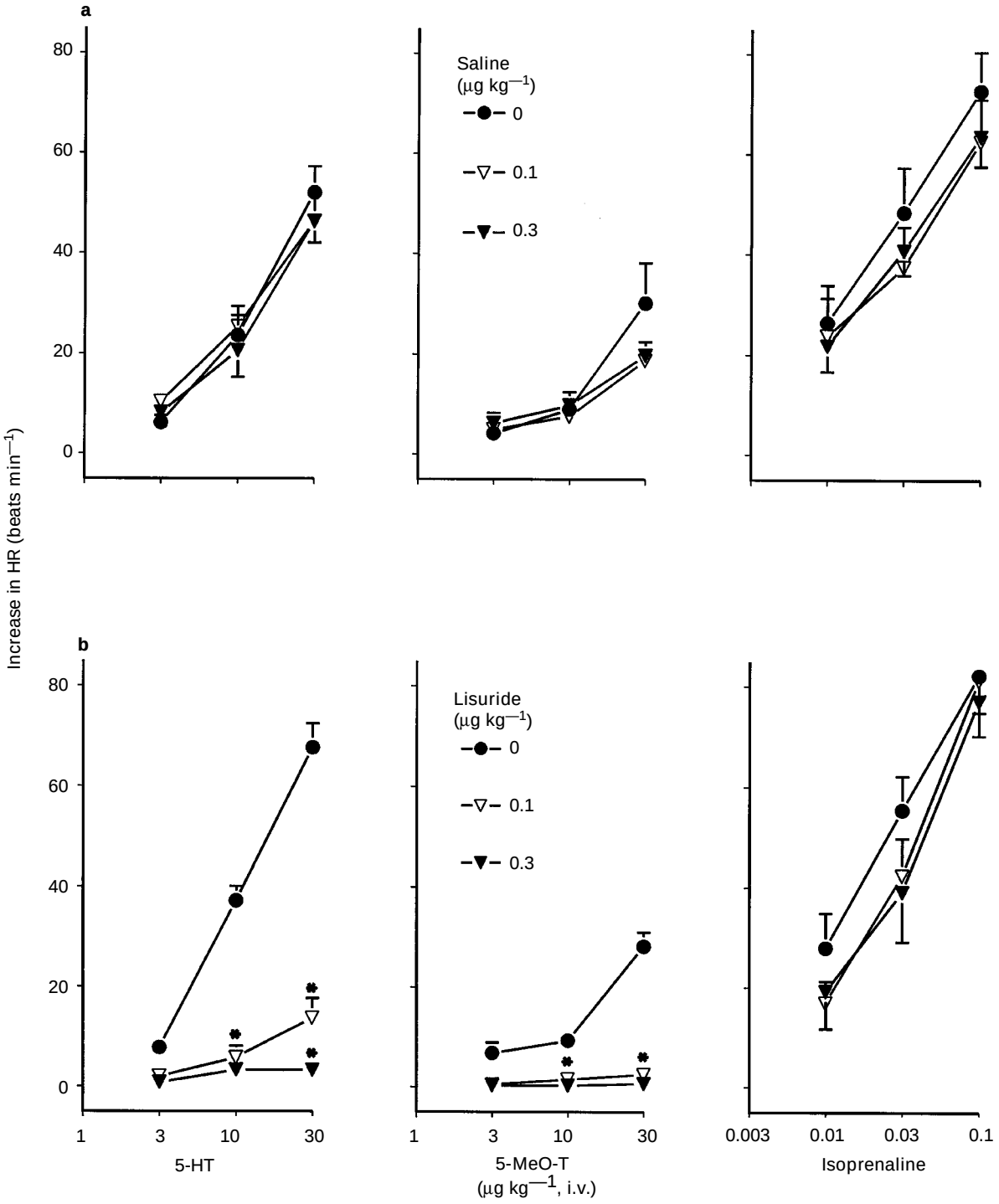

Figure 2 Effects of i.v. bolus injections of (a) physiological saline ($n=5$) or (b) lisuride ($n=6$) on tachycardic responses to 5-HT, 5-methoxytryptamine (5-MeO-T) and isoprenaline in vagosympathectomized spinal cats. HR, heart rate. *$P<0.05$ vs the corresponding control response. Vertical lines show 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 ⩾ 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 ($-17\pm1\%$; $n=4$), ergotamine

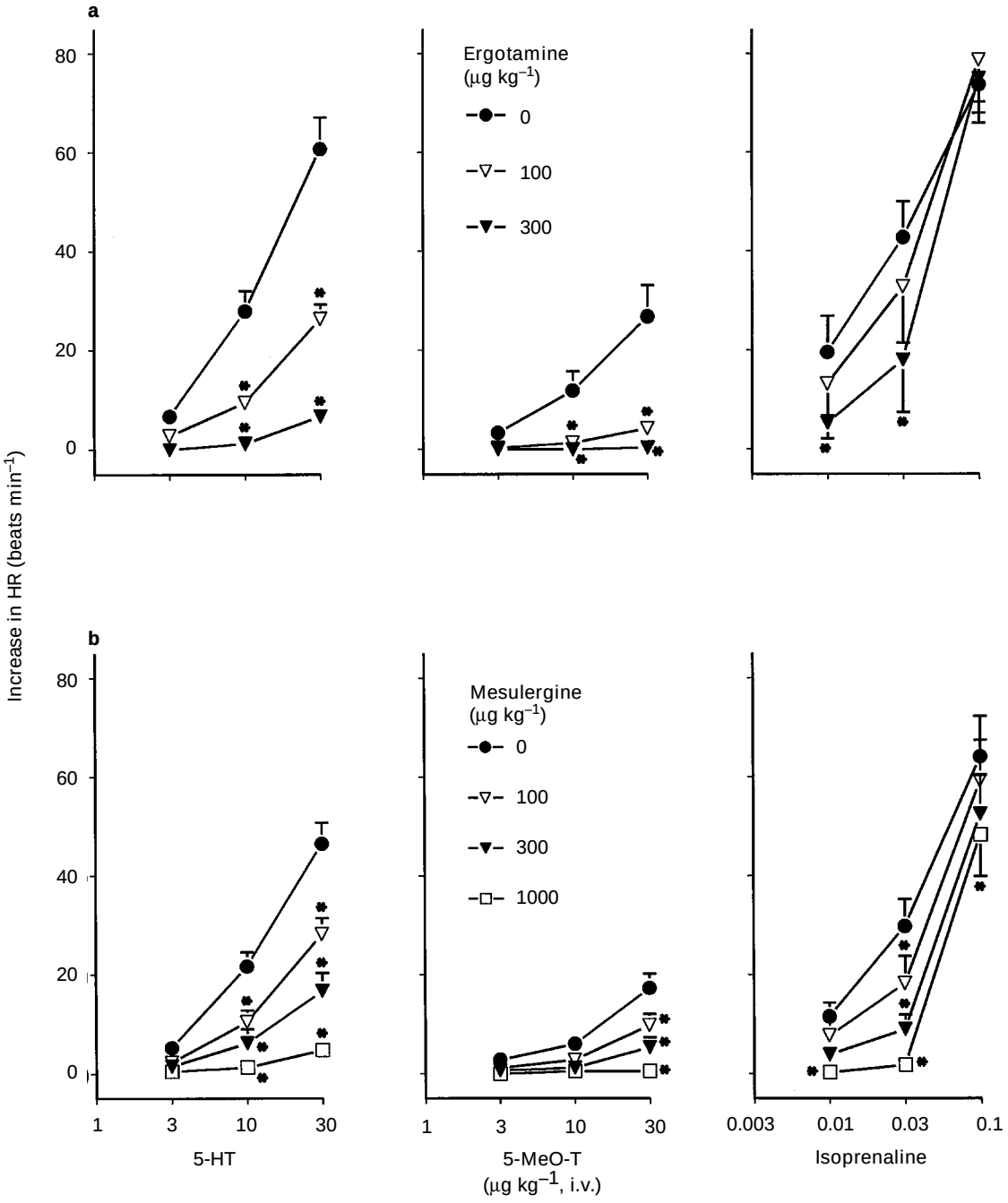

Figure 3 Effects of i.v. bolus injections of (a) ergotamine ($n=6$) or (b) mesulergine ($n=6$) on tachycardic responses to 5-HT, 5-methoxytryptamine (5-MeO-T) and isoprenaline in vagosympathectomized spinal cats. HR, heart rate. $^*P<0.05$ vs the corresponding control response.

($-8\pm2\%$; $n=4$), clozapine ($-22\pm4\%$; $n=3$) or mesulergine ($-25\pm2\%$; $n=6$).

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-HT$_1$-like' receptors on the basis of the resistance to blocking doses of antagonists at 5-HT$_2$ (ketanserin, ritanserin) and 5-HT$_3$ (MDL 72222) receptors. However, it is noteworthy that, in contrast to its constrictor effects via vascular 5-HT$_1$-like receptors (see Saxena & Villalón, 1990), methysergide did not show any agonist activity at the feline cardiac '5-HT$_1$-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-HT$_1$-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

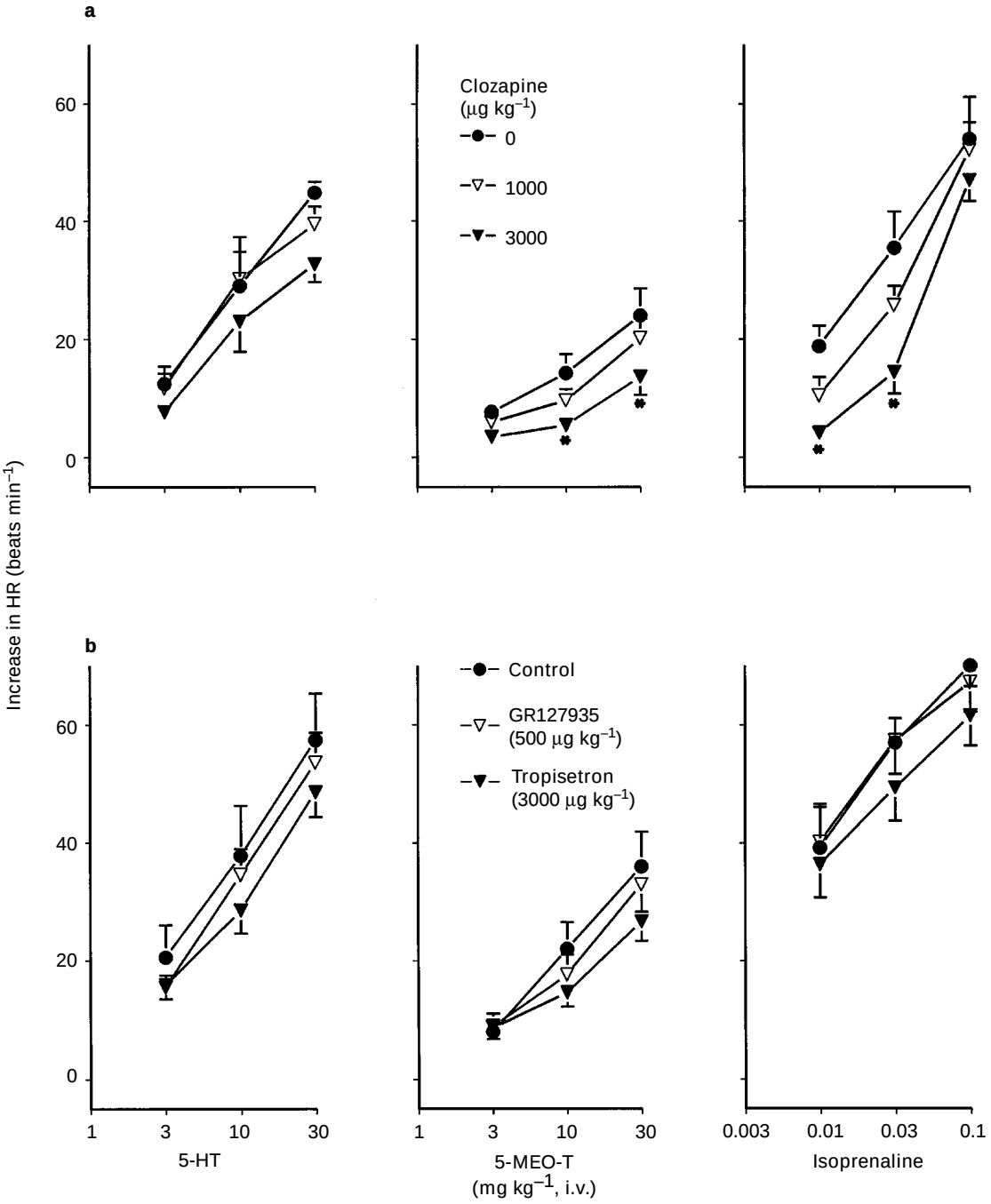

Figure 4 Effects of bolus injections of (a) clozapine ($n=6$) or (b) GR127935 ($n=5$) followed by tropisetron ($n=5$) on tachycardic responses to 5-HT, 5-methoxytryptamine (5-MeO-T) and isoprenaline in vagosympathectomized spinal cats. HR, heart rate. *$P<0.05$ vs the corresponding control response.

receptors were not stimulated by sumatriptan or indorenate (both agonists at typical 5-HT$_1$-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-ht$_7$ 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-ht$_7$ receptor.

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

Admittedly, the rank order of agonist potency (5-CT > > 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-

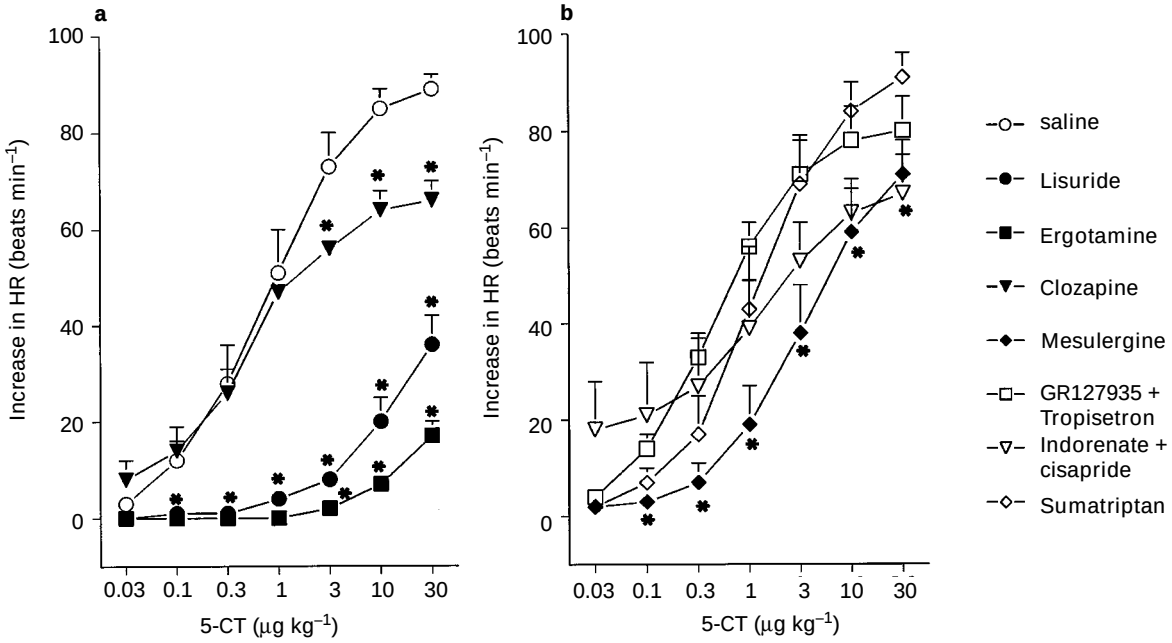

Figure 5 Effects of a number of drugs acting on 5-HT receptors on tachycardic responses to 5-carboxamidotryptamine (5-CT) in vagosympathectomized spinal cats. The cumulative doses of various drugs used were: (a) lisuride 100 μg kg^{-1} ($n=6$), ergotamine 300 μg kg^{-1} ($n=6$), clozapine 4000 μg kg^{-1} ($n=6$); (b) mesulergine 1000 μg kg^{-1} ($n=6$), GR127935 500 μg kg^{-1} + tropisetron 3000 μg kg^{-1} ($n=5$), indorenate 1300 μg kg^{-1} + cisapride 1300 μg kg^{-1} ($n=4$) and sumatriptan 430 μg 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-ht$_7$ receptor subtype; pK_D values obtained for recombinant 5-ht$_7$ 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-HT$_1$ 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-HT$_1$-like receptors, it is well known that the 5-HT$_1$ 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-HT$_1$ 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-HT$_1$ 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-HT$_2$, 5-HT$_3$, 5-HT$_4$, 5-ht$_5$ or 5-ht$_6$ receptors

The 5-HT receptors mediating tachycardia in the cat also seem to differ from the 5-HT$_2$, 5-HT$_3$, 5-HT$_4$ and 5-ht$_6$ 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-HT$_2$ (ketanserin, ritanserin or cyproheptadine) and 5-HT$_3$ (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-HT$_4$ receptors mediating tachycardia in the pig (Villalón *et al*., 1991); (iv) resistance to antagonism by tropisetron (ICS 205-930) at doses (3000 μg kg^{-1}) that block 5-HT$_3$ and 5-HT$_4$ 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-ht$_7$ receptor (p$K_D=8.2$) over the cloned 5-ht$_6$ receptor (p$K_D=5.8$) (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-HT$_3$ receptors (Hoyer *et al*., 1994), cannot be attributed to its agonist properties at 5-HT$_4$ 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-HT$_7$ receptors

Since the involvement of 5-HT$_1$-5-ht$_6$ receptors seems improbable, the possibility has finally to be discussed that the

5-HT receptors mediating tachycardia in the cat resemble the cloned 5-ht$_7$ 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 (Ruat *et al*., 1993; Shen *et al*., 1993) and human (Bard *et al*., 1993) cloned 5-ht$_7$ receptors (5-CT > 5-methoxytryptamine $\geqslant$ 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 musculotropic 5-HT$_7$-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-ht$_7$ 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-ht$_7$ 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-HT$_7$ 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-ht$_7$ 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 (α_1 and α_2)-adrenoceptors, H_2 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-ht$_7$ subtype. Since this cardiac 5-HT receptor represents another functional correlate of the 5-ht$_7$ gene product, the change of the appellation 5-ht$_7$ for 5-HT$_7$ 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-HT$_7$ 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-HT$_7$ receptors.

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