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Effect of a series of 5-HT_4 receptor agonists and antagonists on steroid secretion by the adrenal gland in vitro

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Abstract

We have previously shown that serotonin (5-hydroxytryptamine, 5-HT) stimulates corticosterone and aldosterone secretion from perifused frog adrenal gland in vitro through activation of 5-HT_4 receptors. In the present study, we have used this model to investigate the effect of newly discovered 5-HT_4 receptor agonists and antagonists on corticosteroid secretion. Serotonin, the benzamide derivatives (*R,S*)-zacopride ((*R,S*)-4-amino-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-5-chloro-2-methoxybenzamide, HCl) and its enantiomers, the azabicycloalkyl benzimidazolone derivatives BIMU 1 (endo-*N*-(8-methyl-8-azabicyclo-[3.2.1]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1*H*-benzimidazole-1-carboxamide, HCl) and BIMU 8 (endo-*N*-(8-methyl-8-azabicyclo-[3.2.1]oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1*H*-benzimidazole-1-carboxamide, HCl) were all capable of enhancing corticosterone and aldosterone secretion in a dose-dependent manner. Serotonin was the most potent stimulator of steroidogenesis ($EC_{50} = 1.5 \times 10^{-7}$ M) while the potency of the benzamide and the benzimidazolone derivatives was approximately 10 times lower. The rank order of efficacy of the different 5-HT_4 receptor agonists was: (*S*)-zacopride > BIMU 8 = (*R,S*)-zacopride > BIMU 1 = (*R*)-zacopride = 5-HT. The stimulatory effects of 5-HT and the benzimidazolone derivatives on corticosteroid secretion were not additive, suggesting that they activated the same receptor. The indoleamine derivatives ICS 205930 ((3α-tropanyl)-1*H*-indole-3-carboxylic acid, ester) and GR 113808 ([1-[2-(methylsulphonylamino)ethyl]-4-piperidinyl]methyl 1-methyl-1*H*-indole-3-carboxylate, maleate), and the benzimidazolone derivative DAU 6285 (endo-8-methyl-8-azabicyclo-[3.2.1]oct-3-yl)-2,3-dihydro-6-methoxy-2-oxo-1*H*-benzimidazole-1-carboxylate, HCl), all induced a dose-dependent inhibition of (*R,S*)-zacopride-induced stimulation of corticosteroid secretion. The affinity of GR 113808 ($pK_i = 10.34$) was higher than that of DAU 6285 and ICS 205930 ($pK_i = 7.84$ and 6.20, respectively). Together, these data indicate that the pharmacological profile of the 5-HT_4 receptor in the frog adrenal gland is very similar to those recently characterized in the brain and peripheral organs of mammals. It thus appears that the frog adrenal gland is a valuable model for the investigation of the biochemical characteristics and mode of action of novel 5-HT_4 receptor ligands.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HT_4 receptor; Benzamide derivative; Azabicycloalkyl benzimidazolone derivative; GR 113808; Adrenal gland

1. Introduction

5-HT_4 receptors were originally discovered in mouse embryonic colliculi (Dumuis et al., 1988) and guinea pig hippocampus neurons (Bockaert et al., 1990). The occurrence of 5-HT_4 receptors has since been documented in a number of brain regions (Grossman et al., 1993; Monferini et al., 1993; Waeber et al., 1993) and in various peripheral organs including the oesophagus (Baxter et al., 1991), the ileum (Craig et al., 1990; Eglen et al., 1990) and the atrium (Kaumann et al., 1991). We have recently shown that the stimulatory effect of 5-HT on corticosteroid secretion by frog (Idres et al., 1991) and human adrenal gland (Lefebvre et al., 1992, 1993) is mediated through activation of 5-HT_4 receptors. Since the frog adrenal gland is composed of a single population of steroid-producing cells (Contesse et al., 1993), it appears a valuable model in which to investigate the action of various 5-HT_4 receptor agonists and antagonists on a physiological response which can be easily quantified, i.e. the secretion of corticoste-

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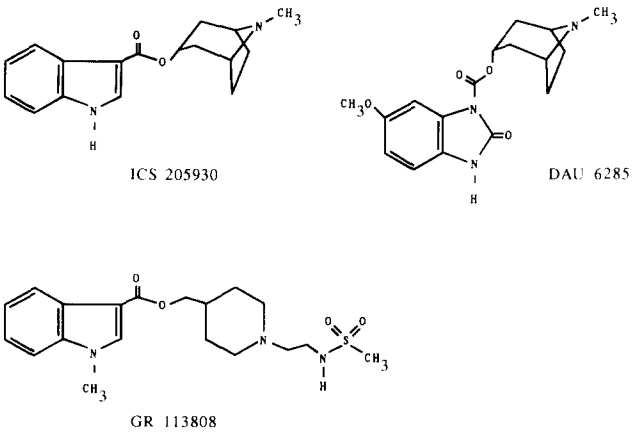

Fig. 1. Structural formulas of three antagonists at the 5-HT_4 receptor.

roids. The use of a perifusion system for monitoring corticosteroid release provides the additional advantage of that secretory dynamics can be followed (Esneu et al., 1994).

The 5-HT_4 receptor agonists available so far belong to three chemical families, i.e. (i) 2-methoxy-4-amino-5-chloro substituted benzamide derivatives such as cisapride, zacopride, BRL 24924, metoclopramide (Dumuis et al., 1989; Bockaert et al., 1990; Elswood et al., 1991; Idres et al., 1991; Villalon et al., 1991), (ii) azabicycloalkyl benzimidazolone derivatives including BIMU 1 and BIMU 8 (Dumuis et al., 1991; Rizzi et al., 1992; Baxter and Clarke, 1992; Monferini et al., 1993), and (iii) indoleamine derivative agonists (e.g. 5-carboxamidotryptamine, 5-methoxytryptamine) which lack selectivity and can activate multiple 5-HT receptor types (Bockaert et al., 1992).

Until recently, studies on 5-HT_4 receptors have been hampered by the lack of selective antagonists. The indoleamine derivative ICS 205930 (Fig. 1) has long been the only available receptor antagonist, although this compound exhibits higher affinity for 5-HT_3 than 5-HT_4 sites (Craig et al., 1990). Characterization of 5-HT_4 receptors should be largely facilitated by the recent disclosure of selective 5-HT_4 receptor antagonists including the benzimidazolone derivative DAU 6285 and the indoleamine derivative GR 113808 (Fig. 1). The antagonistic activity of DAU 6285 at the 5-HT_4 receptor has been demonstrated both in vitro (Tonini et al., 1991; Dumuis et al., 1992; Schiavone et al., 1992) and in vivo (Van Meel et al., 1993). Concurrently, GR 113808 has been shown to act as a highly potent 5-HT_4 receptor antagonist in guinea-pig colon and rat oesophagus preparations (Grossman et al., 1993).

The aim of the present study was to investigate the effects of newly available 5-HT_4 receptor agonists and antagonists on corticosteroid secretion from the frog adrenal gland in vitro.

2. Materials and methods

2.1. Animals and tissue preparation

Adult male frogs (*Rana ridibunda*; body weight 40–50 g) were used as tissue donors. The animals were killed by decapitation, the kidneys were rapidly removed, and the adrenal (interrenal) tissue was dissected free of kidney tissue. For each experiment, six pairs of glands were sliced and preincubated in Ringer's solution (15 mM Hepes, 112 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 15 mM $NaHCO_3$) supplemented with 2 mg glucose/ml and 0.3 mg bovine serum albumin (BSA)/ml. The solution was gassed with a 95% O_2-5% CO_2 mixture, and the pH was adjusted at 7.4. The adrenal slices were rinsed twice with 5 ml fresh Ringer and then transferred into the perifusion chamber.

2.2. Perifusion system technique

The details of the perifusion system have been described previously (Leboulenger et al., 1978). Briefly, the adrenal slices were transferred into a siliconized glass column between several beds of Bio-Gel P2 (Bio-Rad Laboratories, Richmond, CA, USA). The perifusion columns were supplied with gassed Ringer solution at a constant flow rate (200 μl/min) and temperature (24°C). The glands were allowed to stabilize for 2 h before any test substance was added. Secretagogues were dissolved in Ringer's solution and infused into the columns at the same flow rate as Ringer alone by means of a multichannel peristaltic pump (Desaga, Heidelberg, Germany). Fractions of the effluent perifusate were collected every 5 min and the tubes were immediately frozen until corticosteroid assays.

2.3. Corticosteroid radioimmunoassays

Corticosterone and aldosterone concentrations were determined by radioimmunoassays (RIA), without prior extraction, in 100–200 μl aliquots from each fraction of effluent perifusate. The characteristics of both RIAs have been reported previously (Leboulenger et al., 1982). The assays were sensitive enough to detect 20 pg of corticosterone and 5 pg of aldosterone. For both assays, the intra- and inter-assay coefficients of variation were lower than 4% and 10%, respectively. The specificity of the antibodies was evaluated by determining their cross-reactivities with 34 different steroids and related compounds. None of the 5-HT_4 receptor agonists and antagonists tested interfered in the corticosteroid assays at the concentrations used for the experiments.

2.4. Calculations

Each perifusion pattern was established as the mean profile of corticosteroid secretion (±S.E.M.) calcu-

lated over at least three independent experiments. The levels of corticosterone and aldosterone released were expressed as percentages of the basal values, calculated as the mean of eight consecutive fractions (40 min) just preceding the infusion of the secretagogues. EC_{50} refers to the agonist concentrations yielding 50% of the maximal corticosteroid response determined directly on each dose-response curve; pEC_{50} is the negative logarithm of EC_{50}. The K_i values of antagonists were determined from the concentration of the drug reversing 50% of the stimulation of steroid release induced by the agonist, using the Cheng-Prusoff equation (1973); pK_i is the negative logarithm of K_i. Statistical differences were analyzed by using the Student's t-test.

2.5. Reagents

5-Hydroxytryptamine (5-HT, serotonin) and Hepes (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid) were purchased from Sigma Chemical Compagny (St. Louis, MO, USA). BIMU 1 (endo-*N*-(8-methyl-8-azabicyclo-[3.2.1]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1*H*-benzimidazole-1-carboxamide, HCl), BIMU 8 (endo-*N*-(8-methyl-8-azabicyclo-[3.2.1]oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1*H*-benzimidazole-1-carboxamide, HCl) and DAU 6285 (endo-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-6-methoxy-2-oxo-1*H*-benzimidazole-1-carboxylate, HCl) were generous gifts from Boehringer Ingelheim (Milan, Italy). GR 113808 ([1-[2-(methylsulphonylamino)ethyl]-4-piperidinyl]methyl 1-methyl-1*H*-indole-3-carboxylate, maleate) was kindly provided by Glaxo (Greenford, UK). ICS 205930 ((3α-tropanyl)-1*H*-indole-3-carboxylic acid, ester) was given by Sandoz (Basel, Switzerland). (*R*,*S*)-Zacopride ((*R*,*S*)-4-amino-*N*-(1-azabicyclo-[2.2.2]oct-3-yl)-5-chloro-2-methoxybenzamide, HCl) and its enantiomers were obtained through the courtesy of Synthélabo (Rueil-Malmaison, France). [1,2,6,7-^{3}H]Corticosterone and [1,2,6,7-^{3}H]aldosterone were purchased from Amersham International (Buckinghamshire, UK).

3. Results

3.1. Agonist studies

The time course of the response of perifused adrenal slices to graded concentrations of (*R*)- and (*S*)-zacopride (10^{-8} to 10^{-4} M) is illustrated in Fig. 2. Both zacopride enantiomers induced a dose-dependent increase in corticosterone (Fig. 2A) and aldosterone (Fig. 2B) secretion. The minimum effective doses were 10^{-7} M for the (*S*)-enantiomer and 10^{-6} M for the (*R*)-enantiomer. At a dose of 10^{-4} M, (*R*)- and (*S*)-

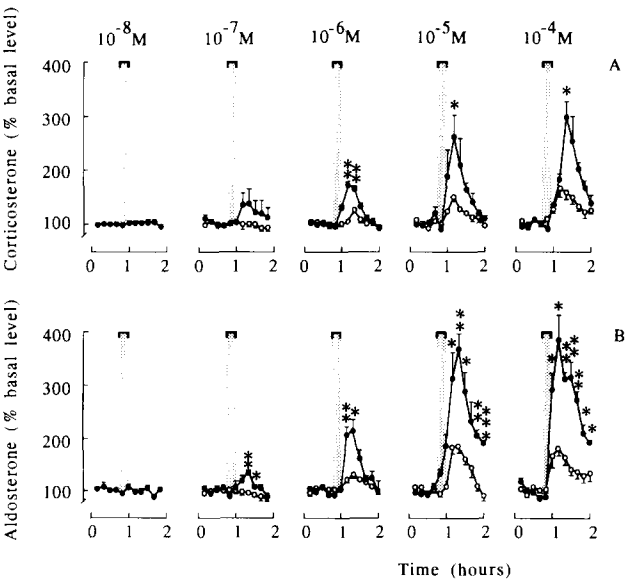

Fig. 2. Effect of increasing concentrations of (*R*)-zacopride (○) and (*S*)-zacopride (●) on corticosterone (A) and aldosterone (B) secretion by perifused frog adrenal slices. After a 120-min equilibration period, graded doses of the zacopride enantiomers (10^{-8} to 10^{-4} M) were administered for 20 min. The data represent the means (±S.E.M.) of at least three independent perifusion experiments. Each point is the mean corticosteroid production of two consecutive fractions collected over 5 min. The spontaneous level of corticosterone and aldosterone release (100% basal level) was calculated as the mean of eight consecutive fractions (40 min) just preceding the administration of the secretagogue. The secretory responses to each enantiomer were compared using the Student's t-test (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$). The mean secretory rates of corticosterone and aldosterone under basal conditions were 69.2±5 and 36.2±3.1 pg/adrenal gland per min, respectively.

zacopride increased corticosterone secretion by 66% and 198%, and aldosterone secretion by 81% and 284%, respectively. The dose-response curves comparing the effects of 5-HT to those of (*R*,*S*)-zacopride and its enantiomers on corticosterone and aldosterone secretion are presented in Fig. 3. Graded concentrations of 5-HT and (*R*,*S*)-zacopride stimulated corticosteroid secretion in a dose-dependent manner. The potency of 5-HT on steroid secretion ($EC_{50} = 1.5 \times 10^{-7}$ M) was higher than that of the benzamide derivatives. In contrast, the efficacy of (*S*)-zacopride, and to a lesser extent (*R*,*S*)-zacopride, was higher than that of 5-HT (Fig. 3). A series of experiments similar to those presented in Fig. 2 was conducted with azabicycloalkyl benzimidazolones. Increasing concentrations of BIMU 1 and BIMU 8 (10^{-7} to 10^{-4} M) gave rise to a dose-related stimulation of corticosterone (Fig. 4A) and aldosterone (Fig. 4B) production. The efficacy of BIMU 1 was similar to that of 5-HT. In contrast, BIMU 8 was significantly more efficient than BIMU 1 or 5-HT to stimulate corticosteroid secretion (Fig. 4). The potency and relative efficacy of the various 5-HT_4 receptor agonists tested are summarized in Table 1.

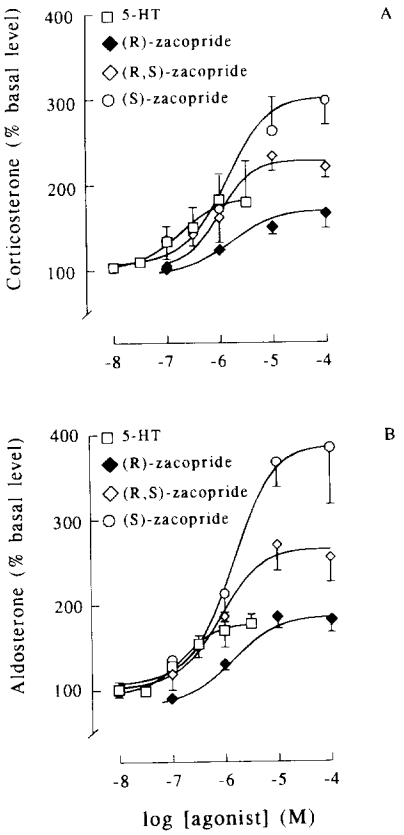

Fig. 3. Semi-logarithmic plot comparing the effect of 5-HT, (*R*,*S*)-zacopride and its enantiomers on corticosterone (A) and aldosterone (B) secretion by perifused frog adrenal slices. All experimental values were calculated from data similar to those presented in Fig. 1. The mean corticosteroid concentration in two consecutive fractions collected just after each pulse of agonist (peak height) was compared to the mean corticosteroid level observed just prior to the infusion of the secretagogue. The data represent the mean (±S.E.M.) of at least three independent experiments.

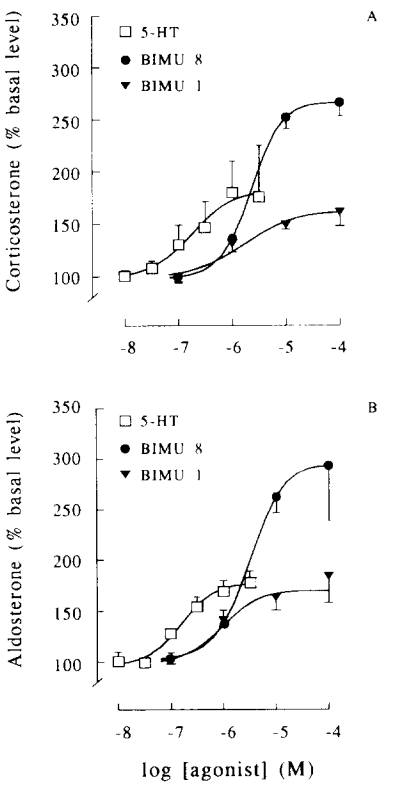

Fig. 4. Semi-logarithmic plot comparing the effect of 5-HT, BIMU 1 and BIMU 8 on corticosterone (A) and aldosterone (B) secretion by perifused frog adrenal slices. See legend to Fig. 3 for other designations.

5-HT was the most potent compound to stimulate corticosterone and aldosterone secretion. In contrast, 5-HT exhibited the lowest intrinsic activity of all the agonists tested, the rank order of efficacy being: (*S*)-zacopride > BIMU 8 = (*R*,*S*)-zacopride > BIMU 1 = (*R*)-zacopride = 5-HT.

Table 1
Relative potencies and efficacies of (*R*)-, (*S*)-, (*R*,*S*)-zacopride, BIMU 1, BIMU 8 and 5-HT to stimulate steroid secretion from frog adrenal gland

Compounds	Corticosterone secretion		Aldosterone secretion	
	pEC_{50}	Efficacy relative to 5-HT	pEC_{50}	Efficacy relative to 5-HT
5-HT	6.58±0.04	1	6.80±0.03	1
(*R*)-Zacopride	5.73±0.18	0.83±0.21	5.96±0.01	1.09±0.15
BIMU 1	5.96±0.02	1.06±0.33	5.87±0.12	1.09±0.34
(*R*,*S*)-Zacopride	6.03±0.17	1.66±0.21	6.06±0.10	2.18±0.38
BIMU 8	5.65±0.02	2.07±0.17	5.52±0.10	2.47±0.73
(*S*)-Zacopride	5.63±0.01	2.46±0.35	5.83±0.05	3.64±0.85

In order to verify that benzimidazolone derivatives actually interacted with 5-HT receptors present in the adrenal tissue, we studied the additivity of the effects

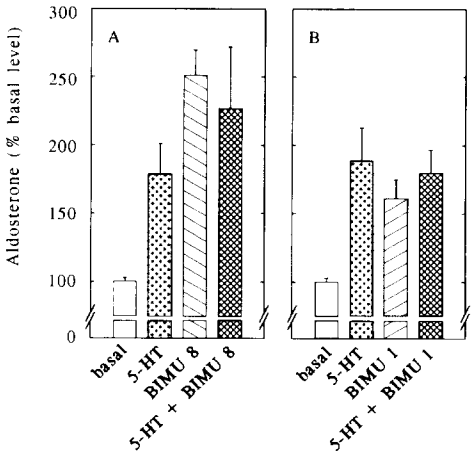

Fig. 5. Effects of BIMU 8 (A) or BIMU 1 (B) and 5-HT on aldosterone secretion by perifused frog adrenal slices. 5-HT (10^{-6} M) and BIMU 1 or BIMU 8 (10^{-5} M) were administered successively and simultaneously. The data were obtained from a series of experiments similar to those presented in Fig. 2. The data represent the mean (±S.E.M.) of at least three independent experiments. The mean secretory rates of aldosterone under basal conditions were 18.3±4 (A) and 19.6±4.5 pg/adrenal gland per min (B).

of 5-HT with those of BIMU 8 or BIMU 1 on corticosteroid secretion (Fig. 5). The magnitude of the stimulation of aldosterone secretion induced by supramaximal concentrations of 5-HT (10^{-6} M) or benzimidazolone derivatives (10^{-5} M) was not enhanced during combined administration of the two compounds. Similar results were obtained with corticosterone secretion (data not shown).

3.2. Antagonist studies

The effects of the three 5-HT_4 receptor antagonists (i.e. ICS 205930, DAU 6285 and GR 113808) on basal and zacopride-stimulated corticosteroid secretion are illustrated in Fig. 6. None of the antagonists tested induced any modification of the basal secretion of aldosterone (Fig. 6). At a concentration of 10^{-5} M, the non-selective 5-HT_3/5-HT_4 receptor antagonist ICS 205930 partially abolished the stimulatory effect of a 10^{-5} M pulse of (*R,S*)-zacopride (Fig. 6A). A 10 times lower dose of DAU 6285 (10^{-6} M) markedly attenuated the response of the adrenal tissue to 10^{-5} M (*R,S*)-zacopride (Fig. 6B). At a concentration as low as 10^{-9} M, GR 113808 significantly reduced (*R,S*)-zacopride-stimulated aldosterone secretion (Fig. 6C). A series of experiments similar to those presented in Fig. 6 was conducted with different concentrations of 5-HT_4 receptor antagonists (Fig. 7). Each antagonist inhibited corticosterone (Fig. 7A) and aldosterone (Fig. 7B) secretion induced by (*R,S*)-zacopride in a dose-dependent manner. Using aldosterone secretion as an index, the pK_i values against (*R,S*)-zacopride were 6.20 ± 0.01 for ICS 205930, 7.84 ± 0.04 for DAU 6285 and 10.34 ± 0.06 for GR 113808.

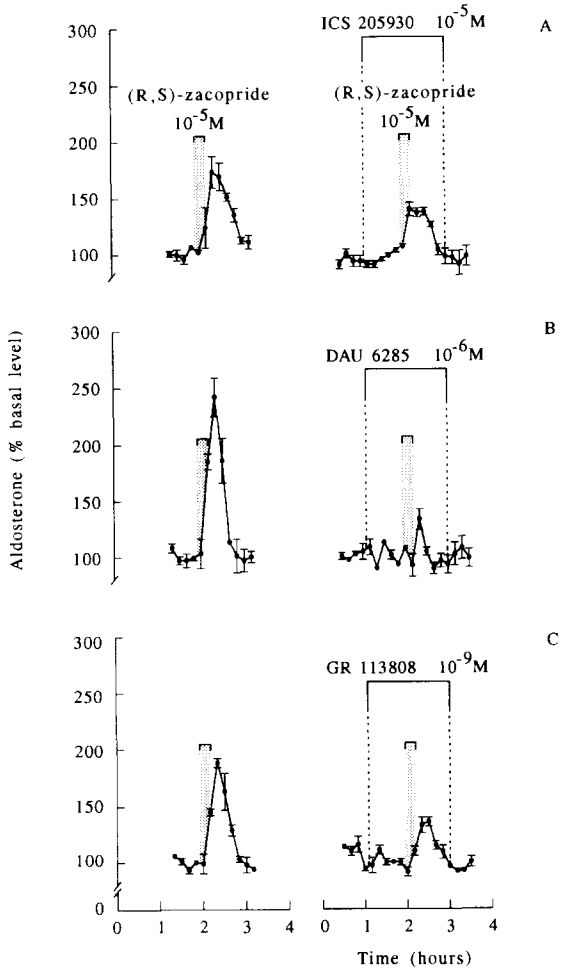

Fig. 6. Effect of (*R,S*)-zacopride alone and during prolonged infusion of ICS 205930 (A), DAU 6285 (B) and GR 113808 (C) on aldosterone secretion by perifused frog adrenal slices. After a 120-min equilibration period, a pulse of (*R,S*)-zacopride (10^{-5} M) was administered for 20 min (control). In the same experiment, one of the 5-HT_4 antagonists was administered for 240 min. During infusion of the antagonist, another pulse of (*R,S*)-zacopride (10^{-5} M) was added for 20 min. The mean secretory rate of aldosterone under basal conditions was 70 ± 13 pg/adrenal gland per min.

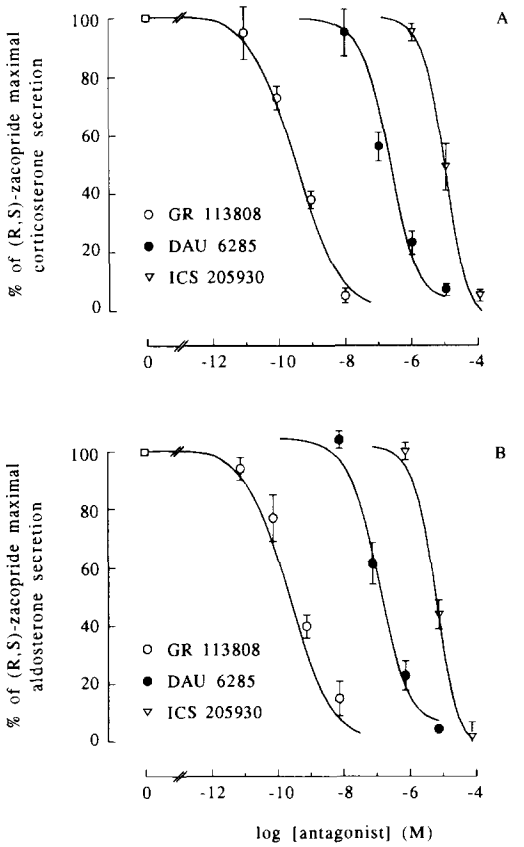

Fig. 7. Semi-logarithmic plot comparing the effect of increasing concentrations of ICS 205930, DAU 6285 and GR 113808 on (*R,S*)-zacopride-induced stimulation of corticosterone (A) and aldosterone (B) secretion by perifused frog adrenal slices. All experimental values were calculated from data similar to those presented in Fig. 6. The results are expressed as a percentage of the response induced by (*R,S*)-zacopride in the absence of antagonist. The data represent the mean ($\pm$S.E.M.) of three independent experiments.

4. Discussion

We have previously reported that the stimulatory effect of serotonin on steroid secretion by the adrenal gland is mediated through activation of 5-HT_4 receptors positively coupled to adenylate cyclase (Idres et al., 1991; Lefebvre et al., 1992, 1993). However, these initial studies could only be performed with the limited number of agonists (zacopride, cisapride and BRL 72222) and the single non-selective antagonist ICS 205930 available at that time. In the present study, we have investigated a series of compounds which have been recently described as 5-HT_4 receptor agonists or antagonists, in order to compare the pharmacological profile of the 5-HT receptor of the frog adrenal gland to that of 5-HT_4 receptors characterized in various mammalian tissues.

Comparison of the corticotropic activity of the two zacopride enantiomers revealed that (*S*)-zacopride was significantly more efficient than the (*R*)-enantiomer or (*R*,*S*)-zacopride, although all three compounds exhibited the same potency. The relative activities of the zacopride enantiomers have previously been compared in vitro on guinea pig ileum (Eglen et al., 1990) and rat oesophagus (Baxter et al., 1991). These studies revealed that the (*S*)-enantiomer is approximately 10-fold more potent than the (*R*)-form. Conversely, in vivo experiments failed to demonstrate any stereoselectivity of (*R*)- and (*S*)-zacopride on the hippocampal EEG spectrum (Boddeke and Kalkman, 1992).

Two novel benzimidazolone derivatives, BIMU 1 and BIMU 8, have been shown to act as 5-HT_4 receptor agonists on brain tissue (Dumuis et al., 1991; Monferini et al., 1993) and peripheral organs (Baxter and Clarke, 1992; Rizzi et al., 1992). In our model, both BIMU 1 and BIMU 8 caused a dose-dependent stimulation of corticosterone and aldosterone secretion. In agreement with results of previous studies on cyclase activity in mouse embryo colliculi neurons (Dumuis et al., 1991), BIMU 8 was found to be significantly more efficient than BIMU 1 in enhancing steroidogenesis. The observation that association of maximum doses of 5-HT and BIMU 1 or BIMU 8 did not produce additive effects on corticosteroid release provides further evidence that the stimulatory effect of 5-HT on adrenocortical cells can be accounted for by activation of 5-HT_4 receptors. It is worth mentioning that BIMU 1 and BIMU 8, like ICS 205930, also act as 5-HT_3 receptor antagonists (Baxter and Clarke, 1992). Since the stimulatory effect of 5-HT was not impaired during combined administration of either BIMU 1 or BIMU 8, it appears that 5-HT_3 receptors are not involved in the corticotropic activity of serotonin on frog adrenal tissue.

The present study showed that the various 5-HT_4 receptor agonists tested were about 5- to 10-fold less potent than 5-HT to stimulate corticosteroid secretion. In contrast, most of these compounds exhibited greater efficacy than 5-HT. This observation is consistent with results of previous studies which showed that zacopride and BIMU 8 are less potent but more efficient than 5-HT to stimulate adenylyl cyclase activity in colliculi neurons (Dumuis et al., 1989, 1991). The lower efficacy of 5-HT, as compared to that of various 5-HT_4 receptor agonists, can be ascribed to the rapid desensitization of adrenocortical cells induced by 5-HT (Idres et al., 1991).

The indoleamine derivative ICS 205930 has long been the only competitive antagonist available for the investigation of 5-HT_4 receptors. However, this compound was of limited value since its affinity for 5-HT_3 receptors is at least 1000-fold higher (Boddeke and Kalkman, 1992). Recently, two novel compounds exhibiting 5-HT_4 receptor antagonistic properties have been described, i.e. the azabicycloalkyl benzimidazolone derivative DAU 6285 (Dumuis et al., 1992; Schiavone et al., 1992) and the indole derivative GR 113808 (Grossman et al., 1993). Both DAU 6285 and GR 113808 were potent inhibitors of zacopride-induced steroidogenesis and the order of potency of the various antagonists (GR 113808 > DAU 6285 > ICS 205930) was identical to that previously determined in various mammalian models. In particular, a good correlation was observed between the potency of ICS 205930 on the frog adrenal gland ($pK_i = 6.20$ against (*R*,*S*)-zacopride) and that reported in mouse colliculi neurons ($pK_i = 6.23$ against (*R*,*S*)-zacopride; Dumuis et al., 1989) and guinea-pig ileum ($pA_2 = 6.4$ against 5-HT; Tonini et al., 1991). Similarly, the relative potency of DAU 6285 to inhibit zacopride-induced corticosteroid secretion ($pK_i = 7.84$ against (*R*,*S*)-zacopride) was in the same range as that reported for the inhibition of cAMP formation in colliculi neurons ($pK_i = 6.74$ against renzapride; Dumuis et al., 1992) or enteric neurons ($pA_2 = 7.2$ against cisapride; Tonini et al., 1991). The high potency of GR 113808 on zacopride-induced corticosteroid secretion ($pK_i = 10.3$) was also in good agreement with binding data obtained for guinea-pig striatum and hippocampus ($pK_i = 9.5$ and 9.6 against GR 113808, respectively; Grossman et al., 1993).

In conclusion, the pharmacological characteristics of the 5-HT receptor present in the adrenal gland are very similar to those of the 5-HT_4 receptor previously described in the central nervous system and in peripheral tissues of mammals. The frog adrenal gland, which is composed of a single population of corticosteroid secreting cells, thus appears as a valuable model in which to investigate the functional properties of the 5-HT_4 receptor. This simple model should also prove useful for the identification of novel 5-HT_4 receptor agonists and antagonists.

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References

Baxter, G.S., D.A. Craig and D.E. Clarke, 1991, 5-Hydroxytryptamine$_4$ receptors mediate relaxation of the rat oesophageal tunica muscularis mucosae, Naunyn-Schmied. Arch. Pharmacol. 343, 439.

Baxter, G.S. and D.E. Clarke, 1992, Benzimidazolone derivatives act as 5-HT$_4$ receptor ligands in rat oesophagus, Eur. J. Pharmacol. 212, 225.

Bockaert, J., M. Sebben and A. Dumuis, 1990, Pharmacological characterization of 5-hydroxytryptamine$_4$ (5-HT$_4$) receptors positively coupled to adenylate cyclase in adult guinea pig hippocampal membranes: effect of substituted benzamide derivatives, Mol. Pharmacol. 37, 408.

Bockaert, J., J.R. Fozard, A. Dumuis and D.E. Clarke, 1992, The 5-HT$_4$ receptor: a place in the sun, Trends Pharmacol. Sci. 13, 141.

Boddeke, H.W.G.M. and H.O. Kalkman, 1992, Agonists effects at putative central 5-HT$_4$ receptors in rat hippocampus by R(+)- and S(−)-zacopride; no evidence for stereo-selectivity, Neurosci. Lett. 134, 261.

Cheng, Y.C. and W.H. Prusoff, 1973, Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction, Biochem. Pharmacol. 22, 3099.

Contesse, V., C. Delarue, F. Leboulenger, H. Lefebvre, F. Héry and H. Vaudry, 1993, Serotonin produced in the adrenal gland regulates corticosteroid secretion through a paracrine mode of communication, in: Cellular Communication in Reproduction, eds. F. Facchinetti, I.W. Henderson, R. Pierantoni and A.M. Polzonetti-Magni (Burgess Science, UK) p. 187.

Craig, D.A., R.M. Eglen, L.K.M. Walsh, L.A. Perkins, R.L. Whiting and D.E. Clarke, 1990, 5-Methoxytryptamine and 2-methyl-5-hydroxytryptamine-induced desensitization as a discriminative tool for the 5-HT$_3$ and putative 5-HT$_4$ receptors in guinea pig ileum, Naunyn-Schmied. Arch. Pharmacol. 342, 9.

Dumuis, A., R. Bouhelal, M. Sebben, R. Cory and J. Bockaert, 1988, A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system, Mol. Pharmacol. 34, 880.

Dumuis, A., M. Sebben and J. Bockaert, 1989, The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-HT$_4$) positively coupled to adenylate cyclase in neurons, Naunyn-Schmied. Arch. Pharmacol. 340, 403.

Dumuis, A., M. Sebben, E. Monferini, M. Nicola, M. Turconi, H. Ladinsky and J. Bockaert, 1991, Azabicycloalkyl benzimidazolone derivatives as a novel class of potent agonists at the 5-HT$_4$ receptor positively coupled to adenylate cyclase in brain, Naunyn-Schmied. Arch. Pharmacol. 343, 245.

Dumuis, A., H. Gozlan, M. Sebben, H. Ansanay, C.A. Rizzi, M. Turconi, E. Monferini, E. Giraldo, P. Schiantarelli, H. Ladinsky and J. Bockaert, 1992, Characterization of a novel 5-HT$_4$ receptor antagonist of the azabicycloalkyl benzimidazolone class: DAU 6285, Naunyn-Schmied. Arch. Pharmacol. 345, 264.

Eglen, R.M., S.R. Swank, L.K.M. Walsh and R.L. Whiting, 1990, Characterization of 5-HT$_3$ and atypical 5-HT receptors mediating guinea-pig ileal contractions in vitro, Br. J. Pharmacol. 101, 513.

Elswood, C.J., K.T. Bunce and P.P.A. Humphrey, 1991, Identification of putative 5-HT$_4$ receptors in guinea-pig ascending colon, Eur. J. Pharmacol. 196, 149.

Esneu, M., C. Delarue, I. Remy-Jouet, E. Manzardo, A. Fasolo, A. Fournier, S. Saint-Pierre, J.M. Conlon and H. Vaudry, 1994, Localization, identification and action of calcitonin gene-related peptide in the frog adrenal gland, Endocrinology 135, 423.

Grossman, C.J., G.J. Kilpatrick and K.T. Bunce, 1993, Development of a radioligand binding assay for 5-HT$_4$ receptors in guinea-pig and rat brain, Br. J. Pharmacol. 109, 618.

Idres, S., C. Delarue, H. Lefebvre and H. Vaudry, 1991, Benzamide derivatives provide evidence for the involvement of a 5-HT$_4$ receptor type in the mechanism of action of serotonin in frog adrenocortical cells, Mol. Brain Res. 10, 251.

Kaumann, A.J., L. Sanders, A.M. Brown, K.J. Murray and M.J. Brown, 1991, A 5-HT$_4$-like receptor in human right atrium, Naunyn-Schmied. Arch. Pharmacol. 344, 150.

Leboulenger, F., C. Delarue, M.C. Tonon, S. Jégou and H. Vaudry, 1978, In vitro study of frog (*Rana ridibunda* Pallas) interrenal function by use of a simplified perifusion system. I. Influence of adrenocorticotropin upon corticosterone release, Gen. Comp. Endocrinol. 36, 327.

Leboulenger, F., C. Delarue, A. Bélanger, I. Perroteau, P. Netchitailo, P. Leroux, S. Jégou, M.C. Tonon and H. Vaudry, 1982, Direct radioimmunoassays for plasma corticosterone and aldosterone in frog. I. Validation of the methods and evidence for daily rhythms in a natural environment, Gen. Comp. Endocrinol. 46, 521.

Lefebvre, H., V. Contesse, C. Delarue, M. Feuilloley, F. Héry, P. Grise, G. Raynaud, A.A.J. Verhofstad, L.M. Wolf and H. Vaudry, 1992, Serotonin-induced stimulation of cortisol secretion from human adrenocortical tissue is mediated through activation of a serotonin$_4$ receptor subtype, Neuroscience 47, 999.

Lefebvre, H., V. Contesse, C. Delarue, C. Soubrane, A. Legrand, J.M. Kuhn, L.M. Wolf and H. Vaudry, 1993, Effect of the 5-HT$_4$ receptor agonist zacopride on aldosterone secretion from the human adrenal cortex. In vivo and in vitro studies, J. Clin. Endocrinol. Metab. 77, 1662.

Monferini, E., P. Gaetani, R.R. y Baena, E. Giraldo, M. Parenti, A. Zocchetti and C.A. Rizzi, 1993, Pharmacological characterization of the 5-hydroxytryptamine receptor coupled to adenylyl cyclase stimulation in human brain, Life Sci. 52, 61.

Rizzi, C.A., T. Coccini, L. Onori, L. Manzo and M. Tonini, 1992, Benzimidazolone derivatives: a new class of 5-hydroxytryptamine$_4$ receptor agonists with prokinetic and acetylcholine releasing properties in the guinea pig ileum, J. Pharmacol. Exp. Ther. 261, 412.

Schiavone, A., E. Giraldo, L. Giudici, M. Turconi and A. Sagrada, 1992, DAU 6285: a novel antagonist at the putative 5-HT$_4$ receptor, Life Sci. 51, 583.

Tonini, M., S.M. Candura, L. Onori, T. Coccini, L. Manzo and C.A. Rizzi, 1991, 5-Hydroxytryptamine$_4$ receptor agonists facilitate cholinergic transmission in the circular muscle of guinea pig ileum: antagonism by tropisetron and DAU 6285, Life Sci. 50, 173.

Van Meel, J.C.A., W. Diederen, R. Haigh, W. Wienen, M. Pairet, M. Turconi and H.N. Doods, 1993, The novel 5-HT$_4$ receptor antagonist DAU 6285 antagonizes 5-hydroxytryptamine-induced tachycardia in pigs, Eur. J. Pharmacol. 233, 295.

Villalon, C.M., M.O. Den Boer, J.P.C. Heiligers and P.R Saxena, 1991, Further characterization, by use of tryptamine and benzamide derivatives, of the putative 5-HT$_4$ receptor mediating tachycardia in the pig, Br. J. Pharmacol. 102, 107.

Waeber, C., M. Sebben, C. Grossman, F. Javoy-Agid, J. Bockaert and A. Dumuis, 1993, [^{3}H]-GR113808 labels 5-HT$_4$ receptors in the human and guinea-pig brain, NeuroReport 4, 1239.