Differential effects of centrally-active antihypertensives on 5-HT_{1A} receptors in rat dorso-lateral septum, rat hippocampus and guinea-pig hippocampus

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1 The electrophysiological responses elicited by 5-hydroxytryptamine_{1A}- (5-HT_{1A}) receptor agonists in rat and guinea-pig CA1 pyramidal neurones and rat dorso-lateral septal neurones were compared *in vitro* by use of conventional intracellular recording techniques.

2 In the presence of 1 µM tetrodotoxin (TTX), to prevent indirect effects, 5-HT, N,N-dipropyl-5-carboxamidotryptamine (DP-5-CT) and 8-hydroxy-2(di-n-propylamino) tetralin (8-OH-DPAT) hyperpolarized the neurones from rat and guinea-pig brain.

3 The hypotensive drug flesinoxan, a selective 5-HT_{1A} receptor agonist, hyperpolarized neurones in all three areas tested; however, another hypotensive agent with high affinity at 5-HT_{1A}-receptors, 5-methyl-urapidil, hyperpolarized only the neurones in rat hippocampus and septum.

4 In guinea-pig hippocampal neurones, 5-methyl-urapidil behaved as a 5-HT_{1A}-receptor antagonist.

5 The relative efficacies (5-HT = 1) of DP-5-CT, 8-OH-DPAT, flesinoxan and 5-methyl-urapidil at the three sites were: rat hippocampus, 1.09, 0.7, 0.5 and 0.24; rat septum, 0.88, 0.69, 0.82 and 0.7; guinea-pig hippocampus, 1.0, 0.69, 0.89 and 0, respectively.

6 It is concluded that the hypotensive agents flesinoxan and 5-methyl-urapidil appear to have different efficacies at 5-HT_{1A} receptors located in different regions of the rodent brain. Whether these regional and species differences arise from receptor plurality or variability in intracellular transduction mechanisms remains to be elucidated.

Keywords: 5-Hydroxytryptamine (5-HT); 5-HT_{1A}-receptors; N,N-dipropyl 5-carboxamidotryptamine (DP-5-CT); 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT); flesinoxan; 5-methyl-urapidil; hippocampus; lateral septum

Introduction

Six subtypes of 5-hydroxytryptamine₁ (5-HT₁) type receptors have been identified in the mammalian central nervous system (Humphrey *et al.*, 1993) and of these, the 5-HT_{1A} receptors have been studied in most detail. Upon activation by 5-HT or synthetic agonists, these G-protein-coupled receptors decrease the activity of adenylate cyclase (DeVivo & Maayani, 1986) and inhibit neuronal activity by increasing membrane conductance to potassium ions (Andrade & Nicoll, 1987a; Colino & Halliwell, 1987). The pharmacology of 5-HT_{1A} receptors is of particular interest since it appears that the anxiolytic drug, buspirone, and the antihypertensive agents, urapidil and flesinoxan, may exert all or part of their beneficial effects via these receptors (see Taylor, 1988; and Saxena & Villalon, 1990, for an overview).

Exactly how and where these compounds act is unknown, but it seems likely that their anxiolytic actions are due to modulation of the activity of 5-HT containing neurones in the raphe nuclei and/or via actions on 5-HT_{1A} receptors located on neurones in the limbic system (Traber & Glaser, 1987). This hypothesis has received considerable support from the results of electrophysiological studies showing that buspirone and other putative anxiolytics such as ipsapirone, gepirone and MDL 73,005EF, do indeed have agonist, partial agonist or antagonist effects at 5-HT_{1A}-receptors on raphe, hippocampal and septal neurones (Andrade & Nicoll, 1987b; Sprouse & Aghajanian, 1987; van den Hooff & Galvan, 1992). In addition, long-term changes in limbic system function have been observed following chronic treatment with anxiolytics of this type (Blier & de Montigny, 1990).

Interest in the anti-hypertensive actions of compounds acting at 5-HT_{1A} receptors arose following the discovery that the selective 5-HT_{1A} receptor ligand, 8-hydroxy-2(di-n-

propylamino)tetralin (8-OH-DPAT; Gozlan *et al.*, 1983) lowers blood pressure in the rat and the cat via an action on the central nervous system (Fozard & Ramage, 1984; Gradin *et al.*, 1985; Fozard *et al.*, 1987). Two other compounds have been identified that appear to act in such a novel manner, urapidil (Sanders & Jurna, 1985; Ramage, 1986; Sanders *et al.*, 1990) and flesinoxan (Wouters *et al.*, 1988). Whilst urapidil itself had a relatively low affinity for 5-HT_{1A} receptors (Fozard & Mir, 1987), 5-methyl-urapidil and flesinoxan have affinities in the low nanomolar range and are potent hypotensive agents in animals (Gross *et al.*, 1987; Mandal *et al.*, 1990; Wouters *et al.*, 1988). More recently, it has been reported that flesinoxan also has anxiolytic and perhaps even anti-depressant activity (Olivier *et al.*, 1991; Schipper *et al.*, 1991).

Although there have been several detailed descriptions of the actions of these antihypertensives on the cardiovascular system and the behaviour of animals (Ramage 1990; Schipper *et al.*, 1991), there are little data available regarding their actions on single neurones in the mammalian brain. The present experiments were therefore performed to compare the actions of the 5-HT_{1A}-receptor agonists 5-HT, N,N-dipropyl-5-carboxamido-tryptamine (DP-5-CT) and 8-OH-DPAT with those of flesinoxan and 5-methyl-urapidil. Preliminary accounts of some of these results have been presented to the Deutsche Gesellschaft für Pharmakologie und Toxikologie (Boeijinga *et al.*, 1992; Leishman & Galvan, 1993).

Methods

Slice preparation

Male Sprague-Dawley rats and albino guinea-pigs (both species 100–300 g; Charles River, France) were decapitated

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and the brains removed and cooled for 1 min in Krebs solution at 2–4°C. The hippocampi were isolated from the surrounding brain tissue and 400 µm slices were cut on a McIlwain tissue chopper and stored prior to recording in a small chamber filled with oxygenated Krebs solution (22–26°C). To prepare septal slices, a block of rat forebrain tissue containing the septum was mounted on a small stage and frontal slices (ca. 400 µm) were cut with a Vibroslice (Campden Instruments, London). The slices were stored in a small holding chamber filled with oxygenated Krebs solution at 36°C.

For recording purposes, slices were transferred to a perspex recording chamber, where they were sandwiched between two nylon nets and continuously superfused (6–8 ml min⁻¹) with a modified Krebs solution at 34°C. The composition of the modified Krebs solution was (mM): NaCl 119, KCl 3.0, NaH₂PO₄ 1.2, MgCl₂·6H₂O 1.2, CaCl₂·2H₂O 2.0, NaHCO₃ 25 and D-glucose 10; all solutions were continuously gassed with 95% O₂/5% CO₂. Compounds were applied in fixed concentrations via the superfusate; due to the design of the perfusion system, there was a delay of about 30–40 s before the new solution arrived in the recording chamber.

Intracellular recording

Intracellular recordings were made with glass microelectrodes filled with 3 M KCl (70 to 100 MΩ). Electrical signals were amplified with a bridge balance amplifier, monitored on a digital oscilloscope and recorded on a chart recorder and a personal computer running pClamp software (Axon Instruments, Foster City, CA, U.S.A.). The neurones included in this study exhibited stable resting membrane potentials more negative than -55 mV and membrane resistances greater than 80 MΩ for septal neurones and ≥ 20 MΩ for hippocampal neurones. In addition, cells not exhibiting a 'robust' response to 5-HT were not further studied. In practice this meant that the minimum acceptable membrane potential changes were as follows (applied concentrations of 5-HT in parentheses): rat septum ≥ 7 mV (10 µM), rat and guinea-pig hippocampus, both ≥ 4 mV (30 µM). In all of the experiments described, tetrodotoxin (TTX; 1 µM) was included in the perfusion medium to block action potential-dependent neurotransmitter release and thus preclude indirect effects of the compounds under study.

Analysis of the results

The response to an agonist was defined as the maximal change in membrane potential attained during superfusion (1–10 min) of the compound. Unless otherwise stated, all numerical data are given as the mean ± s.e.mean. 2-tailed, paired and unpaired *t* tests or ANOVA were calculated as necessary using the programme InStat (Graphpad Software, San Diego, CA, U.S.A.).

Materials

Drug sources were as follows: 5-hydroxytryptamine creatinine sulphate complex and tetrodotoxin (Sigma,

France), fleroxan (Duphar, The Netherlands), 5-methylurapidil, N,N-dipropyl-5-carboxamidotryptamine maleate and (±)-8-hydroxy-2-(di-n-propylamino)tetralin HBr (Research Biochemicals Incorporated, Massachusetts, U.S.A.).

Results

The results described are based on stable intracellular recordings from 77 neurones located in the dorsolateral part of the septum, 51 rat CA1 pyramidal neurones and 44 guinea-pig CA1 pyramidal neurones. The mean resting membrane potential and membrane resistance of these cells are shown in Table 1. The guinea-pig hippocampal neurones had a significantly more negative membrane potential than rat hippocampal CA1 neurones and rat septal neurones had a higher membrane resistance than the hippocampal neurones of both species.

Actions of 5-HT

Bath application of 5-HT (0.3–100 µM) hyperpolarized the membrane potential and reduced the membrane resistance in a concentration-dependent manner in all three brain areas tested (Figures 1 and 2a). The observed hyperpolarizations peaked within about 1–1.5 min and the membrane potential repolarized towards control levels following return to drug-free Krebs solution. The maximum hyperpolarizations and changes in membrane resistance induced by 5-HT are shown in Table 1 as are the reversal potentials measured from current-voltage relationships constructed before and during the application of 5-HT. 5-HT-induced hyperpolarizations in rat septal neurones were significantly larger than those observed in either rat or guinea-pig hippocampal CA1 pyramidal cells. The estimated reversal potentials of between

Figure 1 Concentration-response curves for 5-hydroxytryptamine (5-HT) measured in neurones in the rat dorso-lateral septal nucleus (■; number of determinations in different cells, *n* = 7–66), the rat CA1 area (●; *n* = 8–17) and the guinea-pig CA1 area (○; *n* = 4–6). The applied concentrations (µM) are on the abscissa scale and the ordinate scale shows the hyperpolarization in mV (mean and s.e.mean shown).

Table 1 Passive membrane properties and the changes induced by 5-hydroxytryptamine (5-HT) in rat and guinea-pig hippocampal CA1 neurones and rat dorso-lateral septal neurones *in vitro*

	Control solution		5-HT containing solution		
	<i>Em</i> (mV)	<i>Rm</i> (MΩ)	Δ <i>Em</i> (mV)	Δ <i>Rm</i> (%)	<i>Erev</i> (mV)
Rat hippocampus CA1	-71 ± 5* (51)	41 ± 10* (51)	-7 ± 0.5* (34)	30 ± 1* (8)	-84 ± 3* (6)
Guinea-pig hippocampus CA1	-77 ± 4*† (44)	50 ± 11* (44)	-6.5 ± 0.5* (44)	30 ± 1* (34)	-97 ± 1† (7)
Rat septum	-66 ± 5 (77)	136 ± 55 (77)	-20 ± 2 (8)	72 ± 4 (8)	-98 ± 2 (4)

Resting membrane potentials (*Em*) and membrane resistances (*Rm*) of 3 types of limbic neurones measured in control Krebs solution (means ± s.d.; number of cells in parentheses). The changes (Δ *Em* and Δ *Rm*) as well as the reversal potentials (*Erev*) measured during superfusion of 30 µM 5-HT are given as means ± s.e.means. **P* < 0.01 compared to rat septum. †*P* < 0.01 compared to rat hippocampus; one way ANOVA, Bonferroni corrected *P* values.

– 84 and – 98 mV are consistent with the hypothesis that the underlying conductance change during the action of 5-HT is due to the opening of potassium channels. A contribution from a change in chloride ion conductance cannot be excluded, however under the present recording conditions (employing 3 M KCl-filled microelectrodes), this would be most unlikely (Newberry & Nicoll, 1985). The complete concentration-response relationships are shown in Figure 1 and the estimated EC_{50} values for the pooled data were 1.9, 3.1 and 4.5 μ M, in rat septal, rat hippocampal and guinea-pig hippocampal neurones, respectively.

The responses to 5-HT in rat and guinea-pig CA1 pyramidal neurones differed significantly in their time-courses, particularly in the rates of repolarization (see Figure 2a). The time to 50% recovery in rat hippocampal neurones was 79 ± 6 s ($n = 25$) and 308 ± 16 s ($n = 25$) in guinea-pig hippocampal neurones ($P < 0.01$, unpaired 2-tailed t test). In the rat hippocampus the 5-HT-induced hyperpolarizations were often (19 out of 41 cells) followed by a depolarization and an increase in membrane resistance (Figure 2a; Andrade & Nicoll, 1987a; Colino & Halliwell, 1987). However, in the guinea-pig CA1 neurones, no such changes in membrane potential and resistance were observed ($n = 33$).

Actions of synthetic 5-HT_{1A} agonists

In rat and guinea-pig hippocampus, DP-5-CT, 8-OH-DPAT, flesinoxan and 5-methyl-urapidil were applied to separate groups of neurones at 3 or 30 μ M. This technique was adopted as (1) the hyperpolarizations were only slowly reversible and (2) the slow onset of action and the relatively small maximum response (≤ 10 mV) rendered cumulative drug addition impracticable. In rat septal neurones, full concentration-response relationships were determined by cumulative addition of agonists.

Figure 2b, c illustrates examples of the effects of DP-5-CT and 8-OH-DPAT in rat and guinea-pig CA1 neurones. Each compound induced a hyperpolarization and a reduction in membrane resistance, which were poorly reversible. The onset of action of 8-OH-DPAT was clearly slower than that of DP-5-CT or 5-HT. The antihypertensive agents, flesinoxan and 5-methyl-urapidil, hyperpolarized rat septal and hippocampal neurones; however, only flesinoxan hyperpolarized guinea-pig CA1 neurones (Figure 3). The maximum hyperpolarizations measured during application of DP-5-CT, 8-OH-DPAT, flesinoxan and 5-methyl-urapidil to the three types of limbic neurones are shown in Table 2. Reversal potentials were estimated from current-voltage relationships constructed before and during superfusion with the various agonists. The values obtained (– 84 to – 106 mV; Table 2) are similar to those found during the action of 5-HT (Table 1) and suggest that the synthetic agonists also increase membrane conductance for potassium ions.

In the guinea-pig hippocampus, application of 3 μ M ($n = 5$) and 30 μ M ($n = 5$) 5-methyl-urapidil failed to elicit a hyperpolarization or a reduction in membrane resistance, indicating that in these cells this compound has no important agonist activity. Indeed, further experiments demonstrated that 5-methyl-urapidil (3 μ M) antagonized the action of 5-HT (Figure 4). Control hyperpolarizations elicited by 30 μ M 5-HT were -5.6 ± 0.6 mV and this was reduced to -1.6 ± 0.7 mV ($n = 4$; $P < 0.01$, paired, 2-tailed t test) following superfusion with 3 μ M 5-methyl-urapidil.

Figure 5 illustrates the concentration-response relationships for flesinoxan and 5-methyl-urapidil in rat dorso-lateral septal neurones. The estimated EC_{50} values from the pooled data for these compounds are 25 and 45 nM, respectively. In 2 of the 9 cells responsive to 5-HT, application of 10 μ M 5-methyl-urapidil was without effect on the membrane potential; these cells were not included in the curves shown in Figure 5.

Figure 2 Comparison of the actions of (a) 30 μ M 5-hydroxytryptamine (5-HT), (b) 3 and 30 μ M N,N-dipropyl-5-carboxamidotryptamine (DP-5-CT) and (c) 3 μ M 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in different neurones in the rat (left column) and in the guinea-pig (right column) hippocampal CA1 area. Bath application of the compounds (horizontal bars) hyperpolarized the membrane potential and decreased membrane resistance. The transient downward deflections are membrane responses to intracellular injection of constant current pulses (300 ms, 0.1 Hz, 0.15–0.4 nA). The 30–40 s delay in the perfusion line (see methods) has not been corrected for, in this and all subsequent figures.

Figure 3 Comparison of the actions of flesinoxan (1–30 μ M) and 5-methyl-urapidil (1–3 μ M) on the membrane potential and membrane resistance of neurones in (a) the rat dorso-lateral septal nucleus, (b) the rat CA1 hippocampal area and (c) the guinea-pig CA1 hippocampal area. Note that 5-methyl-urapidil was without effect on the guinea-pig hippocampal neurone.

Table 2 Maximal hyperpolarizations evoked by N,N-dipropyl-5-carboxamido tryptamine (DP-5-CT), 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), flesinoxan and 5-methyl-urapidil in rat and guinea-pig hippocampal CA1 neurones and rat dorso-lateral septal neurones

	DP-5-CT		8-OH-DPAT		Flesinoxan		5-Methyl-urapidil	
	ΔE_m	Erev	ΔE_m	Erev	ΔE_m	Erev	ΔE_m	Erev
Rat hippocampus CA1	-7.5 ± 1.3 (6)	-92 ± 4 (3)	-4.9 ± 0.4 (4)	-8.9 (1)	-3.3 ± 0.6 (7)	-93 ± 8 (3)	-1.8 ± 0.3 (9)	-8.4 (1)
Guinea-pig hippocampus CA1	-6.4 ± 1.7 (30)	-100 ± 4 (5)	-5.2 ± 0.7 (9)	-99 ± 7 (3)	-7.1 ± 0.6 (5)	-104 ± 2 (3)	No effect (10)	
Rat septum	-17.5 ± 1.4 (10)*	-106 (1)	-13.8 ± 0.6 (8)*	ND	-16.4 ± 1.4 (5)	-104 (2)	-14 ± 1.2 (5)	ND

Changes in membrane potential (ΔE_m) and reversal potentials (Erev) are given as means $\pm$ s.e.means, number of cells in parentheses; ND = not determined. The concentrations of agonists applied to the rat hippocampus, the guinea-pig hippocampus and the rat septum were as follows, DP-5-CT: 3, 30 and 1 μ M; 8-OH-DPAT: all 3 μ M; flesinoxan: 30, 30 and 1 μ M and 5-methyl-urapidil: 30, 30 and 10 μ M, respectively. *Data taken from van den Hooff & Galvan (1992).

Figure 4 (a) A hyperpolarization evoked by 10 μ M 5-hydroxytryptamine (5-HT) in a single guinea-pig hippocampal CA1 neurone. (b) The response recorded from the same cell after 60 min superfusion with 3 μ M 5-methyl-urapidil; the 5-HT-induced hyperpolarization was blocked. The electronic potentials were elicited by constant current pulses (-0.25 nA, 250 ms, 0.1 Hz). Note that the response of this cell to 5-HT in control solution was slightly larger than average (see text and Table 1).

5-Methyl-urapidil is a potent ligand at 5-HT_{1A} receptors and is also an effective α_1 -adrenoceptor antagonist (Hanft & Gross, 1989). In order to exclude the possibility that the hyperpolarizations elicited by this compound were due to α_1 -antagonist activity, the actions of the α_1 -receptor antagonist, prazosin, were tested in the septal neurones. In 4 cells, a 5–8 min application of 3 μ M prazosin failed to evoke a significant change in membrane potential (-0.3 ± 0.2 mV).

Relative efficacies of 5-HT_{1A} ligands

The concentration-response relationships for the hyperpolarizations elicited by 5-HT demonstrate that 30 μ M appears to be a just-maximal concentration in hippocampal and septal neurones. To determine the efficacies of the various 5-HT_{1A}-receptor agonists, hippocampal neurones were first exposed to 30 μ M 5-HT and then, following complete recovery, to a 3 μ M concentration of synthetic agonist and in a separate series of experiments to 30 μ M 5-HT followed by 30 μ M synthetic agonist. The largest hyperpolarization induced by the synthetic agonist was taken as the maximum provided that (a) it was not significantly different from

Table 3 Relative efficacies of 5-HT_{1A}-receptor agonists measured in three types of rodent limbic neurones *in vitro*

	DP-5-CT	8-OH-DPAT	Flesinoxan	5-Methyl-urapidil
Rat hippocampus CA1	1.09 ± 0.07 (6)*	0.7 ± 0.08 (4)	0.5 ± 0.11 (7)	0.24 ± 0.04 (9)
Guinea-pig hippocampus CA1	1.0 ± 0 (4)*	0.69 ± 0.07 (9)	0.89 ± 0.08 (6)*	0 (5)
Rat septum	0.88†	0.69	0.82†	0.7

Relative efficacies were estimated in single cells in the rat and guinea-pig hippocampus and the results are expressed as the means ± s.e.means (*n*). Results from the rat septum were estimated from mean concentration-response curves. **P* > 0.05 compared to mean of 1 (One-sample Student's *t* test) and †*P* > 0.05 mean maximum hyperpolarization compared to mean maximum 5-HT hyperpolarization (ANOVA). DP-5CT, N,N-dipropyl-5-carboxamido tryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-n-propyl-amino)tetralin.

Figure 5 Concentration-response curves for flesinoxan (○; *n* = 4–5) and 5-methyl-urapidil (●; *n* = 3–7) measured in the rat dorso-lateral septal nucleus. Ordinates and abscissae are as in Figure 1.

the maximum 5-HT response or (b) the hyperpolarization to the higher concentration of agonists was not significantly larger than that to the lower concentration. The relative amplitudes of the 'maximum' hyperpolarizations allowed an estimate of the relative efficacies of the synthetic ligands. In the case of septal neurones the intrinsic activity was determined from the concentration-response curves.

The relative efficacies of the various agonists are shown in Table 3. These estimates show that DP-5-CT is a full agonist and 8-OH-DPAT a strong partial agonist in all three regions. Flesinoxan was effectively a full agonist in the rat septum and the guinea-pig hippocampus but exhibited reduced efficacy in the rat hippocampus. 5-Methyl urapidil behaved as a strong partial agonist in the rat septum, a weak partial agonist in the rat hippocampus and as an antagonist in the guinea-pig hippocampus.

Discussion

The aim of the present experiments was to compare the action of 5-HT and several hypotensive, 5-HT_{1A}-receptor agonists on single neurones in three different brain regions under identical experimental conditions.

5-HT hyperpolarized the three types of neurone under study most probably via an increase in potassium conductance as has been reported previously (Andrade & Nicoll, 1987a; Colino & Halliwell, 1987; Joëls *et al.*, 1987). The hypotensive agents, flesinoxan and 5-methyl-urapidil, also induced concentration-dependent hyperpolarizations of rat septal and rat hippocampal neurones that reversed at potentials close to the predicted potassium equilibrium potential. The time course of the responses to these compounds was similar to that of the known 5-HT_{1A}-receptor agonists, 8-OH-

DPAT and DP-5-CT (Andrade & Nicoll, 1987b; van den Hooff & Galvan, 1992). The reasons for the observed prolonged recovery phase are unknown; however, it was interesting to note that in guinea-pig hippocampal neurones, the recovery from 5-HT itself took significantly longer than that observed after activation of 5-HT_{1A}-receptors in rat hippocampus (Andrade & Nicoll, 1987a; Beck *et al.*, 1992), rat septum (Joëls *et al.*, 1987; van den Hooff & Galvan, 1992) and rat dorsal raphe nucleus (Sprouse & Aghajanian, 1987).

In the septal nucleus, the rank order of potency estimated from concentration-response curves was flesinoxan > 5-methyl-urapidil > 5-HT. This is in good agreement with the rank order of affinities of these compounds for 5-HT_{1A}-receptors obtained from radioligand binding studies. The reported pK₃₀ or K_i values are 8.8, 8.4 and 8.0, respectively (Gross *et al.*, 1987; Wouters *et al.*, 1988). In addition, radioligand binding experiments have demonstrated that with the exception of 5-methyl-urapidil, which has high affinity for α₁-adrenoceptors, the 4 synthetic agonists under study are relatively selective for 5-HT_{1A}-receptors (Gross *et al.*, 1987; Wouters *et al.*, 1988; Hoyer, 1991). Since the α₁-adrenoceptor antagonist, prazosin, did not mimic the action of 5-HT on rat septal neurones, it can be reasonably concluded that all of these compounds were acting on the same receptor subtype, namely 5-HT_{1A}-receptors. The availability of potent and selective antagonists at 5-HT_{1A}-receptors, such as WAY 100135 (Fletcher *et al.*, 1993) will possibly allow more precise characterization of these agonists in the future.

The actions of compounds with high affinity for 5-HT_{1A}-receptors has been shown to differ, depending on the neurones tested (Gartside *et al.*, 1990) or even on the level of receptor expression in constructed cell systems (Boddeke *et al.*, 1992). For example, the partial agonists, buspirone, ipsapirone and MDL 73,005EF showed changes in efficacy (relative to 5-HT) from 0 to 80% depending on the level of receptor expression in a cell line expressing human 5-HT_{1A}-receptors (Boddeke *et al.*, 1992). The present experiments also sought to resolve the question of whether these compounds exhibit differences in relative efficacy because (1) there are differences in receptor reserve (Meller *et al.*, 1990), (2) there are differences in coupling efficiency to second messenger systems (Fargin *et al.*, 1989) or (3) there are intrinsic differences in the receptors.

The present results show that in the neurones studied here, 8-OH-DPAT behaved as a strong partial agonist in all three cell types and DP-5-CT was a full agonist in both hippocampal neurones and probably a full agonist in the rat septum. However, the estimates of intrinsic activity show that flesinoxan had a higher efficacy in the guinea-pig hippocampus than in the rat hippocampus, whereas 5-methyl-urapidil showed the reverse behaviour. Furthermore, in the case of 5-methyl-urapidil, the efficacy in the guinea-pig hippocampus was so low as to render the compound an antagonist. It is unclear, at present, how these results could be accounted for by changes in receptor reserve or differences in coupling

efficiency to second messengers, which should affect both compounds in a similar manner. Thus, the actions of flesinoxan and 5-methyl-urapidil tend to support the hypothesis that the guinea-pig and rat hippocampal 5-HT_{1A}-receptors are not identical. The human (Fargin *et al.*, 1988) and rat (Albert *et al.*, 1990) 5-HT_{1A}-receptors have already been cloned and sequenced, but to the best of our knowledge, this had not been done with guinea-pig tissue. Since it has already been shown that several 5-HT receptors exhibit small sequence differences between species leading to significant changes in pharmacology (Hartig *et al.*, 1992), it would be of great interest to see if this type of interspecies variation can account for the present findings.

8-OH-DPAT, flesinoxan and 5-methyl-urapidil all lower blood pressure in experimental animals (Fozard & Ramage,

1984; Wouters *et al.*, 1988; Mandal *et al.*, 1991), and the structurally-related compound, urapidil, is an effective antihypertensive drug in man (Schook *et al.*, 1989). The hypotensive actions of these compounds have been postulated to arise from an action at brain stem neurones containing 5-HT_{1A} receptors (Fozard & Mir, 1987; Gillis *et al.*, 1987). The present results show that 8-OH-DPAT, flesinoxan and 5-methyl-urapidil have direct, potent inhibitory actions on mammalian central neurones mediated via 5-HT_{1A}-receptors. If these compounds also hyperpolarize the membrane potential of brain stem medullary neurones, the resulting decrease of tonic drive to spinal preganglionic sympathetic neurones (Ramage, 1990) could contribute to their hypotensive actions.

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