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The effect of the selective 5-HT_{1A} agonists alnespirone (S-20499) and 8-OH-DPAT on extracellular 5-hydroxytryptamine in different regions of rat brain

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1 We have examined the effects of the systemic administration of the selective 5-HT_{1A} agonist alnespirone (S-20499) on *in vivo* 5-hydroxytryptamine (5-HT) release in the dorsal raphe nucleus, the median raphe nucleus and four forebrain areas innervated differentially by both (dorsal striatum, frontal cortex, ventral hippocampus and dorsal hippocampus).

2 Alnespirone (0.1–3 mg kg^{-1}, s.c.) dose-dependently reduced extracellular 5-HT in the six areas examined. In forebrain, the maximal reductions occurred in striatum and frontal cortex (maximal reduction to 23 and 29% of baseline, respectively). Those in dorsal and ventral hippocampus were more moderate (to ca 65% of baseline). In contrast, the decrease in 5-HT elicited in the median raphe nucleus was more marked than that in the dorsal raphe nucleus (to ca 30 and 60% of baseline, respectively). The selective 5-HT_{1A} antagonist WAY-100635 (0.5 mg kg^{-1}, s.c.) prevented the decrease in 5-HT induced by alnespirone (0.3 mg kg^{-1}, s.c.) in frontal cortex.

3 8-OH-DPAT (0.025, 0.1 and 0.3 mg kg^{-1}, s.c.) also reduced extracellular 5-HT in a regionally-selective manner (e.g., to 32% of baseline in striatum and to 69% in dorsal hippocampus at 0.1 mg kg^{-1}, s.c.). In midbrain, 8-OH-DPAT reduced the dialysate 5-HT slightly more in the median than in the dorsal raphe nucleus at all doses examined.

4 Doses of both compounds close to their respective ED_{50} values (0.3 mg kg^{-1} alnespirone, 0.025 mg kg^{-1} 8-OH-DPAT) reduced 5-HT to a comparable extent in all regions examined. However, the reductions attained at higher doses were more pronounced for 8-OH-DPAT.

5 These data show that the reduction of 5-HT release elicited by alnespirone and 8-OH-DPAT is more important in forebrain areas innervated by 5-hydroxytryptaminergic neurones of the dorsal raphe nucleus. This regional selectivity seems unlikely to be accounted for by differences in the sensitivity of 5-HT_{1A} autoreceptors controlling 5-HT release, given the dissimilar effects of these two 5-HT_{1A} agonists in regions rich in cell bodies and nerve terminals. This suggests the presence of complex mechanisms of control of 5-HT release by 5-HT_{1A} receptors.

Keywords: 5-Hydroxytryptamine (5-HT) release; 5-HT_{1A} agonists; antidepressants; dorsal raphe nucleus; dorsal striatum; hippocampus; median raphe nucleus; microdialysis

Introduction

The activation of somatodendritic 5-HT_{1A} receptors inhibits the firing activity of 5-hydroxytryptamine (serotonin, 5-HT) neurones (Sprouse & Aghajanian, 1986; 1987; Blier & de Montigny, 1987). These receptors are coupled to a potassium channel through G_o/G_i proteins (Aghajanian & Lakoski, 1984; Innis & Aghajanian, 1987). 5-HT_{1A} receptors are also localized postsynaptically to 5-HT terminals, mainly in limbic and cortical structures (Marcinkiewicz *et al*., 1984; Pazos & Palacios, 1985; Pompeiano *et al*., 1992). Postsynaptic 5-HT_{1A} receptor activation is also associated with a reduction of neuronal activity in forebrain and brain stem (Andrade *et al*., 1986; Araneda & Andrade, 1991; Stevens *et al*., 1992).

5-HT_{1A} receptor agonists exhibit anxiolytic and/or antidepressant activity in experimental models (see de Vry, 1995 for review) and some members of the azapirone family (e.g., buspirone, gepirone) are used in the treatment of anxiety (Pecknold, 1994). The new aminochroman derivative S-20499 (alnespirone) has a very high affinity for 5-HT_{1A} receptors and shows anxiolytic activity in animal models (Porsolt *et al*., 1992; Kidd *et al*., 1993; Barrett *et al*., 1994). It inhibits the forskolin-activated adenylate cyclase in hippocampal homogenates and suppresses the firing of midbrain 5-hydroxytryptaminergic neurones (Kidd *et al*., 1993). It also reduces 5-HT turnover rate in various brain areas without altering that of dopamine (Kidd *et al*., 1993). Unlike azapirones, alnespirone is not metabolized to 1-(2-pyrimidinyl)-piperazine, a compound which antagonizes α_2-adrenoceptors (Bianchi *et al*., 1988) and therefore it does not directly interact with catecholaminergic neurones (Dugast *et al*., unpublished observations).

The dorsal and median raphe nuclei of the midbrain (DRN and MRN, respectively) contain the vast majority of ascending 5-hydroxytryptaminergic neurones (Dahlström & Fuxe, 1964). There is exhaustive anatomical and neurochemical evidence on a differential innervation of cortical and subcortical structures by neurones of these two nuclei (Jacobs *et al*., 1974; Lorens & Guldberg, 1974; Bobilier *et al*., 1975; Moore & Halaris, 1975; Azmitia & Segal, 1978; Köhler & Steinbusch, 1982; Imai *et al*., 1986; Kosofsky & Molliver, 1987; Mamounas & Molliver, 1988; Vertes, 1991; McQuade & Sharp, 1997). 5-Hydroxytryptaminergic neurones of both midbrain raphe nuclei are endowed with somatodendritic 5-HT_{1A} receptors (Pazos & Palacios, 1985; Weissman-Nanopoulos *et al*., 1985; Sotelo *et al*., 1990). However, despite the role played by the 5-HT system in anxiety and depression, the degree of self-regulation of ascending DRN and MRN 5-hydroxytryptaminergic tracts through 5-HT_{1A} autoreceptors is still controversial. Initial electrophysiological studies demonstrated a greater sensitivity of DRN neurones to the firing-suppressant actions of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (Sinton & Fallon, 1988; Blier *et al*., 1990), a view challenged by more recent data (Hajos *et al*., 1995). Neurochemical studies, in

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which 5-HT$_{1A}$ agonists were employed, have also yielded conflicting results (Hjorth & Sharp, 1991; Invernizzi *et al*., 1991; Kreis & Lucki, 1994; Casanovas & Artigas, 1996).

The present study was aimed at examining the ability of alnespirone to reduce the 5-HT output in six different brain areas, the DRN and MRN of the midbrain and four forebrain areas differentially innervated by axons of one or other nucleus i.e., dorsal striatum (STR), frontal cortex (FC) and hippocampus (dorsal and ventral; DHPC and VHPC, respectively). Given the existing discrepancies on the regional effects of 5-HT$_{1A}$ agonists on *in vivo* 5-HT release, we carried out an additional comparative study with the prototypical 5-HT$_{1A}$ agonist 8-OH-DPAT in four brain areas, DRN, MRN, STR and DHPC.

Methods

Animals

Male Wistar rats (Iffa Credo, Lyon, France) weighing 280–320 g were used. Animals were kept in a controlled environment (12 h light-dark cycle and 22 ± 2°C room temperature). Food and water were provided *ad libitum* before and during the experiments. Animal care followed the European Union regulations (O.J. of E.C. L358/1 18/12/1986).

Surgery and microdialysis procedures

Microdialysis procedures were performed essentially as described in Adell & Artigas (1991). Anaesthetized rats (pentobarbitone, 60 mg kg^{-1}, i.p.) were stereotaxically implanted with one I-shaped probe. The brain regions and coordinates are shown in Table 1 (Paxinos & Watson, 1986). The length of membrane exposed to the brain tissue was 4 mm long (o.d. 0.25 mm) in FC, STR and VHPC, and 1.5 mm long in DHPC, DRN and MRN. Animals were allowed to recover from surgery for approximately 20 h and then probes were perfused with artificial cerebrospinal fluid (CSF) (125 mM NaCl, 2.5 mM KCl, 1.26 mM $CaCl_2$ and 1.18 mM $MgCl_2$) containing 1 μM citalopram at 0.25 μl min^{-1}. Sample collection started 60 min after the beginning of perfusion. Dialysate samples were collected every 20 min (5 μl). Usually 6 fractions were collected before drug administration, of which four were used to obtain the individual basal values. Drugs were administered between 12 h 00 min and 13 h 00 min. At the end of microdialysis experiments, the animals were anaesthetized with pentobarbitone and killed by decapitation. Dialysis probes were perfused with a solution of a dye and their location was checked by visual inspection after cutting the brains at the appropriate levels. The data of animals with probes outside the limits of the brain structures of interest were not included in the calculations.

Table 1 Location and size of the microdialysis probes

	Size (mm)	*AP*	*L*	*DV*
Frontal cortex	4	+3.2	−2.5	−6.0
Dorsal striatum	4	+0.2	−3.0	−8.0
Ventral hippocampus	4	−5.8	−5.0	−8.0
Dorsal hippocampus	1.5	−3.8	−1.8	−4.0
Dorsal raphe nucleus	1.5	−7.8	−3.1[a]	−7.5
Median raphe nucleus	1.5	−7.8	−2.0[a]	−8.9

[a]Probes in the dorsal and median raphe nuclei were implanted with lateral angles of 30° and 13°, respectively, to avoid obstruction of the cerebral aqueduct.

Chromatographic analysis

5-HT was analysed by a modification of a high performance liquid chromatography (h.p.l.c.) method previously described (Adell & Artigas, 1991). The composition of h.p.l.c. eluant was as follows: 0.15 M NaH_2PO_4, 1.3 mM octyl sodium sulphate, 0.2 mM EDTA (pH 2.8 adjusted with phosphoric acid) plus 27% methanol. 5-HT was separated on a 3 μm ODS 2 column (7.5 cm × 0.46 cm; Beckman, CA) and detected amperometrically with a Hewlett Packard 1049 detector (oxidation potential +0.6 V). Retention time was 3.5–4 min. The absolute detection limit for 5-HT was 0.5–1 fmol/sample. Minimal values of dialysate 5-HT during the periods of maximal inhibition were typically above 1.5–2 fmol/sample. Dialysate 5-HT values were calculated by reference to standard curves run daily.

Drugs and reagents

5-HT and 8-OH-DPAT were from RBI (Natick, MA) and 5-hydroxyindoleacetic acid (5-HIAA) from Sigma (St. Louis, MO, U.S.A.). WAY-100635 (*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridyl)cyclohexanecarboxamide 3HCl) (Fletcher *et al*., 1995), alnespirone and citalopram were kindly provided by Wyeth Ayerst, Institut de Recherches Internationales Servier and Lundbeck A/S, respectively. Other materials and reagents were from local commercial sources. Alnespirone (0.1, 0.3, 1 and 3 mg kg^{-1}), 8-OH-DPAT (0.025, 0.1 and 0.3 mg kg^{-1}) and WAY-100635 (0.5 mg kg^{-1}) were dissolved in saline and injected s.c at the doses indicated (1–2 ml kg^{-1}). Control animals received saline.

Data and statistical analysis

Microdialysis results are expressed as fmol/fraction (uncorrected for recovery) or as percentages of basal values (individual means of four predrug fractions). ED_{50} values were calculated by adjusting data points (peak reduction or AUCs) to sigmoidal curves by the GraphPad Prism programme. Statistical analysis (SPSS/PC+) of drug effects on dialysate 5-HT was performed by one- or two-way analysis of variance (ANOVA) for repeated measures of raw data with dose or region as independent factors. Analysis of variance of AUCs or peak reductions was also used to examine further the effect of dose and region factors on the 5-HT output. Data are given as means ± s.e.mean. The number of animals in each group is given in figure legends. Statistical significance has been set at the 95% confidence level (two tailed).

Results

Regional dialysate values

Basal dialysate 5-HT values in the different regions are shown in Table 2. Greater 5-HT values were found in the MRN, followed by the DRN. Lower 5-HT concentrations were found in dialysates from the four different forebrain areas examined.

Dose-dependent reduction of extracellular 5-HT by alnespirone in dorsal striatum

In a first experiment, we studied the effects of alnespirone on extracellular 5-HT in dorsal striatum, an area innervated by 5-hydroxytryptaminergic axons of DRN neurones (Azmitia & Segal, 1978). Alnespirone significantly reduced extracellular 5-HT concentrations in the range 0.3–3 mg kg^{-1} (Figure 1). Two-way ANOVA for repeated measures revealed a significant effect of time ($P<0.001$) and dose ($P<0.003$) and a significant interaction between both ($P<0.001$). The lower dose used (0.1 mg kg^{-1}) caused a moderate but non-significant reduction of extracellular 5-HT (one-way ANOVA). Maximal effects were noted for the dose of 3 mg kg^{-1}, which elicited a long-

lasting reduction of extracellular 5-HT to 25–30% of baseline. The calculated ED_{50} was 0.19 mg kg^{-1} when using maximal reductions of 5-HT and 0.50 mg kg^{-1} when using AUCs for the whole period examined (160 min) (Figure 1). This difference is probably accounted for by the comparable peak reductions at the 0.3 and 1 mg kg^{-1} doses but greater duration of the effect at the latter dose. Further analysis of the regional effects of alnespirone on extracellular 5-HT was then conducted with the doses of 0.3 mg kg^{-1} (close to the ED_{50}) and 3 mg kg^{-1} (maximal effect).

Effect of alnespirone on extracellular 5-HT in forebrain

In addition to STR, we examined the effect of alnespirone in three more forebrain areas, FC, DHPC and VHPC, on the basis of their differential innervation by axons of 5-HT neurones of the DRN and MRN. Alnespirone reduced extracellular 5-HT to a similar extent in STR and FC at 0.3 and 3 mg kg^{-1} (Figure 1). Extracellular 5-HT in DHPC and VHPC was less affected by alnespirone. At 0.3 mg kg^{-1}, the changes in 5-HT were not different from those produced by a saline injection (Figure 1). Moderate and transient 35–40% reductions were noted after administration of 3 mg kg^{-1} alnespirone.

Table 3 shows the F and P values of the repeated factor (time), the independent factor (region or dose) and the interaction between repeated and independent factors, corresponding to the regional effects of alnespirone, analysed by means of two-way repeated-measures ANOVA.

Table 2 Baseline dialysate 5-HT concentrations in different rat brain regions

Region	*5-HT* (fmol/fraction)
Dorsal raphe nucleus ($n=41$)	26.1±1.6
Median raphe nucleus ($n=34$)	41.9±3.5
Dorsal striatum ($n=51$)	20.5±1.2
Frontal cortex ($n=19$)	18.0±1.4
Ventral hippocampus ($n=20$)	17.8±0.1
Dorsal hippocampus ($n=37$)	8.2±0.4

Data are expressed as means±s.e.mean of the number of animals shown in parentheses. Dialysate 5-HT values have not been corrected for length of the dialysis probes (1.5 mm in DRN, MRN and DHPC; 4 mm in FC, STR and VHPC).

Effects of alnespirone on extracellular 5-HT in the midbrain raphe nuclei

Unlike in forebrain, the injection of saline significantly elevated extracellular 5-HT in the dorsal and median raphe nuclei to 163±38% and 211±52% of baseline, respectively ($P<0.0001$, repeated measures ANOVA). This effect is probably attributable to the stress associated to handling and injection (Adell *et al.*, 1997).

At 0.3 mg kg^{-1}, alnespirone reduced extracellular 5-HT similarly in both nuclei with a similar time course (Figure 2). Marked differences were noted at 3 mg kg^{-1}. In the DRN, extracellular 5-HT was maximally reduced to ca 60% of baseline 40 min after administration and persisted at this level until the end of the experiments. This reduction was not different from that achieved at 0.3 mg kg^{-1}. Yet, in the MRN, 3 mg kg^{-1} alnespirone maximally reduced 5-HT to ca 30% of baseline at the end of the experiment (Figure 2). These differ-

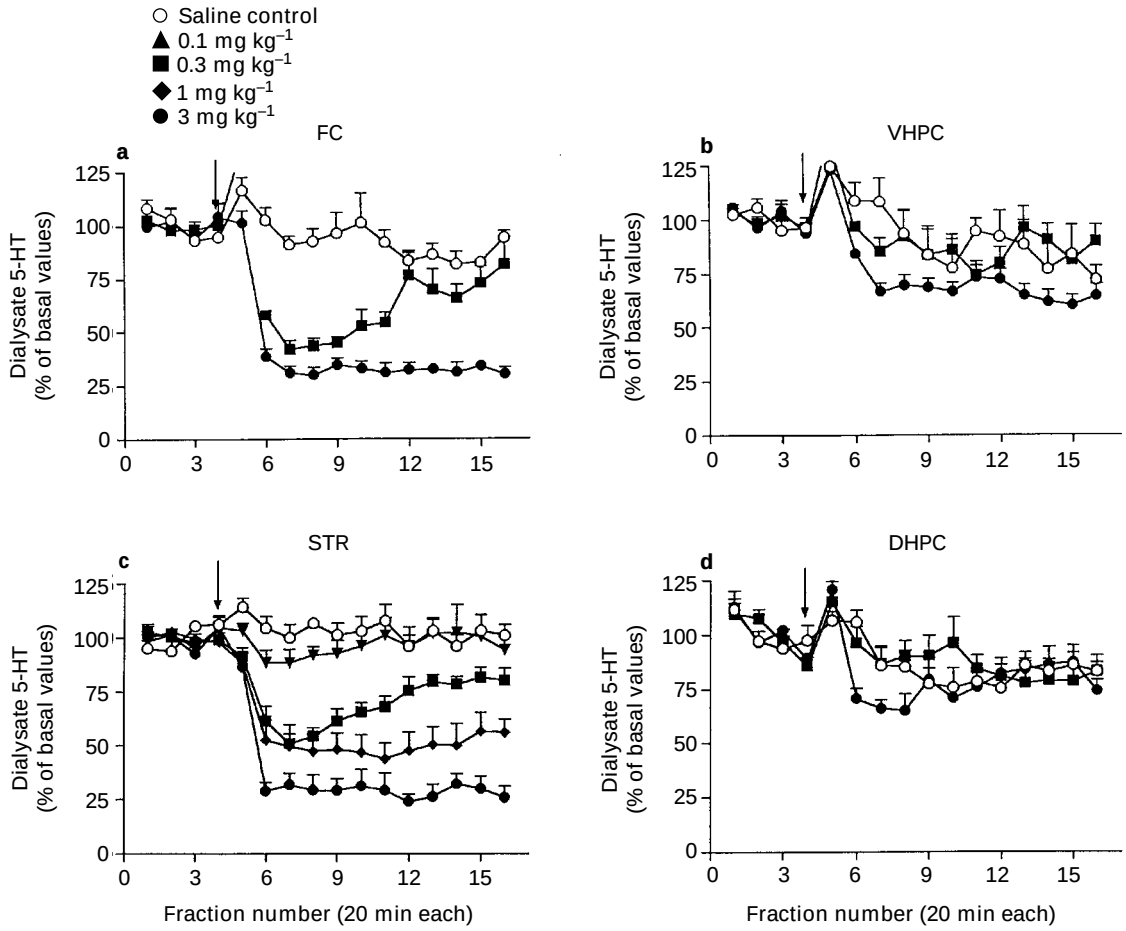

Figure 1 Dose-dependent reductions of the 5-HT concentration in dialysates from forebrain areas after single administration of saline or alnespirone (arrow), 0.1 mg kg^{-1}, 0.3 mg kg^{-1}, 1 mg kg^{-1} and 3 mg kg^{-1}. The number of rats per group (increasing dose order) is as follows, dorsal striatum (STR; $n=6, 7, 6, 6, 6$), frontal cortex (FC; $n=5, 5, 5$), dorsal hippocampus (DHPC; $n=6, 7, 7$) and ventral hippocampus (VHPC; $n=6, 7, 7$). Data points are means and vertical lines show s.e.mean. See Table 3 for statistical details.

Table 3 Significance of two-way repeated measures ANOVA of the effects of alnespirone on dialysate 5-HT concentrations

	F	P *value*
Region factor		
Dose: 0.3 mg kg^{-1}		
Time	18.42	0.001
Region	5.47	0.000
Time × region	1.72	0.001
Dose: 3 mg kg^{-1}		
Time	43.61	0.000
Region	3.76	0.008
Time × region	6.80	0.000
Dose factor		
Dorsal raphe nucleus		
Time	23.90	0.000
Dose	4.58	0.025
Time × dose	1.85	0.012
MRN		
Time	15.88	0.000
Dose	1.34	NS
Time × dose	1.94	0.008
Dorsal striatum		
Time	4.94	0.000
Dose	2.07	NS
Time × dose	2.50	0.000
Frontal cortex		
Time	25.62	0.000
Dose	40.15	0.000
Time × dose	4.32	0.000
Dorsal hippocampus		
Time	6.97	0.000
Dose	0.89	NS
Time × dose	1.60	0.044
Ventral hippocampus		
Time	11.44	0.000
Dose	1.73	NS
Time × dose	1.10	NS

ences in the time course may be possibly accounted for by the spontaneous decline of dialysate 5-HT in median raphe neurones, which amounted to approximately 25% over the 320 min of sample collection (see saline controls in Figure 2). On account of the constant difference between the curves of control rats and those treated with 3 mg kg^{-1} alnespirone, it seems reasonable to assume that the maximal reduction of 5-HT output in the MRN was also achieved soon (40–60 min) after administration of alnespirone and persisted until the end of the experiment. Extracellular 5-HT in DHPC and VHPC also exhibited a moderate spontaneous decline with time, which was more marked than that observed in the DRN or its two projection areas examined, STR and FC (see Figures 1 and 2).

Antagonism of the effect of alnespirone by WAY-100635

The specificity of the effects of alnespirone on 5-HT$_{1A}$ receptors was assessed with the selective 5-HT$_{1A}$ antagonist WAY-100635 (Fletcher *et al*., 1995). Maximal effects of this agent were noted 40 min after s.c. administration and declined rapidly (Romero *et al*., 1996; Romero & Artigas, 1997). As the maximal reduction elicited by 0.3 mg kg^{-1} alnespirone took place at the same time, we administered both agents concurrently. WAY-100635 (0.5 mg kg^{-1}, s.c.) fully prevented the reduction caused by alnespirone in FC ($P<0.000$, significant effect of the time and treatment factors, $P<0.017$, significant treatment × time interaction; two-way repeated measures ANOVA) (Figure 3). AUCs for alnespirone and alnespirone plus WAY 100635 were 66.4±5.4% and 96.3±4.2%, respectively.

Regional comparisons

The AUCs for the effects of alnespirone at 0.3 and 3 mg kg^{-1} during the period 40 min–240 min post-administration were

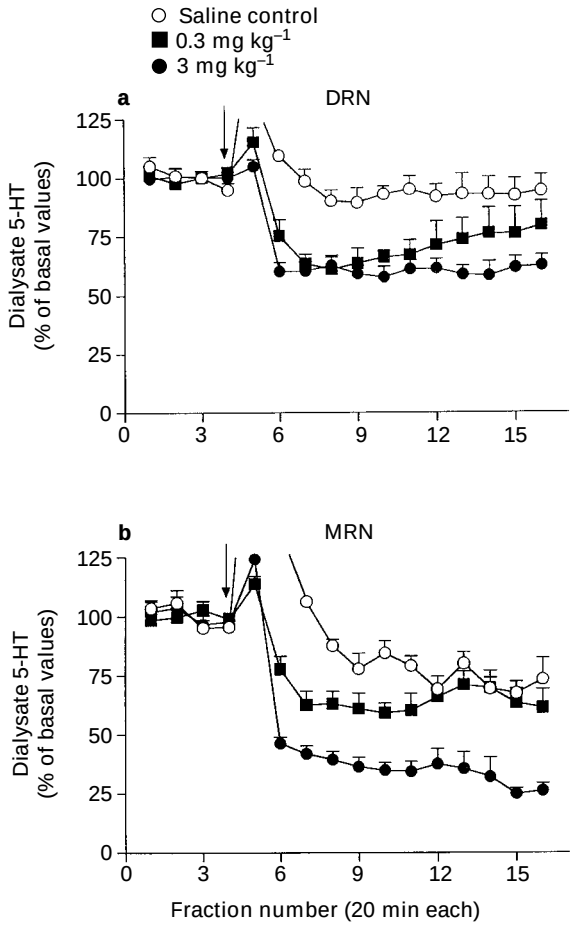

Figure 2 Dose-dependent reductions of the 5-HT concentration in dialysates from the dorsal and median raphe nuclei after a single administration of saline or alnespirone (arrow), 0.3 mg kg^{-1} and 3 mg kg^{-1}. The number of rats per group (increasing dose order) is as follows, dorsal raphe nucleus (DRN; $n=5$, 7, 9), median raphe nucleus (MRN; 5, 6, 6). The maximal value in the ordinates has been set at 125% to enable a visual comparison of the effects of alnespirone and 8-OH-DPAT in all brain areas. Maximal increments produced by saline injection in the dorsal and median raphe nuclei were respectively 163±38% and 211±52% (fraction no. 5). Data points are means and vertical lines show s.e.mean. See Table 3 for statistical details.

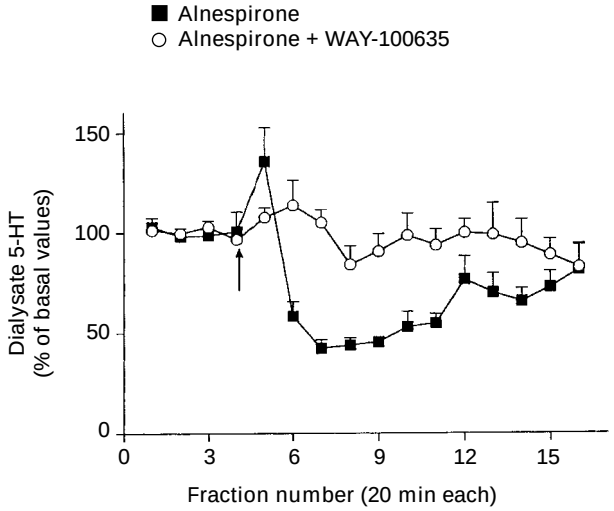

Figure 3 Antagonism of the reduction of dialysate 5-HT concentrations induced by alnespirone in frontal cortex by WAY-100635. Alnespirone 0.3 mg kg^{-1} ($n=5$) or alnespirone 0.3 mg kg^{-1} plus WAY-100635 0.5 mg kg^{-1} ($n=4$) were administered. Injections are shown by an arrow.

calculated and expressed as percentages of control (saline) rats to compensate for the spontaneous decline of 5-HT in some brain areas (see above). The first fraction post-administration was omitted due to the large elevations produced by the injection in the two raphe nuclei. One-way ANOVA revealed a significant effect of the region at the dose of 0.3 mg kg^{-1} ($P<0.0008$). *Post-hoc t* test revealed the existence of two different subgroups, DRN, MRN, STR, FC on one side and DPHC and VHPC on the other. The same analysis conducted on peak reductions also indicated a significant region factor ($P<0.0001$) with the same grouping of brain regions (Figure 4).

The administration of 3 mg kg^{-1} also reduced extracellular 5-HT (AUC values) in a differential manner ($P<0.0001$, one-way ANOVA). Post-hoc *t* test grouped the six regions in a manner different from that at 0.3 mg kg^{-1}, as follows. MRN, STR, FC vs DRN, DHPC and VHPC. The same analysis, conducted on peak reductions also yielded a significant effect of the region factor ($P<0.0001$) and the same *post-hoc* differences between regions.

Reduction of extracellular 5-HT by 8-OH-DPAT

We examined the effects of the systemic administration of 8-OH-DPAT on extracellular 5-HT in STR, DHPC and the two raphe nuclei. Only two forebrain regions, representative of the innervation by DRN and MRN axons, were chosen because of the comparable effects of ipsapirone (Casanovas & Artigas, 1996) and alnespirone in FC and STR on one side, and in the two hippocampal areas on the other. Moreover, in pilot experiments, 8-OH-DPAT reduced extracellular 5-HT to a similar extent in FC and STR.

The s.c. administration of 8-OH-DPAT (0.025, 0.1 and 0.3 mg kg^{-1}) reduced dose-dependently 5-HT in dialysates from these brain areas (Figure 5). At 0.025 mg kg^{-1}, 8-OH-DPAT significantly reduced extracellular 5-HT in DRN (peak reduction: 60±3% of baseline), MRN (59±5% of baseline) and STR (50±4% of baseline) but not in DHPC (93±14% of baseline). These values were similar to those observed after the administration of 0.3 mg kg^{-1} alnespirone (Figure 6). A similar regional pattern was observed at 0.1 mg kg^{-1} 8-OH-DPAT, with peak reductions to 31±3%, 19±3%, 32±2% and 70±11% of baseline, respectively. As with 0.025 mg kg^{-1}, these took place 40 min after 8-OH-DPAT administration. Maximal effects at 0.3 mg kg^{-1} were also noted at this time but persisted for 2–3 more fractions (except in DHPC) (Figure 5). Dialysate 5-HT concentrations were reduced to 24±2%, 14±3%, 20±2% and 41±8% of baseline in DRN, MRN, STR and DHPC, respectively. The reduction of extracellular 5-HT by 0.3 mg kg^{-1} 8-OH-DPAT was superior to that elicited by 3 mg kg^{-1} alnespirone in all areas. The differences in maximal effects were more marked in the two raphe nuclei than in STR or DHPC (Figure 6).

Figure 7 shows the dose-response curves for the 5-HT-reducing action of 8-OH-DPAT in the four brain areas examined. The ED$_{50}$ values calculated for DRN, MRN, STR and DHPC, by use of peak reductions, were 0.024, 0.026, 0.017 and 0.158 mg kg^{-1}, respectively. The curves for the DRN and STR were close to that of MRN but latter had a somewhat more pronounced slope. That corresponding to the DHPC was clearly different from the rest of the brain areas examined, with a lower maximal effect and a greater ED$_{50}$

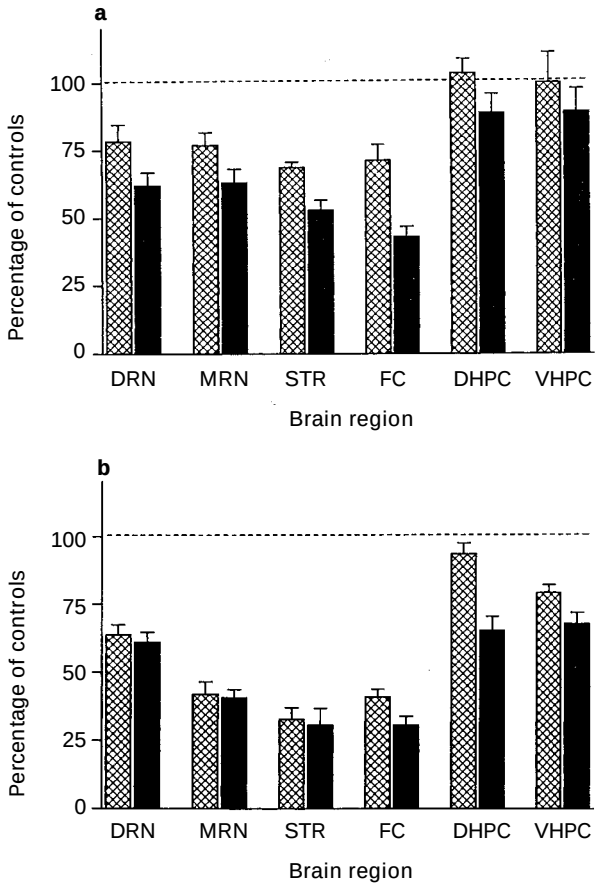

Figure 4 The regional effects of alnespirone (a) 0.3 mg kg^{-1} and (b) 3 mg kg^{-1} on dialysate 5-HT in six rat brain areas: DRN, dorsal raphe nucleus; median raphe nucleus MRN; STR dorsal striatum; FC, frontal cortex; DHPC, dorsal hippocampus; VHPC, ventral hippocampus. Cross-hatched columns express the effect of alnespirone as AUC values. Solid columns refer to maximal percentual reductions of dialysate 5-HT at each dose and region. See text for statistical comparisons.

Discussion

The selective 5-HT$_{1A}$ agonists 8-OH-DPAT and alnespirone reduced the *in vivo* 5-HT release in brain, in agreement with their inhibitory effects on 5-hydroxytryptaminergic cell firing (Sprouse & Aghajanian, 1987; Kidd *et al*., 1993). The extracellular 5-HT reductions effects by alnespirone lasted for more than those by the prototypical 5-HT$_{1A}$ agonist 8-OH-DPAT, in agreement with the longer half-life of alnespirone (Institut de Recherches Internationales Servier, internal data file). The 5-HT-reducing action of alnespirone was fully counteracted by the selective 5-HT$_{1A}$ receptor agonist WAY-100635, which supports the exclusive involvement of 5-HT$_{1A}$ receptors in its action.

A dose of both agonists close to their ED$_{50}$ (0.025 mg kg^{-1} 8-OH-DPAT, 0.3 mg kg^{-1} alnespirone) produced equivalent reductions of extracellular 5-HT. Yet, at higher doses (0.3 mg kg^{-1} for 8-OH-DPAT, 3 mg kg^{-1} for alnespirone), the 5-HT decrease elicited by 8-OH-DPAT was greater than the produced by alnespirone, particularly in the DRN, which may suggest differences in the intrinsic activity of both compounds at high doses. It seems unlikely that the moderate affinity of 8-OH-DPAT for 5-HT$_7$ receptors (Ruat *et al*., 1993) may contribute to the *in vivo* effects of low doses of this agent. Thus, this apparent partial agonist character of alnespirone on DRN 5-HT release is in marked contrast with its full agonistic activity at somatodendritic 5-HT$_{1A}$ receptors controlling cell firing (Kidd *et al*., 1993) and with the greater inhibition of 5-HT release attained in STR or FC, mostly innervated by 5-HT axons of DRN neurones.

The present data add to existing evidence in support of greater inhibitory actions of 5-HT$_{1A}$ agonists in forebrain areas innervated by the DRN, as measured by neurochemical 5-HT indexes (Meller *et al*., 1990; Invernizzi *et al*., 1991; Kreiss & Lucki, 1994); Casanovas & Artigas, 1996). Such a preferential inhibitory effect is also displayed by the SSRIs (Romero & Artigas, 1997; Romero *et al*., 1997). The reasons for the homogeneous reduction of extracellular 5-HT in various

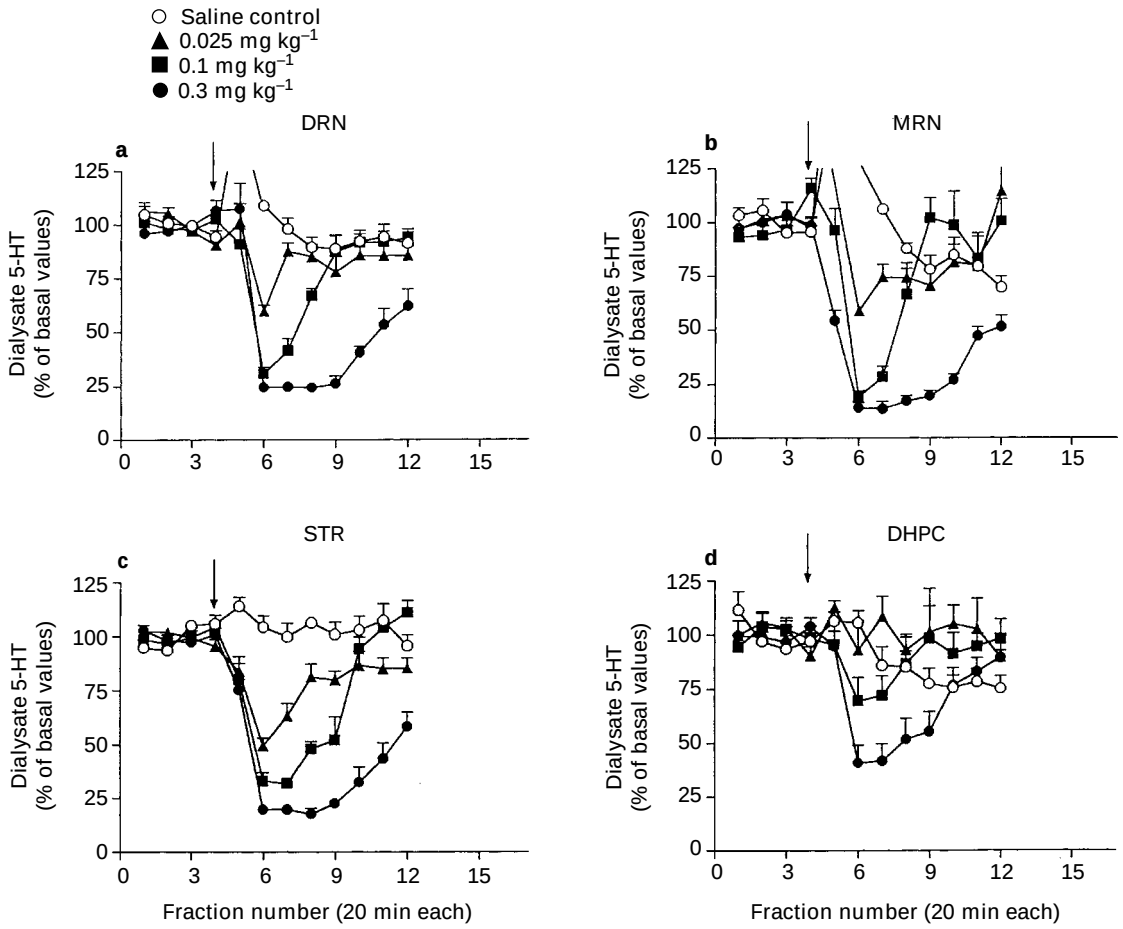

Figure 5 Reduction of extracellular 5-HT in the dorsal and median raphe nuclei as well as in selective projection areas (dorsal striatum and dorsal hippocampus, respectively) by 8-OH-DPAT (0.025 mg kg^{-1}, 0.1 mg kg^{-1}, 0.3 mg kg^{-1}). Saline controls are also depicted. The number of animals per group is as follows (increasing dose order) DRN, 5, 7, 7, 6; MRN 5, 6, 6, 5; STR, 6, 10, 6, 4; DHPC, 6, 6, 6, 5. Data points are means and vertical lines show s.e.mean. See Table 3 for statistical analysis.

forebrain areas after systemic 8-OH-DPAT administration observed in an earlier study (Hjorth & Sharp, 1991) are unclear but may perhaps derive from the use of anaesthetized animals as anaesthetics may change the balance between excitatory and inhibitory inputs to 5-hydroxytryptaminergic neurones (Tao & Auerbach, 1994). During the completion of the present study, McQuade *et al.* (1996) showed a greater inhibition of 5-HT release in FC (vs that in DHPC) of anaesthetized rats at 0.1 and 0.3 mg kg^{-1}, s.c., 8-OH-DPAT but not at 0.025 mg kg^{-1}. In the present study, the greater differences between the effect of 8-OH-DPAT in STR and DHPC were noted at 0.025 and 0.1 mg kg^{-1}. Moreover, we noted greater reductions of 5-HT release in STR at all doses (as compared to those obtained by McQuade *et al.* in FC). In the latter area, 0.025 and 0.1 mg kg^{-1}, s.c., 8-OH-DPAT reduced extracellular 5-HT to 30 and 15% of baseline, respectively (Casanovas & Artigas, unpublished observations). Thus, whereas the 5-HT decreases effected by 8-OH-DPAT appear to be comparable in the hippocampus of freely-moving and anaesthetized rats, marked differences occur in FC and STR.

This regional selectivity adds to previous evidence for a differential regulation of DRN and MRN neuronal groups. Rouquier *et al.* (1994) showed that the α_1-adrenoceptor antagonist prazosin decreased extracellular 5-HT more in hippocampus than in striatum (i.e., contrary to what happens with 5-HT$_{1A}$ agonists) and a recent study described the lack of a tonic inhibitory GABAergic control of MRN 5-hydroxytryptaminergic neurones (Tao *et al.*, 1996), which might account for the greater basal 5-HT output in MRN found in the present and a previous study (Casanovas & Artigas, 1996), despite the lower number of 5-HT neurones in the DRN.

It is unclear whether the more marked reductions of extracellular 5-HT in STR or FC can be accounted for by a greater sensitivity of DRN 5-HT$_{1A}$ autoreceptors (Sinton & Fallon, 1988), as this finding is controversial (Hajos *et al.*, 1995). Indeed, the greater effects of 5-HT$_{1A}$ agonists on extracellular 5-HT in areas innervated by the DRN could result from the greater density of 5-HT$_{1A}$ autoreceptors (Weismman-Nanopoulos *et al.*, 1985) and the receptor reserve in this nucleus (Meller *et al.*, 1990). However, several observations suggest that the control of 5-HT release by 5-HT$_{1A}$ receptors may be very complex. First, the reduction of extracellular 5-HT elicited by 5-HT$_{1A}$ agonists in forebrain does not strictly follow the relative density of innervation by the two raphe nuclei. Alnespirone, ipsapirone and paroxetine (the latter also in conditions of local blockade of the 5-HT uptake with 1 μM citalopram) reduced extracellular 5-HT to a comparable extent in DHPC and VHPC (Casanovas & Artigas, 1996; Romero & Artigas, 1997; Romero *et al.*, 1997), whereas the latter is more densely innervated by 5-hydroxytryptaminergic DRN fibres (Azmitia & Segal, 1978; McQuade & Sharp, 1995; 1997). Secondly, in agreement with a previous study with ipsapirone (Casanovas & Artigas, 1996), the reduction of 5-HT release caused by 8-OH-DPAT and alnespirone was comparable or even slightly greater in the MRN than in the DRN (contrary to what happens in their respective projection areas). Moreover, the larger striatal 5-HT reductions produced by the administration of alnespirone are difficult to reconcile with the more moderate reduction in DRN, particularly at the higher dose examined. Indeed, the origin of the 5-HT in dialysates from the raphe nuclei is far from being fully clarified. While a somatodendritic origin may be attributable, due to the large density of

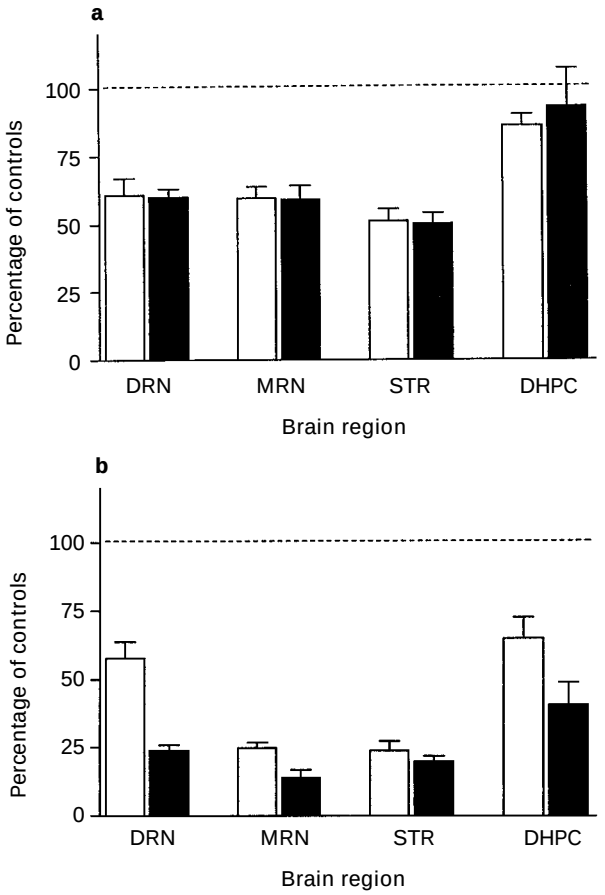

Figure 6 Comparison of the maximal effects of 8-OH-DPAT (solid columns) and alnespirone (open columns) at two different doses (a) 0.025 mg kg^{-1} 8-OH-DPAT vs 0.3 mg kg^{-1} alnespirone and (b) 0.3 mg kg^{-1} 8-OH-DPAT vs 3 mg kg^{-1} alnespirone. Columns (means ± s.e.mean) are maximal percentual reductions obtained after the single administration of each 5-HT$_{1A}$ agonist.

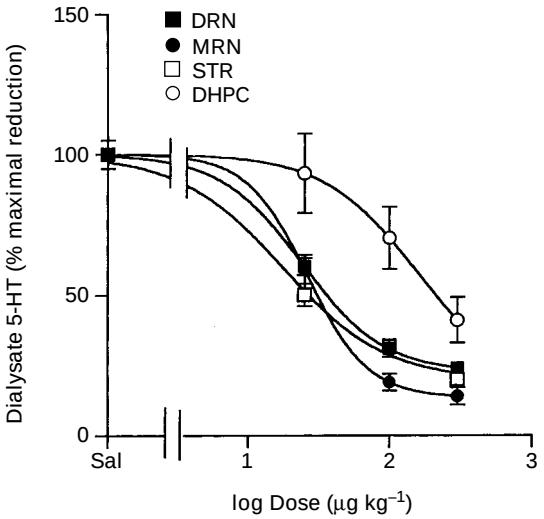

Figure 7 Dose-effect curves for the reduction of 5-HT output by 8-OH-DPAT in the dorsal raphe nucleus (DRN), median raphe nucleus (MRN) dorsal striatum (STR) and dorsal hippocampus (DHPC). Ordinates are the percentage reduction of 5-HT in dialysates during the time of maximal reduction (usually 40 min post-administration).

cell bodies and dendrites (Wiklund *et al*, 1981; Descarries *et al*., 1982), release from the extensive network of efferent fibres within the anatomical limits of these nuclei (see for instance Halliday *et al*., 1995) may contribute as well. Release of 5-HT by afferent fibres from other raphe nuclei could also be considered, since anatomical connections between the various raphe nuclei have been described (see Aghajanian *et al*., 1987; Jacobs & Azmitia, 1992 for review). In particular, Vertes (1991) described a moderate density of dorsal raphe fibres projecting to the MRN, thus raising the possibility that 5-HT in MRN dialysates partly derives from DRN afferents and displays their pharmacological characteristics. A preliminary dual probe study indicates that the infusion of citalopram in the DRN did not reduce extracellular 5-HT in the MRN (Casanovas & Artigas, unpublished observations). As this procedure causes an extensive reduction of extracellular 5-HT in projection areas of the DRN (Romero *et al*., 1994; Romero & Artigas, 1997), the 5-hydroxytryptaminergic character of this DRN-MRN connection can be ruled out. Therefore, 5-HT in MRN dialysates probably derives from cell bodies and fibres of 5-HT neurones in this nucleus. Based on this assumption, it seems logical to conclude that 5-HT$_{1A}$ autoreceptors locally controlling 5-HT release in the MRN have a comparable or even higher sensitivity (see alnespirone data) than those in the DRN. Yet, an opposite profile emerges when considering the changes of 5-HT release in forebrain.

These discrepancies may possibly suggest the additional involvement of local factors modulating the release of 5-HT. For instance, the activation of NMDA receptor enhances 5-HT release (Ohta *et al*., 1994; Fink *et al*., 1995). At the same time, 5-HT attenuates glutamate release through hippocampal postsynaptic 5-HT$_{1A}$ receptors (Matsuyama *et al*., 1996). A reduction of the 5-hydroxytryptaminergic tone on postsynaptic receptors following the activation of cell body autoreceptors by low doses of 5-HT$_{1A}$ agonists might disinhibit glutamate release, which would enhance that of 5-HT, thus counteracting the inhibition achieved at midbrain level. Hippocampal nicotinic heteroreceptors involved in fast synaptic transmission (McGehee *et al*., 1995) are unlikely to participate, as local administration of nicotine (Toth *et al*., 1992) or the selective antagonist mecamylamine did not alter the hippocampal 5-HT output (Casanovas & Artigas, unpublished observations).

One possibility to account for the observed midbrain-forebrain differences in the action of the various 5-HT$_{1A}$ agonists is the hypothesized presence of different subpopulations of 5-HT$_{1A}$ receptors with distinct receptor/effector coupling mechanisms in the DRN (Cox *et al*., 1993). Yet, so far, there are no solid proofs of the existence of subtypes of 5-HT$_{1A}$ receptors, despite the fact that functional differences in the coupling to second messengers and pharmacological differences between pre- and postsynaptic 5-HT$_{1A}$ receptors have been documented (De Vivo & Maayani, 1986; Sprouse & Aghajanian, 1987; Varrault & Bockaert, 1992; Blier *et al*., 1993; Clarke *et al*., 1996; Romero *et al*., 1996). Also pharmacologically dissimilar behavioural actions have been demonstrated for selective 5-HT$_{1A}$ agonists (Scott *et al*., 1994).

The involvement of postsynaptic 5-HT$_{1A}$ receptors controlling the activity of 5-HT neurones might possibly account for such differences as well. Interestingly, alnespirone and ipsapirone, thought to behave as partial agonists at postsynaptic 5-HT$_{1A}$ receptors, reduced the 5-HT release in the DRN to a lesser extent than 8-OH-DPAT, full agonist at pre- and postsynaptic receptors (Casanovas & Artigas, 1996; this study). The existence of long loops involving postsynaptic 5-HT$_{1A}$ receptors has been suggested by some electrophysiological and neurochemical data (Blier & De Montigny, 1987; Ceci *et al*., 1994; Romero *et al*., 1994). The reduction of electrical activity in midbrain and forebrain areas effected by 5-HT$_{1A}$ agonists (Andrade *et al*., 1986; Araneda & Andrade, 1991; Stevens *et al*., 1992) might perhaps result in changes in the balance between excitatory and inhibitory inputs to midbrain 5-hydroxytryptaminergic neurones, as these receive afferents from many brain structures (see Aghajanian *et al*., 1987; Jacobs & Azmitia, 1992 for review). Such a possibility remains speculative but would require careful investigation

given the key role of 5-HT$_{1A}$ receptors in mood control and the existing uncertainties in their mode of action.

In summary, the present study provides extensive evidence in support of a preferential inhibition of 5-HT release in forebrain areas innervated by axons of the DRN after the systemic administration of selective 5-HT$_{1A}$ agonists. Given the comparable (8-OH-DPAT) or smaller (alnespirone) reductions of 5-HT release in DRN (vs MRN) after systemic treatment with these agonists, differences in their effects in forebrain are not likely to be explained by a greater sensitivity of 5-HT$_{1A}$ autoreceptors in the DRN.

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