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Possible *in vivo* 5-HT reuptake blocking properties of 8-OH-DPAT assessed by measuring hippocampal extracellular 5-HT using microdialysis in rats

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1 The 5-hydroxytryptamine (5-HT)$_{1A}$ receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), has been shown to label 5-HT reuptake sites.

2 To study the functional consequences of this property, the effects of 8-OH-DPAT were compared with those of the 5-HT reuptake inhibitors, paroxetine and clomipramine, and of the 5-HT$_{1A}$ receptor agonist flesinoxan, *in vitro* on 5-HT reuptake, and *in vivo* on the extracellular concentration of 5-HT by use of microdialysis, in rat hippocampus. Because 5-HT reuptake inhibitors reportedly attenuate the ability of (+)-fenfluramine to increase the extracellular concentration of 5-HT, the possible reversal of these effects by 8-OH-DPAT and by paroxetine were examined.

3 8-OH-DPAT, paroxetine and clomipramine inhibited [^{3}H]-5-HT reuptake in rat hippocampal synaptosomes (pIC$_{50}$: 6.00, 8.41 and 7.00, respectively). In contrast, flesinoxan did not alter 5-HT reuptake (pIC$_{50}$ < 5).

4 8-OH-DPAT (10 and 100 μM), paroxetine (0.1 μM) and clomipramine (1 μM), administered through the dialysis probe, significantly increased the hippocampal extracellular concentration of 5-HT. In contrast, flesinoxan (100 μM) did not alter extracellular 5-HT. Moreover, the effects of 100 μM 8-OH-DPAT were not blocked by the 5-HT$_{1A}$ receptor antagonist, WAY-100635 (0.16 mg kg^{-1}, s.c.).

5 The increase in extracellular 5-HT induced by 10 mg kg^{-1}, i.p., (+)-fenfluramine was prevented not only by 0.1 μM paroxetine, but also by 100 μM 8-OH-DPAT. In addition, systemic administration of 10 mg kg^{-1}, but not 2.5 mg kg^{-1}, i.p. 8-OH-DPAT attenuated the increase in extracellular 5-HT induced by 2.5 mg kg^{-1}, i.p., (+)-fenfluramine.

6 These findings suggest that the increase in extracellular 5-HT produced by local administration of 8-OH-DPAT does not involve its 5-HT$_{1A}$ receptor agonist properties, but may result, at least in part, from its 5-HT reuptake blocking properties.

Keywords: 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT); 5-HT reuptake; extracellular 5-HT concentration; microdialysis; hippocampus

Introduction

Although known primarily as a 5-hydroxytryptamine (5-HT)$_{1A}$ receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) is not devoid of other properties. Notably, [^{3}H]-8-OH-DPAT has been shown to label 5-HT reuptake sites at concentrations 58–92 times higher than those necessary to label 5-HT$_{1A}$ receptors (Schoemaker & Langer, 1986; Alexander & Wood, 1988) and to inhibit 5-HT reuptake *in vitro* at concentrations 700 times higher than those necessary to inhibit [^{3}H]-5-HT binding to 5-HT$_{1A}$ receptors (Hamon *et al.*, 1984). *In vivo*, 8-OH-DPAT decreases the hippocampal extracellular concentration of 5-HT when given systemically (0.1–0.25 mg kg^{-1}, s.c., or 0.31–1 mg kg^{-1}, i.p.; Sharp *et al.*, 1989b; Kreiss & Lucki, 1994; Assié & Koek, 1996a), when injected in the raphé (0.5–3.2 μg; Sharp *et al.*, 1989a; Kreiss & Lucki, 1994) or when perfused through the dialysis probe in the raphé (at concentrations varying from 30 nM to 10 mM; Bosker *et al.*, 1994; Adell *et al.*, 1993), and is likely to produce these effects through activation of somatodendritic 5-HT$_{1A}$ receptors. Whether 8-OH-DPAT exerts 5-HT reuptake blocking activity *in vivo* is currently unknown.

The present experiments were aimed at examining the ability of 8-OH-DPAT to block hippocampal 5-HT reuptake *in vivo* when administered locally, in an effort to minimize interactions of 8-OH-DPAT with somatodendritic 5-HT$_{1A}$ receptors, as such interactions could conceivably mask its possible reuptake inhibiting properties. By use of *in vivo* microdialysis, extracellular concentrations of 5-HT were measured in the ventral hippocampus of anaesthetized rats. The effects of 8-OH-DPAT, after local administration through the dialysis probe, were compared with those of the 5-HT reuptake inhibitors, paroxetine and clomipramine, and of the 5-HT$_{1A}$ receptor agonist flesinoxan. In addition, the effects of the 5-HT$_{1A}$ receptor antagonist WAY-100635 (Forster *et al.*, 1995) on the 8-OH-DPAT-induced increase in extracellular 5-HT were examined. Further, because 5-HT reuptake inhibitors have been shown to prevent the (+)-fenfluramine-induced increase in extracellular 5-HT (Kreiss *et al.*, 1993), the effects of local administration of paroxetine and 8-OH-DPAT, and of systemic administration of 8-OH-DPAT, on this increase were also examined. Finally, [^{3}H]-5-HT reuptake *in vitro* was measured in rat hippocampal synaptosomes to compare the reuptake blocking properties of the different compounds in the same tissue and the same experimental conditions. Part of this work has been presented to the British Pharmacological Society (Assié & Koek, 1996b).

Methods

Animals

Male Sprague Dawley rats (Ico: OFA SD (I.O.P.S. Caw); Iffa Credo, France], weighing 180–200 g (for [^{3}H]-5-HT reuptake) or 260–340 g (for microdialysis), were group-housed (n = 3 per cage) under controlled illumination (12 h/12 h light/dark cycle: lights on 07 h 00 min) and environmental conditions

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(ambient temperature 21±1°C, humidity 55±5%) with rat food (AO4, UAR, France) and filtered (0.2 μm) tap water available *ad libitum*. At least 5 days were allowed for adaptation before the rats were used in the experiments. The procedures used were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institutes of Health guide for the care and use of Laboratory animals (NIH publication 85–23, revised 1985), and were approved by the institutional Protocol Review Committee ([^{3}H]-5-HT reuptake, protocol 001; microdialysis, protocol 069).

In vitro *[^{3}H]-5-HT reuptake*

The crude synaptosomes were prepared as described by Thomas *et al.* (1987). Rats were killed by decapitation and the hippocampus was dissected out and homogenised in sucrose 0.32 M (50 mg tissue ml^{-1} sucrose) in a Dounce homogenizer. This homogenate was centrifuged at 1000 g for 10 min at 4°C and the supernatant was used immediately for the reuptake assay.

The preincubation medium, consisted of 50 μl of the test compound, 350 μl of oxygenated (5%/95% CO_2/O_2 for at least 30 min) Krebs buffer (composition in mM: NaCl 120, glucose 10, $NaHCO_3$ 25, KCl 4.7, $MgCl_2{\cdot}H_2O$ $1.6H_2O$ 1.2, NaH_2PO_4, ascorbic acid 0.1, EDTA 0.05 and $CaCl_2.2H_2O$ 1.3) containing 25 μM pargyline, and 50 μl of synaptosomal preparation. After a 5 min preincubation period at 37°C, 50 μl of [^{3}H]-5-HT (10 nM final concentration) were added. The incubation was stopped 5 min later by rapid addition of 2 ml cold Krebs buffer followed by immediate filtration, under vacuum, through Whatman GF/C filters with 2 × 2 ml washes of Krebs buffer. The radioactivity retained on the filters was measured by scintillation spectroscopy in 4 ml of scintillation fluid (Emulsifier safe, Packard). Non-specific reuptake was defined by addition of 10 μM citalopram. Of each compound, five concentrations were tested in experiments that were performed in duplicate and that were repeated three times.

Microdialysis procedure

The method used in the present experiments was similar to that described by Sharp *et al.* (1989a). The rats were anaesthetized with 400–500 mg kg^{-1}, i.p., chloral hydrate and maintained under anaesthesia throughout the experiment with supplementary dosing as required (approximately 80 mg kg^{-1} h^{-1}). The body temperature was maintained at 36°C by a homeothermic blanket system (Harvard, Ealing, Les Ulis, France). The rats were mounted in a stereotaxic apparatus (David Kopf), the skull was exposed and a hole was drilled to implant a microdialysis probe (CMA/12, 2 mm length (unless stated otherwise), 0.5 mm outside diameter, CMA, Microdialysis AB), into the left hippocampus (stereotaxic co-ordinates: rostral −4.8 mm, lateral +4.6 mm, ventral −7.5 mm, from bregma and dura surface according to Paxinos and Watson (1986)). The probe was continuously perfused (1.1 μl min^{-1}) with artificial cerebrospinal fluid (aCSF; containing in mM NaCl 140, KCl 3, $CaCl_2$ 2.5, $MgCl_2$1, Na_2HPO_4 1.2, NaH_2PO_4 0.27 and glucose 7.2), via FEP tubing connected to a 1 ml syringe mounted on a microinfusion pump (CMA/100, CMA, Microdialysis AB). In a series of experiments, 1 μM citalopram was added to the aCSF. Starting approximately 2 h after implantation, perfusates were collected every 20 min in small polypropylene tubes inverted over the outlet cannula and directly analysed for 5-HT content by high performance liquid chromatography with electrochemical detection as described previously (Assié & Koek, 1996a). The limit of detection (twice baseline noise) was approximately 0.5–1.5 fmol per 20 μl sample.

Four baseline control samples were collected before the aCSF was replaced by the drug solution, which was present throughout the remainder of the experiment, and samples were collected for 180 min. (+)-Fenfluramine was given i.p., 40 min after the beginning of the perfusion of the drug through the dialysis probe. In the experiments with WAY-100635, the compound was administered s.c., the perfusion of 8-OH-DPAT through the dialysis probe started 40 min later, and samples were collected for a further 140 min. At the end of the experiment, the animal was killed by decapitation and the brain was removed, frozen and cut in a cryomicrotome (Jung Frigocut 2800) to verify the placement of the probe.

In the experiments in which 8-OH-DPAT and (+)-fenfluramine were given systemically, a 3 mm long probe was implanted in order to minimize the number of animals with basal concentrations of 5-HT below the detection limit (see below). (+)-Fenfluarmine 2.5 mg kg^{-1}, i.p. (this dose was chosen because of the mortality observed with 10 mg kg^{-1}; see below) was injected 20 min after i.p. administration of 8-OH-DPAT, and samples were collected for a further 140 min.

In an effort to quantify the proportion of the compounds in the perfusion medium that crossed the probe membrane, [^{3}H]-8-OH-DPAT or [^{3}H]-paroxetine (200-500 μCi l^{-1}) were perfused through the probe *in vitro* and *in vivo* and the perfusion fluid was collected under the same conditions as in the aforementioned microdialysis studies. The radioactivity of each 20 μl sample was measured by scintillation spectroscopy in 4 ml of scintillation fluid (Emulsifier safe, Packard). The probe was either implanted *in vivo* as described above, or was inserted into an eppendorf tube containing a CSF, which allowed the measurement of radioactivity present in the vial at the end of the experiment.

Drugs

[^{3}H]-5-HT (TRK.223: 10–20 Ci mmol^{-1}) and [^{3}H]-8-OH-DPAT (TRK.850: 160–240 Ci mmol^{-1}) were purchased from Amersham (les Ulis, France) and [^{3}H]-paroxetine (NET-869: 15–30 Ci mmol^{-1}) from DuPont NEN (les Ulis, France). 5-HT creatinine sulphate and 5-hydroxyindole-3-acetic acid (5-HIAA) were purchased from Sigma (Saint Quentin Fallavier, France), (±)-8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT), clomipramine hydrochloride from RBI (Bioblock Scientific, Illkirch, France), chloral hydrate from Acros (Geel, Belgium). Citalopram was a gift from Lundbeck (Copenhagen, Denmark). N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide (WAY-100635) dihydrochloride, flesinoxan, paroxetine hydrochloride and (+)-fenfluramine hydrochloride were synthesized by the chemistry department of the Pierre Fabre Research Centre (Castres, France). The doses of compounds are expressed as the base, and the volume of injection was 10 ml kg^{-1}.

Analysis of data

The percentage of specific inhibition of [^{3}H]-5-HT reuptake was calculated at each drug concentration, and the individual values from 3 experiments were used to calculate the pIC_{50} by means of the programme ALLFIT (DeLean *et al.*, 1978).

The concentration of 5-HT in dialysis samples is expressed as fmol per 20 μl sample. The first four samples, collected before any drug administration, were used to determine basal concentrations. Animals for which one or more of the four baseline samples had 5-HT content below the detection limit were not included (9 out of 84 animals tested). Mean basal concentrations of 5-HT were analysed by means of one-way analysis of variance (ANOVA) followed by Newman-Keuls tests. Treatment effects were analysed by means of two-way ANOVA with repeated measures on one factor (time as within-subjects factor, dose as between-subjects factor) performed on measurements that were taken 20 min after the beginning of the perfusion through the probe (or after injection of (+)-

fenfluramine) until the end of the experiment, and followed by Newman Keuls tests. P values less than 0.05 were considered statistically significant.

Results

[^{3}H]-5-HT reuptake inhibition

[^{3}H]-5-HT reuptake in rat hippocampal crude synaptosomes was inhibited by paroxetine ($pIC_{50} \pm$ s.e.: 8.41 ± 0.11), clomipramine (7.00 ± 0.07), (+)-fenfluramine (6.74 ± 0.07), and by 8-OH-DPAT (6.00 ± 0.15). WAY-100635 and flesinoxan were inactive ($pIC_{50} < 5$).

Microdialysis results: basal concentrations of 5-HT in dialysis samples

The overall mean ($\pm$ s.e.mean) basal extracellular concentration of 5-HT in dialysis samples from the rat ventral hippocampus was 4.80 ± 0.27 fmol per 20 μl sample ($n = 60$) when a 2 mm probe was used and was similar (i.e., 5.09 ± 1.01 fmol per 20 μl sample ($n = 15$)) when a 3 mm probe was used. The mean basal concentrations obtained for each of the treatment conditions were not significantly different either with a 2 mm probe (one-way ANOVA: $F_{(11,48)} = 1.15$, $P > 0.10$) or with a 3 mm probe ($F_{(2,12)} = 0.75$, $P > 0.10$). In the presence of 1 μM citalopram in the aCSF, the mean basal concentration was 32.11 ± 2.18 fmol per 20 μl ($n = 10$).

Effects of different compounds, administered through the probe, on hippocampal extracellular 5-HT

When citalopram was present in the aCSF, administration of 8-OH-DPAT (10 μM) through the dialysis probe did not alter the concentration of 5-HT in the dialysis samples (overall effect during a 180 min period: controls, $n = 5$, 29.01 ± 3.62 fmol per 20 μl; 8-OH-DPAT, $n = 5$, 29.64 ± 4.28 fmol per 20 μl).

In the absence of citalopram, however, 8-OH-DPAT increased extracellular 5-HT in a concentration-dependent manner (Figure 1a). In contrast, 100 μM flesinoxan increased extracellular 5-HT only slightly and transiently (Figure 1b). The 5-HT reuptake inhibitors, paroxetine (0.1 μM) and clomipramine (1 μM), both increased extracellular 5-HT (Figure 1b). Two-way ANOVA indicated a significant (treatment × time point) interaction ($F_{(40,192)} = 9.88$; $P < 0.001$). Multiple comparisons showed that 8-OH-DPAT significantly ($P < 0.05$) increased extracellular 5-HT as compared with controls at time points 20 to 80 min and 20 to 180 min after the beginning of the perfusion of 10 and 100 μM, respectively. Flesinoxan did not induce statistically significant effects as compared with controls. Paroxetine and clomipramine significantly increased extracellular 5-HT at time points 40 to 180 min. 8-OH-DPAT (100 μM) produced a significantly larger increase than the other treatments at 20 min, and a significantly smaller increase than paroxetine at 100 to 180 min, and a smaller increase than clomipramine at 140 to 180 min.

Effects of the 5-HT$_{1A}$ receptor antagonist, WAY-100635, on the increase of 5-HT induced by 8-OH-DPAT

WAY-100635, at a dose that did not affect extracellular 5-HT when given alone (i.e., 0.16 mg kg^{-1}), failed to antagonise the increase in extracellular 5-HT induced by 100 μM 8-OH-DPAT (Figure 2). Two-way ANOVA indicated a significant (treatment × time point) interaction ($F_{(12,72)} = 2.99$; $P = 0.02$). Multiple comparisons showed that the 5-HT concentrations were significantly higher in the 8-OH-DPAT- and WAY-100635 + 8-OH-DPAT-treated groups than in the group treated with WAY-100635 alone at all time points. Importantly, the 8-OH-DPAT- and the WAY-100635 + 8-OH-DPAT-treated groups did not differ significantly from each other at any time point.

Effects of 8-OH-DPAT and paroxetine, administered through the probe, on the increase of 5-HT induced by (+)-fenfluramine

The large increase in the concentration of 5-HT induced by 10 mg kg^{-1}, i.p., (+)-fenfluramine was inhibited not only by 0.1 μM paroxetine, but also by 100 μM 8-OH-DPAT (Figure 3). Two-way ANOVA indicated a significant (treatment × time

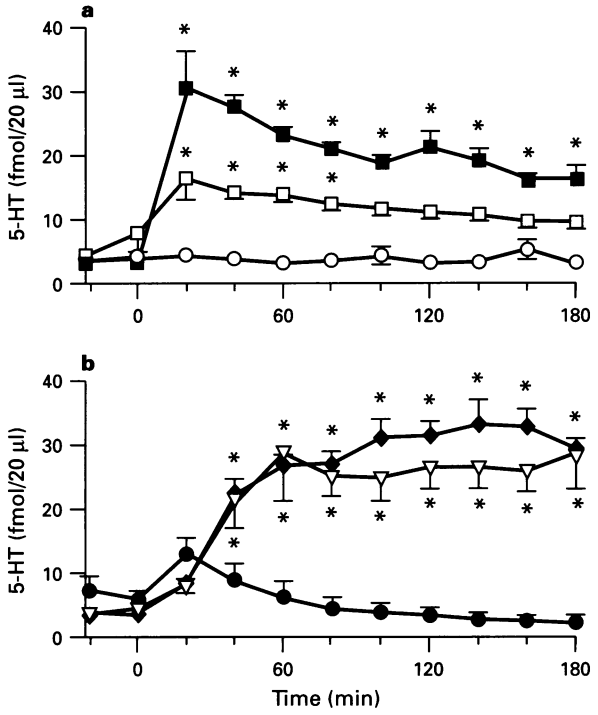

Figure 1 Effects of different compounds, perfused through the dialysis probe, on extracellular 5-HT concentration in rat ventral hippocampus. The compounds were perfused from time = 0 until the end of the experimental session. (a) (○) aCSF, (□) 8-OH-DPAT 10 μM and (■) 8-OH-DPAT 100 μM. (b) (●) Flesinoxan 100 μM, (▽) clomipramine 1 μM and (◆) paroxetine 0.1 μM. Data were analysed by two-way ANOVA followed by Newman-Keuls test; $^*P < 0.05$ compared to aCSF perfused rats. Results are means ± s.e.mean (vertical lines) for 5 animals per group.

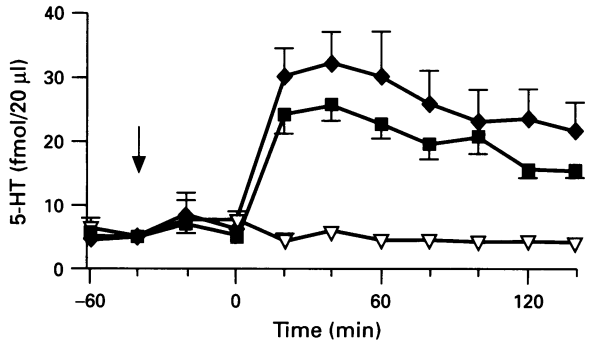

Figure 2 Effects of the 5-HT$_{1A}$ receptor antagonist, WAY-100635 (0.16 mg kg^{-1}, s.c.), on the increase in extracellular 5-HT concentration induced by 8-OH-DPAT (100 μM, perfused through the dialysis probe); (▽) WAY-100635 alone, (■) saline + 8-OH-DPAT and (◆) WAY-100635 + 8-OH-DPAT. The arrow indicates time of injection of WAY-100635, 8-OH-DPAT was perfused from time = 0 until the end of the experimental session. Data were analysed by two-way ANOVA followed by Newman-Keuls test; the groups saline + 8-OH-DPAT and WAY-100635 + 8-OH-DPAT did not differ significantly at any time point, and both differed significantly from the group WAY-100635 ($P < 0.05$) at every time point from 20 min onward. Results are means ± s.e.mean (vertical lines) for 5 animals per group.

point) interaction ($F_{(12,72)}=7.02$; $P<0.001$). Multiple comparisons showed that both paroxetine 0.1 μM and 8-OH-DPAT 100 μM inhibited significantly the (+)-fenfluramine-induced increase in extracellular 5-HT at time points 40 to 100 min after injection of (+)-fenfluramine.

A high incidence of mortality was observed in all groups of animals receiving 10 mg kg^{-1} (+)-fenfluramine (7 animals out of 22 tested); the results obtained with these animals were not included in the analyses.

Effects of systemic administration of 8-OH-DPAT on the increase of 5-HT induced by (+)-fenfluramine

The increase of 5-HT induced by 2.5 mg kg^{-1} (+)-fenfluramine was attenuated by 10 mg kg^{-1} but not by 2.5 mg kg^{-1} 8-OH-DPAT (Figure 4). Two-way ANOVA indicated a significant (treatment × time point) interaction ($F_{(12,72)}=4.82$; $P<0.001$). Multiple comparisons showed that the concentrations of 5-HT from rats treated with 10 mg kg^{-1} 8-OH-DPAT + (+)-fenfluramine were significantly lower than those from rats treated with (+)-fenfluramine alone at time points 40 to 60 min after injection of (+)-fenfluramine.

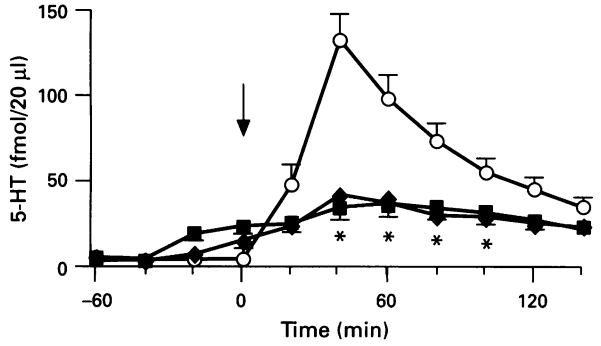

Figure 3 Effects of (+)-fenfluramine 10 mg kg^{-1}, i.p., on extracellular 5-HT concentration in rat ventral hippocampus and its antagonism by 8-OH-DPAT and paroxetine; (○) (+)-fenfluramine alone (■), 8-OH-DPAT 100 μM + (+)-fenfluramine and (◆) paroxetine 0.1 μM + (+)-fenfluramine. The arrow indicates time of injection of (+)-fenfluramine, the other compounds were perfused from 40 min until the end of the experimental session. Data were anlysed by two-way ANOVA followed by Newman-Keuls test; *$P<0.05$ for both 8-OH-DPAT and paroxetine compared to (+)-fenfluramine alone. Results are means ± s.e.mean (vertical lines) for 5 animals per group.

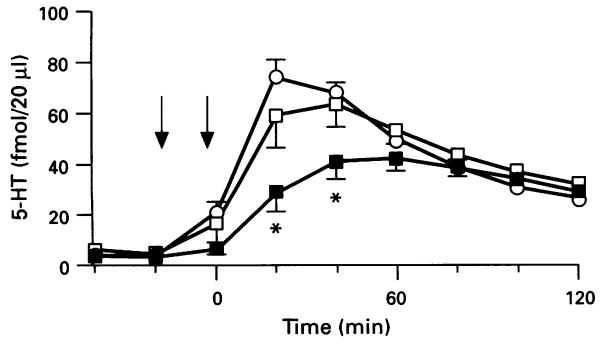

Figure 4 Effects of (+)-fenfluramine 2.5 mg kg^{-1}, i.p., on extracellular 5-HT concentration in rat ventral hippocampus and its antagonism by systemic administration of 8-OH-DPAT; (○) (+)-fenfluramine alone (□), (+)-fenfluramine + 8-OH-DPAT 2.5 mg kg^{-1} and (■) (+)-fenfluramine + 8-OH-DPAT 10 mg kg^{-1}. The arrows indicate time of injection of 8-OH-DPAT and (+)-fenfluramine. Data were analysed by two-way ANOVA followed by Newman-Keuls test; *$P<0.05$ compared to (+)-fenfluramine alone. Results are means ± s.e.mean (vertical lines) for 5 animals per group.

Estimation of the proportion of 8-OH-DPAT and of paroxetine that crossed the probe membrane

When during four, independent experiments, [^{3}H]-8-OH-DPAT was perfused through the probe *in vitro*, the first sample at the outlet of the probe contained 55 ± 2% of the introduced radioactivity, and the mean amount contained in nine consecutive samples was 74 ± 1%. The radioactivity (measured in two experiments) present in the eppendorf tube after nine samples was 20 ± 2% of the total amount introduced. *In vivo* (in two experiments), the percentages were 62 ± 3 and 83 ± 4%, for the first sample and for all nine samples, respectively. When [^{3}H]-paroxetine was perfused through the probe *in vitro*, the first sample contained 16 ± 4% of the introduced radioactivity, and the mean amount for nine consecutive samples was 42 ± 4% ($n=4$). The radioactivity measured in the eppendorf tube after nine samples was 17 ± 0% ($n=2$) of the original amount. *In vivo*, the percentages were 21 ± 1 and 43 ± 2% ($n=2$), for the first sample and for all nine samples, respectively. Although there was no apparent difference between the amount of radioactivity found at the outlet of the probe *in vitro* and *in vivo*, there appeared to be differences between 8-OH-DPAT and paroxetine. For [^{3}H]-8-OH-DPAT, the sum of the radioactivity detected at the outlet of the probe and in the eppendorf tube was close to the total radioactivity that was introduced. In contrast, an important proportion of [^{3}H]-paroxetine (i.e., 41%) could not be recovered.

Discussion

In the present study, local administration of the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, was found to increase the extracellular concentration of 5-HT in the hippocampus, through mechanisms that do not appear to involve its 5-HT$_{1A}$ receptor agonist properties, but that may be related to its *in vivo* 5-HT reuptake blocking properties.

[^{3}H]-8-OH-DPAT labels 5-HT reuptake sites in rat striatum (Schoemaker & Langer, 1986; Alexander & Wood, 1988) and in bovine dorsal raphé (Sprouse *et al.*, 1993) as well as in human platelets (Ieni & Meyerson, 1988). 8-OH-DPAT has been shown also to inhibit [^{3}H]-paroxetine binding in rat whole brain (Wolf & Kuhn, 1991) and in bovine dorsal raphé and hippocampus (Sprouse *et al.*, 1993). The present finding that 8-OH-DPAT inhibited [^{3}H]-5-HT reuptake in hippocampal synaptosomes (IC_{50}: 1 μM) is in agreement with previous work showing 8-OH-DPAT to inhibit 5-HT reuptake in rat cortical synaptosomes (K_i values: 1400 and 339 nM, Hamon *et al.*, 1984; Cheng *et al.*, 1993, respectively). Taken together, these results provide *in vitro* evidence of 5-HT reuptake blocking properties of 8-OH-DPAT at concentrations approximately 1000 times higher than its nanomolar affinity for 5-HT$_{1A}$ receptor binding sites (e.g., Hamon et al., 1984; Schoemaker & Langer, 1986; Alexander & Wood, 1988).

The observation that 8-OH-DPAT did not alter the hippocampal extracellular concentration of 5-HT when it was perfused through the dialysis probe in the presence of the 5-HT reuptake inhibitor, citalopram, is in agreement with previous findings (Sharp & Hjorth, 1990). In the absence of a reuptake inhibitor, however, 8-OH-DPAT (10–100 μM) dose-dependently increased extracellular 5-HT. The maximal increase induced by 8-OH-DPAT was of a magnitude similar to that obtained with the 5-HT reuptake blockers paroxetine and clomipramine, but the time course of its effects appeared to differ from that of paroxetine and clomipramine.

Although the concentrations of the compounds used here are consistent with their respective potencies to inhibit 5-HT reuptake *in vitro*, the actual concentrations in the extracellular fluid surrounding the probe are not known. Indeed, this concentration is dependent on the amount that crosses the probe membrane, which is time-dependent and differs between compounds (see Benveniste, 1989). Here, experiments comparing tritiated 8-OH-DPAT and paroxetine showed that ap-

proximately 42–43% of [^{3}H]-paroxetine and 74–83% of [^{3}H]-8-OH-DPAT were recovered at the outlet of the probe *in vivo* and *in vitro*, and approximately 17% of [^{3}H]-paroxetine and 20% of [^{3}H]-8-OH-DPAT were found in the eppendorf tube, i.e., actually crossed the probe membrane (the latter results are in agreement with those obtained by Kreiss & Lucki, 1994). Thus, for [^{3}H]-8-OH-DPAT almost all the radioactivity was recovered, in contrast, a substantial proportion of [^{3}H]-paroxetine (40%) was not recovered, either because of its binding to the tubing or because of its interactions with the dialysis membrane. The difference in probe recovery between 8-OH-DPAT and paroxetine may contribute to the apparently different time course of their effects. It seems unlikely that activation of the somatodendritic 5-HT$_{1A}$ receptors by 8-OH-DPAT (resulting from possible diffusion to the raphé) is involved in the progressive decrease of its effects on extracellular 5-HT, because this decrease was observed also in the presence of the 5-HT$_{1A}$ receptor antagonist, WAY-100635.

The concentrations of 8-OH-DPAT administered through the probe are similar to those exerting 5-HT reuptake blocking properties *in vitro*. Assuming a probe recovery of 20% (see above), the concentrations of 8-OH-DPAT given through the probe are similar to those necessary to induce 60-100% [^{3}H]-5-HT uptake blockade *in vitro*. Moreover, such an increase in extracellular concentrations of 5-HT, induced by 8-OH-DPAT when administered through the probe, has also been shown to occur in the raphé nucleus, at concentrations of 100 μM and higher (Adell *et al.*, 1993; Bosker *et al.*, 1994) and these authors mentioned the possibility that this effect could be mediated by 5-HT reuptake blocking properties of 8-OH-DPAT. The increase in extracellular concentrations of 5-HT induced by 8-OH-DPAT that was observed here is unlikely to be mediated by an activation of 5-HT$_{1A}$ receptors, because flesinoxan, another high affinity agonist at 5-HT$_{1A}$ receptors (pK_i values 8.77 and 8.55 for flesinoxan and 8-OH-DPAT, respectively; Schipper *et al.*, 1991), did not markedly increase extracellular concentrations of 5-HT when perfused through the probe at 100 μM. Note that flesinoxan was tested at a concentration that was 10 times higher than the concentration of 8-OH-DPAT (i.e., 10 μM) that was found to increase extracellular 5-HT. Because flesinoxan is about 1–3 times less potent as a 5-HT$_{1A}$ receptor agonist than 8-OH-DPAT (Bosker *et al.*, 1996; Assié & Koek, 1996a; unpublished results), its lack of effects on extracellular 5-HT observed here is inconsistent with the involvement of 5-HT$_{1A}$ receptors. Moreover, the 5-HT$_{1A}$ receptor antagonist WAY-100635 (Gurling *et al.*, 1994; Forster *et al.*, 1995), which has been shown to prevent fully, at a dose of 0.16 mg kg^{-1}, the decrease in extracellular concentrations of 5-HT induced by systemic administration of a submaximal dose (0.31 mg kg^{-1}, i.p.) of 8-OH-DPAT (Assié & Koek, 1996c), and which did not alter extracellular concentrations of 5-HT when given alone, failed to antagonize the 100 μM 8-OH-DPAT-induced increase in extracellular concentrations of 5-HT. It is unlikely that the dose of WAY-100635 was insufficient to antagonize a 5-HT$_{1A}$ receptor-mediated effect, because although WAY-100635 was tested in combination with the highest dose of 8-OH-DPAT examined here (i.e., 100 μM), a ten times lower dose of 8-OH-DPAT had a significantly lower effect, allowing the detection of at least an attenuation of the effects of 100 μM 8-OH-DPAT.

Previous studies have shown that the 5-HT releasing agent, (+)-fenfluramine increases extracellular 5-HT and that this increase could be inhibited by 5-HT reuptake blockers administered either systemically (Sabol *et al.*, 1992) or directly through the dialysis probe (Kreiss *et al.*, 1993). These inhibitory effects are believed to be mediated by a blockade of 5-HT reuptake sites which are necessary for (+)-fenfluramine to be taken up into the nerve terminal and, subsequently, to release 5-HT. In the present work, 8-OH-DPAT, like the 5-HT reuptake inhibitor, paroxetine, inhibited the 5-HT releasing effects of (+)-fenfluramine. This provides further evidence that under the present conditions 8-OH-DPAT may act as a 5-HT reuptake inhibitor. (+)-Fenfluramine (at the dose of 10 mg kg^{-1} i.p.) has been widely used to produce increases in extracellular 5-HT (Laferrere & Wurtman, 1989; Sarkissian *et al.*, 1990; Series *et al.*, 1994). Although this dose of (+)-fenfluramine has been described as being high (Laferrere & Wurtman, 1989), to our knowledge lethal effects of this dose have not been obtained previously. It is unlikely that the association of (+)-fenfluramine with the compounds administered through the probe was responsible for the observed mortality, as the lethal effect was observed also in control animals receiving only (+)-fenfluramine. Conceivably, the anaesthetic used here may have potentiated the lethal effects of (+)-fenfluramine; other studies, however, used the compound under similar conditions, yet did not report mortality (Series *et al.*, 1994).

Although the present results suggest that locally administered 8-OH-DPAT has 5-HT reuptake blocking properties, the extent to which these properties can be evidenced after systemic administration is complicated by the 5-HT$_{1A}$ receptor agonist properties of the compound. It is conceivable that the 5-HT$_{1A}$ receptor agonist properties of systemically administered 8-OH-DPAT will mask the expression of its 5-HT reuptake blocking properties in the following manner. Systemic administration of 8-OH-DPAT decreases extracellular 5-HT in the hippocampus (Sharp *et al.*, 1989b; Kreiss & Lucki, 1994; Assié & Koek, 1996a), and systemic administration of the 5-HT reuptake blockers generally increases extracellular 5-HT, although these latter effects may depend on the brain region examined. Indeed, an increase in extracellular concentrations of 5-HT in the raphé after systemic administration of a 5-HT reuptake inhibitor has been observed repeatedly (see: Fuller, 1994); this increase is thought to activate somatodendritic 5-HT$_{1A}$ receptors, thereby decreasing the firing of 5-HT neurones, which would decrease 5-HT release in terminal regions. Such a decrease, however, has been observed only if a 5-HT reuptake inhibitor is present in the perfusion medium (Auerbach *et al.*, 1995); in the absence of a reuptake inhibitor in the perfusion medium, either no effect or an increase in extracellular 5-HT has been observed after systemic administration of 5-HT reuptake inhibitors (see: Fuller, 1994). Indirect evidence of 5-HT reuptake inhibiting properties of 8-OH-DPAT was obtained by exploring its effects on the (+)-fenfluramine-induced increase in extracellular 5-HT, since reuptake inhibitors have been shown to inhibit the effects of (+)-fenfluramine after systemic administration as well as after administration through the probe (see above). Administration of 10 mg kg^{-1}, i.p., but not 2.5 mg kg^{-1} 8-OH-DPAT significantly attenuated the effects of 2.5 mg kg^{-1} (+)-fenfluramine. These inhibitory effects occurred at a dose of 8-OH-DPAT approximately 60 times higher than that necessary to induce 5-HT$_{1A}$ receptor-mediated effects (e.g., lowest i.p. dose to decrease significantly extracellular 5-HT: 0.16 mg kg^{-1}; Assié & Koek, 1996a).

In conclusion, the present results suggest that the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT may also act, *in vivo*, as a 5-HT reuptake blocker. Because the latter effects appear to occur only at doses 60 times higher than those that produce 5-HT$_{1A}$ receptor-mediated effects, the contribution of 5-HT uptake blocking properties to the *in vivo* pharmacological profile of 8-OH-DPAT appears to be limited. However, the present data suggest that results obtained from studies with compounds structurally related to 8-OH-DPAT should be treated with caution, because it is conceivable that some of these compounds may show a separation between 5-HT reuptake blocking and 5-HT$_{1A}$ receptor agonist properties that is smaller than that found here for 8-OH-DPAT.

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