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Role of uptake inhibition and autoreceptor activation in the control of 5-HT release in the frontal cortex and dorsal hippocampus of the rat

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1 Using brain microdialysis, we compared the relative role of 5-hydroxytryptamine (5-HT; serotonin) blockade and somatodendritic 5-HT_{1A} and/or terminal 5-HT_{1B} autoreceptor activation in the control of 5-HT output.

2 Fluoxetine (10 mg kg^{-1} i.p.) doubled the 5-HT output in frontal cortex and dorsal hippocampus. The 5-HT_{1A} receptor antagonist WAY 100635, (0.3 mg kg^{-1} s.c.) potentiated the effect of fluoxetine only in frontal cortex (to $\sim$500 % of baseline).

3 Methiothepin (10 mg kg^{-1} s.c.) further enhanced the 5-HT rise induced by fluoxetine + WAY 100635, to 835 ± 179% in frontal cortex and 456 ± 24% in dorsal hippocampus. Locally applied, methiothepin potentiated the fluoxetine-induced 5-HT rise more in the former area.

4 The selective 5-HT_{1B} receptor antagonist SB-224289 (4 mg kg^{-1} i.p.) enhanced the effect of fluoxetine (10 mg kg^{-1} i.p.) in both areas. As with methiothepin, SB-224289 (4 mg kg^{-1} i.p.) further enhanced the 5-HT increase produced by fluoxetine + WAY 100635 more in frontal cortex (613 ± 134%) than in dorsal hippocampus (353 ± 59%).

5 Locally applied, fluoxetine (10–300 μM; EC_{50} = 28–29 μM) and citalopram (1–30 μM; EC_{50} = 1.0–1.4 μM) increased the 5-HT output two to three times more in frontal cortex than in dorsal hippocampus.

6 These data suggest that the comparable 5-HT increase produced by systemic fluoxetine in frontal cortex and dorsal hippocampus results from a greater effect of reuptake blockade in frontal cortex that is offset by a greater autoreceptor-mediated inhibition of 5-HT release. As a result, 5-HT autoreceptor antagonists preferentially potentiate the effect of fluoxetine in frontal cortex.

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Abbreviations: DRN, dorsal raphe nucleus; 5-HT, 5-hydroxytryptamine (serotonin); MRN, median raphe nucleus; SSRIs, selective serotonin reuptake inhibitors

Introduction

The selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRIs) enhance serotonergic transmission in brain by interacting with the high affinity transporter located in nerve terminals (Hyttel, 1994). However, SSRIs offset the increases in extracellular 5-HT concentration produced in forebrain due to a negative feed-back involving 5-HT_{1A} autoreceptors (see Artigas *et al*., 1996 for review). Using appropriate experimental conditions, such as dual probe microdialysis or systemic administration of SSRIs during local inhibition of the 5-HT reuptake in forebrain, it has been shown that the SSRIs fluoxetine and paroxetine reduce the 5-HT output preferentially in striatum, frontal cortex or amygdala compared to hippocampus (Romero & Artigas, 1997; Romero *et al*., 1997; Hervás & Artigas, 1998). This suggests that the 5-HT release in the latter area is less inhibited by acute SSRI treatments. The hippocampus contains a density of 5-HT reuptake sites greater than that of frontal cortex (D'Amato *et al*., 1987; Hrdina *et al*., 1990). According to the current knowledge on the mechanism of action of SSRIs, both observations suggest that the systemic administration of SSRIs should increase the 5-HT output more in hippocampus than in frontal cortex. However, regional studies suggest that the systemic administration of fluoxetine and paroxetine increase comparably the 5-HT output in frontal cortex, dorsal striatum and hippocampus (dorsal and ventral) (Malagié *et al*., 1995; Romero & Artigas, 1997; Hervás & Artigas, 1998).

The aim of the present study was to examine the reasons for this discrepancy using *in vivo* microdialysis in freely moving rats. To this end, we systemically administered fluoxetine alone or in combination with autoreceptor (5-HT_{1A} and/or 5-HT_{1B}) antagonists and compared the effects with those of its local administration in frontal cortex and dorsal hippocampus. The results suggest that the similar increase in cortical and hippocampal 5-HT output elicited by the systemic administration of fluoxetine is the result of a different balance between the effects of reuptake blockade and autoreceptor activation in these two forebrain areas.

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Methods

Animals

Male Wistar rats (Iffa Credo, Lyon, France) weighing 270–320 g were used. Animals were kept in a controlled environment of a 12 h light-dark cycle (lights on at 0700 h) and 22±2°C room temperature. Food and water were provided *ad libitum*. Animal care followed the NIH guidelines for the care of laboratory animals (publication No. 85-23, revised 1985) and European Union regulations (O.J. of E.C. L358/1 18/12/1986).

Drugs and reagents

Methiothepin mesylate, serotonin hydrochloride and WAY 100635 [*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridyl)cyclohexanecarboxamide 3HCl] were from RBI (Natick, MA, U.S.A.). SB-224289 (2,3,6,7-tetrahydro-1′-methyl-5-{2′-methyl-4′-[(5-methyl-1,2,4-oxadiazole-3-yl)biphenyl4yl]carbonyl}furo[2,3-F]-indole-3-spiro-4′-piperidine oxalate) was kindly donated by SmithKline Beecham (Harlow, Essex, U.K.). The SSRIs citalopramHBr and fluoxetineHCl were kindly provided by Lundbeck A/S (Copenhagen-Valby, Denmark) and Eli Lilly and Co. (Indianapolis, IN, U.S.A.), respectively. Other materials and reagents were from local commercial sources. Drugs were injected i.p. or s.c. at a volume of 1–2 ml kg^{-1}. For the assessment of local effects, citalopram and fluoxetine were dissolved in the perfusion fluid and applied by reverse dialysis. Concentrated solutions (1 mM; pH 6.5–7.0) were stored at −80°C and working solutions were prepared daily with artificial cerebrospinal fluid (aCSF; see below). The doses administered are expressed as free base.

Surgery and microdialysis procedures

Microdialysis procedures were carried out as described in detail in Adell & Artigas (1998). Concentric vertical probes were used. Dialysis membranes were made from hollow Cuprophan fibres with 252 μm OD, 220 μm ID and 5000 daltons molecular weight cut-off (GFE09, Gambro, Lund, Sweden). The total length of the dialysis membrane exposed to the tissue was 1.5 mm in dorsal hippocampus and 4.0 mm in frontal cortex. In experiments assessing the local effects of citalopram and fluoxetine, probes of equal size (1.5 mm) were used in both regions to avoid any methodological source of difference. Anaesthetized rats (sodium pentobarbitone, 60 mg kg^{-1} i.p.) were placed in a David Kopf (Tujunga, CA, U.S.A.) stereotaxic frame and probes were implanted and secured to the skull with anchor screws and dental cement. The stereotaxic coordinates (in mm) for frontal cortex (AP +3.4 DV −6.0 L −2.5) and dorsal hippocampus (AP −3.8, DV −4.0, L −1.8) were taken from bregma and dura mater according to the rat brain atlas of Paxinos & Watson (1986). Rats were allowed to recover from anaesthesia in the dialysis cages (cubic, 40 cm each side) and 20–24 h later the probes were perfused with aCSF (mM: NaCl 125, KCl 2.5, $MgCl_2$ 1.18 and $CaCl_2$ 1.26; pH 6.5–7.0) at 0.25 μl min^{-1}. Dialysate samples of 5 μl were collected at 20-min intervals into polypropylene microcentrifuge vials. After an initial 1-h sample of dialysate was discarded, four to six fractions were collected to obtain basal values before the local or systemic administration of drugs. The 5-HT autoreceptor antagonists WAY 100635, methiothepin and SB-224289 were administered either systemically or locally 180 min after the administration of fluoxetine and dialysate fractions were collected for an additional 120-min period (six fractions). In one experiment, methiothepin was infused after collection of baseline values in presence of citalopram. For the assessment of the local effects of citalopram and fluoxetine, these were perfused at increasing concentrations (1–30 μM citalopram, 10–300 μM fluoxetine; four fractions each concentration). At the end of the experiments, rats were killed by an overdose of sodium pentobarbitone and the placement of the dialysis probes was checked by perfusing Fast Green dye and examination of the probe track after cutting the brain at the appropriate levels.

5-HT was analysed by a modification of a high performance liquid chromatography method previously described (see Adell & Artigas (1998) for details). 5-HT was separated on a 3 μm ODS 2 column (7.5×0.46 cm; Beckman, San Ramon, CA, U.S.A.) and detected amperometrically with a Hewlett Packard 1049 detector set at the potential of +0.6V. Retention time was 3.5–4 min. The detection limit for 5-HT was 0.5–1 fmol. Dialysate 5-HT values were calculated by reference to standard curves run daily.

Data analysis

The concentration of 5-HT in dialysates is expressed as fmol $fraction^{-1}$ and represented in figures as percentages of basal values (average of four pre-drug fractions) to facilitate comparisons between the different experimental groups. The statistical analysis was performed using one- or two-way analysis of variance (ANOVA) for repeated measures of raw data (fmol $fraction^{-1}$). We analysed the effect of the independent factor (treatment or brain region), the repeated factor (time) and the interaction between them. The latter assesses whether the change in 5-HT over time differs between the two treatment groups (or brain regions). Thus, a significant *P* value of the interaction indicates differences in the effects of two treatments (or brain regions) on 5-HT output. The effects of the local or systemic administration of autoreceptor antagonists were assessed by reference of groups treated with fluoxetine alone or fluoxetine plus saline, respectively, using two-way ANOVA of fractions 9–19 (see Figure 1). Student's *t*-test for independent data was also used. EC_{50} values were calculated (GraphPad Prism program) using the averaged 5-HT values of the last two fractions (out of four) at each SSRI concentration. Differences between dose-response curves were assessed by two-way repeated measures ANOVA. Data are given as mean±s.e.mean. Statistical significance has been set at the 95% confidence level (two-tailed).

Results

Baseline 5-HT output and effect of systemic fluoxetine

Baseline values of the 5-HT output in frontal cortex (4-mm probes) and dorsal hippocampus (1.5-mm probes) were, 2.8±0.2 ($n=67$) and 3.0±0.2 ($n=73$) fmol $fraction^{-1}$, respectively (non-significant difference, Student *t*-test). The 5-HT output was unaltered by a saline injection in both brain areas (data not shown). As depicted in Figures 1 to 3, the administration of 10 mg kg^{-1} fluoxetine elevated the 5-HT output to 220±11% of baseline in frontal cortex ($n=44$) and 202±7% of baseline in dorsal hippocampus ($n=50$), expressed as the averaged values of fractions 5–13. The effect of fluoxetine was not significantly different between both regions.

Systemic administration of fluoxetine and autoreceptor antagonists

The administration of 0.3 mg kg^{-1} WAY 100635 to fluoxetine-pretreated rats enhanced the 5-HT output to 506±58% in frontal cortex and to 260±82% in dorsal hippocampus compared to basal predrug values (Figure 1). These effects were significantly different from those of a saline injection in frontal cortex ($F_{10,70}=9.66$, $P<0.00001$, time effect; $F_{10,70}=5.60$, $P<0.00001$, time × treatment interaction) but not in dorsal hippocampus ($F_{10,60}=2.56$, $P<0.015$, time effect, non-significant effect of treatment or time × treatment interaction). As shown also in Figure 1, the 5-HT elevation induced by fluoxetine plus WAY 100635 in frontal cortex was significantly greater than that in dorsal hippocampus ($F_{10,60}=2.79$, $P<0.01$, time × region interaction). A combination of WAY 100635 (0.3 mg kg^{-1} s.c.) and the non-selective autoreceptor antagonist methiothepin (10 mg kg^{-1} s.c.) was administered 3 h after fluoxetine to another group of rats. This combination enhanced the 5-HT output further, to 835±179% in frontal cortex and to 456±24% in dorsal hippocampus (Figure 1). The 5-HT increase was significantly greater in frontal cortex than in dorsal hippocampus (time × region interaction; $F_{10,80}=2.06$, $P<0.04$). Compared to rats treated with fluoxetine plus saline, two-way repeated measures ANOVA revealed the existence of a significant effect of the treatment ($F_{1,8}=31.48$, $P<0.0001$), time ($F_{10,80}=18.71$, $P<0.0001$) and the time × treatment interaction ($F_{10,80}=14.60$, $P<0.0001$), in frontal cortex and of a significant effect of the time ($F_{10,70}=15.57$, $P<0.0001$) and the time × treatment interaction ($F_{10,70}=10.81$, $P<0.001$) in dorsal hippocampus.

The systemic administration of the selective 5-HT_{1B} receptor antagonist SB-224289 (4 mg kg^{-1} i.p.) enhanced significantly the effect of 10 mg kg^{-1} fluoxetine in frontal cortex and dorsal hippocampus (Figure 2). These effects were significantly different from those of a saline injection in frontal cortex ($F_{1,11}=18.73$, $P<0.002$, treatment effect; $F_{10,110}=3.30$, $P<0.00001$, time effect) and dorsal hippocampus ($F_{1,10}=33.09$, $P<0.0002$, treatment effect; $F_{10,100}=6.74$, $P<0.00001$, time effect and $F_{10,110}=4.16$, $P<0.0001$, time × treatment interaction). SB-224289 enhanced the 5-HT output more in frontal cortex than in dorsal hippocampus although no significant differences were found between the two regions (two-way repeated measures ANOVA). The concurrent administration of WAY 100635 (0.3 mg kg^{-1} s.c.) and SB-224289 (4 mg kg^{-1} i.p.) enhanced significantly the effect of fluoxetine in frontal cortex and dorsal hippocampus (613±34 and 393±59%, respectively; Figure 2). These effects were significantly different from those of a saline injection in frontal cortex ($F_{10,80}=11.60$, $P<0.00001$, time effect; $F_{10,80}=6.83$, $P<0.00001$, time × treatment interaction) and dorsal hippocampus ($F_{1,10}=21.86$, $P<0.01$ treatment effect; $F_{10,100}=8.37$,

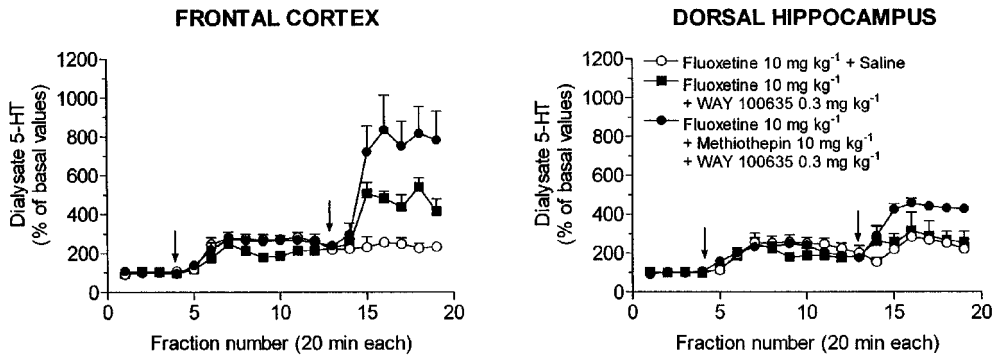

Figure 1 Effects of the systemic administration of 10 mg kg^{-1} i.p. fluoxetine (first arrow) on the 5-HT output in frontal cortex and dorsal hippocampus alone or in combination with the autoreceptor antagonists (second arrow) WAY 100635 (0.3 mg kg^{-1} s.c., $n=4$ in each region) or 0.3 mg kg^{-1} s.c. WAY 100635 and 10 mg kg^{-1} s.c. methiothepin ($n=4-5$). Control rats received fluoxetine and saline ($n=5$ in each region). See text for statistical analysis.

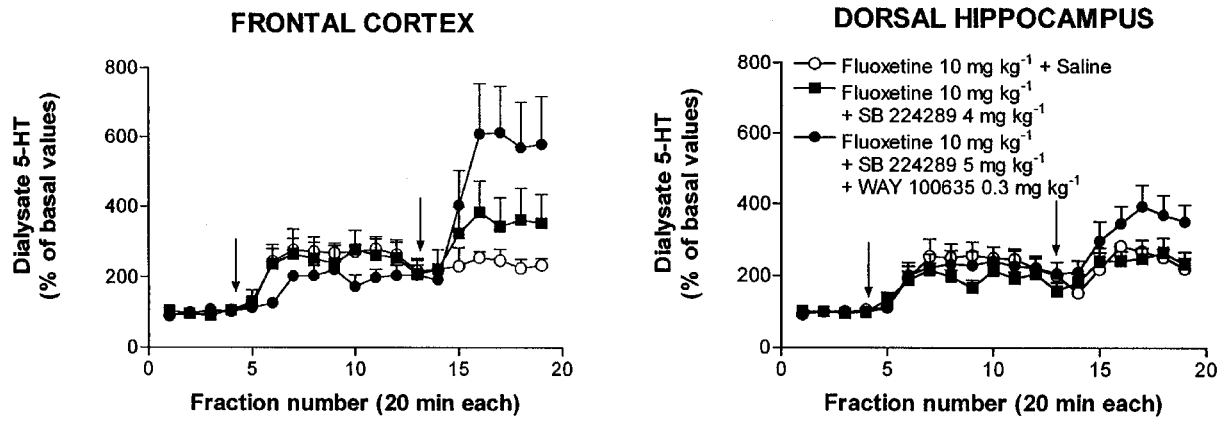

Figure 2 Effects of the systemic administration of 10 mg kg^{-1} i.p. fluoxetine (first arrow) on the 5-HT output in frontal cortex and dorsal hippocampus alone or in combination with the autoreceptor antagonists (second arrow) SB-224289 (4 mg kg^{-1} i.p., $n=8$ in each region) or 0.3 mg kg^{-1} s.c. WAY 100635 and 4 mg kg^{-1} s.c. SB 224289 ($n=5-8$). Control rats received fluoxetine and saline ($n=5$ in each region; same group as in Figure 1).

$P<0.00001$, time effect and $F_{10,80}=3.46$, $P<0.0001$, time × treatment interaction). The increase in 5-HT output was significantly greater in frontal cortex than in dorsal hippocampus ($F_{1,11}=10.78$, $P<0.01$, region effect and $F_{10,110}=12.56$, $P<0.0001$, time × region interaction).

Systemic administration of fluoxetine with local administration of 5-HT autoreceptor antagonists

To examine whether terminal autoreceptors were involved in the potentiation of the effect of fluoxetine by the 5-HT autoreceptor antagonists, we locally perfused WAY 100635 or methiothepin (100 μM each) after fluoxetine (10 mg kg^{-1}). Control rats were perfused with aCSF for the whole experiment. The local administration of WAY 100635 did not significantly alter the 5-HT output in either region (Figure 3). The administration of methiothepin markedly elevated the 5-HT output in frontal cortex to 544±64% of baseline and induced a slight increase in dorsal hippocampus at the end of the perfusion period (Figure 3). The effect of methiothepin addition was significantly different from controls (fluoxetine alone) in frontal cortex ($F_{1,10}=7.32$, $P<0.025$, treatment effect; $F_{10,100}=7.82$, $P<0.001$, time effect and $F_{10,100}=8.71$, $P<0.001$, time × treatment interaction) but not in dorsal hippocampus. The regional difference in the effect of methiothepin was also noted by the significant effect of the time × region interaction ($F_{10,150}=5.19$, $P<0.001$).

Systemic administration of autoreceptor antagonists

The administration of WAY 100635 (0.3 mg kg^{-1} s.c.), methiothepin (10 mg kg^{-1} s.c.) or SB-224289 (4 mg kg^{-1} i.p.) alone did not alter significantly the 5-HT output in either region (Figure 4). The combination of WAY 100635 and methiothepin increased significantly the 5-HT output only in dorsal hippocampus to 160% ($F_{9,36}=7.99$, $P<0.001$). The concurrent administration of SB-224289 and WAY 100635 at the doses indicated elicited a moderate but significant increment of the 5-HT output in frontal cortex in a small group of rats ($F_{9,18}=2.59$; $P<0.05$; Figure 4).

Local administration of SSRIs

The local administration of fluoxetine (10–300 μM) elevated the 5-HT output in a concentration-dependent manner to a maximal value of 619±69% of baseline in frontal cortex and to 351±27% in dorsal hippocampus. Two-way repeated measures ANOVA indicated the existence of a significant effect of the region ($F_{1,10}=15.18$; $P<0.003$), concentration ($F_{3,30}=67.01$, $P<0.001$) and concentration × region interaction ($F_{3,30}=6.66$, $P<0.001$). The calculated EC_{50} values were 28 and 29 μM, in frontal cortex and dorsal hippocampus, respectively (Figure 5). The local administration of citalopram (1–30 μM) also elevated the 5-HT output in a region-dependent manner: 994±246% in frontal cortex

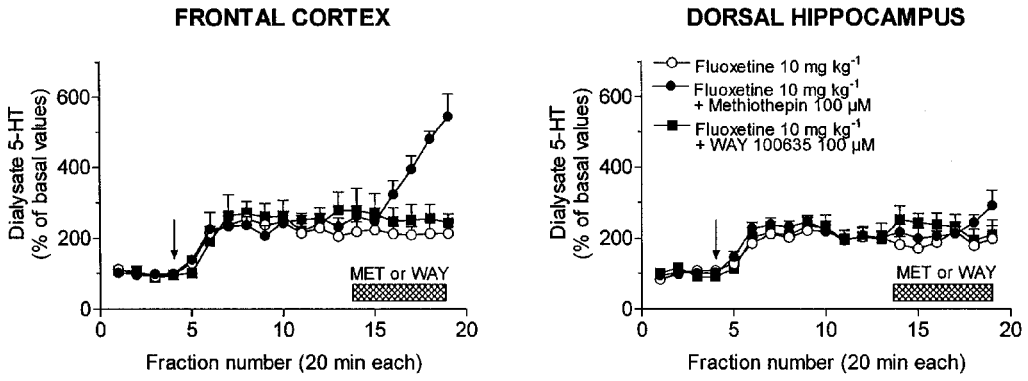

Figure 3 Effects of the local administration of WAY 100635 (100 μM) or methiothepin (100 μM) by reverse dialysis (cross-hatched bar) on the increase in 5-HT output elicited by 10 mg kg^{-1} i.p. fluoxetine (administered at arrow) in frontal cortex or dorsal hippocampus. Control rats received 10 mg kg^{-1} fluoxetine and were perfused with normal aCSF throughout the experiment. Data points are means±s.e.mean of 5–10 rats per group. See text for statistical analysis.

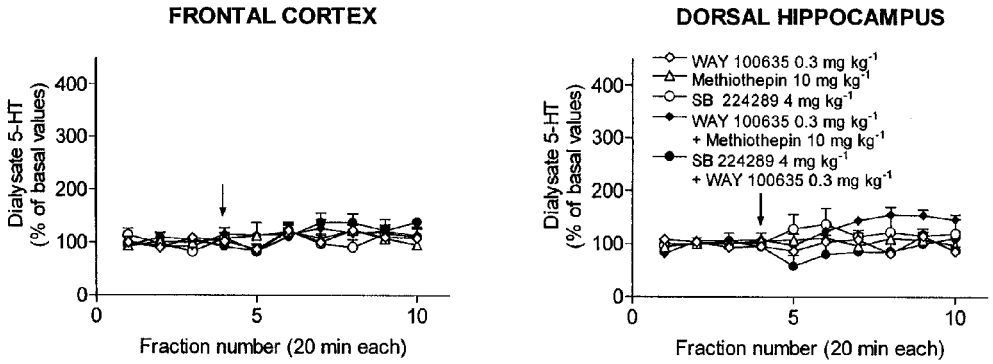

Figure 4 Effects of the administration of the 5-HT autoreceptor antagonists WAY 100635 (0.3 mg kg^{-1} s.c., $n=5$ in each region), methiothepin (10 mg kg^{-1} s.c., $n=5$ in each region), WAY 100635 plus methiothepin ($n=5$ in each region), SB-224289 (4 mg kg^{-1} i.p., $n=5$ in each region) and WAY 100635 (0.3 mg kg^{-1} s.c.) plus SB-224289 ($n=3$). The combinations of WAY 100635+methiothepin and WAY 100635+SB 224289 elevated significantly the 5-HT output in dorsal hippocampus and frontal cortex, respectively (see text for statistical analysis).

(EC_{50} = 1.0 μM); 326±33% in dorsal hippocampus (EC_{50} = 1.4 μM). Two-way repeated measures ANOVA indicated a significant effect of the region ($F_{1,10}$ = 12.32, P<0.006), concentration ($F_{3,30}$ = 16.24; P<0.001) and the concentration × region interaction ($F_{3,30}$ = 3.31, P<0.035) (Figure 5). Citalopram and fluoxetine elicited a comparable 5-HT increase in dorsal hippocampus. The 5-HT increase in frontal cortex was more marked for citalopram but the difference was not statistically significant (P = 0.065, treatment effect; P = 0.984, time × treatment interaction).

In presence of 30 μM citalopram to maximally inhibit the 5-HT reuptake, the local administration (100 μM) of the terminal autoreceptor antagonist methiothepin elevated the 5-HT output comparably in frontal cortex and dorsal hippocampus (to 224±40 vs 181±19%; P = 0.08 effect of the region; P = 0.09, time × region interaction) (Figure 6).

Discussion

Serotonergic neurones are endowed with two different autoreceptor types, 5-HT_{1A} and 5-HT_{1B}. The former are located in the somatodendritic region and their activation reduces 5-HT release by an impulse-dependent mechanism. 5-HT_{1B} receptors are located at nerve endings and control 5-HT release in a local manner. Both receptor subtypes play an important role in limiting the increments in 5-HT output elicited by SSRIs (Artigas *et al*., 1996; Gobert *et al*., 1997; Sharp *et al*., 1997).

The present results concur with previous microdialysis studies showing that systemic fluoxetine enhances the 5-HT output to a similar extent in various forebrain regions in a wide range of doses (Malagié *et al*., 1995; Hervás & Artigas, 1998). A similar effect was observed with paroxetine (Romero & Artigas, 1997). However, in combination with 5-HT_{1A} receptor antagonists, the SSRIs increase preferentially the 5-HT output in frontal cortex or striatum, compared to the hippocampus (Malagié *et al*., 1996; Romero *et al*., 1996a,b; Gundlah *et al*., 1997; Invernizzi *et al*., 1997; Romero & Artigas, 1997; Hervás, & Artigas, 1998). The SSRI-augmenting effect of WAY 100635 is due to an antagonism at raphe 5-HT_{1A} autoreceptors because its local application (100 μM) in the dorsal raphe nucleus potentiated the paroxetine-induced elevation in cortical 5-HT output (Romero & Artigas, 1997). The involvement of postsynaptic 5-HT_{1A} receptors in frontal cortex or hippocampus in this effect seems unlikely since the application of WAY 100635 in these areas at the same concentration did not augment the effect of fluoxetine (this study).

The reason(s) for the preferential effect of such combinations in areas innervated by dorsal raphe serotonergic neurones (Azmitia & Segal, 1978) are not completely understood. A plausible explanation is that dorsal raphe serotonergic neurones would be more responsive to 5-HT_{1A} autoreceptor activation, as suggested previously (Sinton &

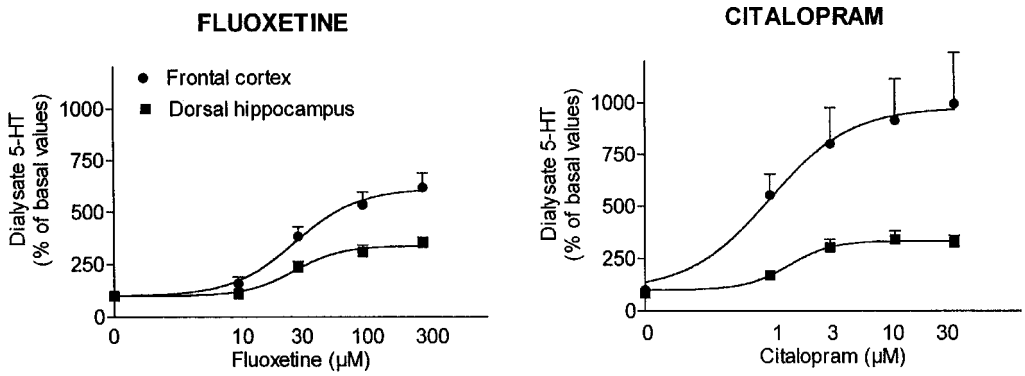

Figure 5 Concentration-effect relationship for the local administration of citalopram and fluoxetine by reverse dialysis in frontal cortex and dorsal hippocampus. Points are average of the last two fractions (out of four) at each SSRI concentration, expressed as percentage of baseline. Both SSRIs enhanced the 5-HT output in frontal cortex significantly more than in dorsal hippocampus. Data points are means±s.e.mean of five rats in frontal cortex (baseline 2.2±0.2 fmol fraction^{-1}), seven rats in dorsal hippocampus (baseline 2.9±0.4 fmol fraction^{-1}) for citalopram and five rats in frontal cortex (baseline 2.0±0.4 fmol fraction^{-1}) and seven rats in dorsal hippocampus (baseline 4.6±0.7 fmol fraction^{-1}) for fluoxetine.

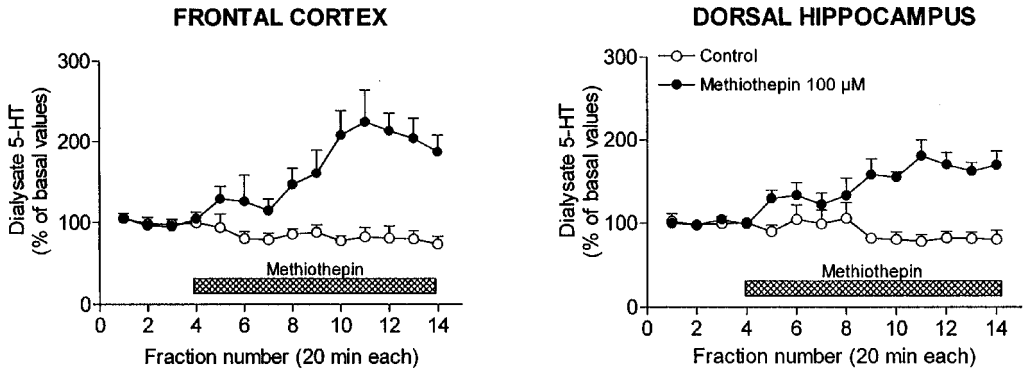

Figure 6 Effect of the local administration of 100 μM methiothepin (shown by a cross-hatched bar) on the 5-HT output. Probes were perfused with aCSF supplemented with 30 μM citalopram. Control rats were perfused continuously with artificial CSF containing 30 μM citalopram. Baseline values (mean±s.e.mean, in fmol fraction^{-1}) in presence of 30 μM citalopram were 15.1±2.1 for controls and 13.2±1.2 for the treated group in frontal cortex, and 8.8±1.8 for controls and 10.7±0.6 for the treated groups in dorsal hippocampus. Data points are means±s.e.mean of 5–6 rats per group. See text for statistical analysis.

Fallon, 1988). In this scenario, the blockade of 5-HT_{1A} receptors in the DRN would conceivably increase extracellular 5-HT produced by these drug combinations more in dorsal raphe-innervated areas as it has been found in the present and previous studies (see above). Other work, however, has shown that 8-OH-DPAT and paroxetine are equally effective in suppressing the firing rate in dorsal and median raphe serotonergic neurones in anaesthetized rats (Hajós *et al*., 1995).

In accordance with the inhibitory role played by 5-HT_{1B} autoreceptors, systemic treatment with the autoreceptor antagonist methiothepin or the selective 5-HT_{1B} receptor antagonist SB-224289 at doses that block 5-HT_{1B} receptors (Gardier *et al*., 1992; Gaster *et al*., 1998) produced an additional potentiation of the effect of fluoxetine + WAY 100635. This effect was greater in frontal cortex than in dorsal hippocampus. We did not examine the effect of the combination of fluoxetine and methiothepin because of the non-selective actions of the latter when administered systemically. Locally applied, methiothepin potentiated the effect of fluoxetine in frontal cortex, which supports the idea that its action was due to the blockade of terminal (5-HT_{1B}) autoreceptors, in accordance with previous evidence (Cerrito & Raiteri, 1979; Feuerstein *et al*., 1987; Hoyer & Schoeffter, 1991). Also, the similarity of the effects of methiothepin and the selective 5-HT_{1B} receptor antagonist, SB-224289, when given in combination with WAY 100635 suggests that the additional potentiation produced by methiothepin involves 5-HT_{1B} autoreceptors. These data further support the idea that terminal autoreceptors also limit the SSRI-induced elevations of the 5-HT output in rodent brain (Rollema *et al*., 1996; Gobert *et al*., 1997; Sharp *et al*., 1997; Gaster *et al*., 1998; Hervás *et al*., 1998).

The systemic administration of SB-224289 and the partial 5-$HT_{1B/1D}$ agonist, GR 127935, to guinea-pigs increased the 5-HT output in the dorsal hippocampus and decreased it (GR 127935) or left it unchanged (SB-224289) in frontal cortex (Roberts *et al*., 1998). Yet SB-224289 did not alter the 5-HT output in either region of rat brain. This may suggest species differences in the endogenous tone of hippocampal autoreceptors in rats and guinea-pigs. Also, due to pharmacological differences between terminal autoreceptors in these two species (Price *et al*., 1995), it is possible that SB-224289 exerts a more potent antagonism at guinea-pig autoreceptors.

Given the different origin of the serotonergic fibres innervating the dorsal hippocampus and the frontal cortex (Azmitia & Segal, 1978) and the different morphological characteristics of dorsal raphe and median raphe fibres (Kosofsky & Molliver, 1987), it is conceivable that 5-HT_{1A} and 5-HT_{1B} autoreceptors can preferentially control the 5-HT release in dorsal raphe and median raphe axons, respectively. However, this view is not supported by current evidence in rat brain. First, the local application of methiothepin elevated the 5-HT output similarly in dorsal raphe- and median raphe-innervated forebrain areas in the rat (Hervás *et al*., 1998). In the present study, local methiothepin potentiated the 5-HT enhancement induced by fluoxetine more in frontal cortex than in dorsal hippocampus and produced a comparable increase when co-perfused with citalopram. Secondly, the systemic administration of methiothepin augmented the effect of fluoxetine plus WAY 100635 more in frontal cortex than in dorsal hippocampus. Moreover, the more selective antagonist SB-224289 displayed the same regional selectivity, i.e. it augmented the effect of fluoxetine and fluoxetine plus WAY 100635 more in frontal cortex than in dorsal hippocampus. Thus, unlike in guinea-pigs (Roberts *et al*., 1998), hippocampal autoreceptors in the rat are not more effective than cortical ones in the control of 5-HT release, in agreement with previous *in vitro* data (Cerrito & Raiteri, 1979; Feuerstein *et al*., 1987). These above observations support the idea that 5-HT autoreceptors (5-HT_{1A} + 5-HT_{1B}) are more effective in restraining the effect of fluoxetine in frontal cortex than in dorsal hippocampus.

Locally administered, both SSRIs increased the 5-HT output more in frontal cortex than in dorsal hippocampus. This regional difference contrasts with the greater density of 5-HT reuptake sites in dorsal hippocampus compared to frontal cortex (D'Amato *et al*., 1987; Hrdina *et al*., 1990), which would suggest the opposite. The origin of this regional difference in the local effect of SSRIs is unknown. Only one type of neuronal 5-HT transporter has been reported in rat brain (Hoffman *et al*., 1991). A glial 5-HT transporter has been partially characterized (Bel *et al*., 1997) which is similar to that present in 5-HT neurones. The comparable EC_{50} values obtained in both regions suggest that both SSRIs interact with the same transporter. Because the SSRIs prevent the reuptake of newly released 5-HT, the greater 5-HT increase in frontal cortex elicited by citalopram and fluoxetine may indicate a greater 5-HT release per volume unit by serotonergic fibres in frontal cortex, compared to those in dorsal hippocampus. It is unclear whether this difference may be accounted for by the distinct morphology and synaptic connectivity of dorsal and median serotonergic fibres (Kosofsky & Molliver, 1987; Blue *et al*., 1988) or other factors, such as the influence of other neurotransmitters on regional control of 5-HT release (e.g., glutamate; Whitton *et al*., 1994).

In summary, the present results support the notion that the similar increase in 5-HT output produced by single fluoxetine administration in frontal cortex and dorsal hippocampus results from a greater increase in 5-HT produced by reuptake blockade in the former region which is compensated by a more marked attenuation of the 5-HT release through somatodendritic and terminal autoreceptors. In this manner, autoreceptor antagonists augment the 5-HT elevations produced by systemic fluoxetine administration more in frontal cortex than in dorsal hippocampus. These results are important for the design of therapeutic strategies for the treatment of depression and other 5-HT-related psychiatric illnesses based on the combination of 5-HT uptake inhibitors and 5-HT autoreceptor antagonists.

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