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Effect of fluoxetine on extracellular 5-hydroxytryptamine in rat brain. Role of 5-HT autoreceptors

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Abstract

Using microdialysis, we examined the effects of the antidepressant drug fluoxetine on 5-hydroxytryptamine (5-HT) output in rat brain. Fluoxetine (1, 3 and 10 mg/kg i.p.) dose dependently increased 5-HT output in the dorsal and median raphe nuclei and four forebrain areas. Maximal elevations were noted in the raphe nuclei. At 1 and 3 mg/kg, fluoxetine elicited minor or no increases of 5-HT output in the forebrain. When citalopram was present in the perfusion fluid, fluoxetine (10 mg/kg) reduced 5-HT output, an effect reversed by the administration of the selective 5-HT_{1A} receptor antagonist {*N*-[2-(4-(2-methoxyphenyl)-1-piperazinyl) ethyl]-*N*-(2-pyridyl) cyclohexane carboxamide · 3HCl} (WAY 100635). This reduction was more marked in the frontal cortex than in the dorsal hippocampus. Consistent with this, WAY 100635 potentiated the effect of 3 and 10 mg/kg fluoxetine more in the frontal cortex than in the dorsal hippocampus. The administration of a combination of WAY 100635 (0.3 mg/kg s.c.) and the 5-$HT_{1B/1D}$ receptor antagonist {*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl),[1,1-biphenyl]-4-carboxiamide} (GR 127935; 5 mg/kg s.c.) potentiated the effect of 3 mg/kg fluoxetine to an extent similar to that of WAY 100635 alone in both areas. These results suggest that somatodendritic 5-HT_{1A} receptors offset the effect of fluoxetine in the frontal cortex but not (or to a lesser extent) in the dorsal hippocampus. GR 127935 may have a partial antagonistic action at terminal 5-HT autoreceptors in vivo.

Keywords: 5-HT_{1A} receptor; Antidepressant; Dorsal raphe nucleus; Median raphe nucleus; Microdialysis; 5-HT (5-hydroxytryptamine, serotonin) uptake

1. Introduction

The selective serotonin reuptake inhibitors have become the most prescribed antidepressant drugs due to their favourable profile of side effects. Work conducted by this and other groups with the microdialysis technique has revealed that these agents increase preferentially the extracellular concentration of 5-hydroxytryptamine (5-HT) in the raphe nuclei of the midbrain (dorsal raphe nucleus and median raphe nucleus), where the cell bodies and dendrites of ascending serotonergic neurones are located (Dahlström and Fuxe, 1964; Wiklund et al., 1981; Descarries et al., 1982). The excess of extracellular 5-HT in the midbrain activates inhibitory somatodendritic 5-HT_{1A} autoreceptors, thus limiting the increase in extracellular 5-HT elicited by blockade of the 5-HT transporter in nerve endings (see Artigas et al., 1996; Stanford, 1996 for review). It was hypothesised that the concurrent antagonism of somatodendritic 5-HT_{1A} receptors should result in a more rapid and effective antidepressant treatment (Artigas, 1993). The results of open-labelled (Artigas et al., 1994; Blier and Bergeron, 1995) and controlled clinical studies (Pérez et al., 1997; Tomé et al., 1997; Zanardi et al., 1997) suggest that the nonselective 5-HT_{1A} receptor antagonist pindolol may accelerate the antidepressant effects of the selective serotonin reuptake inhibitors fluoxetine and paroxetine.

The differential innervation of the forebrain by serotonergic cells of the dorsal raphe nucleus and median raphe nucleus is extensively documented (Jacobs et al., 1974; Lorens and Guldberg, 1974; Bobillier et al., 1975; Azmitia and Segal, 1978; Köhler and Steinbusch, 1982; Imai et al., 1986; Kosofsky and Molliver, 1987; Mamounas and Molliver, 1988; Vertes, 1991; McQuade and Sharp, 1997). The projections of the dorsal raphe nucleus and median raphe nucleus exhibit a differential sensitivity to the inhibitory actions of selective 5-HT_{1A} receptor agonists (Blier et al., 1990; Invernizzi et al., 1991; Kreiss and Lucki, 1995;

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Casanovas and Artigas, 1996; Casanovas et al., 1997). Since selective serotonin reuptake inhibitors behave as indirect agonists at somatodendritic and terminal 5-HT autoreceptors, it is unclear whether their inhibitory actions are equally exerted in dorsal raphe and median raphe projections. This is an important point when devising potentiation strategies based on autoreceptor blockade. We thus conducted a systematic study of the effects of fluoxetine alone or in combination with autoreceptor antagonists, paying special attention to the effects in the frontal cortex and the dorsal hippocampus, areas which are innervated preferentially by fibres of the dorsal raphe nucleus and the median raphe nucleus, respectively (Azmitia and Segal, 1978; McQuade and Sharp, 1997).

2. Material and methods

2.1. Animals

Male Wistar rats (Iffa Credo, Lyon, France) weighing 270–320 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. Animal care conformed with European Union regulations (O.J. of E.C. L358/1 18/12/1986).

2.2. Drugs and reagents

Serotonin hydrochloride was from RBI (Natick, MA, USA). The selective serotonin reuptake inhibitors citalopram · HBr and fluoxetine · HCl were kindly provided by Lundbeck (Copenhagen-Valby, Denmark) and Eli Lilly (Indianapolis, IN, USA), respectively. The 5-HT_{1A} receptor antagonist WAY 100635 {*N*-[2-(4-(2-methoxyphenyl)-1-piperazinyl) ethyl]-*N*-(2-pyridyl) cyclohexane carboxamide · 3HCl} (Fletcher et al., 1995) was kindly supplied by Wyeth-Ayerst Research (Princeton, NJ, USA). GR 127935 {*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl),[1,1-biphenyl]-4-carboxiamide} was kindly provided by Dr. J.M. Palacios (Almirall-Prodes Farma, Barcelona, Spain). 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. The doses administered are expressed as free base. For the assessment of local effects, fluoxetine was dissolved in the microdialysis perfusion fluid and applied by reverse dialysis. In some experiments, 1 μM citalopram was also dissolved in the perfusion fluid (see below).

2.3. Microdialysis experiments

Anaesthetised rats (pentobarbital, 60 mg/kg i.p.) were implanted stereotaxically with 0.25-mm O.D. (1.5–4-mm long) concentric dialysis probes (Adell and Artigas, 1991) equipped with Cuprophan membranes (Gambro, Lund, Sweden) in six different brain regions, the dorsal raphe nucleus, the median raphe nucleus, the dorsal and ventral hippocampus, the frontal cortex and the dorsal striatum. The location of the microdialysis probes is shown in Table 1. Owing to the different size of the brain regions sampled, the length of dialysis membrane exposed to the brain tissue was 1.5 mm in the dorsal raphe nucleus, median raphe nucleus and dorsal hippocampus and 4 mm in all other areas. In one experiment, 1.5-mm probes were used in the frontal cortex to compare the inhibition of 5-HT release elicited by fluoxetine in this area and in the dorsal hippocampus (see Section 3.3). The vertical coordinate of the probe in the frontal cortex was −4.7 mm in this case.

Microdialysis experiments were conducted in freely moving rats about 20–24 h after probe implantation. Rats were implanted with one microdialysis probe in the region of interest. The probes were perfused with artificial cerebrospinal fluid (125 mM NaCl, 2.5 mM KCl, 1.26 mM $CaCl_2$ and 1.18 mM $MgCl_2$) at 0.25 μl/min. Sample collection started 60 min after the beginning of perfusion. Given the long half-life of fluoxetine (Caccia et al., 1990) and the low concentration of 5-HT in dialysates from some forebrain regions, 1-h dialysate fractions were collected in experiments assessing the regional effects. In all other experiments, 20-min fractions were used. After collection of three to four basal fractions, drugs were locally or systemically administered. In experiments devised to assess the inhibition of 5-HT release by the systemic administration of fluoxetine, the probes implanted in the dorsal hippocampus or the frontal cortex were perfused with 1 μM citalopram to locally block 5-HT reuptake. Under these conditions, the administration of a 5-HT uptake inhibitor results in a reduction in 5-HT release due to the activation of somatodendritic 5-HT_{1A} receptors by the excess of extracellular 5-HT produced in the midbrain raphe (Rutter and Auerbach, 1993; Romero and Artigas, 1997).

At the end of microdialysis experiments, the animals were killed by decapitation under pentobarbital anaesthesia and the correct placement of probes was checked by infusing Fast Green dye through the probes and visual inspection after cutting the brains at the appropriate levels.

Table 1
Location of the microdialysis probes

	AP	L	DV
Frontal cortex	+3.4	−2.5	−6.0
Dorsal striatum	+0.2	−3.0	−8.0
Dorsal hippocampus	−3.8	−1.8	−4.0
Ventral hippocampus	−5.8	−5.0	−8.0
Dorsal raphe nucleus	−7.8	−3.1	−7.5
Median raphe nucleus	−7.8	−2.0	−8.9

Coordinates in mm, taken from bregma and dura mater (Paxinos and Watson, 1986). 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.

The data of animals with probes outside the structures of interest were discarded.

2.4. Chromatographic analysis

5-HT was analysed by a modification of a high-performance liquid chromatography (HPLC) method previously described (Adell and Artigas, 1991). The composition of HPLC 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, San Ramón, CA, USA) and detected amperometrically with a Hewlett Packard 1049 detector (oxidation potential +0.6 V). 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 measured daily.

2.5. Data analysis

5-HT concentrations in dialysates are expressed as fmol/fraction and represented in most figures as percentages of basal values (individual means of three to four predrug fractions) to facilitate comparisons between the different experimental groups. To compare the absolute change in 5-HT output in the different brain areas, the increases over baseline were calculated and averaged for the posttreatment period.

Given the different size of the dialysis probes used in the experiments to assess the net effects of fluoxetine, a calibration curve was constructed to compare the baseline 5-HT concentrations and the absolute increases produced by fluoxetine in the different regions. Probes of 1, 2, 3 and 4-mm long were constructed, placed in a solution containing 7 nM 5-HT and 1 μM 5-hydroxyindolacetic acid (5-HIAA) and perfused at 0.25 μl/min. Fig. 1 shows the relationship between membrane length and relative in vitro recovery. Data for 4-mm probes were then corrected (relative factor for 5-HT was 0.66) and expressed as for 1.5-mm probes. The EC_{50} of the inhibitory effect of fluoxetine on 5-HT reuptake was calculated with the GraphPad Prism program (San Diego, CA, USA).

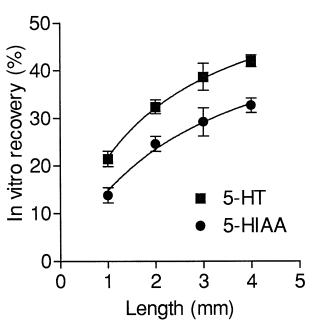

Fig. 1. Relationship between the in vitro recovery of 5-HT and 5-HIAA from concentric dialysis probes and their size. Probes of different membrane length (1, 2, 3 and 4 mm; $n = 4$ each) were perfused at 0.25 μl/min with artificial CSF and placed in Eppendorf tubes containing 7 nM 5-HT and 1 μM 5-HIAA. Four 20-min fractions were collected of which the last three were obtained to calculate the individual recovery (5-HT, ■; 5-HIAA, ●). Data are expressed as means ± S.E.M.

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

Region	5-HT (fmol/1-h fraction)
Dorsal raphe nucleus ($n = 24$)	12.7 ± 0.8
Median raphe nucleus ($n = 24$)	11.9 ± 0.8
Dorsal striatum ($n = 27$)	7.8 ± 0.7
Frontal cortex ($n = 20$)	4.6 ± 0.3
Ventral hippocampus ($n = 23$)	6.2 ± 0.4
Dorsal hippocampus ($n = 26$)	6.4 ± 0.4

Data expressed as means ± S.E.M. of the number of animals shown in parentheses. Dialysate 5-HT levels are for 1-h fractions in the absence of citalopram. Values from frontal cortex, dorsal striatum and ventral hippocampus have been corrected for the length of the dialysis membrane and expressed as for 1.5-mm probes. See also baseline dialysate values in presence of citalopram in legends to some figures.

Statistical analysis of the effects of fluoxetine was performed using one- or two-way analysis of variance (ANOVA) for repeated measures of raw data (fmol 5-HT/fraction) followed by *t*-tests where appropriate. One-way ANOVA followed by Duncan tests were also used to examine regional differences. Data are expressed as means ± S.E.M.. Statistical significance was set at the 95% confidence level (two-tailed).

3. Results

3.1. Baseline values

One-hour baseline 5-HT concentrations in dialysates from the dorsal raphe nucleus, median raphe nucleus and projection areas in the forebrain are shown in Table 2. One-way ANOVA revealed a significant effect of the region on baseline 5-HT ($F(5,138) = 3.99$, $P < 0.01$). 5-HT concentrations were greater in the dorsal raphe nucleus and the median raphe nucleus than in any forebrain area ($P < 0.05$, Duncan test). The lowest 5-HT concentrations were found in the frontal cortex and the dorsal hippocampus.

3.2. Effects of the local and systemic administration of fluoxetine

Fluoxetine elevated significantly the extracellular 5-HT concentration in dialysates from the frontal cortex when applied by reverse dialysis (Fig. 2). Maximal increases were noted at 300 μM (ca. 6-fold). The calculated EC_{50} was 29.7 μM. However, the systemic administration of fluoxetine resulted in smaller dose-dependent increases in

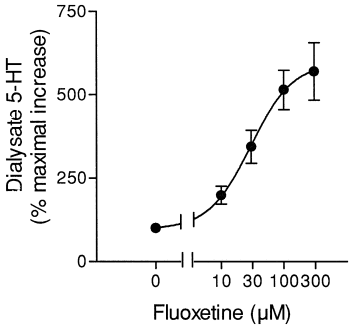

Fig. 2. Concentration-effect relationship for the application of fluoxetine by reverse dialysis through probes implanted in the frontal cortex ($n = 7$; baseline 5-HT: 2.0 ± 0.2 fmol/fraction). Data points are means ± S.E.M. of the increase (percentage of pre-drug levels) at each fluoxetine concentration. The calculated EC_{50} value was 29.7 μM.

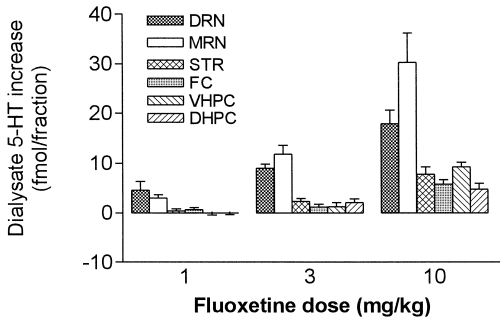

Fig. 4. Absolute increase in dialysate 5-HT concentration produced by the administration of fluoxetine (FLX; 1, 3 and 10 mg/kg i.p.) in the six brain areas examined. Data (expressed as for 1.5-mm probes in all regions) are means ± S.E.M. ($n = 5–8$) of the increases over baseline during the period 1–6 h posttreatment. One-way ANOVA revealed a significant effect of the region at the three fluoxetine doses ($F(5,29) = 5.16$, $P < 0.002$ at 1 mg/kg; $F(5,29) = 21.36$, $P < 0.0001$ at 3 mg/kg; $F(5,35) = 10.95$, $P < 0.0001$ at 10 mg/kg). Duncan test revealed the following significant differences: dorsal raphe nucleus > all other regions at 1 mg/kg; dorsal raphe nucleus = median raphe nucleus > forebrain regions at 3 and 10 mg/kg. At the latter dose, the increase in 5-HT output in the median raphe nucleus was significantly greater than that in the dorsal raphe nucleus.

dialysate 5-HT levels in the various brain areas examined (maximum of about threefold in the median raphe nucleus and the dorsal raphe nucleus at 10 mg/kg). The maximal increase in 5-HT output at 10 mg/kg occurred 2–4 h after the administration of fluoxetine and remained essentially stable until the end of experiments. Lower doses elicited much more moderate increases. Fig. 3 shows the effect of the 3 mg/kg dose in the six brain regions examined. At each dose, the analysis of the data by two-way ANOVA revealed a significant effect of time ($F(6,174) = 4.35$, $P < 0.001$ at 1 mg/kg; $F(6,174) = 16.65$, $P < 0.001$ at 3 mg/kg; $F(6,210) = 32.30$, $P < 0.001$ at 10 mg/kg), region ($F(5,29) = 11.69$, $P < 0.001$ at 1 mg/kg; $F(5,29) = 30.31$, $P < 0.001$ at 3 mg/kg; $F(5,35) = 19.72$, $P < 0.001$ at 10 mg/kg) and time × region interaction ($F(30,174) = 1.94$, $P < 0.004$ at 1 mg/kg; $F(30,174) = 3.61$, $P < 0.001$ at 3 mg/kg; $F(30,210) = 4.11$, $P < 0.001$ at 10 mg/kg). Maximal increases were observed in the dorsal raphe nucleus and the median raphe nucleus.

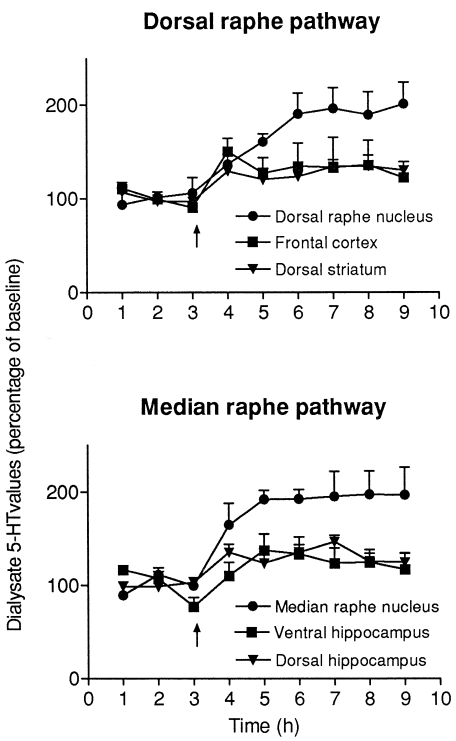

Fig. 3. Effects of the single i.p. administration (arrow) of 3 mg/kg fluoxetine in the dorsal and median raphe nucleus and in respective selective projection areas of the rat brain. Data are means ± S.E.M. from five to seven rats/group. See text for statistical analysis.

The absolute increases in 5-HT output produced by the i.p. administration of fluoxetine (averaged for the 6 h postadministration) are shown in Fig. 4. One-way ANOVA revealed a significant effect of the region at the three fluoxetine doses ($F(5,29) = 5.16$, $P < 0.002$ at 1 mg/kg; $F(5,29) = 21.36$, $P < 0.001$ at 3 mg/kg; $F(5,35) = 10.95$, $P < 0.001$ at 10 mg/kg). At 1 mg/kg, there was no significant increase in 5-HT output in any forebrain area, whereas at 3 mg/kg small increases were noted, but in all cases these were significantly lower than in the dorsal raphe nucleus and the median raphe nucleus ($P < 0.05$, Duncan test). At 10 mg/kg, the increase in 5-HT output in the median raphe nucleus was significantly greater than that in the dorsal raphe nucleus and all forebrain regions ($P < 0.05$, Duncan t-test). The increase in 5-HT output in the dorsal raphe nucleus was also greater than that in all forebrain areas ($P < 0.05$, Duncan test).

3.3. Inhibition of 5-HT release in dorsal hippocampus and frontal cortex

To assess the inhibitory effects of fluoxetine on serotonergic neurones of the dorsal and median raphe nuclei, we administered 10 mg/kg fluoxetine to rats with a single

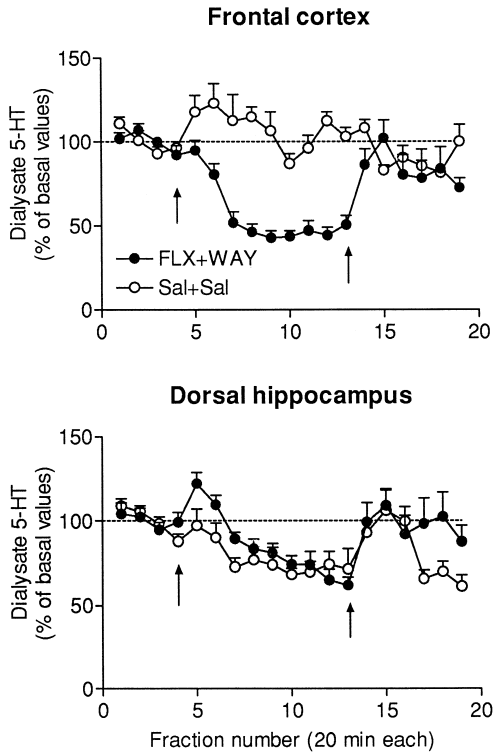

Fig. 5. (Top) Significant ($P < 0.001$) reduction of 5-HT output in the frontal cortex (●, $n = 7$; baseline 5-HT: 9.6 ± 1.0 fmol/fraction) by the i.p. administration of 10 mg/kg fluoxetine (first arrow). Probes (tip: 1.5 mm) were perfused with 1 μM citalopram. The subsequent administration of 1 mg/kg s.c. WAY 100635 (second arrow) reversed the attenuation of 5-HT release elicited by fluoxetine ($P < 0.002$). Control rats (○, $n = 6$; baseline 5-HT 7.9 ± 1.3 fmol/fraction) received two saline injections (arrows). (Bottom) Fluoxetine administration (10 mg/kg i.p.) produced a steady but significant decrease in dialysate 5-HT concentrations in the dorsal hippocampus (●, $n = 9$; baseline 5-HT 8.3 ± 0.8 fmol/fraction) which was not significantly different from that observed in control animals receiving two saline injections (○, $n = 7$; baseline 5-HT: 9.1 ± 0.9 fmol/fraction). See text for statistical analysis.

1.5-mm dialysis probe placed in the frontal cortex or the dorsal hippocampus. We used probes of equal size in both regions to avoid any methodological source of difference. These probes were perfused with an artificial CSF containing 1 μM citalopram to inhibit locally the 5-HT transporter. The i.p. administration of fluoxetine reduced the 5-HT output to 45% of baseline in the frontal cortex ($F(10,60) = 22.48$, $P < 0.0001$, repeated measures ANOVA; Fig. 5, top). This effect was counteracted by the administration of 1 mg/kg s.c. WAY 100635 ($F(7,42) = 9.99$, $P < 0.0001$, repeated measures ANOVA). Representative dialysate values (individual means of 4 fractions) during the basal, fluoxetine, and fluoxetine + WAY 100635 periods were, 9.6 ± 1.0, 4.3 ± 0.5 and 7.9 ± 0.8 fmol/fraction, respectively ($P < 0.001$, fluoxetine effect; $P < 0.002$, WAY 100635 effect; paired Student's *t*-test).

In the dorsal hippocampus, the administration of fluoxetine elicited a significant but slow decline in 5-HT output that was not significantly different from that of saline-injected controls (two-way repeated measures ANOVA). The subsequent administration of WAY 100635 increased 5-HT output in fluoxetine-treated rats, but a second saline injection also increased 5-HT output in the same manner in the control group (Fig. 5, bottom). The averaged 5-HT values for 4 fractions (as above) for the basal, fluoxetine and fluoxetine + WAY 100635 periods were, 8.3 ± 0.8, 6.3 ± 0.7 and 7.2 ± 0.5, respectively ($P < 0.015$ fluoxetine effect; $P = 0.16$, WAY 100635 effect; paired Student's *t*-test).

3.4. Potentiation experiments. Role of 5-HT autoreceptors

The 5-HT_{1A} receptor antagonist WAY 100635 potentiates the increase in 5-HT output induced by 10 mg/kg fluoxetine in the rat frontal cortex (Romero et al., 1996). In view of the distinct inhibition produced by fluoxetine in the frontal cortex and the dorsal hippocampus, we examined the effects of the combined administration of fluoxetine and WAY 100635 in the latter region. For consistency, in the first experiment we used doses equal to those in the previous report (10 mg/kg i.p. fluoxetine and 1 mg/kg s.c. WAY 100635). All subsequent experiments were conducted with lower doses of both agents (3 mg/kg fluoxetine and 0.3 mg/kg WAY 100635). The administration of 10 mg/kg fluoxetine elevated 5-HT output in hippocampal dialysates to about 220% of baseline. The subsequent administration of 1 mg/kg s.c. WAY 100635, a dose devoid of effect on 5-HT output in the forebrain (Romero and Artigas, 1997), moderately increased 5-HT output (from 214 ± 30 to 292 ± 28%; $F(1,8) = 18.16$, $P < 0.003$, treatment effect; $F(8,64) = 4.49$, $P < 0.001$, time × treatment interaction) but this effect was much

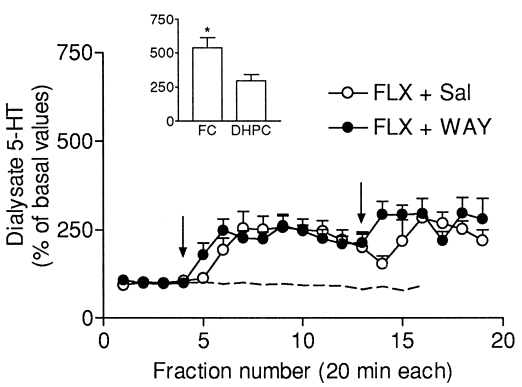

Fig. 6. Effect of the combined administration of 10 mg/kg i.p. fluoxetine (first arrow) and WAY 100635 (1 mg/kg s.c., second arrow) on dialysate 5-HT concentrations in the dorsal hippocampus (●, $n = 6$; baseline 5-HT 1.7 ± 0.2 fmol/fraction). Control rats (○, $n = 4$; baseline 5-HT 4.6 ± 0.9 fmol/fraction) were injected with fluoxetine 10 mg/kg and saline. *Inset*: comparison with the maximal increase elicited by the combination fluoxetine + WAY 100635 in the dorsal hippocampus and frontal cortex (data from the latter area are from Romero et al., 1996). * $P < 0.02$ vs. dorsal hippocampus. The dashed line shows the effect of an injection (arrow) of WAY 100635 (1 mg/kg s.c.).

lower than that produced in the frontal cortex ($P < 0.02$; see inset in Fig. 6).

The administration of 0.3 mg/kg s.c. WAY 100635 potentiated the moderate change in 5-HT output elicited by 3 mg/kg fluoxetine in the frontal cortex (Fig. 7) ($F(7,21) = 6.49$, $P < 0.001$). The elevation induced by WAY 100635 was significantly different from that produced by saline in fluoxetine-pretreated rats ($F(7,42) = 2.49$; $P < 0.03$; treatment × time interaction; two-way repeated measures ANOVA). In contrast, the administration of WAY 100635 induced a moderate increase in 5-HT output in the dorsal hippocampus that was not significantly different from that produced by injection of saline ($P = 0.41$).

To examine whether the blockade of terminal (5-HT_{1B}) autoreceptors could further enhance the 5-HT output in either area, we administered fluoxetine (3 mg/kg i.p.) and a combination of WAY 100635 (0.3 mg/kg s.c.) and GR 127935 (5 mg/kg s.c.). The latter is a 5-$HT_{1B/1D}$ receptor antagonist (Starkey and Skingle, 1994; Skingle et al., 1995a). The administration of WAY 100635 + GR 127935 to fluoxetine-pretreated rats significantly enhanced 5-HT output in the frontal cortex, and this effect was significantly different from that elicited by injection of saline ($F(7,49) = 2.87$, $P < 0.015$; two-way repeated measures ANOVA). The increase in 5-HT output was similar to that achieved with fluoxetine + WAY 100635 but the time course was slightly different, with a more steady increase after the administration of both antagonists (Fig. 7) The average 5-HT value after WAY 100635 alone or in combination with GR 127935 (fractions 14–19 in Fig. 7) was 241 ± 54 and 269 ± 33, respectively, expressed as percentage of baseline ($P = 0.66$).

Similarly, the administration of WAY 100635 + GR 127935 to fluoxetine-pretreated rats significantly enhanced 5-HT output in the dorsal hippocampus more than fluoxetine + saline did ($F(7,49) = 7.02$, $P < 0.001$, time × treatment interaction). The average extracellular 5-HT level after WAY 100635 alone or in combination with GR 127935 was 184 ± 14 and 200 ± 17, respectively, expressed as percentage of baseline ($P = 0.49$).

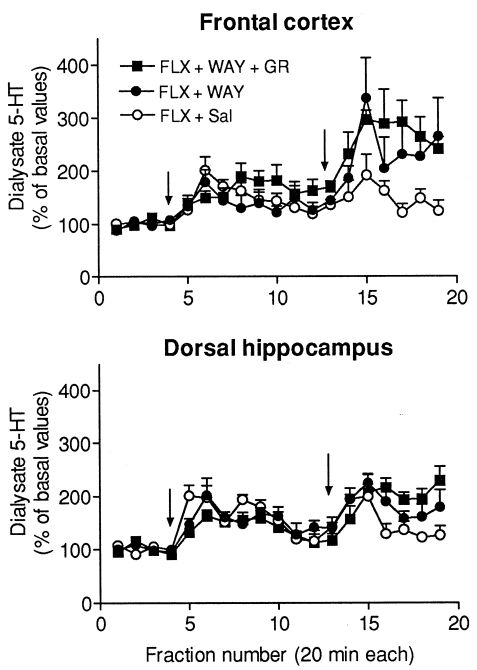

Fig. 7. Comparison of the effects of the administration of fluoxetine (3 mg/kg i.p.) in combination with saline (○), WAY 100635 (0.3 mg/kg s.c.) (●) or WAY 100635 (0.3 mg/kg s.c.) plus GR 127935 (5 mg/kg s.c.) (■) on 5-HT output in the frontal cortex (top) and dorsal hippocampus (bottom). Arrows denote the injection of fluoxetine (first arrow) and saline, WAY 100635 or WAY 100635 plus GR 127935 (second arrow). Baseline 5-HT concentrations were 4.9 ± 0.6, 5.4 ± 1.5 and 4.5 ± 0.6 fmol/fraction for fluoxetine + saline, fluoxetine + WAY 100635 and fluoxetine + WAY 100635 + GR 127935, in the frontal cortex and 2.5 ± 0.3, 4.0 ± 0.9 and 5.0 ± 1.1 fmol/fraction in the dorsal hippocampus, respectively. Data from four to five rats/group. See text for statistical analysis.

4. Discussion

The data of the present study confirm and extend previous observations of a preferential increase in 5-HT output produced by 5-HT reuptake inhibitors in the midbrain raphe nuclei (Adell and Artigas, 1991; Bel and Artigas, 1992; Invernizzi et al., 1992; Gartside et al., 1995; Malagié et al., 1995). Information on the effects of fluoxetine on 5-HT output in the rat brain is fragmentary (Perry and Fuller, 1992; Rutter and Auerbach, 1993; Kreiss and Lucki, 1995; Malagié et al., 1995; Dreshfield et al., 1996). Apart from one study in anaesthetised rats (Malagié et al., 1995), no dose–effect relationships have been examined. The present data, obtained in freely moving rats, indicate that only high systemic doses of fluoxetine (10 mg/kg) consistently enhance 5-HT output in projection areas of the forebrain. Yet, this effect was smaller than that produced by local application of fluoxetine, a difference probably accounted for by inhibitory effects at autoreceptors (see below). Small or no increases in 5-HT output were produced by 3 and 1 mg/kg fluoxetine in the forebrain. These doses are more relevant to understanding the antidepressant effects of fluoxetine because the standard clinical regimen is 20 mg/day.

Interestingly, despite the much lower number of neurones in the median raphe nucleus compared to the dorsal raphe nucleus (Steinbush and Nieuwenhuys, 1983), the median raphe nucleus displayed a marked sensitivity to fluoxetine. Given the uneven innervation of the forebrain by neurones from the dorsal raphe nucleus and the median raphe nucleus and the distinct role of both systems in mood regulation (Graeff et al., 1996), these differences may have functional implications.

The involvement of somatodendritic 5-HT_{1A} autoreceptors in offsetting the increase in 5-HT output induced by selective serotonin reuptake inhibitors is well documented (see Stanford, 1996 and Artigas et al., 1996 for review).

Most research on this issue has focused on the frontal cortex and there is little information on the inhibitory effects of selective serotonin reuptake inhibitors in the dorsal hippocampus, although differences between these two regions have been reported after chronic treatment with these agents (Bel and Artigas, 1993; Invernizzi et al., 1994, 1995; Bosker et al., 1995). More recently, differences in the ability of the 5-HT_{1A} receptor antagonist WAY 100635 to potentiate the effects of the selective serotonin reuptake inhibitors citalopram and paroxetine in the frontal cortex and the dorsal hippocampus have been reported (Invernizzi et al., 1997; Romero and Artigas, 1997). The present data indicate that fluoxetine reduces 5-HT release in the frontal cortex more than in the dorsal hippocampus. Under appropriate experimental conditions (i.e., with an uptake blocker in the perfusion fluid) the systemic administration of selective serotonin reuptake inhibitors reduces 5-HT release in the forebrain, thus showing that 5-HT reuptake inhibitors behave as indirect agonists of somatodendritic 5-HT_{1A} receptors due to the enhancement of 5-HT output in the midbrain raphe nuclei (Adell and Artigas, 1991; Rutter and Auerbach, 1993; Romero and Artigas, 1997). The decrease in 5-HT output seen in this experimental situation is antagonized by the local (in the dorsal raphe nucleus) or systemic administration of the 5-HT_{1A} receptor antagonist WAY 100635 (Hjorth et al., 1996; Romero and Artigas, 1997; this study). With this experimental approach, fluoxetine reduced 5-HT output in the frontal cortex more than in the dorsal hippocampus. In the latter area, fluoxetine elicited a slow and steady reduction, which was indistinguishable from that produced by a saline injection. This spontaneous decrease in 5-HT output was previously observed in the median raphe nucleus and the hippocampus in experiments involving the perfusion of citalopram (Casanovas and Artigas, 1996; Casanovas et al., 1997) whereas no such decrease occurred in absence of citalopram. This decrease may be due to an adaptive change in the synthesis or release of 5-HT in the hippocampus following prolonged blockade of 5-HT reuptake. Also, whereas the effects of the second saline injection and of WAY 100635 were clearly distinct in the frontal cortex, they were superimposed in the dorsal hippocampus. The reasons for such similarity are unclear. The increase in 5-HT output may tentatively be ascribed to some form of sensitization of hippocampal 5-HT release to the injection stress, because the effect of the first saline injection was less marked. This effect was consistently observed in all animals in both groups ($n = 7–9$).

In accordance with the smaller inhibition of 5-HT release in the dorsal hippocampus, WAY 100635 elicited a small potentiation of the effect of 10 mg/kg fluoxetine in this area. The increase produced by WAY 100635 was significantly smaller than that produced in the frontal cortex with the same doses of both agents (Romero et al., 1996). The dose of WAY 100635 used in these experiments (1 mg/kg s.c.) is devoid of effect on 5-HT output in both regions (Romero and Artigas, 1997). At lower doses (3 mg/kg fluoxetine and 0.3 mg/kg WAY 100635) this regional difference also occurred, as WAY 100635 potentiated the increase in 5-HT output in the frontal cortex but not in dorsal hippocampus.

The neurobiological basis for this regional selectivity is common to other selective serotonin reuptake inhibitors (Invernizzi et al., 1997; Romero and Artigas, 1997). Given the preferential innervation of the frontal cortex and the dorsal hippocampus by fibres of the dorsal raphe nucleus and the median raphe nucleus, respectively (Azmitia and Segal, 1978; McQuade and Sharp, 1997), these differences might be accounted for by a greater sensitivity of dorsal raphe neurones to the inhibitory actions of selective serotonin reuptake inhibitors, resulting in a greater reduction of impulse-dependent terminal 5-HT release. Indeed, citalopram reduced 5-HT output more in the frontal cortex than in the dorsal hippocampus when applied in the dorsal raphe nucleus and median raphe nucleus, respectively (Romero and Artigas, 1997). However, it is controversial whether dorsal raphe and median raphe neurones are equally sensitive to the activation of 5-HT_{1A} autoreceptors (Sinton and Fallon, 1988; Blier et al., 1990; Hjorth and Sharp, 1991; Invernizzi et al., 1991; Hajos et al., 1995). Selective 5-HT_{1A} receptor agonists reduce 5-HT release in the forebrain with a regional pattern equal to that produced by the selective serotonin reuptake inhibitors, i.e., a greater effect in the frontal cortex or striatum than in the hippocampus (Kreiss and Lucki, 1995; Casanovas and Artigas, 1996; Casanovas et al., 1997). Yet, unlike in their respective projection areas, the decrease in 5-HT release elicited by 5-HT_{1A} receptor agonists in the median raphe nucleus was comparable or slightly greater than that in the dorsal raphe nucleus (Casanovas and Artigas, 1996; Casanovas et al., 1997). Moreover, the effect of fluoxetine was not potentiated by WAY 100635 in the ventral hippocampus (Malagié et al., 1996), an area receiving a dense innervation from the dorsal raphe nucleus (Azmitia and Segal, 1978; McQuade and Sharp, 1997). This suggests that the inhibition of 5-HT release in projection areas elicited by direct and indirect 5-HT_{1A} receptor agonists is partly unrelated to the dorsal or median raphe origin of the serotonergic fibres innervating a particular forebrain area and that local factors may also be involved. Indeed, it seemed surprising that, in absence of the tight control that 5-HT_{1A} autoreceptors exert on cortical 5-HT release and the greater density of hippocampal (vs. cortical) 5-HT transporter sites (Hrdina et al., 1990), fluoxetine increased 5-HT output in the dorsal hippocampus to an extent comparable to that in the frontal cortex. We therefore examined whether terminal (5-HT_{1B}) autoreceptors in the dorsal hippocampus could be more effective than those in the frontal cortex in controlling 5-HT release during the administration of fluoxetine, using the putative 5-$HT_{1B/1D}$ receptor antagonist GR 127935.

The data obtained with this agent cannot clarify this aspect. The administration of fluoxetine in combination with WAY 100635 and GR 127935 together elicited a maximal increase in 5-HT output comparable to that produced by fluoxetine + WAY 100635 in both areas although the increase in 5-HT output seemed somewhat more persistent. During the completion of the present manuscript, Gobert et al. (1997) reported a large increase in 5-HT output in the frontal cortex of rats for the same drug combination. Also, Sharp et al. (1997) reported a substantial enhancement by paroxetine in the same region when WAY 100635 and GR 127935 were administered concurrently. These discrepancies may be related to the different doses and routes of administration used. Gobert et al. (1997) used 10 mg/kg fluoxetine (vs. 3 mg/kg in the present study) and Sharp et al. (1997) used 5 mg/kg i.v. GR 127935 (these authors used the i.v. route for all compounds). In our study, the failure of GR 127935 to potentiate the increased 5-HT output produced in the frontal cortex by fluoxetine + WAY 100635 cannot be attributed to an insufficient tone of terminal autoreceptors, because this combination increased cortical 5-HT output to 350% of baseline. In separate experiments, GR 127935 (up to 100 μM, applied by reverse dialysis) did not increase 5-HT output in various areas of the rat forebrain when terminal autoreceptors were activated by the concurrent application of citalopram, which increases basal 5-HT output by 4–6-fold (Hervás et al., 1998). Also, the local application of GR 127935 in guinea-pig cortex caused only a very moderate and transient increase in 5-HT efflux (Skingle et al., 1995b) or failed to do so (Hutson et al., 1995) whereas its systemic administration slightly reduced 5-HT release (Skingle et al., 1995b). These data raise doubts about the antagonist character of this agent to prevent the inhibitory action of 5-HT on its own release.

In summary, these findings indicate that 5-HT_{1A} receptors play a pivotal role in limiting the 5-HT release by nerve terminals in the frontal cortex during fluoxetine treatment. The data obtained do not support the notion that GR 127935 is a useful agent to further potentiate the increase in 5-HT output produced by a combination of low-dose fluoxetine and a 5-HT_{1A} receptor antagonist. Whether 5-HT_{1B} receptors are equally effective in modulating the 5-HT release in the frontal cortex and the dorsal hippocampus during the administration of fluoxetine must await further investigation with more potent antagonists.

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