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Short communication

Effects of acute fluoxetine on extracellular serotonin levels in the raphe: an in vivo microdialysis study

Isabelle Malagié, Anne-Cécile Trillat, Christian Jacquot, Alain M. Gardier *

Faculté de Pharmacie, Laboratoire de Pharmacologie JE 92-372, Tour D1, 2ème étage, F-92296 Chatenay-Malabry Cédex, France

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Abstract

Acute administration of fluoxetine (1, 10 and 20 mg/kg i.p.) increased extracellular levels of serotonin (5-hydroxytryptamine, 5-HT) in the frontal cortex, ventral hippocampus and raphe nuclei as measured by in vivo microdialysis in anaesthetized rats. In the frontal cortex, fluoxetine showed a marked dose-response effect whereas in the ventral hippocampus and raphe nuclei the fluoxetine-induced effect was maximum at 10 mg/kg. However, the maximal increase in 5-HT was observed in the cell body-containing area, the raphe nuclei. The order of changes in extracellular 5-HT was raphe nuclei > ventral hippocampus > frontal cortex. Our results add further arguments in favour of the key role played by raphe nuclei in the mechanism of action of serotoninergic antidepressant drugs.

Keywords: 5-HT (5-hydroxytryptamine, serotonin) uptake; Fluoxetine; Frontal cortex; Ventral hippocampus; Raphe nuclei; Microdialysis, in vivo

1. Introduction

Recent in vivo microdialysis studies have shown that systemic administration of a single dose of selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors such as fluvoxamine (Bel and Artigas, 1992) and citalopram (Invernizzi et al., 1992) preferentially increases extracellular levels of 5-HT in the raphe nuclei of the rat: a several-fold increase in extracellular 5-HT was observed nearby the cell bodies and dendrites of serotoninergic neurones compared to that measured in serotoninergic nerve terminal regions such as the frontal cortex. A plausible explanation for this differential effect could involve the high density of the specific 5-HT transporter (Hrdina et al., 1990) and that of somatodendritic 5-HT_{1A} autoreceptors (Sotelo et al., 1990) contained in the raphe. Indeed, 5-HT_{1A} autoreceptors exert a negative influence on the firing of serotoninergic neurones and on the release of 5-HT from nerve terminals. The high extracellular endogenous 5-HT levels produced by the acute blockade of the transporter in the raphe nuclei may activate these autoreceptors, thus leading to a decrease in the firing rate of 5-HT neurones and the terminal release of 5-HT (Artigas, 1993).

Today, there are no data concerning alterations of extracellular levels of 5-HT in the raphe nuclei following acute administration of another 5-HT uptake inhibitor, fluoxetine (see Fuller, 1994 for a review). To know if this property is shared by this 5-HT uptake inhibitor, in vivo microdialysis was simultaneously performed in the frontal cortex, ventral hippocampus and raphe nuclei following acute injection of fluoxetine (1, 10 and 20 mg/kg i.p.) in anaesthetized rats.

2. Materials and methods

Microdialysis experiments were carried out in male Sprague-Dawley rats (200–300 g, Charles River, France). Concentric dialysis probes were made of polyacrylonitrile fibres (HOSPAL AN69, France) and prepared according to Farber et al. (1993). The size of the dialysis membrane was 3.5 mm long × 0.30 mm OD for the three regions studied. Animals were anaesthetized with chloral hydrate (400 mg/kg i.p., plus supplementary doses as appropriate) and placed in a stereotaxic frame. Rats were implanted with three probes follow-

* Corresponding author. Tel.: 1-46-83-54-16; fax: 1-46-83-53-55.

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ing coordinates (in mm) taken from bregma and top of the skull (Paxinos and Watson, 1986): one in the right frontal cortex (A +3.5, L +2.5, V −4), the second in the left ventral hippocampus (A −4.8, L −4.8, V −7.5) and the third in the dorsal and median raphe nuclei (A −7.8, L −0.5, V −9). This latter implantation placed the probe very close to the dorsal and median raphe nuclei, as described by Adell and Artigas (1991). Therefore, mechanical lesioning of both nuclei and perforation of the cerebral aqueduct were prevented. The probes, cemented in place, were continuously perfused with an artificial cerebrospinal fluid (composition in mM: NaCl 147, KCl 3.5, $CaCl_2$ 1.0, $MgCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 25.0, pH 7.4 ± 0.2) at a flow rate of 1.3 μl/min using a CMA/100 Microinjection Pump (Carnegie Medicin, Stockholm, Sweden). Dialysate samples were collected every 15 min in small Eppendorf tubes and were immediately analysed for 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) by high-performance liquid chromatography (HPLC), using a LC-4B amperometric detector (BioAnalytical System), as previously described (Gardier et al., 1994).

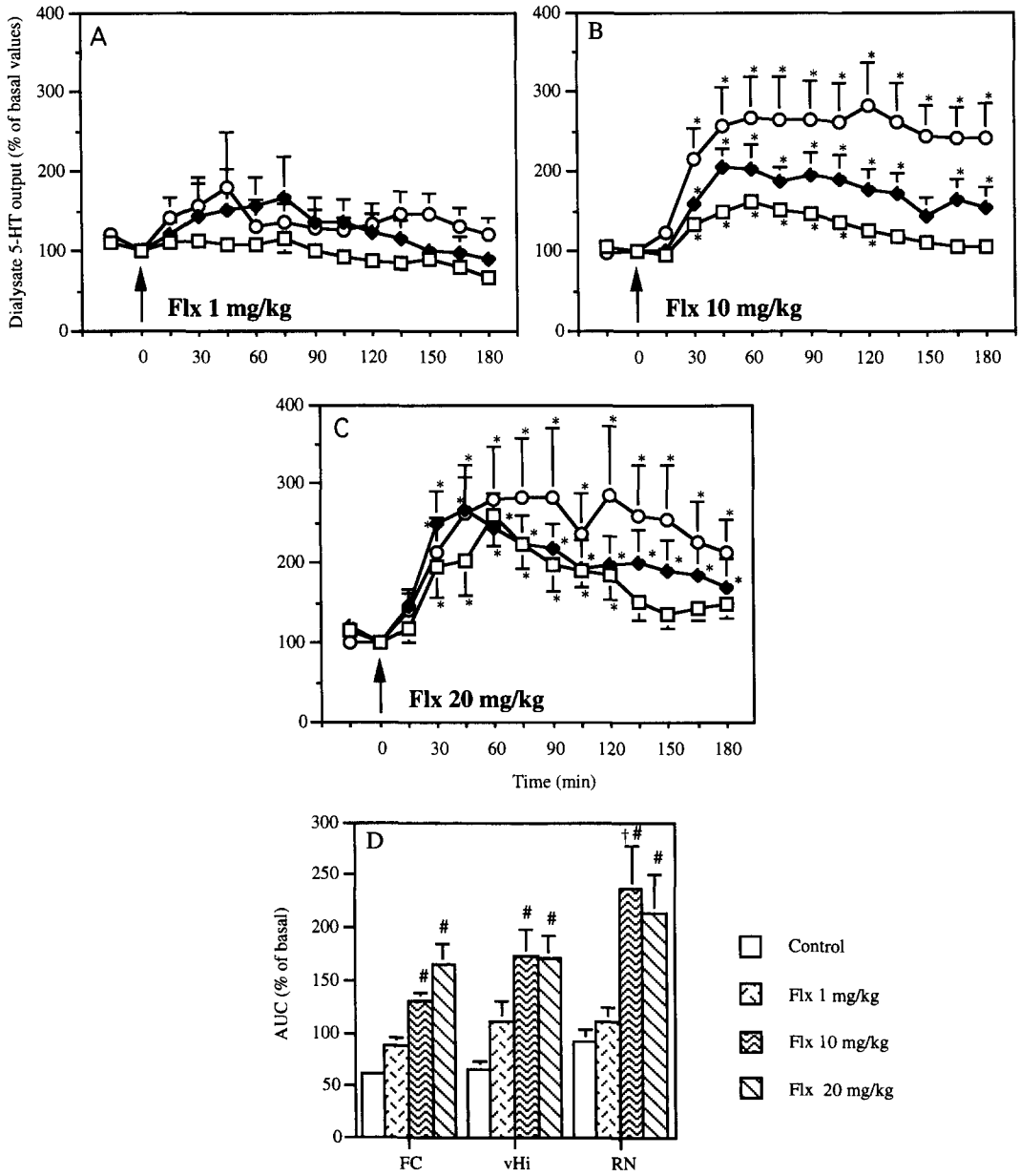

Fig. 1. Effects of the i.p. administration of various doses of fluoxetine (Flx) on extracellular 5-hydroxytryptamine (5-HT) levels in the frontal cortex (FC, □), ventral hippocampus (vHi, ♦) and raphe nuclei (RN, ○). (A) 1 mg/kg; (B) 10 mg/kg; (C) 20 mg/kg. Injection of fluoxetine is shown by the arrow. Each point represents the mean ± S.E.M. for 7–8 animals. See Results for basal levels of 5-HT. * $P < 0.05$, ANOVA for repeated measures and Fisher PLSD multiple comparison test when compared to the respective basal value. (D) AUC (0–180 min) of saline and fluoxetine-treated groups. # $P < 0.05$ one-way ANOVA followed by Fisher PLSD multiple comparison test when AUC values were significantly different from controls and † $P < 0.05$ when AUC values were significantly different from those for the frontal cortex.

The limit of sensitivity was about 1 pg for 5-HT. Fluoxetine hydrochloride (Alchymars, Italy) was dissolved in H_2O and administered in a volume of 2 ml/kg i.p. when dialysate 5-HT levels showed stable values, i.e., 4–5 h 30 following implantation of the probes and once three consecutive samples had shown levels of 5-HT that varied by less than 15%. Controls received the appropriate volume of saline. Data (not corrected for in vitro recovery) were expressed as a percentage of the basal value (means ± S.E.M.), i.e. the fraction just before drug administration. Furthermore, using percentage data, net changes in dialysate 5-HT were determined by means of the area under the curve (AUC) for the 0–180 min period following vehicle or fluoxetine administration. At the end of the experiments, placement of microdialysis probes was verified histologically. Statistical analysis was carried out using a one-way or two-way analysis of variance (ANOVA) or an ANOVA for repeated measures on raw data followed by the Fisher protected least significance difference (PLSD) multiple comparison test. Significance level was set at $P < 0.05$.

3. Results

Basal extracellular levels of 5-HT (in fmol/20 μl) in the frontal cortex, ventral hippocampus and dorsal + median raphe nuclei (mean ± S.E.M. of the various experiments, $n = 25–28$ animals) were 21.1 ± 2.1, 11.5 ± 1.7, and 22.1 ± 3.7, respectively, and those of 5-HIAA (in pmol/20 μl) were 4.3 ± 0.2, 5.8 ± 0.3 and 25.7 ± 1.1, respectively.

When a 1 mg/kg dose of fluoxetine was given (Fig. 1A), the 5-HT output did not increase in the frontal cortex. A small, but not statistically significant 5-HT increase in the ventral hippocampus and the raphe nuclei was induced by this dose of fluoxetine. The maximal increases were to 117 ± 17%, 169 ± 52% and 181 ± 68% of the respective basal values (100%) in the frontal cortex, ventral hippocampus and raphe nuclei, respectively. To study the net effects of fluoxetine on the amount of extracellular 5-HT in the three regions following its acute administration, the AUC values of 12 successive samples (180 min) were considered (Fig. 1D). One-way ANOVA followed by post-hoc comparisons revealed, in each region, that the AUC values for 1 mg/kg fluoxetine-treated rats did not differ from those of controls. Furthermore, the acute effects of 1 mg/kg fluoxetine on extracellular 5-HIAA resulted in significant decreases in the frontal cortex and the ventral hippocampus (−87%, $F(12,72) = 5.6$, $P < 0.001$; −86%, $F(12,84) = 3.3$, $P < 0.001$, respectively) but not in the raphe nuclei (−91%, $F(12,72) = 1.0$, $P > 0.05$).

When a 10 mg/kg dose of fluoxetine was given (Fig. 1B), the 5-HT output exhibited a significant increase in the frontal cortex ($F(12,72) = 9.2$, $P < 0.001$), the ventral hippocampus ($F(12,72) = 6.3$, $P < 0.001$) and the raphe nuclei ($F(12,72) = 3.7$, $P < 0.001$). Thus, in this latter region, fluoxetine almost tripled the extracellular levels of 5-HT (the maximal increase was to 282 ± 55% of the respective basal value (100%)), while its effect was less marked (164 ± 8% and 205 ± 31%) in the frontal cortex and the ventral hippocampus, respectively. In addition, the fluoxetine-induced 5-HT increase in the frontal cortex was short lasting (from 30 to 135 min after drug injection), while it persisted for the duration of sample collection (180 min) in the ventral hippocampus and the raphe nuclei. The AUC values for 10 mg/kg fluoxetine-treated rats were significantly increased relative to those of control rats in the frontal cortex ($F(1,13) = 72.1$, $P < 0.001$), the ventral hippocampus ($F(1,12) = 19.5$, $P < 0.001$) and the raphe nuclei ($F(1,10) = 8.5$, $P < 0.05$) (Fig. 1D). Furthermore, the 5-HT increase in the raphe nuclei was significantly larger than that observed in the frontal cortex ($F(2,18) = 3.7$, $P < 0.05$). The acute effects of a 10 mg/kg dose of fluoxetine on extracellular 5-HIAA did not show marked regional selectivity: the maximal decreases were −65% in the frontal cortex ($F(12,72) = 25.3$, $P < 0.001$), −64% in the ventral hippocampus ($F(12,72) = 15.6$, $P < 0.001$) and −79% in the raphe nuclei ($F(12,72) = 9.4$, $P < 0.001$).

When a 20 mg/kg dose of fluoxetine was given, the 5-HT output (Fig. 1C) exhibited a significant increase in the frontal cortex ($F(12,60) = 5.9$, $P < 0.001$), the ventral hippocampus ($F(12,60) = 6.3$, $P < 0.001$) and the raphe nuclei ($F(12,84) = 4.9$, $P < 0.001$). The maximal increases were to 259 ± 37%, 267 ± 42% and 287 ± 86% of the respective basal value (100%) in the frontal cortex, ventral hippocampus and raphe nuclei, respectively. These effects led to significant increases in the AUC values relative to those of control rats for the frontal cortex ($F(1,12) = 32.5$, $P < 0.001$), the ventral hippocampus $F(1,11) = 22.2$, $P < 0.001$) and the raphe nuclei $F(1,11) = 5.9$, $P < 0.05$) (Fig. 1D). However, no significant difference was found between the AUC values calculated for the three brain areas studied ($F(2,17) = 0.7$, $P > 0.05$). Acute effects of 20 mg/kg of fluoxetine led to maximal decreases in extracellular 5-HIAA efflux, by −70% in the frontal cortex ($F(12,60) = 22.9$, $P < 0.001$), −74% in the ventral hippocampus ($F(12,60) = 17.7$, $P < 0.001$) and −76% in the raphe nuclei ($F(12,84) = 14.1$, $P < 0.001$).

4. Discussion

The present findings show that the systemic acute administration of a selective 5-HT reuptake inhibitor, fluoxetine, to anaesthetized rats preferentially increased the 5-HT content of dialysate from the raphe

nuclei, a brain region mostly containing cell bodies and dendrites of serotoninergic neurones, compared to that of dialysate from two projecting areas of these neurones, the frontal cortex and ventral hippocampus. These results confirm those obtained, using in vivo microdialysis technique, in awake rats following peripheral administration of other potent selective 5-HT reuptake inhibitors like fluvoxamine (Bel and Artigas, 1992) and citalopram (Invernizzi et al., 1992).

Following its systemic administration at the lowest and the intermediate doses (1 and 10 mg/kg), fluoxetine induced region-dependent increases in extracellular 5-HT levels. The order of changes was raphe nuclei > ventral hippocampus > frontal cortex. The difference in the density of 5-HT reuptake sites and the serotoninergic nerve terminal content of the projecting regions may account for quantitative differences between these brain regions. Indeed, the above-described order of changes corresponds to that of the densities of [^{3}H]paroxetine binding to the 5-HT reuptake sites found by Hrdina et al. (1990): the raphe nuclei contain the highest density of [^{3}H]paroxetine binding, while the hippocampus shows an intermediate level and the frontal cortex the lowest level. Furthermore, the densities of [^{3}H]paroxetine sites in rat brain regions found in this study correlate well with the reported 5-HT content (Saavedra, 1977) and localization of serotoninergic innervation in the rat brain (Steinbush, 1981).

Pharmacokinetic studies performed in rats indicate that fluoxetine is metabolized to an active compound, norfluoxetine, which also exhibits 5-HT reuptake inhibiting properties equal to those of the parent drug (Fuller et al., 1991). Thus, the combined effects of both fluoxetine and its desmethylated metabolite on the 5-HT reuptake sites are probably responsible for the changes in extracellular 5-HT measured in vivo.

The regional specificity of the effect of fluoxetine on dialysate 5-HT content disappeared at the highest dosage (20 mg/kg) as similar increases were observed in the three brain regions studied. These results suggest that fluoxetine may have saturated 5-HT reuptake sites mainly in the raphe nuclei and ventral hippocampus in vivo following its acute administration. This dose of fluoxetine is less than 3 times the drug's ED_{50} in producing a serotonin-related behavioural effect, anorexia, in vivo in rats (7.5 mg/kg i.p.; Fuller et al., 1991).

In the present study, the dose-response effect of fluoxetine on extracellular 5-HT levels lasted at least 180 min post-injection in the raphe nuclei and ventral hippocampus, but less than 120 min in the frontal cortex. By contrast, the decreases in 5-HIAA efflux induced by fluoxetine were not dose-related and lasted at least 180 min post-injection in the three regions studied. The short-lasting effect of fluoxetine in the frontal cortex is in contrast with that obtained by Rutter and Auerbach (1993) in the diencephalon: a long-lasting increase in dialysate 5-HT and decrease in 5-HIAA efflux (24 h post-injection) were observed in awake rats following an acute injection of a 10 mg/kg dose of the drug. The discrepancy observed between our study and that of Rutter and Auerbach can be explained in several ways. One explanation could be that while extracellular 5-HT levels measured by using in vivo microdialysis after peripheral fluoxetine reflect a balance between decreased release and inhibition of reuptake, a larger decrease in 5-HT release occurred in the frontal cortex than in other terminal areas. This possibility has to be further investigated, for example, in terms of the inhibitory influence of somatodendritic 5-HT_{1A} autoreceptors located in the raphe (Sotelo et al., 1990) on the terminal output of 5-HT in the frontal cortex compared to that in the ventral hippocampus. It is well known that the frontal cortex receives its serotoninergic innervation predominantly from the dorsal raphe nucleus, which is relatively rich in 5-HT_{1A} binding sites (Weissmann-Nanopoulos et al., 1985), and thus their activation by endogenous 5-HT leads to a greater inhibition of 5-HT release and synthesis in the frontal cortex than in the ventral hippocampus. Another possibility could be that differences in experimental procedures (microdialysis performed in awake rats in Rutter's study or in anaesthetized rats in our study, pH of the artificial cerebrospinal fluid set up at 6.4 in the Rutter's study versus 7.4 in our study) strongly influenced the results.

Although a preferential increase in extracellular 5-HT levels in the raphe nuclei seems to be a general phenomenon induced by acute administration of selective 5-HT reuptake inhibitors, the magnitude of this acute effect is different depending on the 5-HT reuptake inhibitor studied by using the in vivo microdialysis technique. On the one hand, fluoxetine induced only a 2.5-fold increase in extracellular 5-HT levels in the raphe nuclei, while on the other hand, fluvoxamine (Bel and Artigas, 1992) and citalopram (Invernizzi et al., 1992) increase it 5 to 7 times. Furthermore, using the same experimental protocol as that described here, we observed that paroxetine (15 mg/kg dose, subcutaneously) induced large and significant increases in 5-HT output: the maximal 5-HT increases were to $363 \pm 58\%$, $425 \pm 29\%$ and $726 \pm 141\%$ of the respective basal values (100%) in the frontal cortex, ventral hippocampus and raphe nuclei, respectively (data not shown). Differences in the drugs' potency to bind to the 5-HT transporter may explain this phenomenon: of the selective indirect serotoninergic agonists investigated, paroxetine has been found to be the most potent 5-HT reuptake inhibitor in rat brain synaptosomes in vitro, citalopram the most selective and fluoxetine the less potent (Thomas et al., 1987). Thus, it is not surprising that extracellular 5-HT levels increased in vivo to a

greater extent following acute systemic administration of paroxetine than following administration of fluoxetine.

In summary, the present results confirm previous data indicating that the inhibition of 5-HT reuptake elicits a larger increase in extracellular 5-HT levels, as measured by in vivo microdialysis, in a brain region containing mostly cell bodies and dendrites of serotoninergic neurones, the raphe nuclei, than it does in two brain regions containing serotoninergic nerve terminals, the ventral hippocampus and the frontal cortex. The order of changes (raphe nuclei > ventral hippocampus > frontal cortex) correlates well with previous autoradiographic studies showing the regional specificity of densities of [^{3}H]paroxetine binding to 5-HT reuptake sites. Our results, by extending those obtained with other selective 5-HT reuptake inhibitors, add further arguments in favour of the key role played by raphe nuclei in the mechanism of action of serotoninergic antidepressant drugs. We are now investigating the nature of the receptors involved in the less marked effect of fluoxetine in the frontal cortex and the ventral hippocampus.

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