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On the In-vivo Modulation of Neostriatal Dopamine Release by Fluoxetine and 5-Hydroxy-L-tryptophan in Conscious Rats

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Abstract

To help determine the nature of serotonergic regulation of dopamine activity in the brain an in-vivo microdialysis study has been performed in conscious rats to investigate the modulation of dopamine release in the neostriatum by 5-hydroxytryptamine (5-HT).

The 5-HT uptake inhibitor, fluoxetine, and the 5-HT precursor, 5-hydroxy-L-tryptophan (5-HTP), were used to produce an increase in extracellular 5-HT concentration. Systemic administration of fluoxetine (10 mg kg^{-1}, s.c.) produced a 2- to 3-fold increase in extracellular 5-HT concentration but did not change extracellular dopamine concentration in the neostriatum. Co-administration of fluoxetine and 5-HTP (40 mg kg^{-1}, s.c.; 60–90 min after fluoxetine) caused a highly significant tenfold increase in extracellular 5-HT concentration in the neostriatum with a slight but non-significant decrease in extracellular dopamine concentration. Pergolide, a dopamine D_2 agonist, given systemically caused a dramatic decrease in extracellular dopamine concentration demonstrating the responsiveness of the neurons.

These results demonstrate that high concentrations of extracellular 5-HT do not modulate dopamine release in the neostriatum. The possibility that different 5-HT receptor subtypes may mediate different regulation of dopamine release remains to be explored.

Fluoxetine, a selective inhibitor of the 5-hydroxytryptamine (5-HT) transporter (uptake carrier) (Fuller et al 1974; Wong et al 1974), has been used for many years to treat major depression, supporting the hypothesis that diminished central 5-HT function may be involved in the pathophysiology of some types of depression (Fuller 1991, 1992). Recent evidence shows that another important central neurotransmitter, dopamine, may also participate in the etiology of some types of depression (Wilner 1983; Jimerson 1987), which suggests the existence of possible functional interactions between 5-HT and dopamine systems in the brain. Morphologic evidence has been obtained demonstrating that serotonergic neurons originating in the rostral dorsal raphe project to the neostriatum and nucleus accumbens, two projection fields of dopamine neurons (Herve et al 1987). 5-HT-containing terminals also make direct synaptic contact with dopaminergic neurons in the substantia nigra (Nedergaard et al 1988). Electrophysiological studies have shown a modulatory effect of serotonergic agents on the firing rate of dopamine neurons in the ventral tegmental area (Sorenson et al 1989). Chronic administration of 5-HT uptake inhibitors has, furthermore, been found to enhance brain dopamine activity (DeMontis et al 1990).

The nature of serotonergic regulation of dopamine activity in the brain is not, however, clear. Benloucif & Galloway (1991) reported that fluoxetine administered locally via microdialysis probe (4 nmol/20 min) caused a threefold increase in extracellular dopamine concentration in rat neostriatum, whereas in a previous study we found that systemic administration of fluoxetine (10 mg kg^{-1}, i.p.) increased the extracellular 5-HT concentration fourfold, but did not change extracellular dopamine concentration in rat neostriatum (Perry & Fuller 1992). In contrast, another recent study showed that systemic administration of fluoxetine at the same dose as used by Perry & Fuller caused a decrease in extracellular dopamine concentration in the neostriatum (Ichikawa and Meltzer 1995).

One explanation for the seemingly discrepant findings after systemic injection of fluoxetine (no change or a decrease in extracellular dopamine concentration in the neostriatum) and after local application of fluoxetine (threefold increase in extracellular dopamine concentration in the neostriatum) might be that the larger increase in extracellular 5-HT concentration occurring after local application of fluoxetine caused an increase in dopamine release not caused by the smaller increase in extracellular 5-HT concentration after systemic administration of fluoxetine. To explore that possibility, we have co-administered fluoxetine and the 5-HT precursor, 5-L-hydroxy-L-tryptophan (5-HTP), to produce larger increases in extracellular 5-HT concentrations than systemic injection of fluoxetine alone (Perry & Fuller 1993) to determine if extracellular dopamine concentrations in the neostriatum would thereby be changed.

Materials and Methods

Male Sprague–Dawley rats, 260–300 g, (Charles River Laboratories, Portage, MI, USA) were used. The in-vivo microdialysis technique has previously been described in detail (Perry & Fuller 1992). Briefly, a home-made plastic dialysis probe (Perry & Fuller 1992) with an outer diameter of 0·6 mm and containing a 2–3 mm length of dialysis membrane was slowly implanted into the neostriatum of rats anaesthetized with chloral hydrate-pentobarbital (170 and 36 mg kg^{-1} in 30% propylene glycol and 14% ethanol, respectively) according to the following coordinates from the bregma and the dural surface: 1 mm anterior to bregma, 3 mm from the mid-sagittal suture and 7 mm ventral from dura (Paxinos &

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Watson 1986), and then fixed in place with cranioplastic cement (Plastics One, Roanoke, VA, USA). The plastic probe has two advantages over previous types of probe: the plastic tube is flexible enough to move with the brain if the rat shakes its head vigorously, an important consideration when using freely moving rats; and no metal or glue is used in the probe, which eliminates two possible sources of contamination of the neurobiochemical assays because metal and glue may be more likely to catalyse oxidation of dopamine and other catechols.

Two days after surgery the rat was placed in a plastic bowl and connected to a liquid swivel (CMA/120 system for freely moving animals; BioAnalytical Systems, West Lafayette, IN, USA). The input of the dialysis probe was connected to a syringe pump (Harvard Instruments, Model 22, South Natick, MA, USA) which delivered an artificial cerebrospinal fluid (containing 150 mM NaCl, 3 mM KCl, 1·7 mM $CaCl_2$ and 0·9 mM $MgCl_2$) to the probe at a rate of 1·5 μL min^{-1}. The output from the swivel was attached to an electrically actuated switching valve (Valco Instruments, Houston, TX, USA) which was part of an on-line analysis system that assayed the dialysis output from two rats in parallel. Collection of perfusate samples (20 μL) was for 15 min and each experiment lasted between 8 and 12 h. The stable basal samples varying by less than 20% were obtained 2 to 3 h after the start of the perfusion. Fluoxetine (Eli Lilly; 10 mg mL^{-1} in water) was injected after three stable basal samples were obtained and 5-HTP (Sigma; dissolved in water at 20 mg mL^{-1} containing 0·01 M HCl and heated at 100°C for about 5 min) was given 60–90 min after the administration of fluoxetine. The dopamine D_2 agonist, pergolide (Eli Lilly; 0·3 mg mL^{-1} in water; 0·3 mg kg^{-1}, s.c.) was given 4 h after the administration of 5-HTP in two rats. At the end of the experiment the position of the probe was histologically verified.

A ten-port HPLC valve with a 20 μL sample loop and a Prodigy C_8 analytical column (2 × 150 mm, 5 μm particles; Phenomenex, Torrance, CA, USA) was used in conjunction with a small sample clean-up column (Spherisorb ODS2; 2 × 10 mm, 3 μm particles; Keystone Scientific, Bellefonte, PA, USA) which trapped a late-eluting peak contained in the striatal dialysate samples. The mobile phase for both columns was the same and consisted of 80 mM anhydrous sodium acetate, 400 mg mL^{-1} sodium dodecylsulphate, 0·4 mM EDTA and 20% acetonitrile, adjusted to pH 5 with acetic acid. The flow rate for both columns was 0·22 mL min^{-1} and the analytical column was maintained at 40°C. An electrochemical detector (EG&G PARC, Princeton, NJ, USA) with a dual glassy carbon electrode was used. The output of both channels was sent to a Compaq 486/33 chromatography data system (Ezchrom Scientific Software, San Ramon, CA, USA) which calculated peak heights and sample concentrations. The sensitivity for 5-HT and dopamine was approximately 0·1 pmol mL^{-1} dialysate or 2 fmol/sample (20 μL).

A one-factor factorial analysis of variance followed by Fisher's protected least square different test was used to analyse the statistical significance of the difference among more than two samples of the independent data obtained from the in-vivo study. The statistical significance of the difference between two samples was evaluated with Student's paired *t*-test and the Mann–Whitney *U*-test (two-tailed; Winer 1971). The minimum level for statistical significance was set at $P < 0.05$.

Results and Discussion

Fig. 1 shows the extracellular concentrations of 5-HT and dopamine in rat neostriatum after systemic injection of the 5-HT uptake inhibitor, fluoxetine (10 mg kg^{-1}, s.c.), and the subsequent injection of the 5-HT precursor, 5-HTP (40 mg kg^{-1}, s.c.) 90 min later. In most rats fluoxetine alone caused a two- to threefold increase in extracellular 5-HT concentration in the neostriatum (a 224% increase on the basis of the overall mean values; $P < 0.05$ against pooled baseline in the same curve according to Student's paired *t*-test and to the Mann–Whitney *U*-test, two-tailed) but did not change the extracellular dopamine concentration, in agreement with our previous findings (Perry & Fuller 1992).

Subsequent injection of 5-HTP markedly enhanced the increase in extracellular 5-HT concentration (more than tenfold, $P < 0.01$ against pooled baseline according to a one-factor factorial analysis of variance followed by Fisher's protected least square difference test). The extracellular dopamine concentration was not significantly changed by co-administration of fluoxetine and 5-HTP, although a slight but non-significant decrease in dopamine level, on the basis of the overall mean values, was found 2 h after the injection of 5-HTP (Fig. 1). Since such decreases in extracellular dopamine levels appeared at different times (1–2 h) after the administration of 5-HTP in seven out of nine rats tested, Student's paired *t*-test (two-tailed) was used to compare the differences between the baseline and the maximum decrease in individual rats, and a significant difference ($P < 0.05$) was then obtained (data not shown).

In a pilot experiment artificial cerebrospinal fluid alone was found not to change significantly the extracellular dopamine and 5-HT concentrations (data not shown). In previous studies we have demonstrated that 5-HTP alone at the dose tested (40 mg kg^{-1}, s.c.) produced a smaller increase in the extracellular 5-HT concentration than did fluoxetine plus 5-HTP (Perry & Fuller 1993). Because the goal of this study was to compare the difference between the change in extracellular dopamine concentration induced by a small-increase of 5-HT caused by fluoxetine alone, and that induced by a large increase of 5-HT caused by fluoxetine plus 5-HTP, and there is a limit to the number of tests that can be performed in a one-day experiment in one rat, the administration of artificial cerebrospinal fluid alone and 5-HTP alone were not included in the study.

In two rats the dopamine D_2 agonist pergolide was systemically injected (0·3 mg kg^{-1}, s.c.) to test the response of neurons at the end of the experiment. A marked decrease in extracellular dopamine level was found 1 h after administration of pergolide (Fig. 2), possibly because of the activation of dopamine D_2 autoreceptors at presynaptic terminals, resulting in the inhibition of dopamine release, or at cell body regions, resulting in the inhibition of firing rate of the cells, or both. This result indicates that the cells tested were in good shape and responded well to the stimulation.

These results showed that systemic administration of fluoxetine alone did not change dopamine release in the neostriatum, which is consistent with our previous findings (Perry & Fuller 1992), and that co-administration of fluoxetine and 5-HTP which, producing a more than tenfold increase in 5-HT release, only slightly reduced dopamine release in the

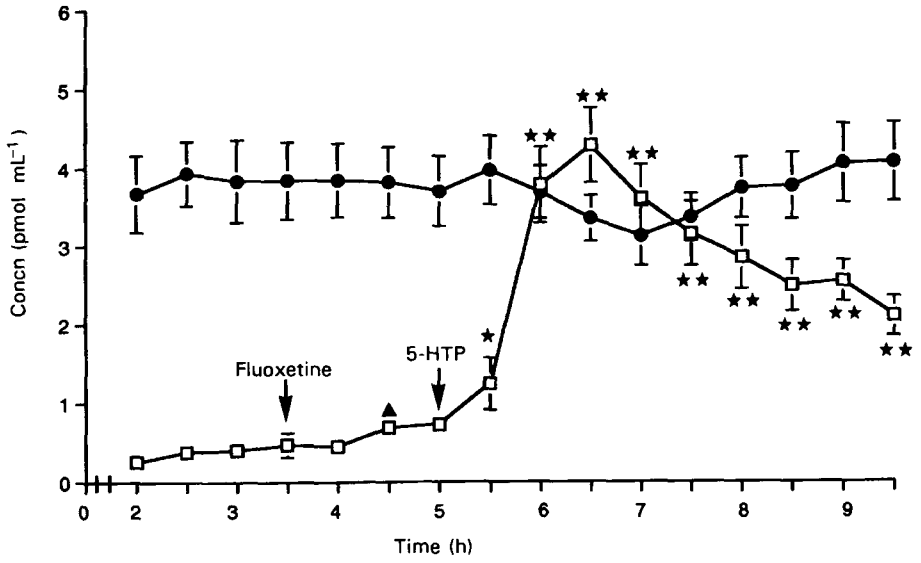

FIG. 1. Effect of systemic administration of the 5-hydroxytryptamine (5-HT) uptake inhibitor, fluoxetine (10 mg kg^{-1}, s.c.) and the 5-HT precursor, 5-hydroxy-L-tryptophan (5-HTP, 40 mg kg^{-1}, s.c.) on the concentrations of 5-HT (□) and dopamine (●) in microdialysate fluid from rat neostriatum. Stable baseline was obtained 2–3 h after the start of perfusion. The drugs were injected at the times shown by the indicators. Each data point represents the mean ± s.e.m. of 5–9 rats. $*P < 0.05$, $**P < 0.01$ compared with the respective baselines according to a one-factor factorial analysis of variance followed by Fisher's protected least square different test. ▲$P < 0.05$ compared with the pooled baseline in the same curve according to Student's paired *t*-test and to the Mann–Whitney *U*-test (two-tailed). No significance was found among the data points of the dopamine curve according to a one-factor factorial analysis of variance test.

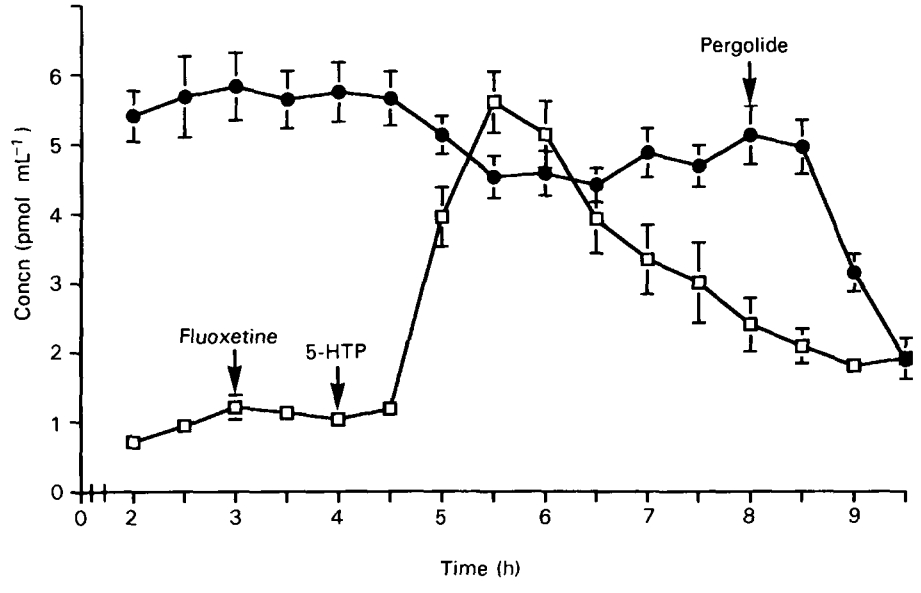

FIG. 2. Effect of systemic administration of the dopamine D_2 agonist, pergolide (0·3 mg kg^{-1}, s.c.) on extracellular concentrations of 5-HT (□) and dopamine (●) in rat neostriatum. The 5-HT uptake inhibitor, fluoxetine (10 mg kg^{-1}, s.c.) and the 5-HT precursor, 5-hydroxy-L-tryptophan (5-HTP, 40 mg kg^{-1}, s.c.) were injected to produce an increase in extracellular 5-HT concentration. The drugs were injected at the times shown by the indicators. Each data point represents the mean ± s.e.m. of two rats.

neostriatum. These findings do not appear to be consistent with the results reported by Benloucif & Galloway (1991) which showed that locally applied fluoxetine increased extracellular dopamine concentration threefold in rat neostriatum. Neither are the results in agreement with a recent report showing that fluoxetine alone reduced extracellular dopamine concentration more than 50% in rat neostriatum (Ichikawa and Meltzer 1995). Although the combination of fluoxetine and 5-HTP may have slightly reduced extracellular dopamine concentration, the change as a percentage of baseline (< 30%) was small compared with the decrease (> 50%) reported by Ichikawa & Meltzer (1995).

In the Benloucif & Galloway (1991) study local application of 5-HT or direct-acting 5-HT_{1A} receptor agonists increased extracellular dopamine concentration in rat neostriatum, as did fenfluramine, a 5-HT-releasing drug. The increase in extracellular dopamine concentration by fenfluramine was antagonized by fluoxetine, consistent with the known dependence of

fenfluramine-induced release of 5-HT on the 5-HT transporter (Fuller et al 1988) and the earlier demonstration that fluoxetine pre-treatment attenuated the fenfluramine-induced increase in extracellular 5-HT concentration measured by microdialysis (Sabol et al 1992; Rutter & Auerbach 1993; Bonanno et al 1994).

The discrepant findings for the modulation of striatal dopamine release by 5-HT may be because of the different striatum areas studied, the different drug doses and routes of administration, the various dialysis probes used, the specific experimental protocol applied, etc. In the study by Benloucif & Galloway (1991) 5-HT and 5-HT_{1A} agonists as well as fluoxetine were given directly into the neostriatum, which mainly activates the terminal 5-HT receptors resulting in the regulation of 5-HT release from the presynaptic terminals, whereas in our studies fluoxetine was given systemically which, by increasing extracellular 5-HT concentration, may also activate other 5-HT receptors at cell-body regions, resulting in changes in the firing rate of the neurons and the synthesis of 5-HT. The increase in extracellular 5-HT concentration after systemic administration of 5-HT uptake inhibitors is limited by the activation of 5-HT autoreceptors as the extracellular 5-HT concentration begins to increase (Hjorth 1993; Hjorth & Auerbach 1994). Uptake inhibitors applied locally to terminal regions cause larger increases in extracellular 5-HT concentration than do uptake inhibitors injected systemically (Rutter & Auerbach 1993; Hjorth & Auerbach 1994), because for inhibitors injected systemically the activation of 5-HT autoreceptors in cell body regions leads to a reduced firing rate of 5-HT neurons. Another possibility may be that systemic administration of fluoxetine and 5-HTP may indirectly influence dopamine neuron activity because dorsal raphe serotonergic neurons project to the neostriatum (Herve et al 1987) and the substantia nigra (Nedergaard et al 1988). Nonetheless, the increase in extracellular dopamine concentration reported by Benloucif & Galloway (1991) was not simply a result of a large increase in extracellular 5-HT concentration when fluoxetine is given systemically, because even when we co-administered fluoxetine and 5-HTP to produce a larger increase in the extracellular 5-HT concentration, no increase in extracellular dopamine concentration was found.

In the study by Ichikawa & Meltzer (1995) probes were implanted into the ventral neostriatum 2–3 days after the cannulation but the measurement was performed immediately after the implantation of the probe, whereas in our study the plastic probes were implanted into the intact neostriatum and the measurement was performed 2 days after the implantation of the probes, to give the animals time to recover.

Finally, activation of different 5-HT receptor subtypes may produce different or even opposite effects on striatal dopamine release. Thus the increase in 5-HT concentrations may neutralize the influence of 5-HT on dopamine release by activating different 5-HT-receptor subtypes at the same time. Using selective 5-HT receptor antagonists to block specific 5-HT receptor subtype(s) or a specific antisense oligonucleotide to inhibit the expression of a certain subtype of 5-HT receptor gene may aid in elucidation of serotonergic modulation of dopamine release.

In conclusion, this study did not provide evidence for the modulation of striatal dopamine release by 5-HT. Further study is needed to clarify the interactions between 5-HT and dopamine neuronal systems in the striatum.

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