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Short communication

Effects of acute olanzapine after sustained fluoxetine on extracellular monoamine levels in the rat medial prefrontal cortex

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Abstract

The combination of selective serotonin reuptake inhibitors with atypical antipsychotic drugs exhibits beneficial effects in treatment-resistant depression. We investigated the effects of a 2-week treatment with a low fluoxetine dose (3 mg/kg per day) plus a single injection of olanzapine (3 mg/kg) on the dialysate concentration of noradrenaline, dopamine and serotonin (5-HT) in the medial prefrontal cortex of the rat. Chronic fluoxetine increased only 5-HT levels whereas single olanzapine administration increased the concentration of catecholamines and decreased that of 5-HT to a comparable extent in vehicle- and fluoxetine-treated rats. Therefore, it is possible that the therapeutic benefit of this pharmacological combination may not be associated to changes in the cortical concentration of monoamines, but to postsynaptic blockade of monoaminergic receptors.

Keywords: Medial prefrontal cortex; Fluoxetine; Olanzapine; Microdialysis; Osmotic minipump

1. Introduction

The combination of the selective serotonin reuptake inhibitor (SSRI) fluoxetine and the atypical antipsychotic drug olanzapine improves significantly the therapeutic response in treatment-resistant depressive patients compared with either treatment alone (Shelton et al., 2001). It is possible that treatment-resistant patients would need a broader pharmacological manipulation that targets more than a single transmitter system. In fact, noradrenaline, dopamine and serotonin pathways converge in the medial prefrontal cortex, which further underscores the importance of such interactions in this forebrain area. Chronic fluoxetine administration predominantly enhances extracellular serotonin (5-HT) in the frontal cortex (Hervás et al., 2001; Gartside et al., 2003), with no parallel changes in the concentration of dopamine (Tanda et al., 1996). On the other hand, olanzapine possesses high affinity for dopamine D1-5, 5-$HT_{2A/2C}$, α_1-adrenoceptor, histamine H_1 receptor and muscarinic M_{1-5} receptor (Bymaster et al., 1996; Zhang and Bymaster, 1999). Recent research has shown that the acute combination of olanzapine and fluoxetine caused a synergistic effect on the extracellular concentrations of noradrenaline and dopamine, particularly in the medial prefrontal cortex (Zhang et al., 2000; Koch et al., 2004). This combination also increased the extracellular level of 5-HT, but not beyond that reached following fluoxetine alone (Koch et al., 2004). In order to mimic the drug administration used in clinical augmentation studies with SSRIs, in the present experiment we have added a single dose of olanzapine to a 2-week fluoxetine treatment, and examined the effects on the dialysate concentrations of noradrenaline, dopamine and serotonin in the medial prefrontal cortex.

2. Materials and methods

2.1. Animals

Male Wistar rats (270–320 g) from Iffa-Credo (Lyon, France) were maintained on a 12-h light/dark cycle (lights on

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at 07:00 h) and housed four per cage before surgery and individually after surgery. Food and water were always freely available. All experimental procedures were carried out in strict accordance with European Communities Council Directive of 24 November 1986 (86/609/EEC) on "Protection of Animals Used in Experimental and Other Scientific Purposes".

2.2. Surgery and microdialysis procedures

Rats were anesthetized with 400 mg/kg chloral hydrate before osmotic minipumps (Alzet model 2002) were implanted subcutaneously. Each osmotic minipump was filled with either vehicle (50% dimethyl sulfoxide in distilled water) or fluoxetine (3 mg/kg per day for 2 weeks) dissolved in vehicle. On the day 13 of treatment, rats were anesthetized with 60 mg/kg sodium pentobarbital and mounted in a Kopf stereotaxic frame, and a dialysis probe equipped with a Cuprophan® membrane (4-mm long) was implanted in the medial prefrontal cortex. Stereotaxic coordinates (in mm) from bregma and duramater were AP +3.2, L −0.8, DV −6.0 (Paxinos and Watson, 1986). Microdialysis experiments were conducted 24 h after surgery (day 14 of treatment) in freely moving rats. Probes were perfused at a rate of 1.5 μl/min with artificial cerebrospinal fluid (CSF) containing 125 mM NaCl, 2.5 mM KCl, 1.26 mM $CaCl_2$ and 1.18 mM $MgCl_2$. Dialysate samples of 55 μl were collected every 35 min in vials containing 5 μl perchloric acid 0.01M to prevent monoamine degradation. The samples were further split in two aliquots of 30 μl to determine the levels of dopamine and 5-HT by high performance liquid chromatography (HPLC) according to described procedures (Ferré et al., 1994; Adell and Artigas, 1998). A separate group of rats was subjected to the same experimental procedures, but dialysate samples were used to determine noradrenaline (Bortolozzi and Artigas, 2003). After a 100-min stabilization period, four dialysate samples were collected to obtain basal monoamine values before the subcutaneous administration of 3 mg/kg olanzapine.

2.3. Chemicals and drugs

All the reagents were of analytical grade and obtained from Merck (Darmstadt, Germany). Fluoxetine hydrochloride and 5-HT oxalate were purchased from Sigma-Aldrich (Tres Cantos, Spain). Olanzapine was donated by Eli Lilly and Co. (Indianapolis, IN).

2.4. Data analysis

The content of monoamines in each sample was expressed as percentage of the average baseline level calculated from four fractions collected before olanzapine injection. Data correspond to mean values±S.E.M. of the percentage obtained in each experimental group. The statistical analysis of raw data was performed using two-way repeated measures analysis of variance (ANOVA) followed by Tukey's post-hoc comparison test. The level of significance was set at $p < 0.05$.

3. Results

3.1. Baseline dialysate values

The basal values (before olanzapine) of noradrenaline, dopamine and 5-HT in dialysate samples of the medial prefrontal cortex are depicted in Table 1. Two-week fluoxetine treatment did not alter the concentrations of noradrenaline and dopamine, but the levels of 5-HT were 2.6 fold higher than those of vehicle-treated rats ($p < 0.005$, Student's t-test).

Table 1
Basal extracellular concentration of noradrenaline, dopamine and 5-HT in chronic vehicle- and chronic fluoxetine-treated rats

	Chronic vehicle	Chronic fluoxetine
Noradrenaline	16.1±0.8	17.3±1.3
Dopamine	15.9±2.0	16.2±1.1
Serotonin	3.1±0.8	8.2±1.1[a]

Data are fmol/fraction, averaged from the four samples before the administration of olanzapine.
[a] Significantly different from the corresponding chronic vehicle-treated rats; Student's t-test ($p < 0.005$).

3.2. Effects of olanzapine

For the three monoamines, there was no significant difference between the vehicle-and fluoxetine-treated groups in the olanzapine effects. Olanzapine increased significantly dialysate noradrenaline ($p < 0.002$, time effect; non-significant effect of group or time × group interaction) and dopamine ($p < 0.00001$, time effect; non-significant effect of group or time × group interaction). In fluoxetine-treated rats, olanzapine (3 mg/kg) increased dialysate noradrenaline (Fig. 1A), but this effect was only significant 20 min after the injection ($p < 0.05$, Tukey's test). The olanzapine-induced increase in the dialysate of dopamine was similar to that of noradrenaline ($p < 0.05$, Tukey's test), but this effect lasted for the 210 min after its administration (Fig. 1B). With regard to 5-HT, there was a significant effect of group ($p < 0.005$) and time ($p < 0.01$), but not of the interaction between both factors. The group effect cannot be appreciated from Fig. 1C since data were expressed as percentage of baseline. However, baseline values in both groups differed markedly (Table 1). The significant effect of time indicates that olanzapine produced an overall decrease in the concentration of 5-HT in vehicle- (−30%) and fluoxetine-treated rats (−15%), although this difference did not reach statistical significance.

4. Discussion

Recent clinical studies have shown that the combination of SSRIs and atypical antipsychotic drugs is an efficacious therapy for treatment-resistant depression (Shelton et al., 2001), and this outcome could be associated to a synergistic effect of both drugs on monoaminergic transmission in the medial prefrontal cortex (Zhang et al., 2000; Koch et al., 2004). In this work we chose 3 mg/kg per day, not to produce maximal increases in extracellular 5-HT, but to mimic the effect of a standard clinical dose of fluoxetine (20 mg per day) in depressed patients. Indeed the human plasma concentration of fluoxetine after this dose regimen (0.14 μM; Pérez et al., 2001) is comparable to that seen in rats after 5 mg/kg (0.15 μM; Caccia et al., 1990).

In the present study, it has been observed that 2-week treatment with fluoxetine (3 mg/kg per day) increased the extracellular levels of 5-HT in the medial prefrontal cortex

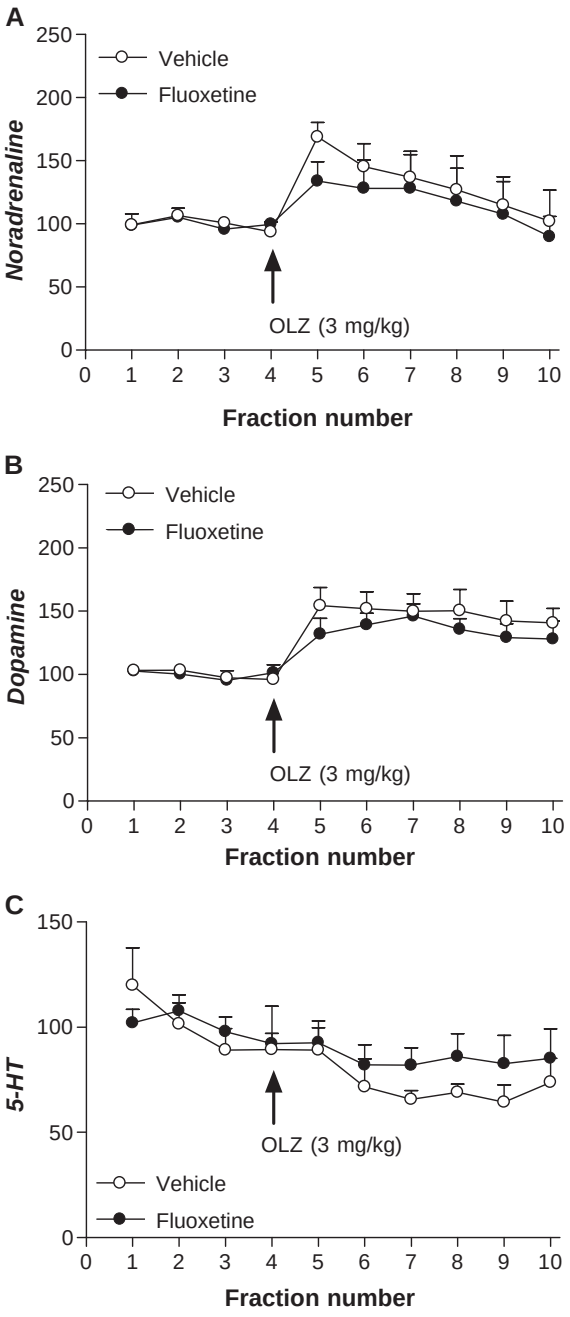

Fig. 1. Effects of 3 mg/kg olanzapine on dialysate noradrenaline (A), dopamine (B) and 5-HT (C) in rats treated for 2 weeks with vehicle or fluoxetine (3 mg/kg per day). Results are expressed as percentage of basal values. The number of animals used ranged from four in A to six or eight in B and C.

without altering those of noradrenaline and dopamine. This was similar to what had been described in previous reports (Tanda et al., 1996; Hervás et al., 2001; Gartside et al., 2003). The increase in dialysate 5-HT after chronic fluoxetine treatment has been attributed to the desensitization of 5-HT_{1A} autoreceptors induced after sustained blockade of the 5-HT transporter (Blier and de Montigny, 1994; Hervás et al., 2001). The overall effects of olanzapine in the medial prefrontal cortex were an increment of the extracellular levels of catecholamines (transient for noradrenaline and sustained for dopamine), and a decrease in the concentration of 5-HT, which is coincident with previous data (Kuroki et al., 1999; Gessa et al., 2000; Amargós-Bosch et al., 2003; Koch et al., 2004). This can result from local effects of the drug as well as its stimulating action of noradrenergic neurons of the locus coeruleus (Seager et al., 2004) and dopaminergic neurons of the ventral tegmental area (Gessa et al., 2000) that project to the medial prefrontal cortex. A predominant effect on dialysate dopamine in the medial prefrontal cortex may be characteristic of atypical antipsychotic drugs such as olanzapine, and seems to be dependent on the binding affinities for 5-HT_{2A} relative to D_2 receptors (Kuroki et al., 1999). In the present work, the combination of long-term fluoxetine with acute olanzapine does not show the synergistic effects on the extracellular levels of catecholamines observed following acute administration (Zhang et al., 2000; Koch et al., 2004). In recent clinical trials, the purported beneficial action of the fluoxetine/olanzapine combination for treatment-resistant depressive patients was observed when both drugs were administered for several days (Shelton et al., 2001). Therefore, it is conceivable that the advantages of this combination therapy may not bear a relationship with the extracellular concentration of monoamines in the medial prefrontal cortex, but with long-term adaptive changes in postsynaptic monoaminergic receptors. In line with this argument, Marek et al. (in press) have shown that blockade of 5-HT_{2A} receptors augment the antidepressant-like effects of fluoxetine without a concurrent increase of 5-HT.

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