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Differential effects of acute and chronic fluoxetine administration on the spontaneous activity of dopaminergic neurones in the ventral tegmental area

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1 Electrophysiological techniques were used to study the effects of fluoxetine and citalopram on the basal activity of dopaminergic neurones in the ventral tegmental area (VTA) and substantia nigra, pars compacta (SNc) of rats.

2 Acute i.v. injection of fluoxetine (20-1280 $\mu g\ kg^{-1}$) caused a dose-dependent inhibition of the firing rate of VTA dopaminergic neurones, but did not affect the activity of dopaminergic cells in the SNc. Citalopram (20-1280 $\mu g\ kg^{-1}$, i.v.) inhibited the firing rate of dopaminergic neurones in the VTA, but its effect (maximal inhibition: 14±7%) was less pronounced than that of fluoxetine (maximal inhibition: 34±7%).

3 Pretreatment with mesulergine (80 $\mu g\ kg^{-1}$, i.v.), a 5-hydroxytryptamine$_{2C/2B}$ (5-HT$_{2C/2B}$) receptor antagonist, blocked the inhibitory effect of fluoxetine on VTA dopaminergic cells. Selective lesions of 5-hydroxytryptaminergic neurones by the neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), abolished the fluoxetine-induced reduction of VTA dopaminergic activity.

4 In a series of experiments, fluoxetine (10 $mg\ kg^{-1}$, i.p.) was administered once daily for 21 consecutive days. Acute i.v. administration of fluoxetine (20-1280 $\mu g\ kg^{-1}$, 72 h after the last i.p. injection) did not cause any change in the basal firing rate of VTA dopaminergic neurones in treated rats, whereas it induced the typical inhibitory effect in control animals. A group of rats chronically treated with fluoxetine, received i.v. *m*-chlorophenylpiperazine (mCPP; 10-320 $\mu g\ kg^{-1}$), a 5-HT$_{2C/2B}$ receptor agonist. This drug significantly inhibited VTA dopaminergic function in control rats, but did not modify the basal activity of dopaminergic cells in animals given chronic fluoxetine.

5 It is concluded that fluoxetine inhibits dopaminergic function in the VTA by enhancing the synaptic levels of 5-HT, which possibly acts through the 5-HT$_{2C/2B}$ receptor subtype. Repeated treatment with fluoxetine induces tolerance to its inhibitory effect on dopaminergic activity, possibly as a consequence of down-regulation of 5-HT$_{2C/2B}$ receptors. The effects of fluoxetine on VTA dopaminergic cell activity might be relevant for its therapeutic actions and may explain the origin of the reported cases of akathisia.

Keywords: Fluoxetine; citalopram; dopaminergic neurones; ventral tegmental area; electrophysiology; 5-HT$_{2C/2B}$ receptors.

Introduction

There is extensive evidence that the activity of midbrain dopamine containing neurones is modulated by the 5-hydroxytryptaminergic system. Neuroanatomical studies indicate that both the substantia nigra, pars compacta (SNc) and the ventral tegmental area (VTA) receive afferent projections from 5-hydroxytryptamine (5-HT)-containing axon terminals originating in the midbrain raphe nuclei (Azmitia & Segal, 1978; Phillipson, 1979; Steinbusch, 1984; Mori *et al.*, 1987). Electrophysiological experiments have shown that selective agonists at specific 5-HT receptor subtypes exert differential effects on the basal firing activity of midbrain dopaminergic neurones. For example, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a prototypical 5-HT$_{1A}$ receptor agonist, which potently inhibits 5-HT neurones in both dorsal and median raphe nuclei (Sinton & Fallon, 1988; Prisco *et al.*, 1993), increased the basal firing rate of dopaminergic cells in both the SNc (Kelland *et al.*, 1990) and the VTA (Prisco *et al.*, 1994). Selective lesions of 5-HT neurones by the neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), abolished the excitatory effects of 8-OH-DPAT on both dopaminergic nuclei (Kelland *et al.*, 1990; Prisco *et al.*, 1994). Mixed 5-HT$_{2C/2B}$ receptor agonists, such as trifluoromethylphenylpiperazine (TFMPP) and *m*-chlorophenylpiperazine (mCPP) (Hoyer, 1988), significantly reduced the activity of dopaminergic neurones in the VTA (Prisco *et al.*, 1994) and weakly inhibited SNc dopaminergic cells (Kelland *et al.*, 1990). These findings indicate that 5-HT exerts a tonic inhibitory influence upon the activity of midbrain dopaminergic neurones. Thus, it is conceivable that the administration of selective 5-HT reuptake inhibitors (SSRIs), which enhance 5-HT levels in the synaptic cleft, may affect dopaminergic function. Fluoxetine was the first compound belonging to the pharmacological class of SSRIs which was found to inhibit potently 5-HT reuptake both *in vitro* and *in vivo* (Wong *et al.*, 1974; Stark *et al.*, 1985). It is now well established that fluoxetine is an effective antidepressant agent (Gram, 1994) which also has good therapeutic activity in the treatment of obsessive-compulsive disorders (Fontaine & Chouinard, 1986; Levine *et al.*, 1989) and eating disorders (Freeman & Hampson, 1987; Kaye *et al.*, 1991). There is evidence that the antidepressant effect of fluoxetine, assessed in the forced swimming test in mice, is antagonized by (±)-sulpiride a dopamine D_2 receptor blocker (Cesana *et al.*, 1993). These data are consistent with the general hypothesis that an enhancement of dopaminergic transmission in the mesolimbic system is involved in the antidepressant effect of several drugs (Cervo & Samanin, 1987; 1988; Cervo *et al.*, 1990). Therefore, elucidation of the mechanisms by which fluoxetine might influence midbrain dopaminergic function will probably be helpful in understanding the pharmacodynamic basis of both its therapeutic actions and untoward effects. For example, administration of fluoxetine to man is associated with several side-effects including parkinsonism (Bouchard *et al.*, 1989; Brod, 1989; Tate, 1989) and akathisia (Lipinski *et al.*, 1989),

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although their incidence has been very limited to date. These extrapyramidal symptoms might be due to a reduced dopaminergic tone (Marsden & Jenner, 1980) which is probably consequent to the enhancement of 5-hydroxytryptaminergic transmission produced by fluoxetine. Of particular interest is the hypothesis about the pathophysiology of akathisia which is thought to derive from a reduced dopaminergic transmission of the mesolimbic system originating in the VTA (Lipinski *et al.*, 1989). Thus, there are several clinical hints that fluoxetine might decrease central dopaminergic function. However, clear experimental confirmation of this hypothesis is still lacking, in that fluoxetine was found to reduce slightly the accumulation of the dopamine precursor L-dihydroxyphenylalanine (DOPA) in various rat brain areas (Baldessarini & Marsh, 1990), but these data were not confirmed in a subsequent study (Baldessarini *et al.*, 1992).

In the present study, single-cell recording techniques were used to assess the effects of acute and chronic fluoxetine on the electrical activity on dopaminergic neurones in both the VTA and the SNc. The effect of acute fluoxetine administration was compared with that of citalopram, another potent SSRI.

Methods

Surgical and recording procedures

Male Sprague-Dawley rats (Charles River, Calco, Italy) weighing 250 to 350 g were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and mounted on a stereotaxic instrument (SR-6, Narishige, Japan). Supplemental doses of anaesthetic were administered *via* a lateral tail vein cannula. Throughout the experiment the animals' body temperature was maintained at 36–37°C by a thermostaticlly regulated heating pad. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, 18 February, 1992) and international laws and policies (*EEC Council Directive* 86/609, OJ L 358,1, Dec. 12, 1987; *NIH Guide for the Care and Use of Laboratory Animals*, NIH Publication N. 85-23, 1985 and Guidelines for the Use of Animals in Biomedical Research, *Thromb. Haemost.*, **58**, 1078–1084, 1987). The coordinates, relatively to the interaural line, for placement of the recording electrode in the areas studied were for the VTA: anterior 2.7 to 3.4 mm, lateral 0.3 to 0.5 mm, 7 to 8 mm ventral to the level of exposed tissue; for the SNc: anterior 2.7 to 3.4 mm, lateral 1.8 to 2.2 mm, ventral 6.5 to 7.5 mm; and for the dorsal raphe nucleus: anterior 0.7 to 1.36 mm, lateral 0, ventral 5 to 6 mm (Paxinos & Watson, 1986). Extracellular recordings were performed with single-barrel micropipettes (4–7 MΩ resistance containing 2% pontamine sky blue dye in 2 M NaCl). Dopaminergic neurones were identified by their location, waveform, firing rate and pattern (Bunney *et al.*, 1973; Grace & Bunney, 1980; Wang, 1981); 5-hydroxytryptaminergic cells were recognized by their location, wide duration (~2 ms), positive-negative spikes, regular rhythm and slow firing rate (0.5–3.5 spikes s^{-1}) (Aghajanian, 1976). Electrical signals of spike activity were passed through a high impedance amplifier the output of which was led into an analog oscilloscope, audio monitor and window discriminator. Unit activity was then converted to an integrated histogram by a rate-averaging computer and displayed as spikes per 10 s intervals.

After each experiment, the recording site was marked by the ejection of pontamine sky blue dye from the electrode with a −20 μA current for 10 min. Brains were removed and placed in 10% buffered formalin for 2 days before histological examination. Frozen sections were cut at 40 μM intervals and stained with neutral red. Microscopic examination of the sections was carried out to verify that the electrode tip was in the VTA, the SNc or the dorsal raphe nucleus.

Drug administration protocols

In all electrophysiological experiments, the drugs were administered i.v. (*via* a lateral tail vein) in exponentially increasing doses every 2 min, and the effect on the activity of dopaminergic and 5-hydroxytryptaminergic neurones was recorded. Only one cell per animal was studied. Fluoxetine (20–1280 μg kg^{-1}) and citalopram (20–1280 μg kg^{-1}) were dissolved in 0.9% saline. The $5\text{-HT}_{2C/2B}$ receptor antagonist mesulergine (80 μg kg^{-1}), administered 10 min before the first injection of fluoxetine (20–1280 μg kg^{-1}), was dissolved in 100–200 μl 10% acetic acid, made up to almost required volume with 0.9% saline and brought to pH 6.5. The dose and the time of mesulergine pretreatment were chosen on the basis of previous findings showing mesulergine antagonism of mCPP inhibitory action on VTA dopaminergic neurones (Prisco *et al.*, 1994). In chronic experiments, i.p. fluoxetine (10 mg kg^{-1}) or saline was administered once daily for 21 consecutive days. Thereafter, electrophysiological recordings of dopaminergic neurones in the VTA were performed to evaluate the effects of acute i.v. fluoxetine (20–1280 μg kg^{-1}), given 24 or 72 h after the last i.p. fluoxetine administration. Preliminary experiments were performed after a 24 h wash-out period which was decided on the basis of previous studies (Kennett *et al.*, 1994a; Hrdina, 1987) but, subsequently, experiments were carried out 72 h after chronic fluoxetine withdrawal, to avoid any possible residual effect of the drug. In a group of rats chronically treated with i.p. fluoxetine the effect of i.v. mCPP (10–320 μg kg^{-1}) on the activity of VTA dopaminergic neurones was tested 72 h after the last injection of fluoxetine. In another series of experiments, the effects of fluoxetine (20–20480 μg kg^{-1}, i.v.) and citalopram (20–1280 μg kg^{-1}, i.v.) on the activity of 5-HT-containing neurones in the dorsal raphe nucleus were evaluated.

Intraventricular injections of 5,7-dihydroxytryptamine

All rats were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.). The 5-HT-selective neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) was dissolved in 0.9% saline solution of ascorbic acid (0.05%). 5,7-DHT (150 μg, free base) in a volume of 20 μl was infused into the right lateral ventricle. Control rats received only the ascorbic acid solution. In order to protect noradrenaline-containing neurones from the action of 5,7-DHT (Baumgarten *et al.*, 1973), 30 min before 5,7-DHT the rats received i.p. 25 mg kg^{-1} desipramine, an inhibitor of noradrenaline uptake into the nerve endings (Samanin *et al.*, 1975). The electrophysiological recordings were performed 7 days after treatment with 5,7-DHT or vehicle.

Biochemical assays

Biochemical determinations of monoamines were performed in the group of rats lesioned with 5,7-DHT. After electrophysiological testing, vehicle and 5,7-DHT-treated rats were killed by decapitation. Brains were rapidly removed, striata and hippocampi were dissected, frozen on dry ice ad stored at −80°C until assay. Tissue samples were homogenized in 400 μl 0.1 N perchloric acid and centrifuged for 15 min at 12000 r.p.m. An aliquot of the supernatant was filtered and transferred to an Eppendorf tube. Levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were measured by reversed-phase high performance liquid chromatography with electrochemical detection. The mobile phase contained 17.5% methanol, 24 mM citric acid, 16 mM Na_2HPO_4, 1.22 mM 1-heptanesulphonic acid sodium salt, 0.19 mM EDTA, (pH 2.8).

Statistical analysis

Data acquisition and analysis was accomplished with an 83286-based PC and an integrated software package for electophysiology (RISI, Symbolic Logic, Dallas, TX, U.S.A.). Dose-response curves were constructed by comparing the

mean firing rate during 2 min, starting immediately after the injection of each dose, with the basal firing rate. The data obtained with the SSIRs were subjected to an analysis of variance (ANOVA) for repeated measures. When significant effects were found, *post-hoc* comparisons were made with Tukey's test. The interactions between the following groups: fluoxetine + mesulergine, fluoxetine + 5,7-DHT; chronic fluoxetine + acute fluoxetine, chronic fluoxetine + mCPP were analysed by two-way ANOVA (split-plot design), followed by Tukey's test. The computer programme Allfit (De Lean *et al.*, 1978) was used to calculate the mean (±s.e.mean) ED_{50} of fluoxetine and citalopram on the activity of 5-HT-containing neurones in the dorsal raphe nucleus. Student's *t* test was used to analyse the effects of 5,7-DHT on brain levels of 5-HT and 5-HIAA.

Burst analysis of dopaminergic neurones was performed by using the RISI programme running on a PC computer. A total of 500 consecutive spikes was recorded for each neurone before and at the peak of drug effect. Burst-firing, when present, was detected with an algorithm similar to that previously described by Grace & Bunney (1984). Comparisons between groups were performed with Student's *t* test or χ^2 (for percentage of neurones exhibiting burst-firing).

Drugs and chemicals

Fluoxetine hydrochloride was kindly provided by Ely Lilly Laboratories (Indianapolis, IN, U.S.A.); citalopram was generously donated by Dr J. Hyttel (H. Lundbeck A/S, Copenhagen- Valby, Denmark); mCPP hydrochloride was from Research Biochemicals Inc. (Natick, MA, U.S.A.); despramine hydrochloride, 5,7-dihydroxytryptamine creatinine sulphate and apomorphine hydrochloride were from Sigma Chemical Company (St. Louis, MO, U.S.A.); mesulergine was kindly provided by Dr F. Franch (Sandoz Pharma Ltd., Basel, Switzerland). All dosages refer to the weight of the salt.

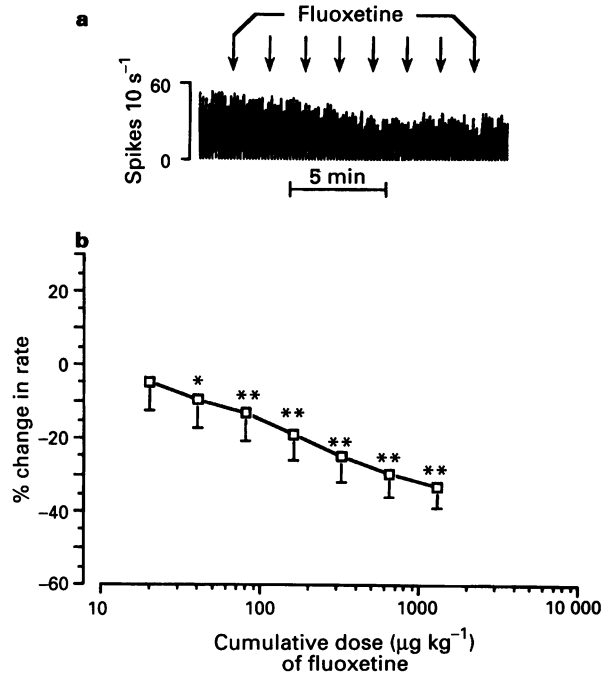

Figure 1 Effect of fluoxetine on the firing rate of VTA dopaminergic neurones: (a) representative rate histogram showing the typical inhibitory effect of i.v. fluoxetine (20, 20, 40, 80, 160, 320, 640, 1280 μg kg^{-1}, at arrows); (b) cumulative dose-response curve showing mean percentage change (±s.e.mean) in firing rate of VTA dopaminergic neurones after i.v. fluoxetine. Mean (±s.e.mean) base-line rate = 54.4 ± 4.5 spikes 10 s^{-1}. *P<0.05; **P<0.01 compared to basal firing rate (one-way analysis of variance, followed by Tukey's test).

Results

Effects of 5-HT-reuptake inhibitors on the basal activity of dopaminergic neurones in the VTA

Intravenous administration of fluoxetine induced a dose-dependent reduction in the firing rate of the VTA dopaminergic cells studied. The typical effect of fluoxetine on dopaminergic neurones is represented in Figure 1a. The inhibitory response varied among different neurones sampled but it did not depend on the basal firing rate of the neurones. Overall, fluoxetine (n = 18) produced a maximal inhibitory effect of 34 ± 7% at the cumulative dose of 1280 μg kg^{-1} (Figure 1b). Administration of higher doses of the drug did not cause additional effects on dopaminergic cell activity (not shown). The effect of i.v. citalopram (n = 16) on the basal firing rate of VTA dopaminergic neurones was less pronounced than that of fluoxetine, in that it produced a maximal inhibition of 14 ± 7% at a cumulative dose of 160 μg kg^{-1} (Figure 2b). The response to citalopram was variable as it clearly inhibited the majority (12 of 16) of dopaminergic neurones tested (Figure 2a), whereas it did not affect the activity of other dopaminergic cells (4 of 16). The differential effect of the drug did not depend on the basal firing rate of neurones sampled. Fluoxetine and citalopram did not induce any changes in the firing pattern of VTA dopaminergic neurones sampled (not shown).

To understand whether the 5-$HT_{2C/2B}$ receptor subtype was involved in the inhibitory effect of fluoxetine on dopaminergic cell activity, mesulergine, an antagonist at this receptor, was given 10 min before the first dose of the SSRI. Injection of mesulergine (80 μg kg^{-1}, i.v.) by itself induced, in some VTA dopaminergic neurones (3 of 7), a slight increase (15 ± 5%) in the firing rate, which returned to base-line levels within a few minutes, i.e. before the first dose of fluoxetine was adminis-

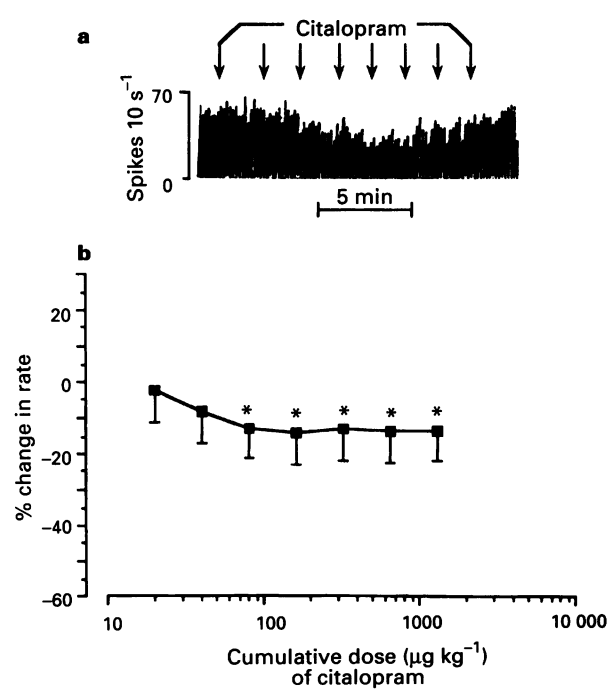

Figure 2 Effect of citalopram on the firing rate of VTA dopaminergic neurones: (a) representative rate histogram showing the typical inhibitory effect of i.v. citalopram (20, 20, 40, 80, 160, 320, 640, 1280 μg kg^{-1}, at arrows); (b) cumulative dose-response curve showing mean percentage change (±s.e.mean) in firing rate of VTA dopaminergic neurones after i.v. citalopram. Mean (±s.e.mean) base-line rate = 48.1 ± 4.4 spikes 10 s^{-1}. *P<0.01 compared to basal firing rate (one-way analysis of variance, followed by Tukey's test).

tered. Pretreatment with mesulergine (80 μg kg^{-1}, i.v.) blocked completely the reduction of VTA dopaminergic activity induced by fluoxetine, as indicated by the findings that administration of fluoxetine in control rats produced the typical inhibitory effect ($n=5$) (Figure 3a, c) which was prevented by pretreatment with mesulergine ($n=7$) (Figure 3b, c).

Selective lesions of brain 5-HT-containing neurones, produced by intraventricular administration of 5,7-DHT, abolished the inhibitory effect induced by acute fluoxetine on the activity of VTA dopaminergic neurones ($n=8$) (Figure 4b, c). Intravenous injection of fluoxetine in vehicle-treated rats produced, as in naïve animals, a significant reduction in the firing rate of dopaminergic cells ($n=7$) (Figure 4a, c). The basal firing rate of VTA dopaminergic neurones in 5-HT-depleted rats was higher than in control animals, but this difference was not statistically significant. Intraventricular administration of 5,7-DHT resulted in significant depletions of both 5-HT and 5-HIAA in the corpus striatum and hippocampus. The effect of 5,7-DHT was particularly evident in the hippocampus, where it produced 90% decrease in 5-HT levels (Table 1).

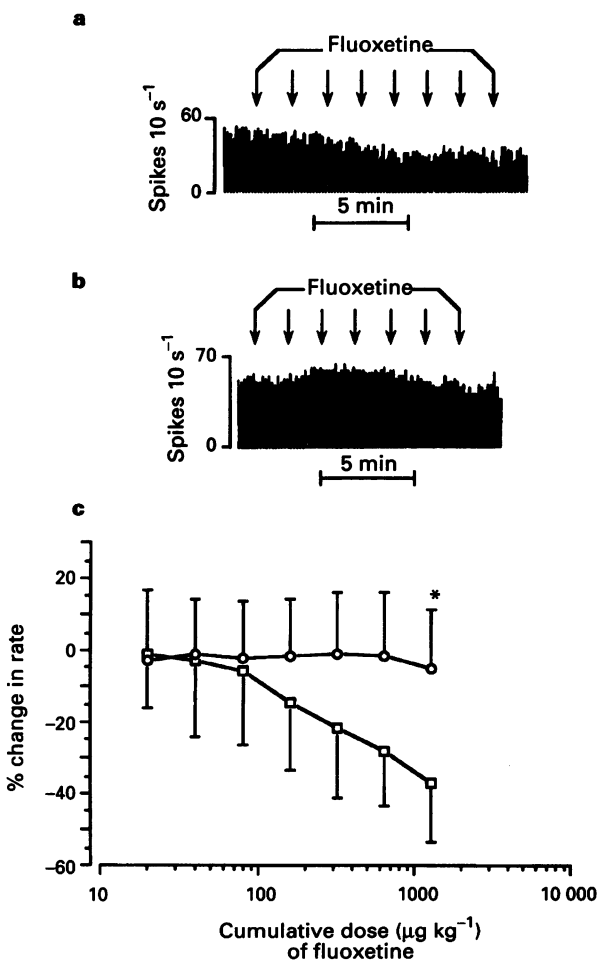

Figure 3 Blockade by mesulergine on the inhibtory action of fluoxetine on the firing rate of VTA dopaminergic neurones: (a) representative rate histogram showing the typical inhibitory effect of i.v. fluoxetine (20, 20, 40, 80, 160, 320, 640, 1280 μg kg^{-1}, at arrows) in a control rat; (b) typical rate histogram showing that i.v. mesulergine (80 μg kg^{-1}) prevents the inhibitory effect of i.v. fluoxetine (20, 20, 40, 80, 160, 320, 640 μg kg^{-1}, at arrows); (c) cumulative dose-response curves showing mean percentage change (±s.e.mean) in firing rate of VTA dopaminergic neurones in control (□) and mesulergine (○) pretreated rats. Mean (±s.e.mean) base-line rate (spikes 10 s^{-1}): saline+fluoxetine = 62.4±12.5; mesulergine+fluoxetine = 56.4±9.2. *$P<0.05$ [$F_{(7,140)}=10.75$, two-way analysis of variance, split-plot design, followed by Tukey's test].

Effect of acute fluoxetine on the basal activity of dopaminergic neurones in the SNc

A group of rats was treated with fluoxetine to investigate the effect of this drug on the basal firing rate of dopaminergic

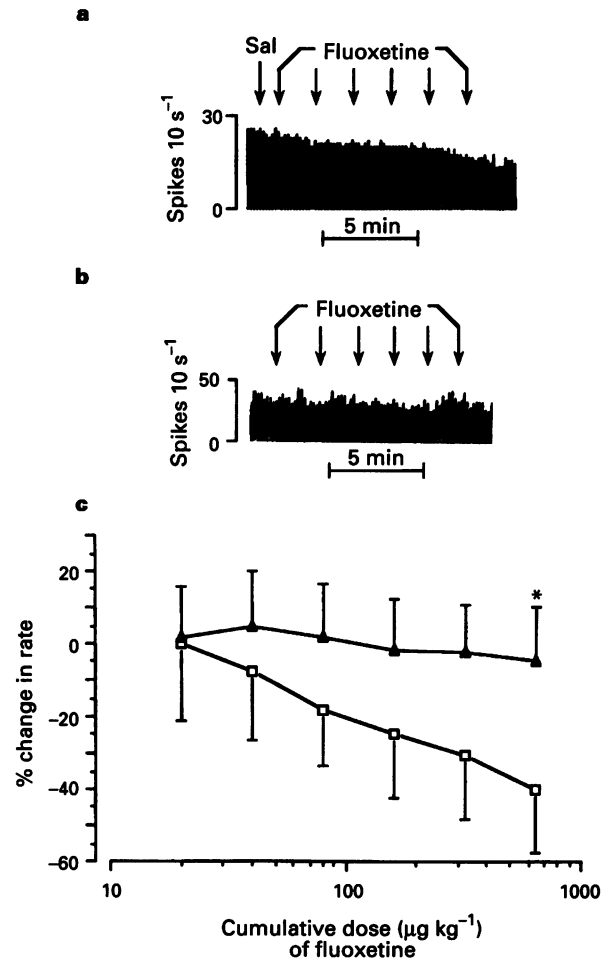

Figure 4 Effects of 5,7-dihydroxytryptamine (5,7-DHT) on the response of VTA dopaminergic neurones to fluoxetine: (a) representative rate histogram showing the typical inhibitory effect of i.v. fluoxetine (20, 20, 40, 80, 160, 320 μg kg^{-1}, at arrows) in a control rat, Sal = i.v. saline injection (0.1 ml, at arrow); (b) typical rate histogram showing the prevention by 5,7-DHT of the inhibitory response to i.v. fluoxetine (20, 20, 40, 80, 160, 320 μg kg^{-1}, at arrows); (c) cumulative dose-response curves showing mean percentage change (±s.e.mean) in firing rate of VTA dopaminergic neurones after i.v. fluoxetine, in control (□) and 5,7-DHT-treated rats (▲). Pretreatment with 5,7-DHT abolished the inhibitory effect of fluoxetine. Mean (±s.e.mean) base-line rate (spikes 10 s^{-1}); vehicle+fluoxetine = 28.8 ± 4.5; 5,7-DHT+fluoxetine = 41.5±5.9. *$P<0.05$ [$F_{(6,78)}=5.47$, two-way analysis of variance, split-plot design, followed by Tukey's test].

Table 1 Effects of 5,7-dihydrixytryptamine (5,7-DHT) on 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels in the hippocampus and corpus striatum

	Hippocampus		*Corpus striatum*	
	5-HT (pg mg^{-1})	*5-HIAA* (pg mg^{-1})	*5-HT* (pg mg^{-1})	*5-HIAA* (pg mg^{-1})
Control	260±45	205±30	322±74	279±33
5,7-DHT	26±6*	14±3*	108±49*	116±65*

Each value is the mean (pg mg^{-1} of tissue)±s.e.mean of 6 animals. *$P<0.01$, as compared with respective control by Student's t test.

neurones in the SNc. Figure 5 represents a typical rate histogram showing that fluoxetine did not modify the spontaneous activity of SNc dopaminergic neurones. Overall, administration of fluoxetine ($n=10$) did not cause any significant change in the activity of SNc dopaminergic cells. The basal firing rate of these neurones was unaffected by doses of the drug (up to 10240 μg kg^{-1}) that were much higher than those which maximally inhibited dopaminergic neurones in the VTA. Moreover, fluoxetine administration did not induce any change in the firing pattern of dopaminergic neurones in the SNc (not shown).

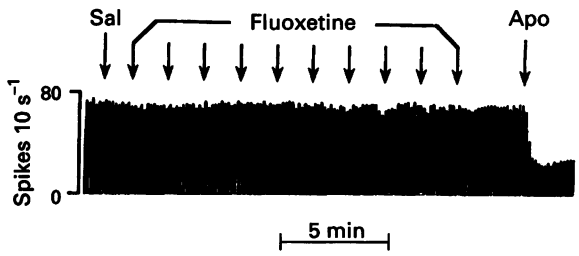

Figure 5 Representative rate histogram showing the lack of effect of i.v. fluoxetine (20, 20, 40, 80, 160, 320, 640, 1280, 2560, 5120 μg kg^{-1}, at arrows) on the basal activity of a dopaminergic neurone in the SNc. Sal = i.v. saline injection (0.1 ml, at arrow); Apo = apomorphine administration (10 μg kg^{-1}, at arrow).

Effects of chronic fluoxetine on the activity of dopaminergic neurones in the VTA

A series of chronic experiments were carried out to test if tolerance developed to the inhibitory effect of fluoxetine on VTA dopaminergic activity. Rats received fluoxetine (10 mg kg^{-1}, i.p.) or saline once daily for 21 consecutive days. At the end of chronic fluoxetine treatment, the body weight of treated rats was significantly reduced as compared with control animals. Chronic fluoxetine did not cause any change in the

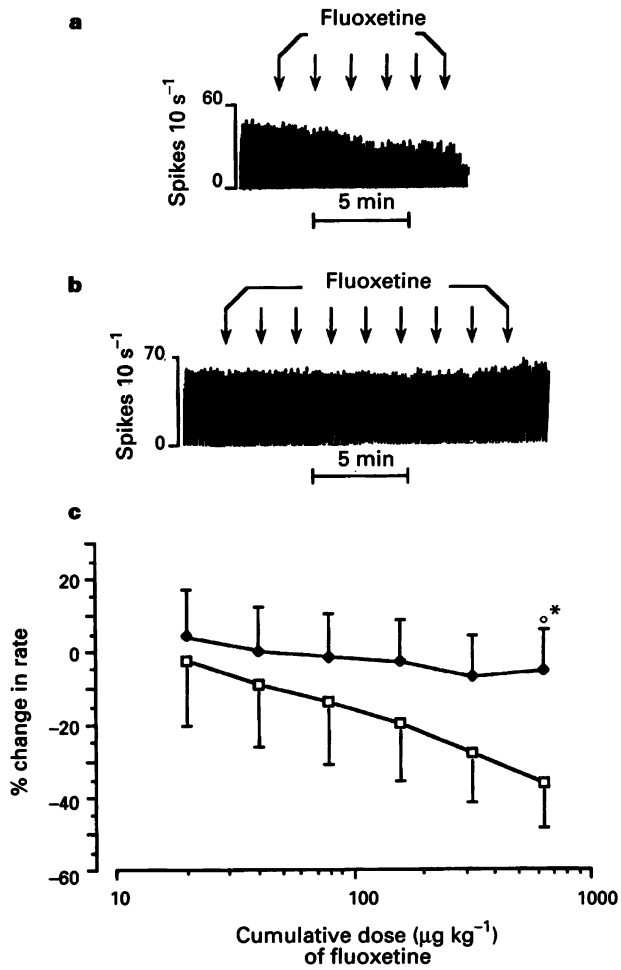

Figure 6 Effects of chronic treatment with i.p. fluoxetine (10 mg kg^{-1}, for 21 days) on the response of VTA dopaminergic neurones to acute i.v. fluoxetine: (a) representative rate histogram showing the typical inhibitory effect of acute i.v. fluoxetine (20, 20, 40, 80, 160, 320 μg kg^{-1}, at arrows) in a control rat; (b) typical rate histogram showing the prevention by chronic i.p. fluoxetine of the inhibitory response to acute i.v. fluoxetine (20, 20, 40, 80, 160, 320, 640, 1280, 2560 μg kg^{-1}, at arrows); (c) cumulative dose-response curves showing mean percentage change (± s.e.mean) in firing rate of VTA dopaminergic neurones after acute i.v. fluoxetine in control rats (□) and in animals treated chronically with fluoxetine (◆). Complete tolerance developed after chronic fluoxetine administration. Mean (± s.e.mean) base-line rate (spikes 10 s^{-1}): control = 41.7 ± 6.6; chronic fluoxetine = 52.5 ± 5.9. *$P<0.05$ [$F_{(6,84)}=6.27$, two-way analysis of variance, split-plot design, followed by Tukey's test].

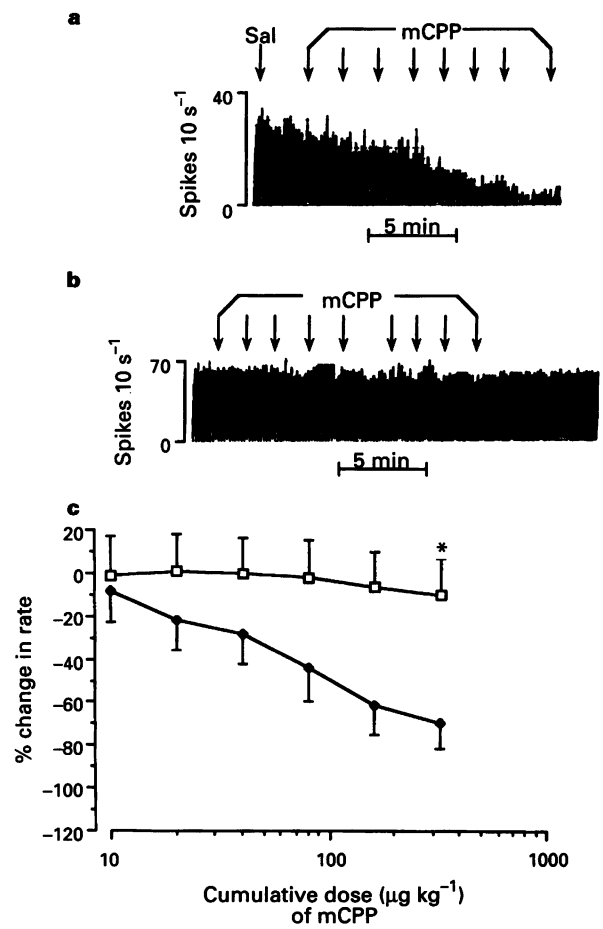

Figure 7 Effects of chronic treatment with i.p. fluoxetine (10 mg kg^{-1}, for 21 days) on the response of VTA dopaminergic neurones to acute i.v. *m*-chlorophenylpiperazine (mCPP): (a) representative rate histogram showing the typical inhibitory effect of acute i.v. mCPP (10, 10, 20, 40, 80, 160, 320, 640 μg kg^{-1}, at arrows) in a control rat, Sal = i.v. saline injection (0.1 ml, at arrow); (b) typical rate histogram showing the prevention by chronic i.p. fluoxetine of the inhibitory response to i.v. mCPP (10, 10, 20, 40, 80, 160, 320, 640, 1280 μg kg^{-1}, at arrows); (c) cumulative dose-response curves showing mean percentage change (± s.e.mean) in firing rate of VTA dopaminergic neurones after acute i.v. mCPP in control rats (◆) and in animals treated chronically with fluoxetine (□). Complete tolerance to mCPP developed after chronic fluoxetine administration. Mean (± s.e.mean) base-line rate (spikes 10 s^{-1}): control = 23.3 ± 3.2; chronic fluoxetine = 43.7 ± 8.1. *$P<0.01$ [$F_{(6,72)}=10.82$, two-way analysis of variance, split-plot design, followed by Tukey's test].

gross behaviour of rats. Acute i.v. challenge with fluoxetine was performed in two separate series of experiments either 24 h or 72 h after the last i.p. fluoxetine injection. Since results obtained after 24 or 72 h were almost superimposable, only data regarding the 72 h wash-out period are presented. Acute i.v. administration of fluoxetine (20–1280 μg kg^{-1}) did not cause any change in the basal firing rate of VTA dopaminergic neurones in the group of rats treated chronically with i.p. fluoxetine ($n=9$) (Figure 6b, c), whereas it induced the typical inhibitory effect in control animals ($n=7$) (Figure 6a, c). In a separate experiment, rats chronically treated with i.p. fluoxetine received i.v. mCPP, a 5-$HT_{2C/2B}$ receptor agonist, to check if a down-regulation of these receptors could be involved in the mechanism of tolerance to fluoxetine. Acute i.v. administration of mCPP (10–320 μg kg^{-1}) 72 h after the last i.p. fluoxetine treatment caused a significant inhibition of VTA dopaminergic cell activity in control rats (maximal inhibition: 69±12%; $n=7$) (Figure 7a, c), but it did not modify the basal firing rate of dopaminergic cells in animals given chronic fluoxetine ($n=7$) (Figure 7b, c). Since the typical inhibitory action of acute fluoxetine and mCPP disappeared in rats treated chronically with fluoxetine, it is likely that prolonged administration of this drug induced a down-regulation of a 5-HT receptor, possibly the 5-HT_{2C} subtype.

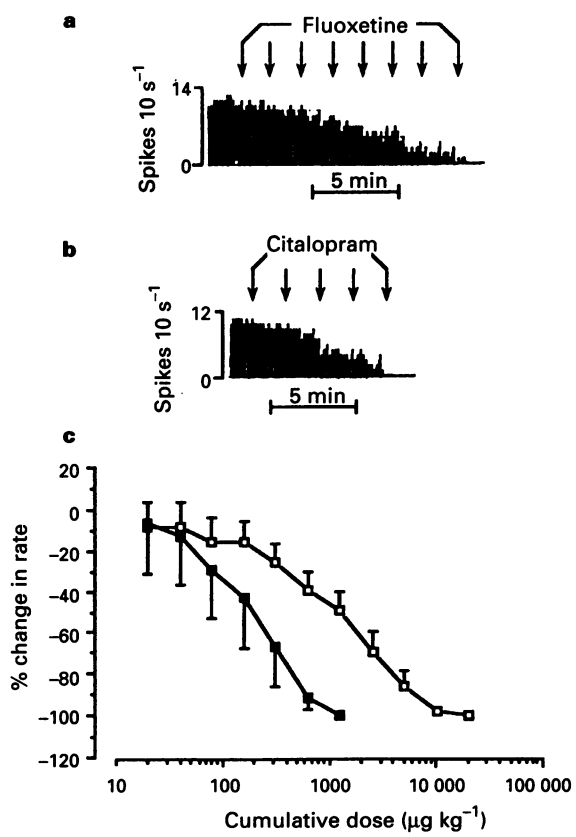

Figure 8 Effects of fluoxetine and citalopram on the basal activity of 5-hydroxytryptaminergic neurones in the dorsal raphe nucleus: representative rate histograms showing the inhibitory effects of (a) i.v. fluoxetine (20, 20, 40, 80, 160, 320, 640, 1280 μg kg^{-1}, at arrows) and (b) i.v. citalopram (20, 20, 40, 80, 160 μg kg^{-1}, at arrows); (c) cumulative dose-response curves showing mean percentage change (±s.e.mean) in firing rate of 5-hydroxytryptaminergic neurones after i.v. fluoxetine (□) or i.v. citalopram (■). Mean (±s.e.mean) baseline rate (spikes 10 s^{-1}): fluoxetine=12.7±1.4; citalopram=12.4±2.8.

Effects of 5-HT reuptake inhibitors on the basal firing rate of 5-hydroxytryptaminergic neurones in the dorsal raphe nucleus

The evidence that selective lesions of 5-hydroxytryptaminergic neurones by 5,7-DHT abolished the inhibitory effect of fluoxetine on VTA dopaminergic cells suggested an indirect action of fluoxetine on dopaminergic neurones of the VTA. Thus, a series of experiments was carried out to investigate the effect of 5-HT reuptake blockers on the basal activity of 5-HT-containing cells in the dorsal raphe nucleus. Intravenous injections of fluoxetine (20–20480 μg kg^{-1}; $n=7$) and citalopram (20–1280 μg kg^{-1}; $n=7$) caused a dose-dependent decrease in the basal firing rate of 5-hydroxytryptaminergic neurones; all the cells studied were completely inhibited, although at different doses (Figure 8). Thus, citalopram stopped the spontaneous firing of 5-hydroxytryptaminergic neurones in the dorsal raphe nucleus at the cumulative dose of 1280 μg kg^{-1} (Figure 8b, c), whereas fluoxetine produced the same effect only at the cumulative dose of 20480 μg kg^{-1} (Figure 8a, c). These differences in potency between the two SSRIs were reflected by the calculated ED_{50} which was lower for citalopram (236.9±35.7 μg kg^{-1}; mean±s.e.mean) than for fluoxetine (1650.2±299 μg kg^{-1}; mean±s.e.mean).

Discussion

The present study shows that acute administration of fluoxetine inhibits the basal firing rate of dopaminergic neurones in the VTA, which is the nucleus of origin of the mesolimbic system (Moore & Bloom, 1978). Interestingly, the effect of acute fluoxetine appears to be selective in that it did not affect the activity of nigrostriatal dopaminergic neurones. The inhibitory action of fluoxetine upon dopaminergic neurones in the VTA was prevented completely by mesulergine, a drug which blocks 5-$HT_{2C/2B}$ and 5-HT_{2A} receptors (Hoyer, 1988). The dose of mesulergine used in the present study was similar to that employed by Prisco *et al.* (1994), who found that 80 μg kg^{-1} i.v. mesulergine affected dopaminergic function in the VTA by acting on 5-$HT_{2C/2B}$ receptors. As reported by Prisco *et al.* (1994), 80 μg kg^{-1} i.v. mesulergine caused a slight excitation of VTA dopaminergic neurones, but it seems unlikely that mesulergine could be acting by blocking dopaminergic receptors because at the dose used in the present study, it did not prevent the inhibitory action of apomorphine (Prisco *et al.*, 1994). Therefore, it is possible to argue that acute fluoxetine reduces mesolimbic dopaminergic function by acting on 5-$HT_{2C/2B}$ receptors. This finding is consistent with a previous report showing that the anti-immobility effect of fluoxetine in the forced swimming test was blocked by mesulergine, but not by the mixed 5-HT_{2A}/5-$HT_{2C/2B}$ antagonist ritanserin (Cesana *et al.*, 1993) thus suggesting that this pharmacological effect was mediated by the 5-$HT_{2C/2B}$ receptor subtype. Therefore, the possibility exists that fluoxetine could exert its effect on the mesolimbic dopaminergic system by acting directly on the 5-$HT_{2C/2B}$ receptors inasmuch as there is evidence that it can bind to this receptor subtype with submicromolar affinity (Wong *et al.*, 1991) acting as an antagonist (Lightowler *et al.*, 1994). However, this possibility seems unlikely since the inhibitory effect of fluoxetine on the activity of VTA dopaminergic neurones was abolished by pretreatment with the neurotoxin 5,7-DHT, which caused a marked and selective reduction of 5-HT levels in the brain. This finding indicates that acute fluoxetine acts presynaptically by enhancing 5-hydroxytryptaminergic transmission which, in turn, reduces the dopaminergic tone in the VTA. That acute administration of fluoxetine can increase the synaptic availability of 5-HT is demonstrated by several studies showing that this drug increases the extracellular concentration of 5-HT, as measured by *in vivo* microdialysis, in different brain areas (Perry & Fuller, 1992; 1993; Ruttler & Auerbach, 1993). Thus, fluoxetine increases the synaptic levels of 5-HT, which inhibits the

activity of the mesolimbic dopaminergic system by stimulating the 5-$HT_{2C/2B}$ receptor subtypes. Like fluoxetine, citalopram, a potent SSRI, decreased the activity of mesolimbic dopaminergic neurones albeit to a lesser extent. It is tempting to speculate that the reduced inhibition of VTA dopaminergic neurones by citalopram, as compared to fluoxetine, might depend on its greater capability of inhibiting 5-hydroxytryptaminergic neurones in the dorsal raphe nucleus. Thus, citalopram was seven fold more potent that fluoxetine in inhibiting the activity of 5-HT-containing neurones in the dorsal raphe nucleus, as calculated from the ED_{50}. This is consistent with previous findings indicating that the ED_{50} for citalopram (0.23 mg kg^{-1}) (Chaput *et al.*, 1986) is much lower than the ED_{50} for fluoxetine (1.8 mg kg^{-1}) (Cunningham & Lakoski, 1990). Probably, the different potencies of these two SSRIs as inhibitors of 5-hydroxytryptaminergic neurones reflect *in vitro* data indicating that citalopram is more effective than fluoxetine in blocking the reuptake of 5-HT from rat brain synaptosomes (Hyttel, 1982; Thomas *et al.*, 1987). However, acute administration of citalopram only slightly increases extracellular 5-HT concentrations in the rat frontal cortex, probably as a consequence of a concomitant reduction in the impulse flow of 5-hydroxytryptaminergic neurones in the dorsal raphe nucleus (Invernizzi *et al.*, 1992). On the basis of these findings, it possible to argue that the slight inhibitory effect of citalopram on the activity of VTA dopaminergic neurones might depend on its low capability of increasing the synaptic levels of 5-HT in terminal regions of the 5-hydroxytryptaminergic system.

The typical inhibitory effect of acute fluoxetine upon the basal activity of VTA dopaminergic neurones disappeared after repeated treatment with this SSRI for 21 consecutive days. This finding indicates that a complete tolerance to fluoxetine's action develops after chronic treatment. The tolerance to the acute challenge with fluoxetine was clearly evident 24 h after the cessation of chronic treatment with this drug. However, a 72 h wash-out period was preferred to avoid possible residual effects of fluoxetine which has been shown to accumulate in the rat brain following repeated treatment (Caccia *et al.*, 1992). Nevertheless, brain levels of fluoxetine fall below detectable limits 72 h after withdrawal from chronic i.p. fluoxetine administration (10 mg kg^{-1} for 21 consecutive days) (Gardier *et al.*, 1993). Interestingly, the results obtained at 24 h and 72 h were very similar, indicating that the putative pharmacodynamic changes induced by chronic fluoxetine persisted for several days. This statement is strengthened by the evidence that chronic fluoxetine administration induced a complete tolerance to the inhibitory action of mCPP, a 5-$HT_{2C/2B}$ receptor agonist (Curzon & Kennett, 1990). Thus, mCPP given to control rats caused a marked inhibition of the basal firing rate of dopaminergic neurones in the VTA, whereas it was ineffective when injected 72 h after the withdrawal of chronic fluoxetine treatment. These findings are consistent with previous data showing that repeated oral administration of fluoxetine and paroxetine for 21 days induces tolerance to the hypolocomotor effect of mCPP in rats (Kennett *et al.*, 1994a). It is unlikely that a reduced disposition of mCPP might have been responsible for its reduced effect, inasmuch as a marked increase in brain levels of mCPP has been found following repeated administration of fluoxetine to rats (Kennett *et al.*, 1994a). Moreover, chronic treatments with citalopram and sertaline, two potent SSRIs, were found to attenuate the hypolocomotor effect of mCPP (Maj & Moryl, 1992; Kennedy *et al.*, 1993). Since it is thought that mCPP reduces locomotor activity in rodents by stimulating the 5-$HT_{2C/2B}$ receptors (Kennett & Curzon, 1988; Kennett *et al.*, 1994b), it has been argued that a down-regulation of 5-$HT_{2c/2B}$ recpetors might be responsible for the behavioural tolerance to mCPP occurring after chronic administration of SSRIs (Kennedy *et al.*, 1993; Kennett *et al.*, 1994a). This is reconcilable with the evidence that mCPP inhibits the activity of dopaminergic neurones in the VTA by acting through the 5-$HT_{2C/2B}$ receptors (Prisco *et al.*, 1994). Thus, it is conceivable that a down-regulation of 5-$HT_{2C/2B}$ receptors might underlie the tolerance to the inhibitory effect of mCPP elicited by chronic fluoxetine.

It is tempting to speculate that the differential effects of acute and chronic fluoxetine on the activity of the mesolimbic dopaminergic system might explain, at least in part, the pharmacodynamic basis of its therapeutic actions. For example, it is known that the antidepressant effect of fluoxetine becomes evident three weeks after the beginning of therapy (Stark & Hardison, 1985; Chouinard, 1985). Thus, in view of the hypothesis that disinhibition of the mesolimbic dopaminergic system underlies the mechanism of action of several antidepressant agents (Cervo & Samanin, 1987; 1988; Cervo *et al.*, 1990), it is conceivable that inhibition by fluoxetine of VTA dopaminergic function may mask its clinical efficacy during the first weeks of treatment. Based on this assumption, it is possible to argue that the antidepressant activity of fluoxetine becomes manifest when tolerance develops to the inhibition of the VTA dopaminergic system.

There is evidence that fluoxetine is an effective agent in the treatment of obsessive-compulsive disorders (OCD) (Turner *et al.*, 1985; Fontaine & Chouinard, 1986; Levine *et al.*, 1989; Jenike *et al.*, 1990). However, there is a latency of about four weeks between the beginning of treatment and the appearance of a significant clinical effect (Levine *et al.*, 1989). It has been suggested that repeated administration of fluoxetine in human subjects induces a down-regulation of 5-$HT_{2C/2B}$ receptors, which is ultimately responsible for its therapeutic effect in OCD (Hollander *et al.*, 1991). This hypothesis is based on the evidence that administration of the 5-$HT_{2C/2B}$ receptor agonist, mCPP, in patients with OCD worsens obsessive-compulsive (OC) symptoms in drug-free subjects, whereas it does not exacerbate OC symptoms in patients treated chronically with fluoxetine (Hollander *et al.*, 1991). These clinical data are consistent with the findings of the present study showing that chronic treatment with fluoxetine induces tolerance to the inhibitory effect of mCPP on the activity of mesolimbic dopaminergic neurones. Although the role of central dopaminergic systems in the pathophysiology of OCD is still unclear (Goodman *et al.*, 1990; 1992), the possibility that the effect of fluoxetine on VTA dopaminergic neurones might play a role in its anti-obsessive activity cannot be ruled out.

The therapeutic use of fluoxetine alone or associated with haloperidol can cause a dysfunction of the extrapyramidal system which may result in parkinsonism (Meltzer *et al.*, 1979; Bouchard *et al.*, 1989; Brod, 1989; Tate, 1989) or akathisia (Lipinski *et al.*, 1989; Baldwin *et al.*, 1991). It has been speculated that the extrapyramidal side-effects of fluoxetine might have been caused by a reduced dopaminergic tone in the basal ganglia (Meltzer *et al.*, 1979; Baldessarini & Marsh, 1990; Baldessarini *et al.*, 1992). However, fluoxetine was found to reduce only slightly dopamine synthesis in the rat striatum and frontal cortex (Baldessarini & Marsh, 1990) and these data were not replicated in a subsequent study (Baldessarini *et al.*, 1992). Moreover, fluoxetine does not change *in vivo* dopamine release either in the striatum (Perry & Fuller, 1992) or in the nucleus accumbens (Tanda *et al.*, 1994). That dopamine release in the nucleus accumbens is unaffected by fluoxetine (Tanda *et al.*, 1994) is apparently in contrast with the findings of this study. The reasons for this discrepancy are presently unknown but, probably, electrophysiological techniques are more sensitive than *in vivo* microdialysis in detecting changes in neuronal function. Thus, the present study provides the first clear evidence of an inhibitory action of fluoxetine on the dopaminergic system originating in the VTA, which might be of relevance in the pathophysiology of akathisia (Marsden & Jenner, 1980). In this regard, it is interesting to note that Lipinski *et al.* (1989) argued, on the basis of clinical data, that fluoxetine might have inhibited dopaminergic neurones in the VTA but not in the SNc, which is

exactly what was found in the present study. However, evidence from this study does not support clinical observations that fluoxetine induces parkinson-like effects since it did not affect the activity of dopaminergic nigro-striatal neurones, whose reduced function is generally thought to be the cause of drug induced parkinson-like syndrome (Marsden & Jenner, 1980).

In conclusion, the present study provides evidence that acute treatment with fluoxetine reduces the activity of dopaminergic neurones in the VTA but not in the SNc. This acute effect of fluoxetine, which is probably mediated by the 5-$HT_{2C/2B}$ receptor subtype, disappears following chronic fluoxetine administration. However, more selective agents are requried to confirm the involvement of the 5-HT_{2C} or 5-HT_{2B} in these responses. It is hypothesized that the differential effects of acute and chronic fluoxetine on the activity of mesolimbic dopaminergic function might be a relevant mechanism underlying the lag in its antidepressant action. Moreover, the inhibition of VTA dopaminergic neurones is probably the cause of fluoxetine-induced akathisia. It is possible to infer that akathisia might disappear after repeated treatment with fluoxetine as tolerance develops to its inhibitory action on the mesolimbic dopaminergic system.

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