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Flibanserin, a potential antidepressant drug, lowers 5-HT and raises dopamine and noradrenaline in the rat prefrontal cortex dialysate: role of 5-HT_{1A} receptors

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1 Using *in vivo* intracerebral microdialysis in conscious, freely moving rats, we examined the effect of flibanserin, a potential antidepressant drug with high affinity for human 5-HT_{1A} receptors and four–50-fold lower affinity for 5-HT_{2A} and D_4 receptors, on basal extracellular concentrations of serotonin (5-hydroxytryptamine, 5-HT), dopamine (DA) and noradrenaline (NA) in selected regions of the rat brain.

2 Flibanserin at 3 and 10 mg kg^{-1} significantly reduced extracellular 5-HT in the prefrontal cortex (by 30 and 45%) and dorsal raphe (35 and 44%), but had no effect on extracellular 5-HT in the ventral hippocampus. The 3 and 10 mg kg^{-1} doses raised extracellular NA to a similar extent in the prefrontal cortex (47 and 50%). In all, 10 mg kg^{-1} raised extracellular DA in the prefrontal cortex (63%) whereas 3 mg kg^{-1} had no significant effect.

3 Pretreatment with the selective 5-HT_{1A} receptor antagonist WAY100,635 (0.3 mg kg^{-1}) 30 min before 10 mg kg^{-1} flibanserin completely antagonized the latter's effects on extracellular 5-HT, DA and NA in the prefrontal cortex. WAY100,635 by itself had no effect on cortical extracellular monoamines.

4 The results show that the stimulation of 5-HT_{1A} receptors plays a major role in the effect of flibanserin on brain extracellular 5-HT, DA and NA.
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Abbreviations: aCSF, artificial cerebrospinal fluid; DA, dopamine; 5-HT, 5-hydroxytryptamine; NA, noradrenaline; SSRI, selective serotonin reuptake inhibitors

Introduction

The serotonergic system has long been implicated in depression and in the response to antidepressant drugs. Among the seven main families of 5-hydroxytryptamine (5-HT) receptors known to date (Hoyer *et al*., 1994; Barnes & Sharp, 1999) attention has focussed particularly on the role of 5-HT_{1A} and 5-HT_2 subtypes in the mechanism of action of antidepressant drugs. 5-HT_{1A} receptor agonists from different chemical classes are active in animal models predictive of antidepressant activity (Cervo & Samanin, 1991; De Vry, 1995) and may have antidepressant effects in man (Stahl *et al*., 1992). The nonselective 5-HT_{1A} receptor antagonists such as pindolol accelerate the antidepressant effect of selective serotonin reuptake inhibitors (SSRI) (Artigas *et al*., 1994; Perez *et al*., 1997) and it has been suggested that the antidepressant effect of both types of substances is related to the desensitization of raphe 5-HT_{1A} autoreceptors with repeated administration (Blier & de Montigny, 1994; Invernizzi *et al*., 1994, 1996; Rutter *et al*., 1994). Thus, 5-HT_{1A} receptor knockout mice give antidepressant-like responses in the tail-suspension (Heisler *et al*., 1998) and forced swimming (Ramboz *et al*., 1998) tests, two models widely used to assess the potential antidepressant effect of drugs (Porsolt *et al*., 1978; Steru *et al*., 1985).

Changes in 5-HT_2 receptor density are observed in depressed patients (Stanley & Mann, 1983; Yates *et al*., 1990; Risch & Nemeroff, 1992) and in response to chronic administration of classical antidepressant drugs (Peroutka & Snyder, 1980). In addition, blockade of 5-HT_{2A} receptors might contribute to the antidepressant effect of mirtazapine, mianserin and nefazodone which potently inhibit 5-HT_{2A} receptors (de Boer *et al*., 1988), and to the antidepressant effect of ritanserin, a 5-$HT_{2A/2C}$ receptor antagonist (Bersani *et al*., 1991).

Recent strategies aimed at developing new antidepressant drugs have focused on compounds acting as 5-HT_{1A} receptor agonists and 5-HT_{2A} receptor antagonists. This approach stems from electrophysiological studies in rats suggesting a functional opposition between 5-HT_{2A} and 5-HT_{1A} receptors in the cortex (Araneda & Andrade, 1991; Ashby *et al*., 1994), one of the brain regions where metabolic alterations have been consistently reported in depressed patients (Drevets, 1998).

Flibanserin is a potential antidepressant drug with high affinity for human 5-HT_{1A} receptors (Ki = 1 nM) and lower

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affinity for 5-HT_{2A} (Ki = 49 nM) and D_4 (Ki = 4–24 nM) receptors, but negligible affinity for a variety of other neurotransmitter receptors and ion channels (Borsini *et al*., 2002). *In vitro* studies showed that flibanserin reduced forskolin-stimulated cAMP formation in cells and rat tissues and antagonized the accumulation of phosphatidyl inositol turnover induced by 5-HT in the mouse cortex (Borsini *et al*., 1995). This suggested that the drug may be a 5-HT_{1A} receptor agonist and 5-HT_{2A} receptor antagonist. *In vivo*, flibanserin displayed some effects compatible with the activation of 5-HT_{1A} receptors. Flibanserin inhibited the firing rate of serotonergic neurons of the dorsal raphe (DR) (Rueter *et al*., 1998) and this effect was antagonized by the selective 5-HT_{1A} receptor antagonist WAY100,635 (Forster *et al*., 1995). Similar to the selective 5-HT_{1A} receptor agonists, flibanserin reduced the accumulation of brain 5-hydroxytryptophan induced by the blockade of aromatic amino-acid decarboxylase ($ED_{50} = 8\,mg\,kg^{-1}$; Brambilla *et al*., 1999). The fact that flibanserin reduced the head-twices ($ED_{50} = 4.1\,mg\,kg^{-1}$) in mice and electrophysiological effects induced by the nonselective 5-HT_{2A} agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) in rats (Rueter & Blier, 1999; Borsini *et al*., 2002) suggests that this compound may block 5-HT_{2A} receptors *in vivo*.

In the present study, we employed the microdialysis technique in conscious rats to investigate the effect of 3 and $10\,mg\,kg^{-1}$ flibanserin on the extracellular concentration of 5-HT in various brain regions. These doses are in the range of those inhibiting brain 5-HT synthesis and antagonizing the effect of DOI (Brambilla *et al*., 1999; Borsini *et al*., 2002). Since 5-HT_{1A} receptor agonists stimulate the activity of noradrenergic cells of the locus coeruleus (Szabo & Blier, 2001) and dopaminergic neurons of the ventrotegmental area (Arborelius *et al*., 1993a; Prisco *et al*., 1994), and increase extracellular noradrenaline (NA) and dopamine (DA) in the respective projection regions (Arborelius *et al*., 1993b; Wedzony *et al*., 1996), we investigated the effect of flibanserin on extracellular DA and NA in the prefrontal cortex. Finally, to prove the involvement of 5-HT_{1A} receptors, in one experiment we studied the effect of flibanserin on cortical monoamine release in rats pretreated with the selective 5-HT_{1A} receptor antagonist WAY100,635. Part of these results has been published in preliminary form (Borsini *et al*., 2002).

Methods

Animals

Male Sprague–Dawley rats (CD-COBS, Charles River, Italy) (250–350 g) were used, housed at constant temperature (21 ± 1°C) and relative humidity (60 ± 5%) under a regular light–dark schedule (light 7:00–19:00 h) with food and water freely available.

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 Febbraio 1992, Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, 12 December 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

Dialysis procedure

Rats were anaesthetized with $3.5\,ml\,kg^{-1}$. Equithesin and placed on a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, U.S.A.). A hole was drilled in the parietal or frontal bone and a small incision was made in the dura with a bent needle tip. The probe was perfused with artificial cerebrospinal fluid (aCSF; see below for composition) and lowered slowly into the rat prefrontal cortex, ventral hippocampus and DR nucleus, then fixed vertically to the skull using two or three stainless-steel anchorage screws and acrylic cement. Stereotaxic coordinates relative to the probe tip were as follows (in mm): prefrontal cortex, AP = 12.7, $L = \pm 0.6$ and $V = 4.6$; ventral hippocampus, AP = 4.2, $L = \pm 4.8$ and $V = 1.6$; DR, AP = 1.1, $L = \pm 0.6$ and $V = 3.0$ with an 8° angle to the sagittal plane. Coordinates were taken from the interaural line according to the Paxinos & Watson (1986) atlas.

The dialysis probes were of the concentric type and were prepared essentially as described by Robinson & Whishaw (1988) except that the dialysis membrane was made of polyacrylonitrile-sodium methallyl sulphonate (AN69, Hospal). Since diffusion of 5-HT through AN69 membrane is markedly delayed (Tao & Hjorth, 1992), we used Cuprophan membranes (216 μm outer diameter, Sorin Biomedica, Italy) for the measurement of 5-HT. The length of the exposed membrane was 4 mm for the prefrontal cortex and ventral hippocampus and 1.5 mm for the DR. *In vitro* recovery was about 8 and 20% respectively for 1.5 and 4 mm Cuprophan membranes and 22–29% for 4 mm AN69 membranes. Each rat was implanted with a single probe in the DR or ventral hippocampus. Bilateral probes were implanted in the prefrontal cortices to allow the detection of changes in extracellular 5-HT and DA or NA in the same subject.

Rats were allowed to recover from anaesthesia, one per cage with free access to food and water. About 24 h after surgery, each rat was placed in a cage and the inlet cannula was connected by polyethylene tubing to a 2.5 ml syringe containing aCSF (composition in mM: 145 NaCl, 3 KCl, 1.26 $CaCl_2 \cdot 2\,H_2O$, 1 $MgCl_2 \cdot 6\,H_2O$ in distilled water and buffered at pH 7.4 with 2 mM sodium phosphate buffer) containing 1 μM citalopram to improve 5-HT detectability. Each probe was perfused at a constant flow-rate of $1\,\mu l\,min^{-1}$ with a microinfusion pump (CMA 100, CMA/Microdialysis, Stockholm, Sweden). After a 30 min washout period, consecutive 30 min samples of perfusate were collected in minivials. Samples were immediately injected into the high-performance liquid chromatograph with electrochemical detection (HPLC-ED) without prior purification for the determination of monoamines as previously described (Invernizzi *et al*., 1992a, b). 5-HT, NA and DA were determined in separate samples of dialysate. Separation of 5-HT was achieved by a reverse-phase column (Supelcosil LC18-DB 3 μm, 150 × 4.6 mm; Supelchem, Italy) and a mobile phase consisting of (mM) citric acid 9, sodium acetate trihydrate 48, Na_2EDTA 0.1, $100\,\mu l\,l^{-1}$ triethylamine and $40\,ml\,l^{-1}$ acetonitrile, pumped at $1\,ml\,min^{-1}$. Separation of NA was obtained using a reverse-phase column (Hypersil-ODS 5 μm, 125 × 3.1 mm, Bischoff, Italy). The mobile phase, consisting of (mM) citric acid 25, sodium acetate 24, sodium octyl sulphate 1.55 and $80\,ml\,l^{-1}$ CH_3OH was pumped at $1\,ml\,min^{-1}$. DA was separated through a 150 × 4.6 reverse-phase column (Supelcosil LC18-DB 3 μm, 150 × 4.6 mm; Supelchem, Milan, Italy) using a mobile phase containing

0.1 M sodium acetate, 60 ml l^{-1} CH_3OH, pH 4.2 with acetic acid, pumped at 1 ml min^{-1}. 5-HT, NA and DA were measured by a Coulochem II electrochemical detector equipped with a 5011 analytical cell at the following potentials (E1/E2): 5-HT 50/180 mV, NA 200/−250 mV and DA 300/−325 mV. Monoamines were read as the second electrode output signal.

Histological procedure

At the end of the experiments, rats were deeply anaesthetized with chloral hydrate (400 mg kg^{-1}) and killed by decapitation, their brains were immediately removed and the correct placement of the probes was checked by examining the probe tracks. Only rats with correct probe placement were considered in the results.

Drug treatment

Flibanserin (previously BIMT17; 1-[2-[4-(3-trifluoromethylphenyl)piperazin-1-yl] ethyl] benzimidazol-[1 H]-2-one) (Boehringer-Ingelheim, Milan, Italy) was dissolved in a vehicle containing 250 ml l^{-1} polyethylene glycol-400 and 22.7 ml l^{-1} 1 M HCl, warmed at about 40°C. On the day of the experiment (24 h after probe implantation), once basal levels of monoamines in the dialysate were stable (no more than 15% difference between three consecutive samples), rats were injected intraperitoneally with vehicle (2 ml kg^{-1}) or 3 and 10 mg kg^{-1} flibanserin (as base). WAY100,635 (*N*-[2-[methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexane carboxamide trihydrochloride) (Pharmacia, Nerviano, Italy) was dissolved in saline and injected subcutaneously 30 min before flibanserin or vehicle.

Statistical analysis

The effects of flibanserin on extracellular 5-HT, NA and DA were analysed by ANOVA for repeated measures (split-plot) with treatment and time as between and within factors, respectively. *Post-hoc* comparisons were made by Tukey–Kramer's test. Values missing because of occasional problems in sample collection or analysis were replaced by the mean of the samples immediately before and after. Statistical analysis was done using the StatView 5.0 statistical package for Apple-Macintosh computer (SAS Institute Inc., SAS Campus Drive, Cary, NC, U.S.A.).

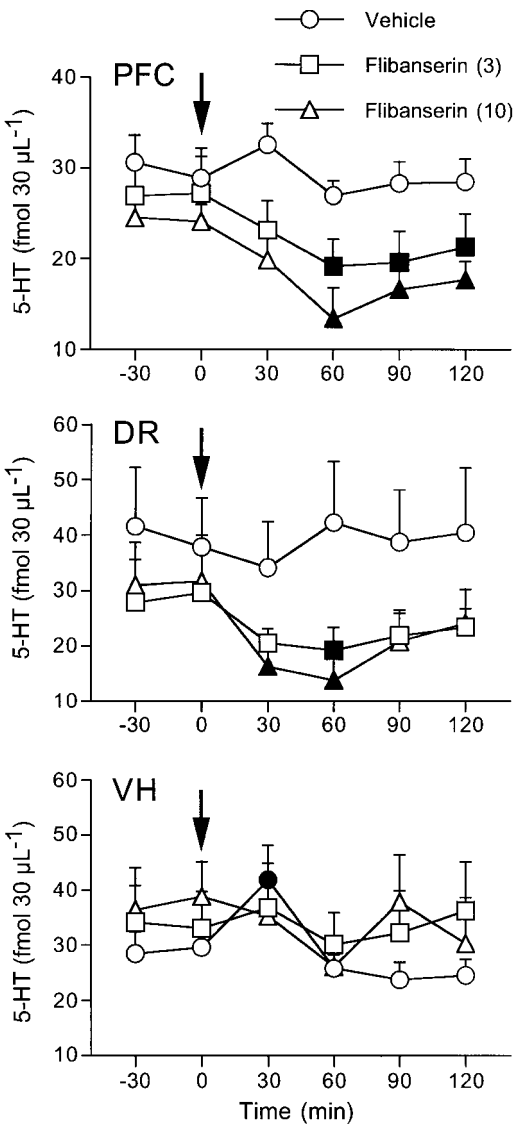

Figure 1 Extracellular 5-HT in the prefrontal cortex (PFC), dorsal raphe (DR) and ventral hippocampus (VH) of rats given 3 and 10 mg kg^{-1} flibanserin or vehicle intraperitoneally. Mean ± s.e.m. of 5–7 rats. The arrows indicate the time of drug or vehicle injection. Solid symbols indicate $P<0.05$ vs basal values (Tukey–Kramer's test).

Results

Effect of flibanserin on extracellular 5-HT in the prefrontal cortex, ventral hippocampus and dorsal raphe

Basal concentrations of extracellular 5-HT (fmol 30 µl^{-1}) measured in the presence of 1 µM citalopram in the perfusion medium were as follows (mean ± s.e.m.): prefrontal cortex 27.6 ± 1.3 ($n=38$), ventral hippocampus 33.6 ± 3.1 ($n=19$), DR 33.3 ± 4.8 ($n=16$). The vehicle had no effect on extracellular 5-HT in the prefrontal cortex and DR but it significantly raised extracellular 5-HT (by 33%) in the ventral hippocampus 30 min after injection. Overall, flibanserin significantly reduced extracellular 5-HT in the prefrontal cortex ($F_{2,16}=4.4$, $P<0.03$) and DR ($F_{8,64}=2.3$, $P<0.03$), but not in the hippocampus ($F_{8,60}=1.9$, $P>0.06$). In all, 3 and 10 mg kg^{-1} flibanserin significantly reduced extracellular 5-HT in the prefrontal cortex, by respectively, 30 and 45% (Figure 1). The reduction was maximal at 60 min and lasted to 120 min after both doses. The effect of flibanserin on extracellular 5-HT in the DR was similar to that on the prefrontal cortex: 3 and 10 mg kg^{-1} reduced extracellular 5-HT by, respectively, 35 and 44% (Figure 1). However, the effect was short-lasting, being significant 30 and 60 min after 10 mg kg^{-1} and only 30 min after 3 mg kg^{-1}.

Effect of flibanserin on extracellular DA and NA in the prefrontal cortex

Basal concentrations of extracellular NA and DA (fmol 30 µl^{-1}) in the prefrontal cortex in the presence of 1 µM

citalopram in the perfusion medium (mean ± s.e.m.) were, respectively, 13.8 ± 0.7 ($n = 35$) and 13.4 ± 0.9 ($n = 36$). This concentration of citalopram had no significant effects on extracellular DA and NA (Pozzi *et al*., 1999 and unpublished results). The vehicle had no effect on extracellular DA and NA in the prefrontal cortex.

As shown in Figure 2, 10 mg kg^{-1} flibanserin significantly increased extracellular DA (63%) and NA (50%) in the prefrontal cortex (F values for DA and NA were $F_{8,56} = 3.2$, $P < 0.005$ and $F_{2,13} = 9.7$, $P < 0.003$). The increases of extracellular DA and NA were significant from 30 to 90 min after injection. The lower dose of flibanserin significantly raised extracellular NA, by 47%, but it had no effect on extracellular DA (Figure 2).

Effect of WAY100,635 on flibanserin-induced changes of extracellular 5-HT, DA and NA in the prefrontal cortex

In rats given saline, 10 mg kg^{-1} flibanserin reduced extracellular 5-HT by 47% at 60 min (Figure 3). Pretreatment with

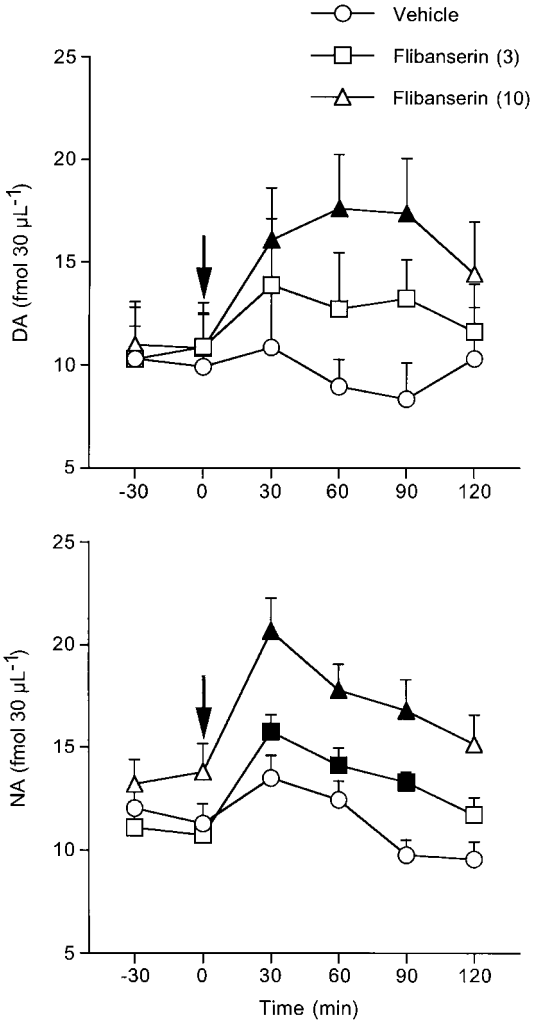

Figure 2 Extracellular DA and NA in the prefrontal cortex (PFC) of rats given 3 and 10 mg kg^{-1} flibanserin or vehicle intraperitoneally. Mean ± s.e.m. of 5–6 rats. The arrows indicate the time of drug or vehicle injection. Solid symbols indicate $P < 0.05$ vs basal values (Tukey–Kramer's test).

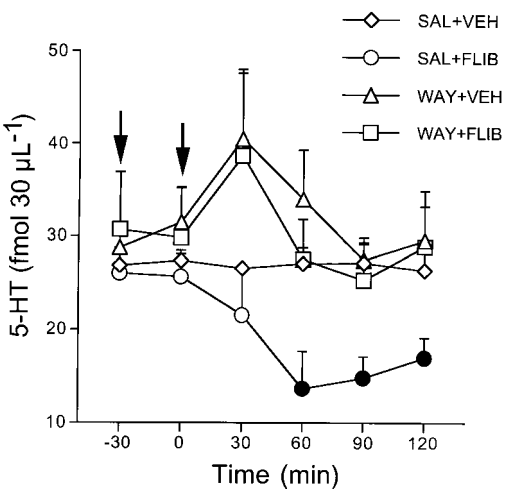

Figure 3 Effect of flibanserin alone and in combination with the selective 5-HT$_{1A}$ autoreceptor antagonist WAY100,635 on extracellular 5-HT in the prefrontal cortex. Rats were pretreated subcutaneously with saline (SAL) or 0.3 mg kg^{-1} WAY100,635 (WAY; first arrow). After 30 min, they received 10 mg kg^{-1} flibanserin (FLIB) or vehicle (VEH) intraperitoneally (second arrow) and extracellular 5-HT was measured for 2 h. Mean ± s.e.m. of 3–6 rats. Solid symbols indicate $P < 0.05$ vs basal values (Tukey–Kramer's test).

0.3 mg kg^{-1} WAY100,635 completely prevented this reduction ($F_{5,50} = 2.6$, $P < 0.01$). As shown in Figure 4, 10 mg kg^{-1} flibanserin significantly increased extracellular DA (58%) and NA (60%) in the prefrontal cortex of rats pretreated with saline. WAY100,635 given 30 min before flibanserin prevented both these rises (DA, $F_{5,50} = 4.8$, $P < 0.001$, NA, $F_{5,55} = 3.1$, $P < 0.01$) (Figure 4). WAY100,635 by itself had no effects on extracellular 5-HT, DA and NA.

Discussion

The present study shows that flibanserin lowered extracellular 5-HT in the prefrontal cortex and dorsal raphe and raised extracellular DA and NA in the prefrontal cortex. The fact that the doses of flibanserin that affected extracellular monoamine concentrations are in the range of those active in chronic mild stress and bulbectomized rat models of depression (Borsini *et al*., 1997; D'Aquila *et al*., 1997), and in the ultrasonic vocalization model of anxiety (Podhorna & Brown, 2000), suggests that the drug's action on monoamines may contribute to these effects. In line with the short half-life of flibanserin (1–2 h) in rats (Borsini *et al*., 2002), changes in extracellular 5-HT, DA and NA peaked at 30–60 min and, except for cortical 5-HT, lasted less than 2 h.

The selective 5-HT$_{1A}$ receptor antagonist WAY100,635 (Forster *et al*., 1995) completely antagonized the effect of flibanserin on extracellular 5-HT in the prefrontal cortex. This finding is consistent with the drug's high affinity for 5-HT$_{1A}$ receptors (Borsini *et al*., 1995) and suggests that the reduction of cortical extracellular 5-HT by flibanserin depends on the stimulation of 5-HT$_{1A}$ receptors. 5-HT$_{1A}$ autoreceptors on 5-HT neurons of the raphe play a pivotal role in regulating the activity of 5-HT neurons, and in previous studies WAY100,635 antagonized the flibanserin-induced decrease of

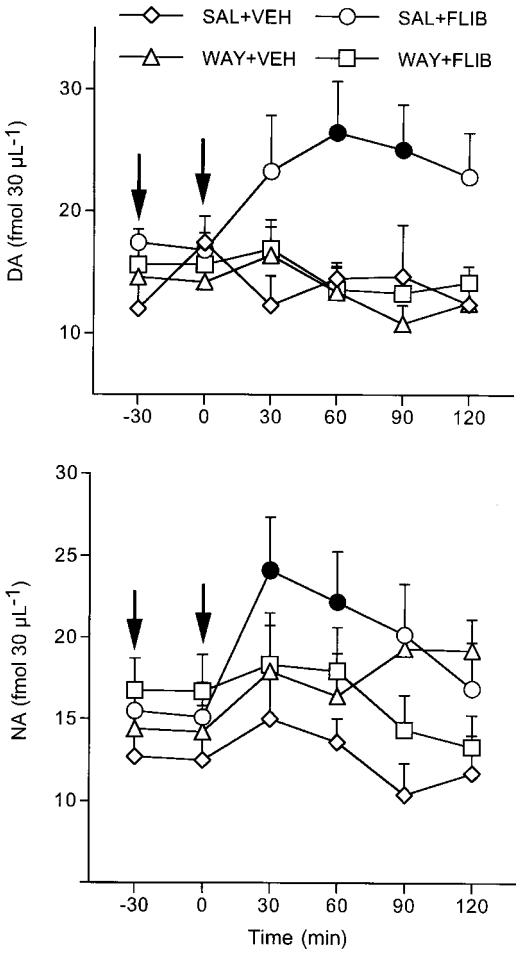

Figure 4 Effect of flibanserin alone and in combination with the selective 5-HT_{1A} autoreceptor antagonist WAY100,635 on extracellular DA and NA in the prefrontal cortex. Rats were injected subcutaneously with saline (SAL) or 0.3 mg kg^{-1} WAY100,635 (WAY; first arrow) and 30 min later received 10 mg kg^{-1} flibanserin (FLIB) or vehicle (VEH) intraperitoneally (second arrow); extracellular DA and NA were measured for 2 h. Mean ± s.e.m. of 2–6 rats. Solid symbols indicate $P<0.05$ vs basal values (Tukey–Kramer's test).

firing rate of 5-HT neurons of the DR (Rueter *et al*., 1998). These results suggest that 5-HT_{1A} autoreceptors of the DR are quite likely involved in the drug's effect on extracellular 5-HT in the prefrontal cortex. 5-HT_{1A} receptors are also present on postsynaptic elements in the prefrontal cortex (Pompeiano *et al*., 1992) and their stimulation may reduce the activity of serotonergic neurons of the DR and the release of 5-HT in the prefrontal cortex (Ceci *et al*., 1994; Hajos *et al*., 1999; Celada *et al*., 2001). Electrophysiological studies have shown that flibanserin inhibits the activity of cortical pyramidal neuron by stimulating 5-HT_{1A} receptors in the prefrontal cortex. The involvement of postsynaptic 5-HT_{1A} receptors is supported by the fact that the effect of flibanserin was anatgonized by WAY100,135 and tertatolol, two 5-HT_{1A} receptor antagonists, but not by the destruction of 5-HT containing neurons with the neurotoxin 5,7-dihydroxytryptamine (Borsini *et al*., 1995). Although in subsequent studies WAY100,635 failed to antagonize the inhibitory effect of flibanserin on the activity of cortical neurons (Rueter *et al*., 1998), a role of postsynaptic 5-HT_{1A} receptors in the effect of flibanserin on cortical extracellular 5-HT cannot be excluded.

Flibanserin at the two doses tested reduced extracellular 5-HT in the prefrontal cortex and DR of conscious rats but had no effect in the ventral hippocampus. In line with this finding, flibanserin was less effective in reducing 5-HT synthesis in the hippocampus than in the prefrontal cortex (Brambilla *et al*., 1999). These findings confirm the results of previous studies showing that selective 5-HT_{1A} receptor agonists such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) preferentially reduced extracellular 5-HT and 5-HT synthesis in the prefrontal cortex in respect to the hippocampus (Invernizzi *et al*., 1991, 1994, 1995; Casanovas & Artigas, 1996; Casanovas *et al*., 1997). It should be considered, however, that the stimulation of 5-HT_{1A} receptors with appropriate doses of the agonist reduced extracellular 5-HT in the ventral hippocampus (Sharp *et al*., 1989; Kreiss & Lucki, 1994). It is conceivable therefore that similar effects may have been observed had we administered flibanserin at higher doses.

The finding that WAY100,635 completely antagonized the flibanserin-induced increase of extracellular DA and NA in the prefrontal cortex strongly supports the role of 5-HT_{1A} receptors in the drug's effect on cortical catecholamines. Accordingly, microdialysis studies have consistently reported that selective 5-HT_{1A} receptor agonists raise extracellular concentrations of DA and NA in the prefrontal cortex (Arborelius *et al*., 1993b; Done & Sharp, 1994; Wedzony *et al*., 1996). 5-HT_{1A} receptor agonists-induced increase of extracellular DA may be secondary to the disinhibition of the activity of mesocortical dopaminergic neurons in the ventrotegmental area (VTA). In support of this hypothesis, such a mechanism has already been shown to account for the disinhibitory effect of 8-OH-DPAT on the firing activity of dopaminergic neurons of the VTA (Prisco *et al*., 1994). Consistently, depletion of endogenous 5-HT by p-chlorophenylalanine attenuated 8-OH-DPAT-induced increase of extracellular DA in the VTA (Chen & Reith, 1995). On the contrary, 5-HT depletion left unaltered the effect of 8-OH-DPAT on extracellular NA (Chen & Reith, 1995). The involvement of presynaptic mechanism in the effect of 5-HT_{1A} receptor agonists on extracellular DA was not confirmed in a subsequent study showing that the increase of cortical extracellular DA induced by MKC-242, a selective 5-HT_{1A} receptor agonist, was not prevented by the neurotoxic lesion of 5-HT containing neurons with 5,7-DHT (Sakaue *et al*., 2000). Therefore, the control exerted by pre- and postsynaptic 5-HT_{1A} receptors on DA and NA release is still controversial and further studies are needed to clarify the mechanism by which flibanserin increased extracellular catecholamines in the prefrontal cortex.

Although *in vitro* studies showing that flibanserin has higher affinity for 5-HT_{1A} receptors than 5-HT_{2A} receptors (Borsini *et al*., 1995) suggest a major role of 5-HT_{1A} receptors in the effect of flibanserin, *in vivo* flibanserin binds 5-HT_{1A} and 5-HT_{2A} receptors to a similar extent (Scandroglio *et al*., 2001). Thus, 5-HT_{2A} receptor blockade may be involved in the effects of flibanserin on cortical monoamines. We did not address this question in the present study, but it is unlikely that blockade of 5-HT_{2A} receptors by itself was responsible for flibanserin's effects on cortical monoamines since the selective blockade of these receptors with M100,907 has no effect on extracellular 5-HT, DA and NA in the prefrontal cortex (Gobert & Millan,

1999; Rollema *et al*., 2000; Ichikawa *et al*., 2001). It cannot be excluded, however, that the blockade of 5-HT_{2A} receptors may have contributed to flibanserin-induced increase of dopamine in the prefrontal cortex since microdialysis studies have shown that 5-HT_{1A} receptor stimulation and 5-HT_{2A} receptor blockade act synergistically to increase extracellular DA in the prefrontal cortex (Ichikawa *et al*., 2001).

5-HT_{2C} receptors exert an important tonic inhibitory control on dopaminergic and noradrenergic neurons innervating the prefrontal cortex and antagonists at these receptors consistently increase extracellular DA and NA in the prefrontal cortex (Gobert *et al*., 2000; Pozzi *et al*., 2002). However, because of the low affinity of flibanserin for 5-HT_{2C} receptors (Borsini *et al*., 2002), these receptors are unlikely involved in the effect of flibanserin on extracellular catecholamines.

In view of the high affinity of flibanserin for D_4 receptors, the interaction with these sites may contribute to some of the effects observed in the present study. *In vitro* studies in cloned cells found that flibanserin behaved as an antagonist or, albeit at higher concentrations, as an agonist or partial agonist at D_4 receptors (Borsini *et al*., 2002). Selective antagonists of D_4 receptors had no effect on extracellular NA and 5-HT (Broderick & Piercey, 1998; Millan *et al*., 1998) in the prefrontal cortex and, although there are reports that selective D_4 receptor antagonists raise extracellular DA in the prefrontal cortex (Millan *et al*., 1998; Broderick & Piercey, 1998), it has been argued that this occurs at doses higher than those believed to block D_4 receptors selectively (Millan *et al*., 1998). Taken together, these findings suggest that blockade of D_4 receptors is unlikely to have contributed to flibanserin-induced changes in extracellular monoamines in the prefrontal cortex.

In summary, the present results show that the stimulation of 5-HT_{1A} receptors plays a major role in the effect of flibanserin on extracellular 5-HT, DA and NA and suggest that these actions could constitute a basis for interpreting the drug's antidepressant-like effects.

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