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Venlafaxine: acute and chronic effects on 5-hydroxytryptamine levels in rat brain in vivo

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Abstract

Venlafaxine is a dual serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline uptake inhibitor which has been claimed to have an onset of antidepressant action which is faster than for other comparable drugs. The effects of venlafaxine on brain 5-HT levels in vivo have not yet been examined. Acute administration of venlafaxine to rats by i.p. injection resulted in dose-dependent increases in cortical and hippocampal 5-HT levels, as measured by in vivo microdialysis, over the range 5–20 mg/kg. The effect of venlafaxine (10 mg/kg i.p.) was potentiated by prior administration of pindolol (10 mg/kg s.c.) in hippocampus but not in frontal cortex. Daily administration of venlafaxine (5 mg/kg i.p.) for 4 weeks did not change basal 5-HT levels in either brain area. The effect of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, 0.2 mg/kg s.c.) to reduce 5-HT levels was unaffected by chronic venlafaxine at this dose, indicating that there was no change in sensitivity of presynaptic 5-HT_{1A} autoreceptors.

Keywords: Venlafaxine; Antidepressant; 5-HT (5-hydroxytryptamine, serotonin); Microdialysis; 5-HT_{1A} receptor; Cortex; Hippocampus

1. Introduction

Venlafaxine is a new antidepressant drug claimed to have superior clinical efficacy to comparable drugs due to a faster onset of action. Although marketed as a dual serotonin and noradrenaline uptake inhibitor, in vitro studies (Muth et al., 1986; Bolden-Watson and Richelson, 1993; Owens et al., 1997; Beique et al., 1998a) have shown the affinity of venlafaxine for the serotonin transporter to be greater than its affinity for the noradrenaline transporter by factors of three-fold, five-fold, 15-fold and 17-fold, respectively. Electrophysiological studies have also shown that the degree of inhibition of cell firing in the dorsal raphe nucleus produced by venlafaxine, as a result of activation of presynaptic somatodendritic 5-HT_{1A} autoreceptors on raphe nucleus serotonergic neurons, was not different from that produced by selective serotonin re-uptake inhibitors such as paroxetine (Gartside et al., 1997). Concomitant blockade of noradrenaline uptake, possibly leading to activation of stimulatory α_1 adrenoceptors on 5-HT (5-hydroxytryptamine) neurons in the raphe nucleus, did thus not offset the effects of activation of the inhibitory somatodendritic 5-HT_{1A} autoreceptors induced by 5-HT uptake blockade.

In vivo microdialysis has been used to determine the effects of antidepressant drugs on brain 5-HT levels after both acute and chronic administration. Acute administration of selective serotonin re-uptake inhibitors in general produced increases in 5-HT levels in terminal areas which were relatively smaller than those produced in somatodendritic areas, e.g., a single dose of 10 mg/kg fluvoxamine led to a two-fold increase in frontal cortex and a six-fold increase in raphe nucleus (Bel and Artigas, 1992; for review see Fuller, 1994). Administration of tricyclic antidepressant drugs with 5-HT uptake blocking properties, e.g., imipramine or clomipramine, produced large increases in terminal areas such as frontal cortex but only at high doses of 10 mg/kg or more (Bel and Artigas, 1996; Romero et al., 1996a). Chronic administration of these drugs, however, led to larger increases of 5-HT levels in terminal areas at low doses, e.g., in frontal cortex chronic fluvoxamine at 1 mg/kg induced a six-fold increase (Bel and Artigas, 1993) and chronic imipramine at 4 mg/kg a four-fold increase (Bel and Artigas, 1996). The drug duloxetine, a structural analog of fluoxetine, which has comparable affinities for the noradrenaline and 5-HT trans-

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porters in vitro but preferentially blocks 5-HT uptake in vivo (Kasamo et al., 1996), produced a rise in 5-HT levels in vivo reaching 340% of baseline after a single injection at 15 mg/kg i.p. (Engleman et al., 1995). After chronic administration of duloxetine at 6.25 mg/kg p.o., basal 5-HT levels in frontal cortex were unaltered, but the effect of a further injection of duloxetine to increase 5-HT levels was augmented (Kihara and Ikeda, 1995).

Co-administration of 5-HT_{1A} autoreceptor antagonists has been shown in many studies to potentiate the increases in 5-HT levels seen in terminal areas after administration of a 5-HT uptake blocking drug. This effect is analogous to the desensitization of the 5-HT_{1A} autoreceptors in the raphe nuclei produced by chronic administration of selective serotonin re-uptake inhibitors (Blier and de Montigny, 1994), and is thought to underlie the therapeutic effect of the 5-HT_{1A} antagonist pindolol as an adjunct to therapy with selective serotonin re-uptake inhibitors (Artigas et al., 1994; Blier and Bergeron, 1995; Blier et al., 1997). There are however very few reports of the effects of co-administration of 5-HT_{1A} receptor antagonists with 5-HT uptake blocking drugs which are not selective serotonin re-uptake inhibitors. Although the tricyclic drug desipramine at 10 mg/kg produced a small increase in 5-HT levels in rat frontal cortex, this increase was not potentiated by the 5-HT_{1A} antagonist {*N*-[2-(4-(2-methoxyphenyl)-1-piperazinyl) ethyl]-*N*-(2-pyridyl) cyclohexane carboxamide · 3HCl} (WAY-100635) (Romero et al., 1996a). Maione et al. (1997) found that while imipramine alone at 5 mg/kg reduced 5-HT levels in prefrontal cortex, co-administration of 5 mg/kg pindolol with this dose of imipramine led to an increase in 5-HT levels. Pindolol at 10 mg/kg also potentiated the effects of milnacipran, a dual 5-HT and noradrenaline uptake blocker, on both 5-HT and noradrenaline levels in guinea-pig hypothalamus (Moret and Briley, 1997). Co-administration of the 5-HT_{1A} receptor antagonist (±)-1-(1*H*-indol-4-yloxy)-3-(cyclohexylamino)-2-propanol maleate (LY 206130) at 3 mg/kg, a dose at which it alone had no effect, with duloxetine at 4 mg/kg, a dose at which it alone also had no effect, similarly produced a five- to seven-fold increase in 5-HT levels in rat hypothalamus (Engleman et al., 1996).

In the present work, we have investigated using in vivo microdialysis the effects of single and repeated administration of venlafaxine, and of single administration of venlafaxine in combination with pindolol, on 5-HT levels in rat frontal cortex and hippocampus.

2. Materials and methods

2.1. Treatment of animals

Male albino rats (Sabra strain) were used in all experiments. The rats were housed in a temperature-controlled environment (24°C) with a regular 12 h light/dark cycle. For the chronic experiment, treatment periods were arranged so that an experiment involving one treated animal and one control animal was performed on each experimental day.

2.2. Implantation and perfusion of the microdialysis probe

Animals were anaesthetised with sodium pentobarbital (60 mg/kg i.p.) and mounted in a stereotaxic apparatus. Guides for dialysis probes (Bioanalytical Systems) were implanted into ventral hippocampus at posterior 5.8 mm from bregma, 4.5 mm lateral and 4.0 mm vertical, or into frontal cortex at anterior 2.8 mm from bregma, 2.5 mm lateral and 2.0 mm vertical. Rats were maintained under anesthesia for approximately 1 h, after which they were free-moving and had unlimited access to food and water. Dialysis probes (4 mm) were inserted into the guides towards the end of the period of anesthesia. The inlet of the probe was connected, through plastic tubing with an internal volume of 12 μl/m, to a 5 ml gas-tight syringe mounted on a microinfusion pump. The inlet and outlet tubing of the probe were mounted to a flexible cable running from the head of the rat to a liquid swivel, allowing the animal to rotate and rear without entangling the fluid tubing. The probes were perfused with Ringer's solution containing 3 mM $CaCl_2$, 4 mM KCl, 130 mM NaCl, pH 6.5, at 0.2 μl/min overnight. The following morning the flow rate was increased to 0.5 μl/min, and 30 min fractions collected. In the experiments involving chronic administration of venlafaxine, 10 μM citalopram was added to the Ringer's solution in order to enhance detectability of 5-HT, since one of the aims of these experiments was to determine whether chronic venlafaxine affected basal 5-HT levels. After each experiment, the dialysis probes were removed under anesthesia, sterilised in alcohol, and if still intact re-inserted into new animals. The animal procedures outlined above received the approval of the Institutional Animal Care and Use Committee of the Hebrew University Faculty of Medicine and Dental Medicine and Hadassah Medical Organization.

2.3. Determination of 5-HT levels

The concentrations of 5-HT were determined by a Bioanalytical systems (BAS) high performance liquid chromatography (HPLC) system. Samples were injected immediately after collection using a Rheodyne 9125 injector with a 5 μl injection loop. The mobile phase was made up of 90 mM sodium dihydrogen phosphate, 10 mM NaCl, 0.5 mM EDTA, 0.15 g/l sodium octyl sulphate and 10.5% acetonitrile, pH 3.9, and was delivered by the HPLC pump at 1.0 ml/min. The mobile phase was passed through a flow splitter and pumped through a 1×100 mm^2 C-18 ODS microbore reversed phase column (particle size 3 μm, BAS) at 0.1 ml/min. 5-HT content was analysed

with a LC-4C electrochemical detector (BAS) with a glassy carbon working electrode set at 550 mV vs. an Ag/AgCl reference electrode. Concentrations of serotonin were calculated by comparing peak levels from the microdialysis samples with those of external standards of known concentrations of serotonin. The detection limit was 0.5–1 fmol. The average of the first four baseline samples was taken as 100%.

2.4. Materials

Venlafaxine was a gift of Wyeth-Ayerst Research, Princeton, NJ, USA. 8-OH-DPAT, pindolol, 5-HT creatinine sulfate complex and sodium octyl sulfate were obtained from Sigma (St. Louis, MO, USA). Citalopram was a gift of H. Lundbeck, Copenhagen, Denmark. HPLC grade acetonitrile was from Frutarom Haifa, Israel. All other chemicals were of analytical grade and were obtained from Merck-Darmstadt, Germany.

2.5. Data analysis

5-HT levels expressed as percentages of the initial levels for each animal were analysed over the whole time course by two-way analysis of variance (ANOVA), with treatment as a 'between groups' variable and time (fraction number) as a 'within groups' variable, i.e., as a repeated measure.

3. Results

Fig. 1 shows the effects of acute i.p. administration of various doses of venlafaxine on extracellular 5-HT levels in frontal cortex. The three doses studied produced transient increases in 5-HT, with a peak at 90 min post-injection and returning to baseline by 3 h post-injection. The effect of venlafaxine was dose-dependent, reaching maximum levels of 155% of basal, 193% of basal and 364% of basal at 5, 10 and 20 mg/kg, respectively. Two-way ANOVA of these data showed a significant effect of time ($F[6,108] = 3.82$, $P = 0.0017$) and a significant interaction between time and dose ($F[18,108] = 2.08$, $P = 0.011$). Post hoc Newman–Keuls tests for the data of fraction 7, i.e., the fraction collected 90 min after injection of venlafaxine, showed that the effect of 20 mg/kg venlafaxine was significantly greater than the effects of saline ($P = 0.002$), 5 mg/kg venlafaxine ($P = 0.013$) or 10 mg/kg venlafaxine ($P = 0.02$).

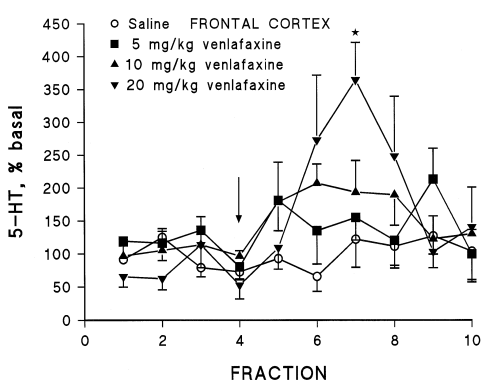

Fig. 1. Effects of varying doses of venlafaxine on 5-HT levels in frontal cortex. The arrow represents the point of injection of venlafaxine. Results are mean ± S.E.M. of observations from six animals treated with saline (– ∘ –), five animals treated with 5 mg/kg venlafaxine (– ■ –), seven animals treated with 10 mg/kg venlafaxine (– ▲ –), and four animals treated with 20 mg/kg venlafaxine (– ▼ –). Basal 5-HT levels in the four groups (mean ± S.E.M.) were: saline, 10.1 ± 4.5 fmol/5 μl; 5 mg/kg, 2.1 ± 0.9 fmol/5 μl; 10 mg/kg, 10.2 ± 6.5 fmol/5 μl; 20 mg/kg, 3.5 ± 2.4 fmol/5 μl. * Significantly different ($P < 0.02$) from other doses by Newman–Keuls post hoc test.

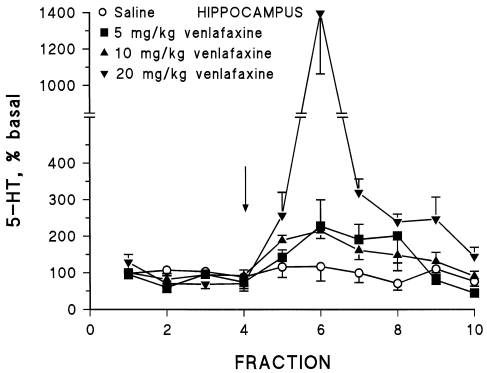

Fig. 2. Effects of varying doses of venlafaxine on 5-HT levels in hippocampus. The arrow represents the point of injection of venlafaxine. Results are mean ± S.E.M. of observations from five animals treated with saline (– ∘ –), four animals treated with 5 mg/kg venlafaxine (– ■ –), five animals treated with 10 mg/kg venlafaxine (– ▲ –), and four animals treated with 20 mg/kg venlafaxine (– ▼ –). Basal 5-HT levels in the four groups (mean ± S.E.M.) were: saline, 9.6 ± 3.2 fmol/5 μl; 5 mg/kg, 5.1 ± 1.4 fmol/5 μl; 10 mg/kg, 7.4 ± 2.7 fmol/5 μl; 20 mg/kg, 4.5 ± 1.8 fmol/5 μl.

Fig. 2 shows corresponding data for the hippocampus. The 5 and 10 mg/kg doses produced increases in 5-HT levels which peaked at approximately two-fold of baseline, while the maximum increase seen with the 20 mg/kg dose was 14-fold baseline. The effects of dose ($F[3,14] = 28.64$, $P = 0.000003$), time ($F[6,84] = 13.14$, $P < 0.000001$) and the interaction between dose and time ($F[18,84] = 7.90$, $P < 0.000001$) were all highly significant.

Figs. 3 and 4 show the effects of s.c. injection of 10 mg/kg pindolol followed 30 min later by i.p. injection of saline or venlafaxine at either 5 or 10 mg/kg, in frontal cortex and hippocampus, respectively. Although the maximum levels of 5-HT obtained with the combination of venlafaxine and pindolol in frontal cortex (Fig. 3; 248% of basal at both doses of venlafaxine) were slightly higher than those obtained with venlafaxine alone (Fig. 1), there was no overall significant effect of treatment, i.e., neither dose of venlafaxine in combination with pindolol significantly increased 5-HT levels above the levels obtained

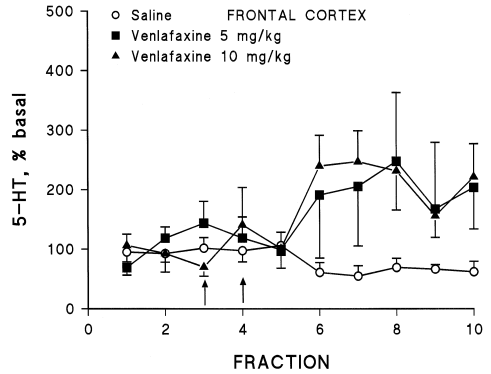

Fig. 3. Effects of pretreatment with pindolol (10 mg/kg s.c.) on 5-HT responses to saline, 5 mg/kg venlafaxine or 10 mg/kg venlafaxine in frontal cortex. Arrows represent points of injection of pindolol and saline/venlafaxine, respectively. Results are mean ± S.E.M. of observations from three animals treated with saline (basal levels 12.1 ± 0.6 fmol/5 μl), five animals treated with 5 mg/kg venlafaxine (basal levels 22.2 ± 7.3 fmol/5 μl), and five animals treated with 10 mg/kg venlafaxine (basal levels 3.0 ± 0.6 fmol/5 μl).

with pindolol and saline. In hippocampus, maximal effects seen with venlafaxine in the presence of pindolol reached 868% and 898% of basal for the 5 mg/kg dose and the 10 mg/kg dose, respectively. Comparison of the effects of 10 mg/kg venlafaxine and saline, both in the presence of pindolol, by two-way ANOVA showed a significant effect of treatment ($F[1,4] = 8.09$, $P = 0.046$), a significant effect of time ($F[6,24] = 3.30$, $P = 0.016$) and a significant interaction between time and treatment ($F[6,24] = 3.178$, $P = 0.019$). Comparison of the effects of 10 mg/kg venlafaxine alone (Fig. 2) with the effects of pindolol followed by 10 mg/kg venlafaxine in hippocampus (Fig. 4) by two-way ANOVA gave a significant main effect of treatment ($F[1,6] = 12.62$, $P = 0.012$), a significant effect of time ($F[6,36] = 7.61$, $P = 0.000025$) and a significant interaction between treatment and time ($F[6,36] = 4.48$, $P = 0.0017$), indicating that pindolol significantly potentiated the action of venlafaxine at this dose. The same comparisons in frontal cortex did not yield any significant effects.

Experiments on rats treated chronically with venlafaxine (5 mg/kg i.p. daily for 4 weeks) were performed on the day following the last injection, i.e., probes and guides were implanted immediately after the last injection and fractions collected the following day. The Ringer's solution used in these experiments contained 10 μM citalopram. Chronic venlafaxine did not affect basal levels of 5-HT in either frontal cortex or hippocampus. In cortex, basal 5-HT values in control (saline-injected) rats were 12.5 ± 2.1 fmol/5 μl fraction (mean ± S.E.M. of data from five animals), and in venlafaxine-treated rats 15.1 ± 4.6 fmol/5 μl fraction (mean ± S.E.M. of data from four animals). In hippocampus, basal 5-HT values in control rats were 10.2 ± 2.2 fmol/5 μl fraction, and in venlafaxine-treated rats 17.1 ± 3.8 fmol/5 μl fraction (mean ± S.E.M. of data from five animals in each case). Fig. 5 shows the effect of peripheral administration of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) on 5-HT levels in frontal cortex of saline- and venlafaxine-treated rats. 8-OH-DPAT induced a maximal 80% decrease in 5-HT levels which persisted for 60 min and only began to return to baseline 120 min after the injection. Two-way ANOVA showed a significant effect of time ($F[6,42] = 8.77$, $P = 0.000003$) but no effect of treatment ($F[1,7] = 0.16$, $P = 0.69$) and no interaction ($F[6,42] = 0.33$, $P = 0.91$). Fig. 6 shows the effect of 8-OH-DPAT in hippocampus, which was similar except that 5-HT levels decreased by only 60% and began to return to baseline almost immediately. Two-way ANOVA showed a significant effect of time

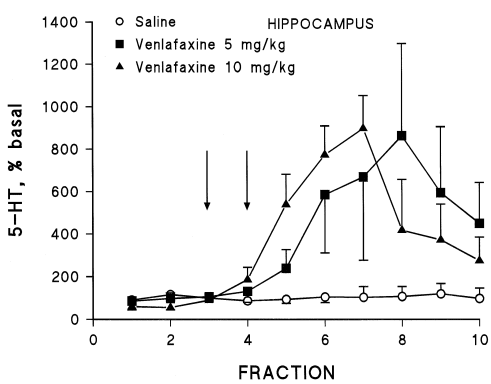

Fig. 4. Effects of pretreatment with pindolol (10 mg/kg s.c.) on 5-HT responses to saline, 5 mg/kg venlafaxine or 10 mg/kg venlafaxine in hippocampus. Arrows represent points of injection of pindolol and saline/venlafaxine, respectively. Results are mean ± S.E.M. of observations from three animals treated with saline (basal levels 17.6 ± 9.4 fmol/5 μl), six animals treated with 5 mg/kg venlafaxine (basal levels 1.9 ± 0.7 fmol/5 μl), and three animals treated with 10 mg/kg venlafaxine (basal levels 2.7 ± 0.9 fmol/5 μl).

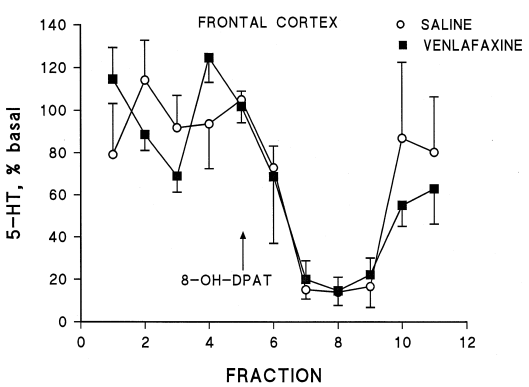

Fig. 5. Effect of systemic 8-OH-DPAT (0.2 mg/kg s.c.) on 5-HT levels in frontal cortex. Results are means ± S.E.M. of observations from five animals treated with saline and four animals treated chronically with venlafaxine (5 mg/kg i.p. daily for 28 days). Measurements were performed on the day following the last venlafaxine injection. Basal levels were 12.5 ± 2.1 fmol/5 μl fraction in saline-treated rats, and 15.1 ± 4.6 fmol/5 μl fraction in venlafaxine-treated rats.

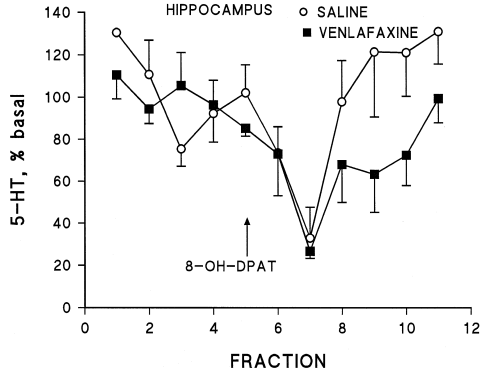

Fig. 6. Effect of systemic 8-OH-DPAT (0.2 mg/kg s.c.) on 5-HT levels in hippocampus. Results are means ± S.E.M. of observations from five animals treated with saline and four animals treated chronically with venlafaxine (5 mg/kg i.p. daily for 28 days). Measurements were performed on the day following the last venlafaxine injection. Basal levels were 10.2 ± 2.2 fmol/5 μl fraction in control rats, and 17.1 ± 3.8 fmol/5 μl fraction in venlafaxine-treated rats.

($F[6,48] = 7.06$, $P = 0.00002$) but no effect of treatment ($F[1,8] = 2.76$, $P = 0.13$) and no interaction ($F[6,48] = 1.29$, $P = 0.28$).

4. Discussion

The effects of acute venlafaxine on 5-HT levels demonstrated in the present study are in keeping with other studies in which dose-responses to 5-HT uptake blocking drugs were examined in vivo. Bel and Artigas (1996) obtained a maximal increase to 390% of basal levels in frontal cortex after injection of imipramine at a dose of 20 mg/kg, while Maione et al. (1997) with the same drug observed an increase to 380% of basal at a dose of 10 mg/kg. The dual uptake blockers milnacipran and duloxetine similarly produced dose-dependent elevations in 5-HT levels, with maximal values of 450% of basal and 340% of basal, respectively, at the highest doses used (Engleman et al., 1995; Moret and Briley, 1996). Malagie et al. (1995), using fluoxetine, found maximal 5-HT accumulations of 259% of basal and 267% of basal in frontal cortex and ventral hippocampus, respectively, at a dose of 20 mg/kg, although at lower doses the effects in ventral hippocampus were greater in magnitude and faster in onset compared to those in cortex. A similar relationship between the two brain areas was observed by Invernizzi et al. (1997) using citalopram. The present results with venlafaxine differ from these observations only in that the peak accumulation of 5-HT in hippocampus was considerably greater than in cortex. This may be due both to the higher density of 5-HT uptake sites in the hippocampus compared to the frontal cortex (Hrdina et al., 1990), and to the greater density of the inhibitory 5-HT_{1A} autoreceptors in the dorsal raphe, which innervates the frontal cortex, compared to the median raphe, which innervates the hippocampus. It would appear that noradrenaline uptake blocking properties of some of these drugs, including venlafaxine, do not interfere with their ability to increase extracellular 5-HT in nerve terminal areas.

In the present work a 10 mg/kg injection of pindolol potentiated the effect of a subsequent injection of 10 mg/kg venlafaxine to raise 5-HT levels in hippocampus but not in frontal cortex. The maximal increase produced by venlafaxine alone in hippocampus was 215% of basal, while after pindolol a nine-fold increase was obtained. These results are in broad agreement with previous observations made with selective serotonin re-uptake inhibiting drugs. Dreshfield et al. (1996) observed that while a 10 mg/kg dose of fluoxetine given alone increased 5-HT levels in rat hypothalamus to 228% of basal, in rats given a subsequent 5 mg/kg dose of pindolol, the maximum level reached was 458% of basal. Hjorth (1996) obtained a maximal rise of 5-HT to 216% of basal in rat ventral hippocampus after 5 mg/kg citalopram, and to 333% of basal after subsequent administration of 8 mg/kg pindolol. In two other studies pindolol was administered before the selective serotonin re-uptake inhibiting drug. Romero et al. (1996b) observed increases to 320% of basal and 280% of basal in rat striatum after 3 mg/kg paroxetine and 1 mg/kg citalopram, respectively, while in the additional presence of 15 mg/kg pindolol 5-HT levels reached 540% of basal and 640% of basal, respectively. Moret and Briley (1997), using guinea-pig hypothalamus, observed a doubling of 5-HT levels after 10 mg/kg milnacipran, while in the additional presence of 10 mg/kg pindolol 5-HT levels reached 650% of basal. In the majority of these studies pindolol alone had no effect on 5-HT levels. The observations in the present study and that of Moret and Briley (1997) that pindolol is able to potentiate the effects of the mixed uptake inhibitors venlafaxine and milnacipran, as well as those of the selective serotonin re-uptake inhibitors, on 5-HT levels, suggest that pindolol could be used clinically to potentiate the therapeutic effects of these drugs too. In clinical studies, potentiative effects have been observed with selective serotonin re-uptake inhibitors but not with tricyclic antidepressants such as trimipramine (Blier et al., 1997). In animal studies too, pindolol potentiated the anti-immobility effects of selective serotonin re-uptake inhibitors in the mouse forced swimming test but was devoid of any activity with the mixed uptake inhibitor imipramine (Redrobe et al., 1996), while a positive effect was observed with venlafaxine (Redrobe et al., 1998).

The lack of a potentiating effect of pindolol on the increase in 5-HT levels induced by venlafaxine in frontal cortex can be explained in the light of recent observations suggesting that pindolol is a partial agonist at presynaptic 5-HT_{1A} autoreceptors, and not a pure antagonist as was originally thought. Clifford et al. (1998) recently showed a decrease in 5-HT levels, as would be expected with a

compound exerting agonist activity at 5-HT_{1A} autoreceptors, in rat frontal cortex after i.v. administration of pindolol at 0.8 or 4 mg/kg. A decrease in 5-HT levels after s.c. injection of 5 mg/kg pindolol was also shown by Dreshfield et al. (1996) in hypothalamus, a tissue similar to frontal cortex in that it receives its serotonergic innervation from the dorsal raphe nucleus which is rich in 5-HT_{1A} receptors. It is noteworthy that the majority of studies in which pindolol injected alone was found to increase basal 5-HT levels were performed in hippocampus, usually also in the presence of a 5-HT uptake blocker (Bosker et al., 1994; Assie and Koek, 1996; Gur et al., 1997), although in one study pindolol alone at 10 mg/kg s.c. induced an increase in 5-HT levels in prefrontal cortex (Maione et al., 1997). This appears to be due to the fact the 5-HT_{1B} autoreceptors are tonically active in hippocampus only, and that pindolol is an antagonist at the 5-HT_{1B} receptor while being a partial agonist at 5-HT_{1A} receptors (Newman-Tancredi et al., 1998).

Chronic venlafaxine did not elevate basal 5-HT levels in either cortex or hippocampus. These results contrast with those of Bel and Artigas (1993, 1996) with fluvoxamine and imipramine, respectively. In both these studies the authors used low doses of the drugs which when given acutely did not produce increases in 5-HT levels in nerve terminal areas. On chronic administration, however, four- to six-fold increases in 5-HT levels in frontal cortex were observed. In our experiments we selected for chronic administration a venlafaxine dose of 5 mg/kg, which produced only a small increase in 5-HT on acute administration. Contrary to expectations, there were no increases in basal levels after chronic administration of this dose. Increases in basal 5-HT levels in striatum and hippocampus after chronic fluoxetine, and in striatum only after chronic desipramine, were also observed by Kreiss and Lucki (1995), but in their experiments high doses of the drugs (15 mg/kg) were used. Increased 5-HT levels in hippocampus were also observed by Auerbach and Hjorth (1995) after chronic administration of 5 or 10 mg/kg citalopram, and in diencephalon and prefrontal cortex by Rutter et al. (1994) and Tanda et al. (1996), respectively, after chronic administration of 10 mg/kg fluoxetine. We have also shown increased 5-HT levels in frontal cortex but not hippocampus after chronic administration of 10 mg/kg clomipramine (Gur et al., 1999). A large number of other studies, however, have failed to show increases in basal 5-HT levels in terminal areas after chronic administration of 5-HT uptake blocking drugs, unless the measurements were performed less than 24 h after the last administration of the drug, in which case the effect is very likely due to the persistence of residual drug (Sleight et al., 1989; Hjorth and Auerbach, 1994; Bosker et al., 1995a,b; Invernizzi et al., 1995, 1996; Arborelius et al., 1996; Moret and Briley, 1996; Gundlah et al., 1997).

Administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT resulted in a decrease in 5-HT levels in both control (saline-treated) and venlafaxine-treated rats. As in a previous study (Gur et al., 1999), the decrease in 5-HT levels produced by systemic injection of 8-OH-DPAT was greater and longer-lasting in frontal cortex than in hippocampus. This is due to the greater density of 5-HT_{1A} receptors in the dorsal raphe nucleus which innervates the cortex, compared to the median raphe nucleus which innervates the hippocampus. A decrease in the degree of reduction of 5-HT levels produced by injection of 8-OH-DPAT, indicative of subsensitivity of the somatodendritic presynaptic 5-HT_{1A} autoreceptors, after chronic administration of fluoxetine was observed by Rutter et al. (1994) in diencephalon, and by Kreiss and Lucki (1995) in striatum and hippocampus, and also after chronic citalopram by Invernizzi et al. (1994) in cortex. Other studies using the 5-HT uptake blocking drugs fluvoxamine, citalopram, fluoxetine and clomipramine (Hjorth and Auerbach, 1994; Bosker et al., 1995b; Invernizzi et al., 1995; Gur et al., 1999) have however failed to show 5-HT autoreceptor subsensitivity by this method. The increase in basal 5-HT levels at terminal areas after chronic administration of these drugs has in general been ascribed to desensitization of both somatodendritic presynaptic 5-HT_{1A} autoreceptors and nerve terminal 5-HT_{1B} autoreceptors. Although 5-HT_{1B} receptor sensitivity was not measured in this study, the failure to find a change in basal 5-HT levels in either of the brain areas examined in the present work, together with the majority of the results quoted above, indicate that microdialysis experiments do not provide evidence for the autoreceptor subsensitivity mechanism proposed largely on the basis of electrophysiological experiments by Blier and de Montigny (1994).

While the present results do not shed any light on mechanisms possibly responsible for the apparently faster action of venlafaxine in treatment of clinical depression, they indicate that the in vivo properties of this drug are very similar to those of the selective serotonin re-uptake inhibitors, and that this similarity extends to potentiation of the action of the drugs on in vivo 5-HT levels by pindolol. Similar conclusions have been reached on the basis of in vitro and in vivo studies of neurotransmitter uptake, and electrophysiological studies (Gartside et al., 1997; Beique et al., 1998a,b). The fact that, at the dose and time period studied here, chronic administration of venlafaxine did not lead to a change in sensitivity of presynaptic 5-HT_{1A} autoreceptors does not detract from the hypothesis that it acts in a manner similar to the selective serotonin re-uptake inhibitors, since microdialysis studies with these drugs (Hjorth and Auerbach, 1994; Bosker et al., 1995b; Invernizzi et al., 1995, 1996; Arborelius et al., 1996; Moret and Briley, 1996; Gundlah et al., 1997) have also failed to show such subsensitivity. Further studies to determine whether such changes occur on administration of higher doses of venlafaxine over a shorter period, which would then explain its apparent fast onset of action, are in progress. Finally, although the effects of venlafaxine on

noradrenaline uptake or in vivo levels of noradrenaline were not measured in the present study, our findings, again in keeping with both in vivo and in vitro results of others (Gartside et al., 1997; Beique et al., 1998a,b) would suggest that the greater part of the antidepressant action of this drug is due to its effects on serotonergic neurons.

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