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Effects of chronically administered venlafaxine on 5-HT receptor activity in rat hippocampus and hypothalamus

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Abstract

The effects of chronic administration of the mixed serotonin [5-hydroxytryptamine (5-HT)]/norepinephrine re-uptake inhibitor venlafaxine (5 mg/kg daily by osmotic minipump for 28 days) on the sensitivity of somatodendritic 5-HT_{1A} autoreceptors on serotonergic neurons innervating the hypothalamus, and on 5-HT_{1B} autoreceptors in both hypothalamus and hippocampus, were determined using in vivo microdialysis in freely moving rats. Venlafaxine induced a reduction in sensitivity of 5-HT_{1B} autoreceptors in hypothalamus, but did not affect the sensitivity of 5-HT_{1A} autoreceptors, or of 5-HT_{1B} autoreceptors in hippocampus. The corticosterone and oxytocin responses to the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT, 0.05 or 0.2 mg/kg), a measure of postsynaptic 5-HT_{1A} receptor activity in the hypothalamus, were reduced in animals administered 5 or 10 mg/kg venlafaxine daily by intraperitoneal injection for 21 days. This desensitization of post-synaptic 5- HT_{1A} receptors in the hypothalamus may be a consequence of increased 5-HT levels induced by desensitization of the presynaptic 5-HT_{1B} receptors. These results taken together with those of previous studies suggest that the hypothalamus might be an important site of drug action, and that venlafaxine has an overall mechanism similar to that of selective serotonin re-uptake inhibitors.

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

1. Introduction

Venlafaxine is a relatively new antidepressant drug that is reported to have a faster onset of action than other comparable drugs and is also effective in treatment of refractory depression (De Montigny et al., 1999), with one report claiming superiority to paroxetine in treatment-resistant patients (Poirier and Boyer, 1999). The pharmacological effects of venlafaxine and its effects on physiology have recently been reviewed (Roseboom and Kalin, 2000). Venlafaxine inhibits the uptake of both norepinephrine and serotonin (5-hydroxytryptamine, 5-HT), with at least a 3-fold greater affinity in vitro for the serotonin uptake site (Bolden-Watson and Richelson, 1983; Muth et al., 1986; Beique et al., 1998a). In vivo electrophysiological studies (Beique et al., 1998b, 1999) have shown that while venlafaxine is more potent at serotonin neurons than at noradrenergic neurons, its potencies at inhibiting firing of dorsal raphe serotonergic neurons and locus coeruleus noradrenergic neurons were similar to those of the specific uptake inhibitors paroxetine and desipramine, respectively, despite the fact that the in vitro affinities of venlafaxine for the uptake sites were at least an order of magnitude lower than those of the specific drugs. These results suggest that mechanisms other than uptake inhibition may be involved in the action of venlafaxine.

There have been three reports of in vivo microdialysis studies in which the acute effects of venlafaxine on transmitter levels were determined. In a previous work from this laboratory, we (Gur et al., 1999a) showed that venlafaxine given acutely at doses of 5–20 mg/kg i.p. increased 5-HT levels in a dose-dependent manner in both frontal cortex and hippocampus, although the increases in the hippocampus were significantly greater than in the cortex. Dawson et al. (1999) tested frontal cortex only and showed that while venlafaxine over the dose range 3–50 mg/kg s.c. increased norepinephrine levels dose dependently up to a maximum of

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7.5-fold of basal, there was no effect at all on 5-HT levels. This finding was surprising considering the greater affinity of venlafaxine for the serotonin uptake site over the norepinephrine uptake site demonstrated both in vivo and in vitro. Hatanaka et al. (2000a,b), who also measured both 5-HT and norepinephrine release in frontal cortex, however, found dose-dependent increases in release of both substances, reaching a maximum of 4.5- to 5-fold at 30 mg/kg venlafaxine given intraperitoneally.

The synthesis and release of serotonin into the synaptic cleft is under the control of two types of serotonergic autoreceptors. Serotonin synthesis and cell firing of serotonergic neurones are primarily controlled by 5-HT_{1A} autoreceptors, which are situated somatodendritically in the raphe nuclei. Recent work has also suggested that activation of postsynaptic 5-HT_{1A} receptors on cortical, probably glutamatergic neurons, may also inhibit firing of serotonergic neurons (Hajos et al., 1999; Casanovas et al., 1999). Release of 5-HT into the synapse is mainly controlled by receptors of the second type, 5-HT_{1B} autoreceptors, which are found at nerve terminals and inhibit 5-HT release on activation. Desensitization of either or both of these autoreceptors has been demonstrated to occur after chronic administration to animals of antidepressant drugs, particularly the selective serotonin re-uptake inhibitors (Blier and De Montigny, 1999; Moret and Briley, 2000), and this process is thought to account for the delay normally observed until the therapeutic effect of these drugs in depressed patients becomes evident.

In the present work, we have examined the effects of chronic venlafaxine on the activity of those 5-HT_{1A} receptors that control 5-HT release in the hypothalamus by determining the degree of reduction of hypothalamic 5-HT levels after administration of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT). In a previous study from our laboratory (Gur et al., 1999a), we found that venlafaxine given daily at 5 mg/kg i.p. for 28 days did not affect the sensitivity of 5-HT_{1A} receptors as measured by the effects of 8-OH-DPAT (0.2 mg/kg) on 5-HT levels in either frontal cortex or hippocampus. In the present work, we have also determined the effect of chronic venlafaxine on the sensitivity of presynaptic 5-HT_{1B} receptors both in hippocampus and in hypothalamus. We have previously shown subsensitivity of these receptors in hypothalamus after administration of clomipramine for 4 weeks (Newman et al., 2000), in hippocampus after administration of fluoxetine for 7 days (Dremencov et al., 2000), and in frontal cortex after administration of transcranial magnetic stimulation (Gur et al., 2000). The activity of postsynaptic 5-HT_{1A} receptors in the hypothalamus was determined in another set of animals by measuring the corticosterone and oxytocin responses to 8-OH-DPAT. Previous work (Li et al., 1993, 1996, 1997) has shown subsensitivity of these responses after long-term administration of the selective serotonin re-uptake inhibitors fluoxetine and paroxetine, but not after the tricyclic norepinephrine uptake inhibitor desipramine (Li et al., 1993). The effects of 5-HT and norepinephrine re-uptake blockers such as venlafaxine on this measure of hypothalamic receptor sensitivity have not been determined.

2. Materials and methods

2.1. Treatment of animals

Male Albino rats (Sabra strain, derived from Sprague–Dawley strain) were used in all experiments. The rats were housed by treatment group in a temperature-controlled environment (24 °C) with a regular 12-h light/dark cycle. Food and water were freely available. Osmotic minipumps (Alzet, 2ML4) filled with either 0.9% saline or 25 mg/ml venlafaxine in 0.9% saline were implanted subcutaneously under anesthesia with a 17:3 mixture of ketamine (100 mg/ml) and xylazine (2%). This concentration of venlafaxine allowed delivery of the drug at 5 mg/kg/day for a period of 28 days, assuming the weight of the rat to be 300 g and given a flow rate of 2.5 μl/h. Implantation of minipumps was performed in a staggered manner so that a 3-day experimental period (1 day for implantation of guides and probes and 2 days for collection of fractions) was available for each pair of animals. Implantation of guides and probes was performed on the 28th day after insertion of minipumps, so that on the days when fractions were collected, the results would not be complicated by the presence of freshly delivered venlafaxine.

In the separate experiments, animals received intraperitoneal injections of either 0.9% saline, 5 mg/kg venlafaxine or 10 mg/kg venlafaxine daily for 21 days. After the last treatment, the animals were moved to individual cages. Twenty-four hours later, each animal received an injection of saline or 8-OH-DPAT at either 0.05 or 0.2 mg/kg s.c., and was decapitated 30 min later. Trunk blood was collected into tubes containing EDTA, which were centrifuged immediately and the plasma stored at −80 °C until assay for hormone levels. Daily injections rather than implantation of minipumps were performed to accustom the animals to handling and injection before they received the final injections of 8-OH-DPAT or saline. This procedure enabled measurement of stress-free basal corticosterone and oxytocin levels.

2.2. Implantation and perfusion of microdialysis probes

Animals were anaesthetised with a 17:3 mixture of ketamine (100 mg/ml) and xylazine (2%) and mounted in a stereotaxic apparatus. Guides for dialysis probes (CMA/12) were implanted into the ventral hippocampus at posterior 5.8 mm from bregma, 4.5 mm lateral and 4.0 mm vertical, and into the lateral hypothalamus at posterior 1.5 mm from bregma, 1.3 mm lateral and 7.0 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 for hippocampus, 2 mm for hypothalamus) were inserted into the guides towards the end of the period of anesthesia. The inlets of the probe were connected, through plastic tubing with an internal volume of 12 μl/m, to 1-ml gas-tight syringes 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 2.25 mM $CaCl_2$, 4 mM KCl, 147 mM NaCl and 10 μM citalopram, 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. 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. 5-HT receptor challenges

On the second experimental day for each animal, fractions from the hypothalamus were injected into high-performance liquid chromatography (HPLC) apparatus immediately after collection for measurement of 5-HT. Once stable baseline 5-HT levels had been obtained, usually after collecting four or five experimental samples, the 5-HT_{1A} receptor agonist 8-OH-DPAT (0.2 mg/kg) was injected subcutaneously. A further six fractions were then collected. On the following day, fractions from both brain areas were used for 5-HT determination. Once stable baseline 5-HT levels had been obtained, the $5\text{-HT}_{1B/1D}$ receptor antagonist (*N*-[4-methoxy-3-(4-methyl-1-piperizinyl)phenyl]-2′ -methyl-4′ -(5-methyl-1,2,4-oxadiazole-3-yl) [1,1′ -biphenyl]-carboxamide (GR 127935, 5 mg/kg) was injected subcutaneously and a further six fractions collected.

2.4. Determination of 5-HT levels

Concentrations of 5-HT were determined by a Bioanalytical systems (BAS) 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 5, 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 10-cm C-18 5 mm reversed phase column 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 5-HT were calculated by comparing peak levels from the microdialysis samples with those of external standards of known concentration of 5-HT. The detection limit was 0.5–1 fmol. The average of the first four baseline samples was taken as 100%.

2.5. Hormone assays

For the corticosterone radioimmunoassay, samples were unextracted plasma in which corticosterone-binding proteins had been denatured by boiling. The assay is based on procedures and antiserum (final dilution 1:11,200, 46% total binding) from ICN Biochemicals (Irvine, CA). The radioactive [^{3}H]corticosterone tracer was obtained from DuPont NEN Research Products (Boston, MA). The sensitivity was 0.02 ng per tube and the intra- and inter-assay variabilities were 4.5% and 11.9%, respectively.

For the oxytocin redioimmunoassay, plasma samples (1 ml) were extracted by mixing with 2 ml ice-cold acetone (Spectranalyzed, Fisher) and centrifuged at 2000 rpm, 4 °C for 30 min. The supernatants were then added to 5 ml cold petroleum ether and mixed immediately. After centrifugation at 2000 rpm, 4 °C for 15 min, the top layers were aspirated and discarded. The remaining solutions were dried by blowing air into the tubes at 4 °C. The dried extracts were then dissolved in 1 ml of assay buffer (0.05 M phosphate buffer, pH 7.4, containing 0.125% bovine serum albumin, 0.01% sodium azide, 1 mM EDTA) and used for the immunoassay. The assay is a double-antibody immunoassay. The plasma extract (20 or 200 μl) and oxytocin standard (0–1 ng) were incubated in triplicate with 0.1 ml of rabbit anti-oxytocin serum (1:50,000 dilution) in a total volume of 0.4 ml for 24 h at 4 °C. [^{125}I]oxytocin (DuPont NEN, 3000 cpm, 0.1 ml) was then added to the tubes and incubated for 72 h at 4 °C. To the tubes was then added 0.1 ml goat anti-rabbit γ-globulin (1:12.5 dilution), followed by 0.1 ml normal rabbit serum (1:120 dilution). After incubation for 24 h, the tubes were centrifuged at 15,000 × *g*, at 4 °C for 20 min. The supernatant was decanted, and the radioactivity in the pellet counted for 5 min by a Micromedic 4/200 plus counter and analysed from the standard curve using the RIA-AID computer program. The concentration of plasma oxytocin was calculated with a correction factor based on the recovery of the extraction. The sensitivity limit of this assay was 1 pg/tube and the intra and inter-assay variabilities 8.1% and 8.6%, respectively.

2.6. Materials

Venlafaxine was a gift of Wyeth-Ayerst Research, Princeton, NJ, USA. 8-OH-DPAT, 5-HT creatinine sulfate complex and sodium octyl sulfate were obtained from Sigma (St. Louis, MO, USA). GR 127935 was a gift of Glaxo Wellcome, Stevenage, UK. 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 form Merck-Darmstadt, Germany.

2.7. Data analysis

5-HT levels expressed as percentages of the initial levels for each animal were analysed over the time course for each challenge by two-way analysis of variance (ANOVA), with treatment as a "between groups" variable and time (fraction number) as a "within groups" variable, that is, as a repeated measure. Hormone levels were examined by two-way ANOVA with both treatment and challenges as "between groups" variables, since different animals were used for the different challenges.

3. Results

Basal 5-HT values in hypothalamus of venlafaxine-treated rats were 23.2 ± 7.0 fmol/5 μl (mean ± S.E.M. of 13 observations) and were not significantly different from those in saline-treated rats (23.1 ± 3.3 fmol/5 μl, mean ± S.E.M. of 12 observations). Fig. 1 shows the effect of 8-OH-DPAT (0.2 mg/kg s.c.) to reduce 5-HT levels in hypothalamus of these rats. There was no difference in the degree of reduction between saline-treated and venlafaxine-treated rats. Two-way ANOVA of the data from fractions 4 to 11, that is, after injection of 8-OH-DPAT, showed a highly significant effect of time ($F[7,70] = 30.46$, $P < 0.000001$), but no effect of treatment ($F[1,10] = 1.36$, $P = 0.27$) or interaction between time and treatment ($F[7,70] = 1.39$, $P = 0.22$).

Fig. 2 shows the effect of GR 127935 (5 mg/kg s.c.) to increase 5-HT levels in hypothalamus of the same animals. In saline-treated rats, 5-HT levels were elevated to approximately 3-fold of basal, while in venlafaxine-treated rats, the degree of stimulation did not exceed 150% of basal. Two-way ANOVA of the data from fractions 4 to 10, that is, after injection of GR 127935, showed a significant effect of time

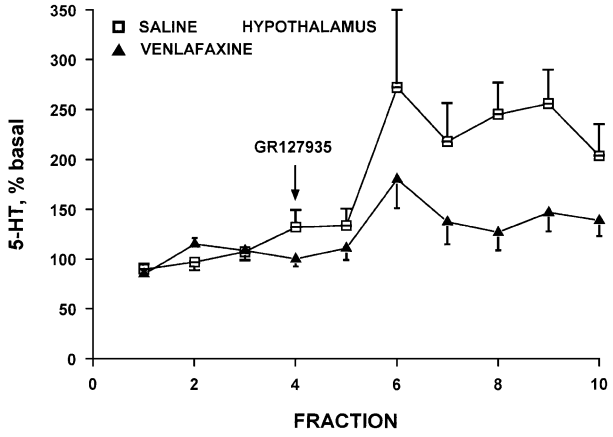

Fig. 2. Effect of GR 127935 (5 mg/kg s.c.) on 5-HT levels in hypothalamus. Results are mean ± S.E.M. of observations from seven animals treated with saline and five animals treated chronically with venlafaxine (5 mg/kg/day for 28 days via osmotic minipumps).

($F[6,60] = 3.24$, $P = 0.008$), a significant effect of treatment ($F[1,10] = 4.68$, $P = 0.05$) but no interaction between time and treatment ($F[6,60] = 0.67$, $P = 0.66$).

Basal 5-HT values in hippocampus of venlafaxine-treated rats were 24.5 ± 8.0 fmol/5 μl (mean ± S.E.M. of seven observations) and were not significantly different from those in saline-treated rats (17.5 ± 2.7 fmol/5 μl, mean ± S.E.M. of six observations). Fig. 3 shows the effect of GR 127935 (5 mg/kg s.c.) to increase 5-HT levels in hippocampus of these animals. As in hypothalamus, the degree of stimulation in saline-treated animals appeared to be greater than that in venlafaxine-treated animals, but in this case, the difference did not reach statistical significance. Two-way ANOVA of the data from fractions 4 to 10, that is, after injection of GR 127935, showed a significant effect of time

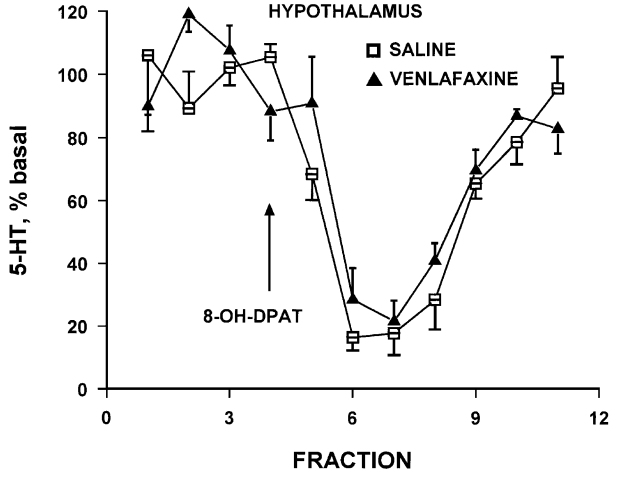

Fig. 1. Effect of 8-OH-DPAT (0.2 mg/kg s.c.) on 5-HT levels in hypothalamus. Results are mean ± S.E.M. of observations from six animals treated with saline and six animals treated chronically with venlafaxine (5 mg/kg/day for 28 days via osmotic minipumps).

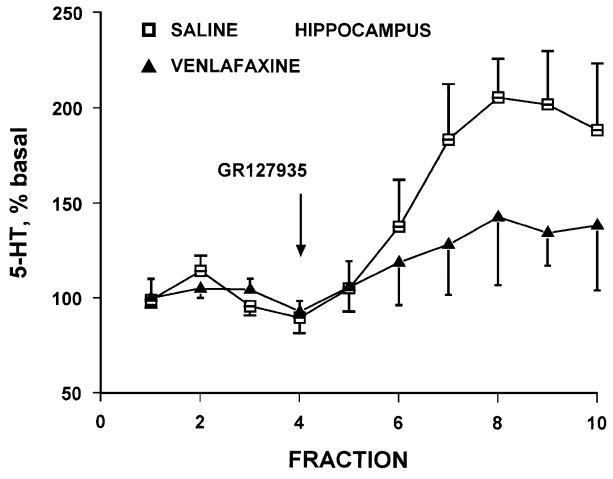

Fig. 3. Effect of GR 127935 (5 mg/kg s.c.) on 5-HT levels in hippocampus. Results are mean ± S.E.M. of observations from six animals treated with saline and six animals treated chronically with venlafaxine (5 mg/kg/day for 28 days via osmotic minipumps).

($F[6,60]=7.82$, $P=0.000003$), but no effect of treatment ($F[1,10]=1.40$, $P=0.26$) or interaction between time and treatment ($F[6,60]=1.83$, $P=0.1$).

Fig. 4 shows the effects of chronic administration of venlafaxine at doses of 5 and 10 mg/kg on the corticosterone and oxytocin responses to challenges with 8-OH-DPAT at doses of 0.05 or 0.2 mg/kg s.c. Both responses were reduced by venlafaxine treatment. Two-way ANOVA of the corticosterone data showed a significant main effect of challenge with 8-OH-DPAT ($F[2,65]=24.79$, $P<0.000001$) and a significant main effect of treatment with venlafaxine ($F[2,65]=3.28$, $P=0.044$) but no interaction between treatment and challenge ($F[4,65]=0.57$, $P=0.68$). Post hoc Newman–Keuls tests for challenge collapsed over treatment showed a significant effect of 0.05 mg/kg 8-OH-DPAT vs. zero concentration of 8-OH-DPAT ($P=0.033$), a significant effect of 0.2 mg/kg 8-OH-DPAT vs. zero concentration of 8-OH-DPAT ($P=0.0001$), and a significant effect of 0.2 mg/kg 8-OH-DPAT vs. 0.05 mg/kg 8-OH-DPAT ($P=0.0001$). When each group of rats was considered separately, significant effects of 0.2 mg/kg 8-OH-DPAT vs. zero concentration of 8-OH-DPAT or vs. 0.05 mg/kg 8-OH-DPAT were observed in all cases. Post hoc Newman–Keuls tests for treatment collapsed over challenge showed a significant difference between saline-treated rats and rats treated with 10 mg/kg venlafaxine ($P=0.033$).

For oxytocin, there was a significant main effect of 8-OH-DPAT challenge ($F[2,66]=10.37$, $P=0.00012$) but no main effect of venlafaxine treatment ($F[2,66]=2.06$, $P=0.135$) or interaction between treatment and challenge ($F[4,66]=1.71$, $P=0.16$). Post hoc Newman–Keuls tests for challenge collapsed over treatment showed a significant effect of 0.2 mg/kg 8-OH-DPAT vs. zero concentration of 8-OH-DPAT ($P=0.0003$) and vs. 0.05 mg/kg 8-OH-DPAT ($P=0.0003$) only. Analysis of each group of rats separately showed that this was due to the changes in the saline-treated rats only (0.2 mg/kg 8-OH-DPAT vs. zero concentration of 8-OH-DPAT, $P=0.00052$; 0.2 mg/kg 8-OH-DPAT vs. 0.05 mg/kg 8-OH-DPAT, $P=0.00054$). There was a significant difference between the effect of 0.2 mg/kg 8-OH-DPAT in saline-treated rats and in rats treated with 5 mg/kg venlafaxine ($P=0.043$), and also between the effect of 0.2 mg/kg 8-OH-DPAT in saline-treated rats and in rats treated with 10 mg/kg venlafaxine ($P=0.03$).

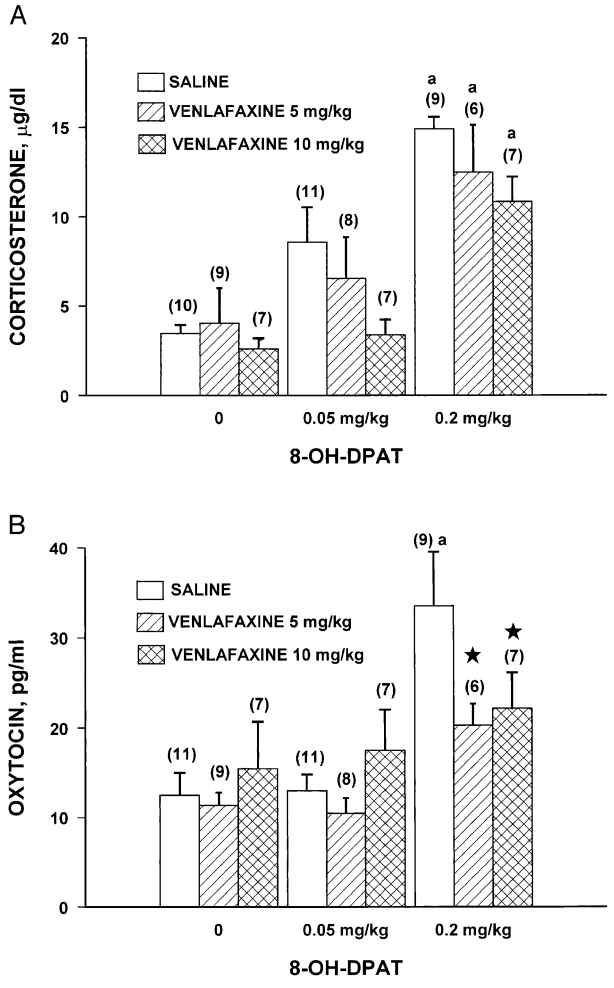

Fig. 4. Effect of 8-OH-DPAT (0.05 and 0.2 mg/kg s.c.) on secretion of (A) corticosterone, (B) oxytocin from animals treated chronically (daily for 21 days) with saline or venlafaxine injections. Results are mean ± S.E.M. of the number of animals given in parentheses above each column. [a]Significant ($P<0.05$) difference from animals with corresponding treatment injected with the zero dose of 8-OH-DPAT. * Significant difference ($P<0.05$) from saline-treated animals receiving 0.2 mg/kg 8-OH-DPAT.

4. Discussion

The effects of venlafaxine administered chronically to animals have been investigated in a few studies. Regarding its effects on the serotonergic system, Maj and Rogoz (1999) found that venlafaxine given at 10 mg/kg twice daily for 14 days did not affect the behavioural syndrome induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT, but reduced the head twitch reactions induced by the 5-HT precursor 5-hydroxytryptophan or by (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane (DOI) and the hyperthermia induced by trifluoromethylphenylpiperazine (TFMPP), all these reactions being mediated by 5-HT_2 receptors. In another study, the same dose regimen of venlafaxine reduced the density of 5-HT_{1A} receptors in the hippocampus but had no effect on 5-HT_2 receptor number in the cortex (Maj et al., 1999). McGrath and Norman (1998) found that venlafaxine given at 7.5 mg/kg once daily for 16 days had no effect on the hypothermic response to 8-OH-DPAT but reduced the density of 5-HT_2 receptors in the cortex. In our previous study (Gur et al., 1999a), we showed that venlafaxine at 5 mg/kg once daily for 4 weeks did not affect the sensitivity of those 5-HT_{1A} receptors that control 5-HT release, as shown by the effect of 8-OH-DPAT (0.2 mg/kg) to reduce 5-HT levels in either frontal cortex or hippo-

campus. Beique et al. (2000a,b) showed using an electrophysiological paradigm that there was no detectable desensitization of hippocampal 5-$HT_{1B/1D}$ receptors after 21 days of venlafaxine at 10 mg/kg, although desensitization was observed at a dose of 40 mg/kg. Finally, an action of chronic venlafaxine beyond the receptor level was reported by Rossby et al. (1999), who observed reduced phosphorylation of the cyclic AMP response-element binding protein (CREB-P) in nuclear lysates of cortex after administration of the drug at 20 mg/kg twice daily for 10 days.

The lack of effect of chronic venlafaxine on basal 5-HT levels in hypothalamus and on the effect of 8-OH-DPAT (0.2 mg/kg s.c.) to reduce 5-HT levels in this brain area observed in the present study is in keeping with our earlier results in cortex and hippocampus (Gur et al., 1999a), and indicates that venlafaxine at the dose given here does not alter the sensitivity of those 5-HT_{1A} receptors controlling 5-HT release, which in addition to their somatodendritic location in the raphe nuclei are also found as postsynaptic receptors in medial prefrontal cortex and the central nucleus of the amygdala (Bosker et al., 1997; Hajos et al., 1999; Casanovas et al., 1999). Although the concentration of 8-OH-DPAT employed in this experiment was high, a decrease in the reduction of 5-HT levels induced by this concentration of 8-OH-DPAT was observed in hypothalamus after administration to rats of chronic electroconvulsive shock (ECS, Gur et al., submitted for publication), considered to be the most effective antidepressant treatment. Kreiss and Lucki (1995) used an even higher concentration of 8-OH-DPAT (1 mg/kg) and found subsensitivity of the response in both hippocampus and striatum after chronic fluoxetine, and in hippocampus only after chronic desipramine, despite the fact that in their experiments, 8-OH-DPAT was administered intraperitoneally and was therefore subject to very rapid first-pass metabolism by the liver. Furthermore, Cremers et al. (2000a) have recently shown a reduction in the effect of 0.2 mg/kg 8-OH-DPAT administered subcutaneously to decrease 5-HT levels in rat ventral hippocampus after chronic administration of citalopram.

The lack of effect of chronic venlafaxine at 5 mg/kg on 5-HT_{1A} autoreceptor activity contrasts with the recent results of Beique et al. (2000a), who observed desensitization of somatodendritic 5-HT_{1A} autoreceptors as determined electrophysiologically after administration of venlafaxine for 21 days, albeit at a higher dose of 10 mg/kg. However, it is interesting that venlafaxine at this dose did not induce an enhanced tonic activation of postsynaptic 5-HT_{1A} receptors in the hippocampus, and such an effect was only observed at a dose of 40 mg/kg at which desensitization of presynaptic 5-HT_{1B} autoreceptors also occurred.

In the present experiments, the sensitivity of the 5-HT_{1B} receptors in hippocampus, as shown by the effect of the 5-$HT_{1B/1D}$ antagonist GR 127935 to increase 5-HT levels, was unchanged after chronic venlafaxine, while the sensitivity of these receptors in hypothalamus was reduced. A similar difference in the responsitivity of 5-HT_{1B} receptors in these two brain areas was observed in animals chronically treated with clomipramine (Gur et al., 1999b; Newman et al., 2000). Furthermore, our results corroborate the results of Beique et al. (2000a,b), who found no desensitization of 5-$HT_{1B/1D}$ receptors in hippocampus after chronic administration of venlafaxine at 10 mg/kg, although desensitization did occur at a higher dose of 40 mg/kg administered for 21 days.

The use of the antagonist GR 127935 to measure 5-HT_{1B} receptor sensitivity has been criticized on the grounds that it has not always been shown to increase 5-HT levels on peripheral administration (Skingle et al., 1995; Hervas and Artigas, 1998). However, Rollema et al. (1996) and Roberts et al. (1998, 1999) reported increases in guinea-pig hypothalamus and hippocampus, respectively, on peripheral administration of GR 127935 in the absence of any other agents. Other investigators have found that systemic GR 127935 only increased 5-HT levels when administered together with an uptake blocker, given either by injection or present in the perfusion medium (Gobert et al., 1997; Davidson and Stamford, 1997; Sharp et al., 1997; Dawson and Nguyen, 2000; Cremers et al., 2000b; Malagie et al., 2001). Since in the present experiments there was no difference in 5-HT_{1A} receptor activities between the two groups of animals, the reduction in the effect of GR 127935 in hypothalamus of venlafaxine-treated animals could have been due either to subsensitivity of the 5-HT_{1B} receptor or a change in 5-HT release or uptake. Since the experiments were performed in the presence of 10 μM citalopram, resulting in total blockade of the 5-HT transporter site, a change in sensitivity of 5-HT_{1B} receptors seems the most likely explanation. A similar interpretation of results with GR 127935 was given by Pineyro and Blier (1996), who found that the effect of GR 127935 to increase 5-HT release from midbrain slices was reduced in animals given chronic paroxetine or befloxatone, and concluded that 5-HT_{1D} receptors in the raphe had been desensitized by the drug treatment. Similarly, Fabre et al. (2000) showed an effect of GR 127935 to increase 5-HT in substantia nigra of normal mice, but not in knockout mice lacking the 5-HT transporter. This was interpreted as functional desensitization of 5-$HT_{1B/1D}$ receptors in the knockout mice, similar to that observed after chronic administration of selective serotonin re-uptake inhibitors. In knockout mice lacking 5-HT_{1B} receptors, the effect of acute paroxetine to increase 5-HT levels in ventral hippocampus was equivalent to the effect of combined administration of paroxetine and GR 127935 in normal mice, indicating that the reduced effect of paroxetine alone in normal mice was due to 5-HT_{1B} receptor-mediated inhibition, and that this inhibition could be alleviated by GR 127935 (Malagie et al., 2001).

It may appear surprising that, despite the desensitization of the 5-HT_{1B} autoreceptors in hypothalamus after chronic venlafaxine, basal 5-HT levels in this brain region were unchanged. In a recent study, Wikell et al. (2001) reported increased basal levels of 5-HT in neocortex after treatment

of rats with 10 mg/kg/day venlafaxine for 2 weeks, administered as in the present study by osmotic minipump. However, measurements in their study were performed with the minipumps in place and still active, while in the present study, measurements were performed on the 29th and 30th days after insertion of the pumps, so that their venlafaxine content would have been entirely discharged, and any changes observed would be due to adaptive processes and not to the presence of freshly delivered venlafaxine.

Another explanation for the lack of change in basal hypothalamic 5-HT levels probably relates to the fact that the perfusion fluid for both saline-treated and venlafaxine-treated animals in our experiments contained 10 μM citalopram. This addition has been found by us and other investigators to stabilize dialysate 5-HT levels, and yields results that more closely approximate 5-HT release rather than a balance between release and uptake. In a recent study, Hervas et al. (2001) observed similar results after chronic administration of 3 mg/kg fluoxetine for 2 weeks. When 5-HT was measured without a 5-HT uptake blocker in the medium, basal frontal cortical levels of 5-HT were higher in fluoxetine-treated than in vehicle-treated rats, while when measurements were performed in the additional presence of 1 μM citalopram in the perfusion fluid, this difference disappeared.

A role for presynaptic 5-$HT_{1B/1D}$ receptors in the pathogenesis of depression and its treatment was suggested by the work of Neumaier et al. (1996, 1997), who found increased levels of 5-HT_{1B} receptor mRNA in dorsal raphe of learned helpless rats, and decreased levels in rats treated with fluoxetine for 7 days. Since presynaptic 5-HT_{1B} autoreceptors are synthesized in the cell bodies of serotonergic neurons and are then transported to the nerve terminal areas, while post-synaptic 5-HT_{1B} receptors are synthesized in the cell bodies of non-serotonergic target neurons, these results imply that the presynaptic autoreceptors undergo specific changes in the learned helplessness model of depression and after antidepressant treatment. Previous work from our laboratory has shown a reduction in sensitivity of presynaptic 5-HT_{1B} receptors in rat hippocampus after subchronic administration of fluoxetine (Dremencov et al., 2000), and in rat cortex after chronic administration of transcranial magnetic stimulation, a novel form of antidepressant treatment that is often compared to ECS (Gur et al., 2000). Subsensitivity of hypothalamic 5-HT_{1B} autoreceptors has also been shown after chronic administration of the antidepressants paroxetine and desipramine (Sayer et al., 1999). Furthermore, in knock-out mice lacking the 5-HT transporter, which can be considered a model for whole-life treatment with a selective serotonin re-uptake inhibitor, the ability of 1 μM GR 127935 administered to the substantia nigra via the microdialysis probe to increase 5-HT levels was completely blunted compared to the response in wild-type mice (Fabre et al., 2000), suggesting that their 5-$HT_{1B/1D}$ autoreceptors were functionally desensitized. Animals, on the other hand, which were chronically administered corticosterone, as a model of the depressed state in human patients which is characterised by high cortisol levels, showed the reverse effect, an increase in presynaptic 5-HT_{1B} receptor sensitivity in the hypothalamus (Gur et al., 2001). The present work thus adds to a body of data which suggest that increased 5-HT_{1B} autoreceptor activity, leading to a reduction in synaptic 5-HT levels particularly in the hypothalamus, may be a characteristic of the depressed state, and that antidepressant drugs and treatments of various types may increase synaptic 5-HT levels by inducing subsensitivity of these receptors. Indeed, the central role of 5-HT_{1B} receptors in psychiatric disorders and their potential as targets for therapeutic intervention was highlighted in a recent review (Moret and Briley, 2000).

Administration of 8-OH-DPAT resulted in increases in plasma levels of both hormones measured, that is, corticosterone and oxytocin. However, the corticosterone response was dose dependent over the range of 8-OH-DPAT concentrations studied, while for oxytocin, only the higher dose of 8-OH-DPAT tested produced significant stimulation. The lack of a clear-cut dose response with oxytocin was almost certainly due to the 30-min interval between administration of 8-OH-DPAT and killing of the animals, which is the optimal time for observing changes in corticosterone but not for oxytocin, for which the optimal time is 15 min (Li et al., 1993).

The reduction in the corticosterone and oxytocin responses to 8-OH-DPAT after repeated injections of venlafaxine shows that, as for the selective serotonin re-uptake inhibitors fluoxetine and paroxetine (Li et al., 1993, 1996, 1997), chronic administration of this drug induced desensitization of post-synaptic 5-HT_{1A} receptors in the hypothalamus. The increased serotonergic tone at these receptors induced by desensitization of the presynaptic 5-HT_{1B} receptors in the hypothalamus may therefore result in an adaptive change at the post-synaptic level. These results contrast with those of Beique et al. (2000a), who found no desensitization of post-synaptic 5-HT_{1A} receptors in hippocampus after administration of venlafaxine at 10 mg/kg daily for 3 weeks. The most likely explanation for this discrepancy is a regional difference, since in recent experiments with chronic ECS (Gur et al., submitted for publication), we showed partial desensitization of post-synaptic 5-HT_{1A} receptor-mediated responses in the hypothalamus, while the response in hippocampus was unchanged. Indeed, regulation of post-synaptic 5-HT_{1A} receptor activity in the hippocampus seems to differ from that observed in other brain areas, since tricyclic antidepressant drugs appear to cause an increase in postsynaptic 5-HT_{1A} receptor-mediated activity in this area (Blier and De Montigny, 1999; Newman et al., 2000) despite their uptake-inhibiting activity that would be expected over time lead to a reduction in the response.

In summary, chronic treatment of animals with venlafaxine appears to induce adaptive changes particularly in the hypothalamus, at both pre-synaptic and post-synaptic sites. Overall, these changes are similar to those observed after

chronic treatment with selective serotonin re-uptake inhibitors. The hypothalamus may be an important site of action for antidepressant drugs.

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