Pharmacological actions of the antidepressant venlafaxine beyond aminergic receptors

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Abstract

The present study examines the effects of the antidepressant venlafaxine, a dual amine reuptake inhibitor, on (a) in vivo regulation of the densities of high- and low-affinity dihydroalprenolol (DHA) binding sites in the cortex of normal and reserpinized Sprague–Dawley rats and (b) targets beyond the beta adrenoceptor. While venlafaxine (30 mg/kg i.p. b.i.d.) administered for 4 d did not alter the DHA-binding parameters in the cortex of normal rats, it significantly reduced, in reserpinized animals, the number of up-regulated low-affinity sites ($R_L$) which have been tentatively identified as serotonin $B$ sites. The drug did not influence the up-regulated high-affinity ($R_H$) DHA-binding sites (beta-adrenoceptor sites). Venlafaxine failed to alter the up-regulated $R_L$ sites in brains of rats depleted of serotonin (5-HT) by p-chlorophenylalanine (PCPA) indicating that the normalization by venlafaxine of the up-regulated $R_L$ receptor population is mediated by increased synaptic 5-HT. Venlafaxine, given for a short period of time, thus mimicked the action of fluoxetine. While venlafaxine (20 mg/kg i.p. b.i.d.) given for 10 d did not change protein kinase A activity as assessed by the phosphorylation of kemptide in the 900 g supernatant or particulate fractions, the drug significantly reduced phosphorylated cAMP response-element binding protein (CREB-P) in nuclear lysates of cortex after chronic but not acute administration. Depletion of 5-HT by PCPA did not alter the venlafaxine-induced change in nuclear CREB-P. Lastly, analysis of reverse transcribed cortical CREB mRNA by competitive PCR indicated that the mean steady-state levels of CREB mRNA in venlafaxine vs. saline-treated animals were not significantly different. Therefore, since the phosphorylation status of CREB determines its transcriptional activity the reduction of nuclear CREB-P may be venlafaxine’s most relevant action beyond the adrenoceptor.

Introduction

Venlafaxine, an ethylcyclohexanol derivative, is a dual amine reuptake inhibitor (Bolden-Watson and Richelson, 1993; Muth et al., 1986) which has been demonstrated to be a clinically effective antidepressant with perhaps a faster onset of therapeutic action when compared to tricyclic antidepressants and selective serotonin reuptake inhibitors (Clerc et al., 1996; Guelfi et al., 1996; Rudolph et al., 1998). Unlike antidepressants with a strong NE component (MAO inhibitors and secondary amines of tricyclics), venlafaxine does not desensitize the beta-adrenoceptor-coupled adenylate cyclase system to isoproterenol in the pineal gland (Moyer et al., 1984; Muth et al., 1991) which is richly innervated by noradrenergic neurons but lacks serotonergic neuronal innervation (Wurtman et al., 1968). These findings have suggested that desensitization of the beta-adrenoceptor-coupled adenylate cyclase system by antidepressants involves ‘cross-talk’ between serotonin (5-HT) and norepinephrine (NE) signal transduction cascades (Nalepa et al., 1998) and perhaps direct action(s) beyond the beta adrenoceptor. In support of this hypothesis, a receptor-independent pharmacological action was demonstrated in vivo for the noradrenergic antidepressant desipramine (DMI) at the level of hippocampal glucocorticoid II receptor mRNA (Eiring and Sulser, 1997; Rossby et al., 1995) and in vitro in transfected LTK$^-$ or neuroblastoma cells (Pepin et al., 1992) and L-929 mouse fibroblasts (Pariante et al., 1997).

The present studies were undertaken (1) to determine the relative in vivo actions of venlafaxine in regulating the beta-adrenoceptor-coupled adenylate cyclase system to isoproterenol in the pineal gland (Moyer et al., 1984; Muth et al., 1991) which is richly innervated by noradrenergic neurons but lacks serotonergic neuronal innervation (Wurtman et al., 1968). These findings have suggested that desensitization of the beta-adrenoceptor-coupled adenylate cyclase system by antidepressants involves ‘cross-talk’ between serotonin (5-HT) and norepinephrine (NE) signal transduction cascades (Nalepa et al., 1998) and perhaps direct action(s) beyond the beta adrenoceptor. In support of this hypothesis, a receptor-independent pharmacological action was demonstrated in vivo for the noradrenergic antidepressant desipramine (DMI) at the level of hippocampal glucocorticoid II receptor mRNA (Eiring and Sulser, 1997; Rossby et al., 1995) and in vitro in transfected LTK$^-$ or neuroblastoma cells (Pepin et al., 1992) and L-929 mouse fibroblasts (Pariante et al., 1997).
density of cortical high (beta adrenoceptors) and low-
affinity (5-HT1B) dihydralpranolol (DHA) binding sites
which are selectively sensitive to the synaptic availability
of NE and 5-HT, respectively (Manier et al., 1989), and (2)
to ascertain venlafaxine’s actions on targets beyond the
beta adrenoceptor.

Materials and methods

Animals, drug treatment, materials

The experiments reported with Sprague–Dawley rats
were conducted in accordance with accepted guidelines
and were approved by the Vanderbilt University Animal
Care Committee. Male Sprague–Dawley rats (250–300 g)
were obtained from Harlan Industries (Indianapolis, IN).
They were housed (4–6 per cage) with a controlled
light–dark cycle. Food and water were available ad
libitum. Rats were treated for 10 d with venlafaxine
(20 mg/kg i.p. b.i.d.) Control rats received saline twice
daily. Twenty-four hours following the last injection the
rats were sedated with a 50/50 mixture of CO2/O2 for
1 min and killed by decapitation. The brains were rapidly
removed, dissected on ice, frozen in liquid nitrogen, and
stored at −70 °C until analysed.

In the studies using the tryptophan hydroxylase
inhibitor dl,L-p-chlorophenylalanine (PCPA), rats received
PCPA (200 mg/kg i.p. per day) on days 1, 2, 3, 5, 7, 9, 11
and 13 of the drug treatment period. Beginning on day 4
half of the PCPA group was treated with venlafaxine
(20 mg/kg i.p. b.i.d.) and the other half was injected with
saline for 10 d. The venlafaxine control group was treated
with saline on days 1, 2 and 3 and beginning on day 4
received venlafaxine (20 mg/kg b.i.d.) to day 13.

Reserpine was given in a dose of 1 mg/kg i.p. every
third day for 14 d, at which time venlafaxine (30 mg/kg
i.p. b.i.d.) was administered for 4 d.

Materials and drugs were obtained as follows: PCPA
(dl,L-p-chlorophenylalanine methyl ester), desipramine HCl
and reserpine from Sigma Chemical Co., St Louis, MO;
[3H]DHA (specific activity 55 Ci/mmol) from NEN Re-
search Products (Boston, MA), fluoxetine HCl was a gift
from Lilly Research Laboratories (Indianapolis, IN), and
venlafaxine HCl was generously provided by Wyeth-
Ayest Research (Princeton, NJ). Polyclonal antibodies for
CREB and CREP-P were obtained from Upstate Bio-
technology (Lake Placid, NY). CREB cDNA was a gift
from Dr Stephen Hyman, NIMH.

Nonlinear regression analysis of agonist-competition-
binding curves

Competition binding curves were constructed as pre-
viously described (Manier et al., 1989) using a con-
centration of 3 nM [3H]DHA and the agonist isoproterenol
over a concentration range of 0.001–100 µM. The
agonist-competition-binding curves were subjected to
nonlinear regression analysis using the program LIGAND
(Munson and Rodbard, 1980).

Assay of protein kinase A (PKA) activity

PKA activity was determined essentially according to the
protocol supplied by Gibco–BRL (Grand Island, NY).
Tissue or cells were disrupted with a glass homogenizer in
1 ml of homogenization buffer (0.32 M sucrose; 10 mM
KH2PO4; 5 mM MgCl2; 150 mM NaCl; pH 6.5). Supernatant
and particulate fractions were separated by centrifugation
at 900 g for 10 min. Four assay conditions
were used: (1) total PKA activity (plus 100 µM cyclic
AMP) with and without PKA inhibitor, and (2) endo-
genous activity (no cyclic AMP added) with and without
PKA inhibitor. Total incubation volume was 80 µl in Tris-
MgCl2 buffer. After incubation for 5 min at 30 °C, 20 µl
aliquots were spotted onto phosphocellulose paper
(2 × 2 cm, Whatman P81). The filter squares were washed
twice for 5 min in 75 mM H3PO4 and twice for 5 min in
H2O and then counted by liquid scintillation spectro-
metry. PKA activity was defined as the transfer of PO4
from ATP (100 µM, 32P-tracer 300 cpm/pmool) to the
heptapeptide Kemptide (50 µM) and normalized per units
protein and time. Proteins were quantitated according to
Lowry et al. (1951).

Preparation of nuclear extracts

Nuclear extracts were prepared according to the method
of Wuarin and Shibler (1990). Briefly, assay material was
homogenized in 5% citric acid in a glass Teflon homogen-
izer. After centrifugation (1500 g, 5 min) the resulting
pellet was resuspended in 5% citric acid containing 0.5%
Triton X-100, and homogenized in a Dounce homo-
genizer. Nuclei were centrifuged through a sucrose
 cushion containing 5% citric acid and 30% sucrose at
1500 g for 5 min. The purified nuclei were resuspended in
1% citric acid and aliquots were taken for protein
determinations. An equal volume of lysis solution (0.1 M
Tris buffer, 2.5% SDS, 0.1 M DTT; pH 7.5) was added
and heated to 90 °C for 10 min. Nuclear lysates were
frozen at −80 °C for later assay or subjected to gel
electrophoresis.

Determination of nuclear CREB and CREB-P

Nuclear CREB and CREB-P were assayed by subjecting
nuclear lysates to gel electrophoresis followed by immu-
noblotting using polyclonal anti-CREB and anti-CREB-P
antibodies (Upstate Biotechnology, Lake Placid, NY)
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Figure 1. Demonstration of identical amplification efficiencies for T7 CREB and T7 CREB-INS plasmids. Three concentrations of the wild-type CREB-containing plasmid T7 CREB (10, 1, 0.1 pg) were each co-amplified with a serial dilution of the competitive standard T7 CREB-INS (100, 10, 1, 0.01 pg). Band densities were quantitated directly from the gel. Two bands corresponding to the expected sizes of the products from T7 CREB (604 bp) and T7 CREB-INS (704 bp) were confirmed. The intensities of the bands derived from T7 CREB-INS decreased while those derived from T7 CREB increased demonstrating competition for primers. At the T7 CREB-INS concentration which produced upper and lower bands of equal density (determined by linear regression analysis, see Materials and methods) the starting concentrations of the two cDNAs (before amplification by PCR) were taken to be equivalent (A, lane 2; B, lane 3, C, lane 4).

According to the supplier's protocol. Antibody–antigen complexes were detected by enhanced chemiluminescence followed by autoradiography and quantitated by laser densitometry.

Competitive PCR (cPCR) analysis of CREB mRNA

Total RNA was isolated and purified from cortex by the method of Chomczynski (1993) utilizing TRI reagent, and RNA quantity and purity were determined by spectrophotometry (Pharmacia Ultraspec III) and RNA gel electrophoresis.

Competitive PCR was performed with primer CREB-N1 of genomic polarity (5'-TTCAGTCTCCAAAAAGTCC-3') and primer CREB-2 of antigenomic polarity (5'-AGGCTGTGTAGGAAGTGC-3'). A competitive template (internal standard) was constructed from the rat T7 CREB plasmid (generously provided by Dr Steven E. Hyman) containing a 1.24 kb CREB coding sequence plus approx. 150 bp of 5' untranslated and 50 bp of 3' untranslated sequences cloned into pGEM β(+)3Zf(-). The plasmid was linearized with StuI which cuts at nt 432 of the CREB sequence producing blunt ends – followed by removal of 5' phosphates with calf intestinal phosphatase (CIP) to prevent self-ligation in the following step. Next the plasmid was recircularized by ligation of a 100 bp DNA insert (isolated from the BRL 100 bp ladder) in low melting temperature (LMT) agarose. The resultant subclone T7 CREB-INS has the same primer annealing sites as the parent clone T7 CREB; but is longer by 100 bp.

Synthesis of cDNA from total cortex RNA was performed in a volume of 20 µl containing 100 U MMLV reverse transcriptase, 0.5 mM dNTP, 5 µM oligo(dT₁₈), 1.5 µg RNA, 10 U placental RNase inhibitor, and 1 x RT transcription buffer. The cDNA product was amplified in a total volume of 40 µl containing 0.1 mM dNTP, 8 µM of each primer, 4 µl T7 CREBS-INS, 4 µl RT product, 0.4 µl Amplitaq and 1 x PCR buffer. The reaction was performed in a PTC Thermal Controller (MJ Research Inc., Watertown, Mass.) as follows: 94°C/20 s, 55°C/30 s, 72°C/40 s, for 25 cycles followed by 72°C/5 min (Ambion kit for RT, Qiagen for Taq polymerase kit). Three amounts of DNA from the wild-type CREB-containing plasmid [T7 CREB (10, 1, 0.1 pg)] were each co-amplified for 30 cycles with a standard series of decreasing amounts of the competitive template, T7 CREB-INS (Figure 1). The amplified reaction mixtures were analysed by electrophoresis in a 2.0% agarose gel containing ethidium bromide and visualized by UV fluorescence. Band densities were quantitated directly from the gel on a digital imaging system (Alpha Innotech Corporation, San Leandro, CA). The two bands corresponding to the expected sizes of the products from T7
CREB (604 bp) and T7 CREB-INS (704 bp) were confirmed. The intensities of the bands derived from T7 CREB decreased while those derived from T7 CREB-INS increased, also as expected, demonstrating competition for primers. At the point where the upper and lower band densities were equivalent, i.e. ratio = 1:1 (determined by linear regression analysis, see below), the known starting concentration (prior to PCR) of T7 CREB was found to be equal to the known starting concentration of the competitor T7 CREB-INS (Figure 1A, lane 2; Figure 1B, lane 3; and Figure 1C, lane 4).

Results

Effect of venlafaxine on the $R_H$ and $R_L$ population of DHA-labelled receptors in frontal cortex: nonlinear regression analysis of agonist-competition-binding curves

In agreement with previous studies (Manier et al., 1989), nonlinear regression analysis of agonist-competition-binding curves, using $[\text{H}]$DHA as the radioligand and (−)-isoproterenol as the agonist, demonstrated that in normal cortical tissue about 77% of beta adrenoceptors labelled by DHA, have high (nanomolar) affinity for isoproterenol ($R_H$) and the remaining 23% of the receptors ($R_L$) display low (micromolar) affinity for isoproterenol (Table 1). Chronic administration of reserpine caused a statistically significant increase in the number of both $R_H$ and $R_L$. While venlafaxine administered for 4 d (30 mg kg b.i.d.) did not change the number of the two sites labelled by DHA, it significantly reduced the number of $R_L$ in reserpine-treated animals to control values without altering the number of $R_H$. Venlafaxine did not alter the values of the dissociation constants $K_H$ or $K_L$.

In order to discover the mechanism of venlafaxine’s effect on $R_L$ in reserpine-treated animals we conducted a series of experiments in which the density of $R_L$ was increased by treatment with PCPA. In the virtual absence of 5-HT (our PCPA treatment schedule selectively depletes brain 5-HT by 95–97%; Nalepa et al., 1998; Rossby et al., 1995), venlafaxine (20 mg/kg i.p. b.i.d.) given for 10 d did not alter the number of up-regulated $R_L$ (Table 2), indicating that the normalization of the up-regulated $R_L$ receptor population by venlafaxine was mediated via 5-HT.

### Table 1. Selective regulation of $R_H$ and $R_L$ of dihydroalprenolol binding in cortex from reserpinized animals

<table>
<thead>
<tr>
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<th>$[\text{H}]$dihydroalprenolol binding (%)</th>
<th>$R_H$</th>
<th>$R_L$</th>
</tr>
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<tr>
<td>Saline</td>
<td>100 ± 3 (11)</td>
<td></td>
<td></td>
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<tr>
<td>Reserpine</td>
<td>128 ± 3 (12)**</td>
<td>100 ± 5 (11)</td>
<td></td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>99 ± 7 (4)</td>
<td>145 ± 6 (12)**</td>
<td>92 ± 13 (4)</td>
</tr>
<tr>
<td>Reserpine + venlafaxine</td>
<td>130 ± 3 (5)</td>
<td>107 ± 12 (5)*</td>
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Effect of venlafaxine on protein kinase A activity in the 900 g supernatant and particulate fractions of cortex

To ascertain whether venlafaxine alters signal transduction beyond the second messenger cyclic AMP, PKA activity was determined in the 900 g supernatant and particulate fractions. The data in Table 3 indicate that venlafaxine (20 mg/kg i.p. b.i.d. for 10 d) did not significantly alter cyclic AMP-stimulated PKA activity in either the 900 g supernatant or the particulate fraction.

### Table 2. Selective regulation of $R_H$ and $R_L$ of dihydroalprenolol binding in cortex from PCPA-treated animals

<table>
<thead>
<tr>
<th></th>
<th>$[\text{H}]$dihydroalprenolol binding (%)</th>
<th>$R_H$</th>
<th>$R_L$</th>
</tr>
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<tbody>
<tr>
<td>Saline</td>
<td>100 ± 9 (4)</td>
<td></td>
<td></td>
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<tr>
<td>Venlafaxine</td>
<td>86 ± 11 (4)</td>
<td>143 ± 42 (4)</td>
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</tr>
<tr>
<td>PCPA</td>
<td>92 ± 4 (5)</td>
<td>413 ± 47 (5)**</td>
<td></td>
</tr>
<tr>
<td>PCPA + venlafaxine</td>
<td>85 ± 10 (5)</td>
<td>426 ± 49 (5)**</td>
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Venlafaxine was administered daily for 10 d (20 mg/kg i.p. b.i.d.) and PCPA was given as described in Materials and methods. Nonlinear regression analysis of agonist-competition-binding curves was accomplished as described in Materials and methods. $R_H$ and $R_L$ designate the binding sites with high (nm) and low (µm) agonist affinity (see Table 1). Control values: $K_H$, 57.4 ± 5.2 fmol/mg protein; $K_L$, 16.4 ± 2.7 fmol/mg protein ($n = 4$). The numbers in parentheses indicate the number of animals.

* $p < 0.01$ (vs. reserpinized); ** $p < 0.001$ (vs. saline).
Table 3. Effect of venlafaxine on PKA activity in frontal cortex

<table>
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<th></th>
<th>Supernatant</th>
<th>Particulate</th>
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<tr>
<td></td>
<td>Endogenous</td>
<td>+cAMP</td>
</tr>
<tr>
<td>Saline</td>
<td>905±60 (11)</td>
<td>2010±93 (11)</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>821±74 (11)</td>
<td>1918±31 (11)</td>
</tr>
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Rats were treated for 10 d with venlafaxine (20 mg/kg i.p. b.i.d.). Animals were killed 16 h after the last drug dose and PKA activity determined in the 900 g supernatant and particulate fractions in the presence or absence (endogenous) of 100 µM cyclic AMP as described in Materials and methods. The numbers in parentheses indicate the number of animals.

![Figure 2](image)

**Figure 2.** Effect of chronic administration of venlafaxine on nuclear CREB-P in the cortex. Male Sprague–Dawley rats were treated with venlafaxine (20 mg/kg i.p. b.i.d.) for 10 d. Nuclear CREB-P was determined in the cortex as described in Materials and methods. (A) Normal animals; (B) PCPA pretreated animals (see Materials and methods). SAL, saline; VEN, venlafaxine; PCPA, p-chlorophenylalanine. Numbers in parentheses indicate the number of animals.

There was also no indication of a translocation of PKA activity from the supernatant to the particulate fraction.

**Effect of venlafaxine on nuclear CREB phosphorylation in cortex**

Previous results have suggested that venlafaxine causes a deamplification of the cyclic AMP cascade by a mechanism beyond the beta adrenoceptor (Nalepa et al., 1998). Accordingly, we analysed nuclear CREB phosphorylation in the frontal cortex of rats treated with venlafaxine for 1 and 10 d (20 mg/kg i.p. b.i.d.). The results in Figure 2A show that treatment for 10 d significantly reduced CREB-P in nuclear lysates. Total CREB in nuclear lysates of frontal cortex was, however, not altered. Also, acute treatment with venlafaxine (1 d) did not change the phosphorylation status of CREB (data not shown).
Since venlafaxine exerts potent serotonergic activity (see previous results in this paper), the action of venlafaxine on nuclear CREB-P was determined in PCPA pre-treated animals. The results (Figure 2B) demonstrate that depletion of 5-HT did not alter the venlafaxine-induced change in nuclear CREB-P, suggesting that this action of the drug does not depend on the synaptic availability of 5-HT.

**Effect of venlafaxine on cortical CREB mRNA using cPCR**

Following the demonstration of identical amplification efficiencies for the T7 CREB and T7 CREB-INS plasmids, samples of cDNA reverse transcribed from cortical RNA isolated from saline- and drug-treated rats were co-amplified with a series of known concentrations of the competitive template T7 CREB-INS. The PCR products were subsequently electrophoresed and band densities determined as described (Figure 3).

Mean steady-state levels of CREB mRNA in rat cortex were almost identical in venlafaxine-treated ($n=7$) vs. saline-treated ($n=8$) animals as determined by a two-tailed Student’s $t$ test ($p=0.7580$).

**Discussion**

Studies with venlafaxine conducted in animals with selective depletion of brain 5-HT by PCPA have suggested cross-talk between norenergic and serotonergic signal transduction cascades at the level of mechanisms involved in the desensitization of the beta-adrenoceptor-coupled adenylate cyclase system (Nalepa et al., 1998). To determine the relative in vivo activity of venlafaxine in increasing the synaptic availability of 5-HT and NE in brain, the reserpine-induced up-regulation of high- and low-affinity sites for the beta-adrenoceptor antagonist DHA was utilized as a biochemical test model. Nonlinear regression analysis of agonist-competition-binding curves has revealed that DHA labels two sites, one with high (nanomolar) affinity for isoproterenol (beta adrenoceptors), and another with low (micromolar) affinity for isoproterenol tentatively identified as 5-HT$_{1B}$ sites (Gillespie et al., 1989). Both sites are up-regulated by reserpine and are very sensitive to the synaptic availability of NE and 5-HT, respectively (Manier et al., 1989).

In the present experiments, venlafaxine normalized the up-regulated 5-HT sensitive low-affinity sites ($R_{L}$) but failed to alter the up-regulated NE sensitive high-affinity sites ($R_{H}$) which in contrast are normalized by the NE reuptake inhibitor desipramine (Manier et al., 1989). Venlafaxine in the doses used thus mimics the pharmacological action of fluoxetine (Manier et al., 1989). The demonstrated lack of effect of venlafaxine on low-affinity sites in the rat brain depleted of 5-HT by PCPA provides evidence that this action of the drug is mediated by 5-HT. It cannot be ruled out, however, that larger doses of venlafaxine or venlafaxine administered for a longer period of time could have affected the NE-sensitive high-affinity beta-adrenoceptor sites. It has been hypothesized that the lack of beta-adrenoceptor down-regulation after chronic administration of venlafaxine might be the consequence of the drug’s activation of protein kinase C via the 5-HT$_{2A/2C}$ receptor cascade (Nalepa et al., 1998). In support of this notion Döbbeling and Berchtold (1996) have demonstrated down-regulation of the PKA pathway in a fibroblast cell line by protein kinase C activation. Since beta adrenoceptor down-regulation and/or desensitization of the beta-adrenoceptor-coupled adenylate cyclase system are accomplished in part by receptor phosphorylation via PKA (Lefkowitz, 1993), down-regulation of PKA could explain, at least in part, the lack of effect on beta adrenoceptors after chronic administration of venlafaxine. However, under the present experimental conditions neither endogenous nor cyclic AMP-stimulated PKA activity was changed in the 900 g supernatant or particulate fractions in vitro following venlafaxine treatment, and there was no indication of a translocation of PKA from the soluble to the particulate fraction, as reported following chronic administration of some antidepressants (Nestler et al., 1989).

However, investigation of CREB phosphorylation (activation) revealed significant decreases in CREB-P in nuclear lysates (cortical) following chronic treatment with venlafaxine. Although the mechanism responsible for this decrease in nuclear CREB-P is presently unclear it is intriguing because the concentration of nuclear CREB-P determines (or strongly influences) the levels of expression of genes containing CREs in their regulatory regions (Gonzalez and Montminy, 1989; Yamamoto et al., 1988).

Finally, whereas phosphorylation/dephosphorylation is generally considered to be the primary mechanism of CREB regulation, the extent to which CREB gene expression plays a role (if any) in its response to second messenger cascades, i.e. induced by antidepressant drugs has been difficult to determine by standard techniques (Meyer and Habener, 1993; Muller et al., 1995), suggesting that the CREB gene is either expressed at barely detectable basal levels or its mRNA is rapidly degraded. In fact data showing extremely low levels of CREB mRNA in many cell types has led to speculation that CREB is constitutively expressed, i.e. unmodulated by signal transduction mechanisms. And although this presumption was invalidated, initially by two studies reporting developmental modulation of CREB gene expression during
spermatogenesis (Ruppert et al., 1992; Waebel et al., 1991), and more recently by Muller et al. (1995) and
Nibuya et al. (1996), assaying changes in CREB mRNA levels in mature, terminally differentiated tissues has
remained problematic. Against this background the cPCR methodology utilized in the present study produced very
robust signals and demonstrated that the mechanism of action of venlafaxine does not involve regulation of CREB
mRNA levels. This lack of effect of venlafaxine on CREB mRNA levels, assayed by sensitive cPCR methodology,
does not support the notion that up-regulation of CREB is a common action of chronic antidepressant treatments
(Nibuya et al., 1996). However, our results may indicate alternatively that the pharmacological action of venla-
faxine beyond the receptors is distinctly different from that of other antidepressants. Moreover, the functional
relevance of any changes in CREB mRNA is difficult to assess, since phosphorylation/dephosphorylation of
CREB rather than its abundance determines its level of transcriptional activity (Meyer and Habener, 1993). The
reduction of nuclear CREB-P reported here may thus be venlafaxine’s most relevant pharmacological action be-
ond the receptors. Accordingly it is tempting to speculate that decreased nuclear CREB-P is responsible for
the not deamplification of amineergic signal transduction cascades following chronic administration of venlafaxine.

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