Naunyn-Schmiedeberg's Arch Pharmacol (2001) 363 : 448–455
DOI 10.1007/s002100100405

ORIGINAL ARTICLE

Cecilia Wikell · Stephan Hjorth · Gustav Apelqvist
Johan Kullingsjö · Jöns Lundmark
Peter B. F. Bergqvist · Finn Bengtsson

Sustained administration of the antidepressant venlafaxine in rats: pharmacokinetic and pharmacodynamic findings

Received: 25 October 2000 / Accepted: 9 January 2001 / Published online: 9 February 2001

Abstract Rats were administered venlafaxine (10 mg/kg per day) for 14 days by using subcutaneously implanted osmotic minipumps. The present study assessed the distribution of VEN in different compartments, whether the VEN concentration in the compartments correlated, the effect of VEN on dialysate monoamine levels and on the spontaneous open-field behavior, and possible relations between the pharmacokinetic and pharmacodynamic parameters.

The venlafaxine level in serum after sustained treatment was about 25% of the concentration in brain parenchyma and much higher than in brain dialysate. There was a clear correlation between venlafaxine concentrations in blood and brain compartments. The sustained venlafaxine challenge resulted in higher neocortical concentration of serotonin and noradrenaline, lower 5-hydroxyindole-3-acetic acid levels and increased locomotor activity in the central part of the test arena as compared to controls. No correlations were found between the venlafaxine concentration and brain monoamine parameters or the open-field behaviors.

We conclude that, although species differences in pharmacokinetic properties for venlafaxine between rat and man exist, the pharmacokinetic correlations found after sustained treatment add information to the in vivo nature of the drug. Also, more studies like the present need to be performed to find the pharmacokinetic/pharmacodynamic interrelations for drugs like VEN.

Keywords Antidepressant · Behavior · Biogenic monoamines · Microdialysis · Pharmacokinetics · Venlafaxine

C. Wikell · G. Apelqvist · P. B. F. Bergqvist · F. Bengtsson (✉)
Department of Clinical Pharmacology, Lund University,
221 85 Lund, Sweden
e-mail: finn.bengtsson@inr.liu.se,
Fax: +46-13-223393

C. Wikell · J. Lundmark · F. Bengtsson
Division of Psychiatry,
Department of Neuroscience and Locomotion,
University Hospital, 581 85 Linköping, Sweden

S. Hjorth
Department of Pharmacology and Clinical Neuroscience,
Umeå University, 901 87 Umeå, Sweden

J. Kullingsjö
Carlsson Research, Box 444, 405 30 Göteborg, Sweden

Introduction

Several novel classes of drugs for the treatment of major depressive disorder have become accessible during the last decade (Preskorn 1995; Möller and Volz 1996). In essence, all of these drugs interact with monoaminergic neurotransmission in brain in one way or the other. Most of the novel agents act by blocking neuronal reuptake mechanisms of serotonin (5-hydroxytryptamine; 5-HT) and/or noradrenaline, thus similar to the drug action of the older tri- and tetracyclic antidepressants (TCA). The new serotonin and noradrenaline reuptake inhibitors (SNRI) are thought to exert their primary pharmacodynamic effects by simultaneously affecting both the 5-HT and the noradrenaline neurotransmitter systems. Venlafaxine (VEN) is an example of an SNRI with a clear-cut in vitro preference for the 5-HT (K_i 19–74 nmol/l) and noradrenaline (K_i 210–1260 nmol/l) vs. the dopamine (K_i 5300 nmol/l) transporter (Bolden-Watson and Richelson 1993; Owens et al. 1997; Béïque et al. 1998). This agent has been reported to possess an advantageous clinical side effect profile and to be better tolerated than the older TCAs (Shrivastava et al. 1994; Holliday and Benfield 1995).

However, the pharmacodynamic and pharmacokinetic properties of VEN, and the interrelationship among these factors – in particular upon sustained drug administration – require further investigation. Thus, the pharmacodynamic activity of VEN and its metabolites differs: *O*-desmethylvenlafaxine (ODV) may be the most important metabolite, with reuptake-blocking properties in vitro similar, albeit not identical, to those of VEN (Muth et al. 1986). Further, two additional metabolites of VEN are *N*-desmethylvenlafaxine (NDV) and *N*,*O*-didesmethyl-

venlafaxine (DDV; Muth et al. 1991). NDV and DDV are only minor metabolites in humans, but there is no data available on their possible contribution to the in vivo pharmacodynamic profile of VEN, neither in rats nor in man.

The present investigation was carried out in rats after sustained VEN administration by means of subcutaneously (s.c.) implanted osmotic minipumps, using in vivo determinations of drug levels, as well as behavioral and microdialysis methods. The aims of the study were to define: (1) the distribution of VEN and its metabolites (if detected) in serum, brain parenchyma and brain dialysate after sustained treatment; (2) the effect of sustained VEN on microdialysate levels of 5-HT, 5-hydroxyindole-3-acetic acid (5-HIAA), noradrenaline and dopamine and on spontaneous open-field behavior; and (3) putative interrelations among the aforementioned pharmacokinetic and pharmacodynamic measures, using both univariate and multivariate analysis methods.

Materials and methods

Animals

Male Sprague-Dawley rats (Möllegaard Breeding and Research Centre, Skensved, Denmark) initially weighing about 300 g were used. Two to three animals were housed in each cage. After implantation of the osmotic minipumps (see below) the animals were housed individually in order to protect the sutures. After 2 days, the same rats were put together again. The animals had unlimited access to tap water and pelleted chow containing 18% crude protein (Altromin no. 1324; Peterson, Ringsted, Denmark) throughout the study. The animals were kept in a temperature-controlled (20°–22°C) room on a 12-h light:dark cycle (lights on from 6.00 a.m. to 6.00 p.m.). The rats were weighed once weekly as well as on the day of the microdialysis probe implantation.

Drug administration

Three weeks after arrival of the rats, osmotic minipumps (ALZET model 2ML4; B&K Universal, Sollentuna, Sweden) were surgically implanted in the s.c. space on the back of the rats under halothane anesthesia (Fluothane; Zeneca, Macclesfield, UK). Prior to the implantation, the pumps were filled with racemic VEN (HCl salt, courtesy Wyeth-Ayerst Research, Rouses Point, N.Y., USA; dissolved in 0.9% NaCl; n=18). The concentration of VEN in each osmotic pump was adjusted so that, at the time of the microdialysis (see below), the rats were receiving an approximate daily dose of 10 mg/kg of VEN (the body weight of each rat at microdialysis was approximated at the osmotic pump implantation by using weight gain curves from previous studies). This dose of VEN has previously been used in rats in vivo and is known to block the reuptake of noradrenaline (Wikell et al. 1998; Dawson et al. 1999), thus representing a pharmacodynamically relevant drug level. The osmotic pumps were left in place until termination of the study, i.e. there was no washout period before the microdialysis and subsequent killing of the rats. For the pharmacodynamic comparisons, i.e. when comparing monoamine levels as well as behavior, a saline control group (0.9% NaCl; n=17) was added. The saline-containing osmotic pumps were implanted in a similar manner as for the VEN-exposed animals.

Open-field behavior

Starting 9 days after the osmotic pump insertion, spontaneous open-field behaviors (comprising locomotion, rearing activity, and time spent rearing) were studied in 60-min sessions for four consecutive nights, spaced 24 h between each test session, by a methodological approach previously described (Apelqvist et al. 1998, 1999). Briefly, the rats were put into one of a set of seven test boxes (34×46×44 cm; w × l × h) on-line connected to a computer. The different movement components were defined as follows: locomotion was expressed in cm, and represents the total distance travelled by the rats inside the test boxes, based on paths which were at least 6 consecutive cm in length. Rearings (number of times the rats stand on their hind limbs) were considered as the sum of the numbers of both so-called on-wall rearings and off-wall rearings (Apelqvist et al. 1998). Further, the floors in the test boxes were divided into two equally large areas, which made it possible to monitor the central (inner) and peripheral (outer) proportion of the behavioral activities. Video cameras placed on top of, and infrared beams placed on the walls of the test chambers detected the locomotion and rearings, respectively, thus recording both behaviors separately but in parallel (for further details, see Apelqvist et al. 1998).

In vivo microdialysis

The microdialysis studies were conducted 14 days after the osmotic pump insertion. The day prior to microdialysis, a microdialysis probe (CMA/12, molecular weight cutoff: 20 kDa, membrane length: 4 mm; CMA/Microdialysis, Stockholm, Sweden) was implanted into the frontal neocortex (rostral +3.2 mm and lateral –3.0 mm relative to bregma, ventral –4.2 mm relative to the dura mater) under halothane (Fluothane) anesthesia. After implantation, the rats were put in individual microdialysis chambers (CMA/120 system for freely moving animals; CMA/Microdialysis) over night. The following day, the microdialysis probe was perfused with isotonic Ringer solution (147 mmol/l NaCl, 4 mmol/l KCl, 2.4 mmol/l $CaCl_2$, pH 6.0) at a flow rate of 2.0 μl/min. Following a 60-min stabilization period, samples were collected in 30-min intervals. Samples 3, 4 and 14 (60–120 min and 390–420 min) were collected for determination of drug concentrations, and samples 5–7 (120–210 min) taken for analysis of monoamine contents. All dialysates were kept frozen (–70°C) until analysis.

At termination of the experiment, all rats were killed by decapitation. In the VEN-treated rats, mixed arterio-venous blood was collected from the neck wound and left for 30 min to allow clotting. Serum was obtained by centrifugation (2000 g for 10 min) of the clotted blood samples. The brains of the VEN-treated rats were quickly removed from the cranium, and the neocortical hemisphere ipsilateral to the probe and the mesencephalon-pons were dissected and frozen. Serum and brain tissue samples were stored at –70°C until subsequent determination of parent drug and metabolite concentrations (see below).

Determination of the concentration of venlafaxine and its metabolites

Serum, brain tissue and brain dialysate levels of VEN, ODV, NDV and DDV were determined by means of high performance liquid chromatography (HPLC) with fluorescence detection as described previously (Björk and Bengtsson 1997; Wikell et al. 1998). Briefly, the brain tissue samples were homogenized with a sonifier (Model B-30; Branson Sonic Power, Danbury, Conn., USA) in 2 ml Milli-Q water and centrifuged at 2000 g for 15 min at 4°C. VEN, ODV, NDV and DDV were extracted from both rat serum and brain supernatant samples with solid-phase extraction. After elution and evaporation, the samples were redissolved in 100 μl of the mobile phase (10% methanol, 20% acetonitrile, 20 mmol/l KH_2PO_4; pH 4.4 set with 0.1 mol/l phosphoric acid) before the HPLC analysis (for further details, see Wikell et al. 1998).

The brain dialysis samples were directly injected to the HPLC system.

Using a 717 automatic injector, 30 µl of the purified serum and brain samples and the brain dialysates was injected to the HPLC system which consisted of a 510 pump and a 474 fluorescence detector (all instruments from Waters, Milford, Mass., USA). The samples were delivered at 1.0 ml/min through a Lichrosphere RP-select B column (250×4.6 mm; Merck, Darmstadt, Germany) and VEN, ODV, NDV and DDV were detected by a 280-nm excitation wavelength and a 310-nm emission wavelength. The data were processed by using the Millennium 2010 chromatography data system (Waters). The limits of quantification for VEN and the metabolites were 20 nmol/l, respectively.

Determination of monoamine and metabolite concentrations

Serotonin and 5-hydroxyindole-3-acetic acid. The dialysate 5-HT and 5-HIAA contents were determined by using HPLC with electrochemical detection according to a method described by Hjorth and Sharp (1993) with minor modifications (Bergqvist et al. 1995). In short, the dialysis samples were directly injected with a Rheodyne 7125 injector (Rheodyne, Cotati, Calif., USA). The mobile phase (0.126 mol/l NaH_2PO_4, 0.85 mmol/l EDTA, 0.01 mmol/l sodium *n*-octyl sulfate, and 13% methanol; pH 4.0 set with phosphoric acid) delivered the samples at 0.2 ml/min through a Pharmacia-LKB HPLC pump (Model 2150; LKB-Produkter, Bromma, Sweden) or a Gynkotek HPLC pump (Model P580; Softron, Gynkotek HPLC, Germering, Germany). The compounds were separated at 30°C on an ODS C_{18} column, 2.0 mm × 15 cm, packed with 5-µm material (Ultrasphere; Beckman Instruments, Fullerton, Calif., USA). The 5-HT and 5-HIAA contents were detected either by a Waters detector (Model 460; Millipore/Waters Chromatography Division, Sundbyberg, Sweden) connected to the LKB pump or by a DECADE detector (ANTEC Leyden, Leiden, The Netherlands) connected to the Gynkotek pump. The detectors consisted of a carbon electrode set at +0.6 V relative to an Ag/AgCl reference electrode. The signals from the detectors were monitored and analyzed either by means of an integrator (Chrom-Jet; Spectra-Physics, San Jose, Calif., USA) or by an on-line computer using the Chromeleon chromatography data sampling/analysis software (Softron). The detection limit for 5-HT was 0.025–0.050 nmol/l (S/N ratio=3).

Noradrenaline and dopamine. The dialysate noradrenaline and dopamine levels were determined by a radioenzymatic method (Peuler and Johnson 1977; Schmidt et al. 1982) with some modifications (Kalén et al. 1988; Bergqvist et al. 1995). In brief, 25 µl of the dialysates was transferred to tubes containing 5 µl 1 mol/l TRIS base, 5 µl 300 mmol/l $MgCl_2$, 2.5 µl 200 mmol/l EGTA, 4 µl catechol-*O*-methyltransferase (prepared from rat liver) enzyme solution, 2.1 µl dithiothreitol/pargyline mix (0.105 mol/l and 25 mmol/l, respectively), and 1 µl *S*-adenosyl-L-[methyl-^{3}H]methionin (TRK 581, 60–85 Ci/mmol; Amersham Sweden, Solna, Sweden). The tubes were incubated at 37°C for 40 min. In order to terminate the reaction, the tubes were placed on ice, and 10 µl phosphotungstic acid (20% in 1.2 mol/l HCl) was added. The product carriers metanephrine and normetanephrine (Sigma Chemical, St. Louis, Mo., USA) were added. This was followed by extraction and washing in several steps for isolation of the [^{3}H]metanephrine and [^{3}H]normetanephrine acid reaction products. The products were separated by using thin layer chromatography. The [^{3}H]metanephrine and [^{3}H]normetanephrine spots (corresponding to dopamine and noradrenaline, in the samples methylated by the catechol-*O*-methyltransferase enzyme) were removed from the thin layer chromatography plates and transferred into scintillation vials for radioactivity counting (1277 Gammamaster; Wallac, Sollentuna, Sweden). The quantification limits for dopamine and noradrenaline were 0.12 nmol/l and 0.04 nmol/l, respectively.

Statistics

All data are presented as means ± standard deviation (SD). Three samples per rat were collected for determination of both monoamine and VEN concentrations in dialysate. For each rat, the average of the three samples represented the concentration of the substance for that rat, and was in turn used to calculate the group means reported. A probability of less than 5% ($P<0.05$) was preset to indicate statistical difference between observations. When NaCl- and VEN-treated rats were compared, a Student's *t*-test was applied. A possible correlation between pharmacokinetic parameters was studied by subjecting the data to Pearson's correlation statistics. Dixon's gap test for outliers was applied where appropriate. All statistical univariate analyses were carried out by means of StatView 4.12 for Macintosh (Abacus Concepts, Berkeley, Calif., USA).

Putative pharmacokinetic/pharmacodynamic correlations were made by multivariate analysis. The main purpose was to investigate possible relationships between the concentration of VEN in serum and the concentrations of the monoamines and the open-field behavior. All multivariate analyses were made by using the software package Simca-P 7.01 (UMETRI, Umeå, Sweden).

Results

Body weight

At decapitation, the body weight increment (initial body weight subtracted from body weight at decapitation) did not differ between the VEN-treated and NaCl-treated control rats (137±37 g vs. 150±47 g), indicating that the 2 weeks of VEN administration had no major impact on normal body weight gain.

Venlafaxine and metabolite levels

The VEN contents in cortex (648±282 pmol/g≈nmol/l, $n=17$) and mesencephalon-pons (527±206 pmol/g≈ nmol/l, $n=18$) were about 300%–400% higher than in serum (157±64 nmol/l, $n=18$). For comparison, the VEN concentration in brain dialysate (33.4±16.9 nmol/l, $n=16$) was found to be about 20% of the VEN concentration found in serum and only about 5% of that in the brain parenchyma. The NDV concentration in serum (27.3±5.1 nmol/l, $n=8$) was about 20% of the VEN concentration in serum. None of the VEN metabolites were found in detectable amounts in either of the two brain regions or in the dialysate samples.

According to univariate statistics, the VEN concentration in serum correlated highly with the VEN concentration in cortex (Fig. 1A) and mesencephalon-pons ($r=0.938$, $P=0.0029$) as did the VEN levels in cortex and mesencephalon-pons ($r=0.993$, $P<0.0001$). Relations were also found between the VEN concentration in serum and dialysate (Fig. 1B), between the VEN concentrations in cortex and dialysate ($r=0.846$, $P=0.0313$), and also between the VEN and NDV levels in serum ($r=0.962$, $P=0.0006$; not shown). Figure 1A,B displays a close intra-individual VEN concentration relation between different compartments. Figure 2 shows the overall interrelations among concentrations in serum, dialysate and cortex, and

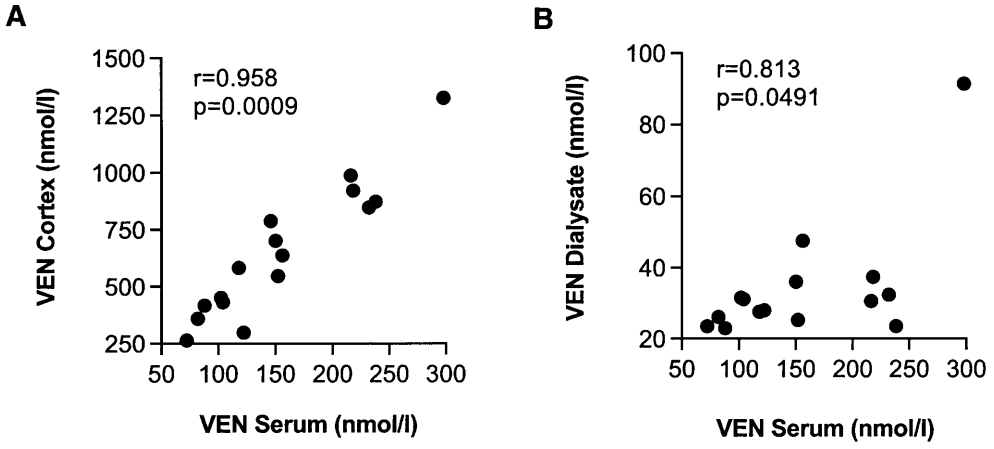

Fig. 1 There was a correlation between the concentration of venlafaxine (*VEN*) in **A** serum and cortex (n=16) and **B** serum and dialysate (n=15). The r- and P-values displayed in the figures result from Pearson's correlation statistics. All values are in nmol/l

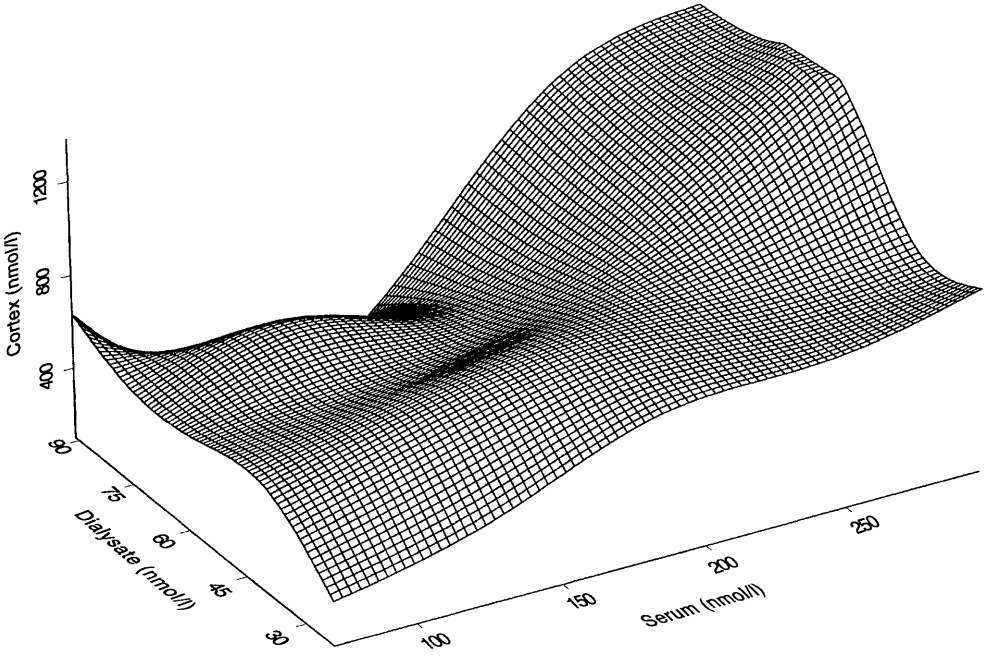

Fig. 2 Gridded spine plot presenting group associations between the venlafaxine concentration (nmol/l) in serum (x-axis), dialysate (y-axis), and cortex (z-axis). The grid was produced by the program (Axum 5; MathSoft, Cambridge, Mass., USA) by multiplying the values of the pharmacokinetic data

demonstrates that there is a disproportionality in the correlation, i.e. when the VEN concentrations in serum and dialysate reached above ~180 nmol/l and ~45 nmol/l, respectively, the increment in cortex was higher than predicted from a simple linear correlation.

Monoamine levels

The neocortical dialysate monoamine levels following the sustained VEN challenge are shown in Table 1. One animal in the VEN-treated group was considered to be an outlier in 5-HT concentration (P<0.01) and was thus excluded. Moreover, due to undetectable 5-HT concentrations in five animals in the control group (below 0.025 nmol/l), data from the reduced number of 12 rats are reported in the NaCl-treated control group. The 5-HT concentration in the dialysates was higher in VEN-treated rats compared with the remaining NaCl rats (P=0.0144). The 5-HIAA concentration was lower in the VEN group compared with the NaCl group (P=0.0008). The dialysate noradrenaline level in the VEN-treated rats was higher compared with the NaCl rats (P=0.0019). The dialysate dopamine concentration did not differ significantly between the two groups investigated.

Table 1 Neocortical dialysate levels of 5-HT, 5-HIAA, noradrenaline and dopamine following sustained venlafaxine (*VEN*; 10 mg/kg per day for 2 weeks) or NaCl administration

	n	NaCl-treated rats[a]	n	VEN-treated rats	P-value[b]
5-HT	12	0.17±0.11	16	0.33±0.19	0.0144
5-HIAA	17	177 ±72	17	109 ±25	0.0008
Noradrenaline	17	0.42±0.26	14	0.84±0.43	0.0019
Dopamine	17	1.16±0.49	14	1.03±0.55	0.4801

[a]All values are in nmol/l, means ± SD
[b]Student's t-test, VEN-treated vs. NaCl-treated control rats

Multivariate analysis failed to uncover any significant correlations between the concentration of VEN in serum

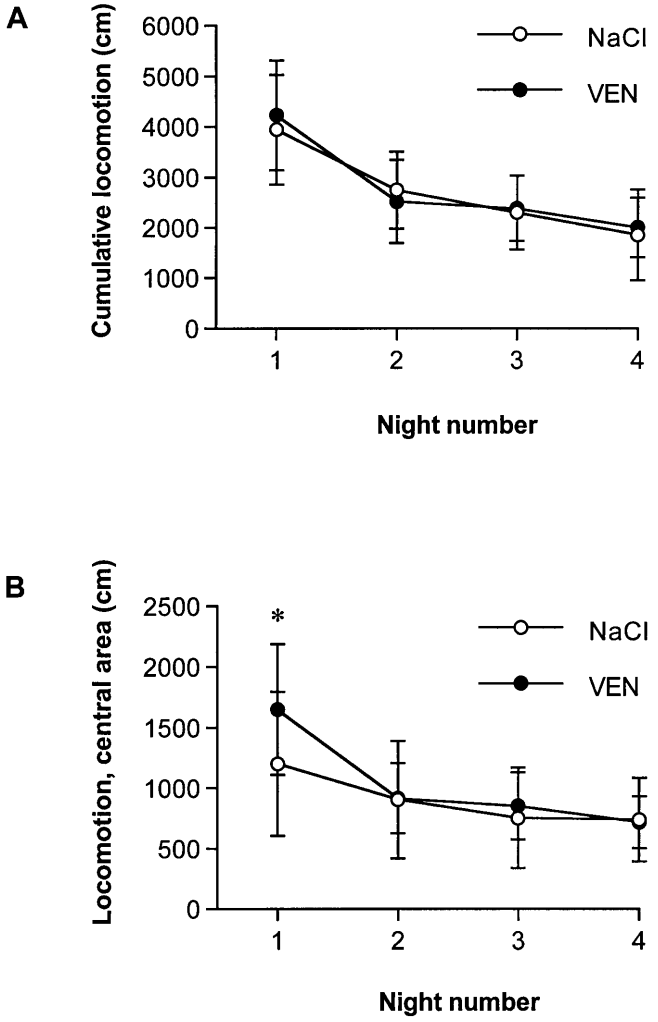

Fig. 3 The **A** cumulative locomotor activity and **B** locomotor activity in the central area (cm) performed during 60 min, four consecutive nights. The rats were treated with venlafaxine (*VEN*; 10 mg/kg per day) or NaCl for 9 days before the first behavioral test session was performed. The rats were unaccustomed to the environment the first night. All values are means ± SD. **P*<0.05, VEN-treated vs. control rats

and the concentration of 5-HT, 5-HIAA, noradrenaline and dopamine (data not shown).

Open-field behavior

The locomotor activity of the VEN-treated and control rats the four nights investigated (i.e. days 9–13 after osmotic pump implantation) is displayed in Fig. 3. Both VEN-exposed and control rats displayed the highest behavioral activity during the first night when they were unaccustomed to the behavioral test equipment. The following three nights both groups showed a continuously diminishing level of behavioral activity, the most clear-cut reduction could be seen between the first and second nights. No differences between VEN group and control group could be seen in cumulative locomotor activity during any of the four nights (Fig. 3A). However, during the first night, the VEN-treated rats displayed a higher level of locomotor activity in the central area than the control rats ($P<0.05$) while no differences in locomotor activity in the central area were evident between the groups during the subsequent second, third and fourth night (Fig. 3B). VEN did not seem to have any effect on the rearing activity compared with the NaCl-treated control group neither regarding number of rearings nor in time spent rearing.

No significant correlations were found when investigating a possible linear relationship between the concentration of VEN in serum and the open-field behavioral parameters by using multivariate analysis (data not shown).

Discussion

The present investigation provides novel data on the pharmacokinetic and pharmacodynamic properties of VEN following sustained administration in rats in vivo. Thus, our findings describe: (1) the distribution of VEN in different compartments in rat, i.a. demonstrating a close correlation between the concentration of VEN in blood and brain compartments; (2) the effects upon monoamine and metabolite levels (elevated 5-HT and noradrenaline and decreased 5-HIAA) in brain dialysates, and on behavior (locomotor activity) under steady-state drug level conditions; and (3) possible interrelations among these pharmacokinetic and pharmacodynamic measures.

VEN was found in serum, brain parenchyma and dialysate samples, but in highly variable amounts. The varying VEN concentrations found in these compartments could be due to a multitude of factors such as i.a. lipid solubility, protein binding and molecular weight. These factors all affect the distribution of the drug and its diffusion or transport across the blood-brain barrier. VEN has a high volume of distribution and a low plasma protein binding in man (6–7 l/kg and 30%, respectively; Klamerus et al. 1992), and more VEN is therefore likely to be found in tissue compartments, like the brain parenchyma, than in blood – thus in accordance with the actual drug distribution pattern observed in the present study in the rat. The serum and dialysate VEN levels were much lower than those found in the brain parenchyma, consistent with the brain tissue compartment being larger in size and more lipophilic as compared to the non-tissue compartments. Interestingly, there was a close correspondence between the dialysate and serum levels of VEN if the relative in vitro recovery rate for VEN over the dialysis membrane is taken into account (16%–21%; data not shown). It should be noted, though, in this context, that the relative dialysis recovery depends on several factors, including substance interaction with the dialysis membrane, speed of perfusion, temperature, diffusion and other processes intrinsic to the local probe environment (for overview, see, e.g., Benveniste 1989; Kehr 1993), and may therefore differ substantially in vitro vs. in vivo. Thus, although the present data might be consistent with the suggestion that VEN has equilibrated across the extracellular and serum compartments under the present conditions, more studies

are needed to establish the recovery of VEN across the dialysis membrane in vivo.

The *O*-desmethylated metabolite of VEN, ODV, was not found in detectable amounts either in brain tissue, serum or dialysate samples. In previous studies, ODV was found in rats after daily intragastric administration of VEN for 14 days (Howell et al. 1994), but the dose was very much higher (120 mg/kg) than in the present investigation (10 mg/kg). ODV was also found in serum after a single dose challenge of 10 mg/kg, but at levels close to the detection limit (Wikell et al. 1998). It should be noted that the ODV level in the urine resulting from a single VEN dose was 29% in humans and only 5% in rats (Howell et al. 1993). Moreover, in the present study, there was a correlation between the concentrations of VEN and NDV in serum. In fact, NDV seems to be more prevalent, but the main urinary VEN metabolite in rat, *cis*-1,4-dihydroxycyclohexyl-VEN, amounted to 15% of the administered dose in rats but could not be detected in humans (Howell et al. 1993). Unfortunately, at present, we have no method available for analysing *cis*-1,4-dihydroxycyclohexyl-VEN, and therefore could not assess the levels of this metabolite in the current study. On a final note, recent reports suggest the presence of a local intracerebral drug metabolism system (Bhamre et al. 1993; Kawashima and Strobel 1995; Ravindranath et al. 1995; Kawashima et al. 1996; Tyndale et al. 1999), able to e.g. *N*-demethylate the tricyclic antidepressant and mixed noradrenaline/5-HT reuptake inhibitor, imipramine. It may thus be speculated that, if an intracerebral metabolism of VEN exists, the VEN concentration in serum will not mirror the concentration found in brain parenchyma, which could therefore underlie difficulties finding clear-cut dose-effect relations in humans.

In the present study, the dialysate 5-HT and noradrenaline concentrations were significantly higher compared with controls after the sustained VEN administration, which confirms the effect on 5-HT and noradrenaline reuptake of the compound in vivo. There is data to suggest that chronic treatment with VEN induces a desensitization of 5-HT_{1A} (and possibly, at higher doses, also 5-HT_{1B}) autoreceptors (Béïque et al. 2000a, 2000b; but see Gur et al. 1999). Since autoreceptor-mediated feedback limits the 5-HT-elevating action of agents blocking the reuptake of the transmitter (e.g. Adell and Artigas 1991; Hjorth 1993; Hjorth and Auerbach 1994; Rutter et al. 1995), it might thus be expected that the baseline output of 5-HT would be increased after chronic VEN administration. However, the literature is not consistent in this regard – nor with respect to the effects of *acute* VEN challenge (see Table 2). Thus, in a previous investigation (Dawson et al. 1999), single s.c. VEN injections of 3–50 mg/kg failed to alter dialysate 5-HT, but resulted in a significantly higher noradrenaline output in the frontal cortex. In the studies by Gur et al. (1999), cortical and hippocampal dialysate levels of 5-HT were increased after administration of single VEN doses (5–20 mg/kg i.p.), but not after chronic treatment (5 mg/kg i.p. once daily). By comparison, in our hands, 5-HT was comparably elevated both after acute and chronic VEN dosing; noradrenaline levels were likewise elevated in both instances (unpublished and present data). The discrepant findings may involve factors such as dose, route of administration, investigated brain area, rat strain, and experimental conditions. Regardless, although some data indicate that prolonged VEN treatment may desensitize 5-HT autoreceptor-mediated feedback, there is so far little to suggest that this results in a greater increase of extracellular 5-HT levels as compared to acute injection of VEN. In this context, it should be noted that there is a great overcapacity of 5-HT autoreceptors (Meller et al. 1990), and that negative feedback mediated by these sites remains functional despite chronic treatment with SSRI agents like citalopram (Gundlah et al. 1997; Hjorth and Auerbach 1999). There was also further support for the sustained effect of chronic VEN administration on the neocortical 5-HT system, in that the dialysate 5-HIAA values were significantly decreased in the VEN-treated group compared with controls. This is what would be expected from a substance with 5-HT reuptake-inhibiting properties. In humans, in a study where patients received VEN for at least 6 weeks, a decreased 5-HIAA concentration was observed in the cerebrospinal fluid compared with before treatment (Little et al. 1999).

With regard to noradrenaline, chronic administration of the SNRI duloxetine has been reported to result in increased output of the transmitter in vitro, tentatively at least partly due to desensitization of α_2 autoreceptors (see e.g. Rueter et al. 1998). By comparison, however, long-

Table 2 Effects of venlafaxine (*VEN*) on dialysate 5-HT and noradrenaline after acute and chronic treatment (*FCx* frontal cortex, *DH* dorsal hippocampus, *ND* not determined)

VEN treatment	5-HT	Noradrenaline	Reference
Acute			
3–50 mg/kg s.c.	No change (FCx)	Increase (FCx)	Dawson et al. 1999
5–20 mg/kg i.p.	Increase (FCx/DH)	ND	Gur et al. 1999
10 mg/kg s.c.	Increase (FCx)	Increase (FCx)	Wikell et al. (unpublished)
Chronic			
5 mg/kg per day (i.p. injection once daily for 4 weeks)	No change (FCx/DH)	ND	Gur et al. 1999
10 mg/kg per day (s.c. osmotic minipumps for 2 weeks)	Increase (FCx)	Increase (FCx)	Present data

term administration of venlafaxine does not appear to cause any significant change in the sensitivity of the α_2 autoreceptors (Béïque et al. 2000b). Consistent with this, the relative increase of noradrenaline vs. baseline values after chronic VEN treatment (approximately 100%; present data) does not seem to be greater than the noradrenaline response to a single challenge dose of the drug (approximately 100%–200%; Dawson et al. 1999; Wikell et al., unpublished data).

Moreover, in the present study, the dialysate dopamine levels remained unaltered following the sustained VEN challenge. In a previous study, employing different experimental conditions to the current, we found an increase of dialysate dopamine after acute administration of VEN (Wikell et al. 1998). It has also been reported that high concentrations of VEN may block dopamine reuptake in vitro (Muth et al. 1986). Thus, while a possible contribution from dopamine reuptake inhibition to the overall pharmacodynamic profile of VEN cannot be entirely excluded, it would appear that it is of limited importance – at least when the drug is given chronically and in moderate dosage.

Monoamines are intimately involved in the control of behavior. In the present investigation, the locomotion in the central area of an open field was higher in the first of four testing sessions in VEN-treated rats compared with untreated controls. The central noradrenergic system is associated with enhanced "drive", reflected in both increased vigilance and general activity of rodents (Foote et al. 1983; Svensson 1987). The noradrenaline-promoting capacity of an antidepressant agent is considered an advantage in treating patients with major depressive disorder displaying passivity as a prominent feature in their illness (see e.g. Carlsson et al. 1969; Dubini et al. 1997; Montgomery 1997). However, the relation between the observed increase in neocortical noradrenaline output and the enhanced locomotion in the chronic VEN-treated rats remains to be clarified, particularly as this behavioral change was rather modest and present only in the first of the four consecutive testing sessions.

In summary, the present investigation in rats subjected to sustained treatment with VEN provides novel pharmacokinetic and pharmacodynamic information on the properties of the compound. Specifically, there was a threefold higher VEN concentration in brain parenchyma than in serum, and a close pharmacokinetic correlation for VEN between blood and brain compartments in vivo. Further, the VEN treatment resulted in increased levels of 5-HT and noradrenaline and decreased levels of 5-HIAA in brain dialysates, consistent with the mode of action of VEN. It remains an important task to uncover the reason(s) as to why there were no significant relations between the pharmacokinetic and pharmacodynamic measures in this study, and further studies are thus needed. Although the model used here is an experimental paradigm, it could be conceptually important to use in the search for human clinical pharmacokinetic/pharmacodynamic interrelations due to ethical problems with clinical studies.

Acknowledgements We gratefully acknowledge Kerstin Fogelström and Prof. Anders Björklund at the Wallenberg Laboratory (Lund University, Lund, Sweden) for excellent technical assistance and generous support with the catecholamine analyses. Rosita Thomasson at the Department of Pharmacology (Göteborg University, Göteborg, Sweden) and Henrik Björk at the Department of Clinical Pharmacology (Lund University, Lund, Sweden) provided excellent technical assistance with the indole derivative determinations and the venlafaxine determinations, respectively. This study was supported by the Swedish Medical Research Council (project no. 14X-12598, F.B. and 14X-7486, S.H.), the Faculty of Health Sciences, Linköping University, the Foundations of M. Bergvall, Golje, Lundbeck, A. Påhlsson, and F. and I. Thuring, the Swedish Society of Medicine, and the Swedish Society for Medical Research, Sweden. The study was approved by the Animal Ethics Committee at Lund University (Lund, Sweden).

References

Adell A, Artigas F (1991) Differential effects of clomipramine given locally or systemically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. An in vivo brain microdialysis study. Naunyn-Schmiedeberg's Arch Pharmacol 343:237–244

Apelqvist G, Hindfelt B, Andersson G, Bengtsson F (1998) Diurnal and gender effects by chronic portacaval shunting in rats on spontaneous locomotor and rearing activities in an open-field. Behav Brain Res 93:25–32

Apelqvist G, Wikell C, Hindfelt B, Bergqvist PBF, Andersson G, Bengtsson F (1999) Altered open-field behavior in experimental chronic hepatic encephalopathy after single venlafaxine and citalopram challenges. Psychopharmacology (Berl) 143:408–416

Béïque J-C, Lavoie N, Montigny C de, Debonnel G (1998) Affinities of venlafaxine and various reuptake inhibitors for the serotonin and norepinephrine transporters. Eur J Pharmacol 349: 129–132

Béïque J-C, Montigny C de, Blier P, Debonnel G (2000a) Effects of sustained administration of the serotonin and norepinephrine reuptake inhibitor venlafaxine. I. In vivo electrophysiological studies in the rat. Neuropharmacology 39:1800–1812

Béïque J-C, Montigny C de, Blier P, Debonnel G (2000b) Effects of sustained administration of the serotonin and norepinephrine reuptake inhibitor venlafaxine. II. In vitro studies in the rat. Neuropharmacology 39:1813–1822

Benveniste H (1989) Brain microdialysis. J Neurochem 52:1667–1679

Bergqvist PBF, Vogels BAPM, Bosman DK, Maas MAW, Hjorth S, Chamuleau RAFM, Bengtsson F (1995) Neocortical dialysate monoamines of rats after acute, subacute, and chronic liver shunt. J Neurochem 64:1238–1244

Bhamre S, Bhagwat SV, Shankar SK, Williams DE, Ravindranath V (1993) Cerebral flavin-containing monooxygenase-mediated metabolism of antidepressants in brain: immunochemical properties and immunocytochemical localization. J Pharmacol Exp Ther 267:555–559

Björk H, Bengtsson F (1997) A racemic HPLC-method for routine TDM of the novel antidepressant venlafaxine and its main relevant metabolites in serum. Eur J Clin Pharmacol 52:A156

Bolden-Watson C, Richelson E (1993) Blockade by newly-developed antidepressants of biogenic amine uptake into rat brain synaptosomes. Life Sci 52:1023–1029

Carlsson A, Corrodi H, Fuxe K, Hökfelt T (1969) Effect of antidepressant drugs on the depletion of intraneuronal brain 5-hydroxytryptamine stores caused by 4-methyl-a-ethyl-meta-tyramine. Eur J Pharmacol 5:357–366

Dawson LA, Nguyen HQ, Geiger A (1999) Effects of venlafaxine on extracellular concentrations of 5-HT and noradrenaline in the rat frontal cortex: augmentation via 5-HT_{1A} receptor antagonism. Neuropharmacology 38:1153–1163

Dubini A, Bosc M, Polin V (1997) Do noradrenaline and serotonin differentially affect social motivation and behaviour? Eur Neuropsychopharmacol 7:S17–S21

Foote SL, Bloom FE, Aston-Jones G (1983) Nucleus locus coeruleus: new evidence of anatomical and physiological specificity. Physiol Rev 63:844–914

Gundlah C, Hjorth S, Auerbach SB (1997) Autoreceptor antagonists enhance the effect of the reuptake inhibitor citalopram on the extracellular 5-HT: this effect persists after repeated citalopram treatment. Neuropharmacology 36:475–482

Gur E, Dremencov E, Lerer B, Newman ME (1999) Venlafaxine: acute and chronic effects on 5-hydroxytryptamine levels in rat brain in vivo. Eur J Pharmacol 372:17–24

Hjorth S (1993) Serotonin 5-HT_{1A} autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor citalopram to increase nerve terminal output of 5-HT in vivo: a microdialysis study. J Neurochem 60:776–779

Hjorth S, Auerbach SB (1994) Further evidence for the importance of 5-HT_{1A} autoreceptors in the action of selective serotonin reuptake inhibitors. Eur J Pharmacol 260:251–255

Hjorth S, Auerbach SB (1999) Autoreceptors remain functional after prolonged treatment with a serotonin reuptake inhibitor. Brain Res 835:224–228

Hjorth S, Sharp T (1993) In vivo microdialysis evidence for central $serotonin_{1A}$ and $serotonin_{1B}$ autoreceptor blocking properties of the beta adrenoceptor antagonist (-)penbutolol. J Pharmacol Exp Ther 265:707–712

Holliday SM, Benfield P (1995) Venlafaxine. A review of its pharmacology and therapeutic potential in depression. Drugs 49: 280–294

Howell SR, Husbands GEM, Scatina JA, Sisenwine SF (1993) Metabolic disposition of ^{14}C-venlafaxine in mouse, rat, dog, rhesus monkey and man. Xenobiotica 23:349–359

Howell SR, Hicks DR, Scatina JA, Sisenwine SF (1994) Pharmacokinetics of venlafaxine and *O*-desmethylvenlafaxine in laboratory animals. Xenobiotica 24:315–327

Kalén P, Kokaia M, Lindvall O, Björklund A (1988) Basic characteristics of noradrenaline release in the hippocampus of intact and 6-hydroxydopamine-lesioned rats as studied by in vivo microdialysis. Brain Res 474:374–379

Kawashima H, Strobel HW (1995) cDNA cloning of a novel rat brain cytochrome P450 belonging to the CYP2D subfamily. Biochem Biophys Res Commun 209:535–540

Kawashima H, Sequeira DJ, Nelson DR, Strobel HW (1996) Genomic cloning and protein expression of a novel rat brain cytochrome P-450 CYP2D18* catalyzing imipramine *N*-demethylation. J Biol Chem 271:28176–28180

Kehr J (1993) A survey on quantitative microdialysis: theoretical models and practical implications. J. Neurosci Methods 48: 251–261

Klamerus KJ, Maloney K, Rudolph RL, Sisenwine SF, Jusko WJ, Chiang ST (1992) Introduction of a composite parameter to the pharmacokinetics of venlafaxine and its active *O*-desmethyl metabolite. J Clin Pharmacol 32:716–724

Little JT, Ketter TA, Mathé AA, Frye MA, Luckenbaugh D, Post RM (1999) Venlafaxine but not bupropion decreases cerebrospinal fluid 5-hydroxyindoleacetic acid in unipolar depression. Biol Psychiatry 45:285–289

Meller E, Goldstein M, Bohmaker K (1990) Receptor reserve for 5-$hydroxytryptamine_{1A}$-mediated inhibition of serotonin synthesis: possible relationship to anxiolytic properties of 5-$hydroxytryptamine_{1A}$ agonists. Mol Pharmacol 37:231–237

Möller H-J, Volz H-P (1996) Drug treatment of depression in the 1990s. An overview of achievements and future possibilities. Drugs 52:625–638

Montgomery SA (1997) Is there a role for a pure noradrenergic drug in the treatment of depression? Eur Neuropsychopharmacol 7:S3–S9

Muth EA, Haskins JT, Moyer JA, Husbands GEM, Nielsen ST, Sigg EB (1986) Antidepressant biochemical profile of the novel bicyclic compound Wy-45,030, an ethyl cyclohexanol derivate. Biochem Pharmacol 35:4493–4497

Muth EA, Moyer JA, Haskins JT, Andree TH, Husbands GEM (1991) Biochemical, neurophysiological, and behavioral effects of Wy-45,233 and other identified metabolites of the antidepressant venlafaxine. Drug Dev Res 23:191–199

Owens MJ, Morgan WN, Plott SJ, Nemeroff CB (1997) Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites. J Pharmacol Exp Ther 283: 1305–1322

Peuler JD, Johnson GA (1977) Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. Life Sci 21:625–636

Preskorn SH (1995) Comparison of the tolerability of bupropion, fluoxetine, imipramine, nefazodone, paroxetine, sertraline, and venlafaxine. J Clin Psychiatry 56:12–21

Ravindranath V, Bhamre S, Bhagwat SV, Anandatheerthavarada HK, Shankar SK, Tirumalai PS (1995) Xenobiotic metabolism in brain. Toxicol Lett 82–83:633–668

Rueter LE, Kasamo K, Montigny C de, Blier P (1998) Effect of long-term administration of duloxetine on the function of serotonin and noradrenaline terminals in the rat brain. Naunyn-Schmiedeberg's Arch Pharmacol 357:600–610

Rutter JJ, Gundlah C, Auerbach SB (1995) Systemic uptake inhibition decreases serotonin release via somatodendritic autoreceptor activation. Synapse 20:225–233

Schmidt RH, Ingvar M, Lindvall O, Stenevi U, Björklund A (1982) Functional activity of substantia nigra grafts reinnervating the striatum: neurotransmitter metabolism and [^{14}C]2-deoxy-D-glucose autoradiography. J Neurochem 38:737–748

Shrivastava RK, Cohn C, Crowder J, Davidson J, Dunner D, Feighner J, Kiev A, Patrick R (1994) Long-term safety and clinical acceptability of venlafaxine and imipramine in outpatients with major depression. J Clin Psychopharmacol 14:322–329

Svensson TH (1987) Peripheral, autonomic regulation of locus coeruleus noradrenergic neurons in brain: putative implications for psychiatry and psychopharmacology. Psychopharmacology (Berl) 92:1–7

Tyndale RF, Li Y, Li N-Y, Messina E, Miksys S, Sellers EM (1999) Characterization of cytochrome P-450 2D1 activity in rat brain: high-affinity kinetics for dextromethorphan. Drug Metab Dispos 27:924–930

Wikell C, Bergqvist PBF, Hjorth S, Apelqvist G, Björk H, Bengtsson F (1998) Brain monoamine output alterations after a single venlafaxine challenge in experimental hepatic encephalopathy. Clin Neuropharmacol 21:296–306