PET neuroimaging with [\textsuperscript{11}C]venlafaxine: serotonin uptake inhibition, biodistribution and binding in living pig brain

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Abstract

The brain binding kinetics and distribution of the antidepressant venlafaxine, labelled with \textsuperscript{11}C in the O-methyl position, was studied by PET after intravenous injection in anesthetized pigs. In addition, venlafaxine’s action on serotonin (5-HT) uptake was studied in vitro in blood platelets obtained from humans or pigs. Venlafaxine resembled imipramine, paroxetine and citalopram in causing a dose-dependent inhibition of 5-HT uptake in blood platelets from pigs and humans. Venlafaxine-derived radioactivity entered the living brain readily and showed higher binding potentials in diencephalic and telencephalic regions than in cerebellum. Acute administration of an antidepressant drug (i.e. imipramine, citalopram or paroxetine) enhanced the distribution and altered the binding of venlafaxine in certain brain regions. The findings show that [\textsuperscript{11}C]venlafaxine is not an ideal PET radiotracer mainly because of its relatively low binding potentials and its lack of specificity for the 5-HT transporter in living brain.

Keywords: Venlafaxine; Antidepressant binding; Imipramine; Paroxetine; Citalopram; Pig brain; Blood platelet; Serotonin reuptake

1. Introduction

Positron emission tomography (PET) is currently being used for investigating brain function in a variety of neuropsychiatric disorders. Studies have shown that depression may be associated with reductions in cerebral blood flow in prefrontal cortical regions (George et al., 1993; Martinot et al., 1990), but the causes of such disturbances are uncertain. One possibility is that depression reflects underlying disturbances in neuronal serotonin (5-HT) transport and that antidepressant drugs act by correcting serotonergic imbalances (Maes and Meltzer, 1995). Venlafaxine has recently been shown to have rapid antidepressant efficacy often in otherwise treatment-resistant cases (Feighner, 1994; Nierenberg et al., 1994). Venlafaxine, in vitro, is several times more potent as an inhibitor of neuronal 5-HT uptake than of neuronal uptake of catecholamines in rat synaptosomes. What is more, venlafaxine lacks affinity for benzodiazepine, opiate and postsynaptic dopamine and serotonin receptors (Muth et al., 1986, 1991). In theory, venlafaxine could be useful as PET radioligand for studying neuronal 5-HT uptake and/or antidepressant actions, provided that its biodistribution, specific binding and selectivity are of sufficient magnitude (Sedvall et al., 1986). The present work was carried out in order to study these aspects of venlafaxine in living brain.

2. Methods

2.1. Experimental animals

Pigs were selected as laboratory animals for the present study for several reasons. Firstly, the limit of resolution of our PET scanner, namely ca. 4 mm, precluded the use of small laboratory animals such as rodents for studying
pharmacokinetic processes in living brain regions. On the other hand, the brain of the pig is large enough for studying regional cerebral pharmacokinetics, hemodynamics and metabolism by PET (Åkeson et al., 1992; Smith et al., 1996; Gee et al., 1996). Secondly, the use of nonhuman primates for PET would have been expensive and potentially dangerous. In contrast, pigs are relatively inexpensive and bear few health hazards for humans (Tumbleson, 1986). Thirdly, pig brain shows sufficient affinity for antidepressant drugs with specific actions on 5-HT uptake sites (Brust et al., 1996). Fourthly, current developments in organ transplantation indicate that the pig may be suitable as brain tissue donor for humans afflicted by neurodegenerative diseases (O’Brien, 1996; Vines, 1996). We plan to do research on such topics and have, therefore, begun using pigs as experimental animals in our studies.

The project was approved by the Danish National Committee for ethics in animal research. Six female Yorkshire Landrace pigs weighing 39–44 kg were used. They were housed singly in a thermostatically controlled (20°C) animal colony with natural lighting conditions. The pigs had free access to water but were deprived of food for 24 h prior to experiments. Pigs were sedated with an i.m. injection of midazolam (0.5 mg/kg) and ketamine HCl (10 mg/kg). After 10–15 min, a catheter was installed in an ear vein through which a mixture of midazolam (0.25 mg/kg) and ketamine (5 mg/kg) was administered. The pig was then intubated and was anesthetized with isoflurane in O₂/N₂. Catheters (Avanti® size 4F–7F) were surgically installed in a femoral artery and vein. Infusions of isotonic saline (ca. 100 cm³/h) and 5% glucose (ca. 20 cm³/h) were administered i.v. throughout experiments. Body temperature was thermostatically maintained in the normal range (39.0–39.4°C) and physiological functions (i.e. blood pressure, heart rate and expired air CO₂) were monitored continuously. Hematocrit and whole blood acid–base parameters (i.e. pH, pCO₂, pO₂, HCO₃ and O₂ saturation) were measured and disturbances in body fluid balance were corrected by appropriate procedures (e.g. forced ventilation and/or changes in infusion rates).

2.2. PET brain imaging

Racemic [O-methyl-¹¹C]venlafaxine HCl ([¹¹C]venlafaxine) was prepared as described in detail elsewhere (Gee et al., 1995). The pigs were studied in the supine position in the scanner (Siemens ECAT EXACT HR) using a custom-made head-holding device. The regional distribution and binding of [¹¹C]venlafaxine in pig brain was studied by administering an i.v. dose (540–1025 MBq) followed immediately by an i.v. injection of heparin solution to flush the catheter. Under control conditions, no further injections were given during the scanning period. Under conditions of antidepressant treatment, an intravenous dose of either paroxetine (5 mg/kg), citalopram (5 mg/kg) or imipramine (8 mg/kg) was administered at 20 min after injection of [¹¹C]venlafaxine in order to determine whether these antidepressant drugs influence the cerebral binding, kinetics and distribution of the radiotracer.

A sequence of twenty-eight arterial blood samples (1–2 cm³) were drawn from the pigs for determination of total plasma radioactivity concentration of [¹¹C]-labelled compounds. Metabolite correction was carried out using samples of arterial plasma (200 μl) alkalized with 10 μl 50% NaOH and to which 400 μl ethyl acetate was added. 200 μl of the organic layer was removed and the quantity of [¹¹C] radioactivity was determined. Plasma levels of unmetabolised [¹¹C]venlafaxine per cm³ plasma were obtained by decay correcting the [¹¹C] count to start of scanning and multiplying by the dilution factor.

Seven brain regions of interest (ROIs) were identified using a neuroanatomical atlas of the pig brain (Yoshikawa, 1968) (Fig. 1). For each region, radioactivity concen-
trations were calculated for the sequence of frames, were corrected for the radioactive decay of $^{11}$C (20.3 min) and were plotted versus time. The data for illustrations were expressed in terms of standard uptake values (SUV), i.e. [radioactivity in ROI (Bq/cc)×body weight (g)/injected dose of radioactivity (Bq)]. Normalization of the data was carried out by dividing SUVs obtained at a particular time in the ROI by SUVs in the cerebellum, a region devoid of neuronal 5-HT transporters (Scheffel and Hartig, 1989). The cerebellum (CB) was also used as reference region for determination of binding potential (Gjedde and Wong, 1991; Nybäck et al., 1994). Binding potential (B.P) was calculated as follows: B.P=$B_{ROI}/F=\frac{(A_{ROI}-A_{CB}/A_{CB})-1}{\{\left(\frac{K_{1,ROI}}{k_{2,ROI}}\right) / \left(\frac{K_{1,CB}}{k_{2,CB}}\right)-1\}}$. Estimation of pharmacokinetic parameters for a two-compartment model was carried out using tailor-made software. $K_{i}$ expresses the unidirectional clearance of the tracer from the circulation to the single tissue compartment, $k_{2}$ in the case of the cerebellum is the true rate constant for clearance from the brain, whereas $k_{2}'$ is an apparent rate constant of clearance from the single tissue compartment, assuming that equilibration between tissue compartments of solution and of binding are so rapid that a single compartment results. The $K_{i}/k_{2}'$ ratio is termed the apparent partition volume and expresses the binding of the compound in the ROI.

### 2.3. Platelet 5-HT reuptake

The reuptake of 5-HT into blood platelets was determined as described in detail elsewhere (Jensen et al., 1994). Briefly, 5-HT reuptake into blood platelets was measured at 4°C and 37°C. Drugs (venlafaxine, imipramine, paroxetine and citalopram) were added to the platelet suspension and the mixture was maintained at either 4°C or 37°C for 10 min with occasional bubbling with gas (95% O$_2$ and 5% CO$_2$) in order to maintain pH between 7.3–7.4. Then, $^{14}$C-5-HT (final concentration of 0.5 μmol/l) was added and incubation was continued for 15 sec. The incubation was terminated by filtration of the platelet suspension under vacuum. Active $^{14}$C-5-HT reuptake (pmol/10$^8$ platelet/min) was calculated by subtracting the reuptake at 4°C from that at 37°C.

### 2.4. Statistical analysis

Nonparametric tests and analysis of variance (ANOVA) were used for determining the statistical significance of the findings with SPSS PC+. Preliminary analysis of the PET data obtained after administration of either imipramine, paroxetine or citalopram failed to disclose reliable differences between their effects on $[^{11}]$Cvenlafaxine pharmacokinetics, so the findings were combined for assessing the statistical significance of antidepressant effects.

### Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug concentration (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>venlafaxine</td>
<td>25±5</td>
</tr>
<tr>
<td>citalopram</td>
<td>31±6</td>
</tr>
<tr>
<td>paroxetine</td>
<td>40±10</td>
</tr>
<tr>
<td>imipramine</td>
<td>20±6</td>
</tr>
</tbody>
</table>

Values shown are percentage inhibition of 5-HT uptake (means±s.e.m., N=4).

### 3. Results

#### 3.1. 5-HT uptake in pig blood platelets

Table 1 presents the effects of venlafaxine, imipramine, citalopram and paroxetine on the uptake of 5-HT into pig blood platelets. The antidepressants inhibited 5-HT uptake significantly and in a dose-dependent fashion ($p<$0.05). Fig. 2 shows the effects of venlafaxine and imipramine on uptake of 5-HT in blood platelets from humans or pigs. The drugs inhibited 5-HT uptake in a dose-dependent manner ($p<$0.001), and their inhibitory action was significantly greater in human blood platelets than in those obtained from pigs ($p<$0.001).

#### 3.2. PET neuroimaging

Fig. 3 shows the time course for $[^{11}]$Cvenlafaxine-derived radioactivity in selected brain regions under control conditions. Table 2 shows that the brain regions differed significantly with respect to the biodistribution of radioactivity (i.e. thalamus$>$frontal cortex$>$brainstem$>$...).
Therefore, we investigated whether 5-HT transport occurs in porcine blood platelets. Our findings show that porcine blood platelets accumulate 5-HT and that antidepressant drugs inhibit that process. Although venlafaxine was less potent as inhibitor of 5-HT transport in porcine blood platelets than in human blood platelets, our findings provide further evidence for 5-HT uptake sites in living pig brain (Brust et al., 1996).

Current interest in neuronal 5-HT transport is mainly related to the notion that disturbances in serotonergic functions are involved in the occurrence of depressive disorders and in the efficacy of antidepressant treatments (Hyttel, 1982; Feighner and Boyer, 1991). Venlafaxine inhibits the neuronal uptake of 5-HT as well as of catecholamines, but it is devoid of affinity for benzodiazepine, opiate and postsynaptic dopamine and serotonin receptors (Muth et al., 1986, 1991). In principle, \(^{11}C\)venlafaxine could be useful as PET radioligand for testing hypotheses concerning certain neurobiological processes, provided that certain criteria are met (Sedvall et al., 1986).

\(^{11}C\)venlafaxine was found to cross the blood-brain-barrier readily; it reached peak levels within 10–20 min after intravenous injection and declined gradually thereafter. The time course shown by \(^{11}C\)venlafaxine in living pig brain is appropriate for pharmacokinetic PET studies using \(^{11}C\)-labelled radioligands (Pike, 1993). The cerebral distribution and binding of \(^{11}C\)venlafaxine-derived radioactivity was primarily in diencephalic (i.e. thalamus and hypothalamus) and telencephalic regions (i.e. basal ganglia and cerebral cortex), which is of interest in light of the notion that disturbances in subcortical and frontal neuo pathways are responsible for depressive disorders (Drevets et al., 1992; Cummings, 1993).

An unexpected finding of the present study was that the binding potential of \(^{11}C\)venlafaxine was enhanced by a large dose of antidepressant drug (i.e. imipramine, paroxetine or citalopram). The reason for this apparent increase in cerebral binding of venlafaxine is unknown, and it would be premature to speculate on possible explanations (Ingle, 1972). It is, nonetheless, noteworthy that a similar finding was obtained in a previous PET study in which humans received fluoxetine together with the 5-HT uptake inhibitor \(^{11}C\)McN-5652-Z (Szabo et al., 1995).

In summary, the present findings show that \(^{11}C\)venlafaxine is not ideal for use as PET radioligand for imaging 5-HT uptake sites in living brain because (1) its binding potential in most brain regions was too low (i.e. < 1) (Pike, 1993; Malison et al., 1995) and (2) antidepressants with potent inhibitory actions on 5-HT uptake failed to displace it markedly from cerebral sites. Although \(^{11}C\)venlafaxine does not fulfil certain criteria as PET radiotracer for mapping cerebral 5-HT uptake sites in living brain, it may be possible to use it for estimating levels of venlafaxine in the brain of humans receiving

4. Discussion

Blood platelets have often been used as a peripheral model for neuronal 5-HT transport (Lingjærde, 1990).
Table 2
Pharmacokinetic parameters of $^{11}$C-venlafaxine-derived radioactivity in brain regions of living swine under control conditions and after administration of an antidepressant drug (Antidep)

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Test condition</th>
<th>$K_i$ (cm$^3$ g$^{-1}$ min$^{-1}$)</th>
<th>Pharmacokinetic parameter</th>
<th>Apparent partition volume$^a$</th>
<th>Binding potential$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$k_2^{a,*}$</td>
<td></td>
<td>$(K_i/k_2)$</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Control</td>
<td>0.41±0.03</td>
<td>0.027±0.005</td>
<td>16.8±4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antidep</td>
<td>0.43±0.04</td>
<td>0.034±0.009</td>
<td>18.3±8.1</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>Control</td>
<td>0.68±0.07</td>
<td>0.025±0.006</td>
<td>32.1±7.0</td>
<td>0.96±0.18</td>
</tr>
<tr>
<td></td>
<td>Antidep</td>
<td>0.90±0.10</td>
<td>0.038±0.011</td>
<td>38.4±18.0</td>
<td>1.01±0.16</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>Control</td>
<td>0.59±0.07</td>
<td>0.021±0.004</td>
<td>31.5±7.5</td>
<td>0.89±0.16</td>
</tr>
<tr>
<td></td>
<td>Antidep</td>
<td>0.83±0.06</td>
<td>0.037±0.011</td>
<td>34.8±15.7</td>
<td>0.87±0.10</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>Control</td>
<td>0.59±0.07</td>
<td>0.025±0.004</td>
<td>26.4±6.2</td>
<td>0.58±0.09</td>
</tr>
<tr>
<td></td>
<td>Antidep</td>
<td>0.73±0.04</td>
<td>0.034±0.009</td>
<td>32.3±14.2</td>
<td>0.75±0.10</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>Control</td>
<td>0.59±0.07</td>
<td>0.024±0.005</td>
<td>27.6±6.0</td>
<td>0.68±0.13</td>
</tr>
<tr>
<td></td>
<td>Antidep</td>
<td>0.66±0.06</td>
<td>0.032±0.008</td>
<td>31.2±14.0</td>
<td>0.66±0.15</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>Control</td>
<td>0.57±0.05</td>
<td>0.021±0.004</td>
<td>29.7±6.2</td>
<td>0.83±0.16</td>
</tr>
<tr>
<td></td>
<td>Antidep</td>
<td>0.82±0.05</td>
<td>0.035±0.010</td>
<td>35.8±14.6</td>
<td>1.00±0.18</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>Control</td>
<td>0.56±0.05</td>
<td>0.030±0.006</td>
<td>22.6±6.0</td>
<td>0.31±0.08</td>
</tr>
<tr>
<td></td>
<td>Antidep</td>
<td>0.64±0.06</td>
<td>0.035±0.009</td>
<td>28.6±14.0</td>
<td>0.47±0.10</td>
</tr>
<tr>
<td>Brainstem</td>
<td>Control</td>
<td>0.56±0.06</td>
<td>0.032±0.007</td>
<td>20.2±4.8</td>
<td>0.20±0.07</td>
</tr>
<tr>
<td></td>
<td>Antidep</td>
<td>0.71±0.05</td>
<td>0.041±0.012</td>
<td>24.7±10.2</td>
<td>0.39±0.08</td>
</tr>
</tbody>
</table>

Values shown are means±s.e.m. ($N=4$).

$^aK_i$ for cerebellum.

$^b$Steady-state volume of distribution.

$^c$Binding potential=[$(K_{1\text{ROI}}/k_2\text{ROI})/(K_i/\text{Cerebellum})/k_2\text{Cerebellum})]^{-1}$. See Section 2 for further explanation of the basis for this equation.

venlafaxine as antidepressant therapy (van Wijk et al., 1977).

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References


Fig. 4. Normalized SUV for level of $[^{11}$C]venlafaxine-derived radioactivity in thalamus of living pig brain under control conditions (unfilled circles) and after administration of an antidepressant drug (filled circles). Values shown are means ($N=4$).


