Vinpocetine: a prospective peripheral benzodiazepine receptor ligand for primate PET studies

Balázs Gulyás, Christer Halldin, Ádám Vas, Richard B. Banati, Evgeny Shchukin, Sjoerd Finnema, Jari Tarkainen, Károly Tihanyi, Géza Szilágyi, Lars Farde

Abstract

Vinpocetine, a synthetic derivative of the Vinca minor alkaloid vincamine, is a widely used drug in neurological practice. We tested the hypothesis that vinpocetine binds to peripheral benzodiazepine binding sites (PBBS) and is therefore a potential ligand of PBBS. Positron emission tomography (PET) measurements in two cynomolgous monkeys showed that pretreatment with vinpocetine markedly reduced the brain uptake of [11C]PK11195, a known PBBS radioligand. On the other hand, whereas pretreatment with PK11195 increased the brain uptake of [11C]vinpocetine due to the blockade of PBBS in the periphery, it significantly reduced the binding potential (BP) values of [11C]vinpocetine in the whole brain and in individual brain structures to PK11195. These findings indicate that, whereas the two ligands have different affinities to PBBS, vinpocetine is a potent ligand of PBBS, which in turn suggests that the pharmacological activity of vinpocetine may involve the regulation of glial functions.

Keywords: Vinpocetine; PK11195; Peripheral benzodiazepine binding site (PBBS); Monkey brain; Glia; Positron emission tomography (PET)

1. Introduction

Vinpocetine (ethyl-apovincaminate) is a synthetic compound, structurally related to the Vinca minor alkaloid vincamine. The drug (Cavinton®, Gedeon Richter, Budapest, Hungary) has since 1978 been widely used as a neuroprotective agent for the prevention and treatment of various cerebrovascular diseases [1].

Whereas the drug’s clinical efficacy has been documented by several trials, the mechanisms of action have been a subject for physiological, pharmacological and neuroimaging studies over the past decades. It has been demonstrated that vinpocetine is a potent inhibitor of the voltage-dependent Na+ channels and the Ca2+/calmodulin-dependent phosphodiesterase 1 [1]. Using positron emission tomography (PET), we have shown in both monkeys and man that [11C]vinpocetine rapidly enters the brain after intravenous injection, the maximal uptake being approximately 4% of the total injected radioactivity [2,3]. The distribution pattern of vinpocetine in the brain is heterogeneous, with the highest uptake in the thalamus, basal ganglia and visual cortex. A similar distribution, with absence of any evident binding to transmitter receptors or transporters, has been demonstrated ex vivo by [11C]vinpocetine autoradiography on cryosectioned
whole hemisphere human brain slices [4]. Brain imaging has shown that vinpocetine administered in pharmacological doses increases the regional cerebral glucose uptake and glucose metabolism in the intact brain tissue in peristroke regions [5,6].

A 2-week-long treatment increased the regional cerebral blood flow especially in the thalamus, basal ganglia and visual cortex of the nonaffected hemisphere [7].

This presence of a region-specific pattern of \( ^{11}C \)vinpocetine binding in brain that corresponds to physiological changes in cerebral blood flow and metabolism in the same brain regions supports that vinpocetine binds to biologically active specific sites that may mediate the known direct clinical effects of the drug.

Recent in vitro studies confirm the receptorogram of vinpocetine and show that the drug has the highest binding affinity to peripheral benzodiazepine binding sites (PBBS) in the rat heart (IC-50=0.2 μM) (Table 1). This affinity is higher than the affinity of other previously reported sites of vinpocetine binding in the brain (cf. e.g., Refs. [2,8]).

Based on the relatively high affinity for PBBS, we hypothesised that in vivo binding of vinpocetine competes with the binding of the isoquinoline PK11195, a reference ligand for the PBBS [9]. To explore this hypothesis, we measured the brain uptake and distribution of \( ^{11}C \)vinpocetine and \( ^{11}C \)PK11195 in two cynomolgous monkeys at baseline conditions and during pretreatment with the unlabelled ligands.

2. Methods

2.1. Animals and ethical committee approval

Two female cynomolgous monkeys (weight: 4.0 and 7.1 kg; age: 4 and 8 years, respectively) were used in consecutive PET measurements. The study was covered by permission of the Animal Research Ethical Committee of the Northern Stockholm Region (1998/14).

2.2. Radiochemistry

2.2.1. Synthesis of \( ^{11}C \)vinpocetine

The synthesis was performed according to a procedure previously described [2]. Vinpocetine (Batch No.: K6B0110) and the precursor for the radiochemical synthesis, apovincaminic acid (Batch No.: H-2190), were obtained from Gedeon Richter. The specific radioactivity obtained at time of injection of \( ^{11}C \)vinpocetine was higher than 2.8 GBq/μmol, mol, corresponding to a total mass injected of less than 20 μg. The radiochemical purity was better than 99%.

2.2.2. Synthesis of \( ^{11}C \)PK11195

The synthesis was performed according to a procedure previously described [10]. The specific radioactivity obtained at time of injection of \( ^{11}C \)PK11195 was higher than 50 GBq/μmol, corresponding to a total mass injected of less than 2 μg. The radiochemical purity was better than 99%.

2.3. The PET system

The PET system used was ECAT EXACT HR47 (Siemens), with a spatial resolution (in-plane) of about 3.8 mm full width at half maximum (FWHM). The system was used in the three-dimensional mode, and the reconstructed volume was displayed as 47 horizontal sections with a center-to-center distance of 3.125 mm. The axial field-of-view was 15 cm. Radioactivity in the brain was measured continuously according to a preprogrammed sequence. Each PET measurement was made according to a 63-min-long data acquisition protocol consisting of 15 time frames (3×1 min, 4×3 min, 8×6 min). Attenuation correction was obtained from a 10-min transmission scan using a \(^{68}Ge \) radiation source.

2.4. Experimental procedures

The monkeys were anaesthetised with repeated intramuscular injections of a combination (50–50%; 0.8–1.0 ml/h) of ketamine (Ketalar®, Parke-Davis; 50 g/ml) and xylazine (Rompun® vet., Bayer; 20 mg/ml). The monkeys were positioned in the PET system with the help of a head fixation device that maintained the same head position during and between the measurements. The image planes were parallel to that defined by the canto-meatal line. In each monkey, a cannula was fixed in a sural vein for intravenous administration of the tracer.

The first monkey received two injections [66 and 66 MBq (1.78 mCi)] of \( ^{11}C \)PK11195. After the first injection, a baseline PET measurement was made. Eight minutes prior to the second injection, 3 mg/kg of unlabelled vinpocetine was injected intravenously (pretreatment measurement). The second monkey received two injections [63 and 66 MBq (1.70 and 1.78 mCi)] of \( ^{11}C \)vinpocetine. After the first

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Tissue</th>
<th>IC-50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzodiazepine, peripheral</td>
<td>Rat heart</td>
<td>0.2</td>
</tr>
<tr>
<td>Adrenergic α1A</td>
<td>Rat kidney cortex</td>
<td>0.9</td>
</tr>
<tr>
<td>Adrenergic α2A</td>
<td>Human recombinant</td>
<td>1.9</td>
</tr>
<tr>
<td>Na(^+) channel, site 2</td>
<td>Rat brain</td>
<td>1.9</td>
</tr>
<tr>
<td>Ca(^{2+}) channel, L-type,</td>
<td>Rat cerebral cortex</td>
<td>2.1</td>
</tr>
<tr>
<td>benzothiazepin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenergic α1A</td>
<td>Rat submaxillary gland</td>
<td>2.9</td>
</tr>
<tr>
<td>Ca(^{2+}) channel, L-type,</td>
<td>Rat brain</td>
<td>4.1</td>
</tr>
<tr>
<td>phenylalkylamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine D4.2</td>
<td>Human recombinant</td>
<td>7.9</td>
</tr>
<tr>
<td>Adenosine A1</td>
<td>Rat brain</td>
<td>8.3</td>
</tr>
</tbody>
</table>
injection, a baseline PET measurement was made. In the pretreatment measurement, 9 min prior to the injection, 1 mg/kg of unlabelled PK11195 was injected intravenously.

2.5. Image analysis and calculations

The individual brain images were stereotactically standardized using the computerized brain atlas system of the Karolinska Institutet [11]. Global and regional radioactivity values were measured in whole brain and selected volumes of interest (VOIs). Global and regional uptake and distribution measurements were made on the basis of the summation images representing an average for the period between 9 and 63 min after tracer administration. The tissue uptake of labelled vinpocetine and PK11195 and the regional and whole brain binding potential (BP) values were determined by using the linear graphical analysis according to Logan et al. [12] using the cerebellar cortex as reference region (cf. Fig. 2) and corresponding to the linear part of the plot covering the last 39–63 min of measurement.

3. Results

3.1. Global brain radioactivity and cerebral kinetics of \([^{11}\text{C}]\text{PK11195}\) and \([^{11}\text{C}]\text{vinpocetine}\)

After iv injection of \([^{11}\text{C}]\text{PK11195}\), 0.77% of total injected activity was found in brain between 9 and 63
min after injection. The maximum fraction of radioactivity in brain was 1.17% at 1.5 min after radioligand injection. In the pretreatment condition, only 0.60% of total injected radioactivity was found in the brain (compared to the baseline condition, 22% change).

The maximum fraction of radioactivity in brain under pretreatment condition was 1.00% at 1.5 min after tracer injection (Fig. 1A).

After iv injection of [11C]vinpocetine, 3.84% of total injected activity was found in brain between 9 and 63 min after injection, and the maximum amount of radioactivity in the brain was 5.90% at 7.5 min after tracer injection. After pretreatment with unlabelled PK11195, the average amount of radioactivity in brain was 5.96% of the total injected radioactivity (compared to the baseline condition: 36% increase). The maximum amount of radioactivity in the brain (6.32%) was at 10.5 min after tracer injection (Fig. 1B).


In Fig. 2, average PET summation images, obtained after the injection of [11C]PK11195 and [11C]vinpocetine, are shown, displaying both baseline and pretreatment conditions. The changes of regional radioactivity values in selected brain volumes of interest (VOIs) are shown in Fig. 3.

3.3. Binding potential values before and after pretreatment

The binding potential (BP) was calculated using a linear graphical approach. The BP for [11C]PK11195 was 0.07 and 0.06 at baseline and pretreatment conditions, respectively. Due to the low brain uptake of the ligand, no reasonable binding potential (BP) analysis could have been performed for individual structures in the brain. On the other hand, the BP values in the parotis, an organ with high PBBS density, were 1.95 and 1.70 (left and right, respectively) at baseline and 1.50 and 1.45 after pretreatment with vinpocetine (−19% change).

The BP values in the case of [11C]vinpocetine are shown in Table 2. As shown in the table, there was a marked decrease in global BP values in the cerebrum (−40%), as well as in all selected brain regions, following pretreatment with PK11195.

4. Discussion

In line with earlier observations [13], the brain uptake of PK11195 was low, and only 0.77% of the total injected radioactivity of [11C]PK11195 entered the brain. This amount was markedly reduced following pretreatment with vinpocetine. This indicates that vinpocetine may bind to binding sites that are occupied by PK11195. However, full examination of regional BP values of [11C]PK11195 is limited due to the low cerebral concentration of the ligand and the low density of the PBBS in the normal brain as demonstrated by autoradiography [14].

In line with earlier observations [2], [11C]vinpocetine passed the blood–brain barrier and entered the brain readily. Of the total injected radioactivity, 3.84% was in the brain, and this fraction markedly increased to 5.94% following pretreatment with the known PBBS ligand PK11195. This behaviour is characteristic for pretreatment conditions of those receptors which have a relatively large number in the periphery, and the blockade of the peripheral receptors by a

Table 2

<table>
<thead>
<tr>
<th>Region</th>
<th>Baseline</th>
<th>Pretreatment</th>
<th>Change in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate nucleus</td>
<td>0.60</td>
<td>0.36</td>
<td>−40</td>
</tr>
<tr>
<td>Putamen</td>
<td>0.33</td>
<td>0.13</td>
<td>−61</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>0.99</td>
<td>0.71</td>
<td>−35</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>0.77</td>
<td>0.50</td>
<td>−30</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>0.97</td>
<td>0.68</td>
<td>−32</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>0.50</td>
<td>0.34</td>
<td>−48</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>0.71</td>
<td>0.26</td>
<td>−32</td>
</tr>
<tr>
<td>Cingulate cortex</td>
<td>0.60</td>
<td>0.48</td>
<td>−45</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.69</td>
<td>0.33</td>
<td>−40</td>
</tr>
</tbody>
</table>

Reference region: cerebellar grey matter, cf. Fig. 2B.
known ligand, administered in pharmacological doses (in the present case by PK11195), results in an increased amount of a radioligand, selective for the same receptor type and administered in tracer doses, in the brain.

The two pretreatment conditions resulted in fundamentally different patterns. Compared to the baseline condition following the injection of \[^{[11C]}\text{PK11195}\], pretreatment with vinpocetine resulted in a lower global brain radioactivity uptake (0.77% and 0.60% of the total injected radioactivity, respectively). Compared to the baseline condition following the injection of \[^{[11C]}\text{vinpocetine}\], in the case of pretreatment with PK11195, the global brain radioactivity uptake was markedly higher (3.84% and 5.96%, respectively). The measured radioactivity uptake in the brain is the result of mainly two factors: (i) the relative relationship between both ligands with regard to their respective ratios between the affinity of ligand binding in the periphery as compared to that in the brain and (ii) the relative differences in the extraction of both ligands. For the cold ligand to displace the hot ligand in the peripheral binding compartment more than in the brain and thus result in relatively higher uptake values in the brain (as was the case of pretreatment with PK11195 prior to the injection of \[^{[11C]}\text{vinpocetine}\]), the affinity of the hot ligand in the periphery has to be less than in the brain. This affinity difference, together with the marked difference in the brain uptake values of the two ligands, may explain the above observation. As at this time no data are available regarding the two ligands’ relative affinity values, further experiments are needed to clarify this issue.

Despite the fact that approximately five times more vinpocetine enters the brain than PK11195, pretreatment with the known PBBS ligand PK11195 reduces markedly the BP values for vinpocetine in the brain, supporting the fact that the two ligands bind to the same receptor site (and that PK11195 does enter the brain albeit much less than vinpocetine).

These observations taken together, the present experiments support the hypothesis that vinpocetine is a ligand with relatively high affinity for PBBS. \[^{[11C]}\text{vinpocetine}\] can be used as a potential PET marker of PBBS and consequently glia cells in the primate brain. Furthermore, the present observations may support the view that, if indeed the PBBS, which is absent in neurons, is a major binding site for vinpocetine, its therapeutic action is likely to involve the modulation of glial cells [15,16).

Acknowledgement

The authors express their gratitude to Ms. Kjerstin Lind and Mr. Julio Gabriel for their technical assistance during the experiments.

References