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AUTORADIOGRAPHIC EVALUATION OF [^{11}C]VINPOCETINE BINDING IN THE HUMAN *POSTMORTEM* BRAIN*

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The main objective of the study was to evaluate with autoradiographic technique whether or not [^{11}C]vinpocetine, a compound widely used in the prevention and treatment of cerebrovascular diseases (Cavinton®, Gedeon Richter Ltd., Budapest), binds to specific sites in the human brain in post mortem human brain sections. Binding was assessed under four conditions: the incubation was performed using Tris-HCl buffer with or without the addition of salts (0.1% (weight/vol) ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$), with or without the addition of excess (10 μM) unlabelled vinpocetine.

Measurements on digitized autoradiograms indicated that [^{11}C]vinpocetine labelled all grey matter areas in the human brain to a similar extent and no significantly heterogeneous binding could be demonstrated among cortical or subcortical regions. The addition of excess unlabelled vinpocetine lowered the binding slightly in all regions. Although these results indicate that [^{11}C]vinpocetine does not bind to human brain transmitter receptors or transporters with a high affinity (Ki < 10 nM), it cannot be ruled out that the compound binds to receptors and/or transporters with lower affinity.

Keywords: Human brain – vinpocetine – autoradiography – post mortem neuroimaging – receptor binding

INTRODUCTION

Vinpocetine (ethyl apovincaminate), is a synthetic compound related to the Vinca minor alkaloid vincamine. The compund was originally discovered and developed by the Chemical Works of Gedeon Richter Ltd., Budapest, and it was introduced and registered in the mid seventies as Cavinton®. The drug is recently used in over 40 countries as a neuroprotective drug in the prevention and treatment of cerebrovascular diseases.

During the past decades several investigations aimed at the exploration of molecular level mechanisms and the results suggest various hypotheses explaining drug action [2]. One hypothesis is that the drug increases cAMP and cGMP levels by inhibition of the phosphodiesterase-1 iso-enzyme system [1, 12, 20].Vinpocetine also inhibits the voltage-dependent Na^+-channels, thereby protecting the surviving neurones from further destruction after ischaemic damage [3, 13, 19, 28, 29].

*Dedicated to Professor József Hámori on the occasion of his 70th birthday.

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Vinpocetine may also interact with glutamate receptors and protect cells against the cytotoxic effect of glutamate [14, 15]. In addition to enzymatic and receptor-related effects, vinpocetine can act directly as an antioxidant [24] and protect from effects of regional and global hypoxia [11, 16, 26, 30]. These studies support the hypothesis that vinpocetine is a centrally acting compound with direct CNS effects.

Alternatively, it has also been suggested that vinpocetine has an effect on blood flow, in general, and on regional cerebral blood flow (rCBF), in particular, and this latter effect does not necessarily reflect a change in neurotransmission and/or increased neuronal activity [17, 18, 22, 25]. Although a number of mechanisms for vinpocetine drug action has already been identified, it is still not clear whether *in vivo* the drug has direct and specific effects on neuronal tissue if its effects are due to non-specific actions, such as changes in cerebral blood flow. In order to understand the primary action of vinpocetine in neuronal tissue, it is of great interest to explore whether the drug enters the brain and binds to specific sites in it.

In the light of these questions, a series of positron emission tomography (PET) studies explored vinpocetine action in monkeys, healthy human subjects and stroke patients. Earlier PET experiments with labelled vinpocetine in monkeys indicated that the drug readily enters the brain and that the uptake in the primate brain is heterogeneous. This result has been supported by human PET studies using labelled vinpocetine with both intravenous and oral administration [5]. Compared with earlier PET studies in stroke patients, the aforementioned investigations on primates and humans also indicated that the major sites for pharmacological actions overlap with the anatomical structures displaying the highest uptake (thalamus, striatum, occipital cortex). In chronic stroke patients, FDG-PET studies have indicated that the drug affects both rCBF and cerebral metabolic rate of glucose (CMRglu), and the major sites of actions are identical with the optimal binding sites with radiolabelled vinpocetine [4, 27].

These observation support the hypothesis that vinpocetine may bind to specific sites in the primate brain. It has, however, not been explored whether this accumulation of vinpocetine is mainly due to an increased vascularisation in these structures [23], resulting in relatively higher blood flow, or some kind of – specific or non-specific – binding.

In order to explore whether vinpocetine may accumulate in, and probably binds to, these structures even when no circulation is present in the brain, we performed the present dedicated *postmortem* human autoradiography study using [^{11}C]vinpocetine.

MATERIALS AND METHODS

Brain tissue

Human *postmortem* brain tissue was obtained by clinical forensic autopsy at the National Institute of Forensic Medicine, Karolinska Institutet, Stockholm, Sweden. The study was approved by the Ethics Committee at Karolinska Institutet and the

Swedish Board of Social Welfare. Whole hemispheres were removed, frozen and cryosectioned as described earlier [6, 7, 9], using a heavy-duty cryomicrotome (Leica cryomacrocut CM3600, Leica, Nussloch, Germany). The tissue cryosections (thickness 100 µm) were transferred to gelatinized glass plates (10×22 cm), dried at room temperature and were then stored with dehydrating agents (–25 °C) until use.

Chemistry and radiochemistry

Vinpocetine (Batch No. K6B0110) and the precursor apovincaminic acid (Batch No. H-2190) were obtained from Gedeon Richter Ltd., Budapest. Other chemicals were obtained from commercial sources and were of analytical grade purity wherever possible. [^{11}C]Ethyl iodide was synthesised from [^{11}C]carbon dioxide utilising an on-pot reaction set-up similar to that reported previously [10].

Semipreparative reversed-phase HPLC was performed using a Kontron 420 pump, an automatic sample injector (Type VICI with a 1 ml loop), a Waters µ-Bondapak-C18 column (300×7.8 mm, 10 µm), and a Kontron 432 UV-detector (wavelength = 254 nm) in series with a GM tube for radiation detection. [^{11}C]vinpocetine was purified using acetonitrile and 0.01 M phosphoric acid (30/70) as the mobile phase with a flow rate of 6.0 ml/min. The radiochemical purity of [^{11}C]vinpocetine was analysed by reversed-phase HPLC using a Kontron 420 pump, a Rheodyne injector (7125 with a 100 µl loop) equipped with Waters µ-Bondapak-C18 column (300×4.6 mm, 10 µm) and an LDC-Milton Roy 300 UV-spectrophotometer (254 nm) in series with a Beckman 170 radioactivity detector. Acetonitrile and 0.01 M phosphoric acid (30/70) were used as the mobile phase with a flow rate of 3.0 ml/min.

The labelling procedure has been described in details earlier [5]. Shortly, [^{11}C]ethyl iodide was trapped at room temperature in a reaction vessel (1.0 ml minivial, Alltech), containing apovincaminic acid (2.0 mg), dimethylformamide (DMF, 250 µl), and sodium hydroxide (4 µl, 5M). The vessel was sealed and heated at 120 °C for 8 min. Mobile phase (700 ml) was added before injection onto the semipreparative HPLC column. [^{11}C]vinpocetine eluted after 13–15 min with a retention time identical to a standard reference sample. After evaporation of the mobile phase, the residue was dissolved in 8 ml sterile physiological phosphate buffered saline (pH = 7.4) and filtered through a Millipore filter (0.22 mm), yielding a solution being sterile and free from pyrogens. The specific radioactivity obtained at time of incubation of [^{11}C]vinpocetine was appr. 135 Ci/mmol (3.7 GBq/µmol).

Autoradiography

For autoradiography using [^{11}C]vinpocetine, four cryosections were mounted separately into a specially designed incubation chamber, which was kept behind lead bricks to minimize radiation exposure to personnel [21]. The cryosections were incubated with [^{11}C]vinpocetine, 0.2 MBq/ml, in Tris-HCl buffer pH 7.4, 0.05 M (incu-

bation volume 200 ml per section) for 20 min at room temperature. For the first two cryosections, the incubation was performed using Tris-HCl buffer without the addition of salts. The third and fourth sections were incubated in a Tris-HCl buffer containing 0.1% (weight/vol) ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. To determine the level of non-specific binding, incubations were performed in the absence or presence of 10 μM unlabeled vinpocetine. After incubation, the radioactivity was pumped out of the incubation chambers, and the sections were rinsed twice for 2 min in Tris-HCl buffer (same conditions as above). The sections were then rapidly rinsed in distilled water and then placed on a warm plate in a warm, dry airflow to facilitate rapid drying. β-sensitive films (Kodak BioMax MR) were applied to the sections overnight before development (developer: Kodak D19, fixation: Kodak Fixer 3000).

Autoradiograms were digitized using a ScanMaker E6 high-resolution scanner (Microtek) and Adobe Photoshop 5.0. Measurements were performed using Adobe Photoshop 5.0. Microsoft PowerPoint was used for preparation of the images.

RESULTS

[^{11}C]Vinpocetine labelled all grey matter areas in the human brain to a similar extent (Fig. 1). The addition of excess (10 μM) unlabelled vinpocetine lowered the binding slightly in all regions (Table 1). As can be seen from the autoradiograms, the accumulation of binding was markedly lower in the white matter areas upon addition of excess vinpocetine. A similar effect has been seen with other radioligands as well, and is normally regarded as an artifact [8].

As the ion dependence of [^{11}C]vinpocetine was unknown, the experiments were performed at two different conditions, with and without the addition of a standard salt composition, used in several other binding assays. No marked differences with or without salts were found.

Table 1

Autoradiographic evaluation of [^{11}C]vinpocetine binding in the human *postmortem* brain

Mean pixel values corresponding to blackness for the different brain regions (in brackets: difference between reference value in the white matter and the value in the given anatomical structure)

Region	46L; 69.5 Total binding	46L; 69.6 +vinpocetine	46L; 69.7 Total binding	46L; 69.8 +vinpocetine
White matter	126	109	134	94
Thalamus	136 (10)	128 (19)	135 (1)	120 (26)
Frontal cortex	162 (36)	159 (50)	175 (41)	142 (48)
Insular cortex	148 (22)	137 (28)	146 (12)	131 (37)
Medial occipital cortex	129 (3)	113 (4)	138 (4)	113 (19)
Temporal cortex	148 (22)	152 (43)	163 (29)	140 (46)
Caudate nucleus	134 (8)	120 (11)	131 (–3)	118 (24)
Putamen	143 (17)	131 (22)	139 (5)	129 (35)

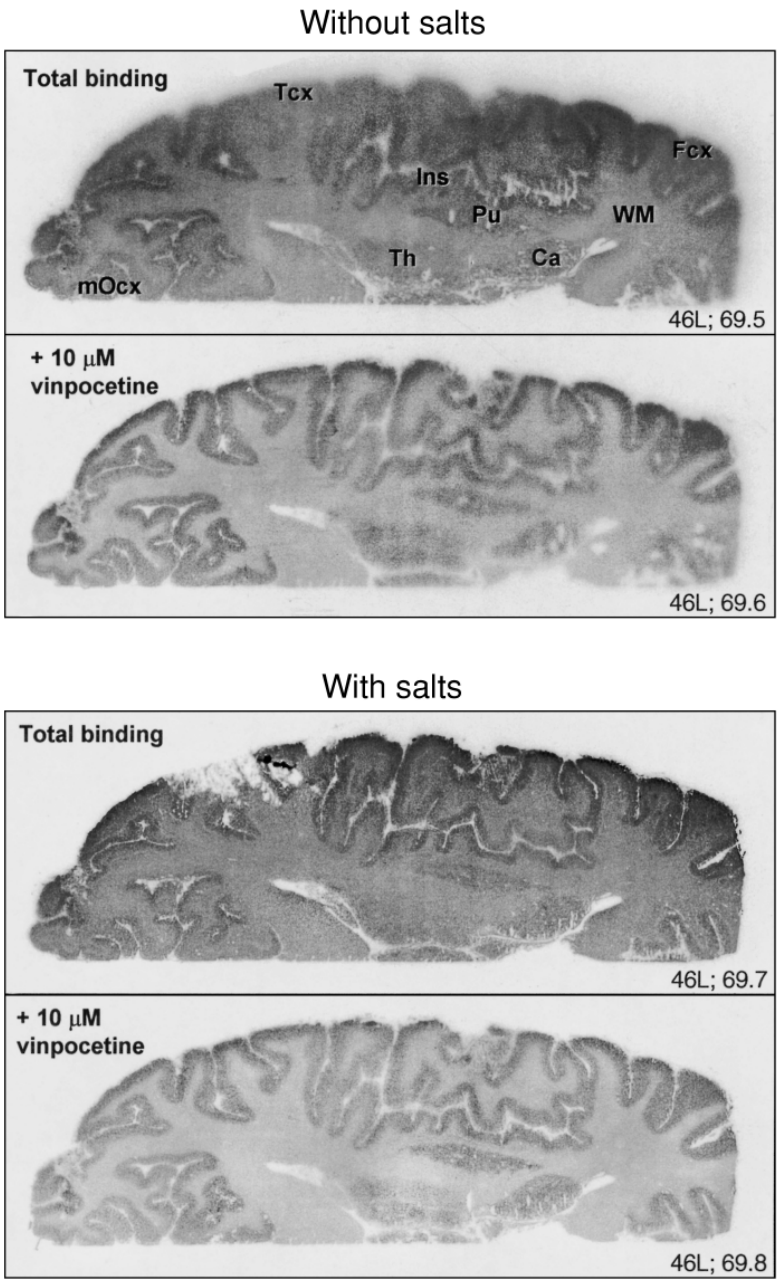

Fig. 1. Autoradiographic evaluation of [^{11}C]vinpocetine binding in the human *postmortem* brain. Ca – caudate nucleus; Fcx – frontal cortex; Ins – insular cortex; mOcx – medial occipital cortex; Pu – putamen; Tcx – temporal cortex; Th – thalamus; WM – white matter

DISCUSSION

The visible and measurable differences in labelling between white matter and grey matter indicate an increased uptake and accumulation of vinpocetine in cortical and subcortical gray matter structures.

The present results suggest that [^{11}C]vinpocetine does not bind to human brain transmitter receptors or transporters with a high affinity (Ki < 10 nM). However, it cannot be ruled out that this compound binds to receptors/transporters with lower affinity, which is not possible to visualize with the *in vitro* whole hemisphere autoradiographic technique.

In light of the *in vivo* data, showing a large difference in uptake values of [^{11}C]vinpocetine between the white matter and certain cortical (occipital cortex) and sub-cortical grey matter structures (thalamus, basal ganglia), it is surprising that the present post mortem autoradiography data display the largest differences in labelling intensities between the white matter and the frontal cortex, the temporal cortex and the insular cortex.

The present findings support the view that vinpocetine has no specific binding sites in the human brain. Uptake and accumulation of the labelled drug in cortical and subcortical structures can most probably be explained by low affinity binding to a number of sites in the human brain, suggested by the drug's receptogram (PanLabs; Table 2). In addition to low affinity binding mechanisms, a number of other *in vivo* mechanisms (local transport facilitation, regional perfusion changes, etc.) may be considered when explaining the relatively large regional differences, seen in *in vivo* studies in the human and non-human primate brain, favouring the thalamus, the basal ganglia and the occipital cortex. The identification and exploration of these mechanisms require further *in vivo* and *in vitro* studies.

Table 2
In vitro receptorial effects of vinpocetine
(PanLabs, SpectrumScreen® results, nine highest affinity values selected)

Receptor	Tissue	IC_{50} (μM)
Adenosine A_1	Rat brain	8.3
Adrenergic α_{1A}	Rat submaxillary gland	2.9
Adrenergic α_{2A}	Human recombinant	1.9
Adrenergic α_{2B}	Rat kidney cortex	0.9
Ca^{++} channel, L-type, benzothiazepin	Rat cerebral cortex	2.1
Ca^{++} channel, L-type, phenylalkylamine	Rat brain	4.1
Dopamine $D_{4.2}$	Human recombinant	7.9
$GABA_A$, benzodiazepine, peripheral	Rat heart	0.2
Na^+-channel, site 2	Rat brain	1.9

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