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Binding characteristics of brivaracetam, a selective, high affinity SV2A ligand in rat, mouse and human brain: Relationship to anti-convulsant properties

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ABSTRACT

Brivaracetam is a novel synaptic vesicle protein 2A (SV2A) ligand reported to be 10 fold more potent than levetiracetam in animal models of epilepsy. This study reports the binding profile of brivaracetam in the brain of several species in relation to its anticonvulsant properties.

The affinity, kinetics and selectivity of brivaracetam and its tritiated form [^{3}H]ucb 34714 have been determined by in vitro binding experiments in rat, human and mouse brain and on recombinant human SV2A. Brivaracetam and levetiracetam ex vivo binding to SV2A and anticonvulsant activities in audiogenic mice were compared in relation to dose and time.

Brivaracetam bound selectively with 20 fold higher affinity than levetiracetam to SV2A. [^{3}H]ucb 34714 bound reversibly and with high affinity to an homogenous population of binding sites in rat and human brain and to human SV2A expressed in CHO cells. The binding sites labeled by [^{3}H]ucb 34714 in brain had the pharmacological characteristics of SV2A and no specific binding could be detected in the brain of SV2A$^{-/-}$ knock-out mice. The time- and dose-dependency of brivaracetam and levetiracetam for binding to brain SV2A and for providing seizure protection in audiogenic mice correlated well; brivaracetam being more potent and faster than levetiracetam.

Brivaracetam is a potent and selective SV2A ligand. From its affinity and pharmacokinetics, simulations predicted that at therapeutically relevant doses, brivaracetam should occupy more than 80% of SV2A in human brain, in line with levels of occupancy observed in pre-clinical models of epilepsy.

1. Introduction

Close to 50 million patients worldwide are suffering from epilepsy, a disease characterized by recurrent spontaneous seizures. About two third of them will be relieved by current therapies. However, despite the introduction over the past fifteen years of several new antiepileptic drugs, one third of patients will show resistance to treatment. While, in general, these newer antiepileptic drugs have improved properties with respect to pharmacokinetic and safety profiles they still fail to provide complete relief of the symptoms, thereby leaving an important medical need unmet (French and Pedley, 2008; Meldrum and Rogawski, 2007).

Levetiracetam (Keppra®), is a novel antiepileptic drug believed to primarily exert its activities through a unique mechanism of action involving binding to SV2A, a protein present in synaptic vesicles (for review see Klitgaard and Verdru, 2007).

Following the discovery of SV2A as the target of levetiracetam (Lynch et al., 2004), a target-based rational drug discovery program was initiated with the purpose of identifying more potent SV2A ligands that provided more complete seizure suppression than levetiracetam. This program led to the identification of brivaracetam (ucb 34714) as a compound displaying at least 10-fold higher affinity than levetiracetam for SV2A (Kenda et al., 2004). Brivaracetam has been extensively evaluated and compared to levetiracetam in a wide variety of in vitro and in vivo preclinical models of seizures and epilepsy. This assessment has demonstrated that brivaracetam exerts a more potent seizure protection than levetiracetam and, in several models, a more complete seizure suppression (Matagne et al., 2008).

Brivaracetam, is currently in phase III clinical studies following positive results in phase IIa trials that showed high efficacy in patients suffering from photosensitive epilepsy (Kasteleijn-Nolst Trenite et al., 2007) and in phase IIb trials that showed efficacy and high tolerability as an adjunctive treatment in adult patients with refractory partial-onset epilepsy (Malawska and Kulig, 2008; van Paesschen and Brodsky, 2007).

Since brivaracetam is differentiated from levetiracetam not only in potency but also in efficacy in several preclinical models of epilepsy, it has been postulated that either additional mechanisms of action for brivaracetam might exist besides binding to SV2A, or alternatively that the binding of brivaracetam to SV2A might lead to a qualitatively different functional response (Matagne et al., 2008). In that context,

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brivaracetam but not levetiracetam has been shown to inhibit voltage-dependent Na^+ channels in rat cortical neurons albeit at high concentrations (Zona et al., 2010).

To further explore the possibility that there may be additional targets for brivaracetam, we extensively investigated the binding properties of radiolabeled and unlabeled brivaracetam in human, mouse and rat brain and to human recombinant SV2A protein expressed in CHO cells. We also performed ex vivo binding experiments in audiogenic mice in order to gain better insight into the pharmacodynamics of brivaracetam and the relationship existing between SV2A binding and protection against seizure. Finally, from the results obtained in this study and others, we extrapolated and simulated SV2A occupancy by brivaracetam in human epilepsy patients.

2. Material and methods

2.1. Chemicals

Levetiracetam (2S-(2-oxo-1-pyrrolidinyl)butanamide), brivaracetam (2S-2-[(4R)-2-oxo-4-propylpyrrolidin-1-yl]butanamide; ucb 34714) and analogs were synthesized at UCB (Braine-l'Alleud, Belgium). Bemegride, bicuculline and chlordiazepoxide were purchased from Sigma-Aldrich (Bornem, Belgium). Pentylentetrazol was purchased from Acros Organics (Pittsburgh, USA). [^{3}H]ucb 34714 (8 Ci/mmol) and [^{3}H]ucb 30889 (2S-2-[4-(3-azidophenyl)-2-oxo-1-pyrrolidinyl]butanamide) (32 Ci/mmol) were custom synthesized by GE Healthcare (UK). All other reagents were of analytical grade and obtained from conventional commercial sources.

2.2. Animals

Sprague–Dawley male rats (200–300 g) from lffa-Credo (Belgium) were used for binding studies.

Male and female genetically sound susceptible mice (Charles River, France) aged 5 weeks, weighing 15–25 g were used in the experiments involving audiogenic seizures and ex vivo binding.

SV2A knock-out (KO) mice from an original colony generated by Dr. S. Bajjalieh, University of Washington (Seattle, USA) were custom bred and genotyped by Charles River (France) to provide us wild-type, heterozygote and homozygote littermates.

All animal experiments were done according to the Helsinki declaration and conducted in accordance with the guidelines of the European Community Council directive 86/609/EEC. A local ethical committee approved the experimental protocol.

2.3. Tissue and cell membrane preparations

2.3.1. Human brain tissue

Human cerebral cortex was purchased from ABS (Wilmington, DE, USA). Samples had been collected from 4 to 6.5 h post-mortem. None of the donors had diagnosed neurological problems. Brain samples were shipped in dry ice and stored at −80 °C until used. Crude membranes were prepared as described in Gillard et al. (2006).

2.3.2. CHO cells expressing human SV2A

Human SV2A was cloned from a fetal brain cDNA library as described in Lynch et al. (2004) and was subsequently stably expressed in CHO cells. Cell culture conditions and membrane preparations were as described in Gillard et al. (2006).

2.3.3. SV2A knock-out and rat brain tissue

Frozen whole brains from 3 to 4 day old SV2A$^{+/+}$, SV2A$^{+/-}$ and SV2A$^{-/-}$ mice littermates were obtained from Charles River.

Crude membranes from rat cerebral cortex or mouse brain were prepared as described in Gillard et al. (2003).

2.4. In vitro binding studies

Mouse, rat and human brain membranes (0.10–0.20 mg proteins/assay) or CHO cells expressing human SV2A (0.05–0.10 mg/assay) were incubated for 120 min at 4 °C (unless otherwise stated) in 0.5 ml of a 50 mM Tris–HCl buffer (pH 7.4) containing 2 mM $MgCl_2$, [^{3}H]ucb 34714 (15 nM) or [^{3}H]ucb 30889 (1–3 nM) and increasing concentrations of unlabelled competing drugs. At the end of the incubation period, the membrane-bound radioligand was recovered by rapid filtration through GF/C glass fiber filters pre-soaked in 0.1% polyethyleneimine. Membranes were washed with 8 ml of ice-cold 50 mM Tris HCl buffer (pH 7.4). The filters were dried and the radioactivity determined by liquid scintillation. The entire filtration step did not exceed 10 s.

Saturation binding studies were carried out by incubating membranes for 240 min at 4 °C (unless otherwise stated) with increasing concentrations of radioligand ranging from 1 to 1000 nM.

[^{3}H]ucb 34714 kinetic association and dissociation experiments were performed in batch mode. Binding was initiated by adding membranes to an adequate volume of binding buffer containing the radioligand. For association kinetics, 500 µl aliquots were collected at increasing time points and filtered as described above. For dissociation kinetics, binding of [^{3}H]ucb 34714 was initiated as for association kinetics and after 30 to 120 min (as stated) radioligand dissociation was induced by addition of levetiracetam (1 mM final concentration). 500 µl aliquots were collected at increasing time points thereafter and filtered as described above.

In all experiments, nonspecific binding of [^{3}H]ucb 34714 or [^{3}H]ucb 30889 was defined as the residual binding observed in the presence of 1 mM levetiracetam.

2.5. Ex vivo binding studies

Mice were injected ip (10 ml/kg) with drug or saline. 60 min post-administration, animals were sacrificed and their forebrains quickly removed and homogenized in 750 µl of ice-cold 50 mM Tris–HCl buffer (pH 7.4) containing 250 mM sucrose. Samples were frozen in liquid nitrogen and stored at −80 °C until required.

10 µl of brain homogenate (650–750 µg prot.) were incubated for 90 min at 4 °C in 0.2 ml of a 50 mM Tris–HCl (pH 7.4) buffer containing 2 mM $MgCl_2$ and 2 nM [^{3}H]ucb 30889. Membrane-bound [^{3}H]ucb 30889 was recovered by filtration as described above. Nonspecific binding was defined as the residual radioactivity measured in the presence of 1 mM levetiracetam.

Percentage of receptor occupancy (RO) is defined as:

$$RO(\%) = 100 - \left\{ 100 \times \left[\frac{(B_D - NSB)}{(B_S - NSB)} \right] \right\}$$

where: B_D and B_S is the total binding measured in the presence or absence of drug respectively.

NSB is the nonspecific binding.

2.6. Audiogenic seizures

Audiogenic seizures were induced by subjecting sound-susceptible mice to a 90-dB, 10- to 20-kHz acoustic stimulus for 30 s. The occurrence of clonic and tonic convulsions was recorded. Mice responding to a first stimulus were retained for subsequent studies carried out the next day. Each experimental group consisted of 10 mice. Mice were used only once for testing the anticonvulsant effects of SV2A ligands.