Inhibition of glutamate release by BIA 2-093 and BIA 2-024, two novel derivatives of carbamazepine, due to blockade of sodium but not calcium channels

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Abstract

We investigated the mechanism(s) of action of two new putative antiepileptic drugs (AEDs), (S)-(−)-10-acetoxy-10,11-dihydro-5H-dibenz[b,f]azepine-5-carboxamide (BIA 2-093) and 10,11-dihydro-10-hydroxyimino-5H-dibenz[b,f]azepine-5-carboxamide (BIA 2-024), by comparing their effects on the release of endogenous glutamate in hippocampal synaptosomes, with those of carbamazepine (CBZ) and oxcarbazepine (OXC). The AEDs inhibited the release of glutamate evoked by 4-aminopyridine (4-AP) or veratridine in a concentration-dependent manner, being CBZ more potent than the other AEDs. Using conditions of stimulation (30 mM KCl), where Na\(^{+}\) channels are inactivated, the AEDs did not inhibit either the Ca\(^{2+}\)-dependent or -independent release of glutamate. The results indicate that BIA 2-093 and BIA 2-024 have sodium channel-blocking properties, but CBZ and OXC are more potent than the new AEDs. Moreover, the present data also indicate that Ca\(^{2+}\) channels coupled to the exocytotic release of glutamate and the activity of the glutamate transporter were not affected by the AEDs. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Antiepileptic drugs; Carbamazepine; Oxcarbazepine; Sodium channels; Calcium channels; Glutamate release

1. Introduction

During the last decade several new AEDs have been developed to treat epilepsy, such as OXC, which is an analogue of CBZ, and is generally accepted as a better tolerated drug [1]. (S)-(−)-10-acetoxy-10,11-dihydro-5H-dibenz[b,f]azepine-5-carboxamide (BIA 2-093) [2] and 10,11-dihydro-10-hydroxyimino-5H-dibenz[b,f]azepine-5-carboxamide (BIA 2-024) [3] are new putative AEDs, chemically related to CBZ and OXC, but specifically designed to prevent the production of toxic metabolites.

The mechanisms underlying the effects of the AEDs are still a matter of debate. It has been shown that CBZ and OXC inhibit voltage-sensitive sodium [4,5] and calcium currents [6,7], and that BIA 2-093 has sodium channel-blocking properties [2]. We recently found that CBZ inhibits L-type Ca\(^{2+}\) channels in hippocampal neurons [8].

Focal application of glutamate to the surface of the cerebral cortex evokes local epileptiform activity. Thus, inhibition of glutamate release may contribute to the efficacy of anticonvulsants against epileptic seizures. In the present work, we investigated the effects of the anticonvulsants on the release of endogenous glutamate from hippocampal nerve terminals, particularly their possible interaction with the Na\(^{+}\) and Ca\(^{2+}\) channels present in nerve terminals, namely P/Q-type channels, which have a predominant role on the exocytotic release of glutamate [9].
2. Materials and methods

2.1. Preparation of synaptosomes

A partially purified synaptosomal fraction (P₂) was isolated from the hippocampi of male Wistar rats (2 months of age) as described previously [9].

2.2. Endogenous glutamate release experiments

The release of endogenous glutamate was followed by using a continuous fluorimetric assay, as previously described [10]. The synaptosomes (1 mg) were incubated for 30 min at 37°C in the following medium (in mM): 132 NaCl, 1 KCl, 1 MgCl₂, 1.2 H₃PO₄, 0.1 CaCl₂, 10 glucose, 10 HEPES-Na, at pH 7.4, with 0.1% fatty acid-free bovine serum albumin (BSA), centrifuged, and then resuspended in 1 ml of the same medium with 1 mM NADP, 50 U of purified L-glutamic acid dehydrogenase type II (EC 1.4.1.3; Sigma), 1 mM CaCl₂ or with 200 nM free Ca²⁺ (without BSA).

Fluorescence was measured by using a luminescence spectrometer (Perkin Elmer model LS-5B) at excitation and emission wavelengths of 340 nm and 460 nm, respectively, with excitation and emission slits of 5 nm and 10 nm, respectively. The data were collected at 0.5 s intervals and the quantitation of glutamate release was performed at the end of each experiment by adding 2.5 nmol of L-glutamate.

2.3. Chemicals and data analysis

Carbamazepine, oxcarbazepine, BIA 2-093 and BIA 2-024 were obtained from BIAL, S. Mamede do Coronado, Portugal. Veratridine, 4-AP, tetrodotoxin, NADP and L-glutamic acid dehydrogenase type II (EC 1.4.1.3; Sigma), 1 mM CaCl₂ or with 200 nM free Ca²⁺ (without BSA).

Fluorescence was measured by using a luminescence spectrometer (Perkin Elmer model LS-5B) at excitation and emission wavelengths of 340 nm and 460 nm, respectively, with excitation and emission slits of 5 nm and 10 nm, respectively. The data were collected at 0.5 s intervals and the quantitation of glutamate release was performed at the end of each experiment by adding 2.5 nmol of L-glutamate.

The data are expressed as means ± SEM. Statistical significance was determined by using an analysis of variance (ANOVA), followed by Dunnnett’s post-test. The dose-inhibition curves for the effects of CBZ, OXC, BIA 2-093 and BIA 2-024 represent the best fit according to nonlinear regression analysis (one site competition), assuming bottom and top values of 0% and 100%, respectively.

3. Results and discussion

Stimulation of hippocampal synaptosomes with 100 μM 4-AP evoked the release of 4.6 ± 0.1 nmol glutamate/mg protein/5 min, and the AEDs inhibited the release of glutamate, as illustrated in Fig. 1, in a concentration-dependent manner (Fig. 2). Carbamazepine was more potent than

![Fig. 1. Illustrative recordings of endogenous glutamate release stimulated by 100 μM 4-AP in hippocampal synaptosomes (1 mM Ca²⁺ present in the external medium). Synaptosomes were stimulated for 5 min with 4-AP, and the AEDs were present since the beginning of the experiment. (A) Control (B) Effect of 300 μM CBZ.](image-url)
(stimulation with 30 mM KCl), indicating that the inhibition caused by the AEDs on the 4-AP- or veratridine-evoked glutamate release was due to the inhibition of sodium channels and not to the inhibition of calcium channels or the glutamate transporter.

It was previously shown that CBZ and OXC inhibit the release of glutamate elicited by veratrine or veratridine [11,12], however it is uncertain whether these effects are relevant in vivo at anticonvulsant doses. It was also reported that CBZ inhibits glutamate release by preferentially blocking sodium channels [13]. In the present work, the release of glutamate stimulated by 4-AP or veratridine was significantly inhibited by CBZ at concentrations similar to those found in plasma levels (17–51 μM) [14]. Since OXC, BIA 2-093 and BIA 2-024 significantly inhibited the release of glutamate at higher concentrations, it is not clear whether these effects are relevant in vivo.

In conclusion, our results show that BIA 2-093 and BIA 2-024 have antagonistic properties on sodium channels, however with lower potency when compared to CBZ or OXC. The AEDs tested neither affected the calcium channels, which are coupled to the exocytotic release of glutamate, nor the glutamate transporter.
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References


Fig. 3. Dose-inhibition curves for the effect of CBZ, OXC, BIA 2-093, or BIA 2-024 on the release of endogenous glutamate evoked by 5 \( \mu \text{M} \) veratridine (1 \( \text{mM Ca}^{2+} \) present in the external medium). The results represent the mean \( \pm \) s.e.m. of three to five independent experiments, in different synaptosomal preparations, and are presented as percentage of control. *\( P < 0.05 \); **\( P < 0.01 \)-Dunnett’s post-test; Statistical significance when compared to control (veratridine stimulation).


