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Biochemical Pharmacology

Biochemical Pharmacology 61 (2001) 1271–1275

Short communication

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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Received 25 July 2000; accepted 3 December 2000

Abstract

We investigated the mechanism(s) of action of two new putative antiepileptic drugs (AEDs), (S)-(-)-10-acetoxy-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (BIA 2-093) and 10,11-dihydro-10-hydroxyimino-5*H*-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.

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-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (BIA 2-093) [2] and 10,11-dihydro-10-hydroxyimino-5*H*-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].

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Abbreviations: AED, antiepileptic drug; CBZ, carbamazepine; OXC, oxcarbazepine; and 4-AP, 4-aminopyridine.

0006-2952/01/$ – see front matter
PII: S0006-2952(01)00584-6

2. Materials and methods

2.1. Preparation of synaptosomes

A partially purified synaptosomal fraction (P_2) 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 $MgCl_2$, 1.2 H_3PO_4, 0.1 $CaCl_2$, 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 $CaCl_2$ or with 200 nM free Ca^{2+} (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) were purchased from Sigma Chemical Co. All other reagents were from Sigma or from Merck-Schuchardt, Germany. CBZ, OXC, BIA 2-093, BIA 2-024 and veratridine stock solutions were prepared in DMSO.

The data are expressed as means ± SEM. Statistical significance was determined by using an analysis of variance (ANOVA), followed by Dunnett'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

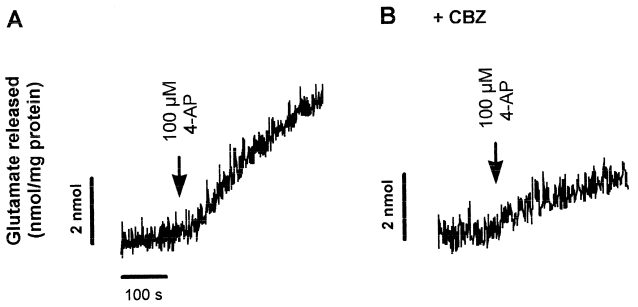

Fig. 1. Illustrative recordings of endogenous glutamate release stimulated by 100 μM 4-AP in hippocampal synaptosomes (1 mM Ca^{2+} present in the external medium). Synaptosomes were stimulated for 5 min with 4-AP, and the AEDs were present since the beginning of the experiment. (A) Control (B) Effect of 300 μM CBZ.

OXC, BIA 2-093 or BIA 2-024. The IC_{50} values for CBZ, OXC, BIA 2-093 or BIA 2-024 were 99.3 ± 1.08 μM, 156.8 ± 1.23 μM, 233.0 ± 1.14 μM or 188.0 ± 1.13 μM, respectively.

Veratridine (5 μM) evoked the release of 6.6 ± 0.2 nmol glutamate/mg protein/5 min. Again, the AEDs inhibited the release of glutamate in a concentration-dependent manner (Fig. 3), and CBZ was again more potent than OXC, BIA 2-093 or BIA 2-024. The IC_{50} values for CBZ, OXC, BIA 2-093 or BIA 2-024 were 123.9 ± 1.07 μM, 156.9 ± 1.15 μM, 232.3 ± 1.07 μM or 190.8 ± 1.11 μM, respectively.

When synaptosomes were stimulated with 30 mM KCl the release of glutamate was 6.8 ± 0.5 nmol glutamate/mg protein/5 min. The Ca^{2+}-independent glutamate release was 36.7 ± 2.9% of total release (data not shown). Moreover, 1 μM tetrodotoxin did not inhibit the release of glutamate evoked by 30 mM KCl (data not shown), indicating that voltage-sensitive sodium channels are inactivated. The AEDs (300 μM) did not inhibit the KCl-evoked (exocytotic plus non-exocytotic) glutamate release (data not shown). Also, the AEDs did not inhibit the non-exocytotic KCl-evoked glutamate release (200 nM external Ca^{2+}, data not shown).

In the present study, we investigated the effects of BIA 2-093, BIA 2-024, CBZ and OXC on the exocytotic and/or carrier-mediated release of endogenous glutamate in hippocampal nerve terminals. As shown previously, P/Q-type Ca^{2+} channels have a predominant role on the exocytotic release of endogenous glutamate [9]. Because CBZ [6,8] and OXC [7] inhibit calcium channels, we investigated whether the AEDs could inhibit P/Q-type calcium channels present in nerve terminals. The inhibitory effect caused by the AEDs on the release of glutamate evoked by 4-AP or veratridine could be due either to the inhibition of sodium channels, to the inhibition of calcium channels coupled to the exocytotic release of glutamate, or in part due to the inhibition of the glutamate transporter. However, the AEDs did not inhibit the release of glutamate when sodium channels are inactivated

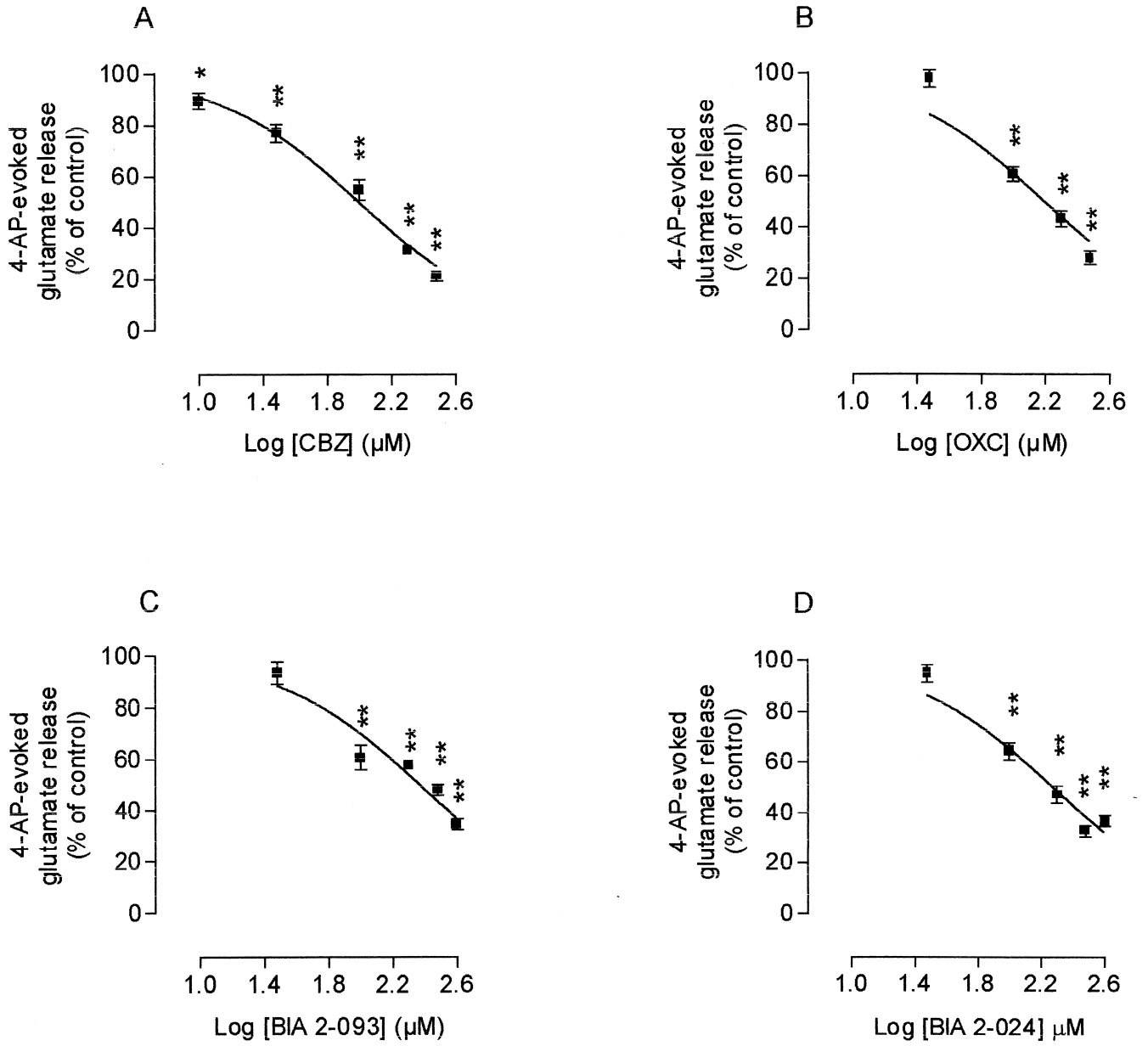

Fig. 2. Dose-inhibition curves for the effect of CBZ, OXC, BIA 2-093 or BIA 2-024 on the release of endogenous glutamate evoked by 100 μM 4-AP (1 mM Ca^{2+} present in the external medium). The results represent the mean ± s.e.m. of three to eight independent experiments, in different synaptosomal preparations, and are presented as percentage of control. *$P < 0.05$; **$P < 0.01$-Dunnett's post-test; Statistical significance when compared to control (4-AP stimulation).

(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.

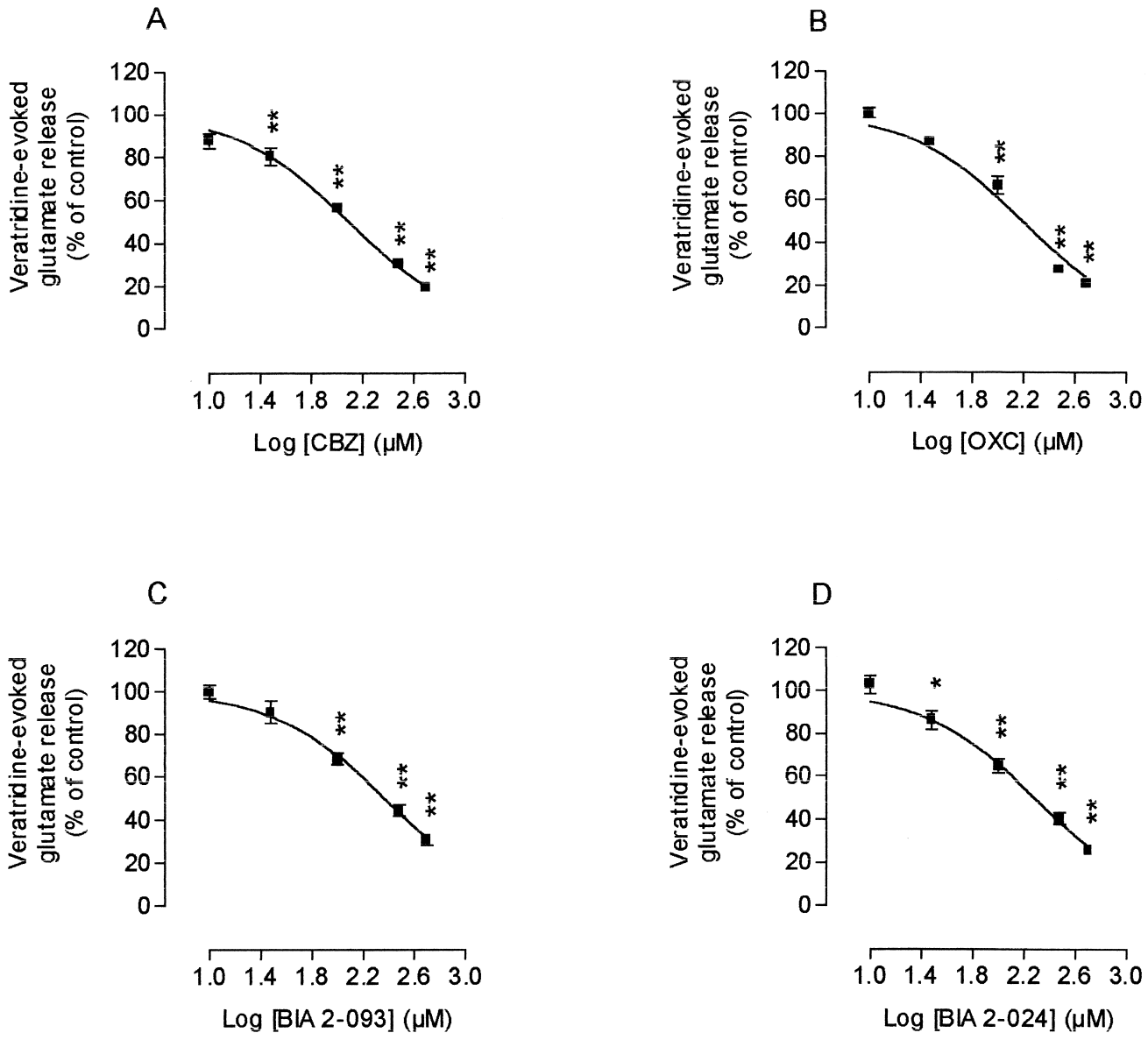

Fig. 3. Dose-inhibition curves for the effect of CBZ, OXC, BIA 2-093, or BIA 2-024 on the release of endogenous glutamate evoked by 5 μM veratridine (1 mM Ca^{2+} present in the external medium). The results represent the mean ± s.e.m. of three to five independent experiments, in different synaptosomal 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).

Acknowledgments

This work was supported by Fundação para a Ciência e a Tecnologia, Programa PRAXIS XXI, Portugal (grant PRAXIS XXI 5604/95) and by BIAL, Portugal.

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