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Cephalosporin antibiotics are weak blockers of GABAa receptor-mediated synaptic transmission in rat brain slices

Dmitry V. Amakhin [a], Elena B. Soboleva [a], Aleksey V. Zaitsev [a, b, *]

[a] Sechenov Institute of Evolutionary Physiology and Biochemistry of RAS, 44, Toreza prospekt, Saint Petersburg 194223, Russia
[b] Almazov National Medical Research Centre, Institute of Experimental Medicine, 2 Akkuratova Street, St. Petersburg 197341, Russia

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ABSTRACT

Cephalosporins are beta-lactam antibiotics that are extensively used in medical practice and are reported to cause epileptic seizures in some patients. The primary cause of cephalosporin-induced convulsions is believed to be their ability to block GABAa receptors. However, direct evidence for the involvement of this mechanism has not yet been provided. The present study aims to investigate the ability of two cephalosporins – cefepime and ceftriaxone – to block inhibitory synaptic transmission in entorhinal cortex slices of rats. Using the whole-cell patch-clamp method, we found that millimolar concentrations of cefepime ($IC_{50} = 1.6 \pm 0.1$ mM) and ceftriaxone (2.0 ± 0.1 mM) were required to block the evoked inhibitory postsynaptic currents (IPSCs). These concentrations are almost two orders of magnitude higher than cerebrospinal fluid concentrations of antibiotics achieved during treatment. We also found that while ceftriaxone did not affect the IPSC decay kinetics, cefepime significantly slowed the decays of the evoked currents, which may be attributed to the diverse mechanisms of the GABAa receptor inhibition of cefepime and ceftriaxone. The experiments involving the fast application of GABA at various concentrations to isolated neurons suggests that cefepime blocks receptors competitively, while ceftriaxone does so noncompetitively. Cefepime, at a concentration of up to 4 mM, was unable to produce seizure-like events in brain slices. However, this antibiotic could induce epileptiform activity in combination with the altered ionic composition of the perfusing media, which may be the case for patients with renal insufficiency. Our results suggest that cefepime and ceftriaxone are weak GABAa receptor blockers and that it is unlikely that the inhibition of GABAa receptors by antibiotics is the primary cause of the seizures.

1. Introduction

Cephalosporins are beta-lactam antibiotics that have a broad spectrum of activity against gram-positive and gram-negative bacteria and good tissue penetration. Although cephalosporins are extensively used in medical practice, pro-epileptic side effects have been reported for most of them [1–3]. It is believed that the primary cause of cephalosporin-induced convulsions is their ability to block GABAa receptors [4–6]. The direct effect of cephalosporins on GABAa receptors, as well as their ability to cause seizures in animal models, have been extensively studied [4,7,8]. However, no quantitative study of their ability to block inhibitory synaptic transmission in brain slices and produce seizure-like events has been performed.

In this study, we investigated the effect of two cephalosporins (cefepime and ceftriaxone) on GABAa receptor-mediated synaptic transmission in the entorhinal cortex, their effects on eIPSC kinetics, the mechanism of GABAa receptor inhibition, and their ability to induce epileptiform activity in brain slices.

2. Methods

2.1. Animals

The experiments were carried out on three-week-old Wistar rats. All animal procedures followed the guidelines of the European Community Council Directive 86/609/EEC. Thirty-five rats were used in this study.

* Corresponding author. Sechenov Institute of Evolutionary Physiology and Biochemistry of RAS, 44, Toreza prospekt, Saint Petersburg 194223, Russia.
E-mail address: aleksey_zaitsev@mail.ru (A.V. Zaitsev).

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2.2. Slice preparation

Rats were sacrificed via decapitation, and their brains removed rapidly. The brain slice preparation was described previously [9]. A vibrating microtome (Microm HM 650 V; Microm; Germany) was used to cut horizontal 300-μm-thick slices that contained the entorhinal cortex and hippocampus. Artificial cerebrospinal fluid (aCSF) with the following composition (in mM) was used: 126 NaCl, 24 $NaHCO_3$, 2.5 KCl, 2 $CaCl_2$, 1.25 NaH_2PO_4, 1 $MgSO_4$, and 10 dextrose. The aCSF was aerated with the gas mixture of 95% O_2 and 5% CO_2. Unless otherwise stated, all chemicals used in this study were purchased from Sigma-Aldrich.

2.3. Whole-cell recordings in brain slices

Recordings were performed at 30 °C. Pyramidal neurons in the deep layers of the medial entorhinal cortex were visualized using a Zeiss Axioscop 2 microscope (Zeiss; Germany) equipped with differential interference contrast optics and a video camera (Grasshopper 3 GS3-U3-23S6M-C, FLIR Integrated Imaging Solutions Inc., USA). Patch electrodes (3–5 MΩ) were pulled from borosilicate glass capillaries (Sutter Instrument; USA). A cesium methanesulfonate-based pipette solution (composition, in mM: 127 $CsMeSO_3$, 10 NaCl, 5 EGTA, 10 HEPES, 6 QX314, 4 ATP-Mg, and 0.3 GTP; pH adjusted to 7.25 with CsOH) was used. Whole-cell recordings were performed with a Model 2400 (AM-Systems; USA) patch-clamp amplifier and an NI USB-6343 A/D converter (National Instruments, USA) using WinWCP 5 software (SIPBS, UK). The data were filtered at 10 kHz and sampled at 20 kHz. In all cells included in the sample, access resistance was less than 15 MΩ and remained stable (≤20% increase) across the experiment. The liquid junction potential was compensated offline for the voltage-clamp recordings by subtracting 7 mV.

The synaptic responses were evoked extracellularly. The stimulating bipolar electrode was placed in the same layer as the recorded neuron at a distance of 100–200 μm. Postsynaptic GABAa receptor-mediated currents were recorded at 0 mV in the presence of DNQX (10 μM, Tocris Bioscience, UK), MK-801 (10 μM, Alamone Labs, Israel), and AP-5 (50 μM, Tocris Bioscience). Cephalosporins were bath-applied at five concentrations (0.5, 1, 2, 4, and 8 mM). In each slice, only one concentration of antibiotics was tested.

A relative block of GABAaR-mediated current was calculated via Eq. (1):

$$\mathrm{Block} = 100\% \times \frac{A_{baseline} - A_{cephalosporin}}{A_{baseline}}, \quad (1)$$

where $A_{baseline}$ and $A_{cephalosporin}$ are the average amplitudes of the evoked IPSCs before and 10 min after cephalosporin administration, respectively.

The concentration-inhibition curves were fitted with Eq. (2) to assess the IC_{50} for cefepime and ceftriaxone:

$$\mathrm{Block(C)} = 100\% \times \frac{C^n}{IC_{50}{}^n + C^n}, \quad (2)$$

where C is the concentration of cephalosporins and IC_{50} and n denote the concentration of antibiotics at a half-maximal current and the Hill coefficient, respectively.

The kinetics of GABAaR-mediated eIPSCs were estimated using a non-linear regression analysis of the decay phase (10–90%). We utilized biexponential function:

$$I\left(t; A_{fast}, \tau_{fast}, A_{slow}, \tau_{slow}\right) = A_{fast} * \exp\left(-\frac{t}{\tau_{fast}}\right) + A_{slow} * \exp\left(-\frac{t}{\tau_{slow}}\right), \quad (3)$$

where A_{fast} and τ_{fast} are the amplitude and time constant of the fast-decaying component and A_{slow} and τ_{slow} are the amplitude and time constant of the slow-decaying component.

The relative contribution of the slowly decaying component was calculated as follows:

$$RC_{slow} = \frac{A_{slow}}{A_{fast} + A_{slow}} \quad (4)$$

The weighted time constants were calculated using the following formula:

$$\tau_{weighted} = \frac{\tau_{fast} * A_{fast} + \tau_{slow} * A_{slow}}{A_{fast} + A_{slow}} \quad (5)$$

In the rat combined entorhinal cortex-hippocampal slices, epileptiform activity was induced via an epileptogenic low-magnesium and high-potassium solution, which contained the following (in mM): 120 NaCl, 8 KCl, 1.25 NaH2PO4, 0.3 MgSO4, 2 CaCl2, 24 NaHCO3, and 10 dextrose. The flow rate in the perfusion chamber was 7 ml/min.

2.4. Whole-cell recordings in isolated neurons

Cell dissociation was performed as described previously [10]. Briefly, after an incubation period of 1–1.5 h, the slices were transferred into an external solution, which contained (in mM) 145 NaCl, 10 HEPES, 10 glucose, 2 KCl, 2 CaCl2, and 1.6 MgCl2 (pH set to 7.4 with NaOH, bubbled with 100% oxygen) for further cell isolation. The slices were digested with protease from Streptomyces griseus, Type XIV (Sigma-Aldrich, P5147, 1 mg/ml for 20 min at 35 °C). After that, the medial entorhinal cortex was removed from each slice under a dissecting microscope. Trituration through heat-polished Pasteur pipettes of progressively smaller tip diameters was used to dissociate single neurons. Isolated neurons were transferred to a recording chamber mounted on an inverted microscope.

The patch electrodes (3–4 MΩ) were filled with a solution containing (in mM): 127 CsF, 10 NaCl, 5 EGTA, 6 QX314, 10 HEPES, 4 ATP-Na, and 0.1 GTP-Na (pH set to 7.25 with CsOH). Whole-cell recordings were performed with a Model 2400 patch-clamp amplifier (AM-Systems, USA) and an NI USB-6211 A/D converter (National Instruments, USA) using WinWCP 5 software (SIPBS, UK). After the formation of the whole-cell configuration, the neurons were clamped at −27 mV. GABA-mediated currents were evoked via the fast-step application of GABA using HSSE-2/3 (ALA Scientific Instruments Inc., USA).

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