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Effects of A1 receptor agonist/antagonist on spontaneous seizures in pilocarpine-induced epileptic rats

Beatriz Oliveira Amorim [a,1], Clement Hamani [a,b,c], Elenn Ferreira [a,1], Maísa Ferreira Miranda [d], Maria José S. Fernandes [e], Antonio M. Rodrigues [d], Antônio-Carlos G. de Almeida [d], Luciene Covolan [a,*]

[a] Disciplina de Neurofisiologia, Universidade Federal de São Paulo, São Paulo, Brazil
[b] Behavioural Neurobiology Laboratory, Centre for Addiction and Mental Health, Toronto, Canada
[c] Division of Neurosurgery, Toronto Western Hospital, University of Toronto, Toronto, Canada
[d] Laboratório de Neurociência Experimental e Computacional, Universidade Federal de São João del-Rei, São João del-Rei, Brazil
[e] Disciplina de Neurologia Experimental, Universidade Federal de São Paulo, São Paulo, Brazil

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ABSTRACT

Adenosine is an endogenous anticonvulsant that activates pre- and postsynaptic adenosine A_1 receptors. A_1 receptor agonists increase the latency for the development of seizures and status epilepticus following pilocarpine administration. Although hippocampal adenosine is increased in the chronic phase of the pilocarpine model, it is not known whether the modulation of A_1 receptors may influence the frequency of spontaneous recurrent seizures (SRS). Here, we tested the hypothesis that the A1 receptor agonist RPia ([R]-N-phenylisopropyladenosine) and the A1 antagonist DPCPX (8-Cyclopentyl-1,3-dipropylxanthine) administered to chronic pilocarpine epileptic rats would respectively decrease and increase the frequency of SRS and hippocampal excitability. Four months after Pilo-induced SE, chronic epileptic rats were video-monitored for the recording of SRS before (basal) and after a 2-week treatment with RPia (25 μg/kg) or DPCPX (50 μg/kg). Following sacrifice, brain slices were studied with electrophysiology. We found that rats given RPia had a 93% nonsignificant reduction in the frequency of seizures compared with their own pretreatment baseline. In contrast, the administration of DPCPX resulted in an 87% significant increase in seizure rate. Nontreated epileptic rats had a similar frequency of seizures along the study. Corroborating our behavioral data, *in vitro* recordings showed that slices from animals previously given DPCPX had a shorter latency to develop epileptiform activity, longer and higher DC shifts, and higher spike amplitude compared with slices from nontreated Pilo controls. In contrast, smaller spike amplitude was recorded in slices from animals given RPia. In summary, the administration of A1 agonists reduced hippocampal excitability but not the frequency of spontaneous recurrent seizures in chronic epileptic rats, whereas A1 receptor antagonists increased both.

1. Introduction

Adenosine is an endogenous brain anticonvulsant known to play a role in mechanisms of seizure arrest and the enhancement of postictal refractoriness to novel epileptiform events [1]. In epilepsy, augmented extracellular levels of hippocampal adenosine increase transporter-mediated neural activity [2]. Possible roles for the extracellular accumulation of adenosine are to activate the Na^+–K^+ ATPase [3] and restore the differential concentration of Na^+ across the membrane. On the other hand, adenosine also acts as a synaptic transmitter through four receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3) [4]. The pharmacological administration of adenosine or A_1 receptor agonists has potent inhibitory effects on neuronal activity [5], suppressing epileptiform discharges and seizure activity [6], and inducing neuroprotection [7,8].

Previous studies have shown that A1 receptor agonists increase the latency for the development of seizures and status epilepticus following pilocarpine administration [9]. Though hippocampal adenosine is increased in the chronic phase of the pilocarpine model [10], the mechanism underlying its modulatory effect on spontaneous recurrent seizures (SRS) is still unknown. Here, we tested the hypothesis that A1 receptor agonists (RPia) and antagonists (DPCPX) administered to pilocarpine epileptic rats would respectively decrease and increase the frequency of SRS and hippocampal excitability.

Abbreviations: ARRIVE, Animal Research: Reporting of *In Vivo* Experiments; CTL, control; DC, direct current; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; Pilo, pilocarpine; PS, population spikes; RPia, (R)-N-phenylisopropyladenosine; SE, status epilepticus; SRS, spontaneous recurrent seizures; TLE, temporal lobe epilepsy.

* Corresponding author at: Departamento de Fisiologia, Universidade Federal de São Paulo, Rua Botucatu, 862, São Paulo SP, 04023-062, Brazil. Tel.: +55 11 55792033.
E-mail address: lucovolan@gmail.com (L. Covolan).

[1] Both authors have equally contributed to the development of this study.

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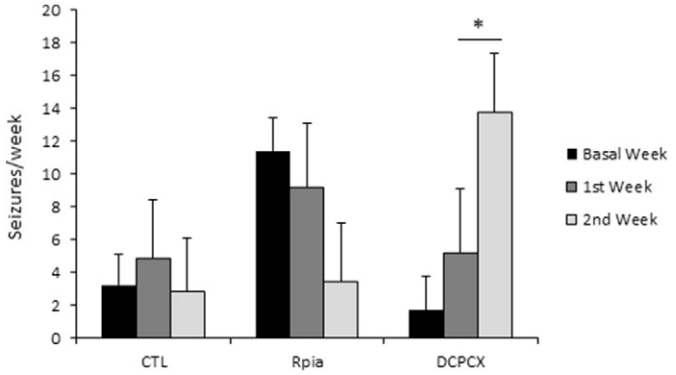

Fig. 1. Frequency of spontaneous recurrent seizures (per hour) in pilocarpine-treated chronic rats. Comparisons were made within each group (CTL: n = 6; DPCPX: n = 5; RPia: n = 5) over time. A high variability in seizure rate can be seen in all groups, including in epileptic controls (CTL). The group injected with DPCPX increased the frequency of seizures over two weeks. Animals injected with RPia had a tendency to reduce the seizure rate in the second week of treatment. Values are expressed as mean ± SEM. * indicates statistically different ($P < 0.02$) from its own baseline.

2. Materials and methods

Protocols were approved by the Animal Care Committee of the Universidade Federal de São Paulo (2070/09). Efforts were made to minimize pain or discomfort of animals. The principles outlined in the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines and the Basel declaration (http://www.basel-declaration.org) were applied. The 3R concept (Replacement, Refinement, and Reduction of Animals in research) was considered when planning the experiments.

2.1. Pilocarpine seizure induction and monitoring

Adult male Wistar rats (250–300 g) were injected with pilocarpine (Pilo; 320 mg/kg i.p.), as previously described [11,12]. Four months after Pilo-induced SE, chronic epileptic rats were videotaped 24 h/day, 7 days/week for 2 weeks to register their baseline frequency of behavioral seizures (Fig. 1). Animals were then paired according to seizure rate and assigned to receive the A_1 receptor agonist RPia (25 μg/kg/day i.p.; n = 5), the antagonist DPCPX (50 μg/kg/day i.p.; n = 5) or saline (n = 6) for 10 consecutive days. Doses of these compounds were selected based on previous data from our laboratory showing treatment-induced increases or decreases in the latency for pilocarpine-induced SE [9].

The number of SRS was monitored as previously described by our group [13]. A blinded investigator visually scored the frequency of behavioral seizures characterized by clonic/tonic/tonic–clonic movements of the forelimbs culminating with rearing and falling (stages III–V according to [14]) from videotapes obtained during recording sessions.

Our working hypothesis was that A_1 receptor agonists would reduce the frequency of SRS, whereas the opposite results would be observed in animals given A1 antagonists. As seizure rate in the pilocarpine model is extremely variable across animals, we decided to treat groups with high, middle, or lower baseline seizure frequencies with RPia, saline, or DPCPX, respectively (Fig. 1). By doing so, our goal was to maximize the chances of testing our hypotheses using the smallest possible number of animals (*i.e.*, having A_1 agonists reduce and antagonists increase the frequency of SRS).

2.2. Electrophysiology

Following 10 days of treatment with RPia, DPCPX, or saline, animals were anesthetized and decapitated, as previously described [15]. Brains were removed from the skull, and 400 μm hippocampal slices were cut on a vibratome. These were individually transferred to an interface-type chamber, placed on a membrane (0.4-μm Millicell culture plate inserts; Millipore, Bedford, MA) and continuously bathed with artificial cerebrospinal fluid (aCSF; 127-mM NaCl, 2-mM KCl, 1.5-mM $MgSO_4$, 1.1-mM KH_2PO_4, 26-mM $NaHCO_3$, 2-mM $CaCl_2$, and 10-mM glucose) at 33 °C under a stream of moisturized 95% O_2–5% CO_2. One hour later, slices were perfused with a zero calcium and 8-mM potassium solution [16].

Extracellular field potentials were recorded from the hippocampal dentate gyrus (DG). Recording electrodes were made of microfilament capillary thin-walled glass (Clark Electromedical Instruments, GC150F-10) pulled with a DMZ-Universal Puller (Zeitz-Instruments, Germany). Electrodes were filled with 1-M NaCl (4–10 MΩ), connected to a head stage (model AI 402 × 50, ultralow *noise* amplifier — Axon Instruments, USA) and a biological amplifier (model CyberAmp 380 — Axon Instruments, USA). Recorded signals were filtered (3 kHz lowpass) and digitized (sampling frequency of 10 kHz) before the analysis.

For each group, slices containing the dorsal hippocampus (3 slices per animal) were analyzed. After the development of epileptiform discharges, recordings were carried out for 20 min. The following parameters were considered for analysis: 1) Latency for recording epileptiform discharges from the moment the bath was perfused with a zero calcium high potassium solution, 2) duration and amplitude of direct current (DC) shifts, and 3) the amplitude of population spikes. A digital Fourier transform was used to quantify DC-shifts. Once in the frequency domain, the event signal was recalculated taking into account only components below 10 Hz. This process allowed the analysis of DC shifts without the interference of population spikes. Event duration was

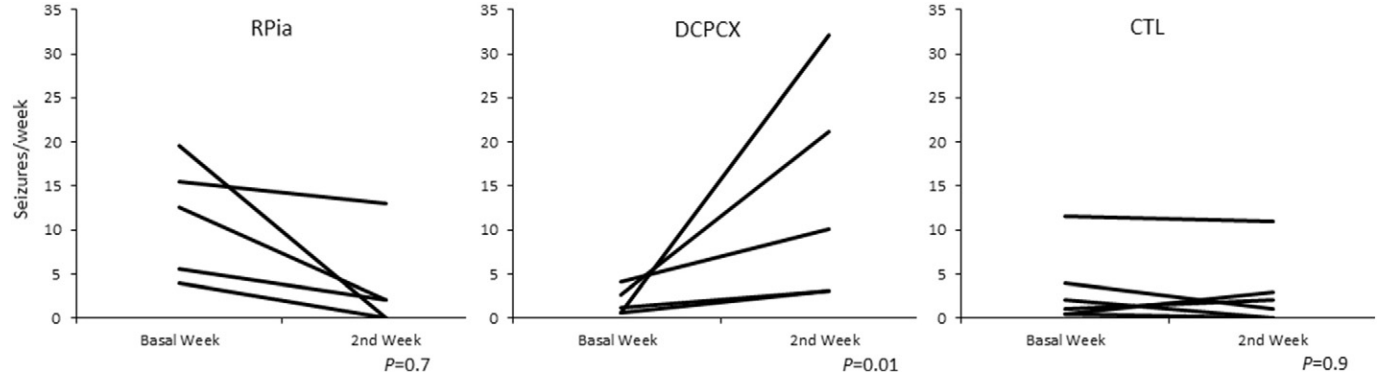

Fig. 2. Effects of the administration of A_1 agonists (RPia), antagonists, (DPCPX) or saline (CTL) on spontaneous seizure rate. All animals treated with RPia had a reduction in seizure rate to some extent (A). DCPCX treatment resulted in opposite effect (B) while no clear pattern was observed in the saline-treated epileptic group (C).