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Research article

Unilateral microinjection of carbenoxolone into the pontis caudalis nucleus inhibits the pentylenetetrazole-induced epileptiform activity in rats

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HIGHLIGHTS

- The gap junction blocker carbenoxolone reduced the incidence of seizures.
- Microinjection of carbenoxolone inhibited the PTZ-induced epileptiform activity.
- The gap junction opener trimethylamine increased the duration of seizures.
- Trimethylamine exacerbated the PTZ-induced epileptiform activity.

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ABSTRACT

Pontine reticular formation (PRF) is involved in the generation and maintenance of generalized epileptic seizures. Carbenoxolone (CBX) is a gap junction blocker with anticonvulsant properties. Therefore, the present study was designed to explore the effects of CBX microinjected into the pontis caudalis nucleus (PnC) on generalized tonic–clonic seizures (GTCS) and epileptiform activity induced by pentylenetetrazole (PTZ).

All control rats presented GTCS after a single dose of PTZ. The microinjection of CBX into the PnC reduced the GTCS incidence induced by PTZ. Moreover, the CBX significantly increased the latency to the first myoclonic jerk. Additionally, CBX significantly decreased the spectral power and the amplitude of the epileptiform activity induced by PTZ. By contrast, the microinjection of a gap junction opener (trimethylamine) did not cause anticonvulsant effects and even increased the duration of the GTCS.

These findings suggest that the PnC is a particular nucleus where the CBX could exert its action mechanisms and elicit anticonvulsant effects.

1. Introduction

Since several years ago, some authors have suggested that PRF is involved in the generation and maintenance of generalized epileptic seizures [1–3]. Specifically, some studies have showed that PnC neurons participate in the generation of behaviors related to seizures events. It has been reported that microinjection of NMDA receptor antagonists into the PnC decreased the incidence of GTCS and decreased the amplitude and frequency of the cortical epileptiform activity induced by PTZ [4].

Gap junctions are clusters of intercellular channels that provide cytoplasmic continuity and permit direct communication between cells. These intercellular channels contribute to the fast exchange of ions and some small biological molecules allowing the electrical coupling and synchronized firing of neurons [5]. It is well known that some chemical agents have the ability to block gap junctions [6]. Between the gap junction blockers, CBX is one of the most widely used because reversibly block the electrical coupling in several neuronal circuits [7,8].

The hypothesis that electrical coupling mediated by gap junctions is a primary mechanism involved in the generation and

Abbreviations: CBX, carbenoxolone; EEG, electroencephalographic recordings; GTCS, generalized tonic–clonic seizures; i.p., intraperitoneal; PnC, pontis caudalis nucleus; PRF, pontine reticular formation; PTZ, pentylenetetrazole; TMA, trimethylamine.

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maintenance of seizures has gained relevance. In fact, the neuronal hypersynchronic activity is a common feature that describes convulsive events. For this reason, it has been described that systemic administration of CBX reduces the clonic and tonic phases of the audiogenic seizures [9]. More recently, Sefil et al. [10] demonstrated that intraperitoneal (i.p.) administration of CBX has anticonvulsant effects by shortening the generalized seizure duration and reducing the total spike number in PTZ kindled rats.

CBX has showed anticonvulsant effects, and there is convincing evidence regarding the presence of gap junctions in pontine nuclei [11,12]. For these reasons, we decided to evaluate the effects of the microinjection of CBX into the PnC on GTCS and the epileptiform activity induced by PTZ in rats.

2. Material and methods

2.1. Animals

Male Wistar rats (270–300 g) were maintained under controlled conditions (24.4 °C; 7:00–19:00 light, 19:00–7:00 darkness; food and water ad libitum). All animals were treated according to regulations specified by the Bioethical Committee and the Mexican Standard for the production, care and use of laboratory animals (NOM-062-Z00-1999).

2.2. Implantation of electrodes and guide cannula

All reagents were purchased from Sigma–Aldrich (St Louis, MO., USA). Animals were anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Two electrodes were deeply implanted in the motor cortex (1.2 mm anterior to Bregma, 2.5 mm lateral to the midline, 1.5 mm below the surface of the skull) for the electroencephalographic (EEG) recordings. One more electrode implanted above the cerebellum was used as a reference (11.9 mm posterior to Bregma, 3.0 mm lateral to the midline, 1.0 mm below the surface of the skull) [13]. Besides, a stainless-steel guide cannula (Becton Dickinson, Mexico) (25 gauge) was stereotaxically positioned 4 mm above the right PnC (10.0 mm posterior to Bregma, 0.8 mm lateral to midline, 9.0 mm below the surface of the skull) [13] for the microinjection of the vehicle, CBX or trimethylamine (TMA).

2.3. Groups

We used 78 rats; however, we only selected those 40 with the precise microinjection into the PnC. Rats were randomly divided into the following groups: control group ($n = 8$) administered with the vehicle (saline solution). Three groups were microinjected with the gap junction blocker: CBX 20 nmol ($n = 8$), CBX 50 nmol ($n = 8$) and CBX 100 nmol ($n = 8$). Another group was microinjected with the gap junction opener: TMA 50 nmol ($n = 8$).

2.4. EEG recordings

The rats were connected to an amplifier (EBNeuro®, Firenze, Italy) by means of flexible insulated cables. Before any manipulation, a basal recording was carried out for 10 min. Then, rats were microinjected with the vehicle, CBX or TMA through an injection needle (32-gauge) 4 mm longer than the guide cannula. Microinjections were carried out with freely moving animals using the injection needle connected to a 10 μL Hamilton syringe (infusion rate 0.2 μL/min). 15 min after the microinjection of the vehicle, CBX or TMA the animals were administered with PTZ (70 mg/kg, i.p.). The video-EEG recordings were stored on the hard drive of a computer for the off-line analysis.

2.5. Histological procedures

At the end of EEG recordings, methylene blue was microinjected (0.2 μL), to localize the injection site and to rule out the animals with microinjections outside of the PnC. Rats were systemically administered with sodium pentobarbital (100 mg/kg, i.p.) and were transcardially perfused with saline-heparin solution (0.9%) followed by formalin (3.7%). Brains were then removed and postfixed in formalin (3.7%). Finally, the injection sites were verified in coronal slices of 80 μm thickness stained with cresyl violet.

2.6. Power spectral analysis

EEG signals analysis was performed with Galileo NT software (EBNeuro®, Firenze, Italy). All EEG signals were filtered with a low-pass at 0.3 Hz and a high-pass at 70 Hz. During rat wakefulness, 60-s epochs of the EEG recordings were extracted. Afterward, the total spectral power (mV^2) as well as the amplitude (mV) and frequency (Hz) of the EEG signal were calculated with a fast Fourier transform method. It has been demonstrated that PTZ induces epileptiform activity characterized by voltage fluctuations mainly at low frequencies (1–16 Hz) and with minimal fluctuations at high frequencies (>30 Hz) [14]. For this reason, the analysis was carried out limiting the frequency range until 32 Hz.

2.7. Statistical analysis

Fisher's exact probability tests ($p < 0.05$) were used to compare the incidence of GTCS and percentage of survival after administration of PTZ. Also, Kruskal–Wallis one-way analysis of variance on ranks with Dunnett's method were used to determine the statistical significance ($p < 0.05$) of the others observed parameters.

3. Results

3.1. Incidence of GTCS and survival

The 100% of the animals administered with the vehicle into the PnC and PTZ presented GTCS. The microinjection of CBX did not induce an apparent dose-dependent effect since the same percentage of animals with GTCS (62.5%) was observed in the groups pretreated with 20 and 100 nmol of CBX. On the other hand, the microinjection of CBX 50 nmol significantly decreased ($p < 0.05$) 75% the incidence of GTCS. By contrast, the gap junction opener TMA, did not protect against the GTCS induced by PTZ (Fig. 1A). The percentage of survival after PTZ administration also was analyzed. It was observed that no rat survived after the treatment with vehicle or TMA and PTZ. By contrast, the animals microinjected with CBX 20, 50 and 100 nmol showed a survival of 50%, 75% ($p < 0.05$), and 37.5%, respectively (Fig. 1B). Similarly, after comparing the duration of the GTCS induced by PTZ, we found that CBX (50 nmol) decreased this parameter. Interestingly, the microinjection of TMA into the PnC induced a significant increase of the GTCS duration (Fig. 1C).

3.2. Latency to the first myoclonic jerk and GTCS

The first seizure parameter observed after the PTZ administration was the myoclonic jerk. This parameter was characterized by a strong shaking of the whole body. In the vehicle group, this characteristic was observed 0.92 ± 0.03 min after PTZ administration. However, the CBX doses significantly ($p < 0.05$) delayed the appearance of this seizure parameter (Table 1).

Later, it was analyzed the latency to the GTCS. All of the control rats presented only one GTCS with a latency of 1.55 ± 0.15 min.