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Reduction of epileptiform activity through local valproate-implants in a rat neocortical epilepsy model

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ABSTRACT

Purpose: Pharmacotherapy of epilepsies is limited due to low concentrations at epileptogenic foci, side effects of high systemic doses and that some potentially efficient substances do not pass the blood–brain barrier. To overcome these limitations, we tested the efficacy of local valproate (VPA)-containing polymer implants in a model of necocortical injected tetanus toxin (TeT) in the rat.
Methods: Tetanus toxin was injected intracortically and cobalt (II) chloride ($CoCl_2$) was applied on the cortical surface. Video-electrocorticography recordings with intracortical electrodes were performed. VPA-containing polymers were implanted above the cortical focus. Antiepileptic effects were evaluated as reductions of epileptiform potentials (EPs) per hour in comparison to saline (NaCl)-containing polymer implants.
Results: Triple 50 ng TeT injections plus $CoCl_2$ application (20/10 mg) showed consistent EPs. NaCl-implanted animals ($n = 6$) showed a mean of 10.5 EPs/h after the first week, the EP frequency increased to 53.5 EPs/h after the second week. VPA-implant animals ($n = 5$) showed a reduction in EP frequency from 71.6 to 4.8 EPs/h after the second week. The EP frequency after the second week was higher in the NaCl-implanted animals than in the VPA-implanted ($p = 0.0303$). The mean EPs/h increase in NaCl-implanted rats (+42.9 EPs/h) was different ($p = 0.0087$) from the mean EPs/h decrease in VPA-implanted rats (−66.8 EPs/h).
Conclusion: Despite former publications no clear seizures could be reproduced but it was possible to establish focal EPs, which proved to be a reliable marker for epileptic activity. Local antiepileptic therapy with VPA has shown efficacy in decreasing EP frequency.

1. Introduction

It is well known that approximately 0.5–1% of the worldwide population suffers from epilepsy [1,2]. About 20–30% of those epilepsies are refractory to pharmacological treatment [1,3,4]. A high percentage of these pharmacoresistant epilepsies are caused by acquired or maldevelopmental cortical lesions. In these cases the development of new antiepileptic drugs (AEDs) has not shown a convincing breakthrough [5]. For many of those patients the surgical removal of these lesions improves the epileptic outcome [6]. However, if the epileptogenic lesion or focus is located in an eloquent brain area, surgery might result in a severe neurological deficit. For these patients, local antiepileptic pharmacotherapy with AED-containing implants could be a treatment option if experimental studies show an effect on epileptic activity.

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Most AEDs can suppress any epileptiform discharges, if the drug is concentrated highly enough. However, in systemic pharmacotherapy, systemic adverse effects arising outside of the epileptic focus may prevent sufficiently high AED concentrations in the focus. Several drugs with potential antiepileptic properties cannot reach the epileptogenic brain area because they do not pass the blood–brain barrier [7,8]. In addition, pharmacoresistant epilepsies are often characterized by multiple drug transporters, which prevent a sufficient AED concentration in the focus [9,10]. In contrast, local application of AEDs would advantageously reduce systemic side effects, bypass the blood–brain barrier and diminish the effect of multiple drug transporters by higher local AED concentrations [11,12].

It was shown that intracerebral or intraventricular application of AEDs as polymer implants or pump systems can reduce seizure frequency or severity [13–16]. In a rat model with intracortical tetanus toxin (TeT) injection Nilsen et al. [17] showed ictal and interictal activity which was quantified by electrocorticography (ECoG) and electromyography (EMG). Thus, these authors demonstrated this model to be potentially suitable for measuring effects of a local antiepileptic therapy. In further experiments, the same group then described an antiepileptic effect of locally applied gap-junction blockers [18].

The aim of the present study was to reproduce the described neocortical TeT-injection epilepsy model by Nilsen et al. [17,18] and establish an ECoG-based quantification method for measuring the epileptic activity in this model. If no stable, quantifiable, epileptic activity could be established, this model should be improved by applying more TeT or multiple TeT-injections; or even combine this model with another neocortical model, as the cobalt(II) chloride ($CoCl_2$)-cortical application. Finally, if a stable, quantifiable, epileptic focus would be established, it could prove a potential therapeutic effect of locally applied valproate (VPA)-containing biodegradable polycaprolactone (PCL)-implants.

2. Materials and methods

Animal experiments were carried out according to the European Community Council Directive (86/609/EEC) and the Declaration of Helsinki and were approved by the local government representative (Regierungspräsidium Baden-Wuerttemberg, Freiburg, registration no.: 35/9185.81/G-09/10). Sixty-three male Sprague-Dawley rats (Animal facility of the University Medical Center Freiburg) at a mean age of approximately two and a half months were included. Four different treatments were performed: no toxin, single injection of 50–200 ng TeT, triple injections of 50 ng TeT or triple injections of 50 ng TeT plus either 20 mg (initially) or 10 mg (afterwards) $CoCl_2$.

2.1. Surgery

Surgery was conducted upon sterile conditions. Sprague-Dawley rats were anesthetized with 1.5–2.5% vol. isoflurane and placed in a stereotactic frame (David Kopf instruments, Tujunga, CA). Borehole trephinations were performed (Proxxon, Niersbach, Germany) and the dura mater was perforated with a needle. Injections were performed over a stainless steel cannula (outer diameter, 0.28 mm), connected to a 2 μL microsyringe (Hamilton, Carl Roth GmbH and Co. KG, Karlsruhe, Germany) *via* PE-20 tubing with a micropump (World Precision Instruments, Sarasota, USA). For ECoG recordings, teflon-coated silver electrodes (diameter = 0.38 mm, World Precision Instruments, Sarasota, USA) with de-insulated endings were utilized.

In rats without injection, two ECoG electrodes were placed into the M1 region of the right hemisphere (coordinates in relation to the bregma; [19]): no. 1: anteroposterior (AP) +1.1 mm, mediolateral (ML) −2.5 mm, dorsoventral (DV) −1.0 mm; no. 2: AP +1.1 mm, ML −1.5 mm, DV −1.0 mm. EMG electrodes were inserted into the right and left temporal muscle. The reference and grounding electrode were initially placed into the cerebellum, later placed intraosseously by attachment to implanted screws, which were positioned over the right and left parietal cranium, serving for additional stability. All electrodes were connected to a plug connector, which was embedded in dental cement together with the screws (Paladur®, Heraeus, Hanau, Germany). All animals in this investigation received 5 mL 0.9% saline (NaCl) solution subcutaneously during surgery for fluid replacement and 0.05 mg/kg bodyweight buprenorphine subcutaneously after surgery to prevent postoperative pain.

In rats receiving a single injection of 50–200 ng TeT, the toxin was injected into the M1 area of the right hemisphere (AP +1.1 mm, ML −2.5 mm; DV −1.0 mm) over a 5-min period. Fifty nanogram TeT were solved in 0.5 μL sterile distilled water with 2% bovine serum albumin resulting in a working concentration of 100 ng/μL. In the case of Evans blue-injection, the toxin was solved in 0.25 μL Evans blue solution and 0.25 μL sterile distilled water with 2% bovine serum albumin. To ensure appropriate toxin diffusion 10 min had to elapse after injection. Then the ECoG and EMG electrodes were positioned as described above.

For rats with triple injections of 50 ng TeT, the additional boreholes were AP +2.1 mm, ML −3.5 mm and AP +0.1 mm, ML −3.5 mm. Each injection was performed over a 10-min period to ensure toxin diffusion. Electrode positioning followed as described above.

For rats with triple injections of 50 ng TeT plus 20 mg or 10 mg $CoCl_2$, a hole (5 mm diameter) was carefully drilled with a mill in the skull above the right primary motor cortex (anterior to the bregma, with edges 1 mm lateral to the midline and bordering the coronal suture; Fig. 1A). Drilling was performed upon steady saline cooling to avoid heat. The dura mater was removed with a sharp miniaturized hook. TeT (50 ng) was injected at injection sites described for the animals with triple injections (Fig. 1B). After the injections, 20 or 10 mg crystalline $CoCl_2 \cdot 6H_2O$ was applied onto the cortex. Afterwards, a biodegradable PCL-implant containing either 0.9% NaCl or VPA (10%, w/w each) was placed on top. Finally, the electrodes and the lesion site were covered with cyanoacrylate (Fig. 1D), that did not get in touch with the cortical surface. Production and release kinetics of the implants were described in detail by Kammerer et al. [20]. Rats treated with TeT or $CoCl_2$ showed an increased irritability to noise, physical contact, toward other rats or the hand of the examiner. In addition, they showed an increased aggression. These signs were also described by Brener et al. [21] and Liang et al. [22].

2.2. Recordings, data analysis and histology

In all animals a combined ECoG/EMG-video-monitoring was performed. Recordings started two days after surgery at the earliest to allow recovery and lasted between 90 min and 6 h each. After amplification, data were acquired using a bandpass filter (1 Hz to 5 kHz) and sampled by CED Spike 2 software version 5 (Cambridge Electronic Design Ltd. Cambridge, U.K.). The recordings were analyzed by blinded analyzers, *i.e.* they did not know which treatment the animal had received. The analysis focused on qualitative and quantitative evaluation of both interictal and ictal activity. Efforts were made not to mistake artifacts for epileptic activity. Interictal epileptic activity was defined as unambiguous epileptiform potentials (EPs) characterized by single or multiple spikes followed by a slow wave and clearly interrupting background activity with respect to amplitude and frequency (Figs. 4B and 5). EPs were previously described after TeT injections