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BASIC RESEARCH

Positive effect of calcitonin on the seizures induced by pentylenetetrazole in rats

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Summary There are many difficulties involved with the treatment of epilepsy, and these problems have driven the search for new agents to control epileptic seizures. Calcitonin is a peptide hormone that has been well studied and shown to have a positive effect on neuropathic and chronic pain. The mechanism by which calcitonin affects these pain syndromes is thought to be similar to the effect of antiepileptic drugs, such as pregabalin, gabapentin and carbamazepine. In this study, we aim to investigate the effects of calcitonin on seizures induced by pentylenetetrazole (PTZ) in rats. The rats were divided into four groups. The first group was the control group, and the rats were given no medications. The second group was given saline + PTZ. The third group was given 50 IU/kg calcitonin + PTZ, and the fourth group was given 100 IU/kg calcitonin + PTZ. EEG traces, Racine's convulsion stages and the time of onset of the first myoclonic jerk were compared between the groups. Between the groups, there were significant differences in the Racine's convulsion stages, the onset of the 'first myoclonic jerk', and the rate of the spikes in the EEG traces. The differences were more pronounced in the 100 IU/kg calcitonin-treated group ($p < 0.001$).

It has been stated that calcitonin relieves pain via regulating voltage-gated Ca^{2+} and/or Na^{+} channels. Calcitonin has a positive effect on convulsions in epileptic rats, possibly using the same mechanisms as is used in the treatment of neuropathic and chronic pain.

Introduction

The main goal in treating patients with epilepsy is to reduce the seizure frequency while avoiding drug interactions and side effects (Garnett, 2000). The new generation epileptic drugs produce favorable results, but in approximately 30% of patients who receive the appropriate treatment seizures persist (Sander, 2004). This demonstrates a continued need for developing new antiepileptic drugs. Neuropeptides have a significant role in the

Abbreviations: PTZ, pentylentetrazole; Ca^{2+}, calcium; GABA, gamma amino butyric acid; EEG, electroencephalography; Na^{+}, sodium; RCS, Racine's Convulsion Scale; FMJ, first myoclonic jerk.

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control of epilepsy and seizures. Neuropeptides, which are different from neurotransmitters, have a longer half-life, and it is thought that they are endogenously neuroprotective (Kovac and Walker, 2013; Dobolyi et al., 2013). The potential advantages of treatments targeting neuropeptide systems compared to neurotransmitter systems revolve around the efficacy and the reduced side effects, making them interesting candidates for the development of new clinical applications (Portelli et al., 2012) in epilepsy.

Calcitonin is a small, 32-amino-acid peptide hormone expressed in the parafollicular C cells of the thyroid gland (Kumar et al., 1963; Sexton et al., 1999; Hamdy and Daley, 2012). Calcitonin has been approved for the treatment of osteoporosis and other diseases involving accelerated bone turnover. Calcitonin has demonstrated analgesic effects against a series of painful conditions, such as reflex sympathetic dystrophy syndrome, adhesive capsulitis, ankylosing spondylitis, rheumatoid arthritis, vertebral crush fractures, metastases, phantom limb pain, and post herpetic neuralgia (Appelboom, 2002; Lyritis and Trovas, 2002; Visser and Kwei, 2006; Eichenberger et al., 2008). Calcitonin receptors are almost ubiquitously expressed in the brain and spinal cord (Tolcos et al., 2003), and exogenous calcitonin is thought to cross the blood-brain barrier and accumulate slowly in the brain, inducing analgesia (Qin et al., 2008). The principal mechanism of the calcitonin analgesic effect is most likely a direct central action mediated by changes in the movement of ionic calcium between nerve tissue and the cerebrospinal fluid (Azria, 2002). Although calcitonin's mechanism of action on neuropathic pain has not been thoroughly explained, it is well understood to have an effect on voltage-gated Ca^{2+} channel, which is similar to the effect of gabapentin and pregabalin (Hagenacker et al., 2011; Horga de la Parte and Horga, 2006; Schulze-Bonhage, 2013).

Pentylenetetrazole (PTZ), a selective blocker of the GABA-A receptor, is a drug that induces chemical kindling characterized by dose-dependent subconvulsions and generalized tonic–clonic seizures (White et al., 2007). In this study, we aim to investigate the effects of different dosages of calcitonin on PTZ-induced seizures in rats.

Materials and methods

Animals and laboratory

All experiments performed in the study were carried out according to the rules listed in the Guide for the Care and Use of Laboratory Animals, as adopted by the National Institutes of Health (USA). The experiments received the Gaziosmanpasa University Animal Ethics Committee's consent. Thirty-two male Sprague-Dawley rats, weighing 200–250 g were used in this study. The rats were kept in quiet rooms with 12-h light–dark cycles (light from 07.00 to 19.00) and an ambient temperature of 22–24 °C. The rats were given standard laboratory food and tap water ad libitum. The rats were between 8 and 12 weeks old at the time of the study.

Experimental procedures

The rats were anesthetized using ketamine (40 mg/kg) (Alfamine®, Ege Vet, Alfasan International B.V. Holland) and xylazine (4 mg/kg) (Alfazyne®, Ege Vet, Alfasan International B.V. Holland) intraperitoneally (i.p.). While the rats were under anesthesia, a small hole was drilled, and an exterior insulated bipolar EEG electrode (100 μm in diameter) was placed in the left thalamic nucleus by a stereotaxic method using the bregma as a reference (coordinates AP: −3.6 mm, L: +2.8 mm, DV: −5.0 mm). The EEG was recorded from thalamic region because generalized seizures begin in the deep structures of the brain such as the thalamus, which have broad projections to all areas of the cerebral cortex. Previous studies showed abnormal changes of the bilateral thalamus. (Blumenfeld, 2012; Wang et al., 2012) A grounding electrode was placed on the bregma via steel screws. The electrodes were fixed using a dental acrylic (numerous alloys are used in the making of dental restorations). All the animals were given single-injection doses of crystallized penicillin (*i.m.*) to prevent postsurgical infections. We confirmed the electrode location histologically after the rats were euthanized. Five days after the electrodes were implanted, the rats were divided into four groups. The first group was described as the control group, and the rats were given no medication. The second group was given saline i.p., the third group was given 50 IU/kg calcitonin (Miacalcic Ampul 100 İU, Novartis) i.p., and the fourth group was given 100 IU/kg calcitonin i.p. The drugs were administered 30 min prior to the pentylenetetrazole (PTZ) injection. All the groups except the control group received 35 mg/kg PTZ i.p. Thalamic EEG recordings were performed in rats while awake in a special container. An EEG recording was taken every 30 min (Souza et al., 2013; Erbas et al., 2013). The EEG signals were amplified 10,000 times and filtered with a range of 1–60 Hz using a Biopac MP 30 amplifier system, and the spike percentages were evaluated. The EEG traces were analyzed using the Biopac MP 150 analysis software. Two clinical neurophysiologists scored the EEG data for the spike percentages. We defined ''spike percentage'' as a reproducible way to quantify epileptiform activity by quantifying the percentage of 1-s bins with at least one spike-wave (Aeby et al., 2005). All the drugs were administered 30 min prior to the PTZ (35 mg/kg, i.p.) injection for evaluation of the EEG recordings. Drugs were administered 30 min prior to the PTZ (70 mg/kg, i.p.) injection for the evaluation of Racine's Convulsion Scale (RCS) and the onset time of the first myoclonic jerk. RCS was used to evaluate the seizures as follows: 0: no convulsion; 1: twitching of the vibrissae and pinnae; 2: motor arrest with more pronounced twitching; 3: motor arrest with generalized myoclonic jerks; 4: tonic–clonic seizure while the animal maintained posture; 5: tonic–clonic seizure with loss of the righting reflex; 6: lethal seizure (Fig. 1).

The onset times of the first myoclonic jerk (FMJ) were measured after the PTZ injections. The observation period for PTZ-induced seizures was limited to a duration of 30 min (Kaputlu and Uzbay, 1997). The Racine score was measured for the maximal seizure intensity observed at 30 min. The animals were then euthanized.