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Effects of thiamine and thiamine pyrophosphate on epileptic episode model established with caffeine in rats

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Summary This study examines the effect of thiamine (TH) and thiamine pyrophosphate (TPP) on epileptic episode model induced in rats with caffeine. Animals were divided into groups and given TH or TPP at doses of 10, 30 or 50 mg/kg intraperitoneally. Subsequently, all animal groups were injected intraperitoneally with caffeine at a dose of 300 mg/kg. Time of onset of epileptic episode was recorded, and the latent period was calculated in seconds. At the end of the experiment, tGSH and MDA levels and SOD and MPO enzyme activities in extracted brain tissues were measured. Latent period duration in rats in the control group was 134 ± 3.2 s, compared to 144 ± 13.9, 147 ± 14.5 and 169 ± 15.1 s, respectively, in the TH10, TH30 and TH50 groups and 184 ± 8.54, 197 ± 9.1, 225 ± 8.37 s, respectively, in the TPP10, TPP30 and TPP50 groups. Latent period duration was 236 ± 6.7 in the diazepam group. Oxidant products were significantly lower in the TPP10, TPP30, TPP50 and diazepam groups compared to the control group ($P < 0.05$), while SOD activity and tGSH levels were significantly higher ($P < 0.05$). There was no significant difference between the TH10, TH30, TH50 groups and the control group in terms of oxidant and antioxidant levels ($P > 0.05$). In conclusions, TPP, especially at a dose of 50 mg/kg, significantly prolonged the latent period from administration of caffeine to time of episode and prevented oxidative damage.

Introduction

Epileptic seizure is defined as a temporary function impairment of the brain, resulting in sudden and temporary motor, emotional, autonomic and psychological injury in association with abnormal discharge, of a repeating character, of hyperexcitable neurons in the brain. This clinical

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picture is generally brief, lasting a few seconds or minutes (Chang and Lowenstein, 2003; Remy and Beck, 2006). Epileptic seizure is the most common neurological disease in developed countries (Chang and Lowenstein, 2003; Lipson et al., 2002). Epileptic animal models provide data concerning the physiopathology of various types of epilepsy, the effectiveness of novel molecules and the functioning of the central nervous system (CNS). In addition, epileptic animal models play an important role in research into and evaluation of novel therapeutic drugs and the discovery of new techniques (Crepeau et al., 2012). Caffeine, a methylxanthine-type alkaloid, can quickly lead to generalized tonic clonic episodes when administered by the appropriate route, the episode-inducing effect of which has been shown in studies not to be attributable to a single mechanism, was used to establish the epileptic model in this study (Georgiev et al., 1993; Johansson et al., 1996). Although there are currently a large number of antiepileptic drugs in use, epilepsy and its complications still pose serious problems (Crepeau et al., 2012). In addition, one-third of patients exhibit resistance to drugs despite receiving appropriate doses of antiepileptic therapy (French et al., 2004; Kwan and Brodie, 2000). Research into novel antiepileptic drugs with few side-effects and high efficacy is therefore continuing. The most commonly accepted view in the pathophysiology of epilepsy is that seizures develop as a result of neuronal hyperexcitability resulting from imbalance between inhibitory and excitatory amino acids. Much research is today being performed into the neurochemical causes of episode development (Georgiev et al., 1993). An excessively prolonged epileptic episode will lead to a series of interconnected changes in neuronal cells (Johansson et al., 1996). Oxidative damage may develop in association with an increase in free oxygen radicals related to mitochondrial dysfunction resulting from changes in neuronal cells (Loscher, 2002; Markowitz et al., 2010). Research is therefore taking place into molecules with a powerful antiepileptic property, that can prevent oxidative stress and that will have few side-effects (Loscher, 2007).

The thiamine (TH) investigated in this study was vitamin B1. Thiamine pyrophosphate (TPP) is an active metabolite of thiamine. Thiamine deficiency leads to neurological disorders, such as Wernicke encephalopathy and beriberi (Ferraro et al., 1999). Previous experimental studies of ours examining the effects of TH and TPP on oxidative damage caused by drugs with toxic effects on the CNS have shown that TPP has positive effects on oxidative damage (Turan et al., 2013a,b). To the best of our knowledge, the literature contains no previous data or findings regarding the protective effects of TH and TPP against epileptic seizure induced with caffeine and against oxidative stress in the rat CNS. The aim of this study was therefore to investigate the effects of TH and TPP on oxidative stress in an epileptic model induced in rats using caffeine.

Materials and methods

Animals

Forty-eight male albino Wistar rats weighing 210—230 g were obtained from the Ataturk University Medicinal and Experimental Application and Research Center, Erzurum, Turkey. Animals were allowed 7 days to acclimatize before the experiments commenced. They were kept in a 12:12 h light/dark cycle (lights on 07:00—19:00 h) in an air-conditioned constant temperature (22 ± 1 °C) colony room, with free access to water and 20% (w/w) protein commercial chow. All studies were performed in accordance with the ethical guidelines set out by the local ethical committee that were fully compatible with the ''NIH Guide for the Care and Use of Laboratory Animals''.

Chemical substances

TH and TPP were provided by Biopharma, Russia. Thiopental sodium and diazepam were obtained from IE Ulagay, Turkey, and Deva, Turkey, respectively.

Pharmacological procedures

Animals were randomly divided into 8 groups of 6 animals each before the experimental procedures began (TH10, TH30, TH50, TPP10, TPP30, TPP50, diazepam and control groups). All doses were administered intraperitoneally (ip) as milligrams per kilogram. The TH10 group was given 10 mg/kg thiamine, the TH30 group 30 mg/kg thiamine, the TH50 group 50 mg/kg thiamine, the TPP10 group 10 mg/kg TPP, the TPP30 group 30 mg/kg TPP, the TPP50 group 50 mg/kg TPP and the diazepam group 2 mg/kg diazepam, all ip. The control group was given saline solution ip. One hour after the administration of TH, TPP, diazepam or saline solution, all animals were injected with 300 mg/kg of caffeine ip, and time of administration was recorded. The 1-h interval was due to the T_{max} (the time after administration of a drug when the maximum plasma concentration is reached) value for diazepam having been reported as 1 h and the elimination half-life as 1.4 h when given ip (Loscher, 2007; Markowitz et al., 2010). Animals' clinical condition was observed, and the time between caffeine administration and seizure onset was defined as the latent period. The animals were observed for the appearance of generalized tonic—clonic convulsive episodes as described by Ferraro et al. (1999). They described generalized convulsions as episodes characterized by generalized whole-body clonus involving all four limbs and tail, and wild running and jumping, followed by sudden loss of upright posture and autonomic signs, such as defecation and hypersalivation (Ferraro et al., 1999). Time of onset of epileptic seizure was recorded, and the latent period was calculated in seconds. Animals dying post-epileptic seizure were recorded. At the end of the study period, immediately after the end of clinical episode, surviving animals were sacrificed with a high dose of anesthesia (50 mg/kg sodium thiopental).

Brains were extracted from both sacrificed animals and those during the study. The cerebrum was used after recovery of the upper layer, and biochemical examination was performed. The results from the TH10, TH30, TH50, TPP10, TPP30 and TPP50 groups were compared with those from the diazepam and healthy groups.