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Different effects of postnatal caffeine treatment on two pentylenetetrazole-induced seizure models persist into adulthood

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Abstract:
Background: Postnatal treatment with caffeine from P7 to P11 (10 or 20 mg/kg daily) resulted in transient changes in two pentylenetetrazole (PTZ)-induced models of epileptic seizures characterized by spike-and-wave EEG rhythm in immature rats. To know if some changes persist into adulthood we studied these models in young adult Wistar rats.
Methods: Caffeine treatment at a daily dose of 10 and/or 20 mg/kg, *sc* was executed during postnatal days 7–11. Rhythmic metrazol activity (RMA, model of human absences) was induced in 60-day old rats by two successive doses of PTZ (20 + 20 mg/kg, *ip*) while for induction of minimal clonic seizures (model of human myoclonic seizures) the second dose of PTZ was 40 mg/kg.
Results: RMA episodes elicited by the 20 + 20 mg/kg dose of PTZ in adult rats exposed to caffeine at P7 to P11 were decreased. This effect was more pronounced in group treated with the higher dose of caffeine. In contrast, the lower dose of caffeine exacerbated minimal clonic seizures (both incidence and intensity were increased). In addition, some animals from the 20-mg/kg caffeine group exhibited transition to generalized tonic-clonic seizures.
Conclusion: Different effects of postnatal caffeine exposure persist into adulthood; the seizure ameliorating effects in a model of absences and seizure exacerbating action in a model of myoclonic seizures are dose-specific.

Key words:
caffeine, postnatal treatment, pentylenetetrazole, spike-and-wave episodes, minimal clonic seizures, rats

Introduction

Caffeine represents one of the most extensively self-administered substance in the world. Caffeine and various analogs, are prescribed as analgesics, stimulant adjuvants, antiinflammatories, antitussives, diuretics/natriuretics, and lipolytics [4]. Moreover, methylxanthines are routinely used in neonatal medicine either acutely or chronically for suppression of apneic episodes in premature infants [3, 28]. Both clinical and experimental evidence on safety and long-term consequences of chronic caffeine administration are scarce [28, 32]. Recent report demonstrated that caffeine improved the rate of survival without neurodevelopmental disability at a corrected age of 18 to 21 months [33]. However, the long-term drug-induced blockade of adenosine receptors at early stages of maturation may modify brain development and have late consequences on brain excitability. There is evidence that neonatal caffeine treatment alters adenosinergic neuromodulation of the respiratory control

system and that this change persists until adulthood [29]. Adenosine is a neuromodulator which plays an important role in epileptic seizures probably as an endogenous anticonvulsant [20]. Chronic exposure to caffeine during peri- and postnatal period in experimental animals was shown to result in adaptive long-lasting neurochemical and behavioral responses that are usually opposite to acute drug effects under normal as well as pathological conditions [23, 30]. Several laboratories, including ours, considered the consequences of chronic caffeine treatment in adult animals as well as during development on seizure susceptibility [2, 13–15, 17, 18, 21, 34, 35]. Numerous experimental data support the idea that the different effects of long-term caffeine treatment in a variety of seizure models cannot be associated with a single specific mechanism of action but might represent a more general phenomenon affecting different neurotransmitter systems [15, 30, 36, 37]. Our previous results revealed that the consequences of postnatal chronic caffeine treatment are more complicated and are dose and model dependent. Thus, in contrast to a decreased sensitivity to convulsant action of drugs interfering with inhibitory systems [37], an increased sensitivity to convulsant effects of glutamate receptor agonists N-methyl-D-aspartate and kainic acid was demonstrated in rats after early-life caffeine administration [37]. Furthermore, caffeine exposure from P7 to P11 caused transient dose-dependent pro- or anticonvulsant action in another model of seizures – epileptic afterdischarges (ADs) elicited by electrical stimulation of sensorimotor cortex [34]. Recently, we have demonstrated that postnatal caffeine treatment exerted different action in two models of experimental seizures in developing rats: rhythmic metrazol activity (RMA) and minimal clonic seizures [34]. These two models can be elicited by different doses of pentylenetetrazole (PTZ); both are characterized by EEG spike-and-wave (SW) rhythm but markedly differ in their behavioral correlates. RMA episodes are characterized by behavioral arrest accompanied by minute rhythmic movements of vibrissae whereas minimal clonic seizures are convulsive seizures involving mostly head and forelimb muscles. Early life caffeine treatment resulted in changes in the model of nonconvulsive (absence) seizures in 18-day-old rats but it did not influence this model in 25-day-old rats, i.e., this effect is only transient [37].

In the present series of experiments we decided to study how postnatal caffeine treatment does or does not affect the two above mentioned seizure models elicited by PTZ in young adult rats. The level of maturation corresponding to full-term human newborn is attained around postnatal day 10 [6], therefore, a stage corresponding to human perinatal period (rats 7–11 days old) was again chosen for repeated caffeine administration.

Materials and Methods

The experiments were carried out on male Wistar rats (breeding of the Institute of Physiology, Academy of Sciences, Prague, Czech Republic). Litters were culled to eight pups at postnatal day 1–2 (postnatal day 0 is a day of birth). The rats were housed together with their mothers in a temperature-controlled environment (22 ± 1°C and humidity 50–60%) with a 12/12 h light/dark cycle (lights on at 6 a.m.). Food and water were provided *ad libitum*. Rat pups were taken from their mothers just before caffeine injections. The animals were weaned on postnatal day 28 (P28) and after this age they were housed in stable social groups under the above mentioned controlled conditions to reach the adulthood (PD60).

All experiments were approved by the Animal Care and Use Committee of the Institute of Physiology to be in agreement with Animal Protection Law of the Czech Republic (fully compatible with European Community Council directives 86/609/EEC).

Rat pups in each litter were randomly assigned to one control (n = 9) and two experimental groups: group with caffeine 10 mg/kg (n = 13) and group with caffeine 20 mg/kg (n = 12). Each group was formed by pups from four or five litters. Caffeine administration started at PD7. Animals assigned to the experimental groups were injected subcutaneously with either 10 or 20 mg/kg caffeine (Sigma, St. Louis, MO, USA) in a volume of 1 ml/kg. Control rats received saline (1 ml/kg). Injections were repeated daily for 5 days.

Epidural cortical electrodes were implanted in 60-day-old rats under ketamine (40 mg/kg, *ip*) and xylazine (20 mg/kg, *ip*) anesthesia. Four cortical recording electrodes were implanted epidurally symmetrically over sensorimotor and occipital areas of both hemispheres with coordinates (AP = 0; L = 2.5 mm

and AP = 6, L = 4 mm, respectively). Both reference and ground electrodes were placed into the occipital bone. All electrodes were connected to a female plug and the whole assembly was fixed to the skull by fast-curing dental acrylic. The surgery lasted 15 to 20 min. After surgery, the rats were allowed to recover for one week before the experiments started.

EEG of 67-day-old rats was amplified and recorded using Vision-Brain video EEG monitoring system (FGU AV ČR, Czech Republic). Digitalization rate was 200 Hz, the signals were filtered at a range of 1.6–50 Hz. All rats were connected to the system and allowed to habituate to the environment for 15 min. Control EEG was registered for 10 min, then two successive PTZ injections were delivered with a 25-min interval and registration continued for at least 30 min after the second injection. Behavior of the rats was continuously registered by video camera. EEG was evaluated off-line with EEG Viewer software (EEG Viewer for MatlabR2007b©, Otáhal 2005–2010).

Pentylenetetrazole (PTZ; free base) (Sigma, St. Louis, MO, USA) was freshly dissolved in 0.9% NaCl solution and administered intraperitoneally at a dose of 20 mg/kg (in a volume of 1 ml/kg) followed after 25 min by the second injection (20 mg/kg).

Latency to the appearance of the first RMA episode and of the first generalized RMA (GRMA, i.e., recorded in all four cortical areas) was measured. RMA episodes were counted between the 10–15 min and 20–25 min (after both the first and the second PTZ injection) and their duration was measured. In addition, both total and mean duration of episodes in the above mentioned intervals were calculated for every dose of PTZ.

Experimental design was the same as in the first experiment, only the second dose of PTZ was 40 mg/kg. The same parameters as in Experiment 1 were registered. The presence of RMA after the first injection served as an index of activity of PTZ. After the second PTZ injection, the incidence and latency of clonic seizures were registered and seizure intensity was calculated using the five-point scale [31].

Statistical analysis

The means and SEM were calculated for all data. Depending on whether the data were normally distributed or not, either parametric or non-parametric test (Mann-Whitney U-test) was used for statistical evaluation. ANOVA with subsequent pairwise comparison using Holm-Sidak test was used to compare all parameters between controls and caffeine-treated groups. The incidence of seizures in Experiment 2 was evaluated by Fisher exact test. All calculations were performed with SigmaStat® (SPSS). The level of statistical significance was set at 5%.

Results

EXPERIMENT 1: **Rhythmic metrazol activity (RMA) induced with successive doses of PTZ (20 + 20 mg/kg)**

The 20-mg/kg dose of PTZ elicited rhythmic EEG activity (RMA) formed by spike-and-wave (SW) rhythm with a frequency of 4–6 Hz accompanied by behavioral arrest in all animals in all three groups (Fig. 1). This SW rhythm predominated in frontal over occipital regions. The difference between the latencies to the first RMA and the first GRMA episode

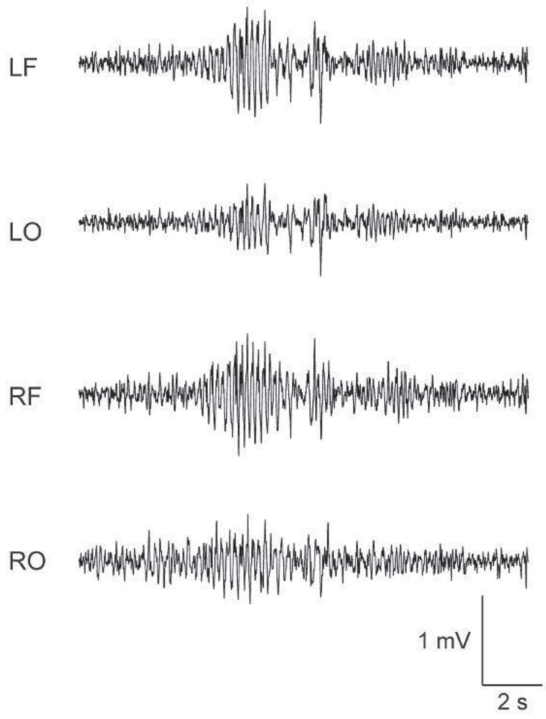

Fig. 1. EEG recording of a spike-wave episode (RMA) from a 67-day-old rat 20 min after the PTZ administration (20 mg/kg, *ip*). Individual leads from top to bottom: LF – left frontal, LO – left occipital, RF – right frontal, RO – right occipital always in reference connection. Amplitude callibration 1 mV, time mark 2 s

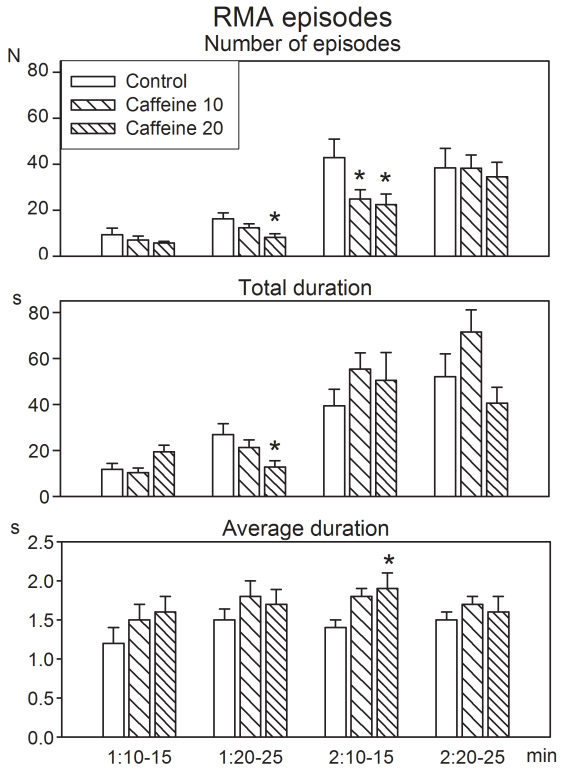

Fig. 2. Effects of chronic treatment with caffeine (10 and 20 mg/kg, *sc*) during P7-P11 on spike-and-wave episodes (RMA) elicited by PTZ injection (20 + 20 mg/kg, *ip*) in adult rats. From top to bottom: number of RMA episodes; total duration of RMA episodes; mean duration of RMA episodes between the 10th and 15th and between the 20th and 25th min after the 1st and after the 2nd 20-mg/kg dose of PTZ, respectively. Abscissa: control animals (n = 9), group with caffeine 10 mg/kg (n = 13), group with caffeine 20 mg/kg (n = 12) (see inset); Ordinates: upper graph – number of episodes; middle and lower graph – duration in seconds. Data are the means ± SEM; * $p < 0.05$ *vs.* controls

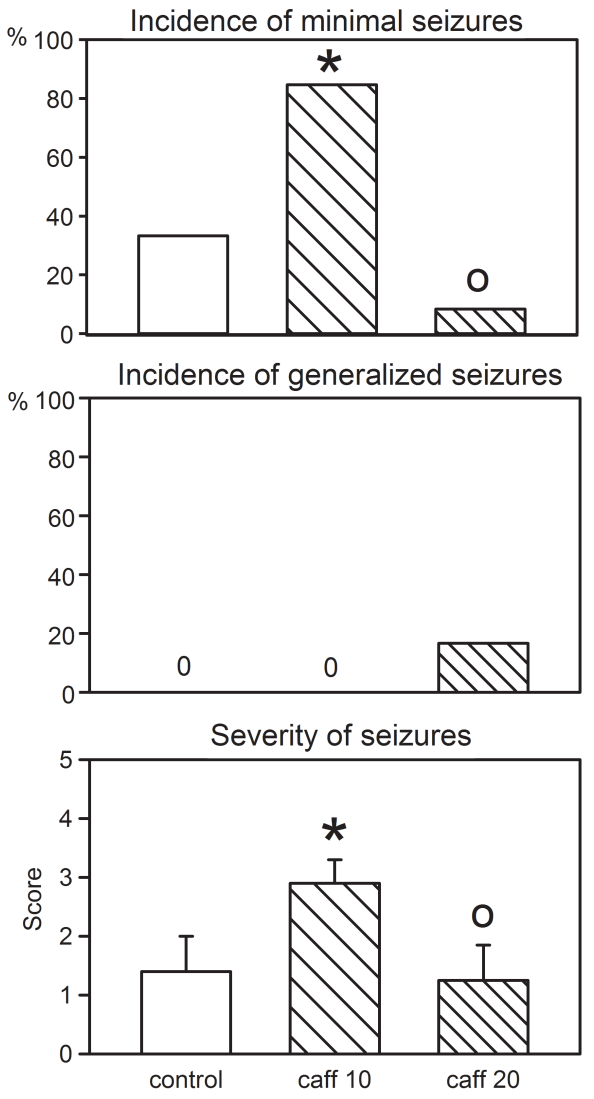

Fig. 3. Effects of chronic treatment with caffeine (10 and 20 mg/kg, *sc*) during P7-P11 on convulsive seizures induced by the 20 + 40-mg/kg dose of PTZ in adult rats. From top to bottom: Incidence of minimal, clonic seizure; incidence of generalized seizures; severity of seizures. Details as in Figure 2. * $p < 0.05$ *vs.* controls; ^{o} $p < 0.05$ *vs.* group with 10 mg/kg caffeine

was minimal; no significant differences were detected (data not shown). Both number and total duration of RMA episodes markedly increased after the second injection in all three groups. Caffeine groups showed a lower number of RMA episodes than their control siblings, the significant level was reached 20–25 min after the 1st PTZ injection (caffeine group with 20-mg/kg injections), 15–20 min after the 2nd PTZ injection (both caffeine groups) (Fig. 2). The duration of RMA episodes elicited by the 1st PTZ injection was shortened at 20–25 min in rats injected postnatally with the 20-mg/kg dose of caffeine (Fig. 2). The average duration of RMA in adult rats remained unchanged after early caffeine treatment in either dose group with the exception of the 10–15-min interval after the second injection in the 20-mg/kg caffeine group (Fig. 2).

EXPERIMENT 2: **Rhythmic metrazol activity and minimal clonic seizures induced with successive doses of PTZ (20 + 40 mg/kg)**

There were no significant changes in the incidence and the latency of RMA episodes after the first dose of PTZ (20 mg/kg) when compared with data from Experiment 1 (data not shown). EEG pattern of minimal seizures observed in 3 out of 9 control rats after the 2nd dose of PTZ was represented by spike-and-wave activity with a frequency of about 3 Hz. Motor

pattern of minimal seizures was formed by clonic seizures of head and forelimb muscles. No marked tonic component was shown in the control group. Caffeine pretreated groups did not exhibit any changes in the EEG and/or motor pattern of seizures. The incidence of clonic seizures was increased in the group treated with 10 mg/kg at P7–P11 (Fig. 3). There was no difference in the latency for the first clonic seizure among groups (data not shown). A significant increase in seizure intensity was observed in the group with lower dose of caffeine (Fig. 3). Two out of 12 rats exposed to the higher dose of caffeine at P7–P11 exhibited generalized tonic-clonic seizures (Fig. 3).

Discussion

In this study, we demonstrated that repeated postnatal treatment with caffeine in doses of 10 and 20 mg/kg results in changes in young adult rats. The effects in the two models of epileptic seizures characterized by SW rhythm in the EEG in adult rats were opposite. Early life caffeine exposure led to a dose-dependent suppression of nonconvulsive seizures (model of human absence seizures) induced by low doses of PTZ in adult rats. On the contrary, the lower dose of 10 mg/kg caffeine injected at P7–P11 exacerbated minimal clonic seizures and the higher dose resulted in an appearance of generalized tonic-clonic seizures (a phenomenon never seen in control animals) in two out of 12 rats.

Recently, we have shown that early life caffeine treatment resulted in age-dependent changes in a model of epileptic absence seizures with complete suppression in 18-day-old rats but without effect in 25-day-old rats [37]. However, postnatal caffeine treatment alleviated these seizures also in adult rats suggesting that this phenomenon is not transient and the development is not so simple as was expected earlier. In addition, various phenomena develop differently – e.g., changes elicited by postnatal caffeine treatment in the first two (transcallosal) components of cortical interhemispheric responses were present in all age groups of immature rats studied (including 25-day-old ones) but not in adult animals [38].

Numerous data have also shown that chronic treatment of adult rats with caffeine and other methylxanthines decreased seizure susceptibility in many acute seizure models with different mechanism of action in adult rats [13, 19, 21]. However, few studies, including ours, demonstrated that seizure susceptibility could be modified as a consequence of early caffeine treatment [14, 16, 35–38].

Caffeine in doses used in our experiments antagonizes both adenosine A1 and A2 receptor subtypes with nearly equal affinities (with Ki values of 29 and 48 μM, respectively) [11], therefore, we can assume that these receptor subtypes are the most likely targets. In the rat, the appearance of the adenosine A1 receptors is gradual and area-specific [16]. Adenosine A2A receptors are also present in the forebrain during the postnatal period, when caffeine is able to modulate the development of the seizure threshold [7]. Adenosine A1 and A2A receptors have opposite actions, therefore, caffeine effects could be due to the diverse activity on adenosine receptor subtypes. Other possible mechanisms of action may be taken into account – the effect on the ryanodine receptor [27] or on heteromeric A(2A)-D(2) receptor complex [10] but they are less probable. El Yacoubi et al. [8] suggested that the protective effects against PTZ-induced seizures, which occurs when adenosine A(2A)R is absent or chronically blocked by a relevant dose of caffeine might be related to a decreased neuronal excitability. The results from experiments on adult rat hippocampus showed an interaction between co-expressed and co-localized A2A and A1 receptors, providing a mixture of excitatory and inhibitory modulation of neuronal excitability [4]. Experiments in transfected cells demonstrated the ability of A1 receptors to heteromerize with A2A receptors and this striatal A1-A2A receptor heteromer provides a "concentration-dependent switch" mechanism by which low and high concentrations of synaptic adenosine can produce opposite effects on glutamate release [9]. The apparent divergence in the effects of the two doses of caffeine on the susceptibility to seizures in our study might be due to disturbed balance between A1 and A2 receptors in brain areas associated with seizure phenomena.

In this regard, Guillet and Kellogg [16] reported that changes in adenosine receptor binding occur as a function of age as well as neonatal caffeine exposure in different brain regions. Neonatal caffeine treatment accelerates the development of adenosine A1 receptors in rats; they reach adult densities at earlier ages and this correlates with altered age-related behavioral development. Similar acceleration was

found in brainstem at P6 after repeated administration of caffeine at P2–P6 [12], i.e., at the same age as in Guillet and Kellog study [16]. Adenosine A1 receptors reach adult density at postnatal day 8 in the brainstem, day 14 in hypothalamus, day 24 in the cerebellum and even later in cortex [12, 16, 19]. Adenosine A2A receptors in striatum increase during the second and third postnatal week [24]. The repeated caffeine injections in our experiments were thus applied at developmental stages when adenosine receptors are present but not yet fully mature.

The two seizure models studied in the present experiments are characterized by spike-and-wave rhythm in the EEG. Our previous reports on immature rats demonstrated a difference in the frequency of the SW rhythm between the two PTZ-induced models (higher frequency in absence model *vs.* lower frequency in minimal clonic seizure model). Motor pattern of the two seizure models markedly differ – minute motor phenomena *vs.* violent clonic seizures [22, 26]. Different motor patterns indicate different spread of epileptic activity into the motor system – only to the nuclei of cranial nerves in a model of absences and to the generator of minimal seizures localized in basal forebrain and to the spinal cord motoneurons (at least in the cervical and upper thoracic segments) in the model of myoclonic seizures elicited by higher doses of PTZ [1, 25]. Not only seizure models but also the intensity of epileptogenic stimulus – the dose of PTZ in our experiments may play a role. Anticonvulsant effect was seen if low dose of PTZ was administered whereas the action of the higher dose of PTZ (40 mg/kg) was potentiated [37].

Similarly to previous finding in 18-day-old rats [37], we have found that young adult rats exposed to the lower dose of caffeine during postnatal period tended to increase the incidence of seizures in a myoclonic seizure model. This enhanced susceptibility was accompanied with higher seizure intensity in the group exposed to the lower dose of caffeine. The proconvulsant effect of a dose of 10 mg/kg caffeine was also demonstrated in another model of myoclonic seizures – epileptic afterdischarges – what agree with our hypothesis that low dose of caffeine is proconvulsant in models of forebrain convulsive seizures [34]. What remains to be solved is if increased sensitivity to PTZ is only in some brain structure (e.g., generator of minimal clonic seizures) or in the whole brain.

There are two main results of the present study:

1. Repeated postnatal caffeine administration led to different results in two models of PTZ-induced epileptic seizures.

2. Changes observed in 18-day-old but not in 25-day-old rat pups [36] were present in young adult animals, i.e., early-life caffeine administration affects reactivity of the brain up to the adulthood.

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