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Postnatal caffeine treatment affects differently two pentylenetetrazol seizure models in rats

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ABSTRACT

Effects of repeated postnatal administration of caffeine (10 and 20 mg/kg s.c. daily from P7 to P11) were studied in two models of epileptic seizures characterized by spike-and-wave EEG rhythm in 18- and 25-day-old rats. Rhythmic metrazol activity (RMA, model of human absences) was induced by low dose of pentylenetetrazol (PTZ, 20 mg/kg or 40 mg/kg, i.p.) and minimal clonic seizures (model of human myoclonic seizures) by two successive doses of PTZ (20 and 40 mg/kg i.p.). Early postnatal caffeine treatment resulted in significant changes of RMA only in 18-day-old rats. Anticonvulsant effects were observed in RMA episodes elicited by the 20-mg/kg dose of PTZ in both caffeine groups whereas latency of RMA episodes induced by the 40-mg/kg dose was shortened and their duration was prolonged. No changes were found in 25-day-old animals. Incidence, EEG and motor pattern of minimal clonic seizures were not changed. Some animals in both control age groups exhibited transition to generalized tonic–clonic seizures. This type of seizures never appeared in caffeine-treated 25-day-old animals. Mixed effects of postnatal caffeine exposure were demonstrated; these predominantly anticonvulsant effects are age- and model-specific.

1. Introduction

Adenosine is commonly accepted as a neuromodulator in central nervous system (CNS) with an inhibitory action on synaptic activity and neurotransmitter release.[1] Low extracellular levels of adenosine in brain activate adenosine A_1 and A_{2a} receptors under physiological conditions.[2] Both receptors are blocked by methylxanthines.[2] The methylxanthine caffeine is the most common CNS stimulant in everyday life. The proconvulsant action of adenosine antagonists such as caffeine, aminophylline and other methylxanthines was described both in adult[3] and in young rats.[4,5] Moreover, it has been shown that immature rats are more susceptible to methylxanthines than the adult animals.[6] Nowadays, methylxanthines are among the most often prescribed drugs in neonatal medicine[7], and in particular, in the treatment of apneic episodes in premature infants.[8] Methylxanthines are given to preterm infants both acutely (loading dose) or chronically.[7] The long-term drug-induced blockade of adenosine receptors at early stages of maturation might modify brain development. The outcome of such blockade cannot be predicted from clinical data therefore experimental studies are necessary. In experimental animals, chronic exposure to caffeine during peri- and postnatal period has been 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.[9–13] Only few reports considered the consequences of repeated treatment with this methylxanthine on seizure susceptibility.[11,14] Similar to the data from adult rodents, repeated caffeine treatment during postnatal period results in a decreased seizure susceptibility in different models of convulsive seizures. Moreover, caffeine effects may delay the decrease in the seizure threshold that occurs for many agents since the late juvenile age.[11,14] The observed attenuation in the seizure susceptibility after repeated administration of caffeine at low doses of 15–20 mg/kg for 5 days both during adulthood and developmental period correlates with an increase in adenosine A_1 receptor density in specific brain structures.[10,15]

Present experiments represent continuation of our recent findings that repeated caffeine administration at postnatal days 7–11 caused both transient and durable age-specific changes in behaviour and seizure susceptibility.[16–18] Furthermore, repeated postnatal caffeine administration attenuated sensitivity to convulsant drugs with different mechanisms of action (aminophylline, pentylenetetrazol (PTZ), picrotoxin, bicuculline) in an age-dependent manner.[18] These data suggest that repeated postnatal caffeine administration affects not only adenosinergic system but at least also (probably indirectly) GABAergic inhibitory system.

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The aim of the present study was to further specify effects of repeated postnatal caffeine treatment in immature rats on seizures in later life. We hypothesized that effects will depend on the type of elicited seizures. Two models of human seizures with different pathophysiological mechanisms were used – a model of generalized absence seizures with an important role of inhibitory mechanisms and a model of myoclonic seizures with marked convulsions (for review[19]). Such two models can be elicited by different doses of pentylenetetrazol (PTZ); both are characterized by EEG spike-and-wave (SW) rhythm but markedly differ in their behavioral correlates. An acute model of absence seizures can be induced by low doses of PTZ. This model is accepted as a valid model of this type of primary generalized human absence seizures.[20] It is characterized only by EEG spike-and-wave rhythm (rhythmic metrazol activity, RMA) but also by the same motor pattern (behavioral arrest and minimal motor phenomena[21]) and pharmacological sensitivity[22] as other experimental models of absences such as feline generalized penicillin epilepsy,[23] gamma-hydroxybutyrate-induced model[24] and genetically determined absence seizure GAERS and WAG/Rij rats.[25,26]

Higher doses of PTZ elicit in rodents clonic seizures usually restricted to head and forelimb muscles without a loss of righting reflexes (minimal metrazol seizures of the older literature); tonic component if present consists mainly of torsion of the trunk.[27] These seizures are also characterized by a spike-wave rhythm in the EEG what is in agreement with the clinical data that this rhythm accompanies not only absences but also other types of seizures in epileptic patients.[28] Motor pattern and pharmacological sensitivity suggest that these seizures could be considered as an experimental model of myoclonic seizures.[29,30] Early postnatal caffeine exposure caused transient dose-dependent pro- or anticonvulsant action in another model of myoclonic seizures elicited by electrical stimulation of sensorimotor cortex – epileptic afterdischarges (ADs).[17,31] Comparison with this model may demonstrate a role of an epileptogenic agent. The 40-mg/kg dose of PTZ never inducing minimal clonic seizures in naïve rats failed to show possible proconvulsant effect of repeated caffeine exposure therefore two successive doses of PTZ (20 and 40 mg/kg i.p.) were administered. Only two age groups (18- and 25-day-old rats) were studied because SW episodes and minimal clonic seizures cannot be induced reliably with PTZ before postnatal day 18.[27,32]

2. Methods

2.1. Animals

The experiments were carried out on immature Wistar rats (breeding of the Institute of Physiology, Academy of Sciences, Prague, Czech Republic). Litters consisted from 10 pups; postnatal day 0 was 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 (with the exception of the test period). Rat pups were taken from their mothers just before testing. 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).

2.2. Caffeine treatment

Rat pups in each litter were randomly assigned to one control and two experimental groups. Each group consisted of at least 10 animals and contained pups from four or five litters. Caffeine administration started at P7; animals assigned to the experimental groups were injected subcutaneously with either 10 or 20-mg/kg caffeine (Sigma, St. Louis, MO, #C 0750) in a volume of 1-ml/kg body weight. Control rats received saline (1 ml/kg). Injections were repeated daily for 5 days.

2.3. Surgery

Four cortical recording electrodes were implanted epidurally over sensorimotor and occipital areas symmetrically over both hemispheres to 18- or 25-day-old rats. Surgical preparation was performed under ether anesthesia. The coordinates were recalculated from the adult brain (AP = 0; L = 2 mm and AP = 6, L = 4 mm, respectively) based on bregma-lambda distance. A reference electrode was placed into the nasal bone and the ground electrode into the occipital bone. All electrodes were fixed to the skull by fast-curing dental acrylic. The surgery lasted less than 10 min. Rats were then placed into plexiglas cages over an electrical heating pad (34 °C, i.e. the temperature in the nest). The animals were allowed to recover for at least 1 h. After the recovery period, righting and placing reflexes were examined and the animals were offered 5% sucrose solution not only as a nutrient but also to check the suckling reflex. Only then the experiment started.

2.4. Recording

EEG was registered in a monitoring system (Kaminskij Biomedical Research Systems, Prague); the rate of digitalization was 200 Hz. All rats were allowed to habituate to the environment for 15 min. Control EEG was registered for 10 min, then PTZ was injected and registration continued for at least 30 min, all behavioral phenomena were coded directly into EEG recording.

2.5. Experiment 1: rhythmic metrazol activity (RMA) induced with the 20-mg/kg dose of PTZ

Pentetrazol (PTZ; free base) (Sigma, St. Louis, Mo., USA) was freshly dissolved in 0.9 % NaCl solution and administered in a dose of 20 mg/kg (in a volume of 1 ml/kg) intraperitoeneally. 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. All RMA episodes (their number and duration) between 10–15 min and 20–25 min after PTZ injection were counted and both total and mean duration of episodes were calculated.

2.6. Experiment 2: rhythmic metrazol activity induced with the 40-mg/kg dose of PTZ

Experimental design was the same as in Experiment 1, only the dose of PTZ was 40 mg/kg i.p. Latency to the appearance of the first and the first generalized RMA episode, number and duration of these episodes were counted in the same two 5-min periods as in the first experiment and total and mean duration of episodes were calculated.

2.7. Experiment 3: seizures induced with successive doses of PTZ (20 + 40 mg/kg)

Experimental design was again the same as in the first and second experiments, the first 20-mg/kg dose of PTZ was followed after 20 min by the second injection (40 mg/kg). Continuous EEG registration was stopped 30 min after the second dose of PTZ. Presence of RMA episodes after the first dose served as an indicator of PTZ action. Attention was focused on incidence of seizures, their motor pattern and duration.

2.8. Statistical analysis

Means and S.E.M. were calculated for all RMA episodes data. The data were subjected to analysis by two-way ANOVA, with