

Short communication

Maternal caffeine intake impairs MK-801-induced hyperlocomotion in young rats

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Abstract

Here we have investigated the effects of maternal caffeine intake (1 g/l) on MK-801-induced hyperlocomotion in rat pups. Animals submitted to caffeine treatment during the gestational and lactational period were separated in two groups: caffeine-treated group (up to 21 days old) and washout group (caffeine treatment up to 7 days old). MK-801 (0.25 mg/kg, i.p.) promoted hyperlocomotion in control rats, but this stimulatory effect was significantly decreased in caffeine-treated and washout groups. The permanent effect after caffeine withdrawal suggests durable or adaptive changes during neurodevelopment, mainly on adenosine receptors or neurotransmitter systems modulated by adenosine, such as the glutamatergic system.

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1. Introduction

Caffeine is a psychostimulant drug constituent of many beverages and foods. The biochemical basis for caffeine effects is blockade of adenosine receptors (Fedholm et al., 1999). Once in the extracellular space, adenosine acts via specific G-protein-coupled receptors that include A₁, A_{2A}, A_{2B} and A₃ adenosine receptors (Sebastião and Ribeiro, 2000). The physiological activation of adenosine receptors exerts modulatory roles on several neurotransmitters such as glutamate, dopamine

and acetylcholine (Sebastião and Ribeiro, 2000). Among their effects, adenosine A₁ and A_{2A} receptors mediate inhibition or facilitation of neurotransmitter release, respectively.

Caffeine non-selectively blocks adenosine A_{2a} and A₁ receptors (Fedholm et al., 1999). One of the main effects of caffeine administration is the regulation of motor activity (Fedholm et al., 1999; Karcz-Kubicha et al., 2003). Caffeine promotes a biphasic dose–response curve and the motor-activating effects of acute caffeine administration involve central blockade of both adenosine A₁ and A_{2A} receptors. When caffeine is chronically administered, tolerance to its locomotor effect develops, probably involving mechanisms related to adenosine A₁ receptors (Karcz-Kubicha et al., 2003).

Antagonists of NMDA (*N*-methyl-D-aspartate) receptors, such as MK-801, phencyclidine and ketamine promote

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hyperlocomotion in rodents and their effects in humans are similar to several clinical symptoms of schizophrenia, being considered a pharmacological model for this disorder (Andine et al., 1999). Studies have shown that adenosine receptor agonists counteract the effects of NMDA receptor antagonists in discriminative behavior (Browne and Welch, 1982) and hyperlocomotor activity (Rimondini et al., 1997). However, chronic treatment with caffeine blunts the hyperlocomotor effect induced by MK-801 (Dall'Igna et al., 2003).

In the immature rat brain, adenosine A₁ receptors can be detected from the second week of embryonic period (Weaver, 1996; Adén et al., 2001). The distribution of adenosine A₁ receptors at 20 days of gestational period is very similar to that observed in adult rat brain (Weaver, 1996). However, the functionality of adenosine A₁ receptors in immature brain is unclear. Adén et al. (2001) have suggested poor coupling to G-protein of this receptor at 7 days old post-natal. However, rats exposed during the neonatal period to CPA (*N*⁶-cyclopentyladenosine), an agonist of adenosine A₁ receptors, have reduced white matter, similarly to brain damage observed after hypoxia (Rivikees et al., 2001; Turner et al., 2002).

Caffeine is able to cross all biological membranes and there are neither blood–brain nor placental barriers to caffeine (Fedholm et al., 1999). Maternal caffeine intake promotes controversial effects on the expression of adenosine receptors in mothers, fetuses and neonates (Adén et al., 2001; Léon et al., 2002). Considering the widespread intake of caffeine during pregnancy and the biological properties of caffeine, we investigated the locomotor activity and the effect of MK-801 in young rats submitted to the chronic caffeine exposure during gestational and post-natal life.

2. Material and methods

2.1. Caffeine treatment

Pregnant Wistar rats were maintained on a 12-h light/12-h dark cycle (lights on at 7:00 a.m.) and with free access to food and drinking water. Dams were treated with caffeine solution (1 g/l) from gestational day 1 onwards during the entire gestational and lactational period. Control pregnant rats received tap water. Pups submitted to caffeine treatment during the gestational and lactational period were divided in two groups: caffeine-treated group, which received caffeine up to 21 days old, and washout group, which received caffeine up to 7 days old. Control pups represented the third group. Experiments were performed with pups at 21 days old (34–38 g) between 9:00 a.m. and 4:00 p.m. (light phase). Procedures for the care and use of animals were adopted according to the regulations of Colégio Brasileiro de Experimentação Animal (COBEA), based on the guide

for the care and use of laboratory animals (National Research Council).

2.2. Locomotor activity assessment

Male rats were allocated to individual black wooden boxes (50 cm×30 cm×30 cm, 50 cm high) placed on the floor of a soundproof and diffusely illuminated room. Motor activity of eight rats was recorded simultaneously by a videocomputerized system, with image analysis at four frames per second. The software tracked the animals by distinguishing their white color from the black background of the floor, registering *X* and *Y* horizontal coordinates (Dall'Igna et al., 2003). MK-801 (0.25 mg/kg, 1 ml/kg) or saline was administered i.p. to rats after habituation to the boxes during 60 min and locomotor activity was recorded during the following 70 min.

2.3. Statistical analysis

Comparisons between locomotor activities at different time points were analyzed using General Linear Model (GLM) repeated measure (drug treatment versus time) with time as the repeated measure. Duncan's post hoc was used to determine the differences between specific groups. A value of $P < 0.001$ was considered significant.

3. Results

Caffeine or water consumption was estimated from the loss of water from drinking bottles. Caffeine intake of the caffeine-treated group was 206.51 ± 23.14 mg/day/kg of body weight and 223.90 ± 63.67 mg/day/kg of body weight for washout group. Tap water intake of control group was 209.45 ± 26.83 ml/day/kg of body weight. Fig. 1 shows locomotor activity of 21-day-old rats submitted to caffeine exposure during gestational and lactational periods. As shown in Fig. 1A, caffeine-treated group did not present alterations in spontaneous locomotion, which can be observed during habituation. As expected, MK-801 induced a significant time-dependent increase in locomotor activity compared to control group, starting 10 min after administration and lasting 70 min (Fig. 1A). However, the development of MK-801-induced hyperlocomotion was significantly reduced in caffeine-treated group (Fig. 1A and B) during the whole experiment. In order to observe if these effects were due to the cross-tolerance or promoted by adaptive changes induced by caffeine treatment, the locomotor activity of washout group was analyzed after the acute administration with MK-801. Interestingly, the results showed that washout group ($n=6$) presented a similar locomotor activity to caffeine-treated group after acute treatment with MK-801, maintaining a diminished response to this NMDA receptor antagonist (Fig. 1A and B). Significant main effects for treatment ($n=9$, $P < 0.001$,

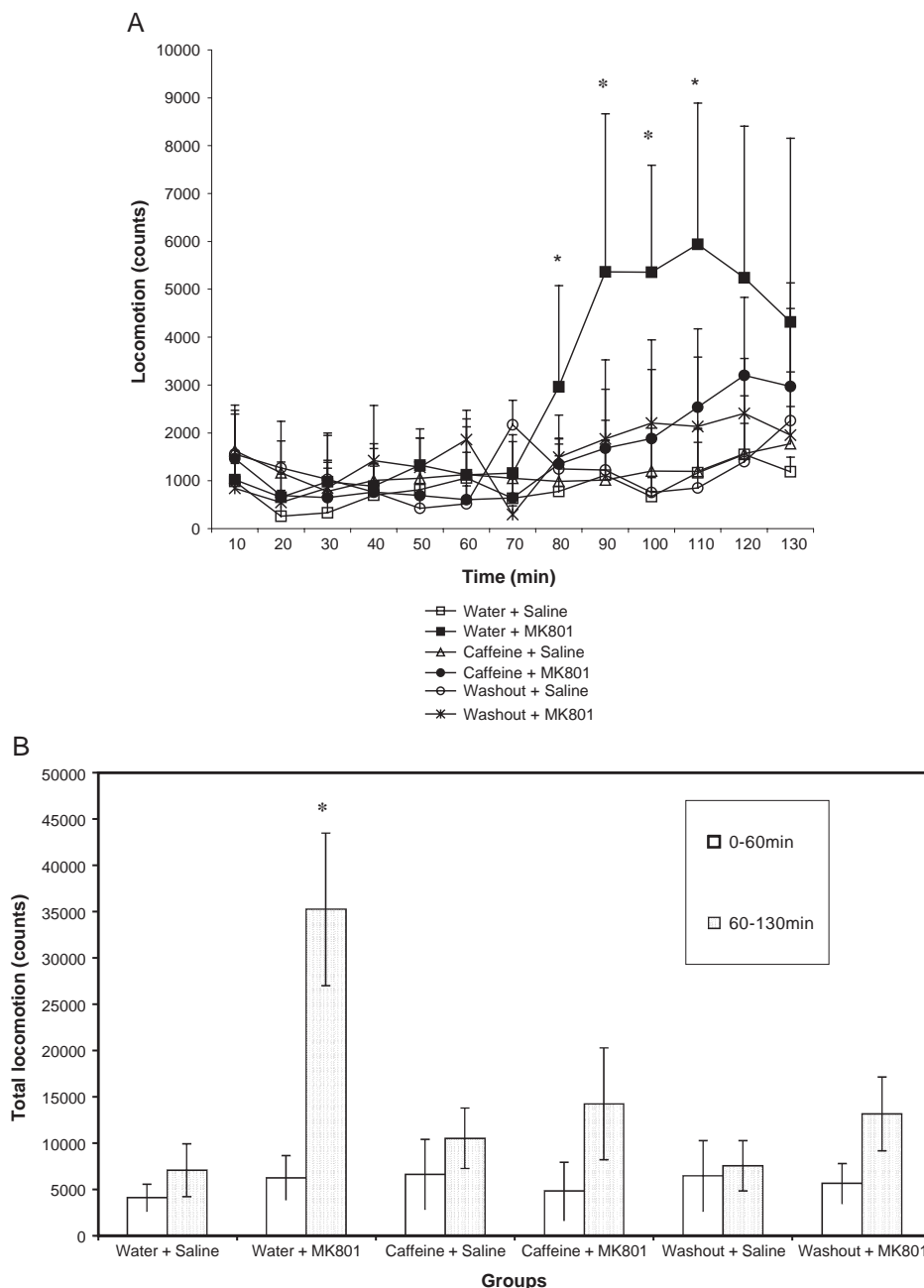


Fig. 1. Effect of maternal caffeine intake on MK-801-induced hyperlocomotion in pups. Control animals, animals that received caffeine (1 g/l) during the gestational period until 21 days old and animals that received the same dose of caffeine until 7 days old were tested when 21 days old. Pups were habituated for 60 min and received intraperitoneal injection of MK-801 (0.25 mg/kg) or saline, and their locomotor activity was assessed for 70 min. A: Locomotion in sets of 10 min. B: Total locomotion during habituation and after MK-801 treatment. Values represent mean \pm S.D. *Significant differences for all groups ($P < 0.001$, GLM repeated measure, post hoc Duncan).

$F_{5,42}=9.717$) and treatment and time interaction ($n=9$, $P < 0,001$; $F_{60,504}=4.465$) were found when comparing locomotor activities for different groups (Fig. 1A and B).

4. Discussion

This study showed that the chronic treatment with caffeine during gestational and lactational periods impairs

the hyperlocomotor response to the NMDA receptor antagonist, MK-801, without affecting normal locomotion. These effects reinforce the idea about the specific involvement of adenosine in locomotor alterations induced by MK-801 (Dall'Igna et al., 2003). Furthermore, it is possible to suggest that permanent and adaptive changes occur in the brain exposed to caffeine during neurodevelopment, mainly involving a specific interaction between adenosine and NMDA receptors.

In the immature rat brain, adenosine receptor expression is quite similar to adult rat brain from the third week of post-natal life (Weaver, 1996). Investigations about the effect of adenosine receptor agonists during early post-natal period showed marked ventriculomegaly, demonstrating the susceptibility of immature brain to pharmacological interventions targeting the adenosinergic system (Rivikees et al., 2001; Turner et al., 2002).

The evaluation of NMDA receptor ontogeny showed immature receptors at the second week of post-natal life (Sircar, 2000). NMDA receptors play a relevant role in synaptic plasticity, being more sensitive in the neonatal than in the adult rat brain (Hestrin, 1992). One possible reason for this higher sensitivity of the developing brain could be a transient increase of NMDA receptor density in rat hippocampus at 6 and 10 days post-natal. The density of these receptors decreases at 13 days toward adult brain levels (Tremblay et al., 1988). Also, alterations in the functioning of regulatory/modulatory sites of NMDA receptor take place during neurodevelopment (Sircar, 2000).

The interaction between adenosine and the glutamatergic system has been investigated and adenosine is a known modulator of glutamate release (Sebastião and Ribeiro, 2000; Ciruela et al., 2001). Activation of adenosine A₁ and A_{2a} receptor reduces NMDA-receptor-mediated effects (De Mendonça and Ribeiro, 1997; Sebastião and Ribeiro, 2000). Reinforcing this assumption, activation of NMDA receptor induces adenosine release in the hippocampus (Manzoni et al., 1994) and striatum (Delaney et al., 1998).

The presence of immature NMDA receptors with higher sensitivity in the developmental brain may be related to the lack of MK-801-induced hyperlocomotion after maternal caffeine treatment. Caffeine exposure during the developmental period may promote an increased release of glutamate by lack of inhibitory tonus induced by adenosine. Taken together, the increased glutamate release and the high sensitivity of NMDA receptor in this phase could promote desensitization of NMDA receptor. This fact could induce the persistent lack of sensitization of NMDA receptors to MK-801 in animals treated with caffeine on the gestational period and first week of post-natal life.

Dall'Igna et al. (2003) have shown cross-tolerance between MK-801 and caffeine in adult mice. To control this cross-tolerance in our results, we performed a washout group, which received caffeine up to 7 days old and were tested 2 weeks later. The similar locomotor profile of washout group in relation to caffeine-treated animals reinforces the idea that the impairment of MK-801-induced hyperlocomotion results from enduring plastic changes rather than a simple pharmacological interaction. Dall'Igna et al. (2003) proposed that a possible mechanism for the stimulant effect of MK-801 is to induce an abrupt reduction of adenosine tone, which would not be relevant after chronic caffeine treatment. Therefore, both results may be related and underscore the important modulating role of adenosine on the glutamatergic system at least in terms of locomotor behavior.

In summary, maternal caffeine intake can induce changes in the immature central nervous system, which are persistent in the young phase even after caffeine withdrawal. Such changes can be related to a deficit in neuromodulation exerted by adenosine in this intense phase of formation of neural connections. An altered inhibitory tonus of adenosine in this condition could facilitate the release of several neurotransmitters, such as glutamate. These results reinforce the influence of adenosine during mammalian neurodevelopment, with implications for the adult behavioral in response to NMDA receptor antagonists, particularly in locomotor activity.

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