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Subchronic-intermittent caffeine amplifies the motor effects of amphetamine in rats

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Summary. Caffeine, the most widely consumed psychostimulant drug, acutely stimulates motor behaviour and enhances dopamine agonists actions whilst chronically it induces tolerance to either caffeine- or dopamine agonist-induced motor activating effects. The present study examined whether subchronic caffeine administration (15 mg/kg, on alternate days for 14 days) induces enduring modifications in caffeine- and amphetamine-mediated motor activity. To this end, motor activation and rotational behaviour stimulated by either caffeine or D-amphetamine (0.5, 2 mg/kg), given 3 days after the last caffeine administration, were evaluated in neurologically intact and unilaterally 6-hydroxydopamine-lesioned rats respectively. Subchronic caffeine resulted in an increase in caffeine-induced motor and turning behaviour. Furthermore, caffeine pretreatment potentiated the motor effects of amphetamine in both intact and 6-hydroxydopamine-lesioned rats. These results suggest that subchronic caffeine treatment results in an enhancement of its motor stimulant effects, rather than in tolerance, and induces neuroadaptive facilitatory changes in dopamine transmission.

Keywords: Caffeine – Amphetamine – Motor behaviour

Introduction

The xanthine-derivate alkaloid caffeine is contained in several dietary sources including coffee, tea, chocolate, soft drinks and over-the-counter medications. The presence of psychostimulant properties, together with its reduced negative side effects and its freely availability, render caffeine one of the most consumed psychoactive substances worldwide.

Solid evidence indicate that psychostimulant actions of caffeine are due to its competitive antagonism at adenosine A_1 and, mainly, A_{2A} receptors (Fredholm et al., 1999; El Yacoubi et al., 2000) and that dopamine is critically involved in the expression of caffeine central effects (Garrett and Griffiths, 1997; Cauli and Morelli, 2005). In rodents, blockade of either D_1 or D_2 dopamine receptors antagonizes caffeine-induced motor stimulation (Garrett and Holtzman, 1994), conversely, sensitization of dopamine receptors potentiates behavioural activation induced by caffeine (Fenu and Morelli, 1998; Fenu et al., 2000). To this end it is interesting to notice that in 6-hydroxydopamine-(6-OHDA)-lesioned rats caffeine induces contralateral turning only in rats repeatedly primed with a dopamine receptor agonist (Herrera-Marschitz et al., 1988; Fenu and Morelli, 1998).

The involvement of dopamine in caffeine effects can be explained through the opposite interactions between A_1-D_1 and A_{2A}-D_2 receptors, located in striatonigral and striatopallidal neurons respectively (Ferré et al., 1997; Fuxe et al., 1998).

Interactions between caffeine and dopamine represent an interesting issue, since the dopaminergic system is critically involved in several physiological processes, such as motor behaviour, associative learning and memory (Jay, 2003). Behavioural and neurochemical evidence indicate that, in rodents, long-term exposure to caffeine influences dopamine transmission. The results obtained, however, critically depend on caffeine doses and protocol of administration. Chronic exposure to high doses of caffeine results in tolerance to its motor stimulant effects, whereas an increase in caffeine-mediated motor behaviour is observed in rats during subchronic and intermittent administration of a moderate dose of caffeine. Moreover, such caffeine treatment enhances behavioural stimulation induced by dopamine D_1 as well as D_2 receptor agonists (Cauli and Morelli, 2002). Interestingly, potentiation of behavioural effects of dopamine agonists induced by re-

peated and intermittent caffeine has been observed 3 days after the interruption of the treatment, suggesting that this phenomenon might reflect the induction of enduring facilitatory neuroadaptive changes in dopamine transmission by caffeine. To this end, sensitization to motor behaviour, consisting in a progressive augmentation in motor activity, is studied as a correlate of enduring changes in neuronal activity induced by drugs.

The present study further examined the interactions between caffeine and dopamine transmission by evaluating the possible sensitization induced by subchronic and intermittent caffeine treatment, at doses approximatively corresponding to the human consumption of four/five cups of coffee (see Fredholm et al., 1999) on the motor effects of the indirect dopamine agonist amphetamine in either neurologically intact and unilaterally 6-OHDA-lesioned rats. Amphetamine administration was preceded by a 3-days washout from last caffeine administration, to avoid the presence of residual caffeine and metabolites (Lau and Falk, 1995).

Materials and methods

Subjects

Male Sprague-Dawley rats were housed in polycarbonate cages and kept under an artificial 12 h light-dark cycle (lights on at 8:00 a.m.), with standard conditions of temperature and humidity. Food and water were available ad libitum, except during measurement of motor behaviour.

All experiments were conducted in accordance with the guidelines for care and use of experimental animals of the European Communities Directive (86/609/EEC; D.L., 27.01.1992, number 116).

Repeated caffeine administration in neurologically intact rats: evaluation of locomotor behaviour

Rats, weighing 180–200 g at the beginning of caffeine or vehicle administrations, were put individually in cages equipped with two pairs of infrared photocell emitters and detectors situated along the long axis of each cage (Opto-Varimex; Columbus Instruments, Columbus, Ohio, USA). Locomotor behaviour along the axes of the cage was recorded by a counter. Locomotor behaviour tests were performed every 10 min for 2 h.

Repeated caffeine administration in 6-hydroxydopamine-lesioned rats

6-OHDA lesions

Rats (275–300 g), anesthetized with chloral hydrate (400 mg/kg, i.p.) were placed on a David-Kopf Instruments (Tujunga, CA, U.S.A.) stereotaxic apparatus and injected unilaterally into the left medial forebrain bundle at coordinates A = −2.2, L = +1.5, V = −7.8, according to the atlas of Pellegrino et al. (1979). 6-hydroxydopamine-HCl (8 μg/4 μl of saline containing 0.05% ascorbic acid) was delivered through a stainless cannula at a rate of 1 μl/min. The injection needle was kept in place for an additional 2 min upon completion of the injection. All rats were pretreated with desipramine (10 mg/kg, i.p.) in order to prevent damage to noradrenergic neurons.

Evaluation of turning behaviour

At 10 days after 6-OHDA infusion, rats were screened on the basis of their contralateral rotations in response to a single administration of apomorphine (0.2 mg/kg, s.c.). Rats not showing at least 100 contralateral rotations during the 1 h testing period were eliminated from the study. Turning behaviour was measured by placing rats in hemispherical bowls with sawdust on the floor and connecting them to an automated rotometer system. A direction-sensitive rotation sensor detected the number of full (360°) rotations in any direction. Rats were placed in each apparatus 30 min before drug administration in order to acclimatize and extinguish any spontaneous rotational behaviour.

Experimental procedure

Experiments consisted of two phases: caffeine subchronic intermittent administration and, three days after treatment, amphetamine administration in caffeine pretreated rats.

Caffeine intermittent administration

After 1 h of habituation to the test cage, neurologically intact rats received caffeine (15 mg/kg, i.p.) or vehicle (i.p.) in spaced injections (one injection every other day) for 14 days (seven total administrations); locomotor behaviour was recorded for 2 h during first and last caffeine administration.

At 2 days after apomorphine challenge, 6-OHDA-lesioned rats received the same treatment described above; turning behaviour was measured in hemispherical bowls for 3 h during first and last caffeine administration.

Amphetamine administration in caffeine pretreated rats

In neurologically intact rats, 3 days after the last caffeine or vehicle administration, amphetamine (0.5 mg/kg, s.c.) was administered and locomotor behaviour was recorded for 2 h.

In 6-OHDA-lesioned rats, 3 days after the last caffeine or vehicle administration, amphetamine (2 mg/kg, i.p.) was administered and turning behaviour was measured for 3 h.

Drugs

6-OHDA hydrochloride, desipramine hydrochloride, *R*-(−)apomorphine hydrochloride, caffeine free base and D-amphetamine sulphate were purchased from Sigma-RBI Co (Milan, Italy). Desipramine and caffeine were administered i.p. (3 ml/kg), whereas amphetamine was given subcutaneously (1 ml/kg) or i.p. (3 ml/kg); all drugs were dissolved in distilled water. Apomorphine was given subcutaneously (1 ml/kg) and was dissolved in distilled water with ascorbic acid.

Statistical analysis

Mean and S.E.M. of locomotor behaviour and of the number of ipsilateral turns in response to caffeine or amphetamine were calculated in caffeine and amphetamine administration phases.

Significance between groups was assessed by one-way ANOVA followed by Tukey's HSD post-hoc test.

Results

Caffeine intermittent administration

In neurologically intact rats, repeated intermittent administration of caffeine (15 mg/kg, i.p.), elicited a locomotor behaviour which was significantly increased on day 7th as compared to day 1st (Fig. 1A).

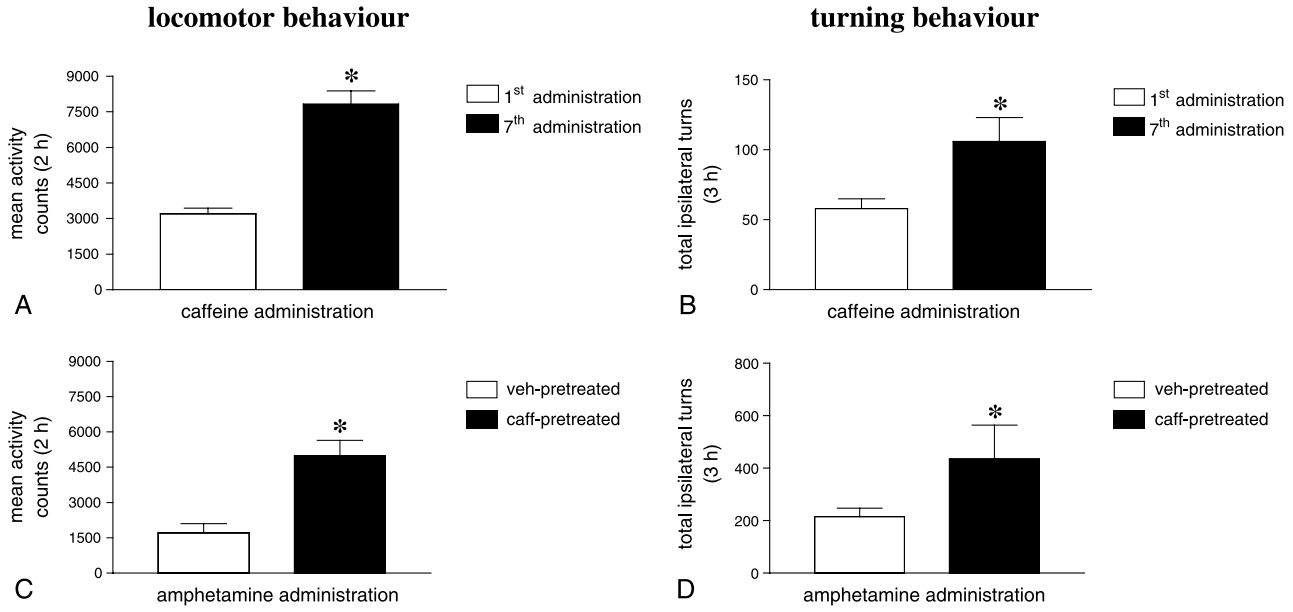

Fig. 1. **A** Effect of caffeine (15 mg/kg, i.p.) on locomotor behaviour recorded for 2 h on the first and the seventh day of the caffeine intermittent treatment. One-way ANOVA revealed a higher locomotor behaviour on 7th day as compared to 1st day ($P < 0.0001$). **B** Effect of caffeine (15 mg/kg, i.p.) on ipsilateral turning behaviour measured for 3 h on the first and the seventh day of the caffeine intermittent treatment. One-way ANOVA revealed a higher total ipsilateral turns on 7th day as compared to 1st day ($P < 0.001$). **C** Effect of amphetamine (0.5 mg/kg, s.c.) on locomotor behaviour recorded for 2 h in caffeine or vehicle pretreated rats. One-way ANOVA revealed a higher locomotor behaviour in caffeine- than in vehicle-pretreated rats ($P < 0.001$). **D** Effect of amphetamine (2 mg/kg, i.p.) on ipsilateral turning behaviour recorded for 3 h in caffeine or vehicle pretreated rats. One-way ANOVA revealed a higher total ipsilateral turns in caffeine- than in vehicle-pretreated rats ($P < 0.05$)

In unilaterally 6-OHDA-lesioned rats subjected to a single apomorphine priming, acute caffeine administration failed in inducing a robust contralateral turning behaviour, as previously observed by Fenu and Morelli (1998), rather resulting in stimulation of ipsilateral rotational behaviour which was significantly increased on administration day 7th as compared to day 1st (Fig. 1B), in agreement with previous reports of our group which showed a progressive increase of caffeine-mediated ipsilateral turning behaviour during its subchronic administration (Cauli et al., 2003, 2005). A few contralateral rotations were performed by 6-OHDA-lesioned rats during subchronic caffeine treatment, but no differences between the 1st and the 7th administration were observed (first caffeine administration, 1.2 ± 0.4 contralateral turns; seventh caffeine administration, 1.4 ± 0.6 contralateral turns).

Amphetamine administration in caffeine pretreated rats

In neurologically intact rats, acute administration of amphetamine (0.5 mg/kg s.c.) induced, three days after the last caffeine or vehicle injection, a significantly higher locomotor behaviour in caffeine- than vehicle-pretreated rats (Fig. 1C).

In 6-OHDA-lesioned rats, acute administration of amphetamine (2 mg/kg, i.p.) induced, three days after the last caffeine or vehicle injection, a significantly higher ipsilateral turning behaviour in caffeine- than vehicle-pretreated rats (Fig. 1D).

Discussion

The results of the present study demonstrate that subchronic and intermittent administration of a moderate dose of caffeine to neurologically intact as well as 6-OHDA-lesioned rats results in an enhanced motor stimulant response to caffeine. Moreover, caffeine pre-exposure increased motor activation induced by amphetamine, given three days after the last caffeine administration, in both intact and 6-OHDA-lesioned rats.

Previous reports showed an enhancement of amphetamine-induced locomotion in rats after a long-term treatment with moderate doses of caffeine (Gasior et al., 2000; Palmatier et al., 2003). In those studies, however, amphetamine was given concomitantly or few hours after caffeine administration; therefore, a direct interaction between caffeine (and/or its metabolites) and amphetamine might have influenced the results observed. To this end, is noteworthy to consider that in the present study potentiation of

amphetamine motor effects has been observed three days after the interruption of caffeine treatment. The washout period used here excludes the presence of residual caffeine and metabolites (see Lau and Falk, 1995), thus suggesting that the enhancement of amphetamine effects observed in caffeine pretreated rats is like to be due to the presence of enduring facilitatory neuronal modifications produced by previous subchronic caffeine treatment. In agreement with this hypothesis, the enhancement of caffeine motor effects observed at the end of subchronic caffeine treatment might reflect the induction of neuronal modifications by long-term caffeine administration.

Although caffeine increases striatal dopamine extracellular concentrations in both naive and subchronically treated rats (Morgan and Vestal, 1989; Cauli et al., 2003) this effect does not seem to be responsible for the potentiation of amphetamine-induced behavioural activation in caffeine-pretreated rats. In fact, in a previous study, we demonstrated that subchronic caffeine did not modify the ability of acute amphetamine in inducing dopamine release (Cauli et al., 2003). On these basis, it can be postulated that neuroadaptive changes induced by subchronic caffeine might take place at post-synaptic level.

As discussed in the introduction, caffeine exerts its motor stimulant effects acting as a competitive antagonist of adenosine receptors, in particular of the A_{2A} subtype (Ledent et al., 1997; El Yacoubi et al., 2000). Adenosine A_{2A} and dopamine D_2 receptors are colocalized on striatopallidal neurons (Schiffmann and Vanderhaeghen, 1993) and negatively interact at receptor-receptor (Hillion et al., 2002) and second messenger level, where D_2 and A_{2A} receptors inhibit and stimulate cAMP formation, respectively (Kull et al., 2000). Therefore, antagonism of A_{2A} receptors by caffeine may, on one hand, directly facilitate the actions of D_2 receptors on striatopallidal neurons and, on the other, through striatal collateral or the basal ganglia circuitry which involves the striatopallidal neurons and the subthalamic-nigro-cortico-striatal projection, indirectly enhance the activation of D_1 receptors located in the striatonigral neurons. On these bases, it is conceivable that prolonged blockade of A_{2A} receptors by subchronic caffeine might produce persistent modifications in dopamine and adenosine receptor interaction and, in turn, result in a facilitation of dopamine transmission which might justify the results described here.

Dopamine critically modulates a large number of brain functions, therefore the ability of caffeine in inducing neuroadaptive long-lasting changes in the dopaminergic system represents an intriguing subject. Interestingly, recent findings of our group showed a potentiation of L-DOPA effects in 6-OHDA-lesioned rats by subchronic intermittent caffeine administration, which have been related to enduring facilitatory neuronal modifications in dopaminergic transmission induced by caffeine (Cauli et al., 2005). Moreover, dopamine influences the expression of glutamate-mediated neuroplasticity, which extensively modulates learning and memory processes (Centonze et al., 2001; Jay, 2003); to this end it has been demonstrated that caffeine reverses memory deficits in animal models of ADHD and aging (Prediger et al., 2005a, b). Taken together, these data suggest that long-term caffeine exposure, by inducing neuroadaptive changes in dopaminergic transmission, might potentially have beneficial effects on dopamine-dependent functions such as motor behaviour, learning and memory.

In conclusion, this study by demonstrating that subchronic caffeine induces enduring facilitatory changes in amphetamine responses underlines the importance of the modulatory role played by adenosine receptors on dopamine-mediated functions.

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