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Psychostimulant pharmacological profile of paraxanthine, the main metabolite of caffeine in humans

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ABSTRACT

Caffeine induces locomotor activation by its ability to block adenosine receptors. Caffeine is metabolized to several methylxanthines, with paraxanthine being the main metabolite in humans. In this study we show that in rats paraxanthine has a stronger locomotor activating effect than caffeine or the two other main metabolites of caffeine, theophylline and theobromine. As previously described for caffeine, the locomotor activating doses of paraxanthine more efficiently counteract the locomotor depressant effects of an adenosine A_1 than an adenosine A_{2A} receptor agonist. In drug discrimination experiments in rats trained to discriminate a maximal locomotor activating dose of caffeine, paraxanthine, unlike theophylline, generalized poorly to caffeine suggesting the existence of additional mechanisms other than adenosine antagonism in the behavioral effects of paraxanthine. Pretreatment with the nitric oxide inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) reduced the locomotor activating effects of paraxanthine, but not caffeine. On the other hand, pretreatment with the selective cGMP-preferring phosphodiesterase PDE9 inhibitor BAY 73-6691, increased locomotor activity induced by caffeine, but not paraxanthine. *Ex vivo* experiments demonstrated that paraxanthine, but not caffeine, can induce cGMP accumulation in the rat striatum. Finally, *in vivo* microdialysis experiments showed that paraxanthine, but not caffeine, significantly increases extracellular levels of dopamine in the dorsolateral striatum, which was blocked by L-NAME. These findings indicate that inhibition of cGMP-preferring PDE is involved in the locomotor activating effects of the acute administration of paraxanthine. The present results demonstrate a unique psychostimulant profile of paraxanthine, which might contribute to the reinforcing effects of caffeine in humans.

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1. Introduction

Caffeine is the most widely consumed psychoactive substance in the world. It is generally believed that caffeine exerts psychostimulant effects acting as a nonselective adenosine A_1 receptor (A_1R) and A_{2A} receptor ($A_{2A}R$) antagonist (Fredholm and Persson, 1982; Nehlig et al., 1992; Fredholm and Lindström, 1999; Fisone et al., 2004; Ferré, 2008). In rats, the trimethylxanthine caffeine is mainly demethylated to the dimethylxanthines paraxanthine, theophylline and theobromine in roughly similar amounts (Arnaud, 1985; Berthou et al., 1988). In humans, caffeine is also rapidly metabolized to the three dimethylxanthines, but with a very different metabolizing rate, with paraxanthine constituting by far the main metabolite (approximately 80% of the three dimethylxanthines) (Lelo et al., 1989; Berthou et al., 1992; for a recent review see Arnaud, 2011). The first studies which compared the pharmacological effects of caffeine and its main metabolites were reported almost 30 year ago when it was shown that caffeine, paraxanthine, theophylline, but not theobromine, were able to increase locomotor activation in mice (Seale et al., 1984). Also, in rats trained to discriminate caffeine from saline, both paraxanthine and theophylline, but not theobromine, were able to generalize to the caffeine-cue (Carney et al., 1985). Moreover, caffeine but not

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paraxanthine, was able to generalize to theophylline in rats trained to discriminate theophylline from saline (Carney et al., 1985). Therefore, those studies already suggested more similarities between caffeine and theophylline than with paraxanthine and even less with theobromine, which was consistently the least active methylxanthine. Paraxanthine has also less anxiogenic activity and toxicity in rodents than caffeine (Stavric, 1988; Benowitz et al., 1995; Okuro et al., 2010).

It is widely assumed that the main mechanism of action involved in the behavioral effects of caffeine and paraxanthine is their antagonism of adenosine receptors, but they have little differences in their affinities for both adenosine A_1Rs and A_{2A}Rs (Snyder et al., 1981), which suggests the existence of additional mechanisms to explain their pharmacological differences. A dopaminergic component for paraxanthine was proposed based on results showing displacement for a low concentration of the labeled dopamine D_1 receptor (D_1R) antagonist [^{3}H]SCH-23390 in the rat striatum (Ferré et al., 1990) and the ability of SCH-23390 to partially counteract the motor activating properties of paraxanthine in reserpinized mice (Ferré et al., 1991). However, the binding experiments with [^{3}H]SCH-23390 could not be replicated by other authors (K.A. Jacobson, personal communication). It is well known that methylxanthines have also modest phosphodiesterase (PDE) inhibitory activity (Essayan, 2001; Francis et al., 2011). PDEs play an important role in intracellular signal transduction pathways (Bender and Beavo, 2006). Among all the different phosphodiesterase families present in the brain, PDE1, PDE2, PDE4, PDE5, PDE9 and PDE10 are the most prevalent (Domek-Łopacińska and Strosznajder, 2010). Some of them, like PDE1, PDE2 and PDE10 hydrolyze both cAMP and cGMP and regulate the duration and the amplitude of cyclic nucleotide signaling activity (Bender and Beavo, 2006), whereas PDE5 and PDE9 isoforms are very selective for cGMP (Domek-Łopacińska and Strosznajder, 2010). The synthesis of cGMP is regulated by a soluble guanylyl cyclase (Garthwaite, 2008) that in turn is regulated by different molecules such as hormones, bacterial toxins, or neurotransmitters like nitric oxide (NO) (Snyder, 1992). In the CNS the neuronal isoform (nNOS) is the main enzyme implicated in the synthesis of NO and it is present in a discrete way in different areas of the brain, and in particular in the striatum (Vincent and Kimura, 1992). Furthermore, NO seems to be also involved in the decrease of basal locomotor activation (Stewart et al., 1994) and in some of the mechanisms responsible for the psychostimulant effect of drugs of abuse (Itzhak, 1997; Przegaliński and Filip, 1997; Li et al., 2002; Zarrindast et al., 2002, 2003; Kayir and Uzbay, 2004).

In the present study we first demonstrate that paraxanthine has a significantly stronger locomotor activating effect than caffeine, theophylline and theobromine in rats. We then present behavioral and biochemical evidences for a selective inhibitory effect of paraxanthine on cGMP-preferring PDEs (most probably PDE9) as a main mechanism responsible for the difference in the locomotor activating effects, which is associated with a significant release of dopamine in the striatum.

2. Materials and methods

2.1. Animals and drugs

Male Sprague–Dawley albino rats (Charles River Laboratories, Wilmington, MA), weighting 300–350 g, were used in all the experimental procedures. Animals were housed 2 per cage and kept on a 12/12-h dark/light cycle with food and water available *ad libitum*. All animals used in the study were maintained in accordance with the guidelines of the National Institutes of Health Animal Care and the animal research conducted to perform this study was approved by the NIDA IRP Animal Care and Use Committee (protocol #: 09-BNRB-73). The methylxanthines caffeine, theophylline, theobromine and paraxanthine, the adenosine A_1R agonist CPA (N^6-cyclopentyladenosine), the adenosine A_1R antagonist CPT (8-Cyclopentyl-1, 3-dimethylxanthine) and the PDE9 inhibitor BAY 73-6691 were purchased from Sigma–Aldrich (St. Louis, MO). The adenosine A_{2A}R antagonist KW-6002 and the adenosine A_{2A}R agonist CGS 21680 (2-p-(2-carboxyethyl) phenethylamino-5′-N-ethylcarboxamidoadenosine) were kindly provided by the CHDI Foundation Inc. (Los Angeles, CA, USA). The NO synthase inhibitor L-NAME (N^G-Nitro-L-arginine methyl ester hydrochloride) and the PDE4 inhibitor rolipram were purchased from Tocris Bioscience (Ellisville, MI). MSX-3, a soluble phosphate pro-drug of MSX-2, was synthesized at the Pharmaceutical Institute, University of Bonn, Germany (Hockemeyer et al., 2004). The doses and the preparation of the adenosine receptor agonists and antagonists were selected based on previous experiments in which they elicited fully significant effects in the behavioral parameters tested, under our experimental conditions (Karcz-Kubicha et al., 2003; Orrú et al., 2011b). Paraxanthine, theophylline, theobromine, CPT, BAY 73-6691 and rolipram were suspended in a solution of 5% dimethyl-sulfoxide, 5% TWEEN80 and 90% ddH_2O; KW-6002 was suspended in a solution of 8% TWEEN80 and 92% ddH_2O. Caffeine was dissolved in saline solution. All drugs were administered i.p. in an injection volume of 2 ml/kg body weight.

2.2. Locomotor activity

Locomotor activity was measured by placing the animals individually in an open field motility soundproof chambers (50 × 50 cm) (Med Associates Inc., VT). Locomotion was measured by counting the number of breaks in the infrared beams of the chambers during consecutive periods of 10 min. Before each testing session, the animals were moved into the experimental room and allowed to habituate to the new environment for at least 2 h before being introduced in the chamber. Recording of the locomotor activity started immediately after placing the animals in the boxes (without habituation) and lasted for 90 min. Data were analyzed as the average of all transformed values (square root) of averaged counts per 10 min during the first hour. This period corresponds to the highest exploratory activity during exposure to a new test environment. All animals were tested only once.

2.3. Surgical procedures and in vivo microdialysis

Rats were deeply anesthetized with 3 ml/kg of Equithesin (NIDA Pharmacy, Baltimore, MD), placed in a stereotaxic apparatus and implanted with concentric dialysis probes (Eicom Corp., Tokyo) in the lateral striatum: anterior = +0.0 mm from bregma, lateral = ±4.5 mm from bregma, vertical = −6.0 mm from dura (Paxinos and Watson, 2006). After surgery, rats were allowed to recover in hemispherical CMA-120 cages (CMA Microdialysis AB, Solna, Sweden) equipped with a swivel (Plastics One, Roanoke, VA). Twenty-four hours after implanting the probe, experiments were performed on freely moving rats in the same hemispherical home cages in which they recovered overnight from surgery. A Ringer solution containing (in mM): 147 NaCl, 4.0 KCl, 2.2 $CaCl_2$ was pumped through the microdialysis probe at a constant rate of 1 μl/min. After a washout period of 90 min, the microdialysate samples of each animal were collected with 30 min of interval. In the first set of experiments, after 3 stable samples for baseline (in a range of 10% of variability), animals received either an injection of 30 mg/kg of L-NAME, caffeine, paraxanthine or vehicle. In a second set of experiments, rats were injected with 30 mg/kg of L-NAME or vehicle followed by a treatment of 30 mg/kg of paraxanthine. The dopamine microdialysate samples were collected for an additional 150 min, loaded in a refrigerated autosampler and analyzed by reverse high-performance liquid chromatography (HPLC) coupled with a coulometric detector (5200a Coulochem III, ESA, Chelmsford). Dopamine values were transformed as percentage of basal values (mean of the three values before vehicle or drug injection) and analyzed using a two-way ANOVA followed by Tukey's multiple comparisons test (Statistica, Stat Soft, Tulsa, OK). At the end of the experiment, rats were given an overdose of equithesin, the brains were extracted, fixed in formaldehyde, and the probe placement was checked using cresyl violet staining.

2.4. Drug-discrimination procedure

Slightly food-restricted rats were trained as described previously (Solinas et al., 2005) under a discrete-trial schedule of food-pellet delivery to respond on one lever after an injection of a training dose of 30 mg/kg of caffeine ($n = 10$) and on the other lever after an injection of 1 ml/kg saline vehicle. Injections of caffeine or saline were given i.p. 30 min before the start of the session. At the start of the session, a white house light was turned on, and in its presence, the rats were required to make 10 consecutive responses (fixed-ratio 10 schedule of food delivery) on the lever appropriate to the pre-session treatment. The completion of 10 consecutive responses on the correct lever produced delivery of a 45-mg food pellet and initiated a 45-s timeout during which lever-press responses had no programmed consequences and the chamber was dark. Responses on the incorrect lever had no programmed consequences other than to reset the fixed-ratio requirement on the correct lever. After each timeout, the white house light was again turned on, and the next trial began. Each session ended after completion of 20 fixed-ratio trials or after 30 min elapsed, whichever occurred first. Discrimination-training sessions were conducted 5 days per week under a single alternation schedule (i.e., DSDSDSDSDS etc., where D = drug and S = saline). Training continued until there were eight consecutive sessions during which rats completed at least 80% of their responses during the session on the correct lever and no more than four responses occurred on the incorrect lever during the first trial. Test sessions with other doses and