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Rapid communication

Reduced appetite for caffeine in adenosine A_{2A} receptor knockout mice

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Abstract

Adenosine A_{2A} receptor knockout mice (A_{2A}R KO) were compared to wild-type controls (A_{2A}R WT) in a caffeine intake paradigm. When mice had ad libitum access to caffeine (0.3 g/l) and water in a two-bottle paradigm for 12 consecutive days, adenosine A_{2A}R KO mice drank less caffeinated solution, demonstrating a reduced appetite for caffeine as compared to adenosine A_{2A}R WT mice. These data reveal an important role for the adenosine A_{2A} receptor in the appetitive properties of caffeine.
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The methylxanthine caffeine, found in coffee, tea and cola, is the most widely consumed behaviorally active substance in the world (Fredholm et al., 1999). The most notable behavioral effects of caffeine occur after low to moderate doses (50–300 mg); they are increased alertness, energy and ability to concentrate. Ordinary caffeine consumption has generally not been considered as a situation of drug abuse, and is indeed not classified as such in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994). However, Strain et al. (1994), based on DSM-IV criteria for substance dependence, found evidence of caffeine dependence in about 16% of adult subjects. Heavy consumers of coffee show a preference for coffee containing caffeine if they have been drinking this type of coffee for 1 week or more, whereas subjects who have been drinking decaffeinated coffee will choose indifferently decaffeinated or caffeine-containing coffee (Griffiths et al., 1986). The main biochemical mechanism that underlies the actions of caffeine at doses achieved in normal human consumption is antagonism of the potent endogenous modulator, adenosine (Fredholm et al., 1999; Dunwiddie and Masino, 2001) acting at adenosine A_1 and adenosine A_{2A} receptors. Conditioned place preference occurs with a low dose of caffeine (3 mg/kg) in rats (Brockwell et al., 1991). Then, the question arises whether the appetitive properties of caffeine may be linked to the blockade of adenosine A_{2A} receptors by the methylxanthine and its metabolites.

The present study aimed to investigate the outcome of the lack of adenosine A_{2A} receptor in A_{2A}R KO mice (Ledent et al., 1997) on caffeine self-administration in the mouse.

Adenosine A_{2A} receptor knockout (A_{2A}R KO) male mice and their wild-type (A_{2A}R WT) controls bred for 30 generations on a CD1 background, and weighing 32–40 g were used after at least two weeks of habituation in our own facilities. Mice were housed in groups of 15–20 in Makrolon cages (38×24×18 cm) with free access to water and food (UAR, France) and kept in a ventilated room at a temperature of 21±1 °C, under a 12:12 h light–dark cycle (light on at 07.00 h). The animals were isolated in small individual cages (20×10×13 cm) one week before and during the experiment. The testing procedure was as follows. First, the basal consumption of water was estimated for two days, then mice were given either water (bottle with short 25 mm tip) or caffeine solution (0.3 g/l, bottle with long 55 mm tip) on alternate days for 6 days. During this conditioning session,

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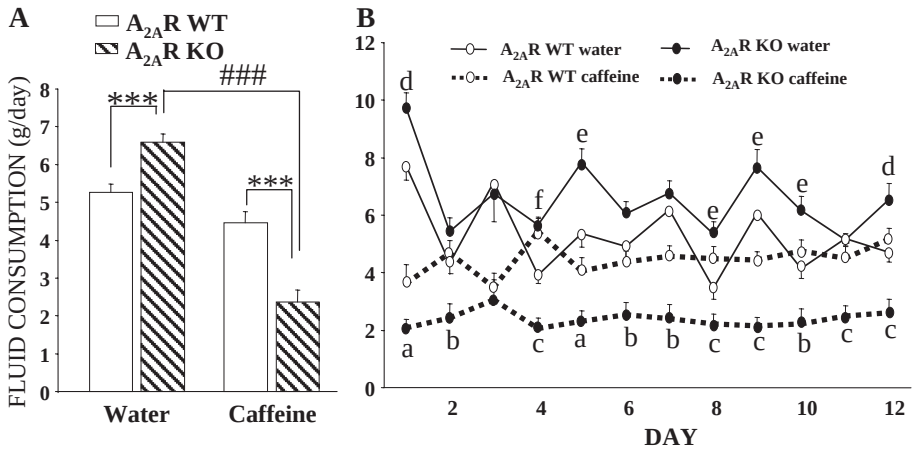

Fig. 1. Effect of the targeted disruption of the adenosine A_{2A} receptor gene on water and caffeinated solution intakes in a two-bottle paradigm. Data are expressed as mean±S.E.M. $N=12$ mice per group. Panel A: Mean fluid consumption. ***$P<0.001$ vs. A_{2A}R WT, ### $P<0.001$ vs. water. Panel B: Daily fluid consumption. (a) $P<0.05$, (b) $P<0.01$, (c) $P<0.001$ vs. A_{2A}R WT caffeine consumption, (d) $P<0.05$, (e) $P<0.01$, (f) $P<0.001$ vs. A_{2A}R WT water consumption.

the gustatory and visual presentation of caffeine or water stimuli allowed the animals to associate caffeine and water to a defined effect. Then, the two bottles of either caffeinated solution or water were made available continuously as a free choice to the animals for twelve days. Fluid consumption (g/day) of caffeine or water from both cylinders was measured daily at 9:00 am. Body weight was recorded every fifth day. Bottle positions were alternated daily to avoid development of a position preference. Data are expressed as mean±S.E.M. Statistical comparisons were made by two-way analysis of variance with post hoc Newman Keuls test (mean consumption) and one-way analysis of variance (daily consumption). The procedures described comply with ethical principles and guidelines for care and use of laboratory animals adopted by the European Community, law 86/609/CCE.

A_{2A}R WT and A_{2A}R KO mice consumption of water did not differ in the basal state (8.21±1.05 g/day vs. 7.21±0.33 g/day, $P>0.05$, 12 mice per group). The experiment with the 0.3 g/l dose of caffeine indicated that A_{2A}R KO mice drank more water ($P<0.001$) and less caffeine ($P<0.001$) than their A_{2A}R WT controls. A two-way ANOVA indicates an interaction [$F(1,44)=41.9$, $P<0.001$] between the factors mutation and caffeine. (Fig. 1, panel A). The time course of caffeine/water consumption across twelve days of free choice session was analyzed (Fig. 1, panel B). A statistical analysis using one-way ANOVAs at every day showed that adenosine A_{2A} R KO mice drank significantly less caffeine than A_{2A} R WT mice throughout the whole duration of the experiment. Body weights following caffeine intake were similar: A_{2A}R WT, 39.7±0.8 g; A_{2A}R KO, 39.7±0.7 g.

These results suggest that invalidation of the adenosine A_{2A} receptor gene results in a decrease of caffeine consumption below that found on the CD1 genetic background. Although the present data do not eliminate the possibility that the adenosine A_1 receptors may also participate in the appetitive properties of caffeine, they strongly suggest that the adenosine A_{2A} receptor plays an important role in these properties. This is in accordance with the previously reported fact that the non-selective adenosine A_1 and A_{2A} receptor antagonist CGS 15943, but not the adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine DPCPX, induced a conditioned place preference (Brockwell and Beninger, 1996), suggesting that adenosine A_{2A} receptors are particularly important in mediating reinforcing effects of caffeine. When considered in a context beyond animal data, these results support a potential role of polymorphisms in this adenosine A_{2A} receptor that may account for interindividual differences in caffeinated beverages consumption by humans.

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