

and occur in classes of organisms other than mammals.

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Caffeine alters plasma adenosine levels

Since the delights of tea were discovered by Emperor Shen Nung in 2737 BC, methylxanthines have been common in the human diet. Today, the methylxanthine caffeine is the most commonly consumed drug in the world¹, with actions mediated primarily by adenosine receptor blockade². We now report that caffeine increases plasma adenosine concentration in a manner that is dose-related, saturable, and mimicked by peripheral adenosine receptor blockade. Opposite effects are seen after caffeine withdrawal, indicative of a receptor-mediated effect.

We studied regulation of basal plasma adenosine by experiments in anaesthetized rats (600 g male Sprague–Dawley retired breeders; Charles River Laboratories)³. We cannulated the carotid artery and jugular vein to allow blood pressure measurement,

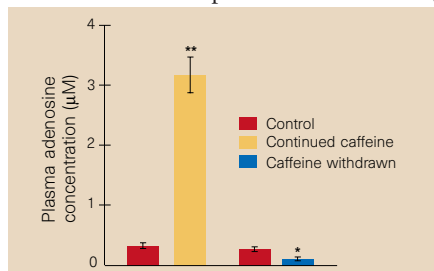
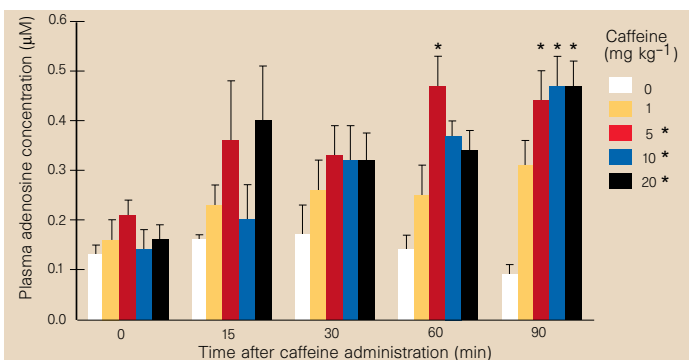


Figure 1 Chronic dietary consumption of caffeine increased the plasma adenosine concentration (** $P < 0.0001$; mean $n = 7$ per group) compared with that in control animals drinking tap water. Caffeine withdrawal had the opposite effect (* $P < 0.05$; mean $n = 12$). Hypotensive animals (<80 mm Hg systolic blood pressure) were excluded from the study.

Figure 2 Caffeine increased plasma adenosine concentration in a dose-related ($P < 0.001$) and saturable manner. Doses of 5, 10 and 20 mg per kg increased the concentration from control levels (* $P < 0.05$). Mean \pm s.e.m. concentration is shown for doses at various times (* $P < 0.05$; mean $n = 9$ per group for each dose). Analysis was by ANOVA then Tukey test. Hypotensive animals were excluded.



arterial blood sampling (0.5 ml samples) and replacement of sampled blood with saline to maintain normal blood pressure. We measured adenosine concentration with a lower detection limit of 0.05 μM , as in ref. 3.

Rats were allowed *ad libitum* access to 0.1% caffeine in drinking water for two weeks (consumption averaged 65 ± 10 mg per kg per day). A control group consumed tap water^{3,4}. On the morning after the two-week period we anaesthetized and cannulated the animals and took samples. Plasma adenosine increased from a control concentration of $0.32 \pm 0.5 \mu\text{M}$ to $3.17 \pm 0.30 \mu\text{M}$ in those rats that continued to drink caffeine (Fig. 1; $P < 0.0001$). When the caffeinated solution was withdrawn and replaced with tap water on the evening before the measurement^{3,4}, the plasma adenosine concentration declined to $0.10 \pm 0.03 \mu\text{M}$ compared to a second control group level of $0.26 \pm 0.04 \mu\text{M}$ (Fig. 1; $P < 0.05$).

Intravenously administered caffeine also roughly doubled the plasma adenosine concentration. We injected caffeine (1, 5, 10 or 20 mg per kg; Sigma) in saline (1 ml per kg body mass); controls received saline alone. The adenosine increase was related to dose (Fig. 2; $P < 0.001$), and was saturable at 5 mg per kg. Increases were observed at 5, 10 or 20 mg per kg (Fig. 2; $P < 0.05$); 1 mg per kg was ineffective when compared with controls. Increases after chronic caffeine administration were of greater magnitude than those following acute intravenous administration: roughly 10-fold as opposed to twofold.

Plasma adenosine did not change after administration of the sympathomimetic amine, tyramine (5 mg per kg intravenously every 10 min for 40 min) ($P > 0.73$), although blood pressure increased by 63 ± 6 mm Hg ($P < 0.0001$), so the increase in plasma adenosine did not reflect sympathetic stimulation. We observed increases in plasma adenosine after intravenous administration of 8-*p*-sulphophenyltheophylline (8SPT; Research Biochemical Inc.), a non-selective adenosine antagonist that crosses neither the blood–brain barrier nor cell membranes⁵. We used a solution of 50 mg 8SPT in 2 ml warm water containing 40 μl of 1 M NaOH; controls received the same

volume of pH-matched saline. A dose of 50 mg per kg 8SPT increased plasma adenosine concentration from $0.11 \pm 0.02 \mu\text{M}$ (baseline) to $0.25 \pm 0.04 \mu\text{M}$ ($P < 0.003$; $n = 12$ per group) 15 min after administration. We found no differences in controls.

The increase in plasma adenosine concentration was, therefore, observed after blockade of peripheral adenosine receptors, and was not specific for caffeine. This finding, as well as the increase in adenosine concentration after antagonist administration and its reduction after antagonist withdrawal, suggests receptor-mediated regulation of the plasma adenosine concentration.

Our data show that adenosine antagonists such as caffeine and 8SPT influence plasma adenosine concentrations, by an unknown mechanism, at caffeine doses approximating those provided to humans by 3–6 cups of coffee per day⁶. Heavy users may easily double this level of caffeine intake (6–12 cups is equivalent to 15 mg per kg per day⁷). This finding may have implications for individuals who cavalierly increase or withdraw from consumption of caffeine, a compound generally considered to be a useful but innocuous stimulant. Sudden changes in methylxanthine consumption — and thus in plasma adenosine concentrations — could dramatically alter the physiology of many organ systems, and provoke bronchospasm, alter blood pressure, change cardiac rhythms, or influence seizure thresholds.

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