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Research report

Differences between the nonselective adenosine receptor antagonists caffeine and theophylline in motor and mood effects: Studies using medium to high doses in animal models

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HIGHLIGHTS

- Caffeine and theophylline have been proposed as therapeutical agents.
- At high doses, both methylxanthines can produce a wide range of side effects.
- Caffeine is more potent at inducing motor suppression, anxiety and stress.
- Only theophylline increased c-Fos immunoreactivity in some brain areas.

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ABSTRACT

Rationale: Caffeine and theophylline are methylxanthines that are broadly consumed, sometimes at high doses, and act as minor psychostimulants. Both are nonselective adenosine antagonists for A_1 and A_{2A} receptors, which are colocalized with dopamine D_1 and D_2 receptors in striatal areas. Adenosine antagonists generally have opposite actions to those of dopamine antagonists. Although the effects of caffeine are widely known, theophylline has been much less well characterized, especially at high doses.
Methods: Adult male CD1 mice were used to study the effect of a broad range of doses (25.0, 50.0 or 100.0 mg/kg) of caffeine and theophylline on measures of spontaneous locomotion and coordination, as well as the pattern of c-Fos immunoreactivity in brain areas rich in adenosine and dopamine receptors. In addition, we evaluated possible anxiety and stress effects of these doses.
Results: Caffeine, at these doses, impaired or suppressed locomotion in several paradigms. However, theophylline was less potent than caffeine at suppressing motor parameters, and even stimulated locomotion. Both drugs induced corticosterone release, however caffeine was more efficacious at intermediate doses. While caffeine showed an anxiogenic profile at all doses, theophylline only did so at the highest dose used (50 mg/kg). Only theophylline increased c-Fos immunoreactivity in cortical areas.
Conclusion: Theophylline has fewer disruptive effects than caffeine on motor parameters and produces less stress and anxiety effects. These results are relevant for understanding the potential side effects of methylxanthines when consumed at high doses.

1. Introduction

Caffeine is the most widely used psychoactive substance worldwide [1,2]. Average consumption ranges from 100 to 400 mg per day, but consumption increased in some groups of consumers with the introduction in the market of energy drinks [2]. Theophylline is a metabolite of caffeine that is also present in teas, as well as some common dietary products [3].

Both methylxanthines exert their psychostimulant effects mainly through adenosine receptor blockade [4,5]. Adenosine is a neuromodulator that is involved in multiple functions such as sleep, attention, locomotion, and anxiety [6–8]. Adenosine acts on four G-protein-coupled receptors: A_1, A_{2A}, A_{2B} and A_3 [4]. A_1 and A_{2A} receptors are the main target for both caffeine and theophylline [4,5]. Whereas A_1 receptors are widely expressed in the brain, A_{2A} receptors are mainly concentrated in the striatal complex [4,9]. On striatal medium spiny neurons, A_1 receptors are colocalized

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with dopamine D_1 receptors while A_{2A} receptors are colocalized and interact with D_2 receptors; adenosine and dopamine receptors can interact by forming heteromeric complexes, and also by convergence onto the same signal transduction pathways [10,11]. Moreover, there is a substantial amount of behavioral and neurochemical data showing that antagonism of adenosine receptors, either with nonselective or A_{2A} selective drugs, can reverse the effects of dopamine D_2 receptor antagonists on motor and motivational functions that involve nucleus accumbens (Acb) and neostriatum [12–15]. Caffeine is being considered as a possible therapeutic agent because of its ability to interact with dopamine receptors and affect signal transduction in striatal neurons. In addition, caffeine has been proposed as a neuroprotective agent to counteract the effects of dopaminergic neural loss [16,17]. Thus, caffeine is potentially useful for the pharmacological treatment of some symptoms of Parkinson disease [18–20], depression [21] and other disorders that involve dopamine transmission or basal ganglia circuitry.

However, although low doses of caffeine stimulate locomotion and do not impair motor coordination in rodents [6,22,23], high doses can suppress locomotion [6,24,25]. High doses of caffeine that are able to suppress locomotion also increase *c-fos* markers throughout the striatum [26,27]. In addition, high doses of caffeine have been shown to increase physiological parameters of stress such as plasma cortisol levels in humans [28], and corticosterone levels in rats [8,29], and also to promote anxiety in humans (for a review see [7]), and anxiogenic-like behaviors in animal models [30,31]. Theophylline, despite its similar therapeutical potential [13,32] has been much less explored, but it has been demonstrated that theophylline can suppress parkinsonian symptoms in humans [33,34]. As is the case with caffeine, low doses of theophylline can induce motor stimulant effects in rodents [32,35]. Nevertheless, there is a general lack of information about the effects of theophylline, especially at higher doses.

Thus, the present experiments were undertaken to explore and compare systematically the effects of moderate to high doses of caffeine and theophylline on measures of motor activity, anxiety and neuroendocrine parameters, as well as their effect on c-Fos immunoreactivity (to provide a marker of neuronal activation in dopamine and adenosine-receptor rich brain areas). The effects of both drugs on different aspects of exploration, vigorous exercise, and motor coordination, as well as the knowledge of their impact on mood and stress responses, could be useful information for understanding their potential side effects at high doses.

2. Materials and methods

2.1. Animals

CD1 adult male mice ($N = 406$) purchased from Harlan-Interfauna Ibérica S.A. (Barcelona, Spain) were 9 weeks old (30–45 g) at the beginning of the study. Mice were housed in groups of three or four per cage, with standard laboratory rodent chow and tap water available ad libitum. Subjects were maintained at $22 \pm 2\,^{\circ}C$ with 12-h light/dark cycles (lights on at 13:00 h). To habituate the animals to the procedures, they were handled and received a single saline injection the day before experimental procedures started. Different groups of animals were used in each experiment, except for the anxiety tests in which the same animals were serially tested in both paradigms. All animals were under a protocol approved by the Institutional Animal Care and Use Committee of Universitat Jaume I, and all experimental procedures complied with European Community Council directive (86/609/ECC).

2.2. Drugs

Caffeine and Theophylline (Sigma-Aldrich, Spain) were dissolved in 0.9% w/v saline. Saline solution was used as the vehicle control. All solutions were administered intraperitoneally (IP) 30 min before behavioral testing, 90 min before brain extraction in the immunohistochemical study and 60 min before blood samples were collected.

2.3. Behavioral apparatus and testing procedures

2.3.1. Locomotion in the open field arena (OF)

The OF apparatus consisted of a clear glass cylinder 25 cm in diameter and 30 cm high. The floor of the cylinder was divided into four equal quadrants by two intersecting lines drawn on the floor. The behavioral test room was illuminated with a soft light, and external noise was attenuated. Tests were videotaped and locomotor activity was registered manually during 10 min. An activity count was registered as horizontal locomotion each time the animal crossed one quadrant with four legs. Animals were not pre-exposed to the OF in order to study novelty-induced exploration and locomotion.

2.3.2. Locomotion in the running wheel (RW)

The RW consists of a stainless steel activity wheel (circumference = 24 cm) situated in a Plexiglas box (35×20 cm) with a magnetic switch attached to a LCD counter for recording number of wheel turns. Animals were exposed to the RW during 30 min in two consecutive days previous to the test. The test day, counts on the wheel were registered during 30 min. The RW generates stable basal high levels of activity when the animals are trained, and thus is useful for evaluating conditions that suppress voluntary self-induced locomotion.

2.3.3. Motor coordination in the rotarod

The rotarod apparatus (UGO Basile, 7650) consisted of an elevated rotating rod that requires coordinated movement in order to avoid falling. Each mouse was placed in the rotating rod accelerating from 4 rpm to 20 rpm in increments of 4 rpm every 30 s. Animals were trained during five trials, and tested for five more trials. A 390 s maximum cut-off on the rod was used. The apparatus automatically recorded the time (in s) at the moment in which the animal fell off the rod.

2.3.4. Anxiety in the elevated plus maze (EPM)

The EPM consists of two open and two enclosed arms arranged in a plus configuration. This anxiety paradigm measures the avoidance that rodents show to elevated open spaces. The behavioral test room was illuminated with a soft light. Animals were placed in the central platform facing the closed arm and assessed during 5 min. Tests were videotaped and a trained observer registered time spent in the open arms, ratio of entries in the open arms to total arm entries, latency to enter the open arms and total entries in the four arms as an index of locomotion. An entry into an arm was recorded when the animal crossed the line that connected that arm with the central platform with all four legs.

2.3.5. Anxiety in the dark and light (DL)

The DL test is based on the conflict between the inherent tendency of mice to explore a novel environment against their natural avoidance of a brightly lighted open field. The DL apparatus consisted of a polypropylene chamber divided in two compartments by a partition containing a small opening. One chamber was open and illuminated while the other was closed and dark. The behavioral test room was illuminated with a soft light. Each subject was placed in the dark chamber. Tests were videotaped and the