Involvement of adenosinergic receptors in anxiety related behaviours

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In the present study, the effect of adenosine (A1 and A2 receptor agonist), caffeine (A2A receptor antagonist), theophylline (A2A receptor antagonist) and their combination was studied in anxiety related behaviours using elevated zero maze and elevated plus maze paradigms and compared their various behavioural profiles. Adenosine (10, 25, 50,100 mg/kg) significantly showed anxiolytic effect at all the doses, whereas caffeine (8, 15, 30, 60 mg/kg) and theophylline (30, 60 mg/kg) showed psychostimulatory action at lower doses and anxiogenic effect at higher doses. Pretreatment with caffeine (8, 15, 30 mg/kg) and theophylline (30 mg/kg) reversed the anxiolytic effect of adenosine. The study suggested the involvement of adenosinergic receptor system in anxiety related behaviours.

Keywords: Adenosine, Caffeine, Zero maze

Adenosine is now generally accepted as the major inhibitory neurotransmitter other than GABA in the mammalian central nervous system1,2. In the central nervous system, its effects are believed to be mainly mediated by two receptors, A1 and A2A3. Adenosine is reported to produce locomotor activity, depressant, anticonvulsant, hypnotic, anxiolytic and analgesic effects4,5. Caffeine and theophylline, the antagonists of adenosinergic receptors3,6, are consumed throughout the world in the form of coffee, tea, chocolate and soft drinks, as well as over-the-counter or prescription drugs2. The popularity of caffeine is thought to be related to its subjective effects, which include increased alertness and CNS stimulation7. However, variations in acute responses to caffeine have been reported, especially in anxiety-inducing effects of the drug. Controlled studies have confirmed that caffeine produces positive effects such as increased stimulation in many, while others experience negative effects such as increased anxiety8,9. There are certain variations in the effect of theophylline also. The exact role of different adenosinergic receptor modulators in anxiety remains unclear, therefore the effect of adenosine (A1 and A2 receptor agonist), caffeine (A2A receptor antagonist), theophylline (A2A receptor antagonist) and their combination on animal behaviours elevated zero maze and elevated plus maze paradigms have been investigated.

Material and Methods

Animals — Laca mice of either sex bred at Central Animal House (CAH) Panjab University, Chandigarh weighing 25-30 grams were used. The animals were housed under standard laboratory conditions, maintained on a 12 hour light and dark cycle, with free access to standard food and water. Animals were acclimatized to laboratory conditions before the test. All the experiments were carried out between 0900 to 1500 hrs. The experimental protocols were approved by the Institutional Animal Ethical Committee and conducted according to the CPCSEA guidelines on the use and care of experimental animals.

Drug treatment and Experimental procedures — Mice were treated with different drugs as caffeine (8, 15, 30, 60 mg/kg), theophylline (30, 60 mg/kg), adenosine (10, 25, 50,100 mg/kg), caffeine (8 mg/kg) + adenosine (25 mg/kg), caffeine (15 mg/kg) + adenosine (25 mg/kg), caffeine (30 mg/kg) + adenosine (25 mg/kg), theophylline (15 mg/kg) + adenosine (15 mg/kg). All the drugs were dissolved in distilled water and were administered 30 min prior to the experimentation. Elevated zero maze and plus maze test were carried out simultaneously in each of the drug treatment groups.

Elevated zero maze — All the animals were analyzed for anxiety levels by using elevated zero maze. This test is a pharmacologically validated assay of anxiety in animal models that is based on the natural aversion of mice to elevated zero maze. It is composed of a 6 cm wide ring with outer diameter of
45 cm containing 4 equal quadrants of alternating walled (closed) or unwalled (open) sections. The entire ring is elevated to the height of 40 cm. Mice were placed in the walled region at the start of 5 min seasons and following parameters were analyzed\(^\text{10}\).

(a) Time spent in open arm;
(b) Latency to enter in open arm;
(c) Total number of entries in open arm;
(d) Number of stretch attend postures (SAP’s).

**Elevated plus maze** — All the animals were also analyzed for anxiety levels by using elevated plus maze. Plus maze is composed of two open arm \((16 \times 5 \text{ cm})\) and two enclosed arms \((16 \times 5 \times 12 \text{ cm})\) with an open roof and is elevated to a height of 25 cm. Each animal was placed individually in the centre of the maze facing towards the open arm and recorded the following parameters during 5 min session\(^\text{11}\).

(a) Time spent in open arm;
(b) Latency to enter in open arm;
(c) Total number of entries in open arm;
(d) First preference of the animal (open/closed);
(e) Number of stretching.

**Statistical analysis** — One specific group of mice was assigned to one specific drug treatment condition and each group comprised six mice \((n=6)\). All the values are expressed as means ± S.E. The data were analyzed by Student’s \(t\)-test. \(P<0.05\) was considered as statistical significant.

**Results**

**Elevated zero maze** — Caffeine showed stimulant effect at lower doses \((8 \text{ mg/kg})\) where as anxiogenic effect (decrease in time spent in open area, increase in latency to enter in open arm, decrease in number of entries in open area) at higher doses \((15, 30 \text{ and } 60 \text{ mg/kg})\). Theophylline also showed stimulant effect at lower dose \((30 \text{ mg/kg})\), whereas anxiogenic effect (decrease in time spent in open area, increase in latency to enter in open arm, decrease in number of entries in open area) at higher dose \((60 \text{ mg/kg})\). Adenosine \((10, 25, 50,100 \text{ mg/kg})\) showed increase in time spent in open area, decrease in latency to enter in open arm, increase in number of entries in open area at initial 3 doses which was decreased at 100 mg/kg. In combination, pretreatment with caffeine \((8, 15 \text{ and } 30 \text{ mg/kg})\) or theophylline \((30 \text{ mg/kg})\) significantly reversed the effect of adenosine \((25 \text{ mg/kg})\), (Fig. 1a, b, c)

**Elevated plus maze** — Caffeine showed stimulant effect at lower doses \((8 \text{ mg/kg})\), whereas anxiogenic effect (decrease in time spent in open area, increase in latency to enter in open arm, decrease in number of entries in open area) at higher doses \((15, 30 \text{ and } 60 \text{ mg/kg})\). Theophylline also showed stimulant effect at lower dose \((30 \text{ mg/kg})\), whereas anxiogenic effect (decrease in time spent in open area, increase in latency to enter in open arm, decrease in number of entries in open area) at higher dose \((60 \text{ mg/kg})\). Adenosine \((10, 25, 50,100 \text{ mg/kg})\) showed increase in time spent in open area, decrease in latency to enter in open arm, increase in number of entries in open area at initial 3 doses which was decreased at 100 mg/kg. In combination, pretreatment with caffeine \((8, 15 \text{ and } 30 \text{ mg/kg})\) or theophylline \((30 \text{ mg/kg})\) significantly reversed the effect of adenosine \((25 \text{ mg/kg})\), (Fig. 2 a, b, c)

**Discussion**

Adenosine is now generally accepted as a main inhibitory neurotransmitter other than GABA in the mammalian central nervous system. It is generated from the adenosine triphosphate (ATP) both intracellularly and extracellularly and transported across the cell membrane by facilitated diffusion transport\(^\text{1,2}\). The functional role of adenosine and related nucleosides and nucleotides in CNS is being actively pursued. Several drug actions have long been explained in relation to purinergic substances\(^\text{3,6}\). In the central nervous system, effects of adenosine are believed to be mainly mediated through \(A_1\) and \(A_{2A}\) receptors, both of which are G-protein coupled receptors\(^\text{12}\). Based on the knowledge of various agonists and antagonists of adenosine receptor system in human as well as behavioural studies in animals the actions of adenosine in CNS have generally been held to comprise locomotor depressant, anticonvulsant, hypnotic, anxiolytics and analgesic effects\(^\text{13,14}\). Role of adenosine in the modulation of anxiety state is still under debate. Existence of interaction between adenosine and benzodiazepines has been suggested in 1980’s. Down-regulation of the number of adenosine \(A_2\) receptors in rat forebrain was found following chronic treatment with benzodiazepines\(^\text{15}\). A more direct involvement of adenosine in the regulation of anxiety states has been suggested by the fact that
Caffeine which is an antagonist of A1 and A2A receptor is known to increase anxiety in humans as well in animals, and papaverine an inhibitor of adenosine uptake produces anxiolytic effects\textsuperscript{15-17}. Also, the A2A knockout mice are anxious in nature and are considered as a model of anxiety. These animals showed higher rates of spontaneous anxiety like responses in two different anxiety like behavioural tests, elevated plus maze and light dark box\textsuperscript{2,18}.

In the present study, the adenosine significantly showed anxiolytic effects, characterized by increase in time spent in open arm, increase in number of entries in open arm and decrease in latency to enter in open arm in both elevated zero maze and plus maze paradigms of anxiety. Caffeine and theophylline, A2A receptor antagonists\textsuperscript{3,6}, at lower doses showed increase in exploratory behaviour on both the paradigms of anxiety suggesting psychomotor stimulant action of them. Further, at higher doses they showed significant anxiogenic effect as suggested by decrease in open arm entries and time spent in open arm as well as increase in latency to enter in open arm.

Fig.1 — Effect of different adenosine receptor agonist and antagonist and their combination on — (a) time spent in open arm (b) latency to enter in open arm (c) number of entries in open in open arm on elevated zero maze paradigm. [Total number of animals in each group is 6 and data is expressed in Mean ± SE. \( ^a P \leq 0.05 \) as compared to control group, \( ^b P \leq 0.05 \) as compared to adenosine (25) group.]
in both the mazes. Several studies in human as well as in animals suggested that the administration of high but not low, doses of caffeine leads to an increase in anxiety. Possibly, at small doses, caffeine binds to and inhibits half of the adenosine receptors. But at higher doses there is more pronounced and appropriate blockade of A2A and A1 receptor and thus produces anxiety state. Methylxanthines (caffeine and theophylline) induced CNS stimulation at lower doses may be accounted for the inhibition of phosphodiesterase enzyme, which is responsible for intracellular metabolism of cyclic AMP. Thus, an increase in the intracellular cyclic AMP and produces effects that mimic β-stimulation which is characterized by increased alertness and psychomotor stimulation. Transgenic mice lacking A2A receptor is abnormally anxious and aggressive and fails to show increased motor activity in response to caffeine suggesting that A2 receptors are involved in anxiety states. Also targeted disruption of A1 receptor in mice produces increase in anxiety behaviour in animals. Further, in combination studies

Fig. 2 — Effect of different adenosine receptor agonist and antagonist and their combination on (a) time spent in open arm (b) number of entries in open in open arm (c) latency to enter in open arm on elevated plus maze paradigm. [Total number of animals in each group is 6 and data is expressed in Mean ± SE. *P ≤ 0.05 as compared to control group, †P ≤ 0.05 as compared to adenosine (25) group]
pretreatment of low doses of caffeine and theophylline prevented the anxiolytic effect of adenosine in both the elevated zero maze as well as elevated plus maze due to competitive blockade of A2A and A1 receptor system. In conclusion, our study demonstrate that caffeine and theophylline (A2A receptor antagonists) produce CNS stimulation at lower doses, whereas profound anxiogenic effect at higher doses by completely blocking A2A receptors. The fact that pretreatment of animals with lower doses of caffeine and theophylline reversed the anxiolytic effect of adenosine suggests the involvement of adenosinergic receptors system in anxiety and related behaviours.

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