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Short communication

Catalepsy and hypolocomotion induced by a nitric oxide donor: attenuation by theophylline

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Abstract

Nitric oxide (NO) promotes adenosine release in the striatum and hippocampus. Behavioral effects of the nitric oxide donor sodium nitroprusside were studied in mice and included an examination of spontaneous locomotion and catalepsy, which are behaviors modulated by adenosine. Sodium nitroprusside caused a dose-dependent (2, 4 and 6 mg/kg) decrease in locomotor activity and catalepsy at the dose of 6 mg/kg. These effects were substantially attenuated by pretreatment with the non-selective adenosine receptors antagonist theophylline (10 and 30 mg/kg). Moreover, combined treatment with theophylline (30 mg/kg) and sodium nitroprusside (6 mg/kg) induced limbic seizures in 23% of animals. The pretreatment with the selective adenosine A_1 receptor antagonist 8-cyclopentyl-1, 3-dimethylxanthine (CPT) (1.2 mg/kg) caused no effect on the spontaneous or sodium nitroprusside-induced behavior. These data suggest that these behavioral effects of sodium nitroprusside are at least partially mediated by adenosine in the striatum and hippocampus, probably via adenosine A_{2A} receptors.

Keywords: Nitric oxide (NO); Adenosine; Sodium nitroprusside; Theophylline; Locomotion; Catalepsy

1. Introduction

Nitric oxide (NO) has been increasingly recognized as an important intercellular and intracellular messenger in the central nervous system. Activation of NMDA receptors is a pivotal stimulus to NO production in neurons, in a Ca^{2+}-calmodulin dependent process (Prast and Philippu, 2001; Kiss and Vizi, 2001). NO, which can diffuse over relatively large distances in brain tissue (Lancaster, 1997), is also able to modulate synaptic activity (Prast and Philippu, 2001; Kiss and Vizi, 2001). Among its extracellular roles, NO has been shown to regulate the release of several neurotransmitters, such as amino acids, dopamine, acetylcholine and serotonin (Prast and Philippu, 2001). Behaviorally, NO modulators exerts somewhat incongruent results, as shown by the similar effects produced by either increase or decrease of NO levels observed in NMDA receptor antagonist-induced hyperlocomotion (Johansson et al., 1997; Bujas-Bubanovic et al., 2000), epilepsy, sleep (Faradji et al., 2000) and brain injury (Iadecola, 1997).

Adenosine is a well-characterized inhibitory neuromodulator in the central nervous system (CNS) (Brundege and Dunwiddie, 1997). Four distinct subtypes of adenosine receptors (A_1, A_{2A}, A_{2B} and A_3) have been cloned and characterized (for review, see Ralevic and Burnstock, 1998). Adenosine A_1 receptors are widely expressed, with high levels in the hippocampus, cerebral cortex, thalamus and cerebellum. By acting on presynaptic adenosine A_1 receptors, adenosine suppresses the release of several neurotransmitters (including glutamate and dopamine) and activation of postsynaptic adenosine A_1 receptors induces neuronal hyperpolarization (Brundege and Dunwiddie, 1997). This inhibitory activity, which is enhanced under neurotoxic conditions, renders adenosine an important endogenous mechanism of neuroprotection (Brundege and Dunwiddie, 1997; Ralevic and Burnstock, 1998). In contrast, facilitatory A_{2A} receptors are particularly expressed in dopamine-rich regions, co-localized with dopamine D_2

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receptors (Fink et al., 1992). Activation of adenosine A_{2A} receptors reduces the affinity of dopamine D_2 receptors for agonists, including the endogenous ligand dopamine (Ferré et al., 1991, 1997). Similar A_1–D_1 interactions also take place in the striatum, where they form heterodymers (Gines et al., 2000). Adenosine provides an inhibitory tone to several brain regions and the behavioral stimulation of caffeine and theophylline are attributed to the non-selective antagonism of adenosine A_1/A_{2A} receptors (Brundege and Dunwiddie, 1997).

NO donors have been shown to stimulate the release of adenosine in hippocampal slices (Fallahi et al., 1996; Broad et al., 2000) and in neuronal cultures through inhibition of adenosine kinase (Rosenberg et al., 2000). Similarly, a NO donor increased, whereas a nitric oxide synthase inhibitor diminished striatal adenosine levels in vivo (Fallahi et al., 1996). This interaction between NO and adenosine has not been assessed behaviorally. We therefore chose two models clearly influenced by adenosine, spontaneous locomotion and catalepsy (Ferré et al., 1997), to test the effect of the NO donor sodium nitroprusside. If the release of adenosine induced by NO is behaviorally relevant, sodium nitroprusside should cause locomotor depression and catalepsy, which should be reversed by adenosine receptor antagonists, such as theophylline.

2. Materials and methods

2.1. Animals

Adult male albino mice weighing 30–45 g were used. The animals were allowed to adjust at least 7 days to a room with a 12-h light/dark cycle (lights on at 7:00 a.m.) and controlled temperature (22 ± 2 °C), with food and water ad libitum.

2.2. Behavioral measurements

All behavioral experiments were conducted between 11:00 a.m. and 05:00 p.m.

2.2.1. Motor activity recording

Mice were randomly allocated to individual black plastic cylinders (diameter = 32 cm, height = 22 cm) placed on the floor of a soundproof and diffusely illuminated room. The setting was adjusted to expose mice to a visually rich and novel room, which stimulates basal locomotion and exploratory behavior compared to standard locomotion boxes with a more restricted environment. Motor activity of eight mice was recorded simultaneously by a video-computerized system, with image analysis at four frames per second. The software Mousetracker, designed by one of the authors (W.N.), tracked the animals by distinguishing their white color from the black background of the cylinders, registering X and Y horizontal coordinates. The method was set to examine horizontal locomotor activity, ignoring small movements, like breathing, head and tail actions, and tremors. The animals were not previously habituated to the cylinder and were observed for 1 h. Sodium nitroprusside (2, 4 and 6 mg/kg, i.p.) or saline was administered to mice 5 min before placing them in the cylinder and pretreatment with theophylline (10 and 30 mg/kg, i.p.), CPT (1.2 mg/kg, i.p.) or saline was administered 15 min before treatment with sodium nitroprusside or saline.

2.2.2. Catalepsy

Mice forepaws were placed over a horizontal glass bar (0.6 cm diameter), elevated 6 cm from the floor. The time in seconds during which mice maintained both forepaws over the bar and both hindpaws on the ground was recorded with a cut-off time of 180 s, allowing three immediate attempts to replace the animal in cataleptic position within the first 10 s (Costall and Naylor, 1974). Catalepsy was determined 10 and 30 min after the injection of sodium nitroprusside or saline. Pretreatment with theophylline, CPT or saline was administered 15 min before sodium nitroprusside or saline injection. Haloperidol 1 mg/kg i.p. (pretreatment time = 30 min) was used as a positive control (catalepsy time for 6 mice = 180 s). The experimenter was blind to drug treatment.

2.3. Drugs

Sodium nitroprusside, potassium hexacyanoferrate [II] (Merck, NJ, USA), 8-cyclopentyl-1, 3-dimethylxanthine (CPT) and theophylline (Sigma, St. Louis, MO, USA) were freshly dissolved in saline solution every day before the experiments and mice were injected i.p. at 10 ml/kg for all treatments.

2.4. Statistical analysis

Comparisons between locomotor activity at different time points were analyzed using two-way ANOVA (drug treatment versus time) with time as the repeated measures. Duncan's post hoc was used to determine differences among specific groups. Catalepsy time was analyzed with Kruskal–Wallis followed by the Mann–Whitney U-test due to cut-off time. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Sodium nitroprusside dose-dependently depresses spontaneous locomotion

Sodium nitroprusside (2, 4 and 6 mg/kg) caused a significant dose-dependent decrease in spontaneous locomotor activity [$F(15,145) = 2,642$; $p < 0,01$] (Fig. 1A).

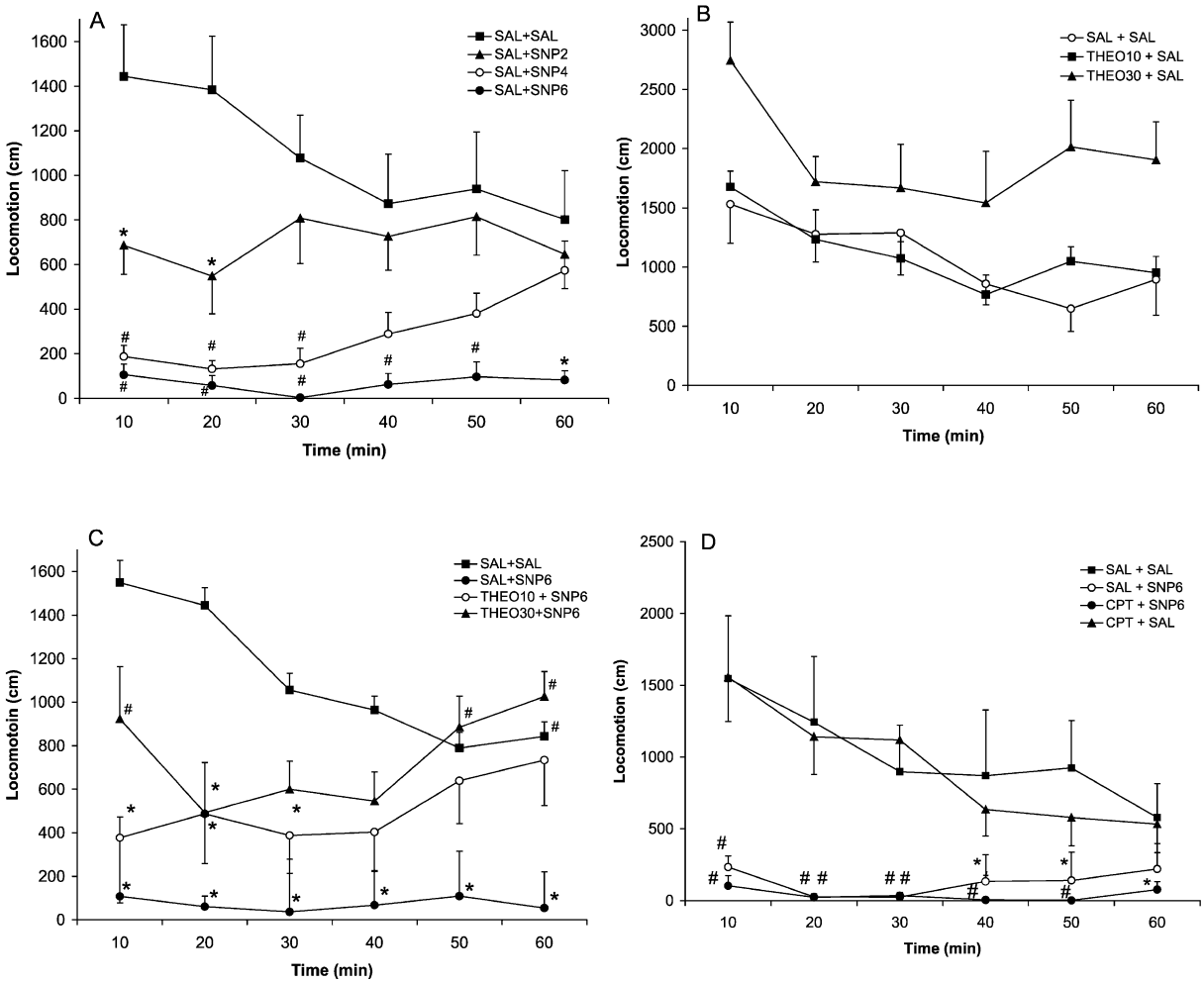

Fig. 1. Effect of sodium nitroprusside and theophylline on spontaneous locomotion. (A) Effect of treatment with saline, sodium nitroprusside 2, 4 and 6 mg/kg; $^{*}p < 0.05$; $^{\#}p < 0.01$ compared to saline ($n = 8$–9 in all groups); (B) effect of saline and theophylline pretreatment (10 and 30 mg/kg), without significant differences between groups ($n = 6$–8 in all groups); (C) effect of pretreatment with saline or theophylline 10 and 30 mg/kg; * and $^{\#}$ denote significant differences ($p < 0.05$) compared to saline and sodium nitroprusside 6 mg/kg, respectively ($n = 7$ in all groups). (D) Effect of pretreatment with saline or CPT 1.2 mg/kg compared to saline and sodium nitroprusside 6 mg/kg; * and $^{\#}$ denote significant differences ($p < 0.05$ and $p < 0.01$, respectively) in relation to saline + saline group. Data expressed as mean + S.E.M.

Sodium nitroprusside 2 mg/kg was partially active for 20 min and 4 mg/kg had significant effects during the first 30 min, whereas mice treated with 6 mg/kg were practically immobile for 60 min. Potassium hexacyanoferrate [II] (6 mg/kg), which is structurally similar to sodium nitroprusside but is not a NO donor, was without effect compared to saline (data not shown).

3.2. Theophylline significantly counteracts sodium nitroprusside-induced hypolocomotion

Theophylline, when administrated before saline, caused a slight but non-significant increased locomotion at 30 mg/kg dose when analyzed every 10 min [$F(15,115) = 0{,}911$; $p > 0{,}05$]. However, if 30 min blocks are analyzed, theophylline 30 mg/kg significantly increased locomotion in both 30 min periods ($p < 0.05$), whereas 10 mg/kg was devoid of effect in either analysis method (Fig. 1B). Theophylline 10 and 30 mg/kg attenuated the effect of sodium nitroprusside 6 mg/kg, particularly at the second half of the experiment (Fig. 1C) in a dose-dependent manner [$F(15,120) = 3040$; $p > 0{,}001$]. In contrast, pretreatment with the selective adenosine A_1 receptor antagonist CPT at a behaviorally effective dose in mice (1.2 mg/kg) (Popoli et al., 1996) failed to affect spontaneous locomotion and locomotor depression induced by sodium nitroprusside 6 mg/kg (Fig. 1D).

3.3. Sodium nitroprusside induced catalepsy and its reversal by theophylline

Sodium nitroprusside 6 mg/kg, but not 4 mg/kg, produced catalepsy in mice at both 10 and 30 min time points (Fig. 2). Theophylline 30 mg/kg, which did not show cataleptic effect when administered alone (data not shown), significantly prevented the effect of sodium nitro-

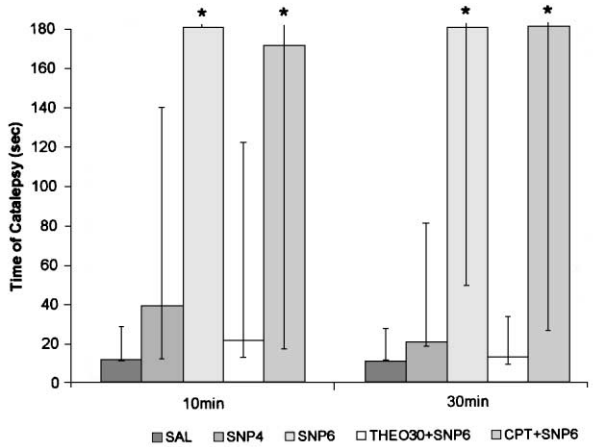

Fig. 2. Effects of sodium nitroprusside and theophylline on catalepsy. Mice were pretreated with saline, theophylline 30 mg/kg or CPT 1.2 mg/kg and treated with sodium nitroprusside 4 mg/kg, 6 mg/kg or saline ($n = 6–13$). The cut-off time was set at 180 s. * denotes significant difference from saline and theophylline + SNP groups ($p < 0.05$). Data expressed as median ± interquartile ranges.

prusside 6 mg/kg at 10 and 30 min, whereas the adenosine A_1 receptor antagonist CPT (1.2 mg/kg) was ineffective (Fig. 2). Potassium hexacyanoferrate [II] (6 mg/kg) was without effect when compared to saline (data not shown).

3.4. Convulsions after treatment with sodium nitroprusside and theophylline

The combined treatment with theophylline 30 mg/kg and sodium nitroprusside 6 mg/kg induced limbic convulsions (rolling, wild running, tonic–clonic movements, loss of balance) in 4 of the 17 animals tested (23%) in both models and their corresponding results were not included in the analysis.

4. Discussion

The main finding of this study was that the NO donor sodium nitroprusside clearly produced locomotor depression and catalepsy, and these behaviors were substantially counteracted by the non-selective adenosine receptor antagonist theophylline, but not by the selective adenosine A_1 receptor antagonist CPT. Moreover, combined treatment with the highest dose of theophylline and sodium nitroprusside produced limbic convulsions in a small number of animals.

These behavioral results are in agreement with previous studies showing that NO donors significantly increased extracellular adenosine levels. More specifically, Fischer et al. (1995) showed that in vivo superfusion of the striatum with a NO donor produced a four-fold increase in adenosine output. This is of particular relevance to our study because the ventral and the dorsal striatum are pivotal regions in the control of locomotor activity and cataleptic behavior, respectively (Ferré et al., 1997). Both behaviors are modulated by adenosine A_{2A} receptors, which are highly expressed in the striatum (Ferré et al., 1997), as evidenced by the locomotor depression and catalepsy induced by adenosine A_{2A} receptor agonists and the locomotor stimulating and anticataleptic effects of adenosine A_{2A} receptor antagonists (Ferré et al., 1997). Thus, if NO donors release adenosine, these behavioral alterations are expected to occur after sodium nitroprusside treatment and to be reversible by theophylline. The modulation of dopamine D_2 receptors by adenosine A_{2A} receptors are likely to contribute to the expression of both behavioral responses (Ferré et al., 1997), but it is noteworthy that adenosine A_{2A} receptor antagonists were recently shown to reverse locomotor impairment in dopamine D_2 receptor-deficient mice (Aoyama et al., 2000; Chen et al., 2001), suggesting that the A_{2A}–D_2 antagonistic actions are at least partially independent.

Adenosine activation of A_1 receptors, which are highly expressed in the hippocampus, reduces presynaptic release of glutamate as well as hyperpolarizes the post-synaptic membrane (Brundege and Dunwiddie, 1997). This inhibition of synaptic activity is important to control seizure threshold (Brundege and Dunwiddie, 1997) and to arrest seizures (During and Spencer, 1992). NO has been shown to induce adenosine release in the hippocampus (Fallahi et al., 1996; Broad et al., 2000), which is a key region in epilepsy. Thus, the release of the anticonvulsant adenosine could counteract the probable epileptogenic effect of NO (Kaku et al., 2001), explaining the occurrence of seizures with the combined treatment with sodium nitroprusside and theophylline. However, this hypothesis could be further tested using higher doses of selective A_1 receptor antagonists than used in our study with proper seizure models.

Treatment yielding increased or decreased NO levels have produced rather contradictory results in models of brain ischemia (Iadecola, 1997), epilepsy, sleep (Faradji et al., 2000), catalepsy (Del Bel et al., 1998; present results) and NMDA receptor antagonist-induced locomotion (Johansson et al., 1997; Bujas-Bubanovic et al., 2000). Adenosine has been repeatedly shown to exert neuroprotective, anticonvulsant, hypnotic (Brundege and Dunwiddie, 1997), cataleptogenic and antipsychotic-like effects (Ferré et al., 1997; Lara and Souza, 2000). Similarly to the putative role of adenosine on sodium nitroprusside-induced hypolocomotion and catalepsy, which are probably mediated by adenosine A_{2A} receptors, nitric oxide may indirectly act by promoting adenosine-mediated activation of adenosine A_1 receptors in models of brain injury and excitotoxicity. Indeed, NO-induced synaptic depression in the hippocampus was blocked by an adenosine A_1 antagonist (Broome et al., 1994). Finally, administration of NO synthase inhibitors may regulate basal levels of adenosine (Fischer et al., 1995), reinforcing a physiological balance

between adenosine and NO actions. This interaction should be further investigated and more frequently considered when studying the effects of NO and adenosine alone.

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