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Stress-Induced Hypothermia Involves Stimulation of Adenosine A_1-Receptors in Rats

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Abstract Subcutaneous (s.c.) injection of adenosine produced hyperthermia in non-stressed rats and potentiated hypothermia in stressed rats. The potent adenosine A_1-receptor stimulants, N^6-cyclohexyl adenosine (CHA) and N^6-(L-phenylisopropyl) adenosine (L-PIA) produced hypothermia even in non-stressed rats, which was markedly potentiated in stressed rats. Furthermore, intracerebral ventricular (i.c.v.) injection of adenosine or adenosine analogues produced potentiation of stress-induced hypothermia relative to that induced with s.c. injection. N^6-(D-phenylisopropyl) adenosine (D-PIA) did not affect core temperature. When rats were treated with adenosine 1 h before stress, and then exposed to stress, the hyperthermia exhibited in the non-stressed state changed to hypothermia in the stressed state. When the rats which were treated with adenosine and then stressed for 1 h were released from stress, the hypothermia observed in the stressed state progressively changed to hyperthermia. Low doses of β-endorphin (i.c.v.) and morphine (s.c.) reversed stress-induced hypothermia which was potentiated by CHA, although high doses of β-endorphin (i.c.v.) and morphine (s.c.) potentiated the stress-induced hypothermia. These results suggest that the effects of adenosine on core temperature in rats are altered depending upon the state of animals. Stress-induced hypothermia may be, at least in part, mediated by endogenous adenosine which seems to selectively activate A_1-receptor in the stressed state.

Key Words: core temperature, adenosine, adenosine analogs, opiate substances, stressed and nonstressed rats

Introduction

The physiological regulatory role of the nucleoside, adenosine in the function of central nervous system has been extensively discussed. Adenosine analogs such as CHA and L-PIA have been developed which have

a high affinity for the adenosine A_1 -receptor and which inhibit adenylate cyclase activity[1)2)]. Exogenous application of adenosine [or N^6-substituted analogs produces antilocomotive, analgesic and sedative effects. Furthermore, L-PIA induces significantly hypothermia in mice[3)4)]. Exogenously given adenosine has, however, only short-lasting action due to its rapid deamination[1)4)] and reuptake[5)]. Since L-PIA is subject to slower deamination and reuptake, this agent is more active and has a more prolonged action[4)6)7)]. There is evidence that both adenosine and morphine inhibit transmitter release[8)], and that the inhibition of neurotransmitter release produced by morphine may be mediated by the initial release of adenosine[9)10)]. Recently, we found that adenosine did not produced gastric lesions in non-stressed rats but did so in stressed rats. Adenosine analogs produce gastric lesions in both the non-stressed and stressed states, and the lesions are modulated by opiate substances such as morphine and β-endorphin. The mechanism involved in gastric lesions induced by these adenosine analogs may be similar to that occurring during stress[11)]. The present study was designed to examine the possible contribution of central purinergic and opiate systems involved in stress-induced hypothermia.

Materials and Methods

Animals.

Healthy male Wistar rats (200–250 g) were obtained from Kyudo Animal Laboratory (Kumamoto, Japan) and maintained in an animal room with a 12 h light-dark cycle (7:00 am – 7:00 pm). Commercial food (MF, Oriental Japan Ltd.) and tap water were available *ad libitum* except during the time of the experiments. All experiments were carried out at an environmental temperature of 23± 1°C. After the rats had been caged in a group of five, they were individually housed in plastic cages and food deprived for 24 h before the experiments. Rats were divided into groups of 5–10 balancing both the body weights and the core temperature measured prior to the experiments.

Measurement of core temperature.

A thermister probe was lubricated with glycerin and inserted approximately 7 cm into the rectum; measurements were read from a digital thermometer. Core temperature was measured for 3 h.

Stress procedure.

Immobilization stress was produced by enclosing rats in a flexible wire mesh (3×3 mm) initially formed into a cone and then bent to conform to the size of the individual animals.

Administration of drugs.

Rats received adenosine (5 and 10 mg/kg s.c.; 5 and 10 μg i.c.v.), CHA (0.1 and 0.3 mg/kg s.c.; 5 and 10 μg i.c.v.), L-PIA (0.3 mg/kg s.c.; 5 and 10 μg i.c.v.) or D-PIA (0.3 mg/kg s.c.; 5 and 10 μg i.c.v.) and core temperature was measured for 3 h. Immediately after drug administration, the rats were exposed to immobilization stress for 3 h.

To further confirm the state-dependent adenosine effects, twenty rats were injected with adenosine 10 mg/kg and left for 1 h and then half of them were stressed by immobilization for 2 h and the remaining half were left without stress for a further 2 h. In addition, twenty rats were immobilized immediately after injection of adenosine at 10 mg/kg for 1 h and then half of them were released from immobilization stress but the remaining half were left immobilized for a further 2 h.

To observe opiate effects on hypothermia induced by CHA in the non-stressed or stressed states, morphine (1–10 mg/kg s.c.) and β-endorphin (5–50 μg i.c.v.) were administered immediately before CHA (0.3 mg/kg s.c.). Rats were exposed immobilization stress immediately after CHA. Core temperature was measured every 30 min for 3 h after drug administration.

Drugs.

Drugs used were adenosine (Sigma, St. Louis), N^6-cyclohexyl adenosine (CHA, Boehringer-Mannheim), N^6-(L-phenylisopropyl) adenosine (L-PIA, Boehringer-Mannheim), N^6-(D-phenylisopropyl) adenosine (D-PIA, Boehringer-Mannheim), morphine hydrochloride (Sankyo K.K. Tokyo) and β-endorphin (Boehringer-Mannheim). CHA, L-PIA and D-PIA were disolved in ethyl alcohol (0.05 ml) and were subsequently diluted with saline (10 ml). All other drugs were dissolved in saline, and an equal volume of vehicle (0.1 ml/kg, s.c.; 5 μl, i.c.v.) was injected to control animals. Intracerebral ventricular injection was performed through

cannulae implanted according to the method of de Weid[12]. Doses are expressed in term of the salt. Injection of vehicles did not produce any abnormal symptoms.

Statistical analysis.

Data were analyzed by means of analysis of variance and subsequent Duncan's test (Siegel, 1956). The level of significance chosen was $P<0.05$.

Results

Effects of adenosine, CHA, L-PIA and D-PIA on core temperature in non-stressed and stres-sed rats.

As shown in Fig. 1, adenosine (5 and 10 mg/kg s.c.) significantly increased the core temperature of rats in a dose dependent manner (A). When rats were restrained, the rectal temperature of animals injected with saline was slightly but significantly decreased. The stress-induced hypothermia was potentiated by adenosine (5 and 10 mg/kg s.c.) (B). CHA (0.1 and 0.3 mg/kg s.c.) and L-PIA (0.3 mg/kg s.c.) markedly decreased core temperature in a dose-dependent manner in the non-stressed state. The maximum response occurred most potently

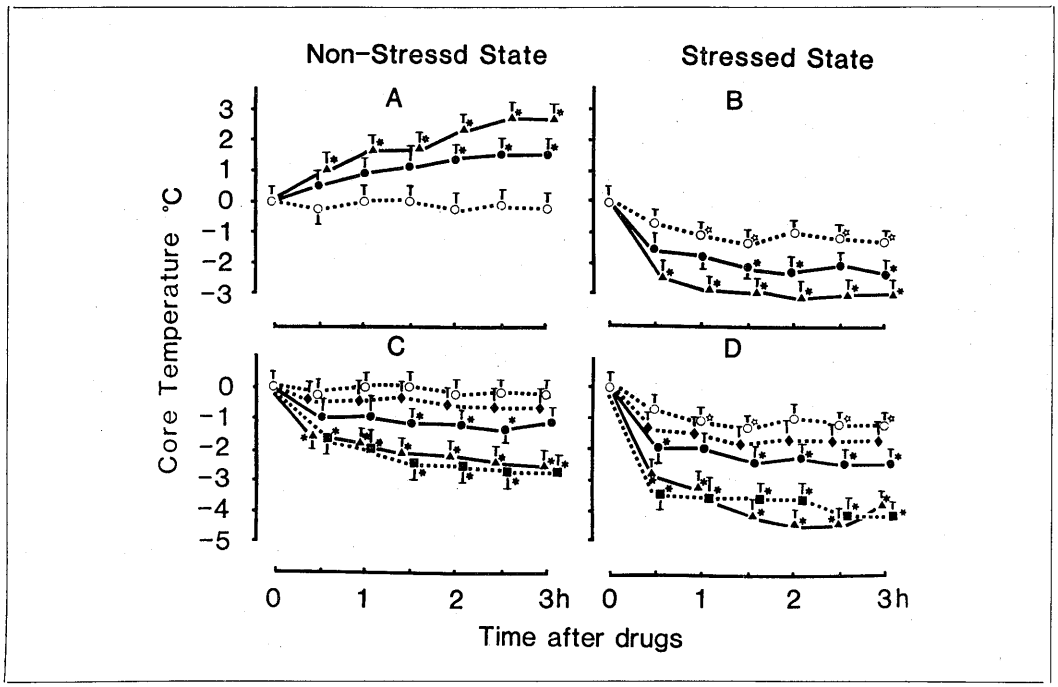

Fig. 1 Effects of adenosine and adenosine analogues on thermic responses in non-stressed and stressed rats. Non-stressed rats were measured 0–3 h after adenosine, CHA, L-PIA, D-PIA and vehicle. Stressed rats were exposed to 0–3 h immobilization stress immediately after adenosine (5 and 10mg/kg s.c.), CHA (0.3 mg/kg s.c.), L-PIA (0.3 mg/kg s.c.), D-PIA (0.3 mg/kg s.c.) and vehicle. Each value shows the mean±SE from 8 rats.
A: Non-stressed rats, B: Stressed rats
○ Vehicle, ● Adenosine 5 mg/kg, ▲ Adenosine 10 mg/kg
C: Non-stressed rats, D: Stressed rats
○ Vehicle, ● CHA 0.1 mg/kg, ▲ CHA 0.3 mg/kg,
◆ D-PIA 0.3 mg/kg, ■ L-PIA 0.3 mg/kg
*; $P<0.05$; significant difference from the non-stressed and stressed vehicle groups, respectively, and ☆; $P<0.05$; from non-stressed vehicle group, determined by ANOVA and subsequent Duncan's test

Table 1 Effect of i.c.v. Injection of Adenosine and Its Analogues on Core Temperature.

Drugs	(μg)	Core Temperature °C	
		Non-stressed rats	StresseS Iats
Vehfcle		37.9±0.1	34.2±0.2 †
AdenoIine	5	39.1±0.1*	33.1±0.2* ††
Adenosine	10	40.8±0.2*	31.2±0.3**††
CHA	5	35.4±0.1**	30.6±0.2**††
CHA	10	32.1±0.2**	27.8±0.3**††
L*f*PIA	5	34.3±0.2*	29.9±0.2**††
L-PIA	10	31.5±0.3**	26.5±0.3**††
D-PIA	5	37.5±0.2	33.9±0.3 †
D-PIA	10	36.5±0.3	33.2±0.3 †

Rats were exposed to immobilization stress immediately after i.c.v. administration of vehicle, adenosine, CHA, L-PIA and D-PIA. In non-stressed rats, core temperaturwas observed 2 h after injection of drugsor vehicle. *; $P<0.05$, **; $P<0.01$; significant difference from vehicle-group in non-stressed and stressed states, respetively, †; $P<0.05$, ††; $P<0.01$, from coresponding doses in non-stressed group, determined by means of analysis of variance and subsequent Duncan's test

1.5 or 2.5 h after injection (C). CHA (0.1 and 0.3 mg/kg) and L-PIA (0.3 mg/kg) markedly potentiated stress-induced hypothermia (D). The effect of D-PIA (0.3 mg/kg s.c.) on thermic responses, however, was weak in both the non-stressed or stressed state (C, D).

In addition, i.c.v. injection of adenosine (5 and 10 μg) produced the most potent hyper- and hypothermic responses in the non-stressed and stressed states, respectively, while CHA (5 and 10 μg) or L- and D-PIA (5 and 10 μg) produced the most potent hypothermic response in both states, relative to SC injection (Table 1).

State-dependent effects of adenosine on core temperature.

When rats were exposed to immobilization stress 1 h after injection of adenosine at 10 mg/kg, the rectal temperature was significantly and markedly decreased during stress. However, once the rats were released from stress, the temperature gradually recovered to control levels and thereafter, increased to the level of rats injected with adenosine without stress. In contrast, when rats which had been injected with adenosine and already exhibited hyperthermia were immobilized their rectal temperature gradually decreased to the levels of stressed rats injected with adenosine and remained at the same decreased temperature level. These results are summarized in Fig. 2.

Effects of morphine and β-endorphin on CHA-induced hypothermia in non-stressed and stressed rats.

As demonstrated in Table 2, the hypothermic response induced by CHA (0.3 mg/kg s.c.) was reversed by low doses of morphine (1 mg/kg s.c.) and β-endorphin (5 and 10 μg i.c.v.) in non-stressed or stressed rats. Higher doses of morphine (10 mg/kg s.c.) and β-endorphin (50 μg i.c.v.) potentiated the hypothermic responses (Table 2).

Discussion

In the present experiments, subcutaneous and intracerebral ventricular injection of adenosine produced hyperthermia in non-stressed rats and potentiated hypothermia in stressed rats. In addition, the selective adenosine A_1-receptor stimulants such as CHA and L-PIA also produced hypothermia in the non-stressed state and potentiated stress-induced hypothermia. The hypothermia occurred in a dose-dependent manner, suggesting that the development of stress-induced hypothermia may at least in part, be mediated by the central purinergic system. Recently, we found that adenosine and these analogs produce gastric lesions, which are in turn potentiated by immobilization stress[11]. Furthermore, intracerebral ventricular injection of adenosine or CHA also produced the rapid and potent exacerbation of stress-induced gastric lesions[11].

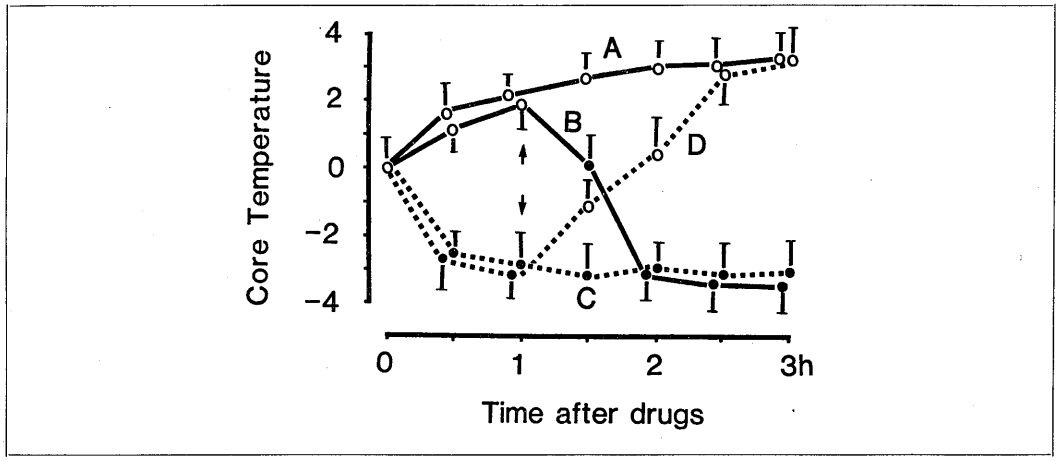

Fig. 2 State dependent effects of adenosine on core temperature in rats. Half of the non-stressed rats (A) were left without stress and the remaining half (B) were exposed to immobilization stress 1 hour after adenosine. Half of the stressed rats (C) were continued to be stressed and the remaining half (D) were released from stress after 1 hour. Each value indicates the mean ± SEM of 10 rats.
○ Adenosine 10 mg/kg, ● Adenosine 10 mg/kg+Stress,
↑ Expesure to stress ↓ Released from stress

Table 2 Effects of Opiate Drugs on Hypothermia Induced by CHA in Non-Stressed and Stressed Rats.

Prejreatment	Core Temperature °C	
	Non-stresseS rajs	StresseS rajs
s.c. Injection (Hg/kg)		
Vehicle	32.4±0.1	29.2±0.2
Morphine 1	37.1±0.2*	35.1±0.2**
Morphine 10	38.2±0.2**	25.2±0.2*
i.c.v. Injection (μg/rat)		
Vehiale	31.9±0.1	28.3±9.1
β-Endorphfn 5	36.3±9.2*	35.1±0.3**
β-Endorphin 10	37.9±0.2*	36.2±0.4*
β-Endorphin 20	37.8±0.3**	36.6±0.3**
β-Endorphin 50	39.1±0.3**	25.5±0.2*

CHA (0.3 mg/kg s.c.) was administered 15 min after vehicle, morphine and β-endorphin. Half of rats were immediately exposed to immobilization stress and the remaining half were left without stressl. Each value indicates the mean±SE of 8 rats. *; $P<0.05$, **; $P<0.01$; signiffcant difference from the vehicle group, determined by analysis of variance and subsequent Duncan's test

It has been reported that adenosine inhibits adenylate cyclase via a "high" affinity receptor, and activates adenylate cyclase via "low" affinity receptor. These receptors are called A_1- and A_2-receptors in brain, respectively. CHA and L-PIA are potent agonists of the A_1-receptor which is a subclass of the p_1-receptor, which is involved in presynaptic autoinhibition mechanisms in peripheral nerves[14)15)]. The stimulatory effect of D-PIA on A_1-receptors is weaker than L-PIA or CHA[1)4)]. The initiation of stress-induced hypothermia may be due to the stimulation of central A_1-purinoceptors because stress-induced hypothermia was markedly potentiated by CHA or L-PIA but only weakly by D-PIA. Accordingly, in the stressed state, adenosine may act to stimulate adenosine A_1-receptor and, as a consequence, produce hypothermia as well as stress gastric lesions.

Body temperature is a balance between heat production and heat loss[16)17)]. These temperature effects may be due to a direct action on the thermoregulatory center in the anterior hypothalamus[18)19)] and to an action on the spinal cord[20)]. The effects of adenosine on core temperature in rats are dependent upon the state of animals; that is, adenosine appears to have a dual action of hyperthermia in the non-stressed state and hypothermia in the stressed state. It has been demonstrated that acute stress results in an increase in endogenous opiate[21)22)], suggesting that endogenous opiate peptide in the brain play a modulating role in stressful situations. In these studies, the opiate agonist morphine and the opiate peptide β-endorphin at low doses reversed the stress-induced hypothermia in combination with adenosine or its analogs but at higher doses, potentiated the hypothermia. High doses of morphine markedly potentiated the stress-induced hypothermia in a dose-dependent manner, with the exception of 2.5 mg/kg which elevated the temperature[23)]. Furthermore, CHA-induced gastric lesions in non-stressed or stressed rats were also antagonized by opiate substances in a dose dependent manner but were not affected by opiate antagonists[23)].

The present results suggest that stress-induced hypothermia may at least in part, involve in the stimulation of presynaptic purinoceptors, probably A_1-receptors, in the brain under stressful situations. β-Endorphin could be opposing the actions of adenosine via a different pathway.

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