Characterization of $N^6$-Cyclohexyladenosine-Induced Hypothermia in Syrian Hamsters

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Abstract. The $N^6$-cyclohexyladenosine (CHA)-induced hypothermia in Syrian hamsters was characterized as follows: intracerebroventricular injection of CHA-induced hypothermia and the potency was increased by lowering the ambient temperature. CHA microinjection into the anterior hypothalamus (AH) elicited the most marked body temperature ($T_b$) decrease compared with other regions such as the preoptic area, dorsomedial hypothalamus, posterior hypothalamus, and hippocampus. In contrast, microinjected CHA into the medial septum, ventromedial hypothalamus, and lateral hypothalamus resulted in negligible changes in $T_b$. These results suggest that CHA-induced hypothermia was probably due to suppression of thermogenesis via the site(s) of CHA action, viz., the AH and medial hypothalamus.

Keywords: adenosine, hypothermia, anterior hypothalamus

Apart from $\gamma$-aminobutyric acid (GABA) (1), adenosine is widely accepted as the major inhibitory neurotransmitter in the central nervous system (CNS). Recent studies in our laboratories have revealed the important role played by the central adenosine system via the adenosine A1 receptor in hibernation entrance characteristic of the profound hypothermia in Syrian hamsters (2). However, characterization of adenosine-induced hypothermia in hamsters remains unclear to date. Adenosine has been reported to induce a hypothermic action in various species (3, 4). It has been confirmed that the adenosine A1 receptor is located in thermoregulation-associated brain sites such as the hypothalamus, hippocampus, and septum (5 – 7). In this study, we attempted to characterize hypothermia induced by $N^6$-cyclohexyladenosine (CHA) (a selective adenosine A1-receptor agonist) and to locate the relevant site(s) of CHA action in Syrian hamsters using brain microinjections.

Adult Syrian hamsters (Shimizu) weighing 90 – 120 g were housed in cages of 5 – 6 animals each in a room maintained at 23 ± 2°C and illuminated with alternating 12-h light/dark cycles (light: 08:00 – 20:00 h). The animals were given food and water ad libitum. All experiments were performed according to the guidelines for animal experiments of Fukuyama University. Syrian hamsters anesthetized with sodium pentobarbital (40 mg/kg, i.p.; Abbott, North Chicago, IL, USA) were each restrained in a stereotaxic device (Narishige, Tokyo) for the placement of guide-cannulae for brain microinjections. According to the hamster brain atlas (8), a 21-gauge stainless guide-cannula was inserted stereotaxically 1 mm above the lateral ventricle (LV), medial septum (MS), preoptic area (PO), anterior hypothalamus (AH), ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH), lateral hypothalamus (LH), posterior hypothalamus (PH), or hippocampal regions (CA1, CA2, and CA3). The guide-cannula affixed to the skull with dental cement (PDDD/Miky red; Nissin, Kyoto) was kept patent prior to microinjection. A telemetory device (TA10TA-F20; Data Science, Inc., St. Paul, MN, USA) was implanted in the peritoneal cavity to monitor changes in the body temperature ($T_b$) before the hamsters were individually housed for 1-week recovery. On the day of the experiments, Syrian hamsters were each placed in a chamber maintained at 25, 15, or 5°C for 3 h before drug injection. Intracerebroventricular (i.c.v.) injection of CHA (Research Biochemical, Inc., Natick MA, USA) was executed through a steel needle connected to teflon tubing filled with pyrogen-free artificial cerebrospinal
fluid (aCSF). The open-end of the teflon tubing was
capable of delivering an injection volume of 10 μl to each administration. Drug admin-
istrations into the MS, PO, AH, DMH, VMH, LH, PH, CA1, CA2, and CA3 by microinjections were respec-
tively performed at 0.5 μl/site per 3 min with a 10-μl syringe driven by an infusion pump (Harvard Apparatus, Inc., South Natick, MA, USA). At the end of the respective experiments, the microinjection sites were histologically identified. Thionin (Merck & Co., Inc., Whitehouse Station, NJ, USA) was injected via the guide-cannula at a rate similar to drug administrations.

Immediately after decapitation, the whole brains of Syrian hamsters were fixed in 10% formalin for 2 – 3 days at 4°C before being subjected to coronal sectioning (thickness: 100 μm) with a microtome (Yamato Koki, Tokyo) and stained with neutral red (Chroma, Muenster, Germany). All values were represented as the mean ± S.E.M.

We confirmed that dose-dependent hypothermia was induced by icv injection of CHA at an ambient temperature of 5°C. This dose-dependent tendency in CHA-induced hypothermia was observed within the dose range of 0.05 – 0.5 nmol per site. The doses of 0.05, 0.1, and 0.5 nmol induced Tb decreases with ΔTb/Δtpeak (Ts

As the respective Ts values before CHA injection in the 25°C, 15°C, and 5°C groups were 37.35 ± 0.20°C, 37.16 ± 0.13°C, and 36.99 ± 0.10°C, CHA-induced hypothermic effects induced were dependent on the ambient temperature (Fig. 1): an i.c.v. dosage of 0.5 nmol induced ΔTs/Δtpeak rates of −6.39 ± 0.25°C/166.7, −14.05 ± 0.71°C/188, and −31.99 ± 0.10°C/465 min at 25°C, 15°C, and 5°C, respectively. However, icv aCSF did not affect the Ts even at the lowest ambient temperature of 5°C.

We further microinjected CHA (0.3 nmol) or aCSF into various brain sites at 5°C to locate the induction site(s) of CHA-hypothermia. According to ΔTs variations after CHA microinjection (Table 1), although sites PO, AH, DMH, PH, CA1, CA2, and CA3 manifested hypothermia (ΔTs: −0.90 ± 0.38°C to −7.37 ± 0.92°C), CHA microinjected into sites MS, VMH, and LH showed negligible ΔTs changes comparable to the null ΔTs effects of all aCSF-microinjected sites studied. Interestingly, site AH manifested significantly pronounced (P<0.05) hypothermia (ΔTs: −7.37 ± 0.92°C) compared with other CHA-sensitive sites (PO, DMH, PH, CA1, CA2, CA3).

![Fig. 1. Time-related hypothermic effects of intracerebroventricular (i.c.v.) CHA (0.5 nmol: opened symbols) or artificial cerebrospinal fluid (aCSF: closed circle) in Syrian hamsters exposed to different ambient temperatures (25°C: triangle; 15°C: rhombus; 5°C: circle). Decreases in body temperature (Ts) are expressed as the mean ± S.E.M. The Ts values until 360 min (at 25°C) and until 450 min (15°C) were significantly lower than those of the aCSF-injected group from 30 min after CHA injection. However, the Ts (at 5°C) values were always significantly lower than those of the aCSF-injected group 30 min after CHA microinjection. Six animals were designated in each experimental group. The Ts values of the CHA-microinjected groups at different ambient temperatures were compared with the aCSF group by repeated measures two-way ANOVA, followed by post hoc test (Dunnett test). The Ts values within each group were statistically analyzed by repeated measures one-way ANOVA, followed by post hoc test (Dunnett test) compared with the value obtained at time 0.]
The role of adenosine on the central thermoregulating system was first investigated by studying the effects of icv CHA on Tb in conscious Syrian hamsters at various ambient temperatures. The extent of Tb decreases induced by icv CHA was correlated with the fall in ambient temperature (Fig. 1). The dosage of 0.5 nmol, in particular, reduced Tb to as low as 6°C, a tendency that is similarly observed in the natural hibernation state. This result suggests that activation of central adenosine A1 receptors is an essential event for hibernation entrance and/or maintenance in Syrian hamsters. However, hamsters with profound CHA-induced hypothermia were eventually dead after having maintained the induced hypothermia for more than 18 h. It is well known that hibernators require a preparatory period before entry into hibernation. As such, hibernators are thought to mediate their physiological mechanisms, including the thermogenation system, during the preparatory period in order to adapt to approaching low Tn. As non-conditioned hamsters were employed in this study, they could not have adapted to hibernation because the thermogenation system under low Tn was probably not developed as yet.

In homiootherms (including the Syrian hamster), it is thought that the thermoregulation center receives afferent sensory inputs from thermoreceptors (warm and cold receptors) distributed ubiquitously all over the body and that normothermia is maintained by the effector responsiveness regulated by the altered activity of thermosensitive neurons in the thermoregulation center (9). Furthermore, the large body surface area against the body mass ratio in small homiootherms affords consistent facilitation of thermogenic reactions involving the brown adipose tissue (10). Thus, ambient temperature-dependent CHA-induced hypothermia may be depressed by decreasing the ambient temperature because of inhibition of the thermogenic reactions. It has been argued that the primary outcome of adenosine A1 receptor activation in many systems is an inhibitory modulation of neurotransmitter releases mediated by the inhibition of Ca2+ influx into the presynaptic nerve terminal in rats (11). Moreover, we have demonstrated in a previous study that the thyrotropin-releasing hormone (TRH) activates thermogenic responses such as non-shivering thermogenesis in Syrian hamsters (12), and Lin et al. have argued that intrahypothalamic administrations of norepinephrine (NE) induce hyperthermia in deer-mice (13). Therefore, hypothermic effects of adenosine via the adenosine A1 receptor may be due to dysfunction of thermosensitive neurons by suppressing the release of analeptic thermoregulating factors like TRH and/or NE.

In the present study, independent CHA microinjections into sites PO, AH, DMH, PH, CA1, CA2, and CA3 induced Tn decreases, although such hypothermic effects were not established with similar administrations into the MS, VMH, and LH of Syrian hamsters. It should be noted that the AH exerted the most predominant response compared with other CHA-sensitive sites. Thermosensitive neurons in rats exist in the AH (14), and this brain site receives afferent sensory inputs from thermoreceptors distributed throughout the body surface in rabbits (15). Therefore, it is thought that the AH is the site that displayed the highest sensitivity to CHA.

In conclusion, CHA-induced hypothermia was probably elicited by the inhibition of thermogenesis. The sites of CHA-induced hypothermia probably comprised the medial hypothalamus and hippocampal regions. Our previous investigations have demonstrated the important role of the central adenosine system (via the adenosine A1 receptor) in the entrance mechanism of hibernation in Syrian hamsters (2). As such, the entrance mechanism of hibernation in Syrian hamsters may be characterized by the said adenosine-induced hypothermia.
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References


