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Research Report

Neuroprotective effects of hibernation-regulating substances against low-temperature-induced cell death in cultured hamster hippocampal neurons

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ABSTRACT

The neuroprotective effects of hibernation-regulating substances (HRS) such as adenosine (ADO), opioids, histamine and thyrotropin-releasing hormone (TRH) on low-temperature-induced cell death (LTCD) were examined using primary cultured hamster hippocampal neurons. LTCD was induced when cultures were maintained at <22 °C for 7 days. ADO (10–100 μM) protected cultured neurons from LTCD in a dose-dependent manner. The neuroprotective effects of ADO were reversed by both 8-cyclopenthyltheophilline (CPT; A_1 receptor antagonist) and 3,7-dimethyl-1-propargylxanthine (DMPX; A_2 receptor antagonist). Morphine (a non-selective opioid receptor agonist) was also effective in attenuating LTCD at an in vitro dose range of 10–100 μM. The neuroprotective effects of morphine were antagonized by naloxone (a non-selective opioid receptor antagonist). In addition, although [D-Ala^2, N-Me-Phe^4, Gly-ol^5]-enkephalin (DAMGO; μ-opioid receptor agonist), [D-Pen^{2,5}]-enkephalin (DPDPE; δ-opioid receptor agonist) and U-69593 (κ-opioid receptor agonist) were also effective, LTCD of cultured hippocampal neurons was not affected by TRH. Furthermore, histamine produced hypothermia in Syrian hamsters and protected hippocampal neurons in vitro at 100 μM. The neuroprotective effect of histamine was reversed by pyrilamine (H_1 receptor antagonist). Apoptosis was probably involved in LTCD. These results suggest that ADO protected hippocampal neurons in vitro via its agonistic actions on both A_1 and A_2 receptors, whereas morphine probably elicited its neuroprotective effects via agonistic effects on the μ-, δ- and κ-opioid receptors. In addition, histamine also protected hippocampal neurons via its agonistic action on the H_1 receptor. Thus, HRS-like adenosine-, opioid- and histamine-like hypothermic actions would most likely induce neuroprotective effects against LTCD in vitro.

1. Introduction

Hibernation is a unique physical condition characterized by depressed body temperature (T_b), respiration, cardiovascular function and general metabolism. Typical hibernating mammals, such as the Syrian hamster and ground squirrel, tend to lower their T_b to near 0 °C during hibernation (Tamura et al., 2005; Wang and Hudson, 1978). Throughout the hibernation period, these hibernators exhibit periodic bouts of torpor marked by dramatic changes in T_b (Tamura et al.,

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2005; Wang and Hudson, 1978). These periodic bouts suggest that functions of the central nervous system (CNS) are retained even at low brain temperatures during hibernation. Investigations of the electroencephalograms (EEG) of hibernating animals have shown that certain limbic structures of the brain, including hippocampus and septum, retain periodic EEG activities during hibernation (Beckman and Stanton, 1982; Chatfield and Lyman, 1954; Strumwasser, 1959; Walker et al., 1977). Moreover, it has been documented that the septohippocampal system plays an important regulatory role associated with hibernation–arousal cycles (Gabriel et al., 1998; Horrigan et al., 1997; Kramarova et al., 1991; Popov and Bocharova, 1992; Popov et al., 1992; Popova, 2004; Spangenberger et al., 1995). These findings therefore suggest that neuronal activities of the hippocampus were retained under low brain temperatures during hibernation. In contrast, excessive hypothermia has been known to cause death in non-hibernating mammals. In short, it is widely accepted that the difference in resistance to hypothermia between hibernators and non-hibernators depends on the difference in resistance to hypothermia.

According to changes in the T_b, the hibernation cycle has been classified conventionally into: (i) entrance, (ii) maintenance and (iii) arousal phases. In our previous studies, T_b during the entrance, maintenance and arousal phases were regulated by central A_1 receptors mediated by the adenosine (ADO) receptor-mediated opioid and type-1 receptor-mediated TRH systems, respectively (Tamura et al., 2005). ADO acts as a neuromodulator in the CNS and the neuroprotective effect of ADO against ischemic or traumatic neuronal injury has been well documented experimentally (Gidday et al., 1995; Schubert et al., 1997; Sweeney, 1997; von Lubitz and Marangos, 1990). Moreover, opioid receptor agonists elicit neuroprotective effects against neurodegeneration induced by ischemia and trauma (Chen et al., 2005; Lyeth et al., 1995; Prince and Feeser, 1988).

In this study, we investigated if hibernation-regulating substances (HRS) such as ADO, opioids and TRH would protect hippocampal cultures from low-temperature-induced neuronal cell death (LTCD). In addition, since it has been reported that histamine is involved in the hibernation mechanism(s) of rodents (Nikmanesh et al., 1996; Panula et al., 2000; Sallmen et al., 1999, 2003a,b,c), the neuroprotective effect of histamine was also elucidated in this study.

2. Results

2.1. *Time-related changes in the cell viability of cultured hippocampal neurons*

One of the aims in this study was to clarify the maintaining system of CNS function during hibernation. For this purpose, it is important to compare the resistance against low temperatures in a nerve cell of a non-hibernator with a hibernator. Thus, cultured rat (non-hibernator) neurons were first maintained at various temperatures in the present study to investigate the influence of low temperatures on non-hibernator neurons. When the culture temperature was above 24 °C (24–37 °C), the cell viability was >90% under all conditions until DIV 21. However, when the hippocampal neurons were maintained at 22 °C from DIV 12, the cell viability was significantly decreased after 6 days in low temperatures. The decrease of cell viability induced by low temperatures was exacerbated by lowering the culture temperature (Fig. 1A).

The resistance against low temperatures of rat and hamster neurons were then compared. Cell viabilities of ca.

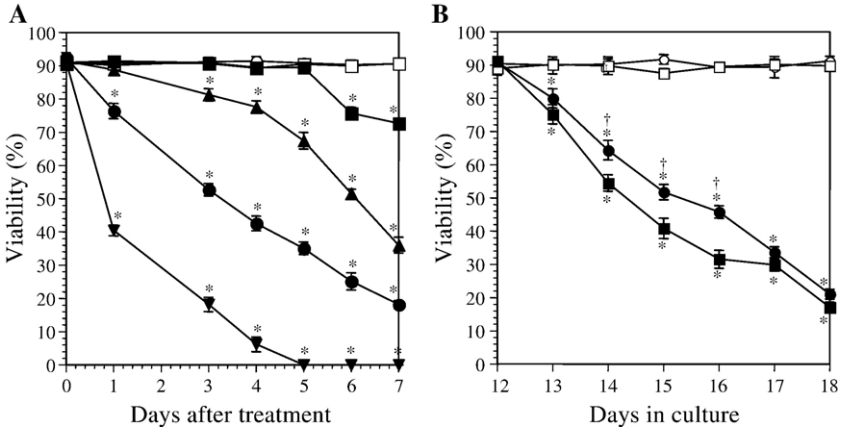

Fig. 1 – Time-related changes in the cell viability of cultured hippocampal neurons. The left plots (graph A) show the temperature- and time-related changes in cell viability of cultured rat hippocampal neurons. Cultures maintained at 37 °C were used as controls (○). Rat hippocampal neurons were maintained at 24 °C (□), 22 °C (■), 20 °C (▲), 18 °C (●) and 16 °C (▼) from day in vitro 12, respectively. The right plots (graph B) show the changes in cell viabilities of hamster (hibernator) and rat (non-hibernator) hippocampal neurons under low temperatures. The sister-cultures of hamster (○) and rat (□) neurons maintained at 37 °C were used as controls. Hamster (●) and rat (■) hippocampal neurons were maintained at 18 °C from day in vitro 12. In graph A, differences where $p < 0.05$ (*) were compared with the control using the Dunnett's-two tailed test. In graph B, differences where $p < 0.05$ (*) were compared with the control, whereas those where $p < 0.05$ (†) were compared with rat neurons using the unpaired Student's *t* test.