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Neuropeptide S increases motor activity and thermogenesis in the rat through sympathetic activation

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ABSTRACT

The central role of neuropeptide S (NPS), identified as the endogenous ligand for GPR154, now named neuropeptide S receptor (NPSR), has not yet been fully clarified. We examined the central role of NPS for body temperature, energy expenditure, locomotor activity and adrenal hormone secretion in rats. Intracerebroventricular (icv) injection of NPS increased body temperature in a dose-dependent manner. Energy consumption and locomotor activity were also significantly increased by icv injection of NPS. In addition, icv injection of NPS increased the peripheral blood concentration of adrenalin and corticosterone. Pretreatment with the β1- and β2-adrenergic receptor blocker timolol inhibited the NPS-induced increase of body temperature. The expression of both NPS mRNA in the brainstem and NPSR mRNA in the hypothalamus showed a nocturnal rhythm with a peak occurring during the first half of the dark period. To examine whether the endogenous NPS is involved in regulation of body temperature, NPSR antagonist SHA68 was administered one hour after darkness. SHA68 attenuated the nocturnal rise of body temperature. These results suggest that NPS contributes to the regulation of the sympathetic nervous system.

1. Introduction

Neuropeptide S (NPS), comprising 20 amino acid residues, was originally identified as an endogenous ligand for the orphan receptor GPR154, now named neuropeptide S receptor (NPSR), in 2004 (Koob and Greenwell, 2004; Xu et al., 2004). The structure of NPS has been highly conserved in terrestrial vertebrates (Reinscheid, 2007). Expression of mRNA for the NPS precursor has been recognized mainly in the peri locus ceruleus, lateral parabrachial nucleus and trigeminal nucleus (Clark et al., 2011; Xu et al., 2007; Xu et al., 2004). On the other hand, it has been reported that expression of mRNA for NPSR is widely distributed in the brain, suggesting that NPS and the NPSR system may be involved in multiple central nervous functions (Clark et al., 2011; Liu et al., 2011; Xu et al., 2007).

In vitro studies have shown that NPS acts through an increase of intracellular cyclic AMP and mobilization of intracellular Ca^{2+} (Xu et al., 2004), and an increase of glutamatergic synaptic transmission to intercalated GABAergic neurons in the amygdala via presynaptic NPSR on connected principal neurons (Jüngling et al., 2008). NPS has also been reported to behave as a potent inhibitor of serotonin and noradrenalin release from mouse frontal cortex synaptosomes (Raiteri et al., 2009). In vivo studies, on the other hand, have examined the effects of intracerebroventricular (icv) injection of NPS on behavior in mice and rats: icv injection of NPS stimulates locomotor activity (Fendt et al., 2011; Mochizuki et al., 2010; Pañeda et al., 2009; Rizzi et al., 2008; Roth et al., 2006; Smith et al., 2006; Xu et al., 2004), increases plasma ACTH and corticosterone (Smith et al., 2006), increases wakefulness and decreases sleeping time (Xu et al., 2004; Zhao et al., 2012), increases the urge for ethanol (Badia-Elder et al., 2008) or cocaine (Kallupi et al., 2010; Pañeda et al., 2009) use, and inhibits or stimulates food intake (Beck et al., 2005; Fedeli et al., 2009; Niimi, 2006). In addition, icv injection of NPS improves spatial memory (Lukas and Neumann, 2012; Okamura et al., 2011; Zhao et al., 2010), time in center entries in an open field (Jüngling et al., 2008; Xu et al., 2004), time on open arms in an elevated plus maze (Jüngling et al., 2008; Rizzi et al., 2008; Xu et al., 2004), and time in the light area in a light/dark box (Pañeda et al., 2009; Xu et al., 2004).

Recently it has been shown that icv injection of several neuronal peptides increases or decreases body temperature, locomotor activity, heart rate and energy consumption through activation of the sympathetic or parasympathetic nervous system (Billington et al., 1991; Currie and Coscina, 1995; Inoue et al., 2013; Lawrence et al., 2002; Mondal et al., 2003; Nakahara et al., 2016; Nakazato et al., 2000; Monda et al., 2006; Messina et al., 2016a). Thermogenesis can be either

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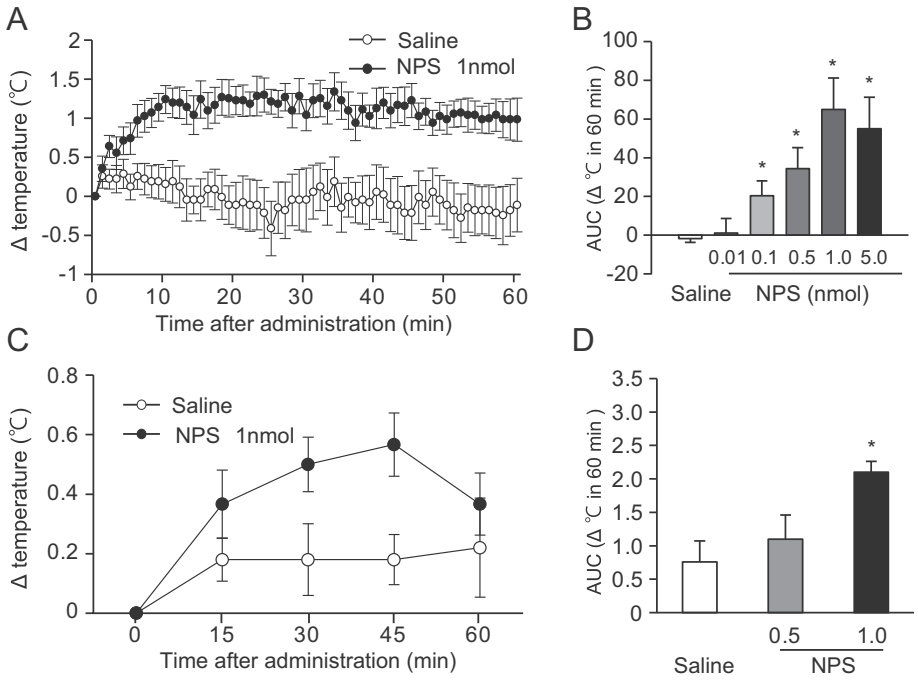

Fig. 1. Icv injection of NPS increased the body temperature. Effect of icv injection of saline and NPS (A, B) (baseline temperature: Saline: 37.07 ± 0.187, NPS 0.01 nmol: 37.06 ± 0.363, NPS 0.1 nmol: 37.05 ± 0.084, NPS 0.5 nmol: 36.74 ± 0.128, NPS 1 nmol: 37.29 ± 0.211, NPS 5 nmol: 37.28 ± 0.076). The difference from baseline temperature is displayed every minute (A) (saline vs 1 nmol: $F = 4.735$ $P < 0.00001$). The area under the curve is displayed in (B) (*saline vs 0.1 nmol: $t = 2.528$ $P = 0.0281$, vs 0.5 nmol: $t = 2.850$ $P = 0.016$, vs 1.0 nmol: $t = 3.757$ $P = 0.003$, vs 5.0 nml: $t = 4.260$ $P = 0.001$) on back surface temperature. Effect of icv injection of saline and NPS on rectal temperature (C, D) (baseline temperature: Saline: 37.85 ± 0.139, NPS 0.5 nmol: 37.86 ± 0.181, NPS 1 nmol: 37.98 ± 0.097). The difference from baseline temperature is displayed every 15 min (A) (saline vs 1 nmol: $F = 2.748$ $P = 0.0414$). The area under the curve is displayed in (B) (*saline vs 1.0 nmol $t = 3.875$ $P = 0.003$). Each bar graph on the right side represents the area under the curve (AUC) of temperature from the baseline for 60 min after injection. Each symbol or bar and vertical line represents the mean ± SEM ($n = 6$). Asterisks indicate significant differences from the saline group (*$P < 0.05$).

exercise-induced or non-exercise-induced. Non-exercise-induced thermogenesis includes all forms of energy expenditure not associated with formal exercise and includes spontaneous physical activity as well as thermogenesis via basal metabolism and brown adipose tissue (Argyropoulos and Harper, 2002; Riley et al., 2016; De Luca et al., 2008; Messina et al., 2012; Messina et al., 2013; Messina et al., 2016c; Messina et al., 2016b). Basal metabolism is regulated by sympathetic activity, whereas thermogenesis by brown adipose tissue is under the control of the sympathetic nervous system and endocrine system (Whittle and Vidal-Puig, 2012). When we preliminarily examined the expression of NPSR mRNA in the brain, relatively high expression was observed in the hypothalamus, which is an important region for homeostasis of body temperature and energy metabolism. Therefore, we speculated that NPS would be involved in thermogenesis or energy consumption. Therefore, in the present study, we examined whether NPS is involved in regulation of body temperature, and if so, whether NPS influences the autonomic nervous system.

2. Materials and methods

2.1. Animals

Male wistar rats (Charles River Japan, Inc., Yokohama, Japan) weighing 300–350 g were housed in individual plexiglas cages (420 × 250 × 200 mm) in an animal room maintained under a constant light-dark cycle (lights on 7:00–19:00 h) and temperature (22 ± 1 ˚C). Food and water were provided ad libitum. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care, and the experiments were authorized by Miyazaki University Animal Experiment Committee (authorization number: 2012-006-5).

2.2. ICV injection of NPS

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (Kyoritsu Seiyaku Corporation, Tokyo, Japan; 50 mg/kg BW) and were then mounted in a Narushige brain stereotaxic instrument (Narushige, Tokyo). A stainless steel cannula (guide cannula, 23-gauge; insert, 27-gauge) was then implanted into the lateral left ventricle. The cannula tip was placed at the following stereotaxic coordinates: 8 mm anterior to the interaural; 1.5 mm lateral to the midline; 3.0 mm below the dura. The guide cannula was anchored to the skull with machine screws and dental acrylic. During a 4-day postoperative recovery period, the rats became accustomed to the handling procedure. NPS (Sigma-Aldrich Co. LLC) was dissolved in saline (0.01, 0.1, 0.5 and 1.0, 5.0 nmol/10 µl), and then injected into each free-moving rat through a 27-gauge injection cannula connected to a 50-µl Hamilton syringe.

2.3. Measurement of body temperature

Rectal temperature was measured with a rectal probe thermocouple thermometer (Unique Medical Co., Ltd., Tokyo, Japan). Until the experiment day, rats habituated to the measurement of rectal temperature well. The experiment began at 7:30 and NPS (0.5, 1 nmol/10 µl) or saline was administered at 8:00, measurements being conducted for the following 60 min. The average value during the 30 min before administration was assumed to be that at zero min, and the values obtained thereafter were indicated as increases or decreases. Room temperature was maintained at subthermoneutrality (i.e., 22 ± 1 ˚C).

Back surface temperature in free-moving animals was recorded using infrared thermographic imaging (FLIR SC620, FLIR Systems, Danderyd, Sweden) as described in our previous paper (Inoue et al., 2013). We started infrared thermographic imaging of the back surface temperature from 7:30 h. Images taken at 1-min intervals were saved