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Research report

Thyrotropin-releasing hormone induced thermogenesis in Syrian hamsters: Site of action and receptor subtype

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Abstract

Early work in our laboratory has revealed the important role played by thyrotropin-releasing hormone (TRH) in the arousal from hibernation in Syrian hamsters. In the present study, we investigated the thermogenic mechanism of TRH in Syrian hamsters. Six to 10 female Syrian hamsters were used in the respective experiments. Intracerebroventricular (icv) injection of TRH elevated the intrascapular brown adipose tissue (IBAT) temperature (T_{IBAT}) and rectal temperature (T_{rec}) in Syrian hamsters. Thermogenic response of icv TRH was suppressed by bilateral denervation of the sympathetic nerve. Icv injection of TRH increased the norepinephrin (NE) turnover rate in IBAT without affecting the total serum triiodothyronine (T_3) level. Moreover, TRH microinjections into the dorsomedial hypothalamus (DMH), preoptic area (PO), anterior hypothalamus (AH) and ventromedial hypothalamus (VMH) induced T_{IBAT} and T_{rec} increases. However, neither T_{IBAT} nor T_{rec} was affected by similar TRH administrations into the lateral hypothalamus and posterior hypothalamus. Interestingly, although TRH-induced hyperthermia was suppressed by pretreatment of anti-TRH-R1 antibodies, no changes were induced by anti-TRH-R2 antibodies. These results suggest that the sites of action of TRH associated with thermogenesis are probably localized in the DMH, PO, AH and VMH. In addition, TRH-induced thermogenesis is probably elicited by facilitation of the sympathetic nerve system via the central TRH-R1 irrelevant of T_3.

Theme: Neural basis of behavior
Topic: Neuropeptides and behavior

Keywords: TRH; BAT; Thermogenesis; Hypothalamus; Syrian hamsters

1. Introduction

Thyrotropin-releasing hormone (TRH), isolated first from the hypothalamus as a hormone, functions to accelerate the thyroid-stimulating hormone (TSH) secretion from the pituitary gland, and its chemical structure has since been determined by Scally and Guillemin in 1970 [3,5]. In the central nervous system (CNS), TRH displays various physiological functions which are independent of endocrine activity in the hypothalamus–pituitary–thyroid axis [12,16] via its extensive distribution network [30]. Thus, TRH most likely plays an important role as a neurotransmitter or neuromodulator. Recent studies in our laboratory have demonstrated the important role of TRH in the arousal mechanism of hibernation in Syrian hamsters [35]. However, the central functions of TRH remain unclear to date. It is widely accepted that non-shivering thermogenesis (NST) in the brown adipose tissue (BAT) is important for arousal from hibernation in hibernating mammals and for heat production in small and infant homoiotherms [4,20,28]. BAT is extensively innervated by the sympathetic nervous system [10]. Thermogenesis in BAT is stimulated by norepinephrine (NE) released from the sympathetic nerve endings via adrenoceptors [2,11,33,34].

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The hypothalamus is known to serve as an autonomic-regulating center for thermoregulation. The hypothalamic nucleus, which includes the preoptic area (PO), anterior hypothalamus (AH), dorsomedial hypothalamus (DMH), ventromedial hypothalamus (VMH), lateral hypothalamus (LH) and posterior hypothalamus (PH), coordinates the regulation of body temperature in mammals [17,19,23,24,27]. Ogawa et al. have located dense TRH receptors specifically in the hypothalamus [30]. However, the thermoregulating capacity of TRH in the hypothalamic nucleus is still unknown in hibernators such as the Syrian hamster.

Recently, two TRH-receptor subtypes, TRH-receptor type-1 (TRH-R1) [37,39] and TRH-receptor type-2 (TRH-R2), have been cloned [7,18,29]. In the CNS, these two receptor mRNAs display defined regional distribution, although both receptors are similarly expressed in certain sites [7,14,29]. These findings may imply the different physiological functions of TRH mediated by these two receptor subtypes.

In this study, we investigated whether centrally administered TRH would induce thermogenesis in the BAT of Syrian hamsters. Furthermore, we attempted to locate the site of TRH action in the brain and the NST-promoting receptor subtype of TRH.

2. Materials and methods

2.1. Animals

Adult female Syrian hamsters (Shimizu, Kyoto, Japan) weighing 90–120 g were housed in cages of 5 or 6 animals each in a room maintained at 23 ± 2 °C and illuminated with an alternating 12-h light/dark cycle. The animals were given food and water ad libitum. All experiments were performed according to the guidelines for animal experiments of Fukuyama University. A total of 124 female hamsters were used in this study, as this gender has been extensively employed and preferred in present investigations. Unless otherwise stated, the number of animals used in each experiment with/without the respective compound injections was 6.

2.2. Surgical procedures

Hamsters anesthetized with sodium pentobarbital (40 mg/kg, i.p.; Abbott, IL, USA) were each placed in a stereotaxic instrument (Narishige, Tokyo, Japan). According to the hamster brain atlas of Lawrence and Ruth (Academic Press, CA, USA; 1st edn, 2001), a 21-gauge stainless guide-cannula was inserted and fixed 1 mm above the respective sites with relevant stereotaxic placements (mm) in the order of anterior–posterior (AP), mediolateral (ML) and dorso-ventral (DV) placements (mm): LV (AP: 0.8, ML: 1.6, DV: 3.5), PO (2.2, 0.4, 6.0), AH (1.3, 0.4, 7.0), VMH (−0.5, 0.4, 8.0), DMH (−0.7, 0.4, 7.5), LH (−0.7, 1.5, 7.5) or PH (−1.0, 0.4, 7.0). The guide-cannula was fixed to the skull with dental cement (Nissin, Kyoto, Japan). The skin incision was sutured and disinfected with povidone iodine (Meiji, Tokyo, Japan). A stylet was inserted into the guide-cannula to keep it patent prior to injections. After surgery, the Syrian hamsters were individually housed and allowed to recover from surgery for 1 week.

2.3. Thermometric procedures

On the day of experiment, Syrian hamsters were anesthetized with isoflurane and placed on a thermal pad to maintain the respective temperature ranges of T_{IBAT} (35.3 ± 0.2 °C) and T_{rec} (36.0 ± 0.1 °C). The T_{IBAT} and T_{rec} were monitored continuously using microthermocouple probes (IT-21, Phisitemp, NJ, USA) connected to Maclab (AD Instruments, Nagoya, Japan), as described by Minokoshi et al. [23].

2.4. Drug administration into the brain

Intracerebroventricular (icv) injection of TRH (Peptide Research Center, Osaka, Japan) was executed through a steel needle connected to teflon tubing filled with pyrogen-free artificial cerebrospinal fluid (aCSF). The open-end of teflon tubing was connected to a 100-μl syringe (Hamilton, NV, USA) designated to deliver an icv injection volume of 10 μl for each administration. TRH administrations into sites PO, AH, DMH, VMH, LH and PH by microinjections were respectively performed at 0.5 μl/site with a 10-μl syringe using an infusion pump (Harvard Apparatus, MA, USA).

2.5. Bilateral denervation of sympathetic nerve in IBAT

IBAT-innervating posterior branches of intercostal nerve bundles that contained sympathetic nerves were isolated and sectioned bilaterally under isoflurane anesthesia, according to the method of Minokoshi et al. [23]. These procedures were performed before inserting a microthermocouple probe in the IBAT for monitoring T_{IBAT}.

2.6. NE turnover experiment

Nine hamsters were designated for each group in this series of experiments. NE turnover rate in IBAT was performed by measuring the NE contents in IBAT after blockade of NE synthesis with DL-α-methyl-*p*-tyrosine methyl ester (α-MPT, 300 mg/kg, i.p.; Sigma, MI, USA), an inhibitory agent of tyrosine hydroxylase. α-MPT or saline (i.p.) and TRH (icv) or aCSF (icv) were respectively injected 5 h and 30 min into the brain of animals before sacrificing the animals for tissue samples.

2.7. Sample preparation and analysis of NE contents

The NE contents in IBAT were measured by high-performance liquid chromatography (CCP&8020, TOSOH,