Central Ventilatory Effects of Thyrotropin-Releasing Hormone in the Conscious Rat

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Abstract—Thyrotropin-releasing hormone was shown to exert potent ventilatory effects after central administration. These data, however, were derived from studies using anesthetized animal preparations. Since TRH elicits strong arousal reactions, the observed ventilatory effects of TRH under anesthesia may have been due to nonspecific reduction in the anesthetic state of the animals. In order to clarify the extent to which the reversal of anesthesia may change ventilatory parameters after TRH application, we investigated the effect of TRH on ventilation rate, relative tidal volume, relative respiratory minute volume, CO₂ production and O₂ consumption, and locomotor activity in the conscious, unrestrained rat. Intracerebroventricular application of TRH induced a dose-dependent, sustained increase in ventilation rate, relative tidal volume, and relative respiratory minute volume of maximally 128%, 890%, and 235%, respectively. In addition, CO₂ production and O₂ consumption were elevated by 4.6 and 11.7 fold, while no significant changes in locomotor activity were observed. The results suggest that TRH stimulates ventilation by a mechanism independent of its analeptic properties.

Introduction

The tripeptide Glp-His-Pro-NH₂, thyrotropin-releasing hormone (TRH), was the first hypothalamic, hypophysiotropic releasing factor to be characterized, marking the initial development of the field of neuroendocrinology (1). More recent evidence, however, revealed that immunoreactive TRH is not only confined to hypothalamic nuclei involved in the regulation of hypophyseal thyrotropin release, but is widely distributed throughout the central nervous system (CNS), including telencephalic and medullary structures (2, 3). Moreover, high affinity TRH receptors have been localized in various CNS sites (4) suggesting that TRH may participate in the mediation or modulation of a variety of CNS functions under physiological conditions. This hypothesis is further validated by the profound effects of TRH on behavioral and autonomic functions after central and peripheral administration in experimental
animals (5, 6, 7). In addition to its potent cardiovascular effects, including increases in arterial pressure and heart rate (5), TRH has been shown to increase ventilation in the anesthetized rat after intracerebroventricular (icv) application (6) or after microinjection into various brainstem structures (8). Moreover TRH was shown to induce rhythmic depolarisations of neurons in the respiratory division of the nucleus tractus solitarii (9). TRH may therefore play a significant role in the central regulation of ventilation. In most of these studies, however, the animals were anesthetized with urethane (10, 11), halothane (6), or urethane-chloralose (12). Since the central application of TRH potently reverses the effects of various centrally acting drugs, including several anesthetics such as barbiturates and ethanol (13, 14), the arousal reaction produced by TRH application (15) may reduce the anesthetic state of the animal leading to an increased respiratory reaction to surgical stress or fixation in a stereotaxic apparatus. In order to determine whether the effects of TRH on respiratory parameters observed in anesthetized rats were due to a specific stimulation of central ventilatory control mechanisms or rather to a general arousal reaction leading to a reversal of the anesthetic state, we investigated ventilatory responses and LA after icv injections of TRH in the conscious unrestrained rat.

Materials and Methods

Male Sprague Dawley rats (375-395 g) were obtained from Taconic Farms (Germantown, NY) and housed at controlled temperature (22°C) and 12h light cycle with free access to food and water. Stainless steel guide cannulas were implanted prior to the experiment (coordinates: −0.8 mm ap, −1.2 mm lat from bregma) under halothane anesthesia (2% in oxygen). The animals were allowed to recover for at least 12h. All experiments were conducted between 8 a.m. and 6 p.m.

Ventilatory parameters were quantified by means of the OXYMAX '85 system (Columbus Instr., Ohio) for measurements of ventilation rate (f), relative tidal volume (rVT), O2 consumption, and CO2 production. The animals were placed in transparent plexiglass chambers of 6.551 volume with a constant flow of 21 of room air per min. Measurements were performed every 2 min.

Ventilation rate (f) and rVT were determined based on the frequency (respirations/min) and amplitude (arbitrary units) of pressure changes due to the ventilatory movements of the animal's thorax. The relative ventilatory minute volume (rVE) was then calculated as the arithmetical product of f and rVT. The O2 and CO2 contents of the animal chambers were measured against a reference chamber with an equal room air flow of 2 l/min. Arithmetical differences between the reference and animal chambers were expressed as O2 consumption and CO2 production respectively.

One hour after the start of the experiment the animals (n = 5) received a control injection of artificial cerebrospinal fluid (CSF) and consecutive injections of 0.25, 1.25, 2.5, 25 and 50nmol of TRH (Bachem) in 10μl of artificial CSF icv. Injections were performed manually over 10–15s, using a 30 gauge needle (7.5 mm length) connected to a 25μl Hamilton syringe by PE-50 tubing. In parallel with the measurements of ventilatory mechanics and gases, the locomotor activity (LA) of the animals was monitored, using the Oxy-Varimex system (Columbus Instr., Ohio). The estimation of locomotor activity is based on the interruption of infrared light beams caused by movements of the animal. The observation period after each injection lasted 1h. After the experiment, the animals were sacrificed and the location of the icv injection was verified by dye application. Baseline conditions were determined after the injection of artificial CSF as means over the last 10–20 min of the respective observation period. Changes (Δ) of f, rVT, rVE, O2 consumption, and CO2 production were calculated using a spreadsheet program (Framework, Microsoft). Dose related effects were assessed by the calculation of approximate curve areas of the parameter changes over the 1h observation periods, using a trapezoidal method.

Statistical evaluation was done according to Kruskal-Wallis followed by Mann-Whitney-U test (16). Unless otherwise stated, the results are expressed as means ± standard error of the mean (S.E.M.) for the indicated number of animals.

Results

TRH icv caused a dose dependent sustained increase of f (curve areas) to a maximum of 128%
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Fig. 1 Effects of TRH on relative ventilatory minute volume (rVE, [respirations (R)], ventilatory rate (f, [R]), and relative tidal volume (rVT, [%]) after consecutive icv administration of 0.25, 1.25, 2.5, 25 and 50nmol in 10 μl artificial CSF. Data are expressed as approximate curve areas of parameter changes over 1 h after each injection. * (p < 0.05) and ** (p < 0.01) denote statistical significance, when compared to vehicle (artificial CSF) injection (Kruskal-Wallis, n = 5).

compared to vehicle control after the highest dose of 50nmol icv (p < 0.01, n = 5; Fig. 1). RV_T was maximally stimulated (8.9 fold) after the lowest dose of TRH (0.25 nmol; p < 0.01). Increasing doses, however, reduced RV_T to levels comparable to those seen after artificial CSF icv (Fig. 1). Nevertheless, rVE was increased to a maximum of 235% compared to vehicle alone after 1.25nmol TRH icv (p < 0.01), but seemed to decrease somewhat after injection of higher doses (Fig. 1).

The maximum effects of TRH on CO2 production and O2 consumption were achieved after the 1.25 nmol dose with sustained increases of 4.6 and 11.7 respectively compared to control injections (p < 0.01; Fig. 2). Larger doses of TRH, however, decreased CO2 production to a higher degree than O2 consumption.

Locomotor activity (LA) after artificial CSF icv was 520 ± 172 counts/h, and increased to 717 ± 274 (0.25 nmol), 1620 ± 229 (1.25 nmol), 1420 ± 448 (2.5 nmol), 1343 ± 248 (25 nmol) and 1101 ± 391 counts/h (50 nmol) after icv injections of TRH. Although there appeared to be a dose-related increase of LA, especially after the injection of 1.25 nmol TRH icv, no statistical significance was found when the TRH treatment was compared to vehicle injections using the Kruskal-Wallis test. Application of the Mann-Whitney-U test alone, however, yielded p values of 0.027 (1.25 nmol) and 0.044 (25 nmol) when the treatment groups were compared to control injections. Since there exists an increased possibility of finding significant results when several tests are performed simultaneously, the adjustment of the statistical α-level according to the Bonferroni procedure (17) or others is strongly advised (18). Since the calculated p values of both dosage groups do not reach or fall below the adjust α-value (α* = 0.01), both measurements have to be considered nonsignificant at α = 0.05. In order to resolve this controversy, another set of animals was prepared as described above. Instead of consecutive, increasing doses of TRH, only one dose (1.25 nmol, n = 6) was injected icv 1 h after the onset of the experiment. The resulting changes in ventilator and locomotor activities were compared to single icv injections of 10 μl artificial CSF (n = 5), which were performed in a similar manner. The t-test for independent samples was used for statistical evaluation. Relative respiratory minute volume (rVE) was increased by 157% (p < 0.05) after 1.25 nmol TRH icv, while f (curve areas: 2510 ± 183 vs. 1547 ± 460 respirations/h after CSF icv), RV_T, and LA activity were not statistically different from CSF injections (Fig. 3).

Discussion

The present study demonstrates the effects of TRH on respiratory mechanics, gas exchange and
The stimulatory effects of centrally administered 
TRH on cardiorespiratory functions have been 
reported in various species (6, 10, 11, 12). In the 
anesthetized rat, TRH was shown to increase f 
after icv injection of pico- to nanomolar doses (6, 10). This stimulatory effect on f was combined with 
a decrease of V_T leading to an either unchanged 
(10) or increased (6) V_E. The underlying mode of 
action may involve an activation of inspiratory 
off-switch mechanisms in the brainstem, possibly 
due to an increased sensitivity to vagal afferent 
inputs (20).

The results of the present study suggest that 
TRH stimulates ventilation unrelated to the state 
of consciousness of the animal. Moreover, a 
striking difference to previous studies using 
anesthetized animal preparations (6, 10) is the 
dose-related stimulation of V_T, which appears to 
be primarily responsible for the elevation of V_E 
over the low dose range. Interestingly, injection of a single dose (1.25 nmol) of TRH per rat produced 
a somewhat reduced effect on all measured para-

ters compared to the effects of the same dose 
distributed after previous injections of CSF and 
0.25 nmol TRH. The mechanism underlying this 
phenomenon is unclear. Repeated icv injections of 
saline at hourly intervals appear to exert signifi-
cantly decreasing responses of f, while no changes 
of V_T or V_E could be detected (unpublished 
observation). This may reflect a behavioral habi-
tuation to stress imposed by the handling of the 
animals during the injection itself. Since TRH was 
shown to enhance the reaction to stress paradigms, 
such as acoustic startle (19), previous administra-
tion of TRH may enhance the ventilatory 
responses to subsequent injections up to a degree 
where tachyphylaxis, presumably due to TRH 
receptor down-regulation exerts a counteracting 
effect.

The discrete mechanisms involved in the effects 
of TRH on respiratory mechanics and gases are 
still unknown. They likely involve specific TRH 
receptors on brainstem nuclei participating in the 
central control of respiration, such as the dorsal 
motor nucleus of vagus and the nucleus tractus 
 solitarii (4). This hypothesis is corroborated by 

studies showing that depletion of brain TRH 
content using the serotonergic neurotoxin 5, 
7-dihydroxytryptamine, results in an upregulation 
of TRH receptors in brainstem and pons (21, 22) 
and an enhanced sensitivity to the respiratory 
stimulation by TRH (23).
The present results further indicate that TRH icv potently stimulates CO₂ production and O₂ consumption. The increase of CO₂ production may be accompanied by a decrease of PₐCO₂ leading to an increase of arterial pH as reported by Hedner et al. (6). However, no significant changes of PₐO₂ could be observed in this study. Additionally, TRH was shown to increase peripheral metabolism by direct and indirect means. Griffiths et al. (24) reported an activation of the metabolic rate of brown adipose tissue of rats which was presumably mediated by a stimulation of sympathetic nerve activity (5). Moreover, the stimulation of LA by TRH as previously shown by Andrews and Sahgal (7) may constitute an indirect mode of metabolic activation due to an increased peripheral energy consumption. It, therefore, appears likely that the increases of CO₂ production and O₂ consumption observed in the present study reflect a ventilatory compensation of enhanced peripheral metabolic demands.

Although a reflex activation of central respiratory control mechanisms through a locomotion-dependent increase of PₐCO₂ leading to a stimulation of Vₑ may be possible, the mediation of the ventilatory effects of TRH by central TRH receptors appears to be the pivotal mechanism of action as shown by the correlation of ventilatory responses of TRH with TRH receptor densities in CNS structures (23). Moreover, in anesthetized animals, TRH icv produces a stimulation of ventilation accompanied by a decrease of PₐCO₂, emphasizing the central mode of action (6). It is yet unclear, however, to what extent peripheral metabolic demands influence the respiratory patterns elicited by TRH. The present study revealed a dissociation of ventilatory and LA effects of TRH icv (Fig. 3). Thus, a single dose of TRH injected icv, clearly stimulated respiration as seen by an increase of rVₑ, while virtually no change of LA occurred. Moreover, the lowest dose of TRH used in this study (0.25 nmol) was able to maximally stimulate Vₑ, CO₂ production, and O₂ consumption (Figs 1 & 2), while mean locomotor activity was increased by only 38% compared to icv injections of artificial CSF. Therefore, it appears unlikely that the ventilatory effects of TRH may primarily constitute a reflexory activation due to changes in metabolic homeostasis following an increased LA. Higher doses of TRH (10 to 100 µg administered in single injections in 2 µl volumes), however, were shown to significantly increase LA, possibly dependent on a diurnal susceptibility rhythm (7). Therefore with administration of higher TRH doses, LA may become a significant factor in the stimulation of respiration via an increased muscular metabolism.

In summary, the results of the present study indicate that TRH after icv administration in conscious rats stimulates ventilation and peripheral metabolism as evidenced by an increase of CO₂ production and O₂ consumption. The stimulatory effect of TRH appears to result from an activation of mechanisms involved in the central control of ventilation. Furthermore, the anesthesia-reversing properties of TRH may be of negligible importance in eliciting the ventilatory effects of TRH in studies using anesthetized animals.

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