RTN TRH causes prolonged respiratory stimulation

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Cream, Carlos L., Aihua Li, and Eugene E. Nattie. RTN TRH causes prolonged respiratory stimulation. J. Appl. Physiol. 83(3): 792–799, 1997.—We injected thyrotropin-releasing hormone (TRH; 10 nl; 0.25, 0.5, 1.0, or 10 mM), its inactive free acid form (TRH-OH; 1 mM), or a metabolite with low TRH-receptor binding affinity, histidine-proline diketopiperazine (cHP; 1 mM), into the retrotrapezoid nucleus of anesthetized rats. Injection location was verified by anatomic analysis. Lower doses (0.25–0.5 mM) significantly increased both the product of integrated phrenic amplitude and frequency (\(\text{Phr} \times \text{f}\)) and \(f\) for 20–30 min compared with artificial cerebrospinal fluid control injections. Higher doses (1.0–10 mM) produced greater and long-lasting stimulation of \(\text{Phr} \times \text{f}\), \(\text{Phr}\), and \(f\) and of blood pressure. This stimulation reached values 150% of baseline and durations of 270 min after a single injection. TRHOH (1 mM) or cHP (1 mM) had no effect on \(\text{Phr}\) but increased \(f\), as did 1 mM TRH. We conclude that TRH has a very powerful stimulatory effect in the retrotrapezoid nucleus region on \(\text{Phr} \times \text{f}\), with the \(\text{Phr}\) response seemingly specific for TRH receptors. Similar responses of \(f\) to TRHOH and cHP suggest it may be nonspecific.

Control of breathing; ventrolateral medulla; thyrotropin-releasing hormone free acid; histidine-proline diketopiperazine; blood pressure; respiratory regulation; retrotrapezoid nucleus

The rostral ventrolateral medulla (RVLM) contains neurons that are important in the control of breathing. Chemoreception occurs there at topographically defined areas within and rostral to, as well as caudal to, the RVLM (5). Cooling of the ventral medullary surface at a topographical location lying between these chemosensitive areas (the intermediate area) causes a significant depression of \(\text{CO}_2\) sensitivity and ventilation, often to apnea, in anesthetized animals (1, 9, 20), with similar but less marked effects in unanesthetized preparations (1, 9).

An important component of the RVLM is the retrotrapezoid nucleus (RTN), a set of superficial neurons near the surface of the RVLM that lies ventral and ventromedial to the facial nucleus at the topographical border of the rostral chemosensitive zone and the intermediate area of the ventral medullary surface. The RTN connects with both the dorsal and ventral respiratory groups (26) and contains neurons that fire in synchrony with the respiratory rhythm (22). Chemical lesions of the RTN in anesthetized animals result in decreased phrenic nerve activity (PNA), often resulting in apnea, and loss of responsiveness to systemic \(\text{CO}_2\) (20). In unanesthetized rats, unilateral lesions affecting ~35% of the RTN neurons reduce \(\text{CO}_2\) sensitivity by 39% but have no effect on baseline ventilation (1). Microinjection of ionotropic glutamate-receptor antagonists into the RTN of anesthetized cats results in decreased PNA and loss of responsiveness to \(\text{CO}_2\) (19); injection of glutamate results in an increase in PNA (16). Injection of metabotropic glutamate-receptor antagonists have little effect on baseline PNA, but injection of metabotropic agonists results in a prolonged increase in PNA sustained for >45 min (16). Muscarinic antagonists also diminish PNA and the response to increased systemic \(\text{CO}_2\) (21).

In this study we ask whether injection of thyrotropin-releasing hormone (TRH; \(L\)-pyroglutamyl-\(L\)-histidyl-\(L\)-prolinamide) into the RTN region of anesthetized rats has any effect on breathing as measured by the product of integrated phrenic amplitude and frequency (\(\text{Phr} \times \text{f}\)). The rationale for this study is as follows. 1) Injection of substance P into the RTN region stimulates respiratory output (3). 2) Substance P-like immunoreactivity colocalizes with TRH-like immunoreactivity in neurons near the RTN region (14, 25). 3) Neurons in the midline raphe and parapyramidal areas nearby to the RTN region contain TRH-like immunoreactivity (25) and have known projections into the RTN region (30). 4) Although no specific observations demonstrate TRH receptors in the RTN region, existing data for rat brain stem show TRH receptors in this general vicinity (17). 5) Administration of TRH via the cerebral ventricles (12, 13, 28) or by microinjection at midline ventral medullary sites caudal to the RTN region (18) stimulates respiratory output. Microinjection of TRH into the more dorsally located nucleus tractus solitarii (NTS) also results in an increase in ventilatory output (12, 18).

We hypothesized that unilateral RTN injections of TRH in artificial cerebrospinal fluid (aCSF) would stimulate our measurement of ventilatory output, i.e., \(\text{Phr} \times \text{f}\). Doses of 0.25 and 0.5 mM TRH increased \(\text{Phr}\) and \(\text{f}\); 1.0 and 10 mM doses increased \(\text{f}\), \(\text{Phr}\), and \(\text{Phr} \times \text{f}\). Control injections of equal volumes of aCSF had no effect, whereas injections of the inactive TRH free acid form (TRHOH; 1 mM in aCSF) and injection of the TRH metabolite (cHP; histidine-proline diketopiperazine), which has essentially no binding to the TRH receptor in vitro (11, 17), increased \(\text{f}\).

METHODS

General preparation. The detailed methods have been described previously (16, 20). Briefly, 28 male Sprague-Dawley rats (weight 275–350 g, Charles River) were anesthetized with 2% halothane in \(\text{O}_2\) while the trachea, femoral artery, and vein were cannulated. The rat was ventilated with 100% \(\text{O}_2\), anesthetized with intravenous chloralose (60 mg/kg) and urethan (550 mg/kg), and paralyzed with gallamine (3 mg/kg) given intravenously.

The VLM surface was surgically exposed, and one phrenic nerve was dissected, sectioned, and placed on a bipolar recording electrode. The nerve and electrode were covered by Sil-gel (Wacker). The signal was amplified, rectified, filtered, and integrated. Blood pressure, integrated PNA, and end-tidal
CO₂ were recorded on a chart recorder. \( \int \text{Phr} \cdot f \), total cycle duration, the duration of inspiration \( (T_I) \) and expiration, and mean arterial blood pressure were computed on line by using locally written software. We also computed the neural equivalent of minute ventilation, i.e., \( \int \text{Phr} \cdot f \).

After surgery, the animal was allowed to stabilize for 20–30 min. A CO₂ response curve was determined by increasing the inspired fraction of CO₂ and monitoring the \( \int \text{Phr} \) and \( f \) measured at end-tidal CO₂ values of 5, 7, and 9%. The \( \int \text{Phr} \) at 9% end-tidal CO₂ was defined as the maximum or 100% CO₂ was then lowered back to an end-tidal value of 4–5% for baseline measurements. All of the animals had baseline \( \int \text{Phr} \) values between 45 and 60% of maximum. Subsequently, for comparisons among animals, all \( \int \text{Phr} \cdot f \) and \( \int \text{Phr} \) values are expressed as a percentage of baseline.

Microinjections (10 nl) were made with a Pico-spritzer (General Valve II) over a 3-s period. All drugs were dissolved in aCSF and equilibrated with CO₂ to pH 7.4. The composition of the aCSF (in mM) was 152 sodium, 3.0 potassium, 2.1 magnesium, 2.2 calcium, 26 chloride, and 25 bicarbonate. The calcium was added after the aCSF was warmed to 37°C and equilibrated with 5% CO₂. For most injections, the movement of the meniscus in the tubing leading to the micropipette was monitored as an index of injection volume. To allow postmortem evaluation of the injection site and determination of the volume injected, fluorescein or rhodamine latex beads (Poly-science) were added to each injection. After the injection, phrenic and blood pressure measurements were taken at 10-min intervals until \( \int \text{Phr} \) returned to baseline.

At the end of the experiment, animals were killed by intravenous infusion of saturated KCl. The brain was rapidly removed and the medulla was dissected and frozen. It was then sectioned at 20 μm in a cryostat. Injection sites were identified by using a fluorescence microscope to localize the center of each injection, and serial sections were stained with cresyl violet to identify anatomic landmarks. The volume of each injection was estimated anatomically by a geometric approach, by which we first measured the total rostral-to-caudal length of the region of fluorescence and the largest cross-sectional area among the serial sections and then calculated volume by using the formula for adjoining cones (1). The center of each injection was also located in millimeters caudal to the bregma.

At each measurement time during the protocol, we assessed the mean value for all variables over a period of 10 respiratory cycles. In preliminary experiments, it was apparent that lower TRH doses produced short-lasting responses, whereas higher doses produced longer duration responses. Accordingly, we used two control groups. The first procedure consisted of four injections of aCSF into the RTN in four animals that were part of the protocols for low-dose TRH, TRH-OH, or chP injections, all groups in which data were collected at 10, 20, and 30 min after the injection. The second control group consisted of six animals that received an aCSF injection of 10 nl into the RTN with measurements made at 10, 20, 30, 60, 90, and 120 min. Responses to 1 and 10 mM TRH were compared with this separate control group. Many of these TRH responses actually lasted for much longer time periods, but the formal statistical analysis is constrained to this 2-h period. These controls were used in a prior study (20) and are included here because the experiments were performed in the same time period and under the same experimental conditions as these TRH injections.

For TRH injections, an increase in \( \int \text{Phr} \cdot f \) that was greater than that observed after any control injection was classified as a response. In practice, this was an increase in \( \int \text{Phr} \cdot f \) that 1) was greater than a 10% change in \( \int \text{Phr} \cdot f \) expressed as a percentage of the maximum and 2) was present at least over 20 min. For mean arterial blood pressure, a response was a >12-mmHg increase sustained over at least 20 min. For these TRH injections, we report only the responders as defined above.

Mean values of \( \int \text{Phr} \cdot f \), \( \int \text{Phr} \), and mean arterial blood pressure for each experimental group at each time period were compared by performing a two-way analysis of variance (Systat) to examine treatment and time with Tukey’s post hoc analysis as appropriate. The first comparison was among the short time period 30-min aCSF controls and the responders to 0.25 and 0.5 mM TRH; the second comparison was among the 120-min aCSF controls and the responders to 1 and 10 mM TRH. The final analysis compared all TRHOH and chP injections (each of 1 mM concentration) with the responders to 1 mM TRH.

RESULTS

There were 43 injections made into the RTN region in 28 rats as determined by postmortem anatomic analysis. These animals maintained a mean arterial blood pressure at 90 mmHg or greater for the duration of the experiments and demonstrated a good initial response to increased CO₂, i.e., a doubling or greater of \( \int \text{Phr} \cdot f \). This indicates a healthy ventral medulla in this type of preparation.

In the low-dose range, four TRH injections (2 with 0.25 mM; 2 with 0.5 mM) produced a significant increase in \( \int \text{Phr} \cdot f \) \( (P < 0.003) \) and in \( f \) \( (P < 0.02) \) compared with four aCSF control injections (Fig. 1). In this, and every other case in this study, the increase in \( f \) resulted from a shortening of \( T_I \). Responses to four TRH injections (1 with 0.25 mM; 3 with 0.5 mM) are indistinguishable from the control data shown in Fig. 1. Figure 2 shows the anatomic location of the center of each of the eight injections shown in Fig. 1. The center of the four control injections was 10.75 ± 0.16 (SE) mm caudal to bregma and that of the four TRH injections was 10.89 ± 0.25 mm; the control injection volume calculated from the fluorescent bead distribution was 9.1 ± 2.6 nl; that of the TRH injections was 21 ± 6.1 nl. Mean arterial blood pressure was unchanged. The locations of the 0.25 and 0.5 mM TRH injections that did not produce a response were also within the RTN region (data not shown).

RTN injection of 1 mM \( (n = 9) \) and 10 mM \( (n = 3) \) TRH produced responses in every case. Figure 3 shows an example of a 1 mM TRH injection. \( \int \text{Phr} \cdot f \) is increased within 10 min to a value of 71% of baseline, reaching a peak value of 150% of baseline at 60 min, then slowly declining to a value of 33% of baseline at 270 min. Both \( \int \text{Phr} \) and \( f \) show similar responses, as does mean arterial blood pressure. The injection site is in the RTN region, 11.06 mm caudal to bregma, and the calculated injection volume is 20 nl. Of the nine 1 mM TRH injections, \( \int \text{Phr} \cdot f \) returned to baseline or the experiment was stopped at 60 \( (n = 2) \), 120 \( (n = 3) \), 180 \( (n = 2) \), and 270 \( (n = 2) \) min. Of the three 10 mM TRH
injections, \( \text{f Phr}\cdot f \) returned to baseline, or the experiment was stopped at 120, 240, and 270 min.

With such long-duration effects, we focused the statistical analysis on the first 120 min by using a control group that received only 10 nl aCSF into the RTN and had measurements made over this time period. These data are shown in Fig. 4. \( \text{f Phr}\cdot f \) was increased significantly compared with control by the 1 mM (\( P < 0.001 \)) and 10 mM TRH injections (\( P < 0.001 \)), and these two TRH injection responses differed from each other (\( P < 0.001 \)).

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\( \text{f} \) was increased significantly by the 1 mM (\( P = 0.03 \)) and 10 mM TRH injections (\( P < 0.001 \)), and these two TRH injection responses differed from each other (\( P < 0.001 \)). Figure 5 shows the anatomic location of the center of each injection summarized in Fig. 4. The center of the six control injections was 11.22 ± 0.12 mm caudal to bregma, that of the nine 1 mM TRH injections, 10.96 ± 0.07 mm, and that of the three 10 mM TRH injections, 11.1 ± 0.29 mm; the control injection volume calculated from the fluorescent bead distribution was 17.4 ± 4.6 nl; that of the nine 1 mM TRH injections, 9.2 ± 1.6 nl; and that of the three 10 mM TRH injections, 23.7 ± 4 nl.

Mean arterial blood pressure was increased in four of nine 1 mM TRH injections and two of three 10 mM TRH injections. The change in mean arterial blood pressure for these six responses is shown on Fig. 6 along with the mean change in blood pressure for the six control animals that received RTN injections of aCSF. All six of these RTN TRH injections with increased blood pressure also had a response in terms of increased \( \text{f Phr}\cdot f \). Their injection locations are among those shown on Fig. 5 and did not differ from the locations of injections that had no associated phrenic or blood pressure response as far as we could tell from our anatomic analysis. The center of the injections that affected blood pressure was 10.93 ± 0.07 mm caudal to bregma; the injection volume calculated from the fluorescent bead distribution was 12.6 ± 2.9 nl.

Responses to five injections of TRHOH at 1 mM concentration are shown in Fig. 7 compared with the two TRH injection responses differed from each other (\( P < 0.001 \)).
responses of the nine 1 mM TRH injections. $\text{EPHr} \cdot f$ is significantly lower after TRHOH injection ($P < 0.03$) as is $\text{EPHr}$ ($P < 0.03$), but the response in terms of frequency is similar after each type of injection. The location of these TRHOH injections is shown (Fig. 7, bottom left). The center of the TRHOH injections was $11.0 \pm 0.23$ mm caudal to bregma; the injection volume calculated from the fluorescent bead distribution was $9.7 \pm 2.8$ nl. None of these injections increased mean arterial blood pressure.

Responses to six injections of the TRH metabolite chHP at 1 mM concentration are shown in Fig. 8 compared, as for TRHOH injections, with the 1 mM TRH responses. $\text{EPHr} \cdot f$ and $\text{EPHr}$ are significantly lower than after equimolar TRH injection ($P < 0.01$), and the change in $f$ is very similar. The chHP injection locations are shown in Fig. 8. The center of the injections was $10.5 \pm 0.13$ mm caudal to bregma; the injection volume calculated from the fluorescent bead distribution was $7.9 \pm 1.8$ nl.

DISCUSSION

Major findings. The major finding of our study is that TRH injection at a volume of 10 nl in the RTN increases $\text{EPHr} \cdot f$ in a dose-dependent manner. With doses in the 0.25 to 0.5 mM range, the effect is on $f$, lasts 20–30 min, and is not associated with changes in blood pressure. With doses in the 1 to 10 mM range the effect is large, up to a 150% increase in $\text{EPHr} \cdot f$, includes increases in $\text{EPHr}$ and $f$, is of long duration, up to 270 min or more and, in some cases, is associated with a similarly long-lasting increase in blood pressure.

Our effective TRH dose range is very similar to that described in experiments with intracerebroventricular injection of TRH in conscious rats (28). We have no evidence as to the actual concentration of TRH released naturally in vivo in the synaptic cleft. In general, it is an interesting feature that in microinjection studies performed in vivo, as was ours, the concentrations needed for physiological effect are larger than those needed for effects in vitro or for binding to isolated receptors. For TRH, in vitro slice experiments use concentrations in the micromolar range (2, 6, 8, 23, 24), and membrane-binding studies use concentrations in the nanomolar range (11), whereas we used concentrations in the millimolar range. It is often assumed that the low concentrations effective in vitro represent the normal physiology but, for glutamate, estimates of the concentration in the synaptic cleft in vivo reach the millimolar range (4). For TRH, the absence of an effective antagonist makes it difficult to conclude which in vivo TRH concentrations reflect an event that could mirror normal physiology.

Controls. Control injections of aCSF had very little effect on $\text{EPHr} \cdot f$, $\text{EPHr}$, or $f$ over either 30 or 120 min. Separate controls used TRHOH, the free acid, with a 1,000-fold lower affinity for TRH receptors in vitro (11, 17), and a TRH metabolite, chHP. At the dose level of 1 mM, part of the increased $f$ response to TRH could be attributable to a nonspecific effect because the free acid produced a similar effect on $f$. chHP, with virtually no affinity for TRH receptors (11), also increased $f$, similarly suggesting an effect that is not specific for TRH receptors. The large $f$ response to RTN TRH injections may represent a mechanism different from that of the $\text{EPHr}$ response and unrelated to specific stimulation of TRH receptors.

Technical issues. TRH can counteract the sedative effects of anesthetic agents (15); it has a net excitatory
effect on the central nervous system, perhaps via the reticular formation. We monitored the level of anesthesia by using blood pressure, respiratory rate, and responses of these variables to a pinch of the footpad or tail. In these TRH experiments we monitored pain-induced responses more frequently and gave additional doses of anesthesia more liberally than we normally do. This possible interaction of TRH with anesthesia depth is a difficult issue given the long-lasting nature of the TRH effects after the 1 and 10 mM doses. We believe that the meticulous attention paid to the depth of anesthesia makes it unlikely that the large and long-duration TRH effects can be attributable to changes in the depth of anesthesia. Experiments in conscious rats with the intracerebroventricular injection of TRH at 0.5 to 5 mM concentrations support this interpretation. In these experiments, ventilation, tidal volume, and f were increased by very large amounts for the 60 min during which they were recorded, with no evidence of a waning response at the end of the 60 min (28).

In monitoring the 10-nl injection volume, in addition to measuring, in some experiments, the movement of the meniscus at the air-liquid interface within the tubing to the micropipette, we evaluated the size of the injections by postmortem measurement of the distribution volume of the injected fluorescent beads. For all 43 injections, the volume estimated by this analysis was 12.8 ± 1.3 nl. This volume did vary among the experimental groups, but the variability was small, with the ChP group having the smallest mean injection volume, 7.9 ± 1.8 nl, and the 10 mM TRH group having the largest mean injection volume, 23.7 ± 4 nl. The actual volume of distribution of the TRH is most likely slightly larger than the injected volume because it will diffuse to a slightly larger region, the degree of this diffusion being determined, in part, by the diffusion coefficient of the injected substance. Of interest here are measure-

Fig. 4. Values for control aCSF RTN injections (●; n = 6), 1 mM TRH injections that produced responses (●; n = 9), and 10 mM RTN TRH injections that produced responses (●; n = 3) are shown for ePhr (A), ePhr (B), and f (C) as a function of time. Values are means ± SE and are expressed as %baseline. Note interrupted time scale. For ePhr, responses of both 1 and 10 mM doses are significantly different from control (P < 0.001; 2-way ANOVA), and responses of these 2 doses differ from each other (P < 0.001; 2-way ANOVA; Tukey’s post hoc test). For f, responses of both 1 and 10 mM doses are significantly different from control (P < 0.001; 2-way ANOVA), and responses of these 2 doses differ from each other (P < 0.001; 2-way ANOVA; Tukey’s post hoc test).

Fig. 5. Anatomic location of control aCSF (top; n = 6), responsive 10 mM TRH (middle; n = 3), and responsive 1 mM TRH (bottom; n = 9) injections shown in Fig. 4. Within rectangles in each section, actual pattern of fluorescent beads that marked center of each injection is shown to scale. Sections chosen are from 1 animal in each group, and each section represents a computer-modified digitized image of actual anatomical section, with fluorescence expressed in black and white (Image-Pro). Bar, 1 mm.
ments by autoradiography of the volume of distribution of radiolabeled TRH injected at 50-, 100-, or 200-nl amounts into the hypothalamic region (29). The 50-nl injected volume had a 77% recovery within the localized injection site; the larger injection volumes showed much greater spread into surrounding tissue. Our much smaller injections most likely have an even more circumscribed effective region. The center of the 43 injections reported in this study were well within the RTN region.

The RTN. The RTN region was defined initially by retrograde tracing studies after injection into the ventral or dorsal respiratory group (26). In the rat (22) this region lies, at its most caudal point, at the level of the rostral aspect of the retrofacial nucleus ventrolateral to the nucleus paragigantocellularis lateralis 100–300 µm dorsal to the ventral medullary surface. It continues in the rostral direction, lying ventral and ventromedial to the facial nucleus. Identified by such retrograde tracing studies, this nucleus may contain neurons that are part of other identified structures like the parapyramidal neurons of the raphe and the juxtafacial portion of nucleus paragigantocellularis lateralis. Although the details of the anatomy of the RTN, including its afferents, remain to be fully described, it is clear from physiologically based experiments that the RTN contains 1) neurons that fire phasically with respiratory output (22), 2) chemoreception (5), 3) ionotropic and metabotropic glutamate receptors (16, 19), 4) cholinergic muscarinic receptors (22), and 5) TRH receptors (the present study). In anesthetized animals, lesions (20) or block of glutamate (19) or muscarinic cholinergic (22) receptors decreases phrenic activity and inhibits the response of the whole animal to CO₂. In awake animals, lesions that affect ~35% of the RTN do not result in apnea but do decrease the CO₂ response by 39% (1). In unanesthetized goats, surface medullary cooling at the RTN region diminishes respiratory output at rest, with exercise, and in response to hypercapnia and hypoxia (9). This small and recently identified region appears to have important influence on respiration and on central chemosensitivity. Peptides also appear to play a role in the function of the RTN. The location of substance P injections that stimulate ventilation appears to include the RTN region (3). The present study shows, with specific and focal RTN injection of TRH, a strong stimulation of breathing and, in some cases, of blood pressure.

Fig. 6. Change (Δ) in MAP for 6 control aCSF injections into RTN (○) and for 6 injections of TRH at 1 mM (n = 4) and 10 mM (n = 2) concentrations into RTN as a function of time. Values are means ± SE.

Fig. 7. Values for ∫Pfr·f(A), ∫Pfr(B), and f(C) for initial 30 min of response to 1 mM TRH injected into RTN (●) and for 5 injections into RTN of 1 mM TRH OH (○), TRH free acid with very little affinity for TRH receptor (see text). Values are means ± SE. ∫Pfr·f and ∫Pfr responses differ significantly between TRH and TRH OH (P < 0.02; 2-way ANOVA), whereas f responses do not differ. Bottom left, center of 5 TRH OH injections as described for Figs. 2 and 5.
Natural source of TRH for the RTN. Several medullary nuclei in the proximity of the RTN contain TRH-like immunoreactivity, including the motor nucleus of VII, raphe magnus, gigantocellular reticular cells, and parapyramidal neurons (2, 7, 14, 25). These would represent nearby sources of TRH input to the RTN because local connections appear to be present at this region (30). NTS and dorsal raphe (2) also contain TRH-like immunoreactivity and have likely connections to the RTN region.

TRH receptors in the RTN. There is no clear demonstration of TRH-receptor binding specific for the RTN. TRH receptor studies of the medulla show their presence near to, if not within, the RTN region, but the level of detail in the provided pictures prevents a clear conclusion (17). In a beautiful study, Sun et al. (27) showed the presence of synaptic boutons containing TRH-like immunoreactivity in ventral medullary neurons identified physiologically by their firing pattern to be respiratory and characterized anatomically by intracellular biotin injection. These neurons are not within the RTN per se, but their processes extend into and through the RTN region. It is possible, then, that some of our RTN TRH injection effects were on these ventral respiratory group neurons via the TRH synapses on their extensive processes.

Mechanisms of TRH effects on neurons. The response to TRH was sustained for prolonged periods, up to 4.5 h in some cases. Earlier studies in our laboratory have also demonstrated a sustained PNA response to 1 mM TRH injected into RTN (16) and for 6 injections into RTN of 1 mM histidine-proline diketopiperazine (cHP; ○), a TRH metabolite with very little affinity for TRH receptor (see text). Values are means ± SE. ±Phr-f and ±Phr responses differ significantly between TRH and cHP (P < 0.01; 2-way ANOVA), whereas f responses do not differ. Bottom left, center of 6 cHP injections as described for Figs. 2 and 5.

In vitro, TRH alters the excitability of several neuronal populations, including rat dorsal motor nucleus of the vagus, NTS, and hypoglossal motoneurons (2, 8, 10, 23, 24). TRH increases the excitability of neurons by inactivating K\(^+\) channels that are normally active at the resting membrane potential (2). The heightened excitability of the neurons was maintained for 5–40 min after TRH removal (2). Our results support the hypothesis that TRH produces sustained changes in neuronal excitability, but these in vitro studies do not explain the very long duration of our higher TRH dose effects in vivo.

TRH effects on respiration at other sites. Injections of TRH at more caudal medullary locations, including the midline raphe and the NTS (18) and the pre-Bötzinger region in the neonatal rat brain stem preparation (10), also result in strong stimulation of respiratory output. Studies during development emphasize the relative amounts of TRH mRNA, TRH-receptor binding, and neuronal excitability in newborn rats with expression of adult values for these variables occurring by 3 wk of neonatal life (2, 8). These observations plus the effects on frequency of TRH injections into the neonatal rat brain stem pre-Bötzinger region (10) raise the possibility that TRH plays a role in rhythm generation, perhaps as a tonic excitatory influence. Reckling et al. (24) described a direct postsynaptic action of TRH depolarizing a subset of inspiratory neurons in the ventral
respiratory group region near the pre-Bötzinger region, data that support this possibility.

Physiological significance. The overall physiological significance of these results will not be clear until we can identify a TRH-receptor antagonist or determine the naturally occurring TRH concentrations. The presence of strong TRH-induced effects on blood pressure and on breathing raises the possibility that it plays a role in a generalized response pattern involving coordination of a number of physiological responses. For example, this could be viewed as a way to control or initiate the “flight or fight” response.

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