Respiratory responses to thyrotropin-releasing hormone microinjected into the rabbit medulla oblongata

Donatella Mutolo, Fulvia Bongianni, Marco Carfi, and Tito Pantaleo
Dipartimento di Scienze Fisiologiche, Università degli Studi di Firenze, I-50134 Firenze, Italy

Respiratory responses to thyrotropin-releasing hormone microinjected into the rabbit medulla oblongata. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1331–R1338, 1999.—We investigated the respiratory role of thyrotropin-releasing hormone (TRH) input to medullary structures involved in the control of breathing in anesthetized, vagotomized, paralyzed, and artificially ventilated rabbits. Microinjections (10–20 nl) of 1 or 10 mM TRH were performed in different regions of the ventral respiratory group (VRG), namely the rostral expiratory portion or Bötzinger complex (Böt. c.), the inspiratory portion, the transition zone between these two neuronal pools, and the caudal expiratory component. TRH microinjections were also performed in the dorsal respiratory group (DRG) and the area postrema (AP). Injection sites were localized by using stereotaxic coordinates and extracellular recordings of neuronal activity; their locations were confirmed by subsequent histological control. TRH microinjections in the Böt. c. and the directly caudally located region where a mix of inspiratory and expiratory neurons were encountered elicited depressant respiratory responses. TRH microinjections were completely ineffective at sites within the inspiratory and the caudal expiratory components of the VRG. TRH microinjections in either the DRG or the AP induced excitatory effects on inspiratory activity. The results show for the first time that TRH may exert inhibitory influences on respiration at medullary levels by acting on rostral expiratory neurons and that not only the DRG, as previously suggested, but also the AP may mediate TRH-induced excitatory effects on respiration.

Bötzinger complex; medullary respiratory groups; area postrema; control of breathing

Medullary respiratory neurons are organized in two main neuronal aggregates, the dorsal respiratory group (DRG), closely associated with the nuclear complex of the solitary tract (NTS), and the ventral respiratory group (VRG), located in the ventrolateral medulla (1, 38). Expiratory neurons are concentrated at the rostral pole of the VRG, in the so-called Bötzinger complex (Böt. c.; see Refs. 1, 4, 5, and 38), and in the caudal part of the VRG (cVRG). Inspiratory neurons are mainly localized in the DRG and in the intermediate portion of the VRG. The transition zone between the Böt. c. and the inspiratory component of the VRG has been defined as the pre-Bötzinger complex (pre-Böt. c.). This subregion of the VRG, which has been suggested to play a crucial role in respiratory rhythm generation both in the neonatal rat and in the adult cat (1, 7, 29, 31, 33), has not yet been investigated and electrophysiologically characterized in the rabbit. In addition, we have recently shown in the rabbit that the area postrema (AP), which lies just adjacent to the NTS, is involved in the control of breathing by exerting excitatory influences on inspiratory activity (35).

Thyrotropin-releasing hormone (TRH) has long been recognized to be involved in the central control of respiration (1). Administration of TRH intraventricularly or its application to the medullary surface stimulates breathing in vivo (14, 15, 26, 39). Systemic injections of TRH have excitatory effects on respiration in humans (25). Studies performed on the rat in an attempt to localize medullary responsive sites have shown that TRH injected using a Hamilton microsyringe in the DRG causes increases in respiratory frequency, whereas similar injections are completely ineffective when performed in the VRG (14, 21); moreover, microinjections of TRH in the retrotrapezoid nucleus proved to induce slowly developing and long-lasting excitatory effects on inspiratory activity (8). In vitro, bath application of TRH increases respiratory frequency in isolated brain stem-spinal cord preparations or medullary slices from newborn rats and mice (13, 23, 28). TRH induces neuronal excitation when applied to brain stem slices (19, 27, 28) and can provoke tonic activation (27) or rhythmic bursting in NTS neurons (10). In vitro experiments have also suggested that TRH-induced excitatory effects on respiration might be mediated by neurons located at the level of the pre-Böt. c. (13) or by inspiratory neurons of the rostral VRG (28). However, at present, it is not settled which of the known medullary structures involved in the respiratory control mediate TRH-induced effects. TRH-immunoreactive terminals and TRH receptors are widely distributed in regions located around the obex, including the NTS and the AP (17, 20, 22), and in the ventral reticular formation (6, 17, 22) where the majority of respiratory neurons are located (1, 38). In addition, a recent study in the rat has shown that expiratory neurons in the Böt. c. receive relatively large numbers of close appositions from TRH-immunoreactive boutons, whereas neurons in the cVRG receive only a small TRH input (36).

This study was undertaken to gain further insight into the role played by TRH input to medullary structures involved in the control of breathing by investigating the effects of TRH microinjections performed in different regions of the DRG and VRG and in the AP of the rabbit.

METHODS

Experiments were carried out on 20 male New Zealand White rabbits (2.8–3.4 kg) anesthetized with a mixture of...
α-chloralose (40 mg/kg iv; Sigma Chemical, St. Louis, MO) and urethane (800 mg/kg iv; Sigma), supplemented when necessary (4 and 80 mg/kg, respectively). The adequacy of anesthesia was assessed by the absence of reflex withdrawal of the hindlimb in response to noxious pinching of the hindpaw. All animal care and experimental procedures were conducted in accordance with the Italian legislation and the official regulations of the European Communities Council on the use of laboratory animals (directive 86/609/EEC). The study was approved by the Ethical Committee for Animal Experiments of the University of Florence.

After cannulation of the trachea, polyethylene catheters were inserted in a femoral artery for the measurement of arterial blood pressure and heart rate (HR) and in a femoral vein for systemic administration of drugs. The animal was placed in a prone position and was fixed in a stereotaxic instrument (model L; Baltimore Instrument, Baltimore, MD) by a stereotaxic head holder and vertebral clamps; the rabbit head holder was made according to the description provided by Sawyer et al. (30). The head was ventroflexed to facilitate access to the rostral part of the medulla. All exposed tissues were covered with warm paraffin oil (37–38°C). Body temperature was maintained at 38°C. The head was ventroflexed to facilitate recordings from the medulla. The dorsal surface of the medulla was widely exposed by occipital craniotomy, and the dorsal surface of the medulla was monitored by an infrared CO2 analyzer (Datex, CD-102; Normocap, Helsinki, Finland). Integrated phrenic nerve activity and the signals of the other variables studied were recorded on an eight-channel rectilinearly writing chart recorder (model 8K20; NEC San-ei, Tokyo, Japan).

Microinjection procedures have been fully described in previous reports (e.g., Refs. 2, 5, and 35). Microinjections (10–20 nl) of 1 or 10 mM TRH (RBI, Natick, MA) or the microelectrode (tip diameter 10–25 μm) by applying pressure using an air-filled syringe connected to the micropipette by polyethylene tubing. TRH concentrations were obviously higher than those employed in vivo (13, 23, 28) and were in the same range as those previously used in vivo (8, 14, 15, 26, 39). The drug was dissolved in 0.9% NaCl solution containing in some experiments 0.1–0.2% Pontamine sky blue to mark the injection site. The pH was adjusted to 7.4 with 0.1 N NaOH. The volume of the injectate was measured directly by monitoring the movement of the fluid meniscus in the pipette barrel with a dissecting microscope equipped with a fine reticule. The time taken to inject the solution ranged from 5 to 10 s. In most cases, the micropipette was glued to a tungsten microelectrode, the tip of which protruded 100–150 μm from the pipette tip; because the glass of the micropipette was glued in close contact with the tungsten microelectrode, the horizontal distance between the tip of the micropipette and the tungsten microelectrode was negligible and approximately equal to the thickness of the micropipette wall at the tip level. Therefore, it was possible to perform TRH microinjections in the same area from which neuronal activity was recorded.

During each experiment, extracellular recordings were first performed to localize the DRG and the different neuronal aggregates of the VRG. The distance between tracks was usually 0.5 mm, but it was reduced to 0.3 mm to more accurately localize some VRG regions such as the transition zone between the condensed population of rostral expiratory neurons and the almost purely inspiratory portion of the rostral VRG. Bilateral microinjections were made in the medullary respiratory regions previously defined by extracellular recordings. In some preparations, the rostral VRG was systematically microinjected along tracks performed at short intervals (0.3 mm) starting from the Böt. c. region, where intense expiratory activity with augmenting discharge pattern was encountered, and continuing until the prevailing inspiratory portion of the VRG was reached (5, 18). TRH microinjections were also performed in the AP (midline or just lateral to the midline, >0.6 mm caudal to the rostral margin of the AP at 150–300 μm depth). In most cases, two micropipettes driven by separate micromanipulators were placed bilaterally in the medulla to execute bilateral microinjections; the injections were made in succession (interval 10–30 s). In a few cases, a single micropipette was used; it was withdrawn after the first microinjection and was reintroduced in the corresponding contralateral site to make the second injection. In this case, the interval between the two injections ranged from 40 to 60 s. Control injections of equal volumes of the vehicle solution were also performed. At the end of the experiment, the brain was perfused with 0.9% NaCl solution and then with 10% Formalin solution via a carotid artery. After at least a 48-h immersion in 10% Formalin solution, the brain was placed in a hypertonic sucrose solution. Frozen coronal sections (20 μm thick) stained with cresyl violet were used for the histological control of pipette tracks and injection sites. The atlas of Shek et al. (32) was used for comparison.

Respiratory frequency (breaths/min), inspiratory time (T1), expiratory time (T2), and peak amplitude (arbitrary units) of the integrated phrenic nerve activity were measured on paper recordings (usual paper speed 5–10 mm/s); the slope of the
straight line drawn from the onset to 90% of the maximum level of the phrenic ramp was considered a reliable estimate of the inspiratory rate of rise (2, 3, 5). Respiratory variables were measured for an average of five consecutive breaths both in the period immediately preceding each trial and at the time when the maximum response to TRH microinjections occurred. In the same periods, systolic and diastolic blood pressure and HR were measured at 2-s intervals; mean arterial pressure (MAP) was calculated as the diastolic pressure plus one-third of the pulse pressure. Because of the small variations in respiratory and cardiovascular variables within a measurement period, average values for each period were taken as single measurements for the purpose of analysis. Statistical analysis of data was performed using Wilcoxon's signed rank tests or Mann-Whitney tests. Changes in respiratory variables were expressed as percentage variations of control values. All values are presented as means ± SE; P < 0.05 was considered significant.

RESULTS

Bilateral microinjections of 1 (10–20 pmol) and 10 (100–200 pmol) mM TRH in the Böt. c. produced depressant effects on inspiratory activity (Fig. 1). All changes in respiratory variables after microinjections of 10 mM TRH were more pronounced than those observed with 1 mM TRH (P always <0.05). After bilateral microinjections of 1 mM TRH (n = 6), respiratory frequency showed a mean decrease of 30.1 ± 5.1% from baseline values of 38.8 ± 1.5 to 27.3 ± 2.4 breaths/min (P < 0.05) mainly due to increases in TEE. Peak amplitude and rate of rise of phrenic nerve activity showed mean reductions of 16.4 ± 4.1% (P < 0.05) and 27.8 ± 8.5% (P < 0.05), respectively. Changes in MAP (from 104.3 ± 2.8 to 106.8 ± 3.6 mmHg) and HR (from 295.1 ± 4.1 to 297.8 ± 5.2 beats/min) were not significant (Fig. 1). Bilateral microinjections of 10 mM TRH (n = 6) in the Böt. c. (Fig. 1) evoked decreases in respiratory frequency of 64.7 ± 3.8% from baseline values of 36.5 ± 1.6 to 12.5 ± 0.9 breaths/min (P < 0.05) due to increases in TEE and, to a lesser extent, in TTI. These effects were associated with reductions in peak amplitude (20.2 ± 1.8%; P < 0.05) and rate of rise (42.3 ± 4.1%; P < 0.05) of phrenic nerve activity. Changes in MAP (from 105.8 ± 3.1 to 109.2 ± 3.7 mmHg) and HR (from 296.7 ± 4.2 to 298.3 ± 5.1 beats/min) were small and inconsistent (Fig. 1).

Similar but less intense responses (P always <0.005) were evoked by TRH microinjections performed in the transition zone where a mix of inspiratory and expiratory neurons were encountered. Slight but inconsistent decreases in respiratory frequency were observed after bilateral microinjections of 1 mM TRH (n = 6); cardiovascular variables did not change. More marked, significant responses were obtained with 10 mM TRH (n = 6); respiratory frequency showed a mean decrease of 27.1 ± 1.9% from baseline values of 36.9 ± 1.2 to 26.9 ± 1.2 breaths/min (P < 0.05) due to increases in TEE. Peak amplitude and rate of rise of phrenic nerve activity did not vary. No significant changes in MAP (from 105.7 ± 2.5 to 106.8 ± 3.1 mmHg) and HR (from 296.7 ± 5.1 to 298.3 ± 4.5 beats/min) were seen.

The onset of respiratory effects both in the Böt. c. and in the directly caudally located region (the putative pre-Böt. c.) was relatively rapid; some appreciable effects were seen already 10–20 s after the end of the
first unilateral injection. Thereafter, the response developed gradually, showing a maximum within 30–120 s after the completion of the second injection; a complete recovery was observed after 20–40 min. Microinjections of 1 (n = 10) or 10 (n = 14) mM TRH performed 0.5 mm or more away from the regions where inspiratory neurons or a mix of inspiratory and expiratory neurons were encountered did not provoke any obvious or consistent effect.

No significant changes in respiratory and cardiovascular variables were observed in response to bilateral microinjections of either 1 or 10 mM TRH in the inspiratory portion of the VRG (17 and 21 trials, respectively) and in the cVRG (14 and 15 trials, respectively). The lack of cardiorespiratory effects in response to 10 mM TRH injected in one site of the cVRG is shown in Fig. 2.

Excitatory effects on inspiratory activity were evoked by TRH microinjections performed in the AP and by bilateral TRH microinjections in the DRG; DRG responsive sites were located close to the obex level. TRH (1 mM) caused only small, inconsistent increases in respiratory frequency (5 trials for each structure) without appreciable changes in cardiovascular variables. However, obvious excitatory effects on respiration (Fig. 2) were elicited in response to 10 mM TRH injected in either the DRG (n = 7) or the AP (n = 6). Respiratory frequency showed mean increases of 34.7 ± 3.8% for the DRG (from control values of 36.1 ± 2.1 to 48.1 ± 2.8 breaths/min; P < 0.05) and 50.4 ± 2.8% for the AP (from control values of 36.8 ± 1.8 to 57.6 ± 2.8 breaths/min; P < 0.05). In both cases, the effects were due to marked reductions in $T_e$ associated with small decreases in $T_i$. Although peak phrenic amplitude did not change, the

---

**Fig. 2.** Responses to TRH (10 mM) microinjections (10 nl) in the dorsal respiratory group (A), the area postrema (B), and the caudal ventral respiratory group (C). TRH microinjections in either the dorsal respiratory group or the area postrema induced increases in the respiratory rate without concomitant changes in arterial BP and heart rate. In A-C, trace on top is IPA, and trace on bottom is arterial BP. TRH-induced effects (A-C, right) were taken 5 min after the injections, when the maximum response occurred. TRH microinjections in the caudal ventral respiratory group did not produce significant cardiorespiratory changes; tracings on right in A-C were recorded 5 min after the injections had been reported.
rate of rise of inspiratory activity showed significant increases (13.9 ± 2.5% for the DRG and 25.1 ± 6.9% for the AP; P < 0.05). Changes in MAP (from 107.5 ± 2.8 to 108.2 ± 3.1 mmHg for the DRG; from 109.5 ± 3.0 to 109.8 ± 3.1 mmHg for the AP) and HR (from 299.5 ± 4.9 to 298.3 ± 4.8 for the DRG; from 298.8 ± 5.0 to 298.7 ± 4.9 beats/min for the AP) were not significant (Fig. 2). TRH-induced respiratory effects in the DRG presented a relatively rapid onset, i.e., 20–40 s after the end of the first injection. The effects developed progressively and reached the maximum within 4–6 min after the completion of the second injection. Respiratory responses elicited by TRH microinjections in the AP displayed a similar time course. A complete recovery was always achieved after 45–60 min. Microinjections of 1 (n = 9) or 10 (n = 12) mM TRH performed 0.5 mm or more away from the responsive sites did not induce any appreciable effect.

Responses to TRH microinjections were reproducible both within and between animals; however, to obtain the same response to repeated injections at the same location, it was necessary to wait from 60 to 90 min. Control injections of the vehicle solution performed at the same locations did not cause appreciable effects. Examples of typical placements of the micropipette within two different medullary regions are shown in Fig. 3. Series of representative coronal sections of the medulla oblongata of the rabbit showing the distribution of sites where 10 mM TRH was injected are represented in Fig. 4.

**DISCUSSION**

This study provides the first description in vivo of the respiratory effects induced by small-volume TRH microinjections in discrete regions of the DRG, VRG, and AP. One of the major findings is that TRH can induce not only excitatory but also depressant effects on respiration. To restrict the spread of the injectate and thereby the number of neurons affected, relatively small volumes (10–20 nl) of TRH solution were injected. Theoretical calculations by Nicholson (24) suggest that volumes of 20 nl should spread ~300 µm in any direction from the injection site. Respiratory effects had a relatively rapid onset, thus indicating that they were mediated at the site of the micropipette tip. Accordingly, injections of TRH in the neighboring region, 0.5 mm away from responsive regions, failed to induce any effect, indicating that TRH injected in this manner did not spread to a distant site. The gradual development of respiratory effects and the long delay in reaching the maximum response, especially at some locations, are not easy to explain. However, they could tentatively be ascribed to the characteristics of TRH receptors and of their transduction mechanisms, which involve second messengers, and may result in slowly developing and protracted changes in neuronal activity (see, e.g., Refs. 6 and 8). It is also very unlikely that TRH-induced responses resulted from nonspecific effects of volume or pressure, since control injections of equal volumes of the vehicle solution performed at the same locations did not alter respiratory activity. Furthermore, because respiratory effects induced by TRH microinjections were not accompanied by appreciable changes in cardiovascular variables, we can exclude any significant role of baroreceptor reflexes in their development (see Ref. 9 for review).

Noteworthy, our study is the first to provide evidence that TRH microinjections in the Böt. c. elicit a depressant effect on respiration. In this context, it seems appropriate to recall that chemical activation of Böt. c. neurons by means of DL-homocysteic acid microinjections provokes depressant respiratory responses in the rabbit (5). The present findings are consistent with the general excitatory effect of TRH on neurons (10, 19, 27, 28, 29).
28) and the relatively large TRH input to rostral expiratory neurons (36), which have inhibitory influences on inspiratory activity (1, 4, 5, 38).

To perform TRH microinjections in the transition zone just caudal to the Bo¨t. c., we used stereotaxic coordinates and extracellular recordings to explore a medullary region where a mix of inspiratory and expiratory neuronal activity was present; the same criterion has been used to identify pre-Bo¨t. c. in the cat (7, 29, 31). We encountered intense multiunit activity of almost purely expiratory and inspiratory neuronal pools in the Bo¨t. c. and the rostral VRG, respectively (5, 18). However, because the pre-Bo¨t. c. has not yet been characterized in the rabbit, the possibility exists that our transition zone may just correspond to the caudal portion of the Bo¨t. c. On the other hand, TRH microinjections in the rostral inspiratory portion of the VRG, just caudal to the transition zone, were completely ineffective. Thus, although there is the possibility that we completely failed to perform TRH microinjections in the pre-Bo¨t. c., the present data may also suggest that TRH microinjections in this region of the rabbit have either no effects or only slight depressant effects on respiration.

Our findings are at variance with the results of previous studies on isolated brain stem-spinal cord preparations or medullary slices from newborn rats and mice (13, 28). Those studies suggest that neurons located in the pre-Bo¨t. c. (13) or inspiratory neurons of the rostral part of the VRG (28) might mediate TRH-induced excitatory effects on respiration. The reasons for these discrepancies are not clear; however, at least some of them are conceivably related to obvious differences in the type of preparation and the animal species employed as well as in its developmental stage. In our opinion, rostral expiratory neurons are the most likely candidates for the mediation of the observed depressant respiratory effects, since they have inhibitory influences on inspiratory activity and receive a large TRH input (see Refs. 5 and 36 also for additional references); however, we cannot rule out that other types of respiratory neurons have been affected by our TRH microinjections.

The present results do not exclude that TRH may exert its respiratory stimulant effects by acting on structures rostral to the brain stem (14, 39); however, they demonstrate that both the DRG and the AP are responsive structures. Our results on the DRG are in agreement with previous findings both in vivo (14, 21) and in vitro (10, 27). The finding that TRH injected in the AP causes excitatory respiratory responses is completely new and is consistent with our previous observations on the excitatory influences of the AP on respiratory activity (35). In this context, it is worth noting that

---

Fig. 4. Series of representative coronal sections of the medulla oblongata of the rabbit showing the distribution of sites where microinjections of 10 mM TRH were performed. TRH microinjections induced excitatory (●) and depressant (▼) respiratory responses. For simplicity, injection sites are projected on the nearest of the reported sections; in addition, representative nonresponsive sites (○) are shown only in the dorsal respiratory group and ventral respiratory group. Outlines of the maps derive from selected sections of one histological preparation (camera lucida redrawing). The atlas of Shek et al. (32) was used for comparison. The distance in mm from the rostral margin of the area postrema (obex) is indicated on the left of each section. NA, nucleus ambiguus; NOI, nucleus olivaris inferior; NRA, nucleus retroambigualis; NV, nucleus tractus spinalis nervi trigemini.
TRH-immunoreactive terminals and TRH receptors have been found both in the NTS and the AP (17, 20, 22). In addition, the localization of DRG-responsive sites is consistent with the occurrence of a high concentration of TRH receptors, especially in the DRG region close to the obex level (20).

Surprisingly, TRH-induced responses at sites of the DRG consisted mainly of increases in frequency and rate of rise of inspiratory activity without significant changes in peak phrenic amplitude. Such changes were to be expected because of the presence of inspiratory premotor neurons in the ventrolateral part of the NTS (1, 38) where the responsive sites were encountered. Furthermore, it seems possible that the injectate could have affected second-order chemoreceptor neurons located in the same area or in close vicinity (see Refs. 1 and 34 for review), thus leading to respiratory responses characterized by increases in frequency.

The lack of significant effects in response to TRH microinjections in the inspiratory portion of the VRG is in agreement with previous results obtained in the rat; the absence of TRH-responsive sites and of TRH-immunoreactive fibers or somata in this region of the rat medulla has been described (21). The absence of respiratory responses to TRH microinjections in the VRG of the rabbit cannot be related to a small volume of the injectate failing to reach a sufficient number of inspiratory neurons. In fact, similar TRH microinjections were effective in other medullary locations. Furthermore, in previous studies (5), 10- to 20-nl microinjections of DL-homocysteic acid in the inspiratory VRG of the rabbit proved to induce strong excitatory effects on phrenic nerve activity. On the other hand, our results are not in contrast to the finding that respiration-related motoneurons in the nucleus ambiguus (i.e., laryngeal and pharyngeal motoneurons) receive a large TRH input, since these motoneurons do not correspond to the respiratory neurons of the VRG (36, 37) and are not involved in respiratory rhythm generation (1, 38).

The absence of responsive sites within the cVRG expiratory neurons is not surprising because of the small TRH input they receive (36) and the scarce involvement, if any, of this population of almost exclusively bulboспinal neurons in rhythm generation (1, 2, 38).

In the present study, injection sites were in most cases adjacent to or within medullary regions involved in cardiovascular regulation, such as the rostral and caudal vasomotor areas, the nucleus ambiguus, and the NTS (see Refs. 9, 11, and 34 also for further references). Nevertheless, our TRH microinjections failed to produce significant changes in cardiovascular variables, thus suggesting that cardiovascular medullary neurons have a small TRH input or even completely lack it. On the other hand, our results are in agreement with previous studies showing that TRH injections in the NTS of the rat (14) or in the nucleus ambiguus of the cat (16) do not significantly affect arterial blood pressure and HR. These findings support the hypothesis that this peptide shows specificity of action at the level of the medullary structures investigated, where it may have physiological significance mainly in terms of controlling respiratory activity. However, this does not exclude that TRH may exert strong effects on both cardiovascular and respiratory variables by acting on other medullary structures (e.g., Refs. 8 and 26).

In conclusion, the results suggest that not only the DRG, but also the AP, could be involved in the mediation of TRH-induced excitatory effects on respiratory activity. In addition, they show for the first time that TRH may also exert inhibitory influences on respiration at medullary levels by acting on rostral expiratory neurons. Although the functional role of rostral expiratory neurons is yet unclear (5), the present results further emphasize that they are an important source of inhibition in the medullary respiratory network, showing that TRH inputs may bring into action their inhibitory influences.

Perspectives

The present findings seem to imply that the medullary regions investigated receive TRH inputs from other neuronal structures that may play either an excitatory or an inhibitory role in respiratory regulation according to the target of their axonal projections. The raphe nuclei are probably the major source of the TRH input to the medullary structures involved in the control of breathing (see, e.g., Refs. 17 and 37). On the other hand, raphe neurons have been shown to exert their activity over the sleep/wake cycle (12). Thus, although the overall physiological significance of TRH-induced respiratory effects is unclear, TRH input to medullary structures may be implicated in sleep/wake modulation of respiratory activity and could be of importance in the pathophysiology of some respiratory disorders such as, for instance, obstructive sleep apnea or some cases of sudden infant death (see, e.g., Ref. 37). TRH input may be particularly relevant to the maintenance of the coordination between muscles controlling airway patency and inspiratory pump muscles after laryngeal stimulation; the combination of augmentation in respiratory hypoglossal activity and depression of phrenic nerve activity for prolonged periods may have a protective role in maintaining airway patency in circumstances that predispose to upper airway collapse, such as sleep and anesthesia (see Refs. 3 and 37 also for further references). Availability of TRH-receptor antagonists would be helpful to get further insights into the physiological significance of TRH-induced respiratory effects.

We thank Salvatore Cammarata and Alessandro Aiazzi for technical assistance and Adrio Vannucchi for preparation of the figures.

This study was supported by grants from the Ministero dell’ Università e della Ricerca Scientifica e Tecnologica of Italy. Address for reprint requests and other correspondence: T. Pantaletto, Dipartimento di Scienze Fisiologiche, Università degli Studi di Firenze, Viale G.B. Morgagni 63, I-50134 Firenze, Italy (E-mail: pantaleo@ces1.unifi.it).

Received 3 December 1998; accepted in final form 18 June 1999.

REFERENCES


