Acta Physiol Scand 1983, 117: 427–437

Effects of TRH and TRH analogues on the central regulation of breathing in the rat

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HEDNER, J., HEDNER, T., WESSBERG, P., LUNDBERG, D. & JONASON, J.: Effects of TRH and TRH analogues on the central regulation of breathing in the rat. Acta Physiol Scand 1983, 117: 427–437. Received 31 Aug. 1982. ISSN 0001-6772. Department of Pharmacology, University of Göteborg, Department of Clinical Pharmacology, Sahlgrenska Hospital, Göteborg and Department of Anesthesiology, University Hospital of Lund, Sweden.

Respiratory activity was studied in rats during light halothane anesthesia. Thyrotropin releasing hormone (TRH) and two TRH analogues: the desamidated form (TRH-OH) and γ-butyrolactone-γ-carbonyl-L-histidyl-L-prolinamide citrate (DN 1417) were administered intracerebroventricularly. TRH 0.5–5 μg induced a marked tachypnoea with a rapid onset and a duration of at least 20 min. DN 1417, a potent analogue of TRH with a very low TSH (thyroid stimulating hormone) releasing activity was more effective in stimulating respiratory frequency, while TRH-OH, regarded to have neither TSH releasing nor extra hypothalamic effects, at equimolar doses was unable to induce any changes in the respiratory pattern. When TRH was given into the fourth ventricle the dose response curve was slightly shifted to the left. In experiments employing the occluded breath tehnique, $P_{0.1}$ was increased in the same magnitude as the mean inspiratory flow (V_T/T_I). The results also indicated an increase in the gain of the inflation reflex loop whereas the central bulbopontine setting for T_I and T_{TOT} were not significantly changed. Local injection of TRH into the nucleus tractus solitarii induced a stimulation of respiratory frequency which was slower in onset compared to the response seen after injection into the lateral or fourth ventricles. Concomitantly to the respiratory changes, i.c.v. TRH injection induced a hypocarbia and an alkalosis. No changes in blood pressure or heart rate were seen. The respiratory stimulant effect of TRH could be potentiated by pretreatment with naloxone, methylatropine or a low dose of GABA. Haloperidol or propranolol did not significantly change the respiratory effects of TRH, while reserpine pretreatment seemed to blunt some of the ventilatory effects of TRH. It seems likely that TRH has few direct effects on brain stem neurones involved in the central regulation of respiration, but the main effects seem to be elicited in areas rostral to the brain stem. The respiratory stimulating effect of TRH is unrelated to TSH. Furthermore, other neurotransmitter systems might also be involved in modulation of the respiratory stimulation evoked by TRH.

Key words: TRH, respiration, carbon dioxide, NTS

In recent years, some basic information concerning the neurochemical control of respiration has been developed which may aid in completing our knowledge how the basic respiratory rhythm is generated. Much of what has been learned is inferential from drug-induced perturbation of respiratory control. The goal has been to relate these drug studies to our knowledge of anatomy and physiology of respiration in order to provide a framework upon which new drugs can be developed to aid in management of respiratory failure patients. Thus, some neurotransmittors and neuromodulators have been shown to have a stimulatory influence on basal respiratory performance, such as, for example, dopamine (Lundberg et al. 1979, Hedner et al. 1982*a*) adrenaline (Bolme et al. 1974) substance P (Euler & Pernow 1956, Hedner et al. 1981*c*) and TRH (thyrotropin releasing hormone) (Kraemer et

al. 1976, Hedner et al. 1981 *b*), while other have been shown to exert a depressive influence, e.g. GABA (Hedner et al. 1981 *d*) glycine (Wessberg et al. 1983) and some endorphins (Florez et al. 1980, Hedner et al. 1981 *a*).

The occurrence of the tri-peptide TRH (pyroglutamyl-histidyl-prolinamide) was first demonstrated in the hypothalamus and shown to have an influence on the release of hormones from the pituitary (Bohler et al. 1969). However, TRH is now known to exist in many other brain areas than hypothalamus (Winokur & Utiger 1974) and relatively high amounts are present in several areas such as the brain stem (Oliver et al. 1974). TRH acts as an ergotropic substance and alters body temperature and locomotion. It has also been shown to induce tachypnea in pentobarbital anesthetized monkey (Kraemer et al. 1976) as well as in halothane (Hedner et al. 1981 *b*, Breese et al. 1981, Mueller et al. 1981) and urethane (Koivusalo et al. 1979) anesthetized rats.

In the present investigation we have studied the respiratory effects of TRH applied at different locations, as well as the effects of some TRH analogues in order to gain a more detailed understanding of the nature of the TRH induced respiratory stimulation. We have also studied the possible interactions between TRH and other putative neurotransmittor systems.

MATERIAL AND METHODS

Male Sprague-Dawley rats weighing 200–300 g were used. Details of the surgical procedures and the pletysmographic set-up for respiratory recordings where the respiratory performance is studied under light halothane anesthesia, have been described in previous publications (Hedner et al. 1982 *a*). In short, in the majority of the experiments the lateral brain ventricles were cannulated under sodium pentobarbitone anesthesia 24–48 h before the respiratory experiments, while in some experiments the fourth ventricle was cannulated according to the technique described by Gomes et al. 1980. Multiple injections (for dose-response studies) as well as single injections were made via the implanted catheters.

In some experiments the tracheal cannula was connected to a low pressure transducer (Grass model PT5A) for registration of the intratracheal pressure. At certain points during the experiments the tracheal cannula was occluded at functional residual capacity (FRC) for 2–3 breaths. The negative intratracheal pressure generated after 0.1 sec, $P_{0.1}$ (Lynne-Davies et al. 1971), was calculated from the registration chart after calibration of the transducer to 0 and 10 cm H_2O. $P_{0.1}$ was always calculated from the first occluded breath in order to minimize the influence of increasing chemical stimuli during the occlusion period.

During the occluded breath an expansion of the pulmonary volume is seen due to decompression of the gas mixture in the respiratory system. The maximum value of this expansion and the corresponding value of the preceding unoccluded breath, V_{max} or V_T respectively, were plotted against the inverse of T_I and T_{TOT} of the same breaths. According to Grunstein et al. (1973), the slope of the function V_{max} vs $1/T_I$ is indicative of vagally mediated volume control of the length of the inspiratory phase, T_I, whereas the T_I value corresponding to the point of intercept of the function with the $1/T_I$ axis represents the inspiratory time set by the bulbopontine pacemaker.

Some animals were exposed to a CO_2 challenge (5% and 10% CO_2 in O_2) before and 30 min after drug injection and the respiratory performance was studied. Arterial blood samples were withdrawn form the ventral tail artery prior to and 20 min after drug administration. The samples were analyzed for the partial pressure (Pa) of CO_2 and O_2, pH and standard bicarbonate in an automatic blood gas analyzer (Instrumentation Laboratories 413).

In a separate set of experiments, the effects of locally applied TRH was studied. The rats were anesthetized with ether to permit cannulation of the trachea and the ventral tail artery and were then mounted in a stereotaxic apparatus (Stoelting Company, USA). A closed plexiglass boxshaped body pletysmograph, measuring 300×140×80 mm (volume 3.4 liters), was attached on the table of the stereotaxic apparatus. During the stereotaxic experiments the animals were continuously kept under a light halothane anesthesia (0.7% halothane in O_2). TRH was injected into the nucleus tractus solitarii (NTS). The volume changes in the body pletysmograph were recorded by a Grass polygraph according to previously described techniques (Hedner et al. 1982 *a*). Tidal volume (V_T) and respiratory frequency (f) were continuously monitored. Minute volume ($\dot{V}_E$) was calculated according to the formula $V_T \cdot f = \dot{V}_E$. Inspiratory time (T_I), expiratory time (T_E) and total cycle duration (T_{TOT}) were calculated from the respiratory curve (Hedner et al. 1980). To gain access to the experimental region, the heads of the rats were flexed to an angle of 45° forward and the dorsal surface of the brain stem was exposed by a limited occipital craniotomy and opening of the atlanto-occipital membrane. The caudal tip of the area postrema, obex, was demarcated as a stereotaxic zero. For microinjections into the area of NTS the following coordinates were used: 0.5 mm rostral, 0.5 mm lateral and 0.5 mm ventral. Drugs were given in a volume of 0.2 µl during 10 s via a glass cannula (outer diasmeter 0.1 mm) connected to a Hamilton microsyringe and a micrometer. After termination of the experiments, the brains were fixed in 5% formalin. Frozen sections were cut (50 µm) and stained with 0.1% thionine. The location of the needle tracts were controlled microscopically.

Drugs used

The following drugs were used in the experiments: TRH (pyroglutamyl-histidyl-prolinamide) (F. Hoffmann-La Roche & Co., Basle, Switzerland), desamidated TRH (pyroglutamyl-histidyl-proline-OH) (F. Hoffmann-La Roche & Co., Basle, Switzerland), DN 1417 (γ-butyrolactone-γ-carbonyl-L-histidyl-L-prolinamide citrate) (Takeda

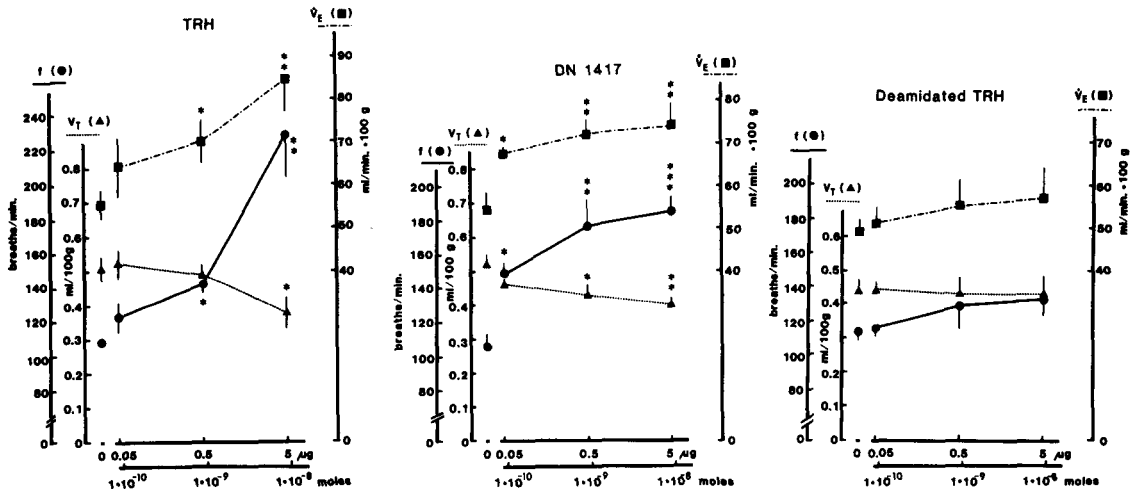

Fig. 1. Dose-response relationships for TRH (*a*), DN 1417 (*b*) and desamidated TRH (*c*) on some respiratory parameters in the anesthetized rat. The drugs were administered into the lateral ventricles. The respiratory parameters were measured at 15 min after drug administration. The values represent the mean of 5 (*a*), 4 (*b*) or 6 (*c*) experiments. The vertical bars represent the SE. Statistical analysis was performed with the one way analysis of variance followed by t-test $*p<0.05$, $**p<0.01$, $***p<0.001$.

Chemical Industries Ltd, Osaka, Japan), haloperidol (AB Leo Helsingborg, Sweden), reserpine (Ciba-Geigy, Basle, Switzerland), GABA (Sigma Chemical Co., St. Louis, Mo., USA), N-methylatropine iodide (Sigma Chemical Co., St. Louis, Mo., USA), naloxone hydrochloride (Endo Laboratories Inc., Garden City, N.Y., USA), DL-propranolol chloride (Ciba-Geigy, Basle, Switzerland).

Statistics

All values were calculated as means±SE. Significant differences were established at the $p<0.05$ level using one way analysis of variance followed by t-test, Student's t-test or paired t-test. The statistical method used is indicated in each figure text.

RESULTS

Effects of TRH and some TRH analogues on basal respiration

Increasing doses of TRH injected into the lateral ventricles induced a dose dependent increase in f and $\dot{V}_E$ while V_T was decreased at the highest dose studied (5 µg approximately corresponding to 10^{-8} mol) (Fig. 1*a*). The changes in the respiratory parameters appeared in the dose interval 0.5–5 µg (10^{-9}–10^{-8} mol), while no significant changes were seen at a lower dose (0.05 µg). The increase in f after 5 µg was approximately twice the control value ($p<0.01$).

DN 1417 is a potent analogue of TRH with a very low TSH (thyroid stimulating hormone)-releasing activity but approximately 3 times more potent than TRH in eliciting the extrahypothalamic effects of TRH (Fukuda et al. 1980). This compound's action on respiration was qualitatively similar to that seen after TRH (Fig. 1*b*); however, significant increases in f ($p<0.05$) and $\dot{V}_E$ ($p<0.05$) were already noted at a dose of 0.05 µg (10^{-10} mol). At 0.5 µg and 5 µg there were marked increases in f and $\dot{V}_E$ (approximately 85% and 35% respectively) while V_T was significantly decreased.

Desamidated TRH has been shown by several authors to have neither TSH-releasing activity nor extrahypothalamic effects (see Breese et al. 1975). In our model no significant changes in the respiratory performance were evoked in the dose interval 0.05–5 µg (approximately corresponding to 10^{-10}–10^{-8} mol) (Fig. 1*c*).

In some experiments TRH was administered i.c.v. in a single dose (Fig. 2). The changes in the respiratory parameters appeared 3–10 min after injection. Maximum stimulation of f, approximately 100%, was reached after 15–30 min and this effect lasted over the 45 min period studied. A decrease in V_T was seen in parallel to the response in f. The time of onset and the duration were similar to that of the f-response. $\dot{V}_E$ tended to increase and was significantly higher than the saline-treated control

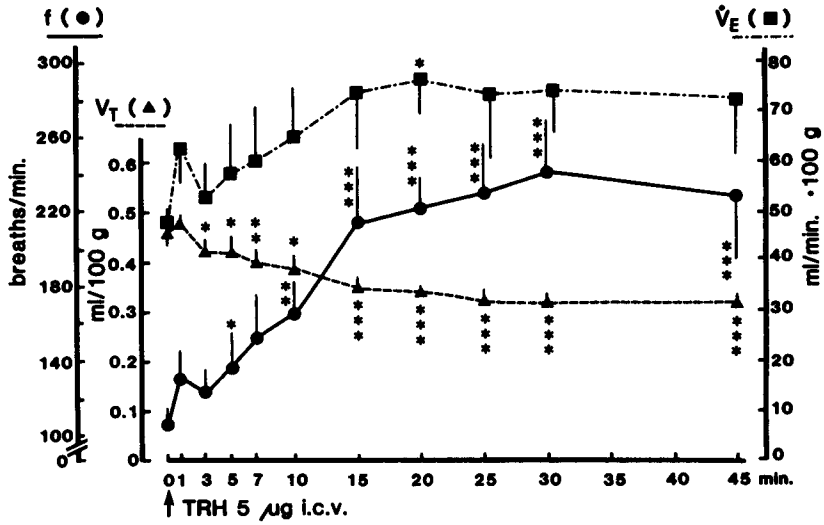

Fig. 2. The effect of TRH (5 µg, i.c.v.) on respiratory performance at different time intervals after i.c.v. administration of the drugs. The values represent the mean of 4 experiments. The vertical bars represent the SE. Statistical analysis was performed with the one way analysis of variance followed by t-test. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

group after 20 min ($p<0.05$). I.c.v. injection of the vehicle (saline) did not alter the respiratory performance (data not shown).

Effects of TRH on inspiratory drive and respiratory timing mechanisms

A significant decrease in T_{TOT} corresponding to the increase in f was initially seen at 7 min after administration of a single i.c.v. dose of TRH. The decrease in T_E however was not seen until after 15 min ($p<0.01$) whereas T_I was significantly shortened already at the 1 min registration (data not shown). After the 15 min registration and at later time intervals, T_I and T_E contributed approximately equally to the total shortening of T_{TOT}. Administration of 5 µg of TRH i.c.v. induced an increase in the quotient V_T/T_I "inspiratory drive", by approximately 55% while "respiratory timing", T_I/T_{TOT} was decreased by approximately 40% (Fig. 3).

In some experiments the occluded breath technique was applied. $P_{0.1}$, which is a parameter reflecting inspiratory drive independent of flow resistance or compliance, increased in the same manner as V_T/T_I, from 3.52±0.22 cm H_2O in the control state to 5.03±0.24 cm H_2O ($p<0.001$) after TRH.

According to Grunstein et al. (1973) the gain of the inflation reflex loop can be quantitated in terms of the slope of the V_T vs. $1/T_I$ relation. This was decreased ($p<0.025$, paired t-test) by TRH indicating an increase in the sensitivity to afferent vagal stimuli (Fig. 4). The intercept with the baseline $1/T_I$ was not significantly changed, however a tendency toward a shift to the left i.e. increase in T_I setting was seen (Fig. 4). Neither the slope nor the intercept were significantly changed for the V_T vs. $1/T_{TOT}$ relation.

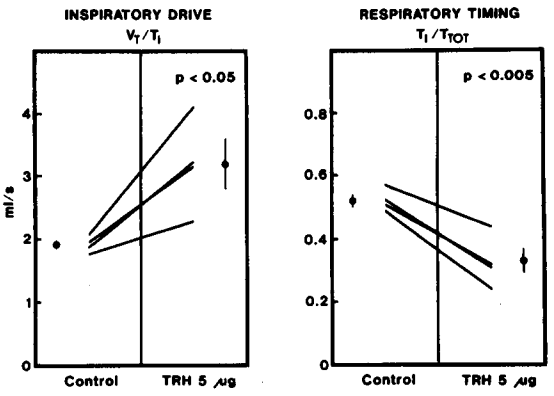

Fig. 3. "Inspiratory drive" (V_T/T_I) and "respiratory timing" (T_I/T_{TOT}) before and after administration of TRH, 5 µg i.c.v. The oblique lines connect the individual values before and after drug administration. Means±SE are also indicated. Statistical comparison by paired t-test.

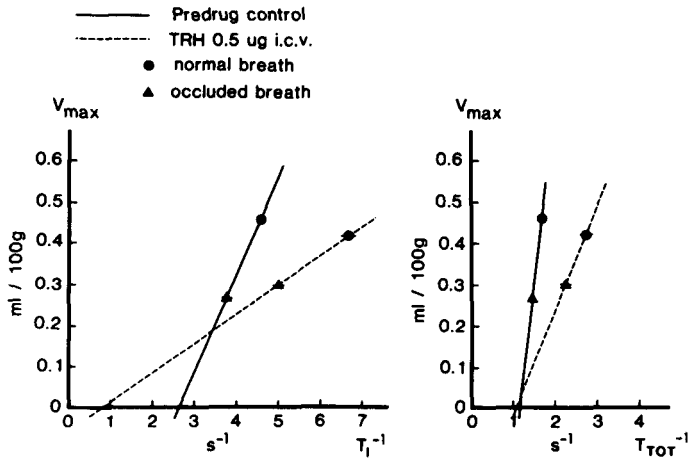

Fig. 4. Relationship between pulmonary volume expansion (in ml/100 g above functional residual capacity) and reciprocal of T_I (left panel), and T_{TOT} (right panel). The solid lines represent untreated animals and the interrupted lines represent animals treated with TRH (0.5 μg i.c.v., 10 min). •, Control breaths; ▲, occluded breaths. Values are mean±SE of 5 animals.

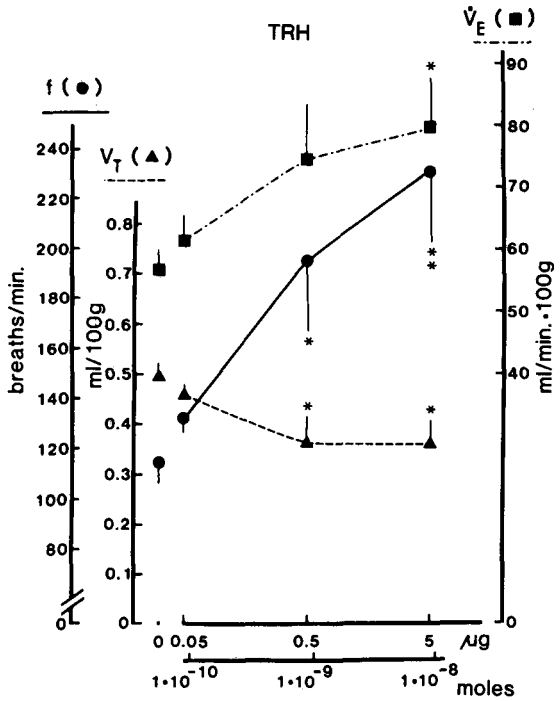

Fig. 5. Dose–response relationships for TRH, administered into the fourth ventricle on some respiratory parameters in the anesthetized rat. The values represent the mean of four experiments. The vertical bars represent the SE. Statistical analysis of the predrug control values was performed with the one way analysis of variance followed by t-test. *$p<0.05$, **$p<0.01$.

Effects of TRH administered into the fourth ventricle or locally into the brain stem

When TRH was applied into the fourth ventricle a respiratory stimulation very similar to that seen after injection into the lateral ventricles was registered (Fig. 5). However, a significant decrease in V_T was already seen at 0.5 μg ($1 \cdot 10^{-9}$ mol) ($p<0.05$). At this dose, the stimulation of frequency was also more pronounced. Thus, injection of TRH into the fourth ventricle tended to shift the dose-response curve to the left.

When TRH was applied locally into the brain stem, no immediate significant changes in the respi-

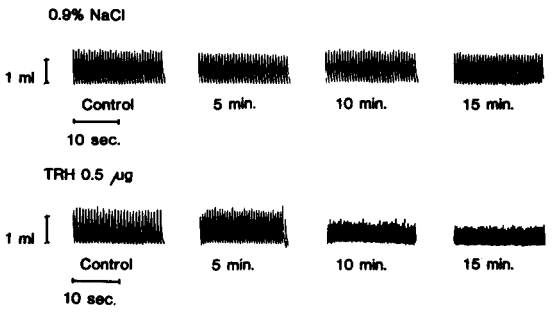

Fig. 6. The effect of TRH given locally into the nucleus tractus solitarii. Shown are replicas of typical respiratory recordings at different time intervals after TRH or saline. Inspiration uppwards.

Table 1. *The effect of TRH (0.5 μg i.c.v.) alone and after pretreatment with methylatropine (0.2 μg i.c.v., 1! min), haloperidol (2 mg/kg i.p., 15 min), GABA (0.1 mg i.c.v., 5 min), propranolol (50 μg i.c.v., 5 min) reserpine (10 mg/kg i.p., 6 h) or naloxone (1 mg/kg i.p., 5 min) on some respiratory parameters at differen time intervals*

		0	1	3	5	10
TRH 0.5 μg (0 min),	f	95±4.6	114±6.9	118±8.4	128±10.5	146±9.1
n=6	V_T	0.46±0.01	0.56±0.04	0.52±0.04	0.50±0.04	0.44±0.02
	$\dot{V}_E$	43.3±2.5	63.0±4.2	61.0±4.7	63.1±5.3	64.2±4.4
Methylatropine 0.2 μg	f	110±7.2 ns	109±11.5 ns	122±7.1 ns	135±6.9 ns	180±31.6 ns
(−15 min)	V_T	0.53±0.03*	0.57±0.06 ns	0.52±0.06 ns	0.50±0.04 ns	0.45±0.03 ns
TRH 0.5 μg (0), n=5	$\dot{V}_E$	58.6±5.1*	60.6±5.1 ns	62.7±3.7 ns	67.3±5.5 ns	78.4±10.7 ns
Haloperidol 2 mg/kg	f	74±4.5*	73±10.7*	78±8.9*	84±8.0**	110±14.0 ns
(−15 min)	V_T	0.46±0.02 ns	0.61±0.08 ns	0.54±0.06 ns	0.54±0.05 ns	0.47±0.02 ns
TRH 0.5 μg (0), n=4	$\dot{V}_E$	33.3±1.1**	41.8±1.8***	41.3±1.1***	44.0±2.8*	51.1±6.0 ns
GABA 0.1 mg	f	74±13.9 ns	98±13.0 ns	121±12.6 ns	146±12.7 ns	170±4.2*
(−5 min)	V_T	0.34±0.03***	0.41±0.05*	0.41±0.02*	0.43±0.03 ns	0.43±0.03 ns
TRH 0.5 μg (0), n=5	$\dot{V}_E$	25.7±5.4**	41.8±7.9*	49.6±5.6 ns	61.8±6.0 ns	73.5±6.1 ns
Propranolol 50 μg	f	101±8.6 ns	122±14.3 ns	128±11.5 ns	127±12.0 ns	127±9.9 ns
(−5 min)	V_T	0.43±0.04 ns	0.49±0.05 ns	0.42±0.04 ns	0.41±0.03 ns	0.40±0.03 ns
TRH 0.5 μg (0), n=5	$\dot{V}_E$	43.6±6.1 ns	58.1±6.4 ns	53.1±6.5 ns	51.3±5.6 ns	52.7±6.0 ns
Reserpine 10 mg/kg	f	110±5.5 ns	113±10.4 ns	127±11.3 ns	127±11.5 ns	125±13.5 ns
(−6 h)	V_T	0.47±0.04 ns	0.44±0.03*	0.40±0.03*	0.38±0.02*	0.40±0.01 ns
TRH 0.5 μg (0), n=4	$\dot{V}_E$	51.8±5.9 ns	49.0±4.3*	50.4±6.3 ns	49.0±4.9 ns	51.2±6.2 ns
Naloxone 1 mg/kg	f	111±4.2*	141±8.1*	145±6.8*	148±8.4 ns	165±10.2 ns
(−5 min)	V_T	0.40±0.03 ns	0.46±0.03 ns	0.43±0.03 ns	0.41±0.02*	0.39±0.02 ns
TRH 0.5 μg (0), n=6	$\dot{V}_E$	43.9±2.8 ns	65.1±5.3 ns	61.3±4.3 ns	61.5±4.4 ns	64.8±4.0 ns

Shown are means±SEM, n represent the number of experiments in each group. Statistical comparison by Student's t-test t the corresponding non-pretreated group. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

ratory performance were seen. In some cases an apnea and a shortlasting hypotension appeared. This may however be an effect of the injection per se as has been reported in other studies (Talman & Reis 1981). After 10 min, however, f was markedly stimulated and followed by a decrease in V_T in 4 out of 5 cases. The respiratory curve was similar to the curve seen after injections into the lateral cerebral ventricles. A representative respiratory recording is shown in Fig. 6.

Effect of pretreatment with different drugs on the TRH induced respiratory stimulation (Table 1)

Pretreatment with propranolol 50 μg i.c.v. (5 min) or reserpine 10 mg/kg i.p. (6 h) did not significantly change the basal respiratory values of the animals. However, in reserpine pretreated animals V_T was significantly decreased after TRH at 1, 3, 5, 15 and 30 min, while no significant increase in $\dot{V}_E$ was seen in these animals. Haloperidol pretreatment 2 mg/kg i.p. (15 min) significantly decreased f and $\dot{V}_E$ while V_T was unaffected. The depression was reversed by TRH and the injection of 0.5 μg i.c.v. restored the values towards normal levels.

GABA pretreatment 0.1 mg i.c.v. (5 min) seemed to depress all the registered respiratory parameters. A recovery was seen after TRH and at the 10 and 15 min registrations f was significantly increased in the GABA pretreated group. This pattern was also seen when GABA was given after TRH injection (Fig. 7). In this case, GABA decreased V_T and increased f.

Pretreatment with methylatropine 0.2 μg i.c.v. (15 min) resulted in an increase in V_T and $\dot{V}_E$, as f was unaffected. However, the TRH induced increase in f was facilitated with a peak value after 30 min ($p<0.05$).

Pretreatment with naloxone resulted in a slight increase in f while V_T and $\dot{V}_E$ were unaffected. The f stimulation after TRH was facilitated at 1, 3 and 30 min ($p<0.05$) and a general decrease in V_T, with a peak value after 5 min, was also seen after naloxone pretreatment.

15	30	45 min
152±10.3	145±13.7	136±14.4
.45±0.02	0.43±0.01	0.47±0.02
8.1±6.7	63.3±7.1	63.4±6.9
184±19.7 ns	202±13.7*	173±15.7 ns
.44±0.02 ns	0.41±0.03 ns	0.42±0.03 ns
0.3±7.4 ns	82.3±5.3 ns	76.8±6.1 ns
122±13.5 ns	129±4.7 ns	133±3.1 ns
.43±0.01 ns	0.40±0.01 ns	0.41±0.01*
2.6±5.7 ns	51.3±2.9 ns	54.6±1.3 ns
177±4.7*	167±8.8 ns	153±9.8 ns
.43±0.02 ns	0.45±0.03 ns	0.49±0.02 ns
5.5±3.9 ns	74.8±2.6 ns	74.0±2.5 ns
140±7.8 ns	153±7.3 ns	132±8.2 ns
.40±0.03 ns	0.39±0.04 ns	0.42±0.04 ns
6.2±4.9 ns	58.1±3.1 ns	54.7±4.4 ns
136±18.5 ns	130±18.7 ns	114±18.1 ns
.38±0.02*	0.36±0.03*	0.40±0.04 ns
2.3±6.9 ns	45.9±5.5 ns	47.0±9.1 ns
179±11.5 ns	190±9.3*	169±9.9 ns
.40±0.03 ns	0.38±0.03 ns	0.41±0.03 ns
1.6±6.4 ns	72.9±5.3 ns	68.7±6.0 ns

Effects of CO_2 on TRH induced respiratory stimulation (Fig. 8)

After adding 5% or 10% CO_2 in the inhalation gas mixture, V_T and $\dot{V}_E$ were significantly increased, while no significant change in f was seen in the control preparation. When the TRH pretreated animals were exposed to increasing CO_2 contents in the inhalation gas mixture the sharp stimulation of f was reversed towards control values while a stimulation of V_T and $\dot{V}_E$ was seen similar to that of the controls.

Effects of TRH on mean arterial blood pressure (MAP) and heart rate (HR)

No significant changes in neither MAP nor HR were seen in the saline injected controls or in the TRH (5 μg i.c.v.) treated animals during the 45 min interval studied. Similarly, MAP and HR were unaffected after single injections of desamidated TRH or DN 1417 (data not shown).

Effects of TRH on arterial blood gases (Table 2)

Before and 20 min after saline or TRH administration blood samples were withdrawn for analysis of arterial blood gases, pH and standard bicarbonate (SB). Saline treated animals showed no significant changes in any of the parameters studied while after

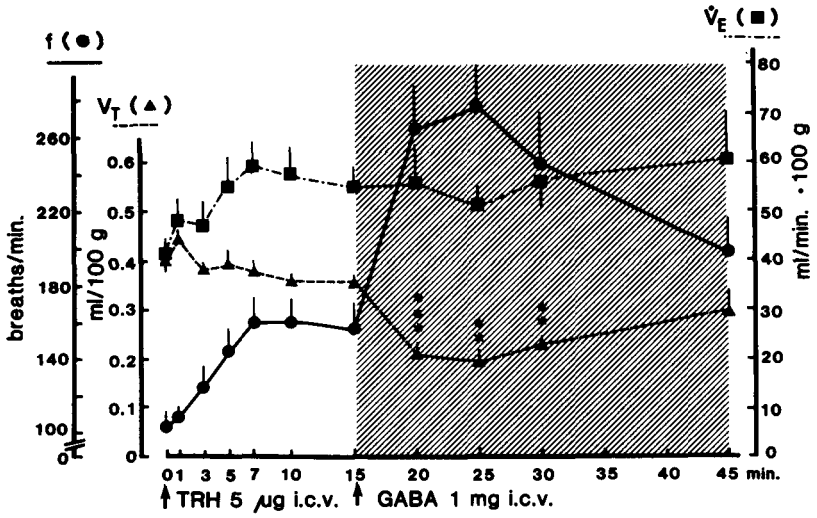

Fig. 7. The effect of TRH (5 μg i.c.v.) followed by GABA (1 mg i.c.v. 15 min) on some respiratory parameters. The lined area represent the time interval after GABA administration. Shown is the mean of 5 experiments. The vertical bars represent the SE. Statistical comparison to animals treated with TRHY alone (Fig. 2) by Student's t-test. $^{**}p<0.01$, $^{***}p<0.001$.

Table 2. *Effects of saline and TRH administered i.c.v. on arterial blood gases, pH and standard bicarbonate (SB) in anesthetized rats*

		pCO_2 (kPa)	pO_2 (kPa)	pH	SB (mmol/l)
Control	n=3	6.0±2.25	29.0±7.90	7.35±0.02	29.6±3.44
Saline		6.6±0.51	23.0±8.74	7.33±0.02	25.1±2.40
Control	n=7	7.2±0.43	22.3±5.49	7.29±0.01	23.8±0.71
TRH		5.8±0.18*	21.3±5.32	7.36±0.01**	24.3±0.67

TRH was given in a single 5 μg i.c.v. injection. Shown are means±SEM of control values and values obtained 20 min after injection. Statistical comparison with paired t-test. *$p<0.05$, **$p<0.01$.

TRH a decrease in pCO_2 ($p<0.05$) and an increase in pH ($p<0.01$) were seen. pO_2 and SB were not changed after TRH administration.

DISCUSSION

A general problem in studies of respiratory regulation are the effects of the anesthetic agents used on the ventilatory performance. In this study we have chosen halothane since, in the rat, this anesthetic agent has a minor depressive influence compared to many other anesthetics (enflurane, pentobarbital, urethane and chloralose) (unpublished results from our laboratory). However, concerning TRH, the qualitative effects of this compound seem to be unrelated to the anesthetic agent used (see e.g. references below).

TRH has been shown to act as a respiratory stimulant in several animal species such as the monkey (Kraemer et al. 1976), cat (Metcalf & Myers 1976, rabbit (Hedner et al. 1982*b*) and rat (Koivusalo et al. 1979, Hedner et al. 1981*b*, Breese et al. 1981). However, no changes were seen after i.c.v. infusion of TRH in the goat (Eriksson & Gordin 1981).

Derivatives of TRH have been synthetized which

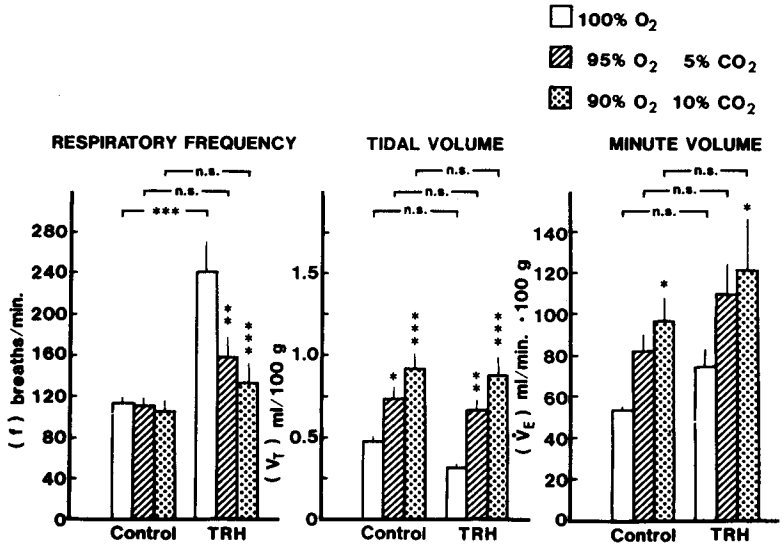

Fig. 8. Effects of exposure to 5% and 10% CO_2 in O_2 on respiratory performance in anesthetized rats. The registrations were made before or 30 min after TRH administration. The values represent the mean of 4 experiments. The vertical bars represent the SE. Statistical analysis was performed with the one way analysis of variance followed by t-test. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

have effects on the central nervous system unrelated to endocrine function. The novel TRH analogue DN 1417 has a very low TSH-releasing activity (Miyamoto et al. 1981), but is about 3 times as potent as TRH in respect of its ergotropic actions (Nagai et al. 1981) while TRH in the desamidated form has been shown to be devoid of both endocrine and extrahypothalamic TRH-like actions (Breese et al. 1975). The potency of DN 1417 as a respiratory stimulant is in agreement with reported results in studies of other parameters such as motor activity or arousal from pentobarbital induced sleep (Miyamoto et al. 1981). As DN 1417 lacks endocrine activity it is very unlikely that the respiratory stimulant effect of TRH is related to the endocrine action of the peptide. As the desamidated form did not induce tachypnoea the respiratory stimulant action of TRH is most probably related to its extrahypothalamic action. The onset of the respiratory stimulation seen after DN 1417 as well as after TRH was short and no differences in duration were seen.

The net respiratory effect of TRH was an increase in inspiratory drive seen as increase in V_T/T_I as well as $P_{0.1}$. Respiratory timing (T_I/T_{TOT}) decreased, however the magnitude of the decrease was proportionally less than the increase in inspiratory drive ($V_E=V_T/T_I \times T_I/T_{TOT}$) (Goldberg et al. 1977). Moreover, the results indicate an increase in the gain of the inflation reflex loop, i.e. an increased sensitivity to afferent vagal impulses which in turn would result in an earlier off-switch of the inspiratory phase. This might be a possible explanation to the decrease in V_T seen after TRH. The increase in f could however not be explained by a change in the bulbopontine setting for T_I or T_{TOT} as these parameters were not significantly changed.

When TRH was administered into the fourth ventricle the dose response curve for the respiratory stimulation was slightly shifted to the left. This effect was seen on f as well as V_T. The shift might be due to an easier access of the drug to areas sensitive to TRH. Therefore, it might be speculated that the primary site of action is in the vicinity of the fourth ventricle. Yamamoto et al. (1981), using a model with urethane anesthetized rabbit pups, reported a tachypnoea after TRH administered i.p. or applied to the surface of the intact brain. These authors also found that this stimulating effect on breathing was abolished after section of the brain stem at the collicular level of unanesthetized animals. However, when TRH was applied locally into the fourth ventricle no respiratory changes were seen. In this model the medulla was exposed in contrast to the method used in our experiments. Thus, different diffusion possibilities for the drug could well account for the differences in results obtained.

In an attempt to further localize the site of action, we have performed local injections into nucleus tractus solitarii which is believed to contain the primary respiratory occilator (Berger et al. 1979). Local injections in some cases led to immediate effects, mainly apnea and hypotension. This has earlier been reported by other authors (Talman & Reis 1981) and is suggested to be an effect of local distortion, as primary afferent baroreceptor nerve fibers terminate in or pass through this region (De Jong & Palkovits 1976). However, in most cases a frequency stimulation and a decrease in V_T was seen after an initial delay of 10 min, suggesting that the respiratory stimulant effects of TRH are elicited in areas rostral to the brain stem possibly, for example, in the hypothalamic region. In preliminary studies, where TRH was given in the lateral ventricles to vagotomized halothane anesthetized animals, $\dot{V}_E$ was still increased. However, in this case, an increase in V_T, not f, was the cause of the increased minute ventilation (Mueller et al., personal communication). Thus, changes in afferent input at the brain stem level can modulate the presumed indirect effects of TRH on respiratory neurones. In fact, the particular pattern of breathing elicited by TRH administration (low V_T and high f) appears to be very similar to the breathing pattern, "hypoxic tachypnea" elicited by oxygen deprivation in carotid body-denervated animals (see e.g. Gautier & Bonora 1980). This type of breathing has been suggested to be elicited by influences from posterior diencephalic and upper midbrain levels on brain stem neurones.

In case of an indirect action, the respiratory effects of TRH are most probably mediated though secondary pertubation with other neurotransmitter systems. Nagai et al. (1981), measuring the interaction of DN 1417 with reserpine and pentobarbital in local cerebral glucose utilization, suggested that dopaminergic and cholinergic mechanisms might be involved. However, in our study neither methylatropine or haloperidol effectively blocked the increase in $\dot{V}_E$ in response to TRH. After reserpine the $\dot{V}_E$ response was more or less abolished, but a significant decrease in V_T was still seen.

The analeptic actions of TRH have been proposed to be related to an inhibition of GABA function (Cott & Engel 1977). GABA given i.c.v. to halothane anesthetized rats induces an immediate depression in f and V_T (Hedner et al. 1981 *d*). In the present study, TRH given to animals pretreated with GABA in a low dose reversed the respiratory depression induced by GABA and even resulted in a relative "overshoot" in f after 10 and 15 min post-injection. When GABA was given to animals pretreated with TRH a potentiation of the TRH effect was noted i.e. a decrease in V_T and an increase in f was seen. It is therefore possible that these two systems are also closely related in terms of their effects on central respiratory regulation.

Pretreatment of animals with naloxone induced a slight increase in f. When TRH was administered to these animals, there was a more rapid increase in f and the peak value was higher. Another putative peptide neurotransmitter, substance P, has been shown to act antagonistically to endorphines (e.g. Jessel & Iversen 1977). Reports exist on potentiation of substance P induced respiratory stimulation by naloxone (Yamamoto et al. 1981). No such interaction has been shown concerning TRH. However, endorphines and enkephalines have a depressive influence on the respiratory performance (Florez et al. 1980, Hedner et al. 1981 *a*) and it may be that the endogenous opioids have a tonic negative influence on respiratory activity. Thus, when this influence is blocked by antagonists one could speculate that respiratory stimulant effects of TRH would be more easily induced.

TRH treatment did not increase the sensitivity to CO_2. In fact, the obvious effect of increasing amounts of CO_2 in the inhalation gas mixture was an abrupt decrease of f. A similar effect has been described in animals with hypoxic tachypnea where hypercarbia restores ventilation (Gautier & Bonora 1980). This observation further supports the concept of a suprapontine site of action for TRH in respiratory regulation.

In conclusion, TRH has a respiratory stimulant action which seems to be localized primarily to areas rostral to the brain stem. Moreover, interference with several other transmitter systems result in a modification of the respiratory stimulant effect of TRH. Therefore, a high degree of integrating mechanisms seem to prevail within the neuronal populations involved in the regulation of respiration.

This work was supported by the Swedish Medical Research Council (grant nos 2464 and 2862). We thank Mrs Birgit Andersson, Mrs Gunilla Jonason and Ms Kristina Kåmark for excellent technical assistance.

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