TRH microdialysis into the RTN of the conscious rat increases breathing, metabolism, and temperature

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Cream, Carlos, Eugene Nattie, and Aihua Li. TRH microdialysis into the RTN of the conscious rat increases breathing, metabolism, and temperature. J. Appl. Physiol. 87(2): 673–682, 1999.—Thyrotropin-releasing hormone (TRH) injected into the retrotrapezoid nucleus (RTN) of anesthetized rats produces a large, prolonged stimulation of ventilatory output (C. L. Cream, A. Li, and E. E. Nattie. J. Appl. Physiol. 83: 792–799, 1997). Here we inject or dialyze TRH into the RTN of conscious rats. In 6 of 17 injections (200 nl, 3.1 ± 1.7 mM), ventilation (Ve) increased 31% by 10 min, with recovery by 60 min. With dialysis, each animal of one group (n = 5) received, in random order, 10 mM TRH, 10 mM TRHOH (a metabolite of TRH), and artificial cerebrospinal fluid (aCSF); each animal of a second group (n = 5) received aCSF and 1 mM TRH. TRHOH, and aCSF had no sustained effects. TRH (1 mM) increased Ve (32%, P < 0.02, by 10 min, with recovery by 60 min), O2 consumption (VO2; 19%, P < 0.03), and body (rectal) temperature (T R; 0.5°C, P < 0.09). TRH (10 mM) increased Ve (78%, P < 0.01, by 10 min, with no recovery at 60 min), VO2 (48%, P < 0.01), and T R (1.0°C, P < 0.01). TRH also induced arousal. The tissue volume affected in dialysis, estimated by spread of dialyzable fluorescent (332.3 mol wt, mol wt of TRH = 362.4), was 1,580 ± 256 nl for 10 mM (n = 5) and 590 ± 128 nl for 1 mM (n = 5). We conclude that 1) the RTN is involved in the integration of Ve, VO2, T R, and arousal and 2) TRH may establish the responsiveness of RTN neurons.

METHODS

General preparation. Animals weighing 300–400 g at the time of surgery were anesthetized with ketamine (100 mg/kg im) and xylazine (20 mg/kg ip). The crown of the skull was shaved and sterilized with alcohol. The rat was placed into a Kopf stereotaxic holder, and for the injection experiments in 12 rats a 0.46-mm-OD stainless steel microinjection cannula was placed into the region of the RTN. For the dialysis experiments in 10 rats a 38-mm-OD dialysis guide tube (no. 11, CMA, Acton, MA) was inserted into the brain stem. The coordinates for probe placement were 2.2 mm caudal and 1.8 mm lateral from lambda and 10.6–10.8 mm below the dorsal surface. The steel cannula or guide tube was secured to the
skull with cranioplast cement, and the wound was sutured. For
the dialysis experiments the abdominal surface was
shaved and sterilized, an incision was made through the linea
alba, and a sterile telemetric temperature probe (model
TA-F-20, Data Sciences, St. Paul, MN) was placed in the
abdominal cavity. The incision was closed, and the animal
was allowed to recover for 3–4 days.
On each experimental day between 0900 and 1500 the rat
would be weighed, then gently held while the dummy cannula
filling the guide tube was removed and the injection cannula
or the dialysis probe was inserted into the guide tube. Animals
were placed into a Pappenheimer-modified plethys
mograph box (19) and allowed 30–40 min to acclimate.
Control measurements were taken in room air. For the
injection experiments, animals received TRH (200 nl, 0.5–10
mM), and VE was monitored by using the whole body plethys
mograph. Measurements were taken over a 20- to 30-s period
at 0, 5, 10, 20, 30, 40, and 50 min. Vo2 and T re were not
monitored during this experiment. T re was measured by
rectal probe at the beginning and end of the experiment.
There were 17 injections in 12 rats. For the dialysis ex-
periments, baseline Ve, Vo2, and T re were measured. The
dialysis pump was run for 30 min at a speed of 4.5 µl/min, and
measurements were taken over a 20- to 30-s period at 0, 5, 10,
20, 30, 40, 50, and 60 min. In the dialysis experiments, each
animal in one group (n = 5) was subjected, on separate days,
to three dialysis conditions selected randomly: artificial ce-
rebrospinal fluid (aCSF), 10 mM TRH in aCSF (pGlù-His-Pro-
NH2, TRH; catalog no. P-216L, Sigma Chemical), or 10 mM
TRH-free acid in aCSF (pGlù-His-Pro-COOH, TRHOH; cata-
log no. P-3905, Sigma Chemical). In the other dialysis group
(n = 5), each animal was dialyzed with aCSF and 1 mM TRH.
TRH and control testing were separated by a 24- to 72-h
recovery period. Separate rats were dialyzed for 30 min
with 1 mM (n = 5) or 10 mM (n = 5) fluorescein dye (332 mol wt;
catalog no. F-1300, Molecular Probes) to estimate the volume
distribution of TRH.
The plethysmograph chamber used in these experiments is
similar to the setup described by Pappenheimer (19). The
analog output of the pressure transducer was recorded on a
strip-chart recorder (model 1200, Honeywell) and a pulse-code
modulation system (model 3000A, Vetter). The animal cham-
ber is designed to operate at atmospheric pressure; thus the
inflow and outflow of inspired gas must be balanced to
prevent hyper- or hypoboric conditions in the box. The inflow
air was humidified, and the flow rate was controlled by a
flowmeter (model 7491T, Matheson). The outflow port
was connected to the in-house vacuum system, and a high-
resistance “bleed” of the outflow line provided ~100 ml/min of
outflow gas to the O2 analyzer (model SA-3, Applied Electro-
chemistry). The flow rate through the plethysmograph was
maintained at ≥1.4 l/min to prevent CO2 rebreathing.
The plethysmograph chamber was calibrated with 0.3-ml injec-
tions. The chamber temperature was measured before each
period of ventilatory data collection. Rat T re was measured at
the beginning and end of each experiment in the injection
group and during each period of ventilatory data collection by
use of the analog output from the telemetric temperature
probe in the peritoneal cavity in the dialysis groups.
At the conclusion of the experiments, animals were killed,
and the medulla was quickly removed and placed in 4%
paraformaldehyde until the time of sectioning. Brain stems
were frozen, then sectioned at 50-µm thickness with a Reichert-J
ung cryostat. Sections were counterstained with cresyl violet to identify anatomic landmarks and the site of
diaryls probe placement with the assistance of a rat brain
atlas (20). The guide tubes were removed postmortem but
before the brain stem was removed and sectioned. Removal of
the guide tubes required manipulation and produced tissue
disruption. This facilitated the anatomic verification of guide
tube and probe tip location but also increased the volume of
tissue disruption compared with that produced by simple
insertion. In the animals dialyzed with fluorescein, the tissue
was frozen and sectioned without fixative, then analyzed for
fluorescence.

Respiratory data were transferred from the Vetter system
to the DataPac III software system. With use of the DataPac
II system, a breath-by-breath analysis was performed, with
the pressure differences and the respiratory cycle time for
each breath being determined over a 20- to 30-s time period.
The data were exported to Sigmaplot 4.0 (Jandel Scientific),
etidial volume (VT) per 100 g body weight, frequency, and
Ve were calculated for each breath. Vo2 was determined by
subtracting the outflow O2 (constant flow rate 1.4–1.5 l/min)
from the inflow O2, dividing by body weight, and multiplying
by 60 to obtain an hourly rate (ml·g⁻¹·h⁻¹). The inflow O2
content was calibrated at the beginning of each experiment,
and the flow rate of gas was set. The outflow content of O2
was read from the O2 sensor.

The results for Ve, VT, frequency, Vo2, and T re were
compared within and between each of the experimental
groups with treatment (aCSF, 10 mM TRH, 10 mM TRHOH
and aCSF, 1 mM TRH) and time as factors with a one-way and
two-way repeated-measures ANOVA (Sigmaplot, Jandel Sci-
entific SYSTAT). A post hoc Tukey test was performed when
significant differences were found.

RESULTS

Controls. Injections of aCSF had no significant sus-
tained effect on Ve, and only 6 of 17 injections of TRH
resulted in an increase in Ve of >10% of baseline, an arbi-
trary definition of a response in these injection experi-
ments. With dialysis the responses of Ve, V02, and T re
to aCSF were small, unsustained, and variable and did
not differ between the two groups of rats (n = 5 in each)
that received dialysis. These data have been pooled
(n = 10) and are shown as the responses to aCSF
dialysis. The responses of Ve, V02, and T re to dialysis
with the TRH metabolite TRHOH (10 mM) were not
significantly different from those obtained with dialysis
with aCSF. These data are not shown.

Ve. Figure 1 shows the responses of Ve expressed
in absolute terms (A) and as percent baseline (B), to
the six injections of TRH that produced a response. In these
responders, Ve increased to 31% of baseline at 10 min
from a baseline value of 74 to 97 ml·min⁻¹·100 g⁻¹. By
60 min, Ve returned to baseline levels. The response
included an increase in VT and frequency. The average
TRH concentration of these six injections was 3.1 ±
1.7 mM.

Figure 2 shows responses of Ve expressed in abs-
olute terms (A) and as percent baseline (B), to dia-
lysis in the RTN of aCSF, 1 mM TRH, and 10 mM TRH. At 1
mM, TRH increased mean Ve by 29 and 32% at 5 and 10
min from a control value of 68.5 to 88.3 and 90.3
ml·min⁻¹·100 g⁻¹, respectively. By 40 min, 10 min after
dialysis was stopped, Ve returned to within control
values. This response was significant (P < 0.02) and
included increases in VT and frequency, although the
latter did not reach significance (P < 0.08). At 10 mM,
TRH dialysis increased mean V˙E by 78% from 79.3 to 141.4 ml·min⁻¹·100 g⁻¹ after 5 min of dialysis. The increase in V˙E was significant (P < 0.01). V˙E remained significantly elevated relative to aCSF for the remaining 55 min. The response was significant (P < 0.01).

T_{re} Figure 3 also shows the responses of T_{re}, expressed in absolute terms, to dialysis in the RTN of aCSF, 1 mM TRH, and 10 mM TRH. At 1 mM, TRH increased mean T_{re} by 0.5°C at 40 min from a control value of 38.1 to 38.6°C. This response was not significant (P < 0.09). At 10 mM, TRH dialysis increased mean T_{re} by 1.0°C from 37.7 to 38.7°C after 30 min of dialysis. T_{re} remained elevated relative to aCSF for the remaining 55 min. The response was significant (P < 0.01).

V˙E and V˙O₂. Figure 4 shows V˙E and V˙O₂ expressed as percent baseline for 10 mM (A) and 1 mM (B) TRH dialysis. Although there is a suggestion that the increase in V˙E is greater than the increase in V˙O₂ in the first 5–10 min with 10 mM TRH dialysis, for the most part the increase in V˙E seems to match the increase in V˙O₂. There is sufficient variability in the V˙E and V˙O₂...
results that arterial blood-gas sampling is needed to show conclusively whether there is hyperventilation.

Arousal. We also made subjective evaluations of the arousal state of each rat. Normally, after the period of acclimatization in the plethysmograph, rats will rest quietly and no longer explore the chamber. After a time they will often appear to fall asleep, curling up with their eyes closed and lying motionless on the floor. We often needed to arouse them by gently tapping on the side of the chamber during control or TRHOH dialysis. We noted that with TRH treatments the rats became more aroused. They would not sleep, they would rise from the prone position, and they would stand on all four legs looking alert or once again exploring the chamber as during their initial acclimatization. They appeared to be more vigilant.

Anatomy. Figure 5 shows single medullary cross sections from each of the five rats in the group that was dialyzed with 1 mM TRH. Each cross section is approximately at the center of the site at which the dialysis probe tip was located in vivo. The cresyl violet stain allows one to easily detect the tissue disruption produced by the probe (rectangles). The size of the disruption is not indicative of the region of tissue disruption in vivo, in that it was difficult to remove the guide tube at the time the animals were killed without enlarging the region of tissue damage. Nevertheless, this anatomic analysis shows the location of the probe tip. In each case it resulted in TRH application to the RTN during dialysis. The probe tips in the five rats that were dialyzed with 10 mM TRH are similarly located (data not shown), and the six injections of TRH shown in Fig. 1 are also similarly located in the RTN (data not shown).

Figure 6 shows in a single rat the spread within the medulla of the fluorescein dialyzed at 10 mM for 30 min. The approximate locations of each section as referenced to the bregma are shown Fig. 6. The bottom right is the same as top right but is stained with cresyl violet to show the traditional anatomic landmarks. The rectangle marks the region of tissue disruption. We...
estimated the volume of distribution by measuring the area for one of each five successive cross sections and calculating the volume for a cylinder of that area and length. All such cylinders were summed to yield a total volume, which for this example is 1,460 nl. Figure 7 shows a similar analysis for dialysis with 1 mM fluorescein. The probe tip was similarly located within the RTN region, but the region of spread was much less; for this example it was calculated to be 700 nl.

In the group results, with dialysis for 30 min with 1 mM fluorescein (n = 5), the largest and smallest radii of the largest cross-sectional area were 631 ± 108 and 478 ± 54 µm, respectively (average 555 µm), and the rostral-to-caudal extent of the distribution was 870 ± 66 µm. The average estimated volume of distribution was 590 ± 128 nl. With dialysis for 30 min with 10 mM fluorescein (n = 5), the largest and smallest radii of the largest cross-sectional area were 854 ± 105 and 553 ± 98 µm, respectively (average 704 µm), and the rostral-to-caudal extent of the distribution was 1,400 ± 127 µm. The estimated volume of distribution was 1,580 ± 256 nl.

DISCUSSION

Conscious rats, V\textsubscript{E}, and TRH. The major findings in this study are that focal application of TRH into the RTN region of the conscious rat increases V\textsubscript{E}, V\textsubscript{O2}, and T\textsubscript{RE} as well as the level of arousal. We used two methods to apply the TRH focally: microinjection and microdialysis. Of 17 microinjections (200 nl), all in the RTN region as judged by postmortem identification of fluorescent
beads mixed into the aCSF, only 6 increased $V_e$. The mean TRH concentration of these six injections was 3.1 mM. The ventilatory response magnitude and time course for these six injections are similar to those with dialysis over 30 min of 1 mM TRH (Figs. 1 and 2), with recovery within 60 min in each case. However, with dialysis, all five rats showed a response suggesting a larger region of TRH distribution. With dialysis of 10 mM TRH, again all five rats showed a response that was greater and longer lasting than that after dialysis with 1 mM TRH or the microinjections.

Results from another study of TRH effects on respiration in conscious rats were qualitatively similar (25). Microinjection of TRH (0.025–5 mM) into the cerebral ventricles increased $V_e$ by 235% (vs. 78% in our study) and $V_{O_2}$ by 11.7-fold (vs. 48% in our study). These quantitative differences are likely due to the route of administration. Injection into the cerebral ventricles affects a much larger region than our local dialysis.

In an earlier study in anesthetized rats with microinjection of TRH (20 nl, 0.5–10 mM) into the RTN, we observed substantial effects (up to 110% increase in ventilatory output) that were long lasting (up to 4 h). The magnitude of this response is similar to that in the conscious rats with 10 mM dialysis and much larger than that observed with 1 mM dialysis or with the larger microinjections (200 vs. 20 nl) of similar TRH concentrations in conscious rats. The responses in the anesthetized rats also lasted longer, up to 4 h, than any of those in conscious rats. This greater effectiveness of the TRH injections in anesthetized rats is unexpected and difficult to explain. We expect that our TRH delivery was at higher concentrations to a wider area in the dialysis experiments, and in the conscious rat microinjection experiments the injection volume was 10 times greater. Slower metabolism and/or clearance of TRH under anesthesia or the presence of suppressive influences from higher brain regions in the conscious rats could be playing a role in these differences in response. Also the anesthetized animals were ventilated to an end-tidal $CO_2$ that was just above the apneic threshold. Consequently, the amplitude of the baseline phrenic burst was relatively small and may have had a larger margin for response than did $V_t$ in the conscious state.

Fig. 6. Tissue distribution of fluorescein dye dialyzed at 10 mM shown as a series of cross sections labeled relative to bregma. Cross section at bottom right is same as that at top right but stained with cresyl violet to demonstrate location of facial nucleus (VII). Rectangle, region of tissue disruption. Volume of distribution of fluorescein is 1,460 nl.
animal. Finally, there is evidence that suggests a greater physiological role of the RTN region in anesthesia. Lesions of the RTN in the anesthetized rat decrease eupneic respiration, often to apnea, and CO₂ chemosensitivity, often abolishing it (16), whereas lesions in conscious rats do not affect eupnea and decrease CO₂ responsiveness only 39% (1). It seems plausible that RTN function is arousal state dependent, and arousal state modulates the level of response to any given RTN manipulation.

Our study in the anesthetized rat (6) also suggested that subpopulations of neurons within the RTN are responsive to TRH. When an injection was ineffective, we could easily reposition the pipette to find a reactive site because of the ventral approach used in those experiments. That only 6 of 17 injections in the conscious rats produced a response supports this interpretation. In fact, we switched to the microdialysis technique to establish a concentration gradient that would facilitate exposure of more neurons of the RTN to TRH in each trial without the tissue disruption produced by large injections. We appear to have achieved this, inasmuch as all animals treated by dialysis responded to TRH.

\( V_{\text{O}_2} \) and \( T_{\text{re}} \) increased significantly in response to dialysis with 1 or 10 mM TRH; it was not measured in the microinjection experiments. The mechanism of the increased metabolic rate is unclear, although such an effect was also observed in prior studies with intracerebral TRH administration (26). In our case, the TRH application was focal, and we still observed these significant changes in metabolic rate.

The \( T_{\text{re}} \) response to TRH administration in the medulla has not been previously described. The response was present with both dialysis concentrations, although the change with 1 mM dialysis did not reach significance. It is difficult to determine whether the temperature response was due to RTN projections with effects in the thermoregulatory centers in the hypothalamus or whether it was merely secondary to increased \( V_{\text{O}_2} \). The time courses of the \( T_{\text{re}} \) and \( V_{\text{O}_2} \) responses were similar. Unpublished findings from our laboratory support a possible direct thermoregulatory action of TRH in the RTN. Retrograde tracing studies demonstrate...
that the RTN receives input from the raphe magnus and the subceruleus. Both of these nuclei receive and integrate thermoreceptor input and, when stimulated, are capable of producing hyperthermia (3, 9). The RTN itself may aid in the integration of this information and couple the thermogenic response to the appropriate level of VE.

In homeotherms, VE, T_{re}, and VO_{2} are tightly linked. For example, exposure to cold temperatures activates thermogenic responses; this increases VO_{2}, and VE increases to meet the increased demand for O_{2} (12). The sites of this integration have been undefined; we suggest that the RTN may be one of these sites.

TRH and arousal. TRH can antagonize the effects of various anesthetics (18, 23). TRH administered intravenously or into the cerebral ventricles decreases anesthetic-induced narcosis and hypothermia (23). A possible site of this TRH action is the brain stem reticular formation (27). Intravenous TRH increases the firing rate of reticular field neurons and decreases the threshold for arousal changes in the frontal cortex (26).

In the conscious rats we noted that during aCSF or TRHOH dialysis the animals would quickly acclimate to being in the plethysmograph and enter sleep, as defined by behavioral criteria. When TRH was dialyzed into these same animals, they did not sleep. They would remain upright on all fours and, with the 10 mM dose, would appear hypervigilant. This effect of TRH on arousal was unexpected, and it may have contributed to the increase in VO_{2}. If arousal and VO_{2} are increased, T_{re} and VE may have increased as a necessary result.

Tissue spread with dialysis. With microdialysis, it is difficult to determine the tissue concentration profile of TRH. It appears certain that the concentration in the dialysate is much higher than that detected outside the probe. Alessandri et al. (2) found 35% delivery into the tissue of glutamate (10–1,000 mM at 2.5 µl/min) during the first 30 min of dialysis. If applied to our case, the TRH concentration next to the probe would be 0.35–3.5 mM for our 1 and 10 mM doses. We used a higher flow rate, 4.5 µl/min, so this estimate is likely to be on the high side. In a study using two dialysis probes, one for delivery and the other for collection and measurement, the steady-state concentrations of drugs 1.5 mm away from the probe were 10–100 times lower than the concentration in the probe (7). Neither of the drugs studied are metabolized in the CNS. TRH, on the other hand, is metabolized, and its concentration drop would be expected to be greater and its spread less. Even so, using these data, for the 1 mM dose we could expect maximum TRH concentrations of 0.35 mM at the probe and between 0.01 and 0.1 mM at 1.5 mm from the probe. For the 10 mM dose, these would be 3.5 mM at the probe and between 0.1 and 1.0 mM at 1.5 mm from the probe.

Our experimentally measured estimate of the tissue volume affected by TRH comes from dialysis of a fluorescent molecule with approximately the same molecular weight as TRH (332.3 vs. 362.4). Because this molecule is not metabolized, the volume of distribution for TRH is arguably smaller. The average (n = 5) volume of dye distribution when dialyzed at 1 mM was 590 nl, the average rostral-to-caudal length of the fluorescein distribution was 870 µm, and the average radius of fluorescein distribution at the cross section with the largest area of distribution was 555 µm. Thus, for 1 mM TRH dialysis, using the fluorescein distribution, we estimate a spread of 555 µm radially in the plane of the cross section and 435 µm in the rostral or caudal direction. For 10 mM TRH dialysis (n = 5) the fluorescein distribution volume was 1,580 nl, with a rostral-to-caudal length of 1,400 µm and an average radius of 704 µm at the cross section with the largest area. For the 10 mM TRH dialysis, we estimate a spread of 704 µm radially in the plane of the cross section and 700 µm in the rostral or caudal direction.

The volume of distribution for the larger dose, 10 vs. 1 mM, was almost three times greater; the radial spread was 27% greater. Our estimate of spread within the medulla, as measured by dialysis and detection of fluorescein dye, is less than that deduced from published tissue concentrations of other substances dialyzed in other brain regions (7). With use of either approach, the distribution of TRH with dialysis of 1 mM TRH is likely to be largely, if not entirely, within the RTN region. With the 10 mM dose, the TRH is focused in the RTN region, but its spread probably involves contiguous regions, including the facial nucleus, the juxtafacial portion of the nucleus paragigantocellularis lateralis, the parapyramidal region, and possibly the medullary raphe.

We believe that the microdialysis approach is a useful method for administration of neuroactive substances to specific brain sites in a conscious animal model. The probe tip containing the semipermeable membrane is 1 mm long and 240 µm diameter, with a volume of 45 nl. Thus its size and the region of tissue disruption are similar to that after an injection of this volume. On the basis of the reliability of the physiological responses and the distribution of the fluorescein dye after dialysis, the volume of tissue affected is greater than that affected by a 10- to 20-nl injection or even a 200- nl injection. Once the probe tip is in place, no further tissue disruption takes place, and repeated dialyses can be performed in the same animal. The major problem with this technique is the delineation of the exact region in the tissue that is affected by the dialyzed substance and the determination of the concentration of the substance at various distances from the probe. This problem also exists for the microinjection approach.

Physiological significance. The RTN receives input from respiratory, raphe, and reticular sources (unpublished observations) and has efferent connections with the respiratory control network and the limbic system (22, 28). Although the RTN has not been identified as a major site containing ir-TRH or TRH receptors, several sources of RTN input contain ir-TRH and are probable TRH connections to the RTN. Approximately 25–30% of serotonin neurons of the caudal raphe contain ir-
TRH and represent the most likely local source of TRH to the RTN (11, 26).

At the cellular level, TRH inactivates K+ channels normally active at the resting membrane potential, which depolarizes the neurons and increases membrane responsiveness (21). This action amplifies the neural response to afferent inputs and may increase subsequent motor output. The physiological settings in which TRH is normally released are unknown. The activity of the caudal raphe is arousal state dependent, being highest with wakefulness and lowest or absent during rapid-eye-movement sleep (10). The change in activity level is presumably associated with variations in the amount of the neurotransmitters released, including TRH. Decreased TRH in the RTN would lead to relative hyperpolarization of the neurons and diminished neuronal activity. This may be manifest most clearly during rapid-eye-movement sleep when V^E, T, and V^O2 become uncoupled, during presumed RTN relative inactivity.

The behavioral arousal effects of TRH in the RTN cannot be adequately explained from our present experiments. Anesthetized rats treated with TRH in the RTN required more frequent additions of anesthesia than in our standard laboratory protocol, and conscious rats did not sleep when dialyzed with TRH in the RTN. This may reflect direct actions of RTN neurons on the reticular activating system to increase arousal. Alternatively, the arousal effects may be secondary to V^O2, T, and V^E changes initiated in the RTN and coupled to arousal at a remote site.

We conclude, that TRH in the RTN can alter the integration of V^E, T, V^O2, and arousal. The lack of a TRH receptor antagonist precludes us from blocking any effects of endogenous TRH, but these striking responses in conscious rats hint at an important role for TRH, possibly as a state-dependent modulator of the control of breathing.

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RECOMMENDATIONS


