Thyrotropin-Releasing Hormone Given Intrathecally to the Rat Increases Arterial Pressure and Heart Rate

Kiran Yashpal, S. Gauthier, and J.L. Henry

In view of evidence implicating thyrotropin-releasing hormone (TRH) as a chemical mediator of synaptic transmission onto spinal sympathetic neurons, this peptide was administered intrathecally, in a dose of 6.5 nmol, at the T9 and T2 spinal levels in the anesthetized rat. At the lower thoracic level TRH increased arterial pressure and heart rate; these effects peaked at 4–7 minutes and decayed over the next 15–20 minutes. At the upper thoracic level the pressor and cardioacceleratory responses were roughly similar in time course but were smaller in magnitude. Hexamethonium (10 mg/kg i.v.) was tested on the responses from the lower thoracic level; both pressor and cardioacceleratory responses persisted after hexamethonium pretreatment. In addition, intravenous administration of 6.5 nmol of TRH failed to alter arterial pressure or heart rate, suggesting that the effects produced by the intrathecal administration of TRH were due to an action of the peptide in the spinal cord. The results also indicate that the pressor effect and the increase in heart rate may be mediated in the sympathetic ganglia at least partly via nonnicotinic transmission. Our results provide physiological support for the possibility that TRH is a chemical mediator of synaptic transmission onto sympathetic preganglionic neurons. This study indicates that the functional sympathetic pathways utilizing TRH as a chemical mediator include those regulating arterial pressure and heart rate. (Circulation Research 1989;65:859–868)
inner tip lay at the T2 or T9 level; spinous processes were used as landmarks. In preliminary experiments, the validity of this method for correct positioning of the inner tip of the catheter was confirmed in radiographs of rats implanted with wire-filled catheters (see Yashpal et al., Figure 1). TRH was administered intrathecally via this catheter.

A second catheter (Intramedic PE-60) was inserted into the left common carotid artery facing the heart for monitoring of arterial pressure via a Statham transducer (model P23 ID, Gould, Cleveland, Ohio) connected to a polygraph (Grass Instruments, Quincy, Massachusetts). Heart rate was calculated from this record. Systolic and diastolic pressures were measured from the ratemeter records, and mean arterial pressure was calculated from these measurements. The number of heart beats in a 10-second period was counted, and that number was multiplied by six to obtain heart rate in beats per minute.

In experiments in which agents were administered intravenously, a third catheter (Intramedic PE-60) was inserted into the left femoral vein facing the heart. Animals respired spontaneously throughout the experiments. Rectal temperature was maintained at approximately 37°C by use of a heating pad.

**Peptide Administration**

After surgical preparation, a 30-minute period was allowed for stabilization. Baseline readings of arterial pressure and heart rate were taken over a 5-minute period, and TRH (Peninsula Laboratories, Belmont, California) was administered intrathecally at either the T2 or the T9 level in a dose of 6.5 nmol delivered over a period of 15–20 seconds in 10 μl artificial cerebrospinal fluid (CSF), an aqueous solution of (mM) 128.6 NaCl, 2.6 KCl, 2.0 MgCl₂, and 1.4 CaCl₂.

In preliminary experiments at T9, 6.5 nmol of TRH was found to be the minimum dose that would reliably alter arterial pressure and heart rate. As one of the objectives of this study was comparison of the relative potency of TRH at T9 and at T2, this was the only dose used in this study; thus, comparison was made on the basis of magnitude of change. After delivery of the peptide, the intrathecal catheter was flushed with 10 μl CSF (the internal volume of the catheter was approximately 8 μl). With zero time being the time of injection of CSF, readings of arterial pressure and heart rate were taken each minute for the next 15 minutes and then at 20 and 30 minutes.

**Block of Ganglionic Nicotinic Transmission**

For determination of the involvement of ganglionic nicotinic transmission in mediation of any changes in arterial pressure and heart rate, the experiments with intrathecal administration were repeated but in animals that had been given hexamethonium; the rationale was that the persistence of a response to TRH would suggest either that the effects were mediated at least partly via nonnicotinic ganglionic transmission or that the peptide was passing into the circulation and expressing its effects via a peripheral site of action. In these experiments, after baseline readings had been taken, hexametho-
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Sodium bromide was administered via the intravenous catheter in a dose of 10 mg/kg body weight (concentration of 10 mg/ml in saline). Three more readings were taken at 1-minute intervals, TRH was administered intravenously as above, and readings were taken each minute for the next 10 minutes.

Intravenous Administration of TRH

Given the possibility that TRH was producing its effects via a peripheral mechanism after passage from the perispinal space into the circulation, the experiments described above were repeated, but TRH was administered intravenously rather than intrathecally. The protocol was otherwise the same as above. The peptide was dissolved in 0.5 ml saline, and the catheter was flushed with 0.5 ml saline.

Statistical Analysis

Results from each rat were tabulated as systolic and diastolic arterial pressures and heart rate. Changes in these parameters from the baseline values were also determined. Data for the figures were summarized by taking the mean±SEM of the values from each group of rats at each minute after administration. To detect evidence of significant treatment/time interaction, a one-between (TRH vs. CSF), one-within (time) analysis of variance was done. Subsequent pair-wise comparisons of means were made by use of the Tukey’s wholly significant difference test.

Results

The rapid onset of the responses in these and in previous experiments in our laboratory supports a local site of action in the vicinity of the level of injection rather than a more remote action elsewhere in the central nervous system. A restricted distribution of the injectant has been reported in various other studies on such varied substances as radiolabeled peptides, morphine, and dyes, and we recently found that the region of penetration of another peptide, substance P, into the spinal cord extends only approximately 1 cm rostrocaudally when the injection procedure was similar to that in the present study.

Intrathecal administration of TRH had no effect on respiratory frequency, which remained at a mean value of approximately 100 breaths/min.

Effects of TRH at the T9 Level

Intrathecal administration of TRH at the T9 level increased both systolic and diastolic arterial pressures. These effects were transient, peaking at about 4–7 minutes after administration. Similar injection of the vehicle failed to alter systolic or diastolic pressure. The mean values of these pressures in the 14 TRH-treated rats and the 13 CSF-treated rats at each sample time are illustrated in Figure 1. When the mean value at each sample time was compared with the mean value before administration, the maximum change in systolic pressure was +16.6±3.5 mm Hg and occurred at 4 minutes; when the changes in systolic arterial pressure were compared between the two groups, the analysis of variance revealed a significant difference between the two groups of animals (p<0.001). The intrathecal administration versus time interaction (p<0.001), indicating that TRH induced an increase in systolic arterial pressure that varied over time. Tukey’s test indicated that the changes in systolic arterial pressure in TRH- versus CSF-treated animals were significantly different at 2–20 minutes (2 minutes, p<0.05; 3–10 minutes, p<0.01; 11–20 minutes, p<0.05). With regard to diastolic pressure, the maximum change was +19.3±4.3 mm Hg and occurred at 4 minutes. Again, the analysis of variance indicated a significant effect of TRH (p<0.001) and a significant intrathecal versus time interaction (p<0.001). Tukey’s test indicated that the changes in diastolic pressure in the two groups of animals were significantly different at 2–20 minutes (2 minutes, p<0.05; 3–20 minutes, p<0.001).

Heart rate was also increased by administration of TRH at the T9 level. The results are summarized in Figure 2. In this case the maximum change, +43.6±11.9 beats/min, occurred at 7 minutes. Before administration the mean heart rate was 332.2±8.0 beats/min in TRH-treated rats and 332.8±15.4 beats/min in CSF-treated rats. The changes from the baseline pressures were compared, the analysis of variance revealed that there was a significant difference between the means of the two groups of rats (p<0.025) but that there was no administration versus time interaction, indicating that the difference between the two groups persisted throughout the experimental period.

Effects of TRH at the T2 Level

With the same dose as used above (6.5 nmol), the effects of TRH at the T2 level were quantitatively and qualitatively different from those at the T9 level.

The effects on arterial pressure are summarized in Figure 3. The analysis of variance revealed no significant effect of TRH administration on systolic pressure (maximum change was +7.6±3.5 mm Hg at 3 minutes in TRH-treated animals and −1.6±4.9 mm Hg in CSF-treated animals). There was a significant difference in diastolic pressure between the two groups (p<0.025), and time was a factor in this difference (p<0.001); however, there was no departure from parallelism between the two groups, indicating that the increase persisted beyond the period of the experiment. In the TRH-treated group the greatest change in diastolic pressure from the mean preadministration value was +12.5±3.5 mm Hg at 6 minutes after administration of the peptide. In the CSF-treated group this change was +12.9±6.3 mm Hg at 6 minutes.

Heart rate increased significantly (p<0.025). Figure 4 shows the changes from baseline heart rate for the TRH- and CSF-treated groups. There
was no administration versus time interaction, indicating that the difference between the two groups persisted throughout the experimental period. During this period the greatest change in the TRH-treated group was +32.5±8.7 beats/min at 3 minutes. At this same time the change in the CSF-treated group was +4.1±2.0 beats/min. Before administration the mean heart rate was 320.0±15.6 beats/min in TRH-treated rats and 361.4±13.2 beats/min in CSF-treated rats.

Effect of Nicotinic Block on Responses to TRH

These experiments were done following the same general procedure as above for administration of TRH at the T9 level, except that 3 minutes before administration of TRH intrathecally, hexamethonium was given in a dose of 10 mg/kg i.v. to block nicotinic transmission in sympathetic ganglia. Mean baseline systolic and diastolic pressures in the six animals tested were 129±4.5 mm Hg and 52.0±0.9 mm Hg, respectively. Administration of hexamethonium decreased these pressures by approximately 50 and 15 mm Hg, respectively.

After the subsequent administration of TRH, systolic arterial pressure increased slowly, from 79.2±4.8 mm Hg just before TRH administration to a peak of 95.0±6.3 mm Hg at 9 minutes after TRH was given. When the two groups of animals were compared, the analysis of variance indicated that administration of hexamethonium had the same

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effect on both groups ($p>0.05$). However, it also indicated that the two groups differed after administration of TRH ($p>0.002$) and that there was a significant intrathecal versus time interaction ($p<0.001$), indicating that the increase in systolic pressure in TRH-treated rats varied over time. Tukey's test indicated that the systolic arterial pressures in the two groups of rats were significantly different at 1–10 minutes (1 minute, $p<0.05$; 2–9 minutes, $p<0.001$). Diastolic arterial pressure was 37.5±1.8 mm Hg just before TRH administration, and it increased to a maximum of 46.6±2.7 mm Hg at 6 minutes after TRH was given. The analysis of variance also indicated that the diastolic arterial pressure differed between the two groups after administration of TRH ($p<0.002$) and that there was a significant intrathecal administration versus time interaction ($p<0.001$). Tukey's test indicated that the diastolic pressures in the two groups were significantly different at 1–10 minutes (1 minute, $p<0.01$; 2 minutes, $p<0.05$; 3–9 minutes, $p<0.01$). These results are illustrated in Figure 5 along with results obtained from six control animals in which CSF replaced the TRH solution.

Mean baseline heart rate in TRH-treated rats was 384.7±19.3 beats/min, and in CSF-treated rats it was 320.2±13.8 beats/min. After administration of hexamethonium, heart rate increased by about 8 beats/min in both groups. After subsequent administration of TRH intrathecally at the T9 level, heart rate increased gradually throughout the following 8 minutes. Changes in heart rate after administration

![Figure 4](http://circres.ahajournals.org/)

**Figure 4.** Change in mean heart rate induced by administration of thyrotropin-releasing hormone (●, 6.5 nmol, $n=11$) or artificial cerebrospinal fluid (○, $n=14$) at second thoracic level. Vertical bars represent ±SEM. bpm, beats per minute.

![Figure 5](http://circres.ahajournals.org/)

**Figure 5.** Effects of intravenous administration of hexamethonium (left arrow, 10 mg/kg) on pressure responses in the rat to intrathecal administration (right arrow) of TRH (6.5 nmol, $n=6$) or CSF ($n=6$) at ninth thoracic level. Vertical bars represent ±SEM. TRH, thyrotropin-releasing hormone; CSF, artificial cerebrospinal fluid.
of CSF followed a different pattern; while the effects of hexamethonium were similar, after CSF was given heart rate did not change. The analysis of variance indicated that systemic administration of hexamethonium had the same effect on heart rate in the two groups of rats (p>0.05). It also indicated that the two groups differed after administration of TRH (p<0.001) and that there was a significant intrathecal administration versus time interaction (p<0.001), indicating that the increase in heart rate in the TRH-treated rats varied over time. Tukey's test indicated that the changes in heart rate in the two groups of rats were different from 7-10 minutes (7 and 8 minutes, p<0.05; 9 minutes, p<0.01; 10 minutes, p<0.05). The results obtained in these experiments are summarized in Figure 6.

**Effects of Intravenous Administration of TRH**

In view of the fact that TRH increased heart rate in rats treated with hexamethonium, the possibility was considered that TRH delivered into the intrathecal space might have passed into the circulation and expressed its effects, at least on heart rate, via a peripheral action. Therefore, the earlier experiments were repeated, except that the same dose of TRH was administered intravenously in a volume of 0.5 ml physiological saline. Before administration of the peptide the mean systolic and diastolic pressures of the four animals used were 124.5±11.2 and 62.0±6.3 mm Hg, respectively. As can be seen in the data summarized in Figure 7, intravenous administration of TRH failed to change either systolic or diastolic pressure.

The results for heart rate are illustrated in Figure 8. The mean heart rate before administration of TRH was 347.3±9.0 beats/min. Intravenous administration of TRH failed to change heart rate.

**Discussion**

This study has indicated that the intrathecal administration of 6.5 nmol of TRH to the T9 spinal segment increases arterial pressure and heart rate in the rat. Interestingly, the similar administration of TRH at the T2 level increased heart rate but had a relatively minor effect on arterial pressure. Systemic administration of the same dose of TRH had no effect on either arterial pressure or heart rate.

Administration of hexamethonium failed to block the increase in heart rate or the increase in systolic or diastolic arterial pressure produced by intrathecal administration of TRH at the T9 level. While it is possible that the effects of TRH on arterial pressure and heart rate were mediated via a peripheral action, this seems unlikely because when the same dose of TRH was given intravenously the peptide had no effect on these parameters. This lack of effect after systemic administration is supported by the earlier evidence that in the rabbit a considerably higher dose of TRH (2 mg/kg i.v., which is 300 times greater than the dose we used) was required to produce an increase in arterial pressure, and, interestingly, even at this relatively high dosage there was no change in heart rate. An increase in heart rate has been reported with intravenous administration of TRH in the Sprague-Dawley rat, but in this case 4 mg/kg was used, a dose 600 times greater than that used in our study.

Therefore, it is suggested that the effects of TRH were due to an action in the spinal cord. In view of the fact that nonnicotinic transmission has been reported to exist in sympathetic ganglia, it is possible that the increase in arterial pressure and heart rate produced by intrathecal administration of TRH is mediated at least partly by pathways using nonnicotinic transmission in the sympathetic ganglia or adrenals. As an alternative explanation, hexamethonium may have increased the sensitivity of peripheral receptors so that subliminal leakage could have had an effect on neurons in these areas. It is unfortunate for comparative purposes that in previous studies in which heart rate increases were observed in response to electrical stimulation of the intermediolateral nucleus, the effects of ganglion block were not studied.

If it is indeed the case, as suggested above, that the cardiovascular responses produced by intrathecal administration of TRH were due to an action within the spinal cord, this action might be considered an excitation of sympathetic preganglionic neurons for several reasons. TRH is contained in fibers and terminals around sympathetic preganglionic neurons in the intermediolateral nucleus. The presence of TRH-binding sites in the spinal cord, the physiological responses observed in...
the present study, and the excitatory effects of TRH on sympathetic preganglionic neurons\textsuperscript{26} raise the possibility that functional TRH receptors may exist in the spinal cord, perhaps on these lateral horn neurons.

The possibility must be considered that if TRH were acting within the spinal cord to elicit the effects, the site of action might be on sensory or motor neurons rather than on sympathetic neurons, thus activating sympathetic output reflexly. Little evidence exists for a possible action of TRH on sensory neurons in the dorsal horn. The TRH levels in this region are relatively low compared with the lateral and ventral horns,\textsuperscript{45,46} and TRH-containing fibers and terminals are not found there.\textsuperscript{24,25,44} TRH has been suggested to play a role in regulation of somatic motor output at the spinal level,\textsuperscript{25,47-49} and this must be considered in the interpretation of the results of the present study. However, such a mechanism can be excluded because motor effects have been observed only with much larger doses.\textsuperscript{47} Additional evidence excluding a somatic motor mechanism lies in the fact that motor effects were not observed in these experiments except for a mild tremor that was seen on some occasions. Finally, in another experimental paradigm in unanesthetized rats, a similar dose of TRH is without effect on reaction time in tail flick test\textsuperscript{50}, a test that involves a local motor response.

It was interesting to note the greater effects of TRH at the T9 than at the T2 thoracic segment, especially in view of the fact that another peptide, substance P, has a greater effect at the T2 level than at the T9 level.\textsuperscript{28} It is possible that at the lower thoracic level TRH stimulates sympathoadrenal preganglionic neurons while at the T2 level neurons in pathways to the heart were activated, and that TRH may have a preferential excitatory effect on the former type of neuron. In fact, if TRH receptors do exist in the spinal cord, as suggested above, the data raise the possibility that there is a greater preponderance of these receptors at the lower level and, therefore, that there is a differential segmental regulation of sympathetic output at the spinal level, at least as far as control via TRH-containing pathways is concerned.

\textbf{FIGURE 7. Effects of intravenous administration of TRH (6.5 nmol, n=4) on mean systolic and diastolic pressures in the rat. Vertical bars represent ±SEM. TRH, thyrotropin-releasing hormone.}

\textbf{FIGURE 8. Change in mean heart rate induced by intravenous administration of TRH (6.5 nmol, n=4) in the rat. Vertical bars represent ±SEM. bpm, beats per minute; TRH, thyrotropin-releasing hormone.}
This study was done in anesthetized animals for several reasons. As delivery of the peptide was at the spinal level, few central synapses were involved. In fact, if TRH acted on sympathetic preganglionic neurons, as is proposed, then no central synapses would be involved and, therefore, anesthetic effects should not affect the pathway. Furthermore, with this approach, arterial pressure could be measured reliably and easily by the direct method, and artificial changes in cardiovascular parameters due to changes in the behavioral states of an awake animal could be avoided. This latter point was considered to be especially important in view of the behavioral responses induced by the intrathecal administration of some peptides.\textsuperscript{51-54} In fact, in an earlier study with intrathecal administration of substance P, it was found that the variation in the results was far greater in unanesthetized rats than it was in anesthetized rats.\textsuperscript{28} Urethane was used as the anesthetic because it has a less disruptive effect on baseline cardiovascular parameters and reflexes as well as on respiratory parameters than do other anesthetics in the rat.\textsuperscript{55-57}

The possibility was considered that TRH may have diffused to brain stem structures to produce the cardiovascular effects reported here. However, this possibility was considered unlikely for a number of reasons. If the action had been in the brain stem, one would have expected a greater effect at the T2 than at the T9 level, and the opposite was the case. Secondly, TRH administration, either intracisternally\textsuperscript{60} or selectively into the nuclei of the tractus solitarius,\textsuperscript{5} induces very different effects: hypotension and either cardioacceleration\textsuperscript{62} or no change in heart rate.\textsuperscript{63} Furthermore, the time course of both the pressor response, which peaked at 4 minutes after injection, and the heart rate response, which peaked at 7 minutes, was faster than might be anticipated for a mechanism that required diffusion of the peptide to the brain stem. A similarly rapid onset of effect on adrenal output of catecholamines has been reported when substance P is administered at the T9 segment.\textsuperscript{27}

While this manuscript was in the review process, a similar paper appeared by Helke and Phillips.\textsuperscript{60} Based primarily on the effects of a TRH-related peptide, the methodological and interpretative differences between their results and ours prompt comment. They used a different peptide, an analogue of TRH that does not share all of the effects of TRH upon intrathecal administration.\textsuperscript{61} In addition, their rats were not breathing spontaneously, but were ventilated artificially. Furthermore, they studied effects at only the T9-10 spinal level, and, therefore, they could not comment on the regional difference reported in our paper. In their case, on the basis of their results after ganglion blockade (with pentolium rather than hexamethonium), they concluded that the effect of the TRH-related peptide was less; however, in their experiments the mean arterial pressure when the peptide was administered was $57\pm3$ mm Hg, indicating that the animals were severely hypotensive. We have chosen a more guarded conclusion—that TRH still produced an effect after ganglion blockade—and we have stayed away from conclusions regarding any change in the magnitude of the response due to the shifted baseline arterial pressure (to $79.2\pm4.8$ mm Hg, in our case). Finally, Helke and Phillips concluded that the cardioacceleratory response to intrathecal administration of the TRH-related peptide was partly due to activation of ascending pathways to vagal output neurons. This interpretation is inconsistent with the evidence outlined above that TRH is without effect in the dorsal horn, although the TRH-related peptide may have effects besides those that mimic TRH.

In summary, our results support evidence implicating TRH as a chemical mediator of synaptic transmission in descending inputs to sympathetic preganglionic neurons, especially in lower thoracic segments.\textsuperscript{2,5,25,26,44} The effects of TRH are central as intravenous administration of this peptide fails to produce any effect on arterial pressure and heart rate. The results with hexamethonium lead to the suggestion that nonnicotinic transmission at the ganglion or adrenal level may participate at least partially in the mediation of these effects. The results from this study prompt the further suggestion that TRH is involved in functional pathways that include at least those regulating arterial pressure and heart rate.

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