Cellular and Molecular Neurobiology, Vol. 25, Nos. 3/4, June 2005 (©2005)
DOI: 10.1007/s10571-005-4008-0

Colchicine Treatment Differently Affects Releasable Thyrotropin-Releasing Hormone (TRH) Pools in the Hypothalamic Paraventricular Nucleus (PVN) and the Median Eminence (ME)

Kiss Alexander,[1] Nikodémová Mária,[1] Kučerová Jana,[1] and Štrbák Vladimír[1,2]

Received December 9, 2003; accepted April 14, 2004

SUMMARY

1. Hypophysiotropic thyrotropin-releasing hormone (TRH) is synthesized in the hypothalamic paraventricular nucleus (PVN) and transported to the median eminence (ME) where it enters the hypophyseal portal blood. TRH in the ME is situated exclusively in nerve terminals, whereas TRH in the PVN and septum is of extrinsic (nerve terminals) as well as intrinsic (perikarya) origin.

2. To determine the source and possible differential regulation of TRH release from these structures, we blocked TRH axonal delivery by i.c.v. administration of colchicine into the lateral cerebral ventricle of euthyroid or hypothyroid rats in doses of 7.5 μg or 7.5, 75 and 100 μg, respectively, two days prior to the evaluation of the TRH secretion from the PVN, ME and the septum *in vitro*.

3. In euthyroid rats a low dose of colchicine did not significantly affect plasma TSH. The secretory response to both ethanol in an isosmolar medium and a high K^+ in the ME as well as the PVN explants was well preserved. However, colchicine treatment resulted in the significant increase of basal secretion of TRH from the PVN.

4. Hypothyroidism induced by 200 mg/l methimazole in drinking water for two weeks resulted in growth arrest, elevated plasma thyrotropin and decreased TRH content in the PVN and the ME. Colchicine partially decreased elevated plasma thyrotropin and increased the TRH content in the PVN and its basal release *in vitro* which was independent of extracellular Ca^{2+}. Interestingly, a TRH release from the PVN could not be further stimulated either by K^+ membrane depolarization or by ethanol. TRH responsiveness to the stimulation remained unaffected in the ME. The effect of colchicine on the septal TRH secretion was intermediate between the effect observed in the PVN and the ME.

5. *In conclusion*, the absence of a TRH secretory response to stimuli in the PVN after colchicine disruption of the microtubules and Golgi system suggests that stimulated TRH release observed from the PVN explants *in vitro* occurs from nerve terminals projecting to the PVN from other brain regions. The independence from extracellular calcium implies that TRH released under the non-stimulating conditions occurs most likely via the constitutive secretory pathway from dendrites and/or perikarya. Regulation of septal TRH is markedly different from the hypophysiotropic one.

KEY WORDS: TRH; paraventricular nucleus; median eminence; septum; colchicine; hypothyroidism; rat; KCl; ethanol; axonal transport; secretion; content.

[1] Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.
[2] To whom correspondence should be addressed at Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, Bratislava 833 06, Slovak Republic; e-mail: vladimir.strbak@savba.sk.

ABBREVIATIONS

ME	median eminence
PVN	paraventricular nucleus
TSH	thyrotropin
TRH	thyrotropin-releasing hormone

INTRODUCTION

Thyrotropin-releasing hormone (TRH), originally isolated and characterized on the basis of its role in the regulation of pituitary thyrotropin secretion, is a wide-spread CNS peptide with numerous other biological functions (Oliver *et al.*, 1974, for review see Lechan, 1993). It is synthesized as a large peptide precursor with its final maturation in secretory granules (Nillni *et al.*, 1993). Hypophysiotropic preproTRH is synthesized in the parvocellular neurons of the hypothalamic paraventricular nucleus (PVN) and is axonally transported as TRH and/or maturing peptide(s) to the median eminence (ME) where it enters hypophyseal portal blood. TRH in the ME is localized exclusively in nerve terminals. No specific signal for preproTRH mRNA was found in the ME after *in situ* hybridization (Segerson *et al.*, 1987). Intense immunolabeling of neurosecretory granules in the PVN neurons supports the assumption that significant processing of pro-TRH occurs in neuronal cell bodies (Liposits *et al.*, 1987). In addition, immunohistochemical studies have demonstrated that the majority of TRH in the PVN is in the perikarya (Merchenthaler *et al.*, 1988; Segerson *et al.*, 1987) with only occasional TRH-immunoreactive terminals (Hisano *et al.*, 1986). Intranuclear autoregulatory events might account for the local presence of the TRH receptors and the occurrence of morphologically identified TRH-TRH interactions (Sharif and Burt, 1985; Toni *et al.*, 1990). In our previous studies we demonstrated the release of TRH from the PVN and the ME explants under basal and stimulating conditions (ethanol or membrane depolarization) *in vitro* (Nikodemova and Strbak, 1995; Nikodemova *et al.*, 1997; Najvirtova *et al.*, 2002) and suggested that this neuropeptide within the PVN might be secreted from the perikarya or dendrites of local neurons.

TRH in the septum is independent from the hypophysiotropic TRH and most likely has a different physiological role. It was shown that TRH in the medial septum may be involved in arousal from ethanol-induced sedation (Morzorati and Kubek, 1993) and that TRH has the ability to antagonize the depressant actions of ethanol (McCown *et al.*, 1986). Septal TRH is also a mixture of both intrinsic and extrinsic origin which comes primarily from the bed nucleus of the stria terminalis (Merchenthaler *et al.*, 1988; Ishikawa *et al.*, 1986).

The aim of this study was to investigate whether TRH release from the PVN and the septum occurs from perikarya and dendrites or from nerve terminals projected to these areas from different brain regions. To distinguish between the secretion from cell bodies and axons, we blocked the axonal delivery of TRH by i.c.v. colchicine pretreatment. Exposure of cells to colchicine causes a disassembly of microtubules which leads to the inhibition of axonal transport and to the disorganization of the Golgi complex which may interfere with the mechanism of exocytosis (Patton, 1978;

Thyberg and Moskalewski, 1985). The effect of colchicine on TRH secretion was studied in both normal and hypothyroid rats which allowed us to compare the TRH responsiveness in normal physiological and pathophysiological conditions characterized by increased TRH expression and release (Liao *et al.*, 1989; Yamada *et al.*, 1992).

MATERIAL AND METHODS

Experimental Animals and Treatment

Male Wistar rats (Charles River, Sulzfeld, Germany) weighing 300–350 g were kept at a controlled temperature (22–24°C) and a constant 12 h light/dark cycle and fed Purina Chow and tap water ad libitum. Forty eight hours prior to the decapitation 7.5 μg colchicine/10 μl saline (Col group) or 10 μl saline (C group) was administered under pentobarbital anesthesia into the central part of the lateral cerebral ventricle.

Hypothyroidism was induced by the treatment with methimazole (200 mg/l in the drinking water) for two weeks. The induction of the hypothyroid condition was confirmed by increased plasma TSH and growth arrest. After two weeks animals were injected with either 7.5, 75 or 100 μg colchicine/10 μl saline (MCol group) or 10 μl saline (MS group) as described above.

After decapitation the brain was rapidly removed and the ME, the PVN area (Nikodemova and Strbak, 1995) and the entire septum (Palkovits and Brownstein, 1988) were dissected under the microscope. Tissues were immediately used for *in vitro* experiments.

TRH Secretion *In Vitro*

The incubation of tissue was performed in stoppered Ependorf tubes at 37°C in a 5% CO_2/95% O_2 atmosphere. After a 30-min preincubation period, the ME (1 ME/tube) and the PVN (1 pair from 1 animal/tube) were incubated for four 30-min periods in a 150 μl medium according to the following sequence: 1) basal medium, 2) stimulating medium, 3) basal medium, 4) stimulating medium. For the septum, a 60 min preincubation period and four successive 15-min incubation periods were used. TRH secretion was stimulated either by 56 mM KCl evoking membrane depolarization or by 80 mM ethanol. All media were assayed immediately for released TRH by radioimmunoassay.

The basal medium contained 6 mM $NaHCO_3$, 130 mM NaCl, 5.6 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM D-glucose, 1.5 mM ascorbic acid, 2 mM HEPES, pH = 7.4. To minimize degradation of the TRH released, 30 mg bacitracin/100 ml was added to the medium. In specified experiments calcium-free medium with 1 mM EGTA was used. The depolarizing medium contained 56 mM KCl and 77.6 mM NaCl (the concentration of NaCl was lowered to maintain physiological osmolarity); the other components were the same as in the basal medium. To test the ethanol stimulation isosmotic medium containing 80 mM ethanol, the concentration of NaCl was lowered to 91.8 mM. Ca^{2+} free medium was prepared by omitting $CaCl_2$ from the medium and adding 1 mM EGTA. All media were gassed with 5% CO_2/95% O_2 atmosphere for 30 min before using.

TRH Assay

TRH was measured by specific radioimmunoassay (RIA, Benicky and Strbak, 2000). The antibody, prepared in our laboratory, is highly specific for TRH and does not exhibit significant cross-reactivity (<0.1%) with pGlu-Glu-Pro-NH_2, TRH free acid, His-Pro diketopiperazine, TRH-Gly or Lys-Arg-Glu-His-Pro-Gly. The cross-reactivity with pGlu-Phe-Pro-NH2 is 8–10%. Synthetic TRH (a gift from Prof. Kasafírek, Research Institute of Pharmacology and Biochemistry, Prague) was labeled with $Na^{125}I$ using the chloramine-T method and purified on Sephadex G 15 column (60 × 1 cm). All assays were performed in a total volume of 400 μl 0.01 M PBS (pH 7.6). After overnight incubation at 4°C bound and free peptides were separated by cold 200 μl dextran coated charcoal (500 mg Norit + 50 mg dextran in 100 ml H_2O). The assay sensitivity was 1 pg of TRH per tube. The intraassay coefficient of variance was 4.2%. TRH released into the medium was directly measured; standards were prepared in each medium utilized and thus correction for medium influence was included.

TRH Extraction from Tissues

After incubation the tissues were placed into 200 μl ice-cold water and sonicated. Aliquots of the homogenates were used for protein determination. An equal volume of 2 M acetic acid was added to the remaining homogenate and the tubes were kept at −20°C overnight. Samples were then centrifuged at 7000 × g for 10 min and the supernatant stored. After addition of 0.5 ml 50% methanol to the sediment, the samples were recentrifuged under the same conditions. Both supernatants were pooled and lyophilized. The lyophilizates were kept at −20°C and reconstituted in an assay buffer on the day of RIA.

Rat TSH Determination

Plasma TSH was assayed by specific RIA with reagents (rat TSH-RP-3, AFP-5512B, rat TSH-I-9 AFP-11542B and anti-rat TSH-RIA-6, AFP-329661 Rb) obtained from the NIDDK through the National Hormone and Pituitary Program.

Data Analysis

Data in each experimental group are expressed as mean ± SE and the number of samples (n) are indicated in the legends to the figures. Data were compared by paired Student's t-test and analysis of variance (ANOVA) for repeated measures. For independent groups, ANOVA followed by Newman-Keuls multiple comparisons was used. Data were considered significantly different at $p < 0.05$.

RESULTS

Euthyroid Animals

Colchicine treatment of intact rats did not affect protein content in the ME, PVN or septum (Table I, Experiment I). Despite a tendency to decrease, the difference

Table I. Protein Content [μg/tissue] after Colchicine (7.5 μg) Treatment

Treatment	*n*	ME	PVN	SEPTUM
Experiment I				
C	9	39.9 ± 4.3	84.1 ± 7.6	779.7 ± 103.8
Col	9	39.0 ± 0.9	79.7 ± 12.6	895.5 ± 59.1
Experiment II				
C	4	53.2 ± 3.8	86.8 ± 5.1	683.9 ± 27.2
MS	5	43.3 ± 3.1	94.8 ± 6.4	603.7 ± 76.5
MCol	4	45.4 ± 4.4	116.7 ± 10.5*	946.5 ± 35.4*,**

Note. C: saline treated controls; Col: colchicine treated animals; MS: methimazole treated and saline injected animals; MCol: methimazole treated and colchicine injected animals; ME: median eminence; PVN: paraventricular nucleus.
*$P < 0.05$ vs. AC, **$P < 0.005$ vs. MS.

in the plasma TSH did not reach statistical significance (Fig. 1) two days after i.c.v injection of 7.5 μg colchicine. The secretory response to both ethanol in isosmolar medium and depolarizing medium was well preserved in the ME as well as in the PVN explants two (Figs. 2–4) or three days after the colchicine injection (not shown). Colchicine treatment resulted in a significant (more than two-fold) increase of the basal secretion of TRH from the PVN (Figs. 2 and 3). The Ca^{2+}-free medium did not affect the basal secretion of TRH either from the PVN or ME (Figs. 3 and 4). The basal secretion from the median eminence was not changed (Fig. 4). Although the total amount of TRH released during four incubation intervals was not affected by the colchicine treatment in any tested tissue (Fig. 5), the TRH content was significantly decreased in the ME and not affected in the PVN (Fig. 6).

Hypothyroid Animals

A 2-week treatment with methimazole induced hypothyroidism, as confirmed by an arrest of growth (Table II) and a ten-fold increase of plasma TSH (Fig. 7).

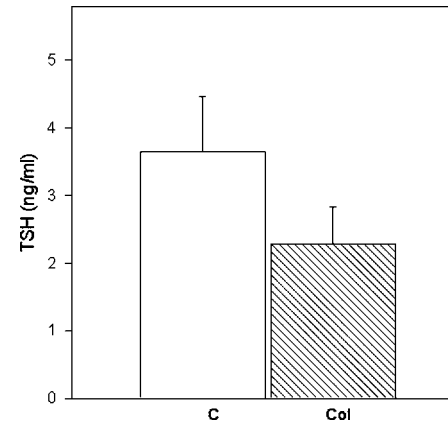

Fig. 1. Effect of colchicine on plasma TSH. Colchicine in the dose 7.5 μg was applied i.c.v. 2 days prior to the decapitation to intact rats. Plasma TSH was determined by RIA. C, saline-treated controls ($n = 8$); Col, colchicine-treated rats ($n = 9$).

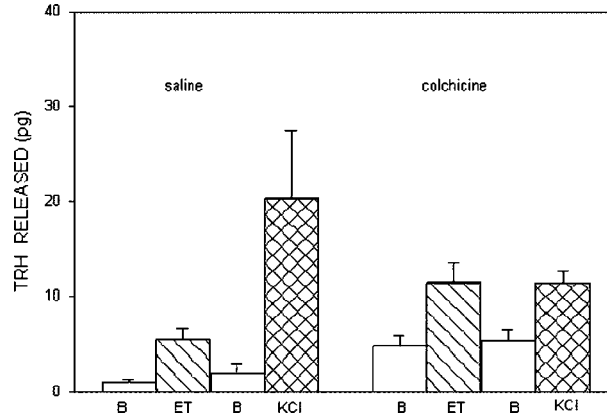

Fig. 2. TRH secretion *in vitro* from the PVN two days after i.c.v. injection of 7.5 μg colchicine ($n = 15$) or saline ($n = 10$). The four bars represent four successive incubations of the same tissue alternatively in basal (B) and ethanol (ET) or high KCl medium. Colchicine treatment resulted in a significant ($P < 0.001$) increase of basal secretion of TRH and did not affect the TRH release in response to the stimulation.

Colchicine treatment increased protein content (Table I, Experiment II) in the PVN and in the septum and decreased the plasma TSH of hypothyroid rats, which, nevertheless, remained markedly higher than that in the euthyroid controls. Similarly, as in euthyroid animals, 7.5 μg colchicine did not affect TRH release from the ME (Fig. 8). The basal secretion from the PVN was significantly increased, however the response

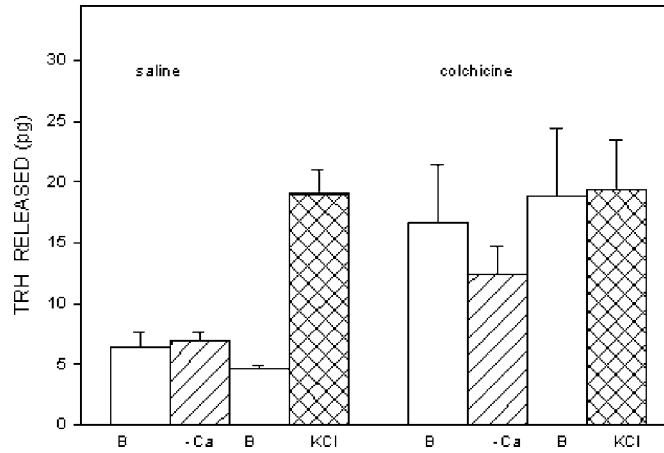

Fig. 3. The role of Ca^{2+} in TRH secretion from the PVN two days after i.c.v. injection of 7.5 μg colchicine ($n = 10$) or saline ($n = 5$). The four bars represent four successive incubations of the same tissue. B—basal medium, –Ca—basal medium without Ca^{2+}, KCl—medium containing 56 mM KCl. Omitting of Ca^{2+} did not affect basal secretion. Colchicine treatment did not affect TRH release in response to the stimulation.

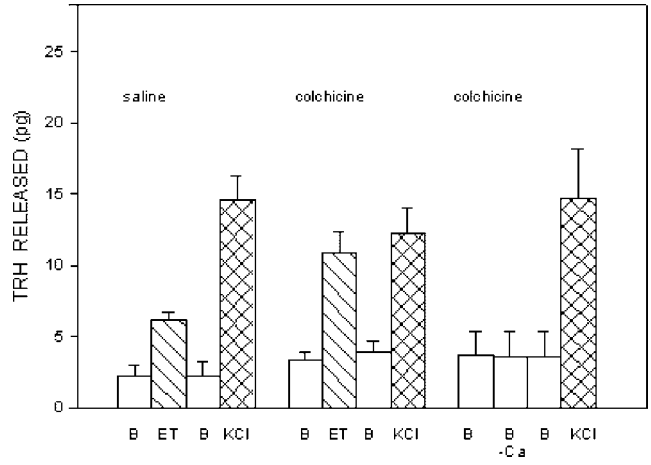

Fig. 4. TRH secretion from the median eminence two days after i.c.v. injection of 7.5 μg colchicine ($n = 15$ or 10 for groups stimulated with ethanol or testing absence of Ca^{2+} in basal medium, respectively) or saline ($n = 10$). The four bars represent four successive incubations of the same tissue. B—basal medium, –Ca—basal medium without Ca^{2+}, ET—isosmolar medium containing 80 mM ethanol, KCl—medium containing 56 mM KCl. Omitting of Ca^{2+} did not affect basal secretion. Colchicine treatment did not affect TRH release in response to the stimulation.

to the stimuli (EtOH or KCl) was absent. Omission of Ca^{2+} from the medium did not affect the basal secretion of TRH either from the PVN or the ME. Administration of higher colchicine doses (75 and 100 μg) showed essentially the same effects; response to permeant ethanol in an isosmotic medium was preserved in the ME and absent in the PVN, whereas in the PVN high basal secretion was again found (Fig. 9).

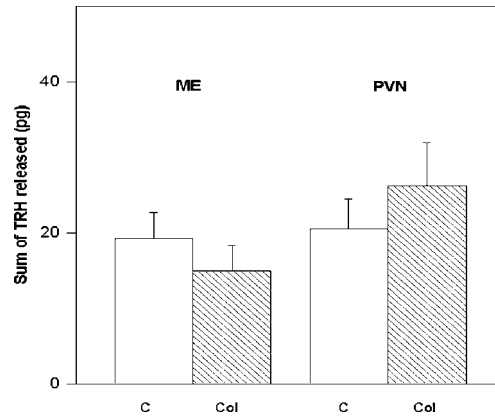

Fig. 5. The total amount of TRH released during four incubation periods from the ME and PVN 2 days after application of 7.5 μg colchicine. Colchicine did not affect the sum of TRH secreted. $n = 15$.

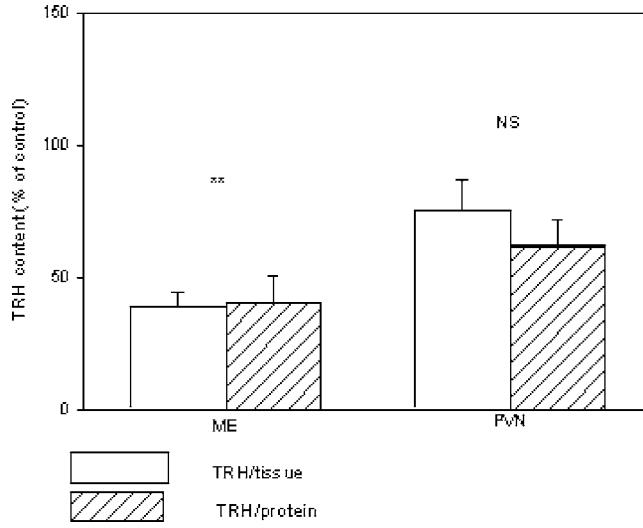

Fig. 6. TRH content 2 days after injection of 7.5 μg colchicine expressed as % of saline injected control. TRH content in the PVN was not changed, while that in the ME significantly decreased (** $P < 0.01$). NS, not significant; $n = 15$.

Septum explants showed an intermediate response—release after the first stimulus was followed by a high secretion persistent till the end of the experiment. Colchicine in the 7.5 μg dose lowered the TRH content in the ME of euthyroid rats by 40% (Fig. 10). Hypothyroidism itself induced a similar decrease of TRH, and this was further lowered by the highest colchicine dose. In the PVN, hypothyroidism resulted in a profound decrease of TRH content which was reversed by colchicine treatment (Fig. 10). Hypothyroidism itself increased the total amount of TRH released by the PVN (Fig. 11) and colchicine treatment further increased this amount three times.

DISCUSSION

We reported previously that PVN explants release TRH *in vitro* in a similar manner as it occurs in the ME. TRH secretion from both structures can be induced by a membrane depolarization and is dependent on extracellular calcium (Nikodemova and Strbak, 1995). While TRH in the ME is localized exclusively in nerve terminals, it

Table II. Effect of Methimazole Treatment on Rat Growth (Body Weight in g, Means ± SE

Treatment	*n*	Initial BW	Final BW	*P*
Control	4	307.5 ± 7.8	351.2 ± 12	<0.005
Methimazole	5	309 ± 6.4	299 ±12.2	N.S.
MCol	4	305 ± 7.1	302.5 ± 11.3	N.S.

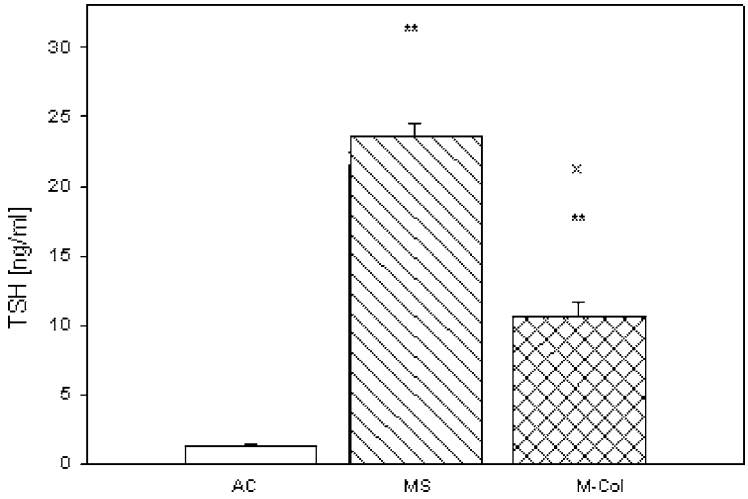

Fig. 7. Effect of methimazole (200 mg/l drinking water) and colchicine (7.5 μg i.c.v. 48 h prior decapitation) treatment on plasma TSH. AC—control rats ($n = 5$), MS—methimazole treated rats injected 10 μl saline i.c.v. ($n = 5$), M-Col—hypothyroid rats injected colchicine ($n = 4$). Both groups of methimazole treated rats have markedly increased plasma TSH as compared to euthyroid rats ($P < 0.01$ and $P < 0.02$ AC vs. MS and M-Col, respectively). Colchicine significantly decreased plasma TSH($P < 0.02$) in hypothyroid rats.

is reasonable to believe that TRH secretion from this structure occurs via a regulated secretory pathway. This pathway allows for an acute increased release of stored materials in secretory granules in response to a specific stimulus (Blazquez and Shennan, 2000). Since TRH in the PVN is of both extrinsic and intrinsic origin, we sought to determine the source of secreted TRH observed *in vitro* from this structure. To deplete TRH from nerve terminals and axons projecting to the PVN from different brain regions, we injected animals with colchicine in order to block an axonal transport.

In the euthyroid rats a decreased TRH content in the ME was found after a low dose of colchicine. Blocking of axonal transport did not result in TRH accumulation in the PVN possibly due to increased basal (unstimulated) TRH secretion from the PVN. It is unlikely that the elevated basal TRH release from the PVN represents a regulated secretion since it was Ca^{2+} independent. We suggest that this phenomenon simply reflects accumulation of TRH in the PVN after inhibition of its axonal transport into the ME and its secretion by the constitutive secretory pathway. It is of interest that the decreased content of TRH in the ME after colchicine treatment did not affect TRH secretion from this structure. This might be a result of TRH maturation in the ME from a small TRH precursor. For instance, TRH-Gly, a TRH precursor, was proven to be present in the axon terminal (Simard *et al.*, 1989). Moreover, we reported earlier (Nikodemova and Strbak, 1995) that the ME explants from euthyroid rats maintained a constant TRH content during a 2.5 h incubation period despite repeated stimulated TRH release.

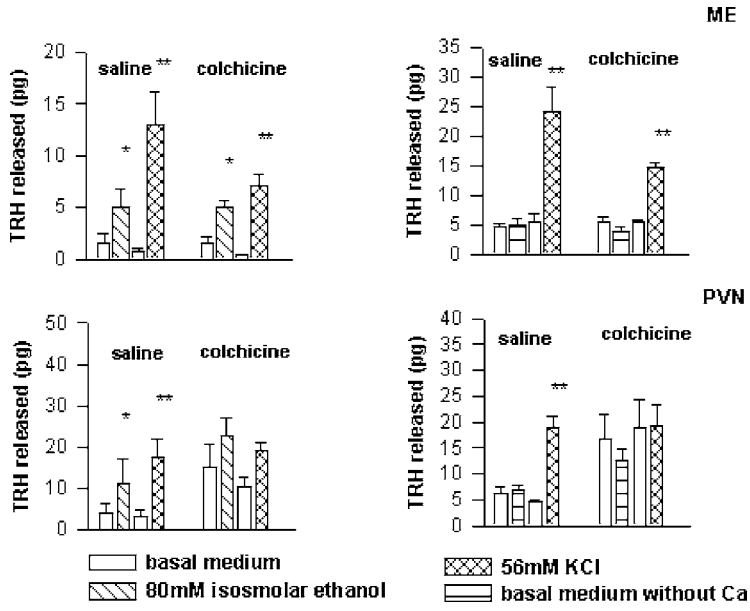

Fig. 8. TRH secretion from the ME and PVN explants two days after i.c.v. injection of 7.5 μg colchicine ($n = 5$) or saline ($n = 4$) to methimazole treated rats. The four bars represent four successive incubations of the same tissue. Omitting of Ca^{2+} did not affect basal secretion. Colchicine treatment did not affect TRH release in response to the stimulation in the ME. In the PVN colchicine induced high basal TRH secretion ($P < 0.001$) and inhibited stimulated secretory response. $^{*}P < 0.05$ and $^{**}P < 0.001$ compared to previous incubation period, $n = 4$ and $n = 5$ for saline and colchicine treated groups respectively.

The effect of the same low dose of colchicine was more obvious when tested in the rats with the TRH system activated by methimazole. A two-week treatment with methimazole arrested rat growth and substantially increased plasma TSH, thus confirming a significant degree of hypothyroidism. In agreement with previous reports (Yamada *et al.*, 1992; Bruhn *et al.*, 1991), hypothyroidism reduced TRH content in the ME and the PVN which in the presence of high TRH biosynthesis (Yamada *et al.*, 1992) could be attributed to its fast transport to and high secretion from axon terminals in the ME *in vivo*. Arrested transport of this neuropeptide from the PVN by colchicine was documented in our experiments by the increased protein and TRH content and high basal release in the PVN, decreased levels of plasma TSH, and lowered TRH content in the ME. It was recently shown that colchicine after i.c.v. application induced corticotrophin releasing hormone mRNA expression in the hypothalamus (Kiss and Aguilera, 2000) and Fos protein in cerebellar and vestibular nuclei (Pirnik and Kiss, 2002, 2003). The possibility that high basal TRH secretion is the result of this kind of stimulation should be therefore considered. However, it was also reported that colchicine even in high doses did not alter preproTRH mRNA in the PVN (Ceccatelli *et al.*, 1991; Fekete *et al.*, 2000). Comparison of the effect of colchicine in euthyroid and methimazole treated rats showed that hypothyroidism

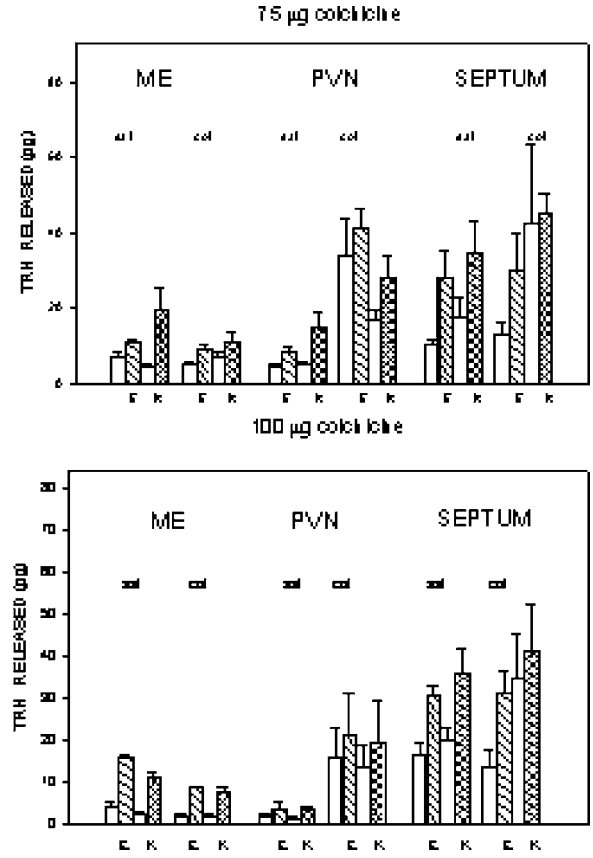

Fig. 9. Effect of high doses of colchicine (upper panel 75 μg; lower panel 100 μg) in hypothyroid rats on TRH secretion from the ME, PVN and septum. Colchicine did not affect TRH secretion from the ME. In the PVN basal secretion was increased and the secretory response to the stimulation was absent. In the septum response only to the first stimulus was present. E—stimulation by 80 mM ethanol in isosmolar medium, K—56 mM KCl. $n = 5$ in each group.

increased sensitivity of the hypophysiotropic TRH system to colchicine and its marked effect was visible even at a very low dose. Moreover, colchicine at this dose did not disturb the exocytotic process itself since secretory response of the ME explants from hypothyroid rats and the ME and PVN explants from euthyroid rats was not affected. An absence of the secretory response to stimuli in the PVN from colchicine treated hypothyroid rats indicates that the TRH synthesized and accumulated inside the PVN is not released in response to stimuli. It is therefore suggested that the secretory response observed from the PVN explants takes place mostly from the extrinsic source delivered to the PVN. After colchicine treatment not enough TRH had been delivered to the PVN to show secretory response.

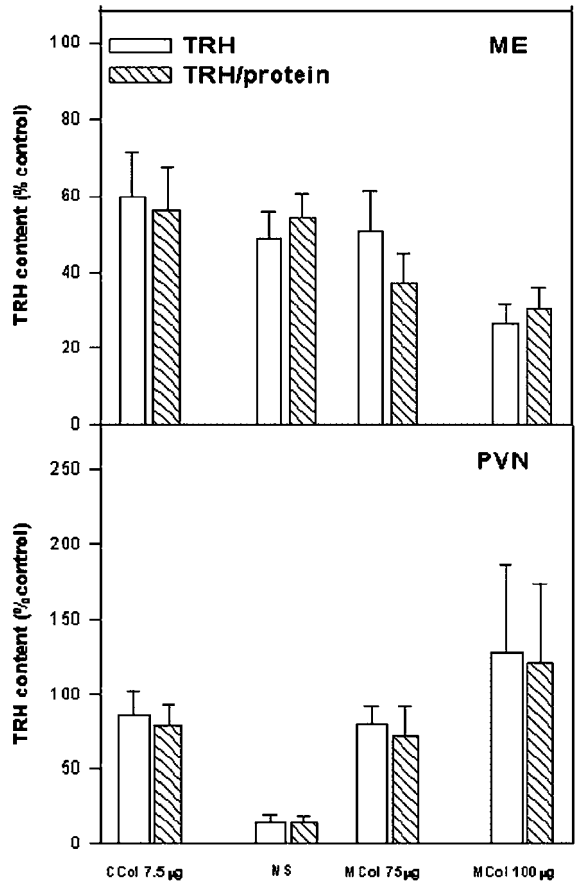

Fig. 10. TRH content expressed as % of intact control. Colchicine (Col) treatment in euthyroid control (C) rats decreased TRH content in the ME ($P < 0.01$), while that in the PVN remained unchanged. Treatment with methimazole (M) resulted in decrease of TRH content in both the ME ($P < 0.01$) and PVN ($P < 0.001$). Colchicine treatment of hypothyroid rats reversed the effect of methimazole in the PVN ($P < 0.001$), while content in the ME was not affected significantly. $n = 5$.

The absence of TRH depletion or accumulation in the brain septum after colchicine suggests that in this structure TRH is partially of local origin and partially imported. This is consistent with morphological findings that colchicine treatment resulted in a dramatic increase of TRH immunostaining of perikarya including the medial septum (Merchenthaler *et al.*, 1988) while TRH immunoreactive fibers and terminals have been seen in the lateral septum. Since we studied the brain septum as one explant, results seem to indicate a balanced combination of inhibition of both import and export of the neuropeptide. Hypothyroidism did not affect the septal TRH (not shown) in our experiments, suggesting its independence from thyroid hormones.

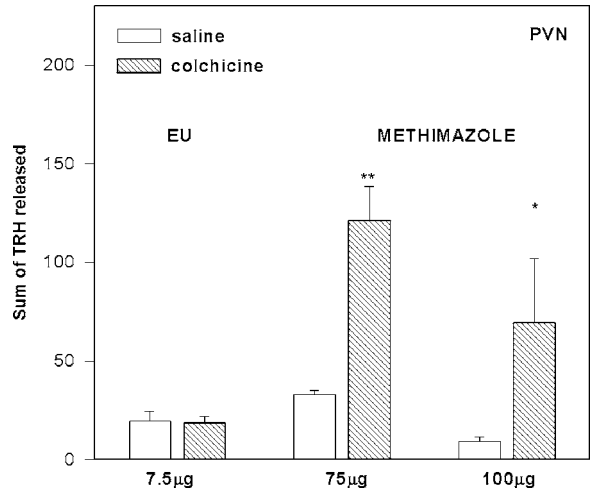

Fig. 11. The total amount of TRH (pg) released from the PVN during experiment. Doses of colchicine are indicated under bars. Methimazole treatment resulted in an significant increase ($P < 0.05$ compared to euthyroid) of total amount TRH released; the change was dramatically potentiated by colchicine treatment PVN ($P < 0.001$ for 75 μg and $P < 0.05$ for 100 μg). EU—euthyroid animals, $n = 5$.

In conclusion, our data showed that the hypophysiotropic TRH system under hypothyroid conditions was more sensitive to colchicine treatment. Colchicine arrested protein transport to the nerve terminals without interfering with the process of exocytosis. Our results suggest that a significant portion of the TRH released from the PVN *in vitro* in response to secretagogues has an extrinsic origin.

ACKNOWLEDGMENTS

The work was supported by projects # 23191/23 and 2/2007 of the Grant Agency of the Ministry of Education and Slovak Academy of Sciences (VEGA), by the project supported by European Commission ICA 1-CT-2000-70008, by the project # 017/2001 of the US- Slovak Science and Technology Program.

REFERENCES

Benicky, J., and Strbak, V. (2000). Glucose stimulates and insulin inhibits release of pancreatic TRH *in vitro*. *Eur. J. Endocrinol.* **142:**60–65.

Blazquez, M., and Shennan, K. I. J. (2000). Basic mechanisms of secretion: Sorting into the regulated secretory pathway. Biochem. *Cell Biol.* **78:**181–191.

Bruhn, T. O., Taplin, J. H., and Jackson, I. M. D. (1991). Hypothyroidism reduces content and increases *in vitro* release of pro-thyrotropin-releasing hormone peptides from the median eminence. *Neuroendocrinology* **53:**511–515.

Ceccatelli, S., Cortes, R., and Hokfelt, T. (1991). Effect of reserpine and colchicine on neuropeptide mRNA levels in the rat hypothalamic paraventricular nucleus. *Brain. Res. Mol. Brain Res.* **9:**57–69.

Fekete, C., Mihaly, E., Luo, L.-G., Kelly, J., Clausen, J. T., Mao, Q., Rand, W. M., Moss, L. G., Kuhar, M., Emerson, C. H., Jackson, I. M. D., and Lechan, R. M. (2000). Association of cocaine-and amphetamine-regulated transcript-immunoreactive elements with thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus and its role in the regulation of the hypothalamic-pituitary-thyroid axis during fasting. *J. Neurosci.* **20:**0224–9234.

Hisano, S., Ishizuka, H., Nishiyama, T., Tsuruo, Y., Katoh, S., and Daikoku, S. (1986). Immunoelectron microscopic observations of hypothalamic TRH-containing neurons in rats. *Exp. Brain Res.* **63:**495–504.

Ishikawa, K., Taniguchi, Y., Kurosumi, K., and Suzuki, M. (1986). Origin of septal thyrotropin-releasing hormone in the rat. *Neuroendocrinology* **44:**54–58.

Kiss, A., and Aguilera, G. (2000). Role of alpha-1 adrenergic receptors in the regulation of CRH mRNA in the paraventricular nucleus of the hypothalamus during stress. *Cell. Mol. Neurobiol.* **20:**683–694.

Lechan, R. M. (1993). Update on thyrotropin-releasing hormone. *Thyroid Today* **16:**1–11.

Liao, N., Bulant, M., Nicolas, P., Vaudry, H., and Pelletier, G. (1989). Thyroid hormone regulation of neuron staining for a pro-TRH-derived cryptic peptide sequence in the rat hypothalamic paraventricular nucleus. *Neuroendocrinology* **50:**217–221.

Liposits, Z., Paul, W. K., Wu, P., Jackson, I. M. D., and Lechan, R. M. (1987). Hypophysiotropic thyrotropin releasing hormone (TRH) synthesizing neurons. *Histochemistry* **88:**1–10.

McCown, T. J., Moray, L. J., Kizer, J. S., and Breese, G. R. (1986). Interactions between TRH and ethanol in the medial septum. *Pharmacol. Biochem. Behav.* **24:**1269–1274.

Merchenthaler, I., Csernus, V., Csontos, C., Petrusz, P., and Mess, B. (1988). New data on the immunocytochemical localization of thyrotropin-releasing hormone in the rat central nervous system. *Am. J. Anat.* **181:**359–376.

Morzorati, S., and Kubek, M. J. (1993). Septal TRH in alcohol-naive P and NP rats following alcohol challenge. *Brain Res. Bull.* **31:**301–304.

Najvirtova, M., Baqi, L., Kucerova, J., and Strbak, V. (2002). Cell swelling induced secretion of TRH by posterior pituitary, hypothalamic paraventricular nucleus and pancreatic islets: Effect of L-canavanine. *Cell. Mol. Neurobiol.* **22:**35–46.

Nikodemova, M., and Strbak, V. (1995). Different regulation of thyrotropin releasing hormone content and release in paraventricular nucleus and median eminence of rat hypothalamus during *in vitro* incubation. *Life Sci.* **56:**1511–1521.

Nikodemova, M., Weismann, P., Filipcik, P., Mraz, P., Greer, M. A., and Strbak, V. (1997). Both iso- and hyperosmotic ethanol stimulate release of hypothalamic thyrotropin-releasing hormone despite opposite effect on neuron volume. *Neuroscience* **80:**1263–1269.

Nillni, E. A., Sevarino, K. A., and Jackson, I. M. D. (1993). Processing of proTRH to its intermediate products occurs before the packing into secretory granules of transfected AtT20 cells. *Endocrinology* **132:**1271–1277

Oliver, C., Eskay, R. L., Ben-Jonathan, N., and Porter, J. C. (1974). Distribution and concentration of TRH in the rat brain. *Endocrinology* **96:**540–546.

Palkovits, M., and Brownstein, M. J. (1988). *Maps and guide to microdissection of the rat brain*, Elsevier, New York.

Patton, S. (1978). Milk secretion at the cellular level: A unique approach to the mechanism of exocytosis. *J. Dairy Sci.* **61:**643–650.

Pirnik, Z., and Kiss, A. (2002). Dexamethasone attenuates by colchicine induced fos expression in the rat deep cerebellar and vestibular nuclei. *Cell. Mol. Neurobiol.* **22:**431–444.

Pirnik, Z., and Kiss, A. (2003). Fos induction in the rat deep cerebellar and vestibular nuclei following central administration of colchicine: A qualitative and quantitative time-course study. *Brain Res. Bull.* **61:**63–72.

Segerson, T. P., Hoefler, H., Childers, H., Wolfe, H. J., Wu, P., Jackson, I. M. D., and Lechan, R. M. (1987). Localization of thyrotropin-releasing hormone prohormone messenger ribonucleic acid in rat brain by in situ hybridization. *Endocrinology* **121:**98–107.

Sharif, N. A., and Burt, D. R. (1985). Limbic, hypothalamic, cortical and spinal regions are enriched in receptors for thyrotropin-releasing hormone: Evidence from [3H] ultrofilm autoradiography and correlation with central effects of the tripeptide in rat brain. *Neurosci. Lett.* **60:**337–342.

Simard, M., Pekary, A. E., Smith, V. P., and Hershman, J. M. (1989). Thyroid hormone modulation of TRH precursor levels in rat hypothalamus, pituitary, thyroid and blood. *Peptides* **10:**145–155.

Toni, R., Jackson, I. M. D., and Lechan, R. M. (1990). Thyrotropin-releasing hormone-immunoreactive innervation of thyrotropin-releasing-hormone-tuberoinfundibular neurons in rat hypothalamus: Anatomical basis to suggest ultrashort feedback regulation. *Neuroendocrinology* **52:**422–428.

Thyberg, J., and Moskalewski, S. (1985). Microtubules and the organization of the Golgi complex. *Exp. Cell. Res.* **159:**1–16.

Yamada, M., Satoh, T., Monden, T., Murakami, M., Iriuchijima, T., Wilber, J. F., and Mori, M. (1992) Influences of hypothyroidism on TRH concentrations and preproTRH mRNA levels in rat hypothalamus: A simple and reliable method to detect preproTRH mRNA level. *Neuroendocrinology* **55:**317–320.