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Cerebrovascular Effects of the TRH Analogues pGlu-3-methyl-His-Pro Amide and pGlu-Glu-Pro Amide: A Comparison with TRH

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ABSTRACT

The goal of the study was to assess whether TRH analogues possess cerebrovascular effects similar to the native peptide. The neuropeptide thyrotropin releasing hormone (TRH) elicits cerebrovasodilation in several species under various conditions. The laser-Doppler method was employed to study the effects of TRH and the analogues pGlu-3-methyl-His-Pro amid (M-TRH) and pGlu-Glu-Pro amide. Intravenous (iv.) injection of 300 μg kg^{-1} of TRH elicited cerebrovasodilation and a 62% increase in blood flow within 1 minute. M-TRH, in a dose of 300 μg kg^{-1} iv., elicited a 80 % increase in cerebral blood flow. Even a minute dose of M-TRH (625 ng kg^{-1}) caused an increase in cerebral blood flow. No clear difference in effects on the cerebral blood flow was observed between spontaneously and mechanically ventilated animals. pGlu-Glu-Pro amide had no cerebrovascular effect.

INTRODUCTION

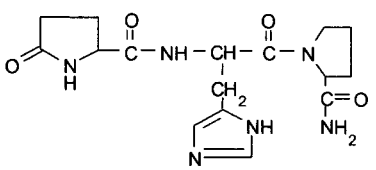
Several peptides have marked effects on the cardiovascular system. One of these peptides is the first hypothalamic hormone to be identified and chemically characterized as pGlu-His-Pro amide, namely thyrotropin releasing hormone (TRH). This neuropeptide is present in the hypothalamic-pituitary system but also, to a great extent, distributed in other regions. TRH and its receptors have been localized in the central as well as peripheral nervous system, and at nonneural sites (20,34,37). The cDNA encoding of the TRH receptor (16,42,49) as well as the distribution of the expression of TRH receptor mRNA have been described (11,22,51,56). TRH administered sc., ip., iv., i.c., and locally in some CNS regions induces a variety of physiological effects, including an increase in blood pressure, effects on the respiratory system, analeptic effects (17,39,55) and cerebrovascular effects (21,24,28,41,46). The mechanism of

effect on brain blood flow is not fully understood. An activation of an intrinsic cerebrovasodilating system has been proposed (27).

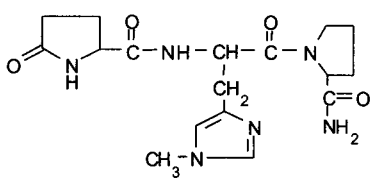
TRH and some of its analogues have been evaluated for beneficial effects in a variety of shock states (18), cerebrovascular disorders (25,48,52), and spinal cord injuries (10). Furthermore, TRH and its analogues are being evaluated for use in humans with cerebrovascular disorders (45,51), and some motor disorders (3,4,5,7,12,15,38). TRH is also able to counteract CNS depressant effects of certain drugs (2,33).

The biological half-time of TRH is short and the affinity to the receptor can be changed by modifying the molecule. It is therefore of great interest to evaluate whether analogues of TRH have similar biological effects to the native peptide.

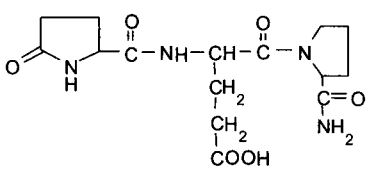
The primary aim of the investigation was to compare the cardiovascular effects of pGlu-3-methyl-His-Pro amide (M-TRH) and TRH. M-TRH is modified at the middle amino acid of the native peptide as depicted in Figure 1. Secondly, a partial dose response relationship of M-TRH was investigated. Thirdly, the possibility that muscle relaxation and artificial ventilation affect the cerebrovasodilation was studied. The effect of a second TRH analogue modified at the middle amino acid residue, pGlu-Glu-Pro amide (GGP) (Figure 1), was also studied. This peptide was orginally recognized as a ferlilization promoting peptide (6).



TRH



M-TRH



pGlu-Glu Pro amide

Fig. 1. The chemical structure of the native peptide TRH (pGlu-His-Pro amide) and the TRH analogues M-TRH (pGlu-3-methyl-His-Pro amide) and GGP (p-Glu-Glu-Pro amide).

METHODS

Male Sprague-Dawley rats (Møllegaard, Ejby, Denmark) weighing 359-475 g were used throughout the study. Animals were housed five rats per cage. The room temperature was 22±2°C and humidity 50±5%. The room was artificially lighted in a 12-hr light/dark cycle. The animals had free access to the pelleted R34 diet (Lactamin, Vadstena, Sweden) and tap water. All experiments were performed between 8 am-2 p.m. The experiments have been approved by the Regional Research Ethical Committee according to the national law.

Anesthesia was induced by injecting 120 mg kg^{-1} thiobutabarbital (Inactin®, RBI, Natick, MA, USA) intraperitoneally. A tracheostomy was performed, and the animal was connected to a small animal ventilator (model 683, Harvard Apparatus, South Natick, MA, USA). Skeletal muscle relaxation was induced by pancuronium bromide (Pavulon®, Organon, Oss, Holland) in a dose of 0.2 mg kg^{-1} iv. with additional doses of pancuronium given to facilitate controlled ventilation. In order to compare the cerebrovascular effects in animals spontaneously breathing with animals with assisted ventilation, a group of animals were not connected to the ventilator and not treated with muscle relaxants. The femoral arteries were cannulated bilaterally. One was used for continuous mean arterial blood pressure (MAP) monitoring with a Gould Statham P 23 ID pressure transducer (Gould Inc., Oxnard, CA, USA) and a SE 120 recorder (ABB-Goerz-Metrawatt, Vienna, Austria). The other arterial cannula was used for blood sampling. Arterial PO_2, PCO_2 and pH were determined at regular intervals with an ABL 500 blood gas analyzer (Radiometer, Copenhagen, Denmark). One femoral vein was cannulated and used for a continuous infusion of 0.5 ml h^{-1} 100g body wt $^{-1}$ of Ringer's solution (25mM $NaHCO_3$, 120mM NaCl, 2.5mM KCl, 0.75mM $CaCl_2$) provided by a syringe pump (341A, SAGE Instruments, Cambridge, MA, USA). The rectal temperature was continuously measured and maintained at 38°C using a CMA 150 servo heating pad (Carnegie Medicine, Stockholm, Sweden).

The "razzed" skin of the head and the outer ear meatuses were locally pre-treated with lidocain hydrochloride (Xylocain® salve 5%, Astra, Södertälje, Sweden). The animal was placed in a stereotaxic frame (I.H. Wells Jr., Mechanical Developments Co., South Gate, CA, USA). The parietal bone was exposed and a small hole, 2-3 mm in diameter, was made using a drill with a flat bottom. The center of the hole was 3 mm caudal to the coronary suture and 4 mm lateral to the sagital suture. Continuous cooling with physiological saline was performed while drilling. Care was taken to leave a very

thin bone layer intact (0.1 mm) in order not to disturb the cortical blood flow. Dural and pial blood vessels were readily recognized through the partial craniotomy.

Cortical microcirculation was continuously measured using a laser-Doppler flowmeter (Periflux PF3, Perimed, Järfälla, Sweden). Using a micromanipulator, the measuring probe (PF 303, outer diameter 1.0 mm, fiber diameter 0.125 mm, fiber separation 0.25 mm) was positioned on the thin bone layer. Large dural and pial vessels were avoided. The method is validated for measurement of the cerebral microcirculation (8,36). However, under some conditions the method can overestimate an increase in blood flow (9). At least 30 minutes elapsed from the positioning of the probe to the start of blood flow recordings. The laser-Doppler signal was recorded on a desktop computer and analyzed with the Perisoft software (Perimed, Järfälla, Sweden). The stability of the flowmeter was determined by measuring the relative flow value in a test vial containing a colloidal suspension of microscopic latex particles in random Brownian motion. Cerebrovascular resistance (CVR) was calculated as CVR=MAP (kPa)/Q (PU) and CVR is reported as vascular resistance unit (PRU), MAP is mean arterial blood pressure and Q is tissue blood flow.

The CBF was monitored continuously before and after the control injection of 200 µl saline iv.. Thereafter 300 µg kg^{-1} TRH (dissolved in 0.9% NaCl, 200 µl) was administered iv. and the effect recorded (n=8). The effect of M-TRH (n=8), in a dose of 300 µg kg^{-1} (dissolved in 0.9% saline, 200 µl) was studied in the same way.

In an attempt to establish a dose-response relationship of M-TRH on the cerebrovascular effect various doses from 625 ng kg^{-1} to 600 µg kg^{-1} were tested. The following doses were studied in more detail, 625 ng kg^{-1}, 10 µg kg^{-1} and 300 µg kg^{-1}. A dose greater than 300 µg kg^{-1} had no additional effect on the cerebral blood flow.

In order to compare whether a major change of the native peptide still possesses cerebrovascular effects the CBF was measured in control situation and during iv. infusion of 50, 100 and 200 µg kg^{-1} min^{-1} of GGP dissolved in 0.9% NaCl. The peptide was infused during 10 minutes. CBF was measured as described above with the exception that laser-Doppler probes (PF403) were placed bilaterally (n=3) and connected to the Periflux 4001 laser-Doppler device. Thus six CBF measurements were performed at each dose.

TRH (Lot No. 33H5820), M-TRH (Lot No. 73H06221) pGlu-Glu-Pro amide (Lot No. 107H0462) from Sigma Chemicals Co. (St. Louis, MO, USA) were used throughout the study. The results were evaluated for statistically significant differences by paired or unpaired Student's t-test or with Wilcoxon signed rank test when appropriate. Results

are reported as means±sem. There was no difference between the animals receiving muscle relaxant and those not receiving. Therefore the results were pooled.

RESULTS

TRH (n=8) and M-TRH (n=8) in a dose of 300 μg kg^{-1} elicited piloerection and lacrimation. In animals without skeletal muscle relaxation, eye lid movements and a discrete muscle activity was observed. In the animals without muscle relaxation a change in respiratory movements was observed after peptide administration. GGP was without visible effects.

The cardiovascular and acid-base parameters are reported in Table 1. There was no significant effect on the blood pressure. TRH tended to increase the heart rate. A very slight but statistically significant increase in P_aO_2 was noted after the administration of TRH (Table 1). No significant effect of M-TRH and GGP was observed.

Table 1 Cardiovascular parameters and blood gas values

	MAP (kPa)	HR (min^{-1})	pH_a	P_aCO_2 (kPa)	P_aO_2 (kPa)
Control(1)	14.3±0.8	374±9	7.39±0.02	5.37±0.27	10.13±0.65
TRH	14.6±0.6	383±11*	7.42±0.02	4.81±0.18	11.20±0.51*
Control(2)	15.5±0.9	363±13	7.39±0.02	4.59±0.39	11.10±0.59
M-TRH (a)	15.1±0.9	364±15	7.41±0.02	4.52±0.26	11.97±0.38
Control (3)	17.1±0.3	382±13	7.42±0.01	4.81±0.09	11.75±0.57
M-TRH (b)	16.8±0.3	381±14	7.42±0.01	4.82±0.13	11.78±0.43
Control (4)	16.4±0.3	382±13	7.43±0.01	4.71±0.18	10.74±0.58
M-TRH (c)	16.6±0.3	379±12	7.42±0.01	5.05±0.20	10.53±0.63
Control (5)	12.3±0.5	354±24	7.46±0.01	4.78±0.19	9.73±0.61
GGP (a)	11.4±1.4	339±34	7.45±0.02	4.48±0.08	10.74±1.63
GGP (b)	12.2±1.4	335±37	7.46±0.01	4.63±0.13	10.33±1.66
GGP (c)	13.0±0.4	338±29	7.46±0.01	4.84±0.22	10.57±1.52

Control (1) is the baseline value before the iv. administration of 300 μg kg^{-1} TRH (n=8), control (2) before 300 μg kg^{-1} M-TRH (a) (n=8), control (3) before 625 ng kg^{-1} M-TRH (b) (n=7), control (4) before 10 μg kg^{-1} M-TRH (c) (n=7) and control (5) before 10 min. infusion of 50 μg kg^{-1} (a) followed by 100 μg kg^{-1} (b) and 200 μg kg^{-1} GGP (c) (n=3, two probes, 6 measurements). *$p<0.05$ as compared to control value (paired Student's t-test). Values are mean±sem.

A stable laser-Doppler signal was recorded throughout the experiments. TRH elicited an increase in cortical blood flow by 62±16% ($p<0.01$), see Figure 2. The corresponding value for the same dose of M-TRH was 80±18% ($p<0.01$). This

represents a vasodilatation resulting in a decrease in the CVR from 0.093±0.012 to 0.061±0.008 PRU ($p<0.01$) after TRH administration and from 0.103±0.017 to 0.068±0.014 ($p<0.01$) after M-TRH. When comparing the effects of the two peptides no statistically significant difference in the percentile increase in CBF or decrease in CVR was observed (unpaired Student's t-test). The cerebrovasodilation appeared at 1.02±0.12 minutes after TRH administration and 0.62±0.10 minutes after M-TRH. Thus, the vascular effect of M-TRH appeared faster than the effect of TRH ($p<0.05$, unpaired Student's t-test).

In an attempt to study a dose response relationship of M-TRH on the CBF, various amounts of the peptide was administered. The lowest dose with a detectable effect was 625 ng kg^{-1}. MAP, HR, pH_a, P_aCO_2 and P_aO_2 were unaffected, see Table 1. The CBF increased by 9±2% ($p<0.001$, n=7) and the CVR decreased from 0.109±0.006 to 0.097±0.005 PRU ($p<0.01$). The increase in CBF appeared at 0.63±0.13 minutes after M-TRH administration and lasted for several minutes. Doses between 625 ng kg^{-1} and 300 µg kg^{-1} elicited vasodilatation but sometimes with great variations. Thus 10 µg kg^{-1} caused an increase in CBF by 25±16% ($p<0.02$, n=7, Wilcoxon signed rank test) and the CVR decreased from 0.106±0.014 to 0.087±0.010 PRU ($p<0.02$). Absolute blood flow values are given in Figure 2. The highest dose tested, 600 µg kg^{-1} of M-TRH had no additional effect as compared to 300 µg kg^{-1}.

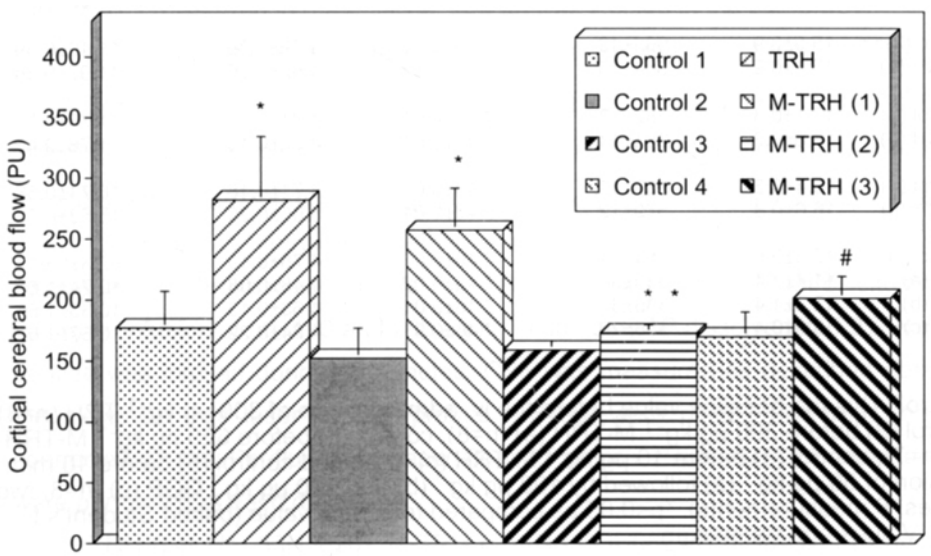


Fig. 2. Cortical cerebral blood flow. Control 1 is the blood flow before the iv. administration of 300 µg kg^{-1} TRH (n=8), control 2 before 300 µg kg^{-1} M-TRH (1) (n=8), control 3 before 625 ng kg^{-1} M-TRH (2) (n=7) and control 4 before 10 µg kg^{-1} M-TRH (3) (n=7). *$p<0.05$, **$p<0.01$ (paired Student's t-test), #$p<0.02$ (Wilcoxon sign rank test) as compared to the respective control value. Values are mean±sem.

There was no marked cerebrovascular effect of GGP. The baseline CBF was 173.8±15.7 PU on the right side and 189.2±15.8 PU on the left side. Comparing the control CBF (100%) to that after 10 minutes infusion of 50 μg kg^{-1} min^{-1}, 100 μg kg^{-1} min^{-1} and 200 μg kg^{-1} min^{-1} of GGP the corresponding values are 95±8%, 100±10% and 99±8% on the right side and 88±12%, 91±15% and 89±15% on the left side, respectively. Baseline CVR on the right side was 0.071±0.004 PRU and after pGlu-Glu-Pro amide 0.071±0.014, 0.071±0.006 and 0.077±0.006 PRU respectively. The corresponding values for the left side was 0.065±0.003, 0.070±0.011, 0.073±0.005 and 0.082±0.012 PRU, respectively. Thus no drastical effect of the peptide was observed.

DISCUSSION

We and others have previously shown that TRH has pronounced cerebrovasodilating properties in the rabbit, rat and monkey (19,21,23,25,26,28,46). Conflicting results of TRH on the CBF in the cat have been presented (35,46). Furthermore, a low TRH dose seems to elicit a slight increase in CBF in humans (41). In this study TRH elicited a pronounced cerebrovasodilation of the same magnitude as previously reported (21,28). A more pronounced response has been shown in conscious rats after higher doses of TRH (23).

Interestingly, one of the modified TRH peptide (M-TRH) used in this study has a similar effect on CBF as TRH. It has been shown that M-TRH has a very high affinity for the TRH receptor in the CNS (13). M-TRH is more potent in eliciting behavioral effects as well as being more potent in the release of both growth hormone (14) and thyroid stimulating hormone (13). The two peptides had very similar effects on CBF. It is therefore not probable that the above mentioned differences in the effects of the peptides affected our results. It seems clear that a major change in the peptide structure as in the TRH analogue pGlu-Glu-Pro amide results in lack of marked cerebrovascular effects.

The final mechanism of the cerebrovasodilation is not settled. Clearly an intrinsic cerebrovasodilating pathway seems to be involved (27). It has been proposed that the increase in CBF elicited by TRH in the rat is counteracted by atropine (21) but not by methylatropine (28) and not by muscarinic blockade in the rabbit (30). In another study it was proposed that the increase in CBF is counteracted by indomethacin (23). This is not the case in the rabbit (30). In the rabbit, the major part of the cerebrovasodilation after TRH is not blocked by naloxone (29). An α_2-receptor mechanism is proposed in

the cerebrovasodilating effect (47). Some part of the effect of TRH on the CBF in the rat is mediated by nitric oxide (31,32). Interestingly, TRH and the analogues DN-1417, RX 77368, YM-14673, JTP 2942 have been shown to have beneficial effects in experimental models of stroke and brain injuries (25,40,44,48,50,53,54). Some of the beneficial effects of these peptides can be mediated by vascular effects as shown in the present study.

TRH has been evaluated in human beings for its effect on motordisorders (7) as well as in the treatment of spinal cord injuries (43). The peptide appears to elicit different hemodynamic effects in vegetative patients as compared to brain-dead patients (1). In a recent study it was shown that TRH reduces the duration of anesthesia with propofol, which is widely used as an iv. anesthetic agent (33). This effect was probably due to a pharmacodynamic effect, and was not counteracted by atropine. Thus, TRH seems to have a broad potential spectrum for clinical use. It is therefore of great importance to evaluate analogues of TRH for their biological effects in order to specify which part of the molecule is active. This may generate new treatment regimes not only in the field of neurosurgery and neurology but also in patients with general trauma.

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