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Antinociceptive properties of thyrotropin releasing hormone in mice: comparison with morphine

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1 To investigate the antinociceptive activity of thyrotropin releasing hormone (TRH) in mice, different nociceptive stimuli were used. TRH (i.p.) was active in the phenyl-*p*-benzoquinone or acetic acid-induced writhing tests (chemical stimuli) and in Haffner's test (mechanical stimulus); its action decreased rapidly 15 min after intraperitoneal injection .

2 To determine whether the activity of TRH has a peripheral or central origin, we administered TRH intracerebroventricularly via cannulae previously implanted in mice. The results provide evidence that a central mechanism is involved in the analgesic effect of TRH since when given intracerebroventricularly it was 10,000 and 1,000 times more active against chemical and mechanical stimuli respectively than intraperitoneally. The action of TRH decreased rapidly 5 min after i.c.v. injection. Morphine was studied in these tests and it was found that at the peak effect TRH analgesia (i.c.v.) was greater than that of morphine (i.c.v.) on a molar basis.

3 To investigate the mechanisms involved in the antinociceptive action of TRH, the effects of pretreatment with either agonists or antagonists of noradrenaline (NA), dopamine and 5-hydroxytryptamine (5-HT), or with naloxone were studied. TRH activity was generally resistant to modifications of NA, dopamine and 5-HT systems. The TRH effect was not antagonized by naloxone, but TRH at a non-analgesic dose prevented the hyperalgesia induced by naloxone.

4 In conclusion, TRH i.c.v. possessed a short, strong antinociceptive activity against chemical and mechanical stimuli. This analgesia was at least equipotent to that of morphine i.c.v.

Introduction

Thyrotropin releasing hormone (TRH) is one of the endogenous peptides for which an effect on the central nervous system independent of its endocrine activity has been demonstrated (Nemeroff, Loosen, Bissette, Manberg, Wilson, Lipton & Prange, 1979). Cuenca, Serrano, Gibert-Rahola, Carrasco & Esteban have shown that TRH at doses above 6.25 mg/kg given intravenously has an antinociceptive effect on abdominal writhing induced by acetylcholine bromide.

The aim of this work was to determine whether TRH also had analgesic activity in tests used to detect central analgesics (Haffner's test, hot plate test, tail-flick test). In addition TRH was administered intracerebroventricularly (i.c.v.) to determine whether the TRH-induced analgesia was peripheral or central in origin. The antinociceptive activity of TRH was compared to that of morphine for the tests where TRH was effective. Finally, in order to investigate the possible mechanisms involved in the antinociceptive action of TRH, the effects of pretreatment with drugs that act as agonists or antagonists on noradrenergic, dopaminergic and 5-hydroxytryptaminergic systems and naloxone (an opiate receptor antagonist) on the response to TRH, were studied.

Methods

The tests used to evaluate antinociceptive activity were selected to include three different types of noxious stimuli: chemical, mechanical and thermal.

Animals

Female OFl and male CDl mice, 6 weeks old weighing approximately 25 g on the day of the experiment, were used. The number of animals in each experimental group varied from 7 to 25. Each animal was used only once.

All animals were maintained at an ambient temperature of 22 ± 1°C.

Injections

The peripheral injections were given intraperitoneally (i.p.) or subcutaneously (s.c.) in a volume of 0.1 mg/10 g body wt. The control animals received the solvent in which the test drugs were dissolved.

For the central injections, a cannula was implanted into the right lateral ventricle of mice aged 5 weeks (Boschi, Launay & Rips, 1981). The drugs were injected in a volume of 0.5 µl over a period of 50 s. The treated animals received TRH dissolved in artificial cerebrospinal fluid (CSF) or morphine dissolved in saline. The control animals received vehicle only. After the experiments histological controls were performed for all the treated and control animals using 0.5% methylene blue solution which was injected via the cannula in a volume of 0.5 µl over a period of 50 s. The animals were killed and the brains removed, sectioned frontally at the level of the cannula, and examined microscopically for the presence of dye in the ventricles.

Drugs

TRH was synthesized in our laboratory.

The phenyl-*p*-benzoquinone (PBQ) solution was freshly prepared before injection: 12.5 mg of 1,4-phenylbenzoquinone (Sigma) was dissolved in 50 ml of 5% ethanol in water.

Drugs acting on the synthesis, release or uptake of NA, dopamine and 5-HT or on their receptors: nomifensine acid maleate (Hoechst), fluoxetine hydrochloride (Lilly), *p*-chlorophenylalanine ethyl ester hydrochloride (PCPA) (Aldrich), DL α-methyl-*p*-tyrosine methyl ester hydrochloride (α-MT) (Aldrich), phenoxybenzamine (SKF), phentolamine methane sulphonate (Ciba-Geiby), yohimbine (Sigma), apomorphine hydrochloride (Sigma), haloperidol (Lebrun), imipramine hydrochloride (Geiby), nortriptyline hydrochloride (Lilly), iprindole hydrochloride (Wyeth), mianserin (Organon), 5-hydroxytryptophan (5-HTP) (Sigma). All drugs were dissolved in distilled water except nomifensine, α-MT and 5-HTP which were dissolved in 2% w/v Tween 80 in water and apomorphine hydrochloride which was dissolved in a solution of 0.2 mg/ml ascorbic acid.

Morphine sulphate (Francopia) was dissolved in 0.9% w/v NaCl solution (saline).

The morphinomimetic antagonist, naloxone hydrochloride (Endo), was dissolved in distilled water.

CSF was prepared according to Chermat & Simon (1967) without urea.

Experimental procedures

Chemical stimuli

PBQ-induced writhing test adapted from the method of Hendershot & Forsaith (1959) An intraperitoneal injection of PBQ solution (0.25 ml) produced a characteristic syndrome: contraction of the abdominal muscles accompanied by extension of the hind limbs. The TRH and solvent both peripherally and centrally were administered simultaneously with the PBQ solution. Five minutes after the injection of the drugs the number of writhes occurring during a period of 10 min was counted for each animal. A 50% reduction in the number of writhes for treated animals compared to control animals represents antinociceptive activity.

Morphine (s.c.) was injected 20 min before the PBQ solution and morphine (i.c.v.) was administered simultaneously with the PBQ solution to observe the mice at the time of peak effect.

The various drugs likely to modify the effect of TRH (i.p.) in the PBQ writhing test were given 30 min before the simultaneous injection of TRH and PBQ for nomifensine, fluoxetine, phenoxybenzamine, yohimbine, imipramine, nortriptyline, iprindole, mianserin and 5-HTP; 48 and 24 h before the PCPA; simultaneously with the injection of TRH and PBQ for naloxone, phentolamine and apomorphine; and 3 h before for α-MT and haloperidol.

A low dose (0.125 mg/kg i.p.) of TRH was given to investigate any potentiation and a higher dose (2 mg/kg i.p.) of TRH was given to investigate any antagonist action. The doses of the different drugs tested were those which on their own induced the smallest effect in the PBQ writhing test. But in the case of PCPA, 5-HTP and α-MT doses large enough to obtain a decrease or an increase of the 5-HT levels or a decrease of the NA and dopamine levels were required respectively.

Acetic acid-induced writhing test adapted from the method of Koster, Anderson & De Beer (1959) A 0.6 % acetic acid (HOAc) solution was injected i.p. in mice (0.2 ml/10 g body wt.) 5 or 25 min after TRH injection i.c.v. The acetic acid-induced abdominal contortions and contractions were defined as writhes. Five minutes after the acetic acid injection the number of writhes occurring during a period of 10 min was counted for each animal.

Mechanical stimulus

Haffner's test adapted from the method described by Bianchi & Franceschini (1954) An artery clip (force = 650 g) with the arms covered by thin plastic was applied to the base of the mouse's tail to induce a quick turning reaction and efforts to dislodge the clip by biting it. Each mouse was tested before drug administration and those mice not reacting to the clip within 10 s were excluded. If a mouse did not respond to the clip within 30 s after drug administration it was

considered to have a positive antinociceptive response and the clip was removed. The test was made at 15 min intervals after i.p. injection or at 5 min intervals after i.c.v. injection of TRH or solvent. For morphine the test was made at 30 min intervals after s.c. injection and at 15 min intervals after i.c.v. injection (peak effect). The duration of the TRH effect was studied by testing the mice 15, 30, 60 and 90 min after i.p. injection and 5, 15, 30, 60 and 90 min after i.c.v. injection.

Thermal stimuli

Direct contact: hot plate test adapted from the method of Eddy & Leimbach (1953) A mouse placed on a metallic plate at 55°C reacted by licking its forepaws or by jumping off the plate. Each mouse was tested twice, 15 min apart, before drug administration. Each mouse had a control latency between 4 and 10 s and if a mouse did not respond to the thermal stimulus within 20 s after injection of the drug it was considered to have a positive antinociceptive response. The test was performed at 15 min intervals after i.p. injection or at 5 min intervals after i.c.v. injection of TRH or solvent.

Radiant heat: tail-flick test adapted from the method of D'Amour & Smith (1952) The mouse was placed in a restraint cage and radiant heat was applied approximately 1.5 to 2.0 cm from the tip of the tail. This stimulus produced a reflex characterized by a lateral displacement of the tail. The intensity of the heat was adjusted to give a baseline latency of about 5 s. Three determinations of the latency were made in succession and their average was defined as the control latency. Only mice with a control latency of less than 7 s were used. To avoid tissue damage a cut-off time of 10 s was imposed on those animals failing to remove their tail from the light beam. After injection of the drug three further determinations of the latency were made in succession. If the mean latency was greater than 10 s the animal was considered to have a positive antinociceptive response. The tests were performed at 15 min intervals after i.p. administration. The effects of TRH injected centrally have not been investigated using this test since the construction of a restraint cage for an implanted mouse remains a problem.

Calculation of results

The effective doses for 50% of the animals (ED_{50}) were calculated according to the method of Litchfield & Wilcoxon (1949) and the 95% confidence limits found.

The percentage of the maximum possible effect (% MPE) was calculated as follows:

$$\%\,\mathrm{MPE} = \frac{\text{test latency} - \text{control latency}}{\text{cut-off time} - \text{control latency}} \times 100$$

The variability of the results was expressed by the

Table 1 Antinociceptive effects of TRH injected i.p. and i.c.v. in the PBQ-induced writhing test and Haffner's test, compared to morphine

Drugs		ED_{50} (peak effect)		*Molar relative potency in comparison to morphine*	
		Writhing test	*Haffner's test*	*Writhing test*	*Haffner's test*
Morphine					
s.c.	mg/kg	0.20 (0.148–0.27)	4.41 (3.18–6.18)	1	1
i.c.v.	μg/mouse	2.47×10^{-3} (0.63×10^{-3}–9.46×10^{-3})	2.47 (1.07–5.66)	1	1
	μg/kg	99×10^{-3} (25×10^{-3}–380×10^{-3})	98.8 (42.8–226.4)		
TRH					
i.p.	mg/kg	0.3 (0.18–0.48)	40 (17.6–93.2)	0.847	0.14
i.c.v.	μg/mouse	0.85×10^{-3} (0.14×10^{-3}–5.2×10^{-3})	1.5 (0.69–2.18)	3.69	2.09
	μg/kg	34×10^{-3} (5.6×10^{-3}–208×10^{-3})	60 (27.6–87.2)		

In parentheses, 95% fiducial limits

standard error of the mean (s.e.mean); the significance of the results when TRH was injected peripherally was calculated using Student's *t* test; the values before TRH injection were compared to those obtained afterwards for Haffner's test, the hot plate test and the tail-flick test (in these tests each animal acted as its own control); for the PBQ writhing test, the animals treated with TRH were compared with control animals, and animals treated with TRH plus drug were compared to animals treated with TRH or drug alone. When the TRH was administered centrally the significance was calculated using the Mann-Whitney non parametric test or Student's *t* test; for Haffner's test and the hot plate test, the differences between the values obtained before and after injection for the treated and control animals were compared; the treated animals were compared with control animals for the PBQ and acetic acid writhing tests.

Results

Chemical stimuli
Phenyl-p-benzoquinone-induced writhing test

Thyrotropin releasing hormone The antinociceptive activities (ED_{50}) of TRH and of morphine administered peripherally and centrally are given in Table 1. The dose-response curve for TRH (i.c.v.) is shown in Figure 1. If the TRH was injected i.p. 25 min before the PBQ solution, it was much less active (ED_{50}: 3.33 < 5 < 7.50 mg/kg) than if it was injected simultaneously with the PBQ solution (ED_{50}: 0.18 < 0.3 < 0.48 mg/kg); therefore the latter procedure was chosen.

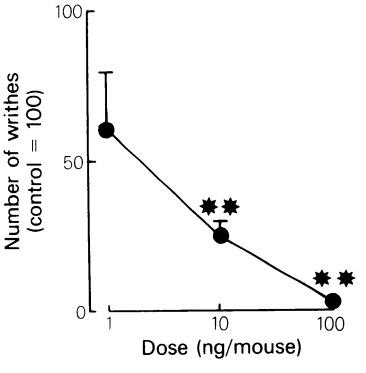

Figure 1 The effect of thyrotropin releasing hormone (TRH) i.c.v. on nociception in the phenyl-*p*-benzoquinone (PBQ)-induced writhing test. Ordinate scale: number of writhes as a percentage of corresponding control mice. Abscissa scale: doses of TRH in ng/mouse (log scale). The mice were treated with PBQ i.p. (0.25 ml) and TRH (1, 10 and 100 ng dissolved in 0.5 µl of CSF) or CSF (0.5 µl) which were given simultaneously. Five minutes after the injection of the drugs the number of writhes for each animal was counted during a period of 10 min. Vertical bars represent s.e.mean. The values obtained for the treated and control animals were analysed using the Mann-Whitney U test. ** Indicates a significant difference ($P < 0.01$). The number of mice was between 14 and 17 for each experimental group.

The ratio of the ED_{50} for TRH i.p. to that for TRH i.c.v. is high:

$$\frac{ED_{50}\,\text{i.p.}}{ED_{50}\,\text{i.c.v.}} = \frac{0.3 \times 10^3\ \mu g/kg}{0.034\ \mu g/kg} = 0.88 \times 10^4$$

This result suggests a central origin for the TRH action.

The potency of TRH relative to morphine calculated at peak effect is given in Table 1 for peripheral and central administration. TRH was approximately as active as morphine when injected peripherally but 3.5 times as active when given centrally.

Effect of various drugs injected i.p. which act upon noradrenergic, dopaminergic or 5-hydroxytryptaminergic systems and their interactions with TRH i.p. TRH (2 mg/kg i.p.) significantly reduced the number of writhes compared to the controls by 84.3 ± 0.55%. None of the tested drugs (Table 2) significantly antagonized this analgesic effect. With the lower dose of TRH (0.125 mg/kg i.p.) the number of writhes was not significantly reduced and therefore this dose was used to investigate possible potentiation of the TRH effect. The α-MT (tyrosine hydroxylase inhibitor) which depresses both noradrenergic and dopaminergic systems by decreasing their synthesis significantly potentiated ($P < 0.01$, Student's *t* test) the TRH effect (Table 3). This result suggests that noradrenergic or dopaminergic systems may be implicated in the TRH-induced analgesia. However, the involvement of the noradrenergic system was not clear since α-adrenoceptor antagonists such as phenoxybenzamine (8 mg/kg i.p.), phentolamine (4 mg/kg i.p.) and yohimbine (0.5 mg/kg i.p.) did not significantly modify the TRH effect. In addition some antidepressants which increase the synaptic level of NA such as nortriptyline and mianserin did not alter the TRH effect. In the investigation of the role of the dopaminergic system, apomorphine (0.05 mg/kg i.p.) which stimulates the presynaptic dopamine receptors at this dose, significantly potentiated ($P < 0.05$, Student's *t* test.) the TRH effect (Table 3), whereas haloperidol (0.5 mg/kg i.p.) which blocks the postsynaptic dopamine receptors, and nomifensine (20 mg/kg i.p.) which inhibits the uptake of dopamine, did not alter the TRH effect. This apparent discrepancy made it difficult to demonstrate that the dopaminergic system was implicated in the an-

Table 2 Drugs used to investigate potentiation or antagonism of the thyrotropin releasing hormone (TRH) activity in the phenyl-*p*-benzoquinone (PBQ) writhing test

Drugs	*Doses* (mg/kg i.p.)
5-HT modulators	
5-HTP	200
PCPA	300
Fluoxetine	10
NA modulators	
α-MT	250
Phenoxybenzamine	8
Phentolamine	4
Yohimbine	0.5
Dopamine modulators	
Apomorphine	0.05
Haloperidol	0.5
Antidepressants	
Imipramine	5
Nortriptyline	1
Mianserin	2
Iprindole	20
Nomifensine	20
Opiate receptor antagonist	
Naloxone	1 (s.c.)
	2.5

tinociceptive action of TRH. None of the 5-HT modulators or other antidepressants tested, significantly potentiated the TRH effect.

Interactions between TRH and naloxone Naloxone at a dose of 1 mg/kg s.c. did not produce hyperalgesia, potentiation or antagonism of the TRH effect. At the higher dose of 2.5 mg/kg i.p. it produced hyperalgesia (Table 3) which was antagonized by the simultaneous injection of 0.125 mg/kg i.p. of TRH ($P<0.01$, Student's *t* test).

Acetic acid-induced writhing test When the HOAc solution was injected i.p. 25 min after injecting TRH i.c.v. the TRH was inactive. In contrast, if acetic acid was injected i.p. simultaneously with TRH i.c.v., the number of writhes was reduced significantly by 65.5% ($P<0.05$, Mann-Whitney U test).

Mechanical stimulus

The antinociceptive activities (ED_{50}) of TRH and morphine administered both peripherally and centrally are given in Table 1. The dose-response curve for TRH i.c.v. is shown in Figure 2. The ED_{50} values for TRH were greater than those calculated in the PBQ writhing test, but their ratio remained high.

$$\frac{ED_{50}\,\text{i.p.}}{ED_{50}\,\text{i.c.v.}} = \frac{40\times10^3\,\mu g/kg}{60\,\mu g/kg} = 0.66\times10^3$$

The potency of TRH relative to morphine was calculated at peak effect (Table 1). When the drugs were administered peripherally, morphine was approximately seven times more active than TRH, but after central administration the TRH activity was twice as great as that of morphine.

The duration of the analgesic effect of TRH i.p. and i.c.v. has been studied. When the peptide was injected i.p. the dose which produced an analgesic effect in 50% of the animals 15 min after the injection had no effect at 30 min. When TRH was injected i.c.v. the time course for the more effective doses (8 and 16 μg/mouse) was as shown in Figure 3. Five minutes after injection the % MPE were 70 and 95%

Table 3 Drugs which affect the activity of thyrotropin releasing hormone (TRH)

Drugs	*Dose* (mg/kg i.p.)	*% inc. or dec. of the number of writhes compared to controls*	
α-MT	250	+17.9	(24)
TRH	0.125	− 3.8[a]	(24)
α-MT + TRH		−50 **†	(24)
Apomorphine	0.05	−13	(24)
TRH	0.125	− 8[a]	(24)
Apomorphine + TRH		−46 *†	(24)
Naloxone	2.5	+64.7	(12)
TRH	0.125	−24.1[a]	(12)
Naloxone + TRH		−35.2 **‡	(12)

Student's *t* test: $*P<0.05$; $**P<0.01$.
Comparison between (TRH + drug) and TRH (†) or drug (‡).
In parentheses, number of mice.
[a] Effect obtained on the same day as the drug and (TRH + drug).

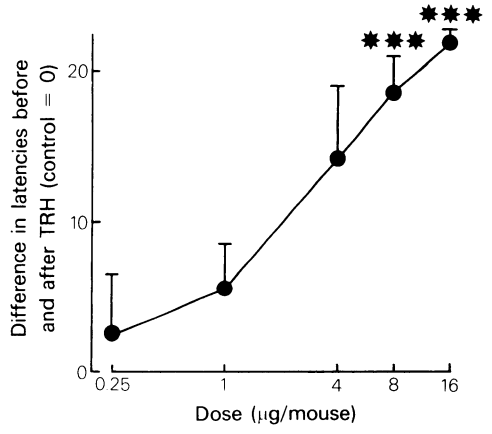

Figure 2 The effect of thyrotropin releasing hormone (TRH) i.c.v. on nociceptive reaction latencies in Haffner's test. Ordinate scale: difference in latencies before and after TRH. Abscissa scale: doses of TRH in μg/mouse (log scale). The mice were treated with TRH (0.25, 1, 4, 8 and 16 μg dissolved in 0.5 μl of CSF) or CSF (0.5 μl), 5 min before the test. Vertical bars represent s.e.mean. The differences between the values obtained before and after injection for the treated and control animals were analysed using the Mann-Whitney U test for doses of 0.25–1 and 4 μg/mouse and Student's *t* test for 8 and 16 μg/mouse. *** Indicates a significant difference ($P < 0.001$). The number of mice was between 7 and 25 for each experimental group.

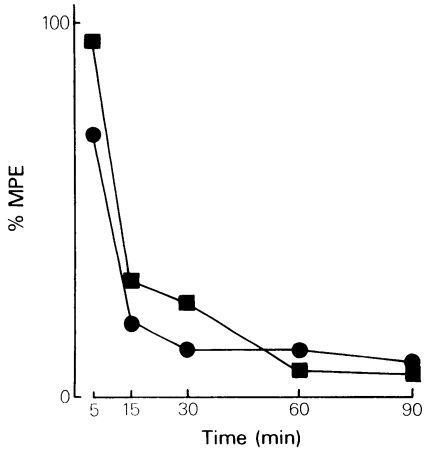

Figure 3 Variation of the percentage of the maximum possible effect (% MPE) with time in Haffner's test. The mice received 8 μg (●) and 16 μg (■) thyrotropin releasing hormone i.c.v. dissolved in 0.5 μl of CSF. The number of animals in each experimental group was 25 and 14 respectively.

respectively, and 15 min after the injection the analgesic effect was reduced to about one third of these values.

TRH was less effective in Haffner's test than in the PBQ-induced writhing test and therefore the interaction between TRH and other drugs was only studied in the latter.

Thermal stimuli

Hot plate test None of the animals was protected by TRH injected i.p. (1, 10 or 40 mg/kg) (Table 4). In contrast, at the higher dose TRH induced a significant hyperalgesia ($P < 0.001$, Student's *t* test). When TRH was injected i.c.v. 1 μg/mouse produced a significant increase of the latencies ($P < 0.05$, Student's *t* test), but a higher dose of TRH (20 or 40 μg) did not produce a significant increase (Table 4).

Tail-flick test None of the animals was protected by TRH i.p. (40 mg/kg) and we were unable to investigate the effect centrally in the tail-flick test.

Discussion

TRH injected peripherally or centrally possesses antinociceptive properties in Haffner's test and the PBQ writhing test. TRH i.p. is not effective in the hot plate test even though a slightly significant increase of the latencies was observed after 1 μg/mouse of TRH i.c.v. We cannot conclude that TRH possesses an analgesic effect in this test since higher doses of TRH i.c.v. did not produce any antinociceptive effect. The lack of TRH activity i.p. in the tail-flick test confirms that TRH does not protect the mice against thermal stimuli. The effect of TRH i.c.v. in Haffner's test and the PBQ writhing test was approximately 1,000 and 10,000 times greater respectively than that of TRH i.p. This provides evidence that a central mechanism is involved. TRH analgesia is strong since its effect when given i.c.v. is twice as great on a molar basis as that of morphine i.c.v. in Haffner's test and 3.5 times as great in the PBQ writhing test. The duration of the TRH action was very short: in Haffner's test the peak effects of TRH i.c.v. and i.p. were at 5 and 15 min respectively, after which the activity rapidly decreased; in the PBQ writhing test, peripheral injections of TRH were more active when injected simultaneously with the PBQ rather than being given 25 min beforehand; TRH exhibited an antinociceptive response in the acetic acid-induced writhing test only if it was injected simultaneously with the acetic acid. This short duration of analgesic activity for TRH against acetic acid-induced writhing would explain the results observed by Osbahr, Nemeroff, Lutinger, Mason & Prange (1981) who found that TRH

Table 4 Nociceptive reaction latencies before and after injecting thyrotropin releasing hormone (TRH, i.p. and i.c.v.) and CSF (i.c.v.) in the hot plate and tail-flick tests

	Hot plate		*Tail-flick*	
	(mean ± s.e.mean of latencies)			
	Before	*After*	*Before*	*After*
TRH i.p. (mg/kg)				
1	6.30 ± 0.54	8.20 ± 1.35 (10)		
10	7.35 ± 0.41	5.80 ± 1.16 (10)		
40	6.25 ± 0.35	1.90 ± 0.31*** (10)	4.67 ± 0.54	4.69 ± 0.39 (10)
TRH i.c.v. (μg/mouse)				
1	8.47 ± 0.25	12.8 ± 1.19* (16)		
20	7.28 ± 0.25	11.2 ± 1.52 (14)		
40	6.64 ± 0.36	10.86 ± 2.04 (7)		
CSF i.c.v. (μl)				
0.5	8.00 ± 0.38	8.33 ± 1.07 (9)		

Student's t test; $^{*}P < 0.05$; $^{***}P < 0.001$.
In parentheses, number of mice.

injected intracisternally 25 min before acetic acid had no effect. The short action of TRH has already been evoked for its other properties: Heal & Green (1979) have shown that bilateral injection of TRH (10 μg) into the nucleus accumbens of rats produces a short increase in coordinated locomotor activity lasting only 20 min after injection; Boschi & Rips (1981) have shown that TRH-induced hyperthermia (40 mg/kg i.p.) is maximal at 15 min and then decreases. This short action of TRH could reflect a rapid degradation which makes the peptide a good candidate for having a neurotransmitter function. Such lability has already been shown for the enkephalins and is considered to be a prerequisite for all neurotransmitters (Frederickson, 1977).

TRH is not equiactive against the different nociceptive stimuli. The ED_{50}s i.p. and i.c.v. are smaller in the PBQ writhing test than in Haffner's test. The same observation was made with morphine (morphine s.c. and i.c.v. is more active in the PBQ writhing test than in Haffner's test). This has already been shown for morphine s.c. by Umans & Inturrisi (1981). TRH was inactive against thermal nociception (hot plate test, tail-flick test). This lack of antinociceptive activity in the tail-flick test has already been found in mice by Delbarre, Senon & Doreau (1978) after administration of 0.5 μg of TRH i.c.v. and by Martin, Dewey, Chau-Pham & Prange (1977) after i.p. administration of 1, 10 and 25 mg/kg of TRH. Dennis & Melzack (1980) investigated the modulation of pain by 5-hydroxytryptaminergic adrenergic agents and morphine using three pain tests (tail-flick, hot plate and formalin tests). These authors suggested that the differences in the type of noxious stimulus and in the motor response to these stimuli were crucial in determining the observed pharmacological profile of pain and analgesia.

Investigation of the possible mechanisms involved in the TRH effect using the PBQ writhing test led us to conclude that the activity of TRH is resistant to alteration of the NA, dopamine and 5-HT systems since effectively no antagonism of the TRH action was observed. Only α-MT and apomorphine produced a significant potentiation of the TRH effect ($P < 0.05$ and $P < 0.01$, respectively, Student's t test) but this fact remains unexplained since α-receptor blockers, a dopamine receptor blocker and some antidepressants did not significantly modify the TRH effect. The mechanisms involved in the TRH analgesia are therefore different from those involved in the TRH hyperthermia where a peripheral adrenergic system has been implicated in mice (Desiles & Rips, 1979; Boschi & Rips, 1981). TRH analgesic activity is not antagonized by naloxone, either 1 mg/kg s.c. or 2.5 mg/kp i.p., but TRH at a non-analgesic dose antagonizes the naloxone-induced hyperalgesia. This result leads us to believe that there is a possible interaction of TRH with opiate receptors even though their blockade by naloxone does not prevent the TRH analgesia. Our results differ from those of Cuenca *et al.* (1978) who have shown that a 5-hydroxytryptaminergic system is implicated in the antinociceptive effect of TRH against acetylcholine bromide-induced writhing; they also found that naloxone alone exhibits hyperalgesia in doses above 0.5 mg/kg s.c. and that prior administration of naloxone (1 mg/kg s.c.) partially blocks the effect of TRH whereas 2 mg/kg s.c. gives complete blockade. This discrepancy could be explained by differences in experimental design.

Injection of TRH or other drugs into precise brain areas may provide useful information as to the mechanism of TRH action regardless of the antinociceptive action against mechanical or chemical stimuli. The short duration of action and the resistance to pharmacological modification of the TRH-induced analgesia suggest that TRH may be an endogenous analgesic, as has been suggested for other short-acting peptides (Hughes & Kosterlitz, 1977). The discovery that TRH coexists with substance *P* and 5-HT (both of which are involved in pain conduction) in the neurones of the medulla oblongata which project into the spinal cord (Johansson, Hökfelt, Pernow, Jeffcoate, White, Steinbusch, Verhofstad, Emson & Spindel, 1981) suggests that the mechanisms involved in TRH analgesia are complex.

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