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Effect of low intravenous doses of TRH, acid-TRH and cyclo(His-Pro) on cerebral and peripheral blood flows

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1 Local cerebral and peripheral blood flow in conscious and anaesthetized rabbits were investigated with the microsphere method, before and after the i.v. administration of 25 or 50 $\mu g\,kg^{-1}$ thyrotropin-releasing hormone (TRH). Before the experiment, the cervical sympathetic chain was sectioned on one side in order to evaluate the possible effect of the sympathetic nerves on cranial and extracranial blood flows.

2 Blood flow was also determined in anaesthetized rabbits before and after the administration of the TRH metabolites cyclo(His-Pro) and acid-TRH and after subsequent administration of 50 $\mu g\,kg^{-1}$ TRH.

3 TRH caused an increase in mean arterial blood pressure (MAP) of about 1 to 2 kPa whereas cyclo(His-Pro) and acid-TRH had no effect on MAP.

4 In the anaesthetized animal an increase in total cerebral blood flow (CBF_{tot}), from 71 ± 7 to $107 \pm 12\,g\,min^{-1}\,100g^{-1}$ ($P < 0.05$) was observed on the sympathetic intact side after 25 $\mu g\,kg^{-1}$ TRH and a further increase to $130 \pm 9\,g\,min^{-1}\,100g^{-1}$ ($P < 0.01$) after 50 $\mu g\,kg^{-1}$ TRH. A similar effect was observed on the sympathotomized side.

5 An effect on CBF in the conscious animal was not detected. The control CBF_{tot} ($104 \pm 8\,g\,min^{-1}\,100g^{-1}$) was higher in these animals than in the anaesthetized animals ($P < 0.02$).

6 Neither cyclo(His-Pro) nor acid-TRH mimicked the effect of TRH on CBF.

7 In several peripheral tissues, e.g. skin, pancreas and gastric mucosa, a reduction in blood flow was noted after the administration of TRH in both anaesthetized and conscious rabbits.

8 It was concluded that TRH can induce cerebral vasodilatation in animals with a depressed CBF, whereas the vasoconstrictor effect of TRH in peripheral organs is not markedly affected by the state of consciousness.

Introduction

Thyrotropin-releasing hormone, or TRH, a tripeptide widely distributed in the CNS and peripheral organs (Hökfelt *et al.*, 1975a,b; Morley *et al.*, 1977; Leppäluoto *et al.*, 1978) has, in addition to endocrine functions, complex effects on, for example, cardiovascular functions, respiration, behaviour and cerebral neuronal activity; for reviews see Morley (1979) and Yarbrough (1979). Beneficial effects of TRH in a variety of shock states (Faden *et al.*, 1981, Holaday *et al.*, 1982) and other pathological conditions e.g. spinocerebellar degeneration (Sobue *et al.*, 1983) and depression (Prange *et al.*, 1972; Ogawa *et al.*, 1984) have been reported. The precise mechanisms of action of TRH in these conditions are not yet understood.

Circulatory effects in the CNS of TRH have been evaluated in various models and under a variety of conditions with conflicting results (Faden *et al.*, 1982, 1983; Hanko *et al.*, 1982; Naftchi & Gennaro, 1982). Recently it was reported that TRH caused cerebral vasodilatation and peripheral vasoconstriction in the anaesthetized animal (Koskinen & Bill, 1984). It has also been shown that TRH can abolish the decrease in cerebral blood flow in cerebral ischaemia (Koskinen, 1985).

TRH is metabolized by at least two routes; two of the metabolites are pyroglutamyl-histidyl-proline (acid-TRH) and histidyl-proline diketopiperazine (cyclo(His-Pro)); see Peterkofsky & Battaini (1980). The latter in particular may be responsible for eliciting some of the effects attributed to TRH and in addition

to have effects different from those of TRH. There is evidence that cyclo(His-Pro) inhibits some of the effects elicited by TRH (Peterkofsky & Battaini, 1980). Interestingly, this metabolite is distributed throughout the CNS and in peripheral organs, sometimes in excess of TRH (Prasad *et al.*, 1982; Lamberton *et al.*, 1984). For this reason it has been proposed that cyclo(His-Pro) may have origins other than TRH. Effects elicited by acid-TRH have also been described (Boschi *et al.*, 1980).

The experiments presented here were undertaken in order to characterize the effects of low intravenous doses of TRH in anaesthetized and conscious animals. Furthermore, acid-TRH and cyclo(His-Pro) were administered in animals anaesthetized with urethane in order to elucidate whether these metabolites of TRH had biological activity in the cardiovascular system, particularly on the microcirculation in the brain.

Methods

New Zealand white rabbits of either sex weighing 1.9–2.5 kg were used.

Anaesthetized animals

Anaesthesia was induced by injecting 7 ml kg^{-1} of a 25% solution of urethane into a marginal ear vein. Both femoral arteries were cannulated and used as described below. The left ventricle was cannulated retrogradely via the left brachial artery. The animal was tracheotomized and ventilated by a Palmer pump. Body temperature was recorded by a rectal thermistor and maintained at 38–39°C. Pancuronium bromide (Pavulon) 0.05–0.2 mg kg^{-1} i.v. was given in order to induce skeletal muscle relaxation. To elucidate sympathetic effects to the head region the cervical sympathetic chain was unilaterally sectioned about 1 cm below the upper cervical ganglion. The side of sectioning was varied.

Conscious animals

To prepare these rabbits for the experiment they were first anaesthetized with an initial dose (40 mg kg^{-1}) of pentobarbitone sodium (Mebumal) via a marginal ear vein. Both femoral arteries were cannulated with polyethylene catheters, the catheters being fixed and tunnelled beneath the skin and exteriorized on the hind legs. A catheter was also inserted into the left ventricle as described above and exteriorized in the neck. The sympathetic chain on one side was sectioned as described above. All catheters were filled with a heparin solution of 800 i.u. ml^{-1} to prevent clotting. The wounds were treated with chlortetracyline (Aureomycine) powder and then sutured. The rabbit was placed in a cage and allowed to rest for at least 24 h before blood flow measurements were made. Only rabbits in good condition were used for the measurements.

General

One of the arterial catheters was used for the determination of mean arterial blood pressure (MAP) and heart rate (HR) with a Druck PDCR 75/1 transducer and a Servogor 460 recorder. The second catheter was used for blood sampling. The heart catheter was used to introduce microspheres for regional blood flow determinations (Wagner *et al.*, 1969; Alm & Bill, 1972). Spheres, 15 μm in diameter (NEN), labelled with ^{103}Ru, ^{113}Sn and ^{141}Ce were used. Cardiac output (CO) was calculated as $CO = Q_r \times CPMi \times CPMr^{-1}$ where Q_r = reference blood flow, CPMr = reference activity and CPMi = injected activity. Arterial P_{CO_2}, P_{O_2} and pH were determined with an ABL2 acid-base analyser (Radiometer, Copenhagen). When needed, i.v. sodium bicarbonate was given in order to maintain a normal pH. Heparin, 500 i.u. kg^{-1} i.v. was injected in order to prevent clotting.

First a control blood flow measurement was performed. Then 25 μg kg^{-1} TRH ($n = 6$ anaesthetized, $n = 7$ conscious), or in the anaesthetized animal either 50 μg kg^{-1} acid-TRH ($n = 6$) or cyclo(His-Pro) ($n = 6$), was injected intravenously and about 3 min later the second blood flow measurement was made. About 15 min later TRH 50 μg kg^{-1} i.v. was injected. Three minutes later a third blood flow measurement was made. After the last microsphere injection the animal was killed by an i.v. dose of anaesthetic followed by saturated KCl.

Organs and tissue samples were dissected out and placed in preweighed plastic tubes. Samples included grey matter from frontal and occipital cortex, white matter, hippocampal region, caudate nucleus, thalamic region, hypothalamic region, collicles, pons-mesencephalon, medulla oblongata, cerebellum and spinal cord. Total cerebral blood flow (CBF) was calculated to include all regions except medulla oblongata, cerebellum and spinal cord. The eye was dissected into the choroid, iris and ciliary processes and another extracranial tissue, the masseter muscle, was sampled in addition to the following peripheral tissues; skeletal muscle, skin, duodenum, jejunum, gastric mucosa, pancreas, spleen, adrenal glands, kidney cortices and cardiac muscle. The radioactivity of blood and tissue samples was determined by gamma spectrometry. Blood flows were calculated according to the free flow method (reference blood collected from an artery into pre-weighed plastic tubes in 10 s portions during 60 s starting at the time of injection of the microspheres) (Alm & Bill, 1972).

TRH, lot no. GA 3558, kindly donated by Ferring

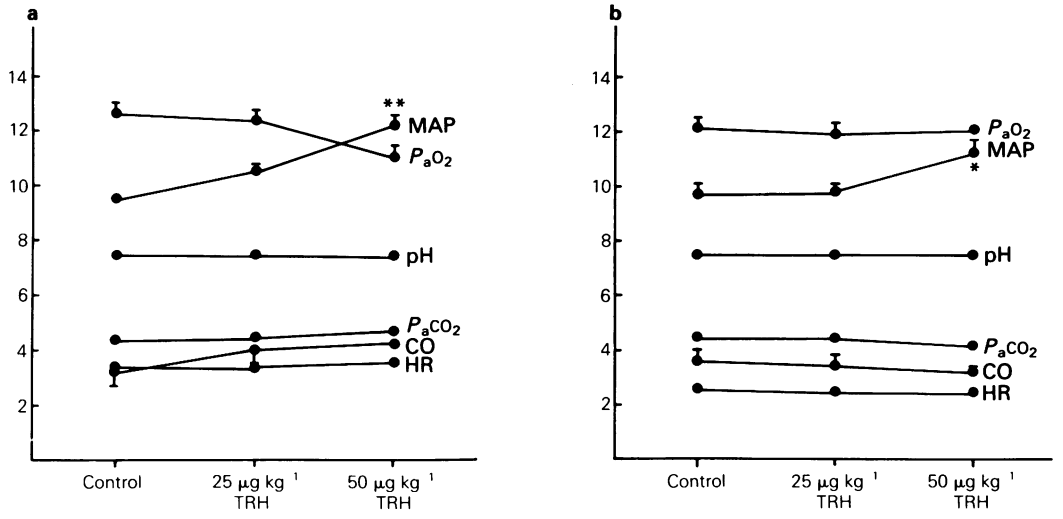

Figure 1 Arterial acid-base values, mean arterial blood pressure (MAP), heart rate $\times 10^{-2}$ (HR) and cardiac output $\times 10^{-2}$ (CO) in (a) anaesthetized ($n = 6$) and (b) conscious ($n = 7$) animals during the control situation and after 25 and 50 $\mu g\,kg^{-1}$ TRH administration. Pressures are given in kPa and CO in $g\,min^{-1}$. $^*P < 0.05$ and $^{**}P < 0.01$ compared with control values.

(Malmö, Sweden) or lot no. 100F-5880 (Sigma), acid-TRH lot no. 34F-0374 and cyclo(His-Pro) lot no. 14F-0003 (Sigma) were used.

The results were evaluated with Student's two-tailed t test for paired or unpaired observations or with analysis of variance (ANOVA). Dunnet's test was used as a post ANOVA test for the comparison of treatments with control. Two levels of significance were considered in Dunnet's test, $P < 0.05$ and $P < 0.01$. If not otherwise stated P-values were generated by Dunnet's test. Results are presented as means ± s.e.

Results

Effects of TRH in anaesthetized and conscious animals

Figure 1 shows the cardiovascular variables before and after the administration of TRH. Blood gases, CO and HR were not greatly affected although a decrease in P_aO_2 was seen in the anaesthetized animal. TRH 50 $\mu g\,kg^{-1}$ caused a statistically significant increase in MAP in both conscious ($P < 0.05$) and anaesthetized ($P < 0.01$) animals.

In the anaesthetized rabbit a marked increase in regional cerebral blood flow (rCBF) was observed after the administration of TRH, see Table 1. The lower dose of TRH caused increases ranging between 20–90% while the higher dose caused increases from 30 to 100%. Increases of more than 70% were observed in the cortical gray matter, caudate nucleus, thalamic and hypothalamic regions on both the intact and sympathotomized side (all statistically significant) after 50 $\mu g\,kg^{-1}$ TRH. Total cerebral blood flow increased from 71 ± 7 to 107 ± 12 ($P < 0.05$) after 25 $\mu g\,kg^{-1}$ TRH and to 130 ± 9 $g\,min^{-1}\,100g^{-1}$ ($P < 0.01$) after 50 $\mu g\,kg^{-1}$ on the intact side. Corresponding values for the sympathotomized side were 73 ± 7, 112 ± 13 ($P < 0.05$) and 135 ± 11 $g\,min^{-1}$ $100g^{-1}$ ($P < 0.01$) respectively, see Figure 3.

Table 2 shows the local cerebral blood flows in the conscious animals. There was no obvious increase in rCBF after the administration of TRH. As expected, the basal total CBF, 104 ± 8 $g\,min^{-1}\,100g^{-1}$ on the intact side, was higher in the conscious animals than in the anaesthetized animals ($P < 0.02$, unpaired t test). After 25 $\mu g\,kg^{-1}$ TRH the flow was 103 ± 6 and after 50 $\mu g\,kg^{-1}$ TRH 93 ± 4 $g\,min^{-1}\,100g^{-1}$. Values for the sympathotomized side were 108 ± 10, 102 ± 5 and 93 ± 2 $g\,min^{-1}\,100g^{-1}$ respectively, see Figure 3.

Figure 4 shows the blood flows, expressed as a % of the initial values, in some peripheral tissues after TRH administration. In the anaesthetized animal 50 $\mu g\,kg^{-1}$ TRH caused a statistically significant decrease in blood flow in the pancreas ($P < 0.05$), spleen ($P < 0.01$) and gastric mucosa ($P < 0.01$). The most pronounced effect was manifest in the spleen and gastric mucosa with decreases of 50% or more. TRH 50 $\mu g\,kg^{-1}$ tended to cause an increase in blood flow in the adrenal gland and duodenum whereas the most pronounced effect was in the cardiac muscle in which there was an increase of 137% ($P < 0.01$). In the conscious animal decreases in local peripheral blood

Table 1 Regional cerebral and spinal cord blood flows in urethane anaesthetized rabbits ($n = 6$) in the control situation and after TRH 25 and 50 $\mu g\,kg^{-1}$ i.v.

	Control		*TRH 25 $\mu g\,kg^{-1}$*		*TRH 50 $\mu g\,kg^{-1}$*	
	I	*S*	*I*	*S*	*I*	*S*
Gray matter	78 ± 6	81 ± 6	119 ± 13*	122 ± 13*	158 ± 11**	163 ± 13**
White matter	57 ± 6	62 ± 7	74 ± 8	91 ± 9	92 ± 5**	99 ± 9*
Hippocampal region	46 ± 6	50 ± 6	69 ± 11	73 ± 11	75 ± 6*	77 ± 8
Caudate nucleus	75 ± 6	81 ± 7	119 ± 16*	129 ± 13**	139 ± 10**	148 ± 8**
Thalamic region	73 ± 8	74 ± 9	127 ± 21*	135 ± 22*	150 ± 11**	153 ± 13**
Hypothalmic region	46 ± 4	47 ± 6	73 ± 7*	93 ± 18*	88 ± 8**	93 ± 4*
Collicles	88 ± 10	88 ± 10	116 ± 11	116 ± 13	123 ± 9	128 ± 10
Pons-mesencephalon	64 ± 9	65 ± 8	86 ± 10	88 ± 10	99 ± 6*	97 ± 7*
Medulla oblongata	60 ± 11	59 ± 9	86 ± 11	81 ± 13	85 ± 7	83 ± 7
Cerebellum	99 ± 19	97 ± 18	122 ± 14	125 ± 15	151 ± 14	149 ± 17
Spinal cord (C_1–C_3)	51 ± 10	48 ± 9	66 ± 6	66 ± 10	65 ± 4	62 ± 5

I = intact side, S = sympathotomized side. Statistical comparisons were made with control by Dunnet's test. *$P < 0.05$ and **$P < 0.01$. Values are g min^{-1} $100g^{-1}$ tissue.

flow by more than 30% were elicited by 50 $\mu g\,kg^{-1}$ TRH in the skin ($P < 0.01$), jejunum ($P < 0.01$), duodenum ($P < 0.05$), pancreas ($P < 0.01$) and gastric mucosa ($P < 0.05$). However in none of the peripheral tissues investigated was there a pronounced increase in blood flow after TRH.

The vascular resistance in the masseter muscle, calculated as MAP/blood flow min^{-1} per 1 g tissue, is shown in Figure 6. There was a clear decrease in resistance on the sympathotomized side ($P < 0.01$, *t* test), which was accentuated in the anaesthetized, compared with the conscious animal ($P < 0.05$, unpaired *t* test). TRH caused a small increase in resistance on the side with an intact sympathetic supply. There was no statistically significant effect of TRH on the ocular blood flow (Table 5). However, in the animal under urethane anaesthesia there was a clear effect of the sympathotomy resulting in a statistically significant higher blood flow compared with the intact side. This difference seemed to be accentuated by TRH administration. This was not observed in the conscious animal, see Table 5.

Effects of acid-TRH and cyclo(His-Pro) in anaesthetized animals

Figure 2 shows the cardiovascular parameters before and after the administration of the peptides. Blood gases, CO and HR were within the normal range. Acid-TRH and cyclo(His-Pro) had no effect on MAP.

Table 2 Regional cerebral and spinal cord blood flows in conscious rabbits ($n = 7$) in the control situation and after TRH 25 and 50 $\mu g\,kg^{-1}$ i.v.

	Control		*TRH 25 $\mu g\,kg^{-1}$*		*TRH 50 $\mu g\,kg^{-1}$*	
	I	*S*	*I*	*S*	*I*	*S*
Gray matter	140 ± 14	141 ± 20	141 ± 10	133 ± 10	124 ± 8	123 ± 8
White matter	54 ± 9	51 ± 6	52 ± 7	46 ± 6	46 ± 6	43 ± 5
Hippocampal region	55 ± 4	56 ± 4	52 ± 2	48 ± 2	48 ± 1	45 ± 2
Caudate nucleus	114 ± 12	122 ± 17	116 ± 6	130 ± 14	108 ± 6	109 ± 5
Thalamic region	89 ± 6	92 ± 6	93 ± 5	93 ± 6	84 ± 4	85 ± 3
Hypothalamic region	58 ± 3	58 ± 2	60 ± 3	57 ± 4	48 ± 1*	55 ± 4
Collicles	106 ± 7	106 ± 11	110 ± 7	101 ± 4	97 ± 4	88 ± 3
Pons-mesencephalon	77 ± 6	83 ± 6	74 ± 4	77 ± 4	66 ± 2	68 ± 3
Medulla oblongata	52 ± 3	55 ± 4	51 ± 3	52 ± 4	44 ± 1	45 ± 3
Cerebellum	99 ± 8	98 ± 6	99 ± 6	98 ± 4	89 ± 4	89 ± 3
Spinal cord (C_1–C_3)	36 ± 3	38 ± 3	36 ± 2	37 ± 2	31 ± 1	31 ± 2

I = intact side, S = sympathotomized side. Statistical comparisons were made with control by Dunnet's test. *$P < 0.05$. Values are g min^{-1} $100g^{-1}$ tissue.

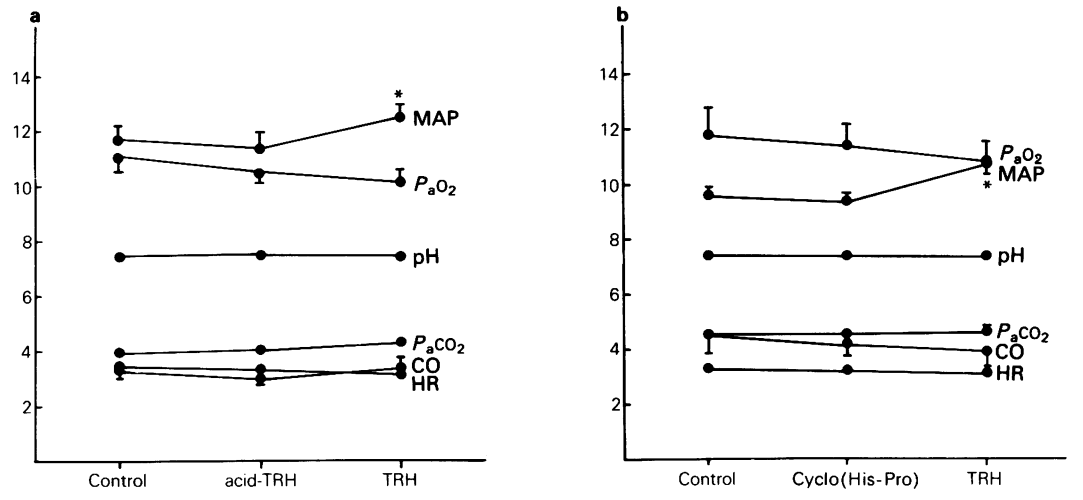

Figure 2 Arterial acid-base values, mean arterial blood pressure (MAP), heart rate $\times 10^{-2}$ (HR) and cardiac output $\times 10^{-2}$ (CO) in anaesthetized animals during the control situation, (a) after 50 $\mu g\,kg^{-1}$ acid-TRH ($n = 6$) or (b) cyclo(His-Pro) ($n = 6$) and after 50 $\mu g\,kg^{-1}$ TRH administration. Pressures are given in kPa and CO in $g\,min^{-1}$. $^*P < 0.05$ compared with control values.

The subsequent administration of 50 $\mu g\,kg^{-1}$ TRH elicited an increase in MAP by about 1 kPa ($P < 0.05$).

Regional cerebral blood flow was not significantly altered by acid-TRH, see Table 3 but the subsequent administration of TRH caused an increase. Total CBF on the side with an intact sympathetic supply was 74 ± 6 in the control situation, 76 ± 5 after acid-TRH and 107 ± 7 $g\,min^{-1}\,100g^{-1}$ ($P < 0.01$) after TRH administration. The values for the sympathotomized side were 75 ± 7, 80 ± 6 and 114 ± 10 $g\,min^{-1}\,100g^{-1}$ ($P < 0.01$), respectively, see Figure 3.

In gray matter and caudate nucleus there was a tendency for an increase in blood flow after the administration of cyclo(His-Pro), see Table 4. After TRH administration rCBF was statistically significantly increased in a number of regions. However, compared with the acid-TRH experiments the response to TRH was smaller and occurred in fewer regions. Total CBF on the intact side increased from 64 ± 4 to 74 ± 6 $g\,min^{-1}\,100g^{-1}$ after cyclo(His-Pro) and to 87 ± 7 $g\,min^{-1}\,100g^{-1}$ ($P < 0.05$) after TRH. The corresponding values for the denervated side were 70 ± 4, 76 ± 5 and 94 ± 8 $g\,min^{-1}\,100g^{-1}$ ($P < 0.05$), respectively, see Figure 3.

A statistically significant effect of acid-TRH was not found in any of the peripheral tissues investigated

Table 3 Regional cerebral and spinal cord blood flows in anaesthetized rabbits ($n = 6$) in the control situation and after acid-TRH 50 $\mu g\,kg^{-1}$ i.v. and TRH 50 $\mu g\,kg^{-1}$ i.v.

	Control		*acid-TRH*		*TRH*	
	I	*S*	*I*	*S*	*I*	*S*
Gray matter	72 ± 8	75 ± 7	75 ± 7	84 ± 8	126 ± 13**	142 ± 16**
White matter	66 ± 10	68 ± 4	71 ± 8	70 ± 10	80 ± 8	85 ± 5*
Hippocampal region	47 ± 2	47 ± 4	50 ± 4	50 ± 4	64 ± 4**	65 ± 6*
Caudate nucleus	82 ± 6	82 ± 8	87 ± 7	81 ± 8	117 ± 14*	123 ± 11**
Thalamic region	75 ± 6	79 ± 8	80 ± 6	84 ± 6	120 ± 9*	126 ± 10**
Hypothalamic region	42 ± 4	53 ± 5	42 ± 3	59 ± 5	62 ± 5	73 ± 8**
Collicles	90 ± 8	88 ± 8	95 ± 5	98 ± 7	104 ± 5	106 ± 8
Pons-mesencephalon	65 ± 6	65 ± 6	68 ± 4	69 ± 5	80 ± 5	78 ± 5
Medulla oblongata	61 ± 4	61 ± 6	64 ± 2	68 ± 5	72 ± 7	76 ± 6
Cerebellum	92 ± 10	91 ± 10	96 ± 7	100 ± 7	108 ± 8	108 ± 8
Spinal cord (C_1–C_3)	50 ± 5	51 ± 4	53 ± 4	54 ± 4	55 ± 5	60 ± 6

I = intact side, S = sympathotomized side. Statistical comparisons were made with control by Dunnet's test. $^*P < 0.05$ and $^{**}P < 0.01$. Values are $g\,min^{-1}\,100g^{-1}$ tissue.

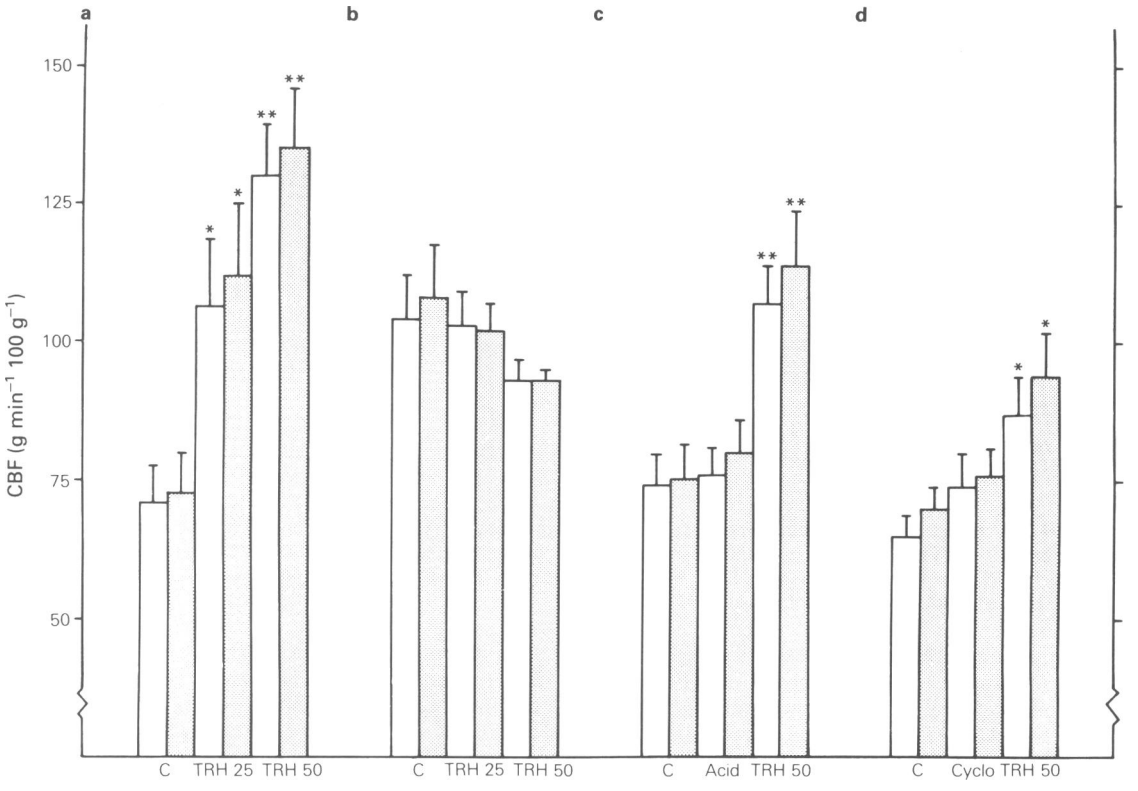

Figure 3 Total cerebral blood flow in (a) anaesthetized ($n = 6$) and (b) conscious ($n = 7$) animals during control situation (C) and after 25 or 50 $\mu g\,kg^{-1}$ TRH administration, and in (c and d) anaesthetized animals after 50 $\mu g\,kg^{-1}$ acid-TRH (acid; $n = 6$) or cyclo(His-Pro) (cyclo; $n = 6$) and after 50 $\mu g\,kg^{-1}$ TRH administration. Open columns = intact side, shaded columns = sympathotomized side. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with control.

Table 4 Regional cerebral and spinal cord blood flows in anaesthetized rabbits ($n = 6$) in the control situation and after cyclo(His-Pro) 50 $\mu g\,kg^{-1}$ i.v. and TRH 50 $\mu g\,kg^{-1}$ i.v.

	Control		*cyclo(His-Pro)*		*TRH*	
	I	*S*	*I*	*S*	*I*	*S*
Gray matter	55 ± 5	61 ± 5	70 ± 8	74 ± 9	91 ± 11*	104 ± 14*
White matter	52 ± 3	53 ± 3	65 ± 5	59 ± 5	62 ± 5	62 ± 6
Hippocampal region	46 ± 2	45 ± 3	48 ± 3	49 ± 2	54 ± 4	55 ± 5
Caudate nucleus	76 ± 6	72 ± 3	96 ± 9	93 ± 9	107 ± 12	109 ± 8**
Thalamic region	68 ± 4	72 ± 4	76 ± 6	77 ± 5	94 ± 10	98 ± 9*
Hypothalamic region	50 ± 2	55 ± 5	61 ± 4	58 ± 5	73 ± 14	70 ± 11
Collicles	82 ± 8	86 ± 6	90 ± 9	94 ± 8	99 ± 9	103 ± 10
Pons-mesencephalon	60 ± 4	63 ± 3	64 ± 2	65 ± 4	72 ± 5	77 ± 7
kedulla oblongata	76 ± 6	75 ± 5	76 ± 3	75 ± 3	82 ± 7	82 ± 6
Cerebellum	98 ± 6	103 ± 6	97 ± 7	103 ± 8	110 ± 12	117 ± 12
Spinal cord (C_1–C_3)	47 ± 4	50 ± 5	52 ± 4	52 ± 5	53 ± 4	56 ± 4

I = intact side, S = sympathotomized side. Statistical comparisons were made with control by Dunnet's test. $^{*}P < 0.05$ and $^{**}P < 0.01$. Values are g min^{-1} $100g^{-1}$ tissue.

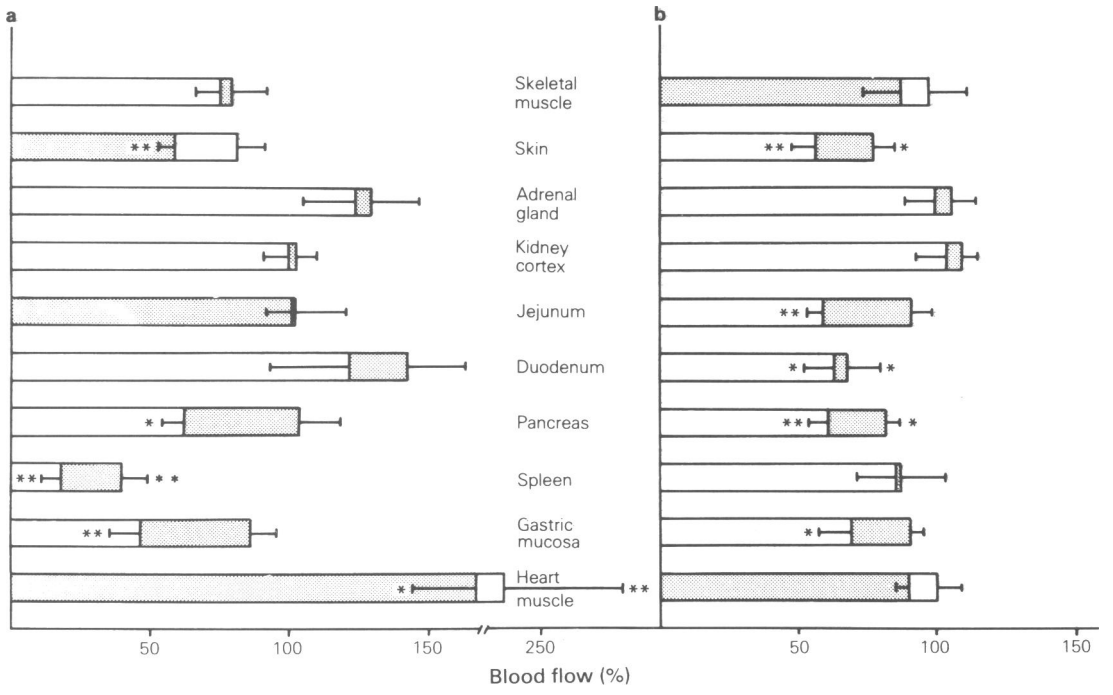

Figure 4 Blood flow as percentage of control values in some peripheral tissues in (a) anaesthetized ($n = 6$) and (b) conscious ($n = 7$) animals. Shaded columns = after $25\,\mu g\,kg^{-1}$ TRH, open columns = after $50\,\mu g\,kg^{-1}$ TRH. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with control.

whereas cyclo(His-Pro) caused a small but statistically significant decrease in blood flow in the gastric mucosa ($P < 0.05$), see Figure 5. The subsequent administration of $50\,\mu g\,kg^{-1}$ TRH caused a statistically significant decrease in blood flow in pancreas, spleen and gastric mucosa, while an increase in blood flow was observed in the kidney cortex and heart muscle in both the acid-TRH and cyclo(His-Pro) experiments.

A tendency to an increase in resistance in the masseter muscle on the sympathetic intact side was observed after the administration of TRH while acid-TRH and cyclo(His-Pro) had no such effect (Figure 6). The ocular blood flow seemed not to be greatly effected by the metabolites or by TRH itself (Table 5). Sympathectomy resulted in a statistically significant increase in ocular blood flow in the control situation. This pattern was sustained throughout the various treatments.

Discussion

Previous studies have shown that the effect of TRH on cardiovascular parameters is probably of an intracerebral origin, for reviews see Yarbrough (1979), Morley (1979). TRH causes an increase in peripheral resistance, in part due to an activation of the sympathetic nervous system (Koskinen & Bill, 1984). This could explain the pressor effect of TRH. However, it has also been suggested that low doses of TRH cause an increase in blood pressure by a non-catecholaminergic mechanism in man (Zaloga *et al.*, 1984) and a pressor response, probably due to a vasopressin mechanism has been shown (Kunos *et al.*, 1984). The present results indicate that low doses of TRH can cause an increase in blood pressure in conscious and urethane anaesthetized animals. However, there are conflicting results. In pentobarbitone anaesthetized rabbits 10–100 μg TRH administered intravenously did not affect the blood pressure whereas the same doses elicited an effect when administered intracerebroventricularly (Horita *et al.*, 1979). The two metabolites of TRH had no effect on the blood pressure or other cardiovascular parameters. This indicates that TRH itself is required to elicit the blood pressure effect when the peptide is administrated intravenously.

Peripheral vasoconstriction elicited by higher doses of TRH has previously been reported (Koskinen & Bill, 1984; Koskinen, 1985). In accordance with these results the present study indicates that TRH, even in lower doses, can increase vascular resistance in many

Table 5 Ocular blood flows in conscious ($n = 7$) and urethane anaesthetized ($n = 6$) rabbits in the control situation and after TRH 25 and 50 $\mu g\,kg^{-1}$ i.v.

	Choroid		*Iris*		*Ciliary processes*	
	I	*S*	*I*	*S*	*I*	*S*
Conscious						
Control	1014 ± 101	1053 ± 93	58 ± 6	61 ± 6	100 ± 17	118 ± 21
TRH 25	953 ± 84	993 ± 84	61 ± 10	61 ± 11	88 ± 13	99 ± 15
TRH 50	981 ± 85	995 ± 74	55 ± 6	52 ± 8	79 ± 13	97 ± 13
Urethane						
Control	585 ± 126	771 ± 166*	39 ± 12	65 ± 15*	52 ± 10	94 ± 23*
TRH 25	499 ± 114	736 ± 145*	25 ± 8	66 ± 17*	39 ± 8	86 ± 21*
TRH 50	531 ± 76	924 ± 129**	23 ± 2	101 ± 22*	32 ± 8	104 ± 15**
Control	609 ± 113	909 ± 135**	22 ± 7	52 ± 12*	47 ± 19	85 ± 22*
Acid	535 ± 124	809 ± 153**	24 ± 8	71 ± 22*	42 ± 18	81 ± 29*
TRH 50	424 ± 74	779 ± 112**	16 ± 6	76 ± 31*	25 ± 9	64 ± 18*
Control	578 ± 114	944 ± 154*	35 ± 10	74 ± 20*	59 ± 18	99 ± 22*
Cyclo	553 ± 68	897 ± 120*	36 ± 12	75 ± 17*	57 ± 20	92 ± 22*
TRH 50	659 ± 101	1043 ± 109**	36 ± 13	85 ± 20*	49 ± 18	88 ± 20**

Values for anaesthetized rabbits treated with either acid-TRH (acid) ($n = 6$) and after 50 μg^{-1} TRH are also shown. I = intact side, S = sympathotomized side. Statistical comparisons between the intact and sympathotomized side were made with Student's t test. * $P < 0.05$ and ** $P < 0.01$. Values are mg min^{-1}.

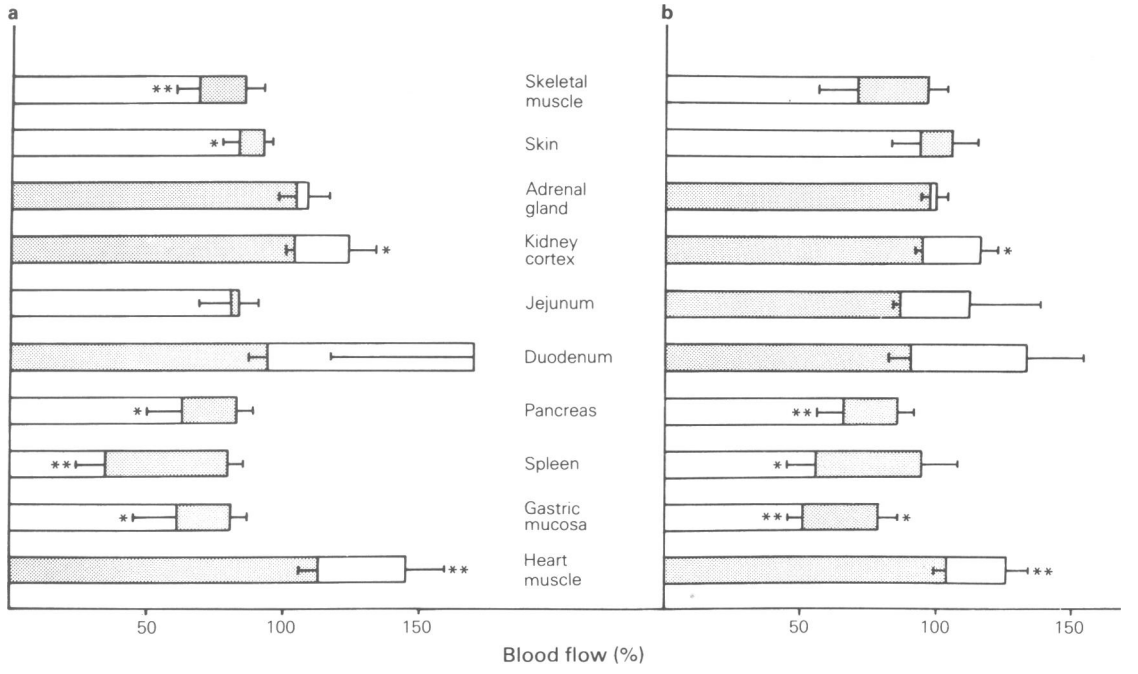

Figure 5 Blood flow as percentage of control values in some peripheral tissues in anaesthetized animals treated with (a) acid-TRH ($n = 6$) or (b) cyclo(His-Pro) ($n = 6$) followed by TRH. Shaded columns = after either 50 $\mu g\,kg^{-1}$ acid-TRH or cyclo(His-Pro), open columns = after 50 $\mu g\,kg^{-1}$ TRH. * $P < 0.05$ and ** $P < 0.01$ compared with control.

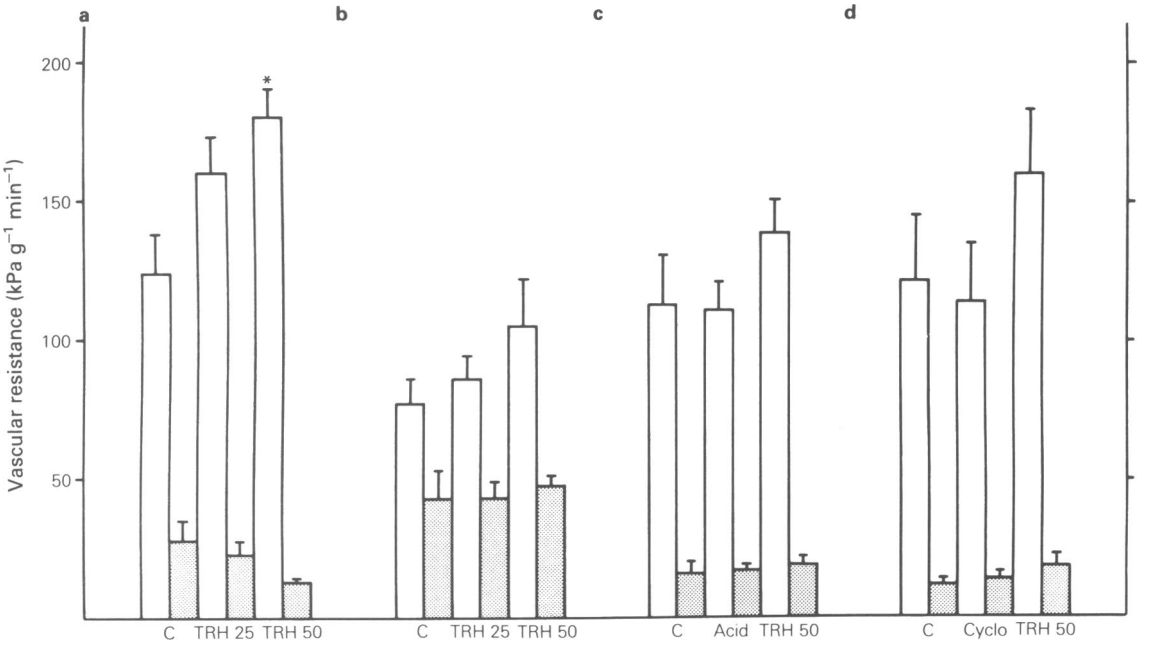

Figure 6 Vascular resistance in the masseter muscle in (a) anaesthetized ($n = 6$) and (b) conscious ($n = 7$) animals during the control situation (C) and after 25 or 50 $\mu g\,kg^{-1}$ TRH administration. Values are also shown for anaesthetized animals treated with (c) 50 $\mu g\,kg^{-1}$ acid-TRH ($n = 6$) or (d) cyclo(His-Pro) ($n = 6$) followed by 50 $\mu g\,kg^{-1}$ TRH. Open columns = intact side, shaded columns = sympathotomized side. $^*P < 0.05$ compared with control values.

organs. Indeed, it has previously been shown that TRH activates sympathetic nerves (Backman & Henry, 1984; Koskinen & Bill, 1984). In the present study, using lower doses the same tendency was observed in the head region, with an increase in vascular resistance on the side with an intact sympathetic supply. However, acid-TRH and cyclo(His-Pro) had no clear effect on peripheral blood flow; the only exception was the decrease in gastric mucosa blood flow elicited by cyclo(His-Pro). The subsequent administration of TRH caused vasoconstriction in many peripheral organs indicating that acid-TRH or cyclo(His-Pro) had no obvious antagonistic effects. In the anaesthetized animal there was an increase in the heart muscle blood flow after the administration of TRH. A similar effect has been described after larger doses in animals with cerebral ischaemia (Koskinen, 1985). CO was not affected by TRH and the increase in MAP was not greater than 10–25%, indicating that the dilatation of the coronary arteries was not entirely due to an increase in cardiac work. To decide whether this effect is of neurogenic origin requires further experiments.

It has previously been shown in the urethane anaesthetized animal that there is an active sympathetic influence on the vessels of the eye (Koskinen & Bill, 1984; Koskinen, 1985). However, in the conscious animal the sympathetic tone to the eye does not seem to be pronounced (Koskinen & Bill, 1983). In the present study the same pattern of sympathetic influence was observed.

Various effects of TRH on cerebral blood vessels and blood flow have been reported. Thus, no response to TRH was found in the *in vitro* preparation of cat cerebral vessels (Hanko *et al.*, 1982) nor was there any effect on CBF in the dog with experimental stroke (Faden *et al.*, 1982). On the other hand, in the anaesthetized rabbit a marked cerebral vasodilatation was observed (Koskinen & Bill, 1984). In this study TRH elicited an increase in total and regional CBF in the anaesthetized animal indicating a cerebral vasodilatation. In the conscious animal no such effect was found. As expected, the cerebral blood flow was higher in the conscious than in the anaesthetized animals. It is known that various pharmacological agents produce different effects in animals under anaesthesia compared with conscious animals. Thus it has been shown that other peptides, e.g. enkephalins elicit a pressure response in conscious, but a decrease in blood pressure in anaesthetized animals (see Lang *et al.*, 1982). It has also been shown that morphine can cause an increase in cerebral blood flow in conscious animals (Miller *et al.*, 1972; Koskinen & Bill, 1983)

whereas under anaesthesia a decrease was seen (Takeshita *et al.*, 1972; Buchweitz *et al.*, 1984). The effects of various pharmacological agents on the single unit activity in the CNS is also affected by the consciousness of the animal (Trulson, 1984). There was no obvious effect of the sympathotomy on the cerebral blood flow which is in accordance with previously reported results (for a review see Heistad *et al.*, 1981).

The vasodilatation elicited by 2 mg kg^{-1} TRH has previously been shown to be at least in part primary, and not entirely due to increased cerebral metabolism (Koskinen & Bill, 1984; Koskinen & Sperber, 1986). It is known that TRH has an analeptic effect (Prange *et al.*, 1974) and this could explain part of the increase in blood flow after TRH administration. However, it has also been shown that TRH has no effect on the anaesthesia induced by 1000 mg kg^{-1} urethane (Smith *et al.*, 1976). Thus it seems unlikely that most of the action of TRH in the present study was due to an analeptic effect. Indeed, intrinsic cerebral vasodilatory pathways have been shown to exist (Nakai *et al.*, 1982, Reis *et al.*, 1982) and these or other pathways may be activated by TRH. There is some evidence that TRH elicits the cerebral vasodilatation from submesencephalic structures (Koskinen & Bill, 1985). TRH containing neurones as well as receptors for TRH have been demonstrated in these areas (Hökfelt *et al.*, 1975a; Taylor & Burt, 1982). It is possible that TRH is involved in an intrinsic cerebrovasodilating system tonically operating in the conscious animal at such a high level that extrinsic TRH had no clear effect. The activity of this system on the cerebral circulation might be reduced by general anaesthesia. Thus the cerebrovasodilatation effect of TRH could be unmasked in the animal under anaesthesia.

Acid-TRH had no clear effect on the cerebral vascular resistance. Cyclo(His-Pro) has been shown to elicit a variety of effects proposed to be of CNS origin (Morley, 1979; Peterkofsky & Battaini, 1980). One of the effects is an analeptic action greater than that of TRH on ethanol-induced sleep, while TRH has a more potent effect on barbiturate sleep. The effect of cyclo(His-Pro) on the cerebral circulation was not at all as great as that of TRH. However, the effect elicited by the subsequent administration of TRH seemed to be somewhat reduced compared to the acid-TRH pretreatment experiments. This may indicate that cyclo(His-Pro) is a partial agonist to TRH in this system.

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