injection of melatonin (250 μg/kg) nearly doubled the dopamine content and significantly ($p < 0.05$) increased the norepinephrine concentration 1 h following its administration (Table). No significant changes in dopamine or norepinephrine content were observed when rats were injected with melatonin and sacrificed immediately.

In order to circumvent the blood-brain barrier, melatonin was given intracisternally[4]. Melatonin (40 μg/kg) given in this manner resulted in significant increases in both brain dopamine and norepinephrine (Table). The increase in dopamine and norepinephrine levels induced by melatonin given intracisternally was noted within 15 min after administration, achieved maximal levels between 30 min and 1 h, and returned to control levels approximately 2 h later. A slight, though insignificant increase in brain dopamine and norepinephrine content was noted 1 h after sham injection in control animals (Table). This is probably related to the ether anesthesia since the increase was not seen in control animals which were injected intraarterially in which ether was not used.

The intracisternal injection of 6-hydroxymelatonin (40 μg/kg), the main metabolite of melatonin did not result in significant changes in either dopamine or norepinephrine content one hour following its administration. The i.p. injection of melatonin is known to result in its rapid metabolism to 6-hydroxymelatonin[3]. Perhaps the inability of previous investigators[2,6] to show melatonin induced changes in brain catecholamine content may be due to its rapid conversion to 6-hydroxymelatonin which we find is inactive when given intracisternally.

In summary, our results demonstrate that melatonin given both intraarterially and intracisternally results in significant increases in both rat brain dopamine and norepinephrine concentration. Also, the intracisternal administration of comparable amounts of 6-hydroxymelatonin does not alter brain catecholamine content.

Zusammenfassung. Nachweis, dass Melatonin die Konzentration von Dopamin und Noradrenalin im Rattenhirn erhöht, wenn es intraarteriell oder intracisternal injiziert wird.

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Tremorogenic Effect of Thyrotropin Releasing Hormone in Rats

In 1972 PRANGE et al.[1] reported on antidepressant activity of thyrotropin releasing hormone (TRH) in man. They assumed that the beneficial effect of TRH in depressive patients was not thyroid-mediated, but rather due to a direct central nervous stimulating activity[2]. These reports and interpretations have encouraged the study of the central nervous effects of TRH in experimental animals. PLOTNIKOFF's group[3] has described an L-DOPA-potentiating effect of TRH in pargyline pretreated mice. HINE et al.[4] have noted pharmacological effects of TRH in the 'conscious' dog which they suggested might be related to a general sympathetic activation by the hormone.

In the context of a study of the endocrinological activity of TRH, we have observed a number of effects which appeared immediately after the i.v. injection of the hormone. In additional experiments we attempted to define the mechanism and specificity of these effects.

Effects of TRH in the rat. Shortly after an i.v. injection of TRH (0.3 to 3.0 mg/kg) muscle tremor, excitation, tail lifting, and pilo-erection were observed in the non-anaesthetized rat. The tremorogenic effect only lasted a few minutes. In addition, immediately after the injection the amplitude of respiration increased and signs of augmented cutaneous blood flow appeared. In rats anaesthetized with pentobarbital (50 mg/kg i.p.). all these effects were more pronounced. The latency of onset, the amplitude and the duration of the tremor were dose-dependent (Table I and Figure 1a). After some min the tremor ceased, but it could still be elicited by touching the animal.

After the arousal phase, within 5 to 60 min after the injection, the animals returned to the inert sleeping position. The duration of anaesthesia was not shortened significantly. After waking, the animals appeared normal.

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Table I. Tremorogenic effect of TRH in rats. Latency and duration of tremor

TRH (mg/kg)	No. of rats reacting	Latent period until onset of tremor (sec)	Duration of spontaneous tremor (min)	Total time of spontaneous + elicitable tremor (min)
0.3	7/7	123 ± 12	3.4 ± 0.9	18.7 ± 4.9
1.0	5/5	54 ± 4	5.4 ± 0.5	16.4 ± 2.0
3.0	6/6	24 ± 1	21 ± 4	44 ± 8
		$\bar{X} \pm SE$		

Rats were anaesthetized with pentobarbital (50 mg/kg) by i.p. injection. TRH (pGlu-His-Pro-NH_2) was dissolved in 0.5 ml physiological saline and injected over exactly 2 min into the jugular vein. The onset of tremor was recorded in sec after beginning of the injection.

Tremorogenic effect of TRH in hypophysectomized and thyroidectomized rats. Acutely hypophysectomized or thyroidectomized rats reacted to TRH in the same way as intact animals. If the animals were tested 4 weeks after thyroidectomy, TRH only elicited tremor in high doses (3 mg/kg). If these animals were given 1 mg L-thyroxine by i.v. injection 24 h before testing, the full reaction to TRH was observed, as in intact animals.

Obviously the tremorogenic effect of TRH is not mediated by the hypophysis and thyroid. However, the rapid phasic muscular contraction characteristically provoked by TRH is evidently impaired after prolonged thyroid deficiency.

Specificity of the tremorogenic effect of TRH. Several short-chain peptides structurally related or unrelated to TRH, were administered i.v. to rats anaesthetized with pentobarbital. Peptides closely related in structure to TRH such as the mono-iodo-TRH and di-iodo-TRH also elicited tremor, but only in doses 30 to 100 times higher than those of TRH (Table IIa). All the other peptides, as well as pyroglutamine, histidine and prolinamide, thyroxine or di-iodo-thyronine were inactive (Table IIb).

Table II.

(a) Peptides with tremorogenic activity[b]

Substances	Tremorogenic potency in comparison to TRH = 1
pGlu-His-(I)-Pro-NH_2[a]	0.01
pGlu-His-(I_2)-Pro-NH_2	0.01

(b) Peptides and amino acids without tremorogenic activity in the pentobarbital-anaesthetized rat

Substances	Injected dose (mg/kg)
pGlu-His-Pro-OH[a]	30
pGlu-His-NH_2	30
pGlu-His-OMe	30
pGlu-NH_2	10
H-His-OH	10
H-Pro-NH_2	10
H-Lys-Pro-Val-NH_2	10
H-Lys-Lys-Pro-OH	10
H-Met-Glu-His-Phe-Arg-Trp-Gly-OH [ACTH-(4-10)]	30
pGlu-Tyr-Arg-Trp-NH_2	30
H-Pro-Leu-Gly-NH_2 (MIF)	100
H-Pro-Ile-Gly-HN_2	10
H-Gly-His-Lys-OH	30
H-Gly-Lys-His-NH_2	30
Thyroxine	1
Di-iodo-thyronine	10
pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 = LH RH	4
H-Leu-Arg-Pro-Gly-NH_2	4

[a] All amino acid residues except glycine in the L-form. Abbreviations used for amino acid residues according to the recommendations of the IUPAC-IUB commission on biochemical nomenclature, J.B.C. 247, 977 (1972); pGlu, pyroglutamic acid; MIF, melanocyte stimulating hormone release inhibiting factor; LH-RH, luteinizing hormone releasing hormone. [b] Peptides were dissolved and injected as described for Table. I

Mechanism of action. The tremorogenic effect of TRH was not altered if injections were given consecutively at 30-min intervals. It was also not influenced by pretreatment of the rats with L-DOPA (100 mg/kg i.p.), melanocyte stimulating hormone release inhibitory factor (MIF) (30 mg/kg i.p.), DL-*O*-methyltyrosine methylester (300 mg/kg i.p.), or atropine (5 mg/kg i.p.) nor blocked by pretreatment with propranolol (10 mg/kg i.p.) phentolamin (2 mg/kg i.p.) or reserpine (0.2, 0.3 and 1 mg/kg s.c. successively on 3 days).

The tremorogenic effect of TRH in the rat does not seem to be mediated by stimulation of cholinergic or adrenergic receptors. In fact amphetamine (3 mg/kg i.v.), L-Dopa (100 mg/kg) and tremorine (20 mg/kg i.v.) do not elicit tremor in the pentobarbital-anaesthetized rat.

Discussion. The tripeptide pGlu-His-Pro-NH_2, thyrotropin releasing hormone, has marked central nervous effects in the rat resulting in tremor and in a general picture of arousal. These effects are elicited by doses of TRH about 100 times higher than those stimulating thyrotropin release. They usually appear during or shortly after the slow injection of TRH; they are fully reversible and no signs of toxicity have been observed. TRH has been reported to be non-toxic in rats in doses up to 100 mg/kg i.v. daily[5].

The neurotropic effects of TRH in the rat do not depend on an intact pituitary-thyroid-axis. Similarly, PLOTNIKOFF et al.[6] have shown that the L-DOPA-potentiating effect of TRH also occurs in the hypophysectomized mouse. Recently, KRUSE[7] described tremorogenic and other neurotropic effects of TRH in mice which could also be elicited in hypophysectomized animals. Studies now in progress show that TRH induces, with a latent period of a few seconds only, signs of EEG arousal in the conscious cat and facilitates the stretch reflex in the decerebrate cat.

All these observations indicate that TRH has central nervous effects which are not mediated by stimulation of the pituitary-thyroid axis. The tremorogenic effect appears to be linked to the tripeptide pGlu-His-Pro-NH_2 structure. Another tripeptide, Pro-Leu-Gly-NH_2 (MIF), which in mice potentiates the effect of L-DOPA[6], is not tremorogenic in the rat. The ACTH sequence 4–10. which has been reported to have central nervous effects[8], is also inactive under the test conditions described here.

There is no doubt that TRH has direct central nervous effects when injected in high doses in animals. The mechanism of its action has not yet been elucidated, all the results suggest that these effects are not mediated through the known cholinergic or adrenergic pathways.

It also remains to be shown whether the antidepressant effect of TRH in patients, as described by PRANGE and others, is due to a direct central nervous stimulation or to an indirect action via thyroid activation.

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Fig. 1a. TRH-induced tremor Rat (Nembutal) Right forepaw

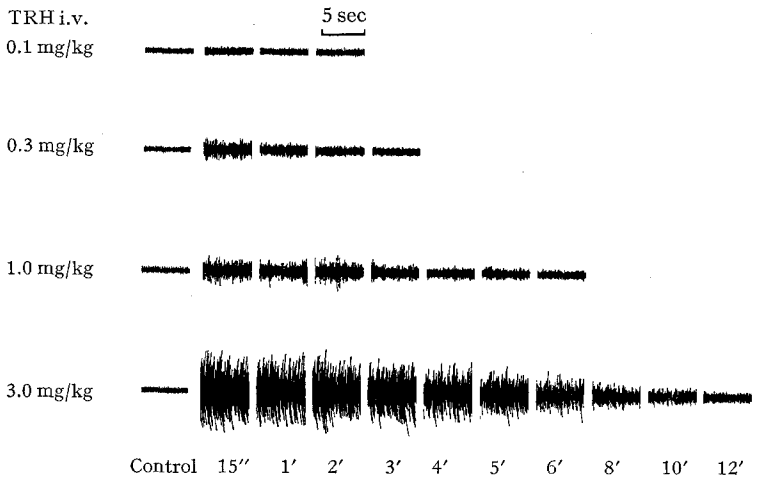

Fig. 1b. TRH-induced tremor Rat (Nembutal) Right Forepaw

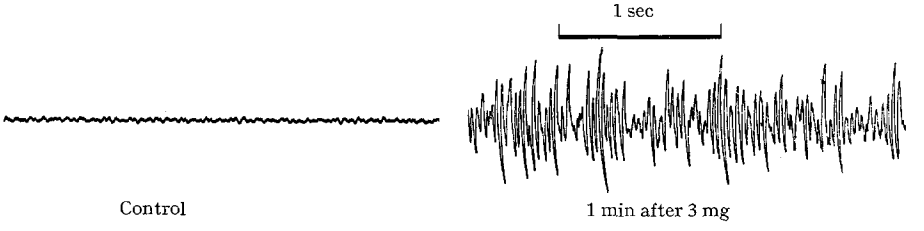

Fig. 1. TRH-induced tremor. (a) Dose-response relationship. (b) Registration of the tremor at a higher paper speed. Male rats (200–300 g body weights, Sprague-Dawley from Tierfarm Sisseln) were anaesthetized with pentobarbital (50 mg/kg i.p.) TRH dissolved in 0.5 ml physiological saline was injected during exactly 2 min into the jugular vein. Thereafter the tremor was recorded by means of a force-transducer attached to the right forepaw and a Polygraph. The time after the end of the injection is indicated at the bottom.

Zusammenfassung. Thyrotropin-Releasing Hormon (TRH) erzeugt unmittelbar nach i.v. Injektion an Pentobarbital-narkotisierten Ratten einen feinschlägigen Tremor und Haarsträuben. Diese Symptome werden durch eine direkte zentralnervöse Wirkung von TRH und nicht durch Stimulation der Schilddrüse ausgelöst. Die Wirkung ist dosisabhängig und spezifisch für das Tripeptid pGlu-His-Pro.

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Local α-Adrenoceptor Mediated Feed-Back Inhibition of Catecholamine Release from the Adrenal Medulla?

An α-receptor mediated feed-back mechanism controls neurotransmitter secretion from noradrenergic nerve endings[1–8]. Liberated noradrenaline and exogenous sympathomimetic drugs decrease the secretory response to nerve impulses by activation of neuronal α-receptors. α-Adrenolytic agents block the receptors, interrupt the feed-back loop and thus enhance noradrenaline release. The question arises whether a similar mechanism exists in the adrenal medulla. Therefore, the influence of an α-receptor activating drug, oxymetazoline, and 2 α-adrenolytic agents, phentolamine and phenoxybenzamine, on potassium-evoked catecholamine output from perfused adrenal glands was investigated. In addition, the effect of desipramine has been tested. Potassium was used as a stimulant rather than the physiological secretagogue acetylcholine, since it depolarizes chromaffin cells directly[9]; any possible interaction of the drugs tested with acetylcholine receptors probably does not influence potassium-induced secretion. In nerve endings, release of noradrenaline evoked by high potassium and that evoked by nerve impulses are influenced by drugs with affinity to α-receptors in a similar way[10, 11].