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

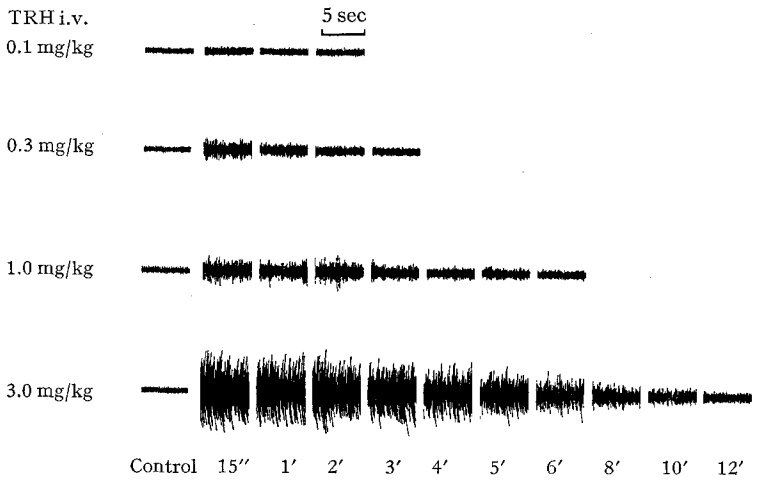

Fig. 1b. TRH-induced tremor Rat (Nembutal) Right Forepaw

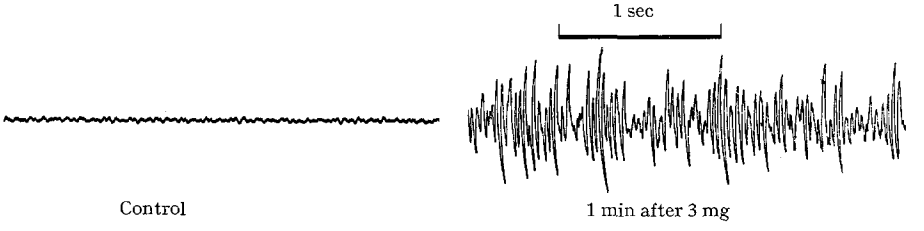

Fig. 1. TRH-induced tremor. (a) Dose-response relationship. (b) Registration of the tremor at a higher paper speed. Male rats (200–300 g body weights, Sprague-Dawley from Tierfarm Sisseln) were anaesthetized with pentobarbital (50 mg/kg i.p.) TRH dissolved in 0.5 ml physiological saline was injected 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].

Effect of desipramine, oxymetazoline, phentolamine and phenoxybenzamine on potassium-evoked outflow of catecholamines from the adrenal medulla

Group	Drug concentration	Catecholamine outflow evoked by K_2	Catecholamine outflow evoked by K_3	n
1	—	107.4 ± 11.9	124.5 ± 20.1	7
2	Desipramine (10^{-6} M)	88.1 ± 11.9	83.4 ± 25.6	5
3	Oxymetazoline (10^{-6} M)	96.8 ± 4.3	77.3 ± 7.7	4
4	Oxymetazoline (10^{-5} M)	111.5 ± 10.1	88.4 ± 11.9	5
5	Phentolamine (10^{-6} M)	80.2 ± 8.4	76.9 ± 7.9	5
6	Phentolamine (10^{-5} M)	119.1 ± 8.9	113.9 ± 12.7	7
7	Phenoxybenzamine (10^{-5} M)	160.0 ± 18.6[a]	135.3 ± 25.7	8
8	Phenoxybenzamine (10^{-6} M)	146.9 ± 26.5	160.8 ± 48.9	6
9	Phenoxybenzamine (10^{-5} M)	153.3 ± 8.8[a]	140.0 ± 20.2	5

Normal Tyrode solution was replaced by a solution containing 40 mM potassium 3 times for 12 min (K_1, K_2, K_3). Drugs were infused (0.275 ml/min) either from 18 min before the onset until the end of K_2 (groups 2–7), or from 18 min before the onset of K_2 until the end of the experiment (groups 8 and 9). The outflow of catecholamines evoked by K_2 and K_3, respectively, was expressed as percentage of that evoked by K_1. Significant differences from group 1 (t-test): [a] $p < 0.05$.

Methods. Bovine adrenal glands were obtained from a slaughter house 20–30 min after the death of the animals. The glands were immediately flushed with ice-cold Tyrode solution (137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.1 mM $MgCl_2$, 11.9 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, 5 mM glucose). The surface of the glands was sacrified by making multiple incisions. Retrograde perfusion with Tyrode solution at 22 °C was performed according to HECHTER et al.[12]. The flow rate was kept constant (6 ml/min). From 30 min after the start of perfusion onwards, the effluent was collected in 6-min samples. Normal Tyrode solution was switched over 3 times for 12 min to potassium-rich solution (40 mM; equimolar replacement of NaCl by KCl). Stimulation by high potassium started 36 (K_1), 72 (K_2) and 108 min (K_3) after the onset of perfusion. Catecholamines were estimated photometrically[13]. Experiments in which amine outflow 36–42 min after the start of perfusion, i.e. during K_1, was less than twice the spontaneous outflow (30–36 min) were excluded.

Results. The spontaneous efflux of catecholamines was 14.3 ± 0.7 µg/min (n = 52). It was not changed by the drugs tested. 40 mM potassium increased amine outflow. 18–24 min after the end of stimulation by high potassium, efflux had returned to the pre-potassium level. Therefore, potassium-evoked catecholamine outflow was calculated as the difference between *total* outflow during, plus the first 18 min after, high potassium perfusion, and the *spontaneous* outflow. The output of catecholamines evoked by the first perfusion with 40 mM potassium (K_1) was 1065 ± 131 µg (n = 52). In control experiments, the outflow evoked by the 2nd and 3rd stimulation with potassium was slightly higher than that evoked by the first one (Table). Desipramine, oxymetazoline and phentolamine did not cause any change. In contrast, 10^{-5} M phenoxybenzamine increased the response to potassium.

Discussion. At a concentration which strongly inhibits the neuronal uptake of noradrenaline, and thereby enhances noradrenaline outflow from sympathetically innervated tissues[14], desipramine did not change catecholamine outflow from the adrenal gland. The observation confirms previous conclusions that there is no quantitatively relevant re-uptake of catecholamines in the adrenal medulla[15,16].

The increase of potassium-evoked amine outflow caused by phenoxybenzamine cannot be explained by inhibition of re-uptake, since desipramine was ineffective. Rather it seems probable that phenoxybenzamine facilitates potassium-evoked secretion from chromaffin cells. Similarly, phenoxybenzamine in vivo facilitates hormone release from dog adrenal glands induced by splanchnic nerve stimulation[15]. The *spontaneous* amine outflow was unchanged in the present experiments. KIRPEKAR and CERVONI[15] observed that phenoxybenzamine also increased spontaneous outflow; however, in their experiments the splanchnic nerves were left intact, and impulses from the central nervous system may have contributed to the 'resting' amine output. Thus, it seems that phenoxybenzamine specifically enhances the secretory response to depolarizing stimuli (high potassium or acetylcholine). This effect is analogous to phenoxybenzamine's action on noradrenergic nerves, and may also result from the blockade of α-receptors mediating an inhibition of secretion by extracellular catecholamines. An α-receptor mediated feed-back inhibition of the secretory response to stimulation, comparable to that postulated for nerves[1–8], may also operate in the adrenal medulla.

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The lack of effects of phentolamine and oxymetazoline is not incompatible with this assumption. The catecholamine concentration in the vicinity of the chromaffin cells is very high. The amines may prevent access of the competitive antagonist phentolamine to the α-receptors, and maximal activation of the feed-back inhibition by catecholamines may prevent any additional inhibitory effect of oxymetazoline. Only the irreversible antagonist phenoxybenzamine effectively blocks the receptors and disinhibits secretion.

Zusammenfassung. Der Einfluss von Desipramin, Oxymetazolin, Phentolamin und Phenoxybenzamin auf die durch 40 m*M* Kalium hervorgerufene Abgabe von Katecholaminen aus isoliert perfundierten Rindernebennieren wurde untersucht. Desipramin, Oxymetazolin und Phentolamin bewirkten keine Änderung; 10^{-5} *M* Phenoxybenzamin erhöhte die Freisetzung. Dies deutet auf eine durch α-Adrenoceptoren vermittelte Rückkopplungshemmung der Freisetzung von Katecholaminen, ähnlich der an noradrenergen Nerven.

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Influence of 2-Br-α-Ergokryptine-Mesilate (CB 154) on the Pituitary Prolactin Content of Prooestrus Rats

In cycling female rats, an increased release of LH (ovulatory surge) from the pituitary occurs during the afternoon of the prooestrus phase. This is accompanied by a similar surge of prolactin. In the late afternoon of prooestrus, the pituitary contains much less prolactin than in the morning[1], while the serum prolactin shows several times higher levels in the afternoon than in the morning[2]. We have used the prooestrus rat to test CB 154 for its inhibitory action on a spontaneously occurring pituitary prolactin depletion.

Methods. From a stock of female Ivanovas-Wistar rats with a history of regular 4-day vaginal cycles, 6–7 synchronous animals were allocated to one of the following groups: I, controls with solvent treatment; II, treatment groups: a) 0.1 mg/kg s.c. CB 154, b) 0.3 mg/kg s.c. CB 154, c) 1.0 mg/kg s.c. CB 154.

Treatment was given at 17 h of postoestrus day and again on the next morning at 09.30 h, the prooestrus day. Animals that showed a clear prooestrus smear were killed at 17.00 h of this same day by decapitation. The pituitaries were quickly removed, dissected, the anterior lobe weighed and deepfrozen until used for analysis.

For assessing the prolactin content of the pituitaries, the disc-electrophoresis procedure, as mentioned by YANAI and NAGASAWA[3], was used. The prolactin bands were measured photodensitometrically and the results compared with those of a standard sheep prolactin preparation (Ferring) run in parallel at several concentrations.

The results are calculated in mU/mg anterior pituitary, assuming the standard to contain 1000 IU per ampoule.

Results and discussion. The results are presented in the Table. They show that pretreating 4-day cycling rats with CB 154 late in postoestrus and again in the morning of prooestrus produces a higher pituitary prolactin content on the afternoon of prooestrus than in untreated control animals. On the basis of observations by others[1] on the prolactin depletion of rat pituitaries during the prooestrus phase, this effect of CB 154 is interpreted as demonstrating its inhibitory effect on pituitary prolactin release. These observations corroborate indirect evidence for the inhibitory effect of CB 154 on prolactin secretion in the rat, using other experimental situations[4–6].

Résumé. Le traitement des femelles par le CB 154 a produit des taux de prolactine accrus, comparés à ceux des individus non traités. Cet effet dépendait de la dose de CB 154. Il a démontré que ce composé est également efficace en état de stimulation endogène de la prolactine.

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Prolactin content of rat adenohypophysis (mU/mg) at 17.00 h of prooestrus

	Controls	Treatment groups		
CB 154 (mg/kg s.c.)	0	0.1	0.3	1.0
	29.3	37.0	44.0	53.5
	25.2	35.1	43.2	54.0
	27.6	38.4	45.3	55.1
	25.8	38.3	46.0	55.0
	29.4		43.1	52.0
	28.9			
m	27.7	37.2	44.3	53.9
± SD	1.8	1.5	1.3	1.3
n	6	4	5	5
P		<0.001	<0.001	<0.001

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