findings[15]. We shall here only give a preliminary report on the syntheses of the compounds II, III, and V (Fig. 1–3); detailed papers are to appear elsewhere.

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Zusammenfassung

Einige Peptide, strukturell ähnlich den gemeinsamen Aminosäuresequenzen der drei Hypophysenhormone ACTH, α- und β-MSH, wurden synthetisiert. Sie zeigen eine sehr schwache Wirkung auf die Melanophoren (V wurde nicht geprüft); das Heptapeptid (III) besitzt keine ACTH-Aktivität. Die drei Verbindungen II, III und V vergrössern die ACTH-Ausschüttung im *in vitro*-Versuch von Saffran, Schally und Benfey[7] stark. Damit sind die ersten synthetischen Peptide mit dieser CRF-Aktivität gefunden.

Adrenaline-Noradrenaline Content of the Submaxillary Gland of the Cat

It has been suggested that some sympathicomimetic amines, e. g. tyramine, exert their effect by release of adrenaline-noradrenaline (Burn and Rand[1]). In the present experiments, the effect on the adrenaline-noradrenaline content of the submaxillary gland caused by infusion of tyramine has been studied.

Methods. Ten cats were used for the experiments. One series consisted of three normal cats. In a series of five cats, the effect of an intravenous infusion of tyramine was studied. In two cats, the superior cervical ganglion of one side was excised 3 weeks before the experiment.

In the acute experiment, the cats were given chloralose (80 mg/kg) after preliminary ether anesthesia. To facilitate a quick removal, the glands were dissected, leaving nerves, vessels, and secretory duct intact. The excised gland was immediately put into a cooled homogenizer containing 10 cm³ of ice – chilled 0.4 *N* perchloric acid. The homogenate was kept in a deep-freeze (– 20°C) until the estimation of the catechols was made one or two days later.

Estimation of adrenaline-noradrenaline[2]. The noradrenaline and adrenaline content of the homogenates was estimated according to the fluorometric method described by Bertler, Carlsson, and Rosengren[3]. The simplified extraction variant of the procedure was used. A slight error is admittedly introduced hereby, especially since the glands were not weighed, in order to reduce the time delay between removal and homogenization. The error introduced is, however, only of the order of 1%, even if it is assumed that the weight of an organ weighing about 1 g can be estimated by sight not better than within ± 0.5 g. The elution was made with 8 ml 1 *N* HCl and the estimation made on 2 ml of the eluate.

Further technical details are given below.

Results. The normal submaxillary gland was found to contain only small amounts of adrenaline in comparison with the amounts of noradrenaline. The two normal glands from a single animal were very similar in their noradrenaline content (Table), and therefore the effect of tyramine infusion or of ganglionectomy on the noradrenaline content could be studied on pairs of glands, using one gland as a control.

Infusion of tyramine was started after one gland had been removed and homogenized. A cannula was tied into the secretory duct of the remaining gland. Each animal was then given 150 mg of tyramine intravenously during approximately 1 h. At a constant rate of infusion, the secretory rate declines. In order to keep up a secretion the rate of infusion was increased intermittently. In spite of this, the gland can not be made to secrete for a long time. At the end of infusion, when the gland was removed, there was no secretion in any of the cases even though huge doses were given (up to 10 mg/min).

The infusion was found to lower the noradrenaline content of the gland to some 50% (Table).

Excision of the superior cervical ganglion caused a complete disappearence of noradrenaline, while adrenaline was found in seemingly normal quantities (Table).

Expressed in μg, the noradrenaline and, within brackets, the adrenaline content of the submaxillary gland of the cat.

Cat. No.	Procedure	Right gland	Left gland
1	Normal glands	0.71 (0.04)	0.71 (0.10)
2		0.78 (0)	0.74 (0.06)
3		0.69 (0)	0.66 (0)
		Mean value: 0.73 (0.01)	0.70 (0.05)
4	Right superior cervical ganglion excised 3 weeks earlier	0 (0.06)	0.45 (0.07)
5		0 (0.06)	1.24 (0.06)
		Mean value: 0 (0.06)	0.85 (0.07)
6	Left gland removed before and right gland removed after infusion of tyramine	0.49 (0.06)	1.16 (0.04)
7		0.59 (0.02)	1.29 (0)
8		0.51 (0.02)	0.85 (0)
9		0.40 (0.07)	1.01 (0)
10		0.48 (0.05)	0.91 (0)
		Mean value: 0.49 (0.04)	1.04 (0.01)

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[2] I am greatly indebted to Drs. Å. Bertler, A. Carlsson, and E. Rosengren for facilities to make the estimations.

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Discussion. The decrease in the noradrenaline content of the submaxillary gland following an infusion of tyramine supports the hypothesis that tyramine causes a release of noradrenaline. It may therefore well be that the effects of tyramine are exerted via a release of noradrenaline. The total disappearence of noradrenaline from the gland, as well as of the secretory response to tyramine (STRÖMBLAD[4,5]) after removal of the sympathetic supply, is also consistent with such a mode of action of tyramine.

At the end of the infusion of tyramine, no secretory response was obtained, but the gland was found to retain some 50% of its noradrenaline. No explanation can be given; but it could be pointed out that the sympathetic innervates also the vessels of the gland and therefore the remaining noradrenaline could be associated with terminals around the vessels. On electrical stimulation of the sympathetic, the nerve terminals in contact with the secretory cells seem to be more easily depleted of transmitter than those in contact with the vessels (EMMELIN and ENGSTRÖM[6]).

The figures for the adrenaline content of the glands were low and varied much more than those for the noradrenaline content. It is, however, of interest to note that adrenaline was present in apparently normal quantities after excision of the superior cervical ganglion. It could be that adrenaline is located outside the nerve terminals in the submaxillary gland.

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Zusammenfassung

Der Noradrenalingehalt in der Submaxillarisdrüse der Katze nahm nach der Infusion von Tyramin ab, wodurch die Auffassung unterstützt wird, dass diese Substanz durch Freisetzung von Noradrenalin wirkt. Sympathische Ganglionektomie verursachte das vollständige Verschwinden von Noradrenalin in der Drüse.

In Vivo Uptake of Dopamine and 5-Hydroxytryptamine by Adrenal Medullary Granules

In 1953 the adrenaline and noradrenaline of the adrenal medulla were shown to be localized within intracellular granules of about the same size as mitochondria but not identical with these[1,2]. Subsequent observations[3,4] indicate that also catechol amines in other tissues are kept in similar particles. In studies of this storage mechanism, the adrenal medulla is often the tissue of choice, because it is easy to isolate and prepare. However, it has the drawback of a comparably slow turnover, which makes investigations of the kinetics of the storage mechanism difficult.

In an attempt to overcome the difficulty, we have studied the uptake of catechol amines of the catechol amine containing granules after administration of 3,4-dihydroxyphenylalanine (DOPA). This amino acid penetrates into the cell, where it is decarboxylated to dopamine by means of DOPA-decarboxylase. This enzyme is located in the cytoplasmic sap[5]. It was observed that most of the new-formed dopamine was present in the granular fraction after differential centrifugation. The procedure is briefly described below.

L-DOPA in a dose of 100 mg/kg was administered i. v. into rabbits weighing about 2 kg. The animals were killed at varying intervals of time after the injection. The adrenals were rapidly removed, and the adrenal medulla was dissected free from cortical tissue. The medulla was homogenized in ice-cooled 0.3 M sucrose in a Potter-Elvehjem homogenizer. From the homogenate three fractions were prepared by means of differential centrifugation according to HILLARP *et al.*[1]: (1) the low speed sediment, containing cell nuclei and cell debris, (2) the high speed sediment, containing the mitochondria and catechol amine storing granules, (3) the high speed supernatant, containing the cytoplasmic sap. The catechol amine contents of the fractions were determined as previously described[6]. As will be seen in the Table, the granular fraction contained 3.7 µg dopamine 30 min after the administration of DOPA, whereas less than 1 µg was localized in the cytoplasmic sap. The newly-formed dopamine is thus rapidly taken up by the granules. In the kidney cortex, however, which is rich in DOPA-decarboxylase but lacks catechol amine storing granules, most of the dopamine formed was found in the cytoplasmic sap. Pretreatment of the animals with reserpine impaired the uptake of dopamine by adrenal medullary granules after DOPA administration. Thus the primary effect of reserpine which is known to deplete the body stores of catechol amines[7–9] may be its interaction with the uptake of catechol amines by the granules.

In-vivo uptake of dopamine and 5-hydroxytryptamine by adrenal medullary granules after administration of DOPA and 5-hydroxytryptophan respectively (µg per pair of adrenals. Each value represents the mean of two determinations. DA: dopamine; NA: noradrenaline; A: adrenaline; 5-HT: 5-hydroxytryptamine; 5-HTP: 5-hydroxytryptophan).

		DA	NA	A	5-HT
DOPA, 100 mg/kg (30 min)	Cytoplasmic sap	0.8	0.9	8.2	—
	Granules	3.7	4.8	72	—
DOPA, 100 mg/kg (1 h)	Cytoplasmic sap	1.4	2.7	14.5	—
	Granules	5.4	15.5	127	—
Reserpine, 5 mg/kg (3 h) + DOPA, 100 mg/kg (30 min)	Cytoplasmic sap	1.5	2.0	11.6	—
	Granules	2.0	–0.5	69	—
Reserpine, 5 mg/kg (20 h) + DOPA, (30 min)	Cytoplasmic sap	4.0	1.3	1.1	—
	Granules	0.8	1.0	1.9	—
5-HTP, 100 mg/kg (1 h)	Cytoplasmic sap	—	—	—	0.45
	Granules	—	—	—	1.40
Control	Cytoplasmic sap	0.0	0.2	6.7	0.0
	Granules	0.3	1.2	105.3	0.0

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