actions from the pyramidal tract on reflex paths to motoneurones[1].

Zusammenfassung. Es konnte gezeigt werden, dass Überleitungen in spinalen Reflexsystemen, die für die präsynaptische Depolarisation gewisser primärer Afferenzen verantwortlich sind, von der sensorisch-motorischen Grosshirnrinde über die Pyramidenbahn gefördert werden kann.

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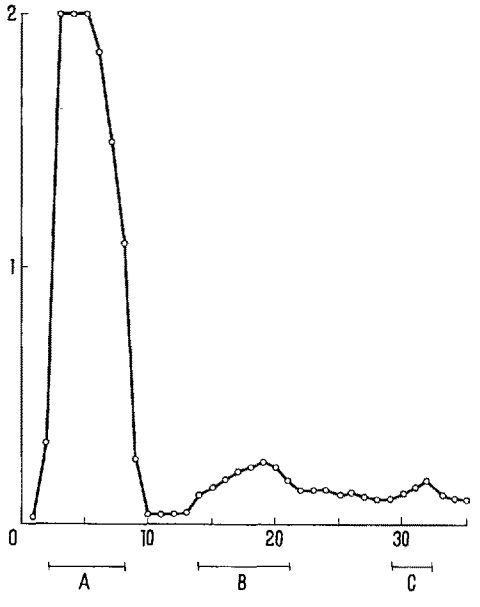
Isolation of a Hypothalamic Peptide with TRF (Thyreotrophin Releasing Factor) Activity *in vitro*

A hypothalamic factor activating adenohypophysial acid phosphatases *in vitro* was prepared from acetic acid extracts of bovine hypothalami by high voltage electrophoresis and its TSH-releasing activity was demonstrated *in vitro*[1,2]. A review of previous work on the relationship between adenohypophysial acid phosphatase and TSH secretion and on the characteristics of the activating factor was published elsewhere[3].

The active factor was now further purified by gel filtration on a Sephadex G-25 column and the probable peptide character was demonstrated by a combined electrophoretic and chromatographic analysis.

Fractions HH_{18+1} and HH_{20+1}, prepared by high voltage electrophoresis from lyophilized acetic acid extracts of 85 and 120 bovine hypothalami respectively, were dissolved (170 mg and 700 mg respectively) in 1 cm³ 0.225% NaCl and applied to a Sephadex G-25 column (1.7 cm × 12.0 cm), saturated with 0.225% NaCl. The column was eluted in an automatic fractions collector with 0.225% NaCl and 35 and 50 fractions respectively (3 cm³ each) were collected (flow = 6 cm³/h). The extinction of these fractions was measured at 280 mμ and three peaks were found, as shown for fraction HH_{18+1} in the Figure. Separation of the individual peaks in gel filtration of fraction HH_{20+1} was less distinct, probably because a larger amount of material was used. The fractions giving the individual peaks were pooled to give subfractions $HH_{18+1,A}$ (85 mg after lyophilization and subtraction of NaCl), $HH_{18+1,B}$ (45 mg), $HH_{18+1,C}$ (15 mg), $HH_{20+1,A}$ (410 mg), $HH_{20+1,B}$ (150 mg) and $HH_{20+1,C}$ (68 mg).



Gel filtration of fraction HH_{18+1} on a Sephadex G-25 column. Abscissa: Number of fraction; ordinate: extinction at 280 mμ. Fractions corresponding to individual peaks of extinction were pooled as indicated to give fraction $HH_{18+1,A}$, $HH_{18+1,B}$, and $HH_{18+1,C}$.

Tab. I. Acid phosphatase activity in μg phenol liberated by 1 mg adenohypophysial tissue in 30 min. Concentration of the fractions 10 μg/cm³.

1st experimental series				
Group	I	II	III	IV
Number of tests	24	24	24	18
Fraction	0	$HH_{18+1,A}$	$HH_{18+1,B}$	$HH_{18+1,C}$
Acid phosphatase activity	3.0 ± 0.06	3.3 ± 0.09	3.1 ± 0.09	3.6 ± 0.08[a]

2nd experimental series				
Group	I	II	III	IV
Number of tests	18	18	18	18
Fraction	0	$HH_{20+1,A}$	$HH_{20+1,B}$	$HH_{20+1,C}$
Acid phosphatase activity	2.5 ± 0.18	3.1 ± 0.13	2.1 ± 0.12	3.3 ± 0.09[a]

[a] Comparison with group I in Fisher's *t*-test $p < 0.01$.

Acid phosphatase activity was measured in rat adenohypophysial homogenate[3] in the presence of subfractions A, B, and C in concentration of 10 μg/cm³. The results are shown in Table I. Slight elevation of activity was found in the presence of the A fractions, but more important elevation of activity occured in the presence of the C fractions. Both C fractions ($HH_{18+1,C}$ and $HH_{20+1,C}$) were therefore used to test their effect on TSH release *in vitro* and for electrochromatographic analysis.

For electrochromatographic analysis of the C fractions, the method described by JIRGL[4] was used. 1 mg of fraction $HH_{18+1,C}$ or $HH_{20+1,C}$ was dissolved in 1 cm³ H_2O and applied to the middle of a 12 × 30 cm strip of Whatman 3 paper (native specimen). The same amount of the fractions was dissolved in 0.5 cm³ H_2O, 0.5 cm³ 6*N* HCl was added and the specimen was heated at 115°C for 24 h in a sealed beaker (hydrolyzed specimen). The hydrolyzate was then dried *in vacuo* at 100°C, dissolved in 1 cm³ H_2O

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Tab. II. Activity = % of the dose of radio-iodine found in the thyroid gland

	1st experimental series			
Group	1	2	3	4
Administered material	0	medium with fraction $HH_{18+1,C}$	medium after incubation of hypophyses without $HH_{18+1,C}$	medium after incubation of hypophyses with $HH_{18+1,C}$
Number of tests	10	10	10	10
Concentration of the fraction in the medium	—	90 μg/cm³	—	90 μg/cm³
Total dose of tested material in cm³	0	2	2	2
Mean weight of adenohypophyses incubated in 1 cm³ medium in mg	—	—	3.80	3.80
Activity, means $\pm \sigma_m$	11.2 ± 1.26	11.7 ± 2.0	14.4 ± 1.93	18.6 ± 3.4
	2nd experimental series			
Group	1	2	3	4
Administered material	0	medium with fraction $HH_{20+1,C}$	medium after incubation of adenohypophyses without $HH_{20+1,C}$	medium after incubation of adenohypophyses with $HH_{20+1,C}$
Number of tests	9	8	8	8
Concentration of the fraction in the medium	—	200 μg/cm³	—	200 μg/cm³
Total dose of tested material in cm³	0	2	2	2
Mean weight of adenohypophyses incubated in 1 cm³ medium in mg	—	—	5.02	4.96
Activity, means $\pm \sigma_m$	12.6 ± 1.09	16.3 ± 1.07	23.9 ± 1.5	30.5 ± 3.06[a]

[a] Comparison of groups 3 and 4 by Fisher's t-test: $p < 0.05$.

and applied to the paper in the same way. Both specimens were then subjected to paper electrophoresis (pyridine-acetate buffer, pH 4.1, 250 V, 20 mA, 90 min). The electrophoreograms were then dried at 90° C and the longer side of the strip was sewn to the shorter side of another strip (38 × 44 cm) of Whatman 3 paper on a sewing machine. Descending chromatography in a butanol-acetic acid-water system (4:1:5) was then carried out for 22 h (until the tropeolin standard spot reached the end of the paper). The electrochromatogram was then dried and detected by 0.5% ninhydrin solution in acetone. The native specimens of both fractions gave no ninhydrin positive spots, indicating that they do not contain free amino acids. After hydrolysis several ninhydrin positive spots were found, corresponding to the following amino acids: lysine, valine serine, glycine, alanine, asparagine, glutamic acid, leucine, isolencine. It thus follows that the active fractions contain a small peptide, composed of the 9 given amino acids. The possibility that the fraction contains another ninhydrin negative substance cannot be excluded. From previous work we know, however, that the factor is probably not identical with histamine, adrenaline, noradrenaline, oxytocin, vasopressin, acetylcholine, and serotonin[5]. Detection of the chromatograms of the C fractions for phenolic acids (diazotised *p*-nitroaniline) and for indol compounds (*p*-dimethyl aminobenzaldehyde in acid ethanol) gave negative results.

For the examination of the TSH-releasing activity of the fractions $HH_{18}{}^{+}{}_{1,C}$ and $HH_{20+1,C}$ *in vitro*, the same method as in previous reports[1,2] was used. Male albino rats (Wistar descendants) weighing 130–200 g, acclimatized at 23 ± 1°C and fed on a standard Larsen diet and water *ad libitum* were killed by decapitation and their adenohypophyses removed and cooled to 0°C. In each of four successive experiments, 13 adenohypophyses were incubated for 1 h at 37 ± 0.1°C in the two following media: (A) 13 cm³ Krebs-Ringer phosphate; (B) 13 cm³ Krebs-Ringer phosphate containing 90 μg fraction $HH_{18+1,C}$ or 200 μg fraction $HH_{20+1,C}$ in 1 cm³. Both media contained 300 mg glucose/100 cm³. The TSH concentration in the media was determined by the radioiodine uptake method described in detail elsewhere[6]. Female albino rats weighing about 100 g were hypophysectomized by the parapharyngeal route under ether anaesthesia. Three days after hypophysectomy administration of the tested material was started: once daily every rat received one subcutaneous injection of 0.5 cm³. Four groups of hypophysectomized rats were used in each experiment: (1) untreated rats served as controls; (2) rats given four doses of 0.5 cm³ Krebs-Ringer phosphate medium containing 90 μg/cm³ fraction $HH_{18+1,C}$ or 200 μg/cm³ fraction $HH_{20+1,C}$; these rats served as a second control group; (3) rats given four doses of 0.5 cm³ medium A (after incubation of adenohypophyses without fraction); (4) rats given four doses of 0.5 cm³ medium B (after incubation of adenohypophyses in the presence of the fraction).

1 h after the last injection, 4 μC of carrier-free $Na^{131}I$ was administered to each rat subcutaneously; 24 h later the rats were killed by ether anaesthesia, the thyroids were removed and their radioactivity was measured by a Geiger-Müller counter. The activity of each thyroid was expressed as a percentage of the radioiodine dose administered, a model of the rat thyroid containing 4 μc $Na^{131}I$ being used as the indicator of 100% accumulation. The results are shown in Table II. The radioiodine uptake in the thyroids of group 2 was not statistically different from group 1, indicating that no TSH was present in fractions $HH_{18+1,C}$ and $HH_{20+1,C}$. After the administration of medium A the uptake was somewhat higher, indicating some release of TSH into the medium even in the absence of the fractions. After the administration of medium B a further increase in the uptake occured; the difference between group 3 and 4 was statistically significant only on using fraction $HH_{20+1,C}$. The results nevertheless show an increase in TSH release in the presence of fractions

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$HH_{18+1,C}$ and $HH_{20+1,C}$. As there was no significant difference between the TSH content of adenohypophyses incubated with and without the fractions[7], it seems unlikely that the differences in TSH content in the media were caused by a block of TSH break-down[8].

Zusammenfassung. Die elektrophoretisch isolierte Fraktion des hypothalamischen Extraktes, die die adenohypophysären sauren Phosphatasen *in vitro* und die Sekretion des TSH aktiviert, wurde mittels Gel-Filtration an einer Sephadex G-25 Säule weiter gereinigt. Elektrochromatographische Analyse der gereinigten Fraktion zeigte, dass sie in nativem Zustand Ninhydrin-negativ ist und dass nach der sauren Hydrolyse folgende Aminosäuren aus ihr entstehen: Lysin, Serin, Glycin, Alanin, Asparagin, Glutamsäure, Valin, Isoleucin und Leucin. Dieselbe Fraktion erhöht die Sekretion des TSH aus Rattenhypophyse *in vitro*. Die Fraktion enthält also wahrscheinlich den hypothalamischen Peptid, der die Sekretion des TSH aus der Hypophyse stimuliert (thyreotrophin releasing factor, TRF).

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Interactions of a Chemical Carcinogen with Neuro-Endocrine Factors in Mouse Breast Cancer[1]

Interactions in breast cancer development between pituitary implants[2–5], the hypothalamus[5,6], and a chemical carcinogen[7–15] were explored in recipients of the inbred Dilute Brown subline 8 (D_8) stock, while male D_8 mice, 24–28 weeks of age, served as donors. Three groups, each composed of 30 female, 5–6 week old mice, received once one of the following treatments by trocar into the right axilla: (a) three pieces of brain surface—the control treatment; (b) three pituitaries and three pieces of brain surface; or (c) three pituitaries and three 'hypothalami'[5].

Starting about $1-{}^1/_2$ months after these implantations, 20 mice of each treatment group were force-fed six times, at intervals of about one week, with 1 mg of 3-methylcholanthrene in 0.2 cm^3 of olive oil per 20 g body weight. The remaining 10 animals of each group served as controls and received only 0.2 ml olive oil per 20 g of body weight, also by oral intubation.

Thereafter, twice a week, the animals were inspected and palpated, if indicated, for breast tumors. The mice were killed for complete autopsy when the tumors extended at least in one dimension over more than 1.0 cm. Tumors and other gross pathology were also examined histologically[16].

The effect of 3-methylcholanthrene on cancer incidence in the pituitary isografted D_8 mouse is shown in Table I,

Tab. I. Breast cancer development in groups investigated

Tissue inserted	Tumor incidence with MCh[a]	without MCh[b]
Brain surface only	2/20	0/10
Pituitary only	19/20	0/10
Pituitary and hypothalamus	9/20	0/10

[a] 1 mg 3-methylcholanthrene/0.2 cm^3 olive oil/20 g body weight; oral intubation, 1/week, for six weeks.

[b] 0.2 cm^3 olive oil/20 g body weight; oral intubation, 1/week, for six weeks.

summarizing results obtained within 227 days after implantation and 160 days after the last intubation. By this time, all animals in the group given pituitary plus brain surface as well as the carcinogen had developed breast cancer, except for one mouse that died noncancerous at the age of 251 days. A significantly smaller number of mice had developed breast cancer in the other carcinogen-fed groups whether they were implanted with both pituitary and hypothalamus or only with brain surface. During the same time period, no tumors were observed in the several implanted control groups fed only olive oil (Table I).

The first breast cancers of the 3-methylcholanthrene treated isografted mice appeared at about the same time in the pituitary-hypothalamus group and in the pituitary-brain surface group. Nonetheless, within the period of study, the addition of hypothalamic tissue to the pituitary

[1] This investigation was aided by U.S.P.H.S. grants (C-5748, C-4359), the American Cancer Society (Minnesota Division and E-155), and Elsa U. Pardee Foundation.

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