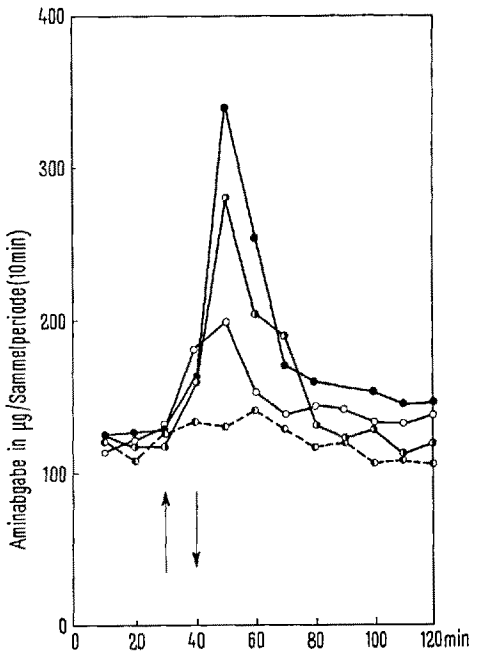

Abgabe von Katecholaminen aus der isoliert durchströmten Rindernebenniere. Beginn ↑ und Ende ↓ der Durchströmung mit thrombinhaltiger Tyrodelösung. Bei o—o mit 100 NIH-E, bei ⊙—⊙ mit 200 NIH-E, bei ●—● mit 300 NIH-E Thrombin/ml und bei ⊙---⊙ = 200 NIH-E Thrombin + 30 µg Hirudin/ml.

nicht an den granulären Aminspeichern liegen, sondern ist vermutlich an den Membranen der aminhaltigen Zellen zu suchen.

Wie die vorliegenden Versuche zeigen, ist die durch Thrombin ausgelöste Freisetzung biogener Amine nicht auf die Blutplättchen beschränkt. Es werden sich voraussichtlich noch weitere Objekte finden, an denen sich diese Wirkung des Fermentes nachweisen und studieren lässt. Für die Frage nach dem der aminfreisetzenden Wirkung des Thrombins zugrunde liegenden Mechanismus ist von Bedeutung, dass diese Wirkung an die spezifische proteolytische Aktivität des Fermentes geknüpft ist, die bekanntlich in der Spaltung bestimmter Peptidbindungen beruht. Die Aminfreisetzung könnte daher durch Reaktion des Thrombins mit einem proteinartigen Substrat an der Oberfläche der aminhaltigen Zellen eingeleitet werden.

Summary. Experiments on isolated perfused suprarenals have shown that the injection of thrombin caused a significant rise in the liberation of catechol amines. The specific thrombin-inhibitor, Hirudin, was able to abolish the thrombin effect. When isolated medullary granules from suprarenals were incubated with thrombin, no increase of catechol amine release was observed.

F. Markwardt und W. Nuernbergk

Pharmakologisches Institut der Medizinischen Akademie Erfurt (DDR), 21. Oktober 1964.

Effect of Win 18501-2 on the Content of Catecholamines and the Number of Catecholamine-Containing Granules in the Rabbit Hypothalamus

It is well established that reserpine causes sedation with concomitant loss of serotonin, norepinephrine, and dopamine from the nervous tissues[1-5]. Spector et al. have reported that Win 18501-2 (Oxypertin, 1-(5,6-dimethoxy-2-methyl-3-indole)-ethyl-4-phenylpiperazine) also produced sedation accompanied by the loss of norepinephrine without affecting serotonin content in the brain[6]. Matsuoka has also reported that Win 18501-2 depleted only norepinephrine without loss of dopamine, suggesting that sedative effect of the drug is closely related to loss of brain norepinephrine[7]. The present study was undertaken on the rabbit hypothalamus better to understand the effect of Win 18501-2, both by biochemical and electronmicroscopic techniques.

By electronmicroscopy, De Iraldi et al. demonstrated the existence of a large number of granulated vesicles supposed to be the site of storage of norepinephrine in the synapses of the anterior hypothalamus[8]. Shimizu and Ishii have demonstrated that the number of the granulated vesicles decreased markedly in the anterior hypothalamus after reserpine injection[9]. De Iraldi et al. showed the complete disappearance of the granulated vesicles in the pineal body after reserpine injection[10]. These studies might lead to the conclusion that norepinephrin is contained in the granulated vesicles. However, it is undecided whether or not other important amines, such as serotonin and dopamine, might also be contained within the granulated vesicles.

As shown in the Table, Win 18501-2 (70 mg/kg, intraperitoneal injection) caused a decrease in norepinephrine content and in the number of granulated vesicles, whereas an increase in dopamine content in the rabbit hypothalamus, 3 h after a single injection, was observed when the

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Effect of Win 18501-2 on the content of catecholamines and the number of the granulated vesicles in the rabbit hypothalamus

Region	Treatment	No. of samples	Norepinephrine (μg/g)		Dopamine (μg/g)		Number of granulated vesicles per area (200 μ^2)		
			Range	Mean	Range	Mean	No. of the area	Range	Mean
Medial part	Control	5	1.27–2.12	1.61	0.69–1.03	0.86	10	53–116	77
	Win 18501-2	3	0.33–0.82	0.50	1.12–1.42	1.26	10	6–20	12
Lateral part	Control	5	0.24–1.26	0.55	0.54–1.18	0.64	10	7–36	19
	Win 18501-2	3	0.31–0.55	0.34	1.20–1.61	1.43	10	6–16	9

The animals were killed by decapitation 3 h after the intraperitoneal injection of Win 18501-2 (70 mg/kg), and the tissues of the hypothalamus were collected from 8–10 animals and assayed for catecholamines as one sample. Catecholamines were extracted and separated by the method reported from our laboratory[11].
The hypothalamus was divided into the medial and the lateral part by the dorso-ventral line passing through the fornix and mamillothalamic tract, the medial part containing mainly anterior and ventromedial nuclei, and the lateral part the lateral nucleus in the same level. By the electronmicroscopic method reported by us[9], the 10 areas (200 μ^2) were observed at random in the neuropil of both the medial and lateral parts obtained from 5 control and 3 treated animals.

animal showed complete sedation. Action of Win 18501-2 on the submicroscopic morphology of the hypothalamus is demonstrated in Figure 2. In electronmicroscopic observation the number of granulated vesicles was markedly decreased in the anterior hypothalamus 3 h after Win 18501-2 injection (compare Figures 1 and 2). It is of interest to note that the effect of the drug on the content of norepinephrine and the number of granulated vesicles was more pronounced in the medial part than in the lateral part of the hypothalamus. No difference in the number of granulated vesicles of the neuropil was observed between the nucleus anterior and nucleus ventromedialis of the hypothalamus[12]. Reserpine is known to release serotonin[1], norepinephrine[3], and dopamine[4], while Win 18501-2 releases norepinephrine alone[6,7]. Both drugs caused sedation accompanied by the same changes in submicroscopic morphology of the hypothalamus, namely the decrease of granulated vesicles and the disappearance of dense core within the vesicles. A parallelism

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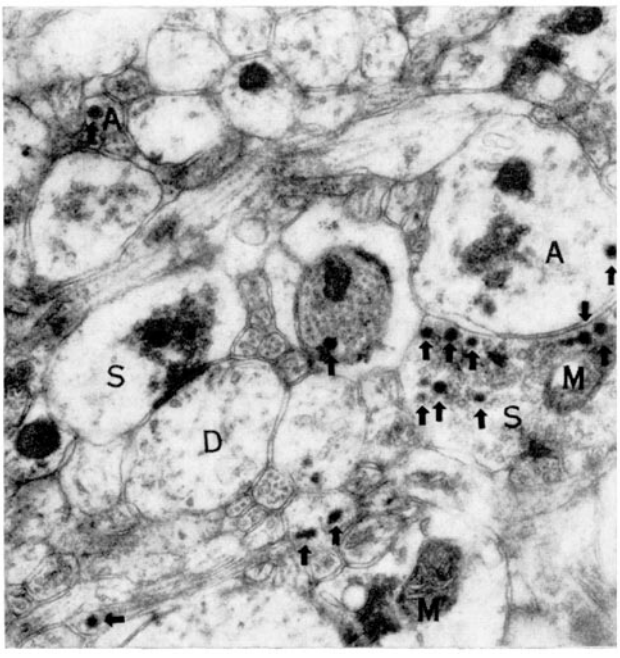

Fig. 1. The neuropil of the nucleus hypothalamicus anterior. Many granulated vesicles (arrow) are seen within the synaptic terminal (S) and the small axon (A). × 18000.

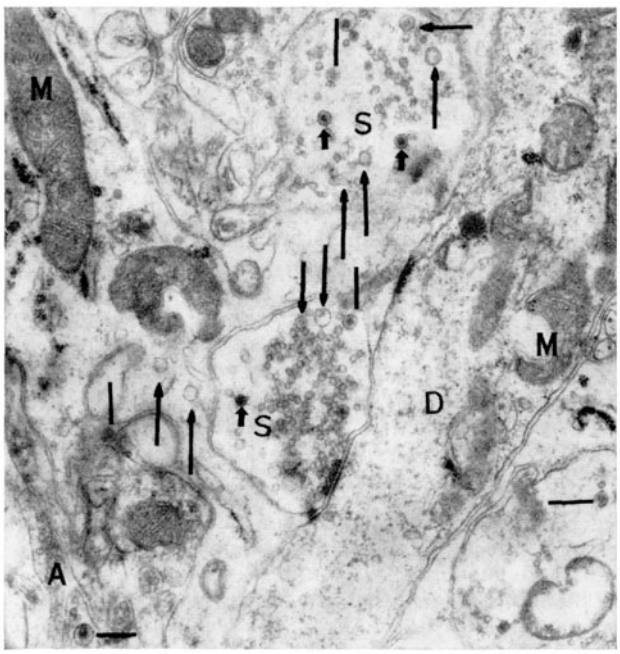

Fig. 2. The neuropil of the nucleus hypothalamicus anterior of the rabbit injected with Win 18501-2 (70 mg/kg) 3 h before sacrifice. Most granulated vesicles change in appearance, either the granulated vesicles disappearing to leave empty vesicles (marked by ↑) or remaining vesicles decreasing in electron density of dense core (marked by |). A few vesicles are of similar density to normal vesicles (marked by ↑). M, mitochondria; S, synaptic terminal; A, axon; D, dendrite. × 18000.

between norepinephrine content and the number of granulated vesicles in the hypothalamus observed in the present study suggests that the granulated vesicles contain only norepinephrine. It is also suggested that the sedation induced by Win 18501-2 or reserpine is closely related to loss of norepinephrine, which is contained within the granulated vesicles in the synapses and the axons of the hypothalamus[13].

Zusammenfassung. Der Hypothalamus von Kaninchen wurde nach Win 18501-2-Injektion biochemisch und elektronenmikroskopisch untersucht. Die Behandlung erzeugte auffallende Veränderungen im Noradrenalingehalt und in der Anzahl der Katecholamine enthaltenden granulierenden Vesiculae im Hypothalamus. Die Resultate scheinen zu zeigen, dass diese charakteristischen Vesiculae nur Noradrenalin enthalten und dass die Sedation des Kaninchens nach der Injektion von Win 18501-2 auf der Verminderung des Noradrenalins und der charakteristischen Vesiculae beruht.

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Effects of Starvation on the Free Amino Acid Pools of *Ciona intestinalis*

FERRINI et al.[1] have recently given quantitative data on the free amino acids of ovarian eggs of *Ciona intestinalis* with the help of amino acid analyser. This line of investigation, using such a modern and quantitative technique, has surpassed the method of paperchromatography, which gives only a qualitative indication of the more abundant amino acids present and is misguiding when the amount of material is small[2–4]. However, even this provides certain clues in connection with certain specific questions.

FERRINI et al. found a high concentration of taurine and sarcosine, unusual amino acids, in eggs of *Ciona*. Earlier, ACKERMANN and JANKA[5] had also detected taurine in *Ciona*. Taurine has also been detected in other marine invertebrates[6]. The excessive quantities of certain amino acids present in marine invertebrates is an interesting fact which has not yet been fully explained, but AWAPARA[6] has discussed the various possibilities. It is not clear whether the pattern is dependent on environment, i.e. if it can change with altered concentration of salt in the medium. On the other hand, there is some evidence by ROBERTS and SIMONSEN[7], BRAHMACHARY[8], and AWAPARA[6] that some organisms maintain a starvation-resistant genetically-fixed pattern of amino acids. In tadpoles of the toad *Bufo melanostictus* and in the Sicilian frog Discoglossus[9,10], BRAHMACHARY found a considerable loss in the quantity of free amino acids in tails following starvation, though there was no appreciable change due to metamorphosis, and by comparison with a larger quantity of tails from starved tadpoles, he detected that the pattern remained qualitatively the same. Other workers detected a loss of amino acids in starving insect larvae[11].

In view of these facts, it may be of some interest to follow the free amino acid patterns, especially taurine and sarcosine, in starved and unstarved *Ciona*.

Two batches of *Ciona* were starved for one month and three weeks respectively by keeping them in filtered sea water which was changed every day. The water was not pasteurized and some microorganisms might have dropped into it from the air, and thus a very small amount of nourishment might have been available to the organisms. But as the creatures became perceptibly very much emaciated (some of them died), the process of catabolism had far exceeded that of anabolism. The free and bound amino acid patterns in this condition were compared with those of normal *Ciona*. *The patterns turned out to be consistently very similar.*

The muscle tissues of *Ciona* were cut and removed and, after drying, homogenized in 70–80% methanol or ethanol. One-dimensional chromatograms developed in the solvent *n*-butanol : acetic acid : water (4 : 1 : 1) showed three very intense spots with some trailing. This condition was highly