ity attainable with this compound was somewhat less than that observed with several other agents, possibly because of the use of the indirect recording method. On the other hand, the compound was active at lower doses than reserpine, guanethidine or methyldopa, the 3 clinically most widely used substances. Wy-8678 also lowered pressure substantially in hypertensive dogs. A marked decrease in heart rate was also observed. The dose of guanethidine used was the maximum tolerated in these animals.

The above findings clearly indicate that Wy-8678 shows promise as a potentially useful therapeutic agent. A recent report showed that the compound also reduces pressure in anesthetized animals after an initial pressor phase[4]. The mechanisms responsible for the hypotensive action are currently under investigation. Preliminary experiments suggest that inhibition of sympathetic tone is a major feature of the compound[5].

Zusammenfassung. Bei unanästhesierten Ratten und Hunden wurden die antihypertensiven Eigenschaften von 2,6-Dichlorobenzyliden-Aminoguanidin-Azetat (WY 8678), welches zu einer neuen Reihe von aktiven Substanzen gehört, sowie noch einige andere klinisch wirksame Substanzen an zwei experimentellen Modellen für Hypertension geprüft und dabei eine wesentliche Blutdrucksenkung festgestellt.

T. Baum, D. K. Eckfeld, N. Metz, J. L. Dinish, G. Rowles, R. van Pelt, A. T. Shropshire, S. P. Fernandez, M. I. Gluckman and W. F. Bruce

Pharmacological Evaluation and Organic Medicinal Chemistry Sections, Wyeth Laboratories, Inc., Radnor (Pennsylvania 19087, USA), 14 April 1969

[4] I. L. Natoff, B. G. Katzung, F. Weir and J. K. Kodama, The Pharmacologist *10*, 157 (1967).

[5] St 155 was kindly supplied by Boehringer Ingelheim GmbH, guanethidine and hydralazine by Ciba Pharmaceutical Co., and methyldopa by Merck, Sharp & Dohme.

Electron Microscopic Observations on the Granular Vesicles in the Ciliary Ganglion of the Rat

On the basis of fluorescent microscopical studies, adrenergic nerve terminals have been postulated to exist in the ciliary ganglion of the cat, the goat, and the rat[1–3]. In addition, similar structures have been observed in the avian ciliary ganglion[4]. However, recent reports on electron microscopy fail to mention the existence of granular or 'dense core' vesicles in the avian ciliary ganglion[5–9]. Small granular vesicles (about 900 Å in diameter) have been presumed to store biogenic amines, which take part in the transmission of impulses in the nervous tissue, while larger granular vesicles (about 2000 Å in diameter) are generally interpreted as neurosecretory ones[10,11]. The present study was designed to investigate the mode of distribution of granular vesicles in the ciliary ganglion of the rat, a subject not previously studied.

About 20 male and female albino rats of Sprague-Dawley strain were used. The animals were decapitated under light ether anaesthesia. The fresh ganglia were quickly removed and fixed by immersion in 3.5% glutaraldehyde solution for 1–2 h, followed by immersion in 1% osmium tetroxide solution for 1–2 h. Both fixatives were ice-cold and buffered to pH 7.2 with 0.1 *M* phosphate. After rinsing in the buffer solution, the specimens were dehydrated in the graded alcohol series and embedded in Epon. The ultrathin sections were stained with lead citrate, and for electron micrography a Philips EM-200 apparatus was used. About 10 rats were injected i.p. with reserpine (Serpasil®, Ciba), 1 mg daily, 3 doses altogether, which treatment depletes the catecholamines from all autonomic synapses including those in the ciliary ganglion, as was demonstrated by formaldehyde-induced fluorescence[3]. Both ciliary ganglia of the injected animals were treated equally as those of the control rats. No difference in the results were observed between the sexes in the present study.

In the ciliary ganglion of the rat, granular vesicles were observed in 2 different regions. Firstly, granular vesicles were found in some nerve endings and synapses surrounding the nerve cell bodies. In these synapses, which are mostly of axodendritic type, the granular vesicles were localized in the presynaptic or axonal part of the synapse (Figure 1). In addition to these apparently

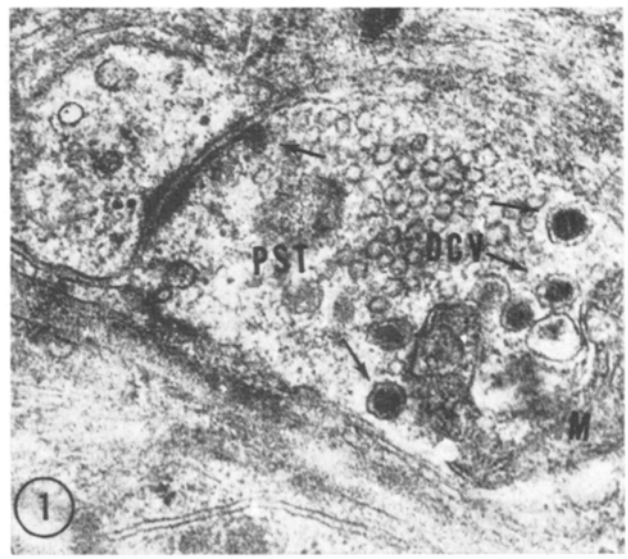

Fig. 1. Adrenergic synapse. Several granular vesicles in the presynaptic terminal. Ca. × 55,000.

Abbreviations used in the figures: A, Axon; DCV, Dense core vesicle = Granular vesicle; M, Mitochondrion; NC, Neuronal cytoplasm; NE, Nerve ending; NG, Neurosecretory granule; PST, Presynaptic terminal; S, Synapse.

[1] B. Hamberger, K.-A. Norberg and U. Ungerstedt, Acta physiol. scand. *64*, 285 (1965).
[2] B. Ehinger, Acta physiol. scand. *67*, Suppl. 268 (1966).
[3] K. Huikuri, Acta physiol. scand. *69*, Suppl. 286 (1966).
[4] B. Ehinger, Z. Zellforsch. *82*, 577 (1967).
[5] A. J. de Lorenzo, J. biophys. biochem. Cytol. *7*, 31 (1960).
[6] K. Takahashi and K. Hama, Z. Zellforsch. *67*, 174 (1965).
[7] K. Takahashi and K. Hama, Z. Zellforsch. *67*, 835 (1965).
[8] A. Hess, J. Cell Biol. *25*, 3, Part 2, 1 (1965).
[9] K. Takahashi, Z. Zellforsch. *83*, 70 (1967).
[10] M. A. Grillo, Pharmac. Rev. *18*, 387 (1966).
[11] O. Eränkö, Ann. Rev. Pharmacol. *7*, 203 (1967).

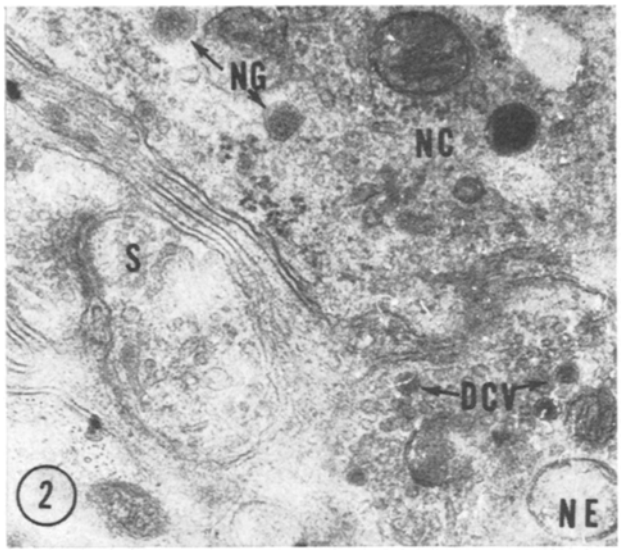

Fig. 2. Neuronal cytoplasm containing both granular vesicles and neurosecretory granules. Ca. × 25,000.

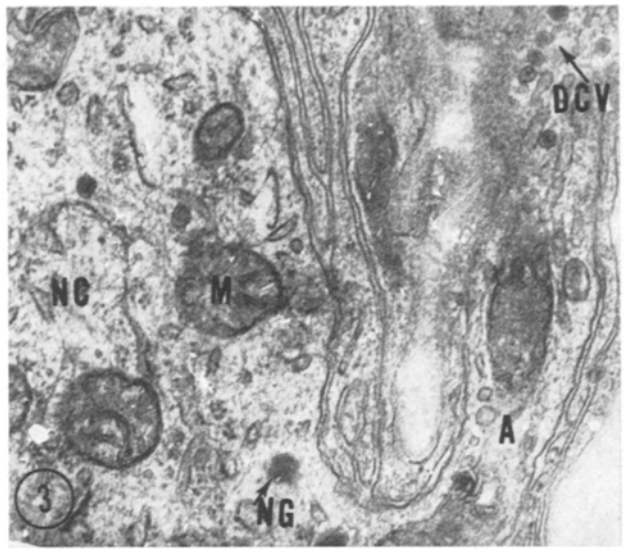

Fig. 3. Several granular vesicles within the nerve fibre (axon) departing from the respective perikaryon, and some neurosecretory granules in the cytoplasm. Ca. × 17,000.

adrenergic synapses, there were more numerous other synapses which displayed only agranular vesicles both on the pre- and postsynaptic side. Secondly, granular vesicles were also observed in the cytoplasm of some neurones. These granular vesicles were equal in size to those found in the nerve endings, but their concentration was smaller in the perikaryon (Figure 2). However, in some nerve fibres connected with the nerve cell body the granular vesicles showed much greater concentration than in the cytoplasm of the respective perikaryon (Figure 3).

While the granular vesicles in the nerve fibres, in the presynaptic terminals of some synapses and in the cytoplasm of some neurones varied in diameter in the to approximate range of 900–1200 Å, of which about 700 800 Å was occupied by the granule inside the vesicle, the agranular vesicles found in the synapses measured about 400–500 Å in diameter.

In the cytoplasm of all neurones, also larger granular vesicles were found. These vesicles had much larger diameter (about 2000 Å or more) than the synaptic granular vesicles. They were most often found in the vicinity of the Golgi apparatus, and they were diagnosed as neurosecretory granules surrounded by a membrane.

In the ciliary ganglion of the reserpinized rats, the number and localization of the granular vesicles had not been changed to any noteworthy extent. However, the density of the core seemed less clearly visible than in the controls. This suggests that the granules contained a catecholamine.

Zusammenfassung. Im Ganglion ciliare normaler ausgewachsener Ratten werden elektronenmikroskopisch granulierte oder «dense core» Vesikula von etwa 900 bis 1200 Å Durchmesser festgestellt (in Nervenfasern, im Zytoplasma von Nervenzellen und in Präsynapsen). Reserpin (Serpasil®, Ciba) verursachte keine nennenswerte Änderung in Zahl oder Lokalisation der Vesikula, hingegen eine Abnahme ihrer Kerndichte.

K. HUIKURI

Department of Anatomy, University of Helsinki, Helsinki (Finland), 14 May 1969

Intracellular Localization of Acid Phosphatase and Aryl Sulfatase in Rat Metrial Gland Cells

The granulated metrial gland cells of the pregnant rat uterus are complex structures, the function of which is at present unclear. The cells are rich in glycogen[1] and their granules are acidophilic and metachromatic[2]. Furthermore they give a diastase-fast reaction with the PAS-technique[3]. From these tinctorial properties as well as the incorporation of ^{35}S by the cells, it has been concluded that the cytoplasmic granules contain a lysine-rich protein component[3] as well as a sulfated mucopolysaccharide[1,4] which, however, does not appear to be heparin[5]. It has also been postulated that the hormone relaxin may be produced by these cells[3,6]. This hormone has recently been demonstrated in the cells by immunofluorescence techniques[7].

More recently, light microscopic and histochemical studies on the granulated cells of the metrial gland of the pregnant rat have shown these cells to have a high content of hydrolytic enzymes[8,9]. On these grounds it has been suggested that the metrial gland cell granules may be lysosomal in nature[9]. It was therefore considered of interest to investigate the nature of the metrial cell granules – at the electron microscopic level – with respect to the lysosomal enzymes acid phosphatase and aryl sulfatase.

For the experiments 10 pregnant Sprague-Dawley rats were used. The animals were sacrificed at late stages of gestation (15–20 days). After ether anesthesia and exsanguination small pieces of the sub-placental uterine