Zusammenfassung. Die Wirkung von Phenylephrin und Orciprenalin auf die Freisetzung von Noradrenalin wurde an isolierten, perfundierten Kaninchenherzen mit ^{14}C-markierten Noradrenalin-Vorräten untersucht. Beide Stoffe verminderten die Abgabe von ^{14}C-Noradrenalin bei Sympathicusreizung. Diese Hemmwirkung wirde durch Vorinfusion von Phenoxybenzamin, nicht aber durch Vorinfusion von Propranolol verhindert.

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On the Origin of Small Adrenergic Storage Vesicles: Evidence for Local Formation in Nerve Endings after Chronic Reserpine Treatment

The axonal enlargements (varicosities, nerve endings) of peripheral autonomic nerves contain large amounts of vesicles supposed to represent the intraneuronal storage sites for the transmitter substance[1]. In this paper some preliminary findings are presented, demonstrating that chronic treatment with reserpine, a drug known to deplete monoamine stores in the peripheral and central nervous system[2], causes certain fine structural changes in peripheral adrenergic nerve endings in the dilator muscle of the rat iris. These changes are discussed in relation to the formation of storage vesicles.

Material and methods. Ten male albino rats (Sprague-Dawley, b. wt. 200 g) were used, 8 of which received 1 daily dose of reserpine (Serpasil®, 1 mg/kg i.p.) for 6 days. 4 rats were sacrificed 24 h and 4 rats 72 h after the last injection. 2 untreated rats served as controls. The irides were fixed in ice cold 3% potassium permanganate ($KMnO_4$)[3] and processed for electron microscopy as previously described[4].

Results and discussion. Two types of axons were seen in untreated rats, adrenergic and presumably cholinergic, characterized respectively by their content of granular (vesicles with an electron dense core) (DCV) and agranular vesicles (Figure 1) with a spherical or slightly flattened shape and a diameter mainly of about 500 Å. In addition, both types of varicosities contain mitochondria and a few tubular or irregular membrane structures probably belonging to the axonic smooth endoplasmic reticulum (ASER) (Figure 1).

Both 24 and 72 h after the last injection of reserpine, several varicosities are seen containing many 'elongated vesicles'[5], tubular and/or slightly hour-glass formed membrane structures (thickness about 150–300 Å) (Figures 2 and 3) from which vesicles occasionally seem to bud off. Sometimes an electron dense precipitate is found within these structures (Figure 3). Such varicosities contain in addition 'normal' spherical vesicles, some of which may have an electron dense core (Figure 2). Other varicosities have almost exclusively spherical vesicles many of which may contain an electron dense core, especially 72 h after the last reserpine injection. The presence of DCV strongly suggests that these nerve endings belong to adrenergic neurons (for ref. see[6]). In varicosities with many tubular structures and 'elongated vesicles' the proportion of spherical DCV is low. Since the number and proportion of DCV probably parallel the recovery of NA levels after the reserpine treatment, it may be assumed that such nerve endings are in an early phase of recovery. Further varicosities contain vesicles only of the agranular type and are indistinguishable from those present in untreated rats and presumed to belong to cholinergic neurons. No obvious increase in the number of tubular structures and 'elongated vesicles' was found in this type of varicosity, indicating that reserpine causes such changes only in adrenergic neurons.

Although several explanations may be advanced we would like to discuss, hypothetically, the present results in relation to the formation of storage vesicles. Almost all components of the neuron have been proposed as the origin of the synaptic vesicles: Mitochondria[7], microtubules[8], nerve cell membrane and complex vesicles[9–13], large granular vesicles[14], the Golgi apparatus[15] and finally the ASER[15–19]. For an extensive discussion and further ref. see[18].

Flat vesicles have previously been described in normal[20] and reserpine treated[21] animals. In the present study an increase in the number of 'elongated vesicles' and tubular structures probably belonging to the ASER was observed specifically in adrenergic nerve terminals 24 and 72 h after chronic treatment with reserpine, a drug which is known to cause a long-lasting depletion of amine levels. Since this depletion probably is due to an irreversible destruction of the Mg^{++}-ATP dependent storage mechanism, the

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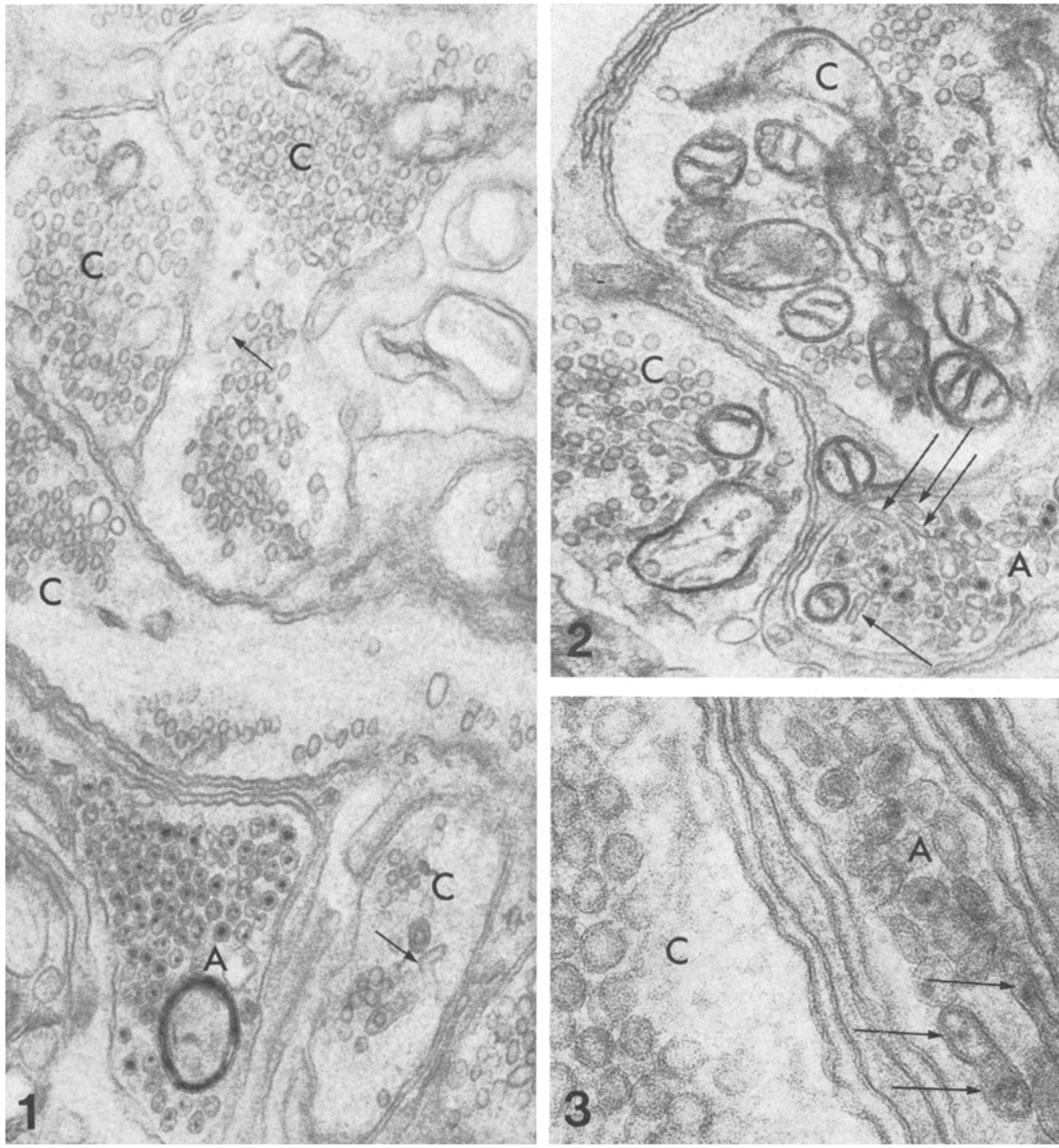
recovery of amine levels should depend on the formation of new storage sites confined to new storage vesicles[22]. In fact, DAHLSTRÖM and HÄGGENDAHL[23] have presented evidence for an increased formation of storage vesicles after reserpine treatment. Also the results of THOENEN et al.[24] and MOLINOFF et al.[25], demonstrating marked increases in tyrosine hydroxylase and dopamine-β-hydroxylase activity respectively after reserpine treatment, may be interpreted as a sign of a compensatory increase in synthesis processes after depletion of amines caused by this drug. Thus, it may be assumed that the increase in tubular structures found in this study occurs during a period of increased formation of storage vesicles. Since these tubules occasionally contain an electron dense material, which may reflect the presence of an amine at the moment of fixation[6], and since vesicles seemingly bud



Figures 1–3. Axonal enlargments close to the dilator muscle of the rat iris. 1. Untreated rat. Both adrenergic (A) and probable cholinergic (C) nerve endings contain many vesicles mainly with a diameter of 500 Å and mostly with a round or slightly flattened shape. Almost all vesicles in A contain an electron dense core. Note tubular structures (arrows) in some varicosities. × 61,000. 2. Rat chronically treated with reserpine, sacrificed 24 h after the last injection. Several tubules or 'elongated vesicles' (arrows) are seen in one axon (A). Note that some of the round vesicles contain an electron dense core characteristic of adrenergic nerve endings. The other varicosities (C) contain mainly round vesicles without an electron dense core. × 58,000. 3. Same treatment as in Figure 2. In one axon (A) tubular structures containing a weak electron dense precipitate is seen (arrows). Only round vesicles are seen in the second axon (C). × 150,000.

off from the tubules, it is temping to relate the tubules to amine storage, maybe as precursors of storage vesicles. Our findings would thus favour the possibility of a local formation of storage vesicles from the ASER as suggested in previous studies[17–19, 26]. In other studies formation of storage vesicles from microtubules has been described[8]. However, since microtubules are not visualized in $KMnO_4$ fixed tissue[27] and since the tubules and 'elongated' vesicles in the present study clearly have a typical triple-layered membrane structure, considerable transformational changes would have to occur during such a process.

It should be emphasized that the present data do not exclude the formation of storage vesicles in the cell body. It has been clearly shown that both small and large DCV are present in the cell bodies of peripheral[4] and central (to be published) NA neurons as well as in the axons[4]. Thus, vesicles produced in the cell body and transported down with the axoplasmic flow in all probability contribute to the vesicular population in the varicosities[28]. It is at present impossible to evaluate the relative importance of these two possible sources of vesicles, i.e. such produced in the cell body and those locally in the nerve endings from ASER. Furthermore, other sites of origin may also exist as discussed above[29].

Zusammenfassung. Die adrenergen Nervenendigungen der von Ratten, die während 6 Tagen Reserpin Iris erhalten haben, enthalten eine höhere Anzahl von länglichen synaptischen Vesikeln und Tubuli als diejenigen unbehandelter Kontrolltiere. Die Möglichkeit, dass die adrenergen Bläschen unter gewissen Umständen in den Nervenendigungen gebildet werden könnten, wird diskutiert.

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Electron and Fluorescence Microscopy of the Hamster Atrium After Administration of 6-Hydroxydopamine

6-Hydroxydopamine (6-OH-DA) can induce selective degenerative lesions in terminal adrenergic nerves, as demonstrated by electron microscopy[1], by the rapid disappearance of monoamine fluorescence in these nerves, and by a pronounced decrease in the norepinephrine content of various tissues[2]. The catecholamine content of the adrenal medulla, after administration of 6-OH-DA, remains normal but a marked increase in the activity of tyrosine hydroxylase, dopamine-B-oxidase and phenylethanolamine-N-methyl transferase suggests enhanced secretion of adrenal catecholamines[3]. The present work was undertaken to observe the behavior of the atrial specific granules[4] in the hamster heart after chemical sympathectomy with 6-OH-DA.

Materials and methods. Forty male MHA/SsLAK hamsters (Lakeview Hamster Colony, Newfield, New Jersey, USA) were used for two experiments. Each experiment consisted of 20 animals, half of which served as controls. The hamsters were kept in an air conditioned room and were maintained on Purina Laboratory Chow and tap water ad libitum. 6-Hydroxydopamine hydrobromide(6-OH-DA) (Regis Chemical Co., Chicago, USA) was dissolved in cold (0 °C) demineralized water containing 1% NaCl to which had been added 1% ascorbic acid (Fisher Scientific Co., Montreal, Canada) to decrease oxidation, and injected slowly (1 min) into the jugular vein under light ether anesthesia in a volume of 0.5 ml. In the first experiment, hamsters avering 60 g (range: 50–70 g) received 10 mg of 6-OH-DA on the first day and were sacrificed 24 h later together with the controls. In the second experiment, the hamsters averaged 135 g (range: 120–145 g) and were given 6-OH-DA at the dose of 10 mg per 100 g body weight on the 1st, 6th, 12th and 18th day. Experimental and control animals were killed together 24 h after the last injection.

For electron microscopy, 8 animals from each group were anesthetized with ether and the chest opened by an anterior midline incision. The right atrium was perfused with 2% cold glutaraldehyde buffered with cacodylate HCL (0.1 *M* at pH 7.1) using a needle inserted into the right ventricle[5]. During perfusion, the same fixative was dripped onto the surface of the right atrium. After perfusion, thin fragments of the anterior portion of the right atrium were placed in the fixative for 1 h, then washed for 3 periods of 15 min each in cacodylate to which 2% sucrose had been added. Subsequently, the specimens were left in the cacodylate solution for 12 h, post-fixed in osmium tetroxide buffered with veronal acetate for 1 h, and embedded in Araldite. Thick sections were stained with toluidine blue for orientation and thin sections were mounted on grids coated with formvar and carbon, stained with uranyl acetate and lead citrate, and examined with a Philips 200 electron microscope.

Monoamine fluorescence was assessed on two controls and two 6-OH-DA-treated animals at the end of each experiment. After sacrifice, the right atrium was immediately removed, rapidly quenched in liquid nitrogen-cooled isopentane and freeze-dried for 3 days. The tissues were exposed to paraformaldehyde vapors of optimal humidity for 1 h at 80 °C, followed by vacuum infiltration in paraffin for 15 min and sectioning at 8 µm. The fluorescence microscope was equipped with an Osram

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