Ultrastructural evidence of amantadine and amphetamine noradrenaline releasing action

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Summary. Amantadine, like amphetamine, exerts a depletory effect upon noradrenaline containing vesicles of the adrenal medulla. The microtubular system seems to play a role in the releasing process.

Introduction. Since the first study realized about the pharmacological and toxicological properties of adamantanamin hydrochloride by VERNIER and coworkers in 1969[2], a great number of investigations have appeared in literature on the pharmacological effect of amantadine upon various neurotransmitters in the CNS[3–11]. From these data, the drug appears to be able, at high concentrations, to inhibit the uptake of catecholamines[12,13]; at low concentrations, it increases the release of these neurotransmitters from their neuronal storage pools[3,5,9]; an increase is also observed in the activity of dopamine and noradrenaline receptors[3,14,15].

On the other hand, there are few data on the adrenergic potentiating activity exerted by amantadine at peripheral level[16–19]; furthermore these studies are always realized by measuring the arterial pressure of the animal or using isolated organ techniques.

We thought it interesting to investigate the catecholamine releasing action of amantadine upon adrenal medulla in vivo, using electron microscope methods in order to obtain a morphological evidence of this effect. Amphetamine has been reported to exert an effect similar to amantadine upon catecholamine turnover and its action on the adrenal medulla is well known[6,9,11,15,20,21]; we therefore used this drug as a reference compound in our study.

We have mainly focused our attention on the doses which are able to induce a depletory effect, the period of action of the drugs and the ultrastructural changes observed in the adrenal medullary cells after administration of amphetamine and amantadine.

Material and methods. The drugs (amantadine in increasing doses of 2, 5, 10, 20 and 30 mg/kg and amphetamine in increasing doses of 0.5, 1.5 and 2.5 mg/kg) were administered i.p. to Wistar rats. The animals were killed 30 min, 1 h, 6 h, 12 and 24 h later.

Within 30 sec after killing, the adrenal tissue was fixed in glutaraldehyde at 2.5% in Millonig's buffer[22]. The inclusion was realized following the Spurr technique[23].

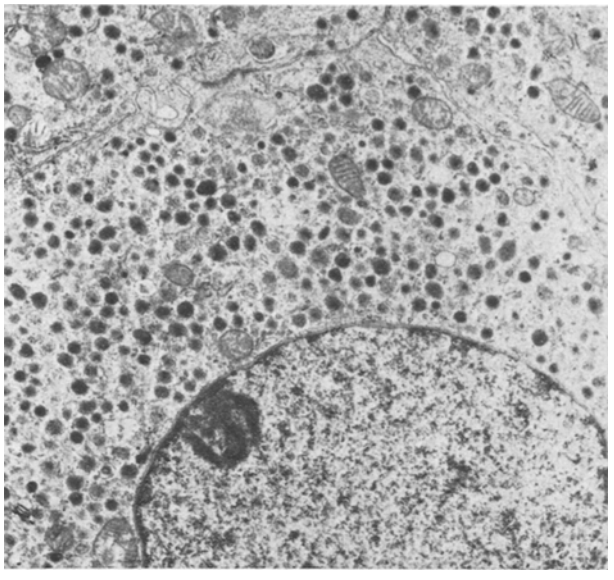

Fig. 1. Normal adrenaline-containing cells. Granule contents presents a medium optical density and it is placed centrally in relation to the surrounding membrane. × 20,000.

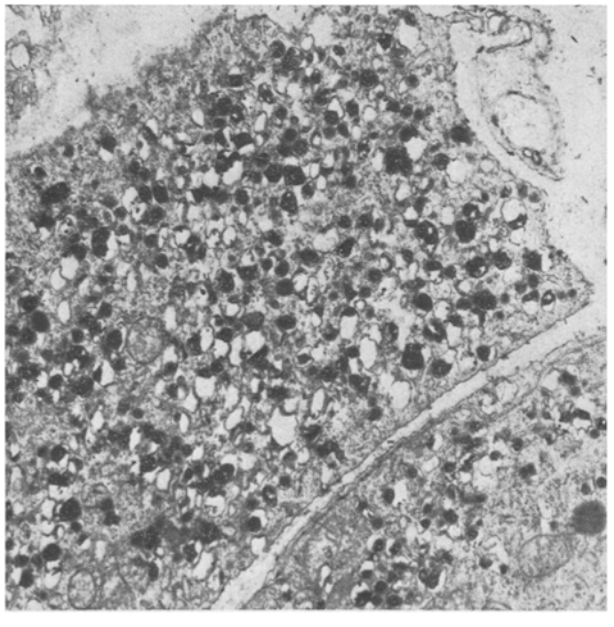

Fig. 2. Normal noradrenaline-containing cells. Granule contents shows a high optical density and it contacts partially with the surrounding membrane, leaving an empty space on the opposite place. × 20,000.

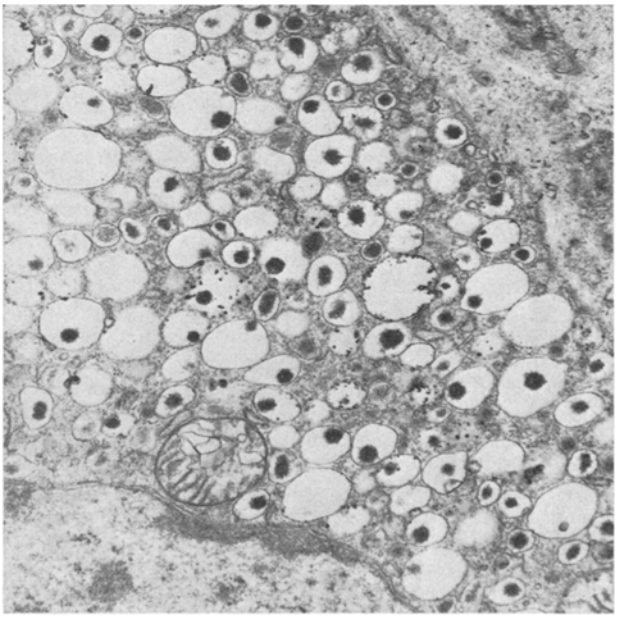

Fig. 3. Noradrenaline-containing cell 6 h after administration of 5 mg/kg of amantadine. The majority of the noradrenaline vesicles are empty and many others show a significant decrease of granule contents. × 30,000.

Later the slides were stained with uranyl acetate and lead citrate[24].

Results. Amantadine, in doses of 2 and 5 mg/kg, exerts a remarkable depletion of the noradrenaline-containing vesicles. On increasing the administered dose, this effect decreases progressively, being weakly discernible at dose of 10 mg/kg and really negligible at doses of 20 and 30 mg/kg. The greatest intensity of the releasing action has been observed from 1 to 6 h after administration of the low doses and, in some areas, it is still detectable after 12 h.

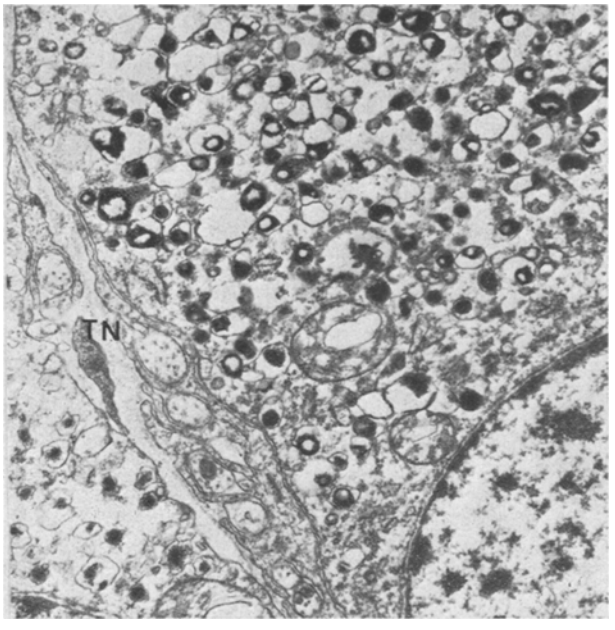

Fig. 4. Noradrenaline-containing cells 30 min after administration of 1.5 mg/kg of amphetamine. The noradrenaline depletory process is clearly advanced but it has not reached its maximum. 20,000 ×.

Amphetamine also induces the extrusion of noradrenaline but not of adrenaline, at doses varying from 0.5 mg/kg to 1.5 mg/kg; it is remarkable the shortness of the amphetamine liberatory action, which reaches its maximum at 30 and 60 min and 6 h after administration has completely disappeared.

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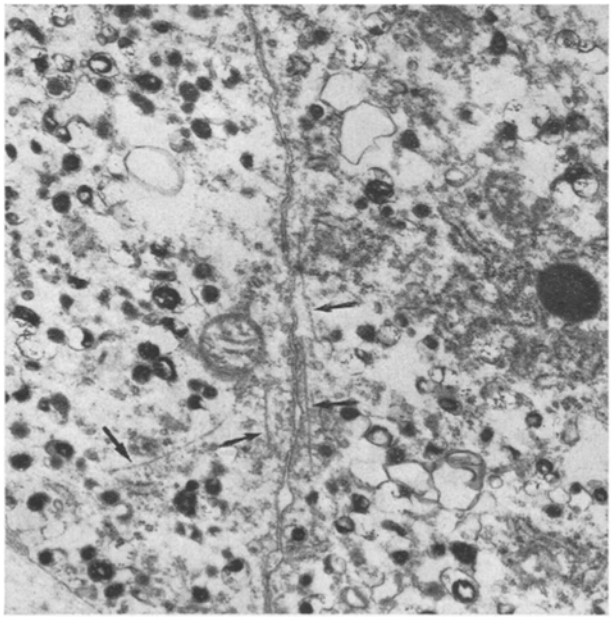

Fig. 5. Noradrenaline-containing cells 30 min after administration of 2 mg/kg of amantadine. The depletory process is beginning. We can see a great number of cytoplasmatic microtubules (arrows). × 20,000.

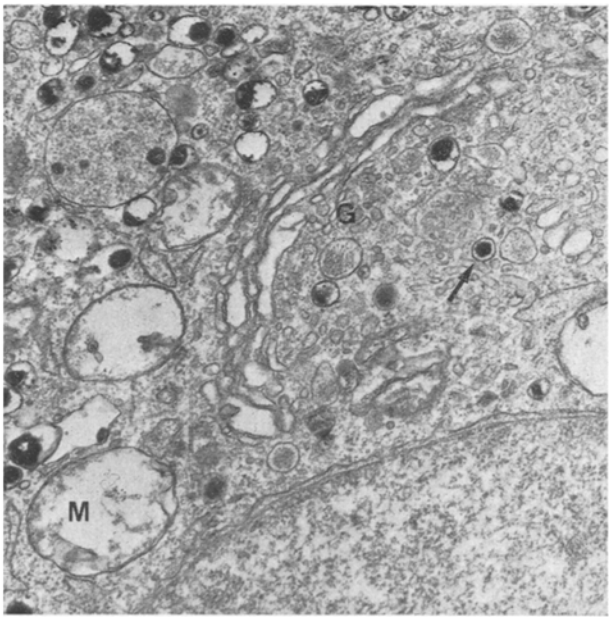

Fig. 6. Noradrenaline-containing cell 12 h after administration of 10 mg/kg of amantadine. It is remarkable the hypertrophy of the Golgi apparatus. × 25,000.

The extent and duration of amantadine and amphetamine effects are confirmed by the observation of significant signs of hyperactivity in noradrenaline producing medullary cells. The Golgi apparatus shows a clear hypertrophy and it is surrounded by a great number of new formation vesicles, characterized by their low optical density and by the concentric disposition of the granule contents respect to the membrane. We have not found an increase of exocytosis with any of the two drugs employed in the study. On the contrary, we have observed an obvious increase in the number and size of cytoplasmatic microtubules during the releasing phase.

Comments. Morphological observation proves to be a reliable rule for investigating the pharmacological effect of some drugs. The peripheric action of amantadine is better clarified by the fact that the drug has a definite and specific activity at medullary level via a release of noradrenaline.

The morphological data in our hands seems to support that the mechanism of release of catecholamines from the adrenal cells is due to a process of hemiocytosis. This is in contrast with the observations realized by SCHNEIDER[21], SMITH and VAN ORDEN[25] and TRIFARO and coworkers[26]; but, on the other hand, our data confirm the results obtained by other authors[27–29] who think that the microtubular system is involved in the noradrenaline and adrenaline release from adrenal medullary cells.

The actions of amantadine seems therefore to be the overall results of an effect on the CNS and of the activity at peripheric level.

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An indirect demonstration of the substructure of the lamina basalis in the branchial heart of Sepia officinalis L. by means of cholinesterase reaction[1]

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Summary. Using a cytochemical cholinesterase reaction, a filamentous substructure of the lamina basalis of the peripheral epithelium in the branchial heart of Sepia officinalis L. can be demonstrated. The reaction product can be identified between the probable collagenous microfilaments, so that a form of negative staining is given.

The basement lamina has been identified as a fundamental structure of many epithelia with transporting functions. In excretory and blood vessel systems, this lamina is a structure which, as a 'semipermeable' barrier, separates the blood, hemolymph, of excretory fluid from the fluid which washes around the basal plasmalemma of the epithelial cells. Biochemical and cytochemical findings[2–4] have produced some indications of the chemical composition of the basement lamina in vertebrates; but its substructure is not well clarified[5].

In our cytochemical and electron microscopical investigations concerning the circulatory and excretory system of dibranchiate Cepahlopods, we were able to see that the epithelia and endothelia of these organs (the branchial and systemic heart, the blood vessels, the renal and pancreatic appendages, the gills, etc.) always have a very large and distinct basement lamina of 200 nm thickness which very often builds up the only closed border of the blood sinus in the periphery of the organs[6,7]. With normal electron microscopical fixations and staining with uranyl acetate and lead citrate, the lamina shows a granular, amorphous, or partially a grid-like substructure (figure, a) in higher magnifications.

Cytochemical identification of the non-specific cholinesterase in the peripheral epithelium of the branchial heart of Sepia officinalis will provide new aspects of the fine structure of this extracellular layer in cephalopods.

Material and methods. The tissue was taken from the branchial heart of juvenile Sepia officinalis (L.) anaesthetized with 0.5–1% Ethanol Seawater. Fixation of specimens (⌀ 0.5–1 mm): 4% glutaraldehyde in phosphate buffer (Sørensen), pH 7.4; 1050 mOsm; 2 h; 6 °C; rinsing: in 10% sucrose for 30 min at 20 °C; incubation[8]: for 30 min in acetylthiocholineiodide 70 mg, 0,1 M sodium acetate 22 ml, 0,1 N acetic acid 3 ml, 3.75% glycine 1 ml, 0.1 M $CuSO_4$ 1 ml, 0.5% $PbNO_3$ 1 ml; the reaction can be inhibited with 10^{-6} M Mipafox; rinsing: seawater 5 min, H_2S saturated sea water 5–6 min, seawater 5 min; postfixation: 1.5% OsO_4 in phosphate buffer; embedding: Durcupan® ACM (araldit); thin sections were cut on a LKB ultramicrotome and viewed – without staining with uranyl acetate or lead citrate – in a Zeiss EM 9A or a Philips EM 300. – In addition some samples were stained with alcian blue and the PAS-reaction for light microscopic analysis.

Results. The branchial heart sections primarily show reaction products on the surface of the ovoid cells[9] and in the lamina basalis of the peripheral brush border epithelium bordering the organ to the pericardial coeloma (figure, b). Some precipitations can be seen in the alcian blue and PAS-positive basic region of the epithelial cells and also in the microvilli of the brush border.

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