Electron Microscopic Localization of 5-Hydroxydopamine, a 'False' Adrenergic Neurotransmitter, in the Autonomic Nerve Endings of the Rat Pineal Gland

The major innervation of the rat pineal gland is sympathetic[1], consisting of fibres which originate from cell bodies in the superior cervical ganglia. In the perivascular terminals of these nerve fibres, both small agranular and granular vesicles (~500 Å units in diameter) and large granular vesicles (~1000 Å units in diameter) can be demonstrated by electron microscopy[2]; the dense cores of the small granular vesicles represent biogenic amines[3-6]. There is some question concerning the meaning of empty vesicles which are visualized in terminals containing mostly granular vesicles, one interpretation being that the empty vesicles contain acetylcholine[7]. Recently, however, evidence has been put forward that empty and dense core vesicles, of peripheral sympathetic nerve endings, represent a homogeneous population differing only in the degree of amine filling[8].

In a previous study[9] on the rat pineal gland, BONDAREFF reported the first direct visualization of a 'false neurochemical transmitter'. He showed that Metaraminol, an electron lucent 'false' neurotransmitter[10], could displace endogenous noradrenaline (NA) from its storage site in the vesicles and that subsequent repletion of the empty-looking vesicles occurred after treatment with α-methylnoradrenaline an osmiophilic 'false' neurotransmitter.

5-Hydroxydopamine (5-HO-DA), another 'false' neurotransmitter[11], has proved useful as a marker for aminergic nerve endings in the peripheral sympathetic system[12]. The present fine structural study reports the replacement of the physiological transmitter(s) by 5-HO-DA in the autonomic nerve endings of the rat pineal gland, which are thought to contain both NA[3-5,13,14] and 5-hydroxytryptamine (5-HT)[6,15-17] in the physiological state.

Rats pretreated with α-methylmetatyrosine (α-MMT) 200 mg/kg, 20 h and 4 h before the experiment, and non-treated controls, were given a single i.v. injection of 5-HO-DA (10 mg/kg) 20 min before sacrifice. Pineal glands, from control, α-MMT and α-MMT + 5-HO-DA treated rats were removed under light ether anaesthesia, fixed by immersion in 4% glutaraldehyde, post-fixed in osmium tetroxide and embedded in Epon for electron microscopy. Ultrathin sections were double stained with uranyl acetate and lead citrate.

Examination of thin sections of the pineal glands from untreated controls confirmed previous findings namely that approximately half of the small vesicles contained a dense core (Figure 1). Pre-treatment of animals with α-MMT, whose action is largely mediated through the 'false' sympathetic transmitter Metaraminol, produced a complete depletion of the small granular vesicles (Figure 2). 5-HO-DA administered to control and pre-treated rats lead to the accumulation of a large quantity of strongly osmiophilic material in the vesicles (Figure 3). Moreover, virtually all the vesicles were filled to capacity. After such treatments no detectable morphological alterations were observed in the pinealocytes. Similar results were obtained under the following conditions: i.p. injection of 5-HO-DA (4 × 20 mg/kg) over a period of 48 h, intraventricular injection of 5-HO-DA (5 mg in 50 µl saline) over 3 h, and incubation of thin tissue slices in a Krebs-Henseleit solution containing 1 mg/ml 5-HO-DA, for 30 min.

In contrast to the results obtained with α-MMT, pre-treatment with reserpine (5 mg/kg i.p. 20 h before the experiment) followed by the administration of 5-HO-DA, resulted in a continued depletion of the vesicles in the nerve endings.

It is proposed that the dense material in the vesicles represents the exogenous 5-HO-DA. The results after reserpine pre-treatment are in support of previous findings[13] which imply that the amine uptake and storage mechanisms are impaired. Thus 5-HO-DA and its eventual metabolites are taken up and stored in a similar way to the physiological transmitter(s) NA and/or 5-HT. Our observations therefore confirm and extend those of others[9,12].

The evidence concerning the specific amine(s) normally occurring in pineal nerve endings is conflicting. Although it is generally believed that these nerve endings contain NA[3-5,13,14], they take on the fluorescent histochemical staining indicative of 5-HT[15]. In addition they concentrate tritiated 5-hydroxytryptophan as shown by electron microscopic radioautography[16] and become depleted of their granulated vesicles upon treatment with *p*-chlorophenylalanine[6], a specific inhibitor of 5-HT biosynthesis[18], while unaffecting the pineal NA content. The possibility that both amines may be stored in the same nerve terminal and in the same organelle has also been proposed[17]. Using the techniques of electron[19] and fluorescence[20] microscopy, it has been shown that autonomic neurons of both the peripheral and central nervous systems which normally contain catecholamines, are capable of concentrating exogenous 5-HT. The present findings are therefore additional evidence that the uptake and storage of exogenous biogenic amines by adrenergic nerves and their vesicles is not completely specific. This implies also that from experiments using an exogenous amine (e.g. a 'false' neurotransmitter or labelled amine) as a tracer, one cannot equate the exoge-

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Figs. 1–3. Autonomic nerve endings of the rat pineal gland.

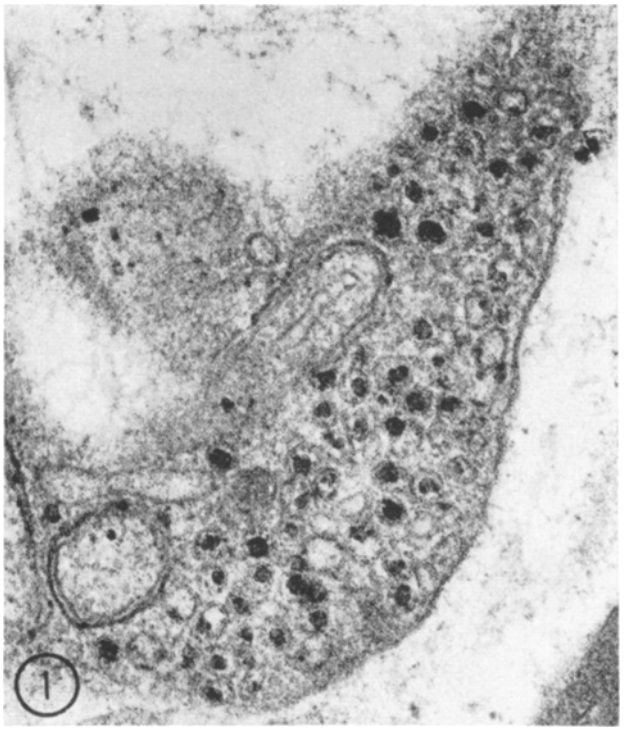

Fig. 1. Untreated control. In the perivascular nerve ending approximately half of the small vesicles contain an electron dense core (the physiological transmitter(s) NA and/or 5-HT). × 75,000.

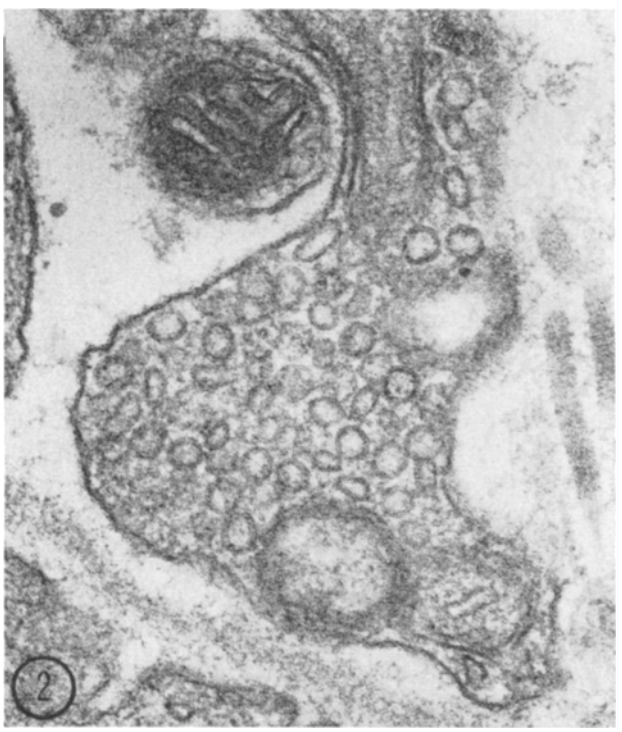

Fig. 2. Treatment with α-MMT. All the small vesicles appear empty (the electron lucent 'false' transmitter has displaced the osmiophilic, physiological transmitter(s)). × 75,000.

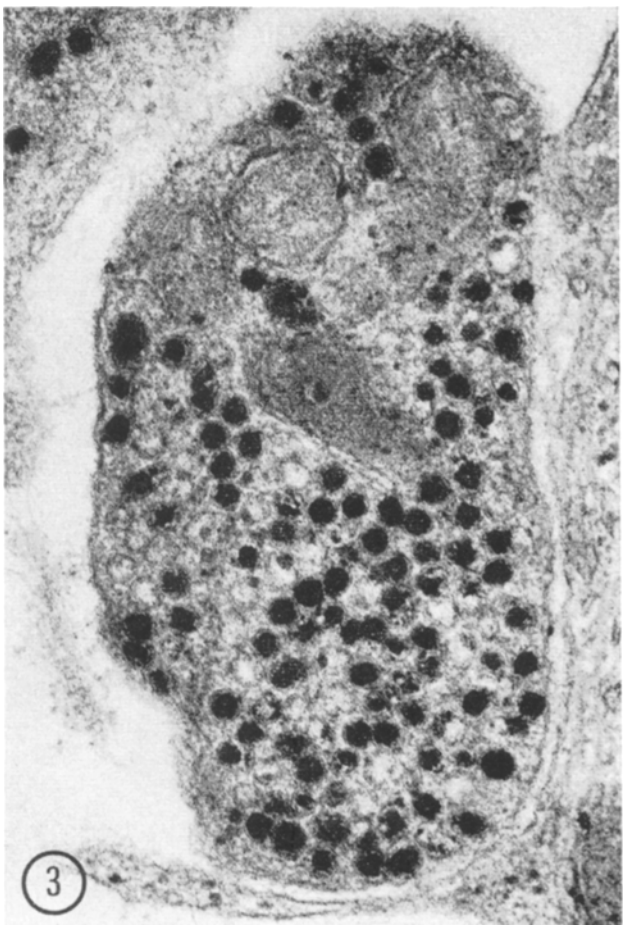

Fig. 3. Treatment with α-MMT followed by 5-HO-DA. Virtually all the vesicles are filled to capacity with a strongly osmiophilic material (the 'false' neurotransmitter 5-HO-DA). × 75,000.

nous amine localization with the endogenous amine storage site.

Since 5-HO-DA has proven to be a useful marker for aminergic nerves in the peripheral sympathetic system, efforts to visualize small granular vesicles as the amine storage sites in the central nervous system are now being undertaken.

Résumé. La 5-hydroxydopamine (5-HO-DA), un «faux» neuro-transmetteur adrénergique, a été localisée dans les vésicules des terminaisons nerveuses sympathiques de la glande pinéale du rat, par examen au microscope électronique, après traitement des animaux par cette amine.

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