Ultrastructural changes in the nerve endings of the median eminence after nialamide-DOPA administration

With the histochemical fluorescence technique for the demonstration of catecholamines a strong accumulation of primary monoamines has been found in the external zone of mammalian median eminence[2,3]. Electron microscopic studies of this zone in different species had revealed the presence of numerous nerve terminals surrounding the capillary loops of the hypophyseal portal plexus. These terminals contained a mixed population of clear vesicles of 400–700 Å and granulated vesicles whose diameter ranged from 800 to 1300 Å. Based on the clear correspondence between catecholamine content and presence of granulated vesicles demonstrated in other territories, some authors, who had previously considered the granulated vesicles of these terminals as representing releasing factors or neurosecretory products, interpreted them as carriers of catecholamines (for literature see refs. 8, 10, 11, 16, 18). More recently, this hypothesis received experimental support because together with a decrease in the monoamine content of the median eminence induced by reserpine administration, as revealed by the histochemical reaction, the number of granulated vesicles present in them diminished significantly[17,18]. However, other authors have failed to observe such changes after a similar experimental approach[10,11].

The administration of the precursor amino acid of catecholamine synthesis, L-dihydroxyphenylalanine, to animals pretreated with a monoamine oxidase inhibitor results in a remarkable enhancement of the intensity of the specific fluorescence in the external zone of the median eminence[4]. In order to investigate further the nature of the granulated vesicles present in these nerve endings we studied their fine structural appearance following a similarly induced increase in their monoamine content.

The material consisted of 15 male adult rats of the Wistar strain, 5 animals serving as controls. The other 10 rats were injected with 400 mg/kg nialamide (Niamid, Chas. Pfizer and Co.) intraperitoneally 5–6 h prior to sacrifice and 30 min before sacrificing with 100 mg/kg L-β-3,4-dihydroxyphenylalanine (L-DOPA, Sigma Chemical Co.) subcutaneously. The animals were sacrificed by decapitation, their brains quickly exposed and thin slices comprising the median eminence fixed with 3% glutaraldehyde and postfixed with 1.5% osmium tetroxide. Blocks stained with aqueous uranyl acetate were subsequently dehydrated in ethanol and embedded in Epon 812. Light microscopic observations of toluidine blue stained sections of 1–3 μ in thickness were made to localize the zone under study. Thin sections stained with lead citrate were observed in a Zeiss EM 9-A electron microscope. The photographs of the basal median eminence were obtained from an area extending no more than 2–3 μ from perivascular space. This zone was charted planimetrically and vesicles were counted in about 400 sq. μ in photographs taken at random from control as well as from treated animals. The total count in each photograph was expressed as number of granulated vesicles per surface unit. The statistical analysis of these data was performed using Student's t test for significance of differences between mean values. Confidence limits for $P = 0.05$ were also obtained.

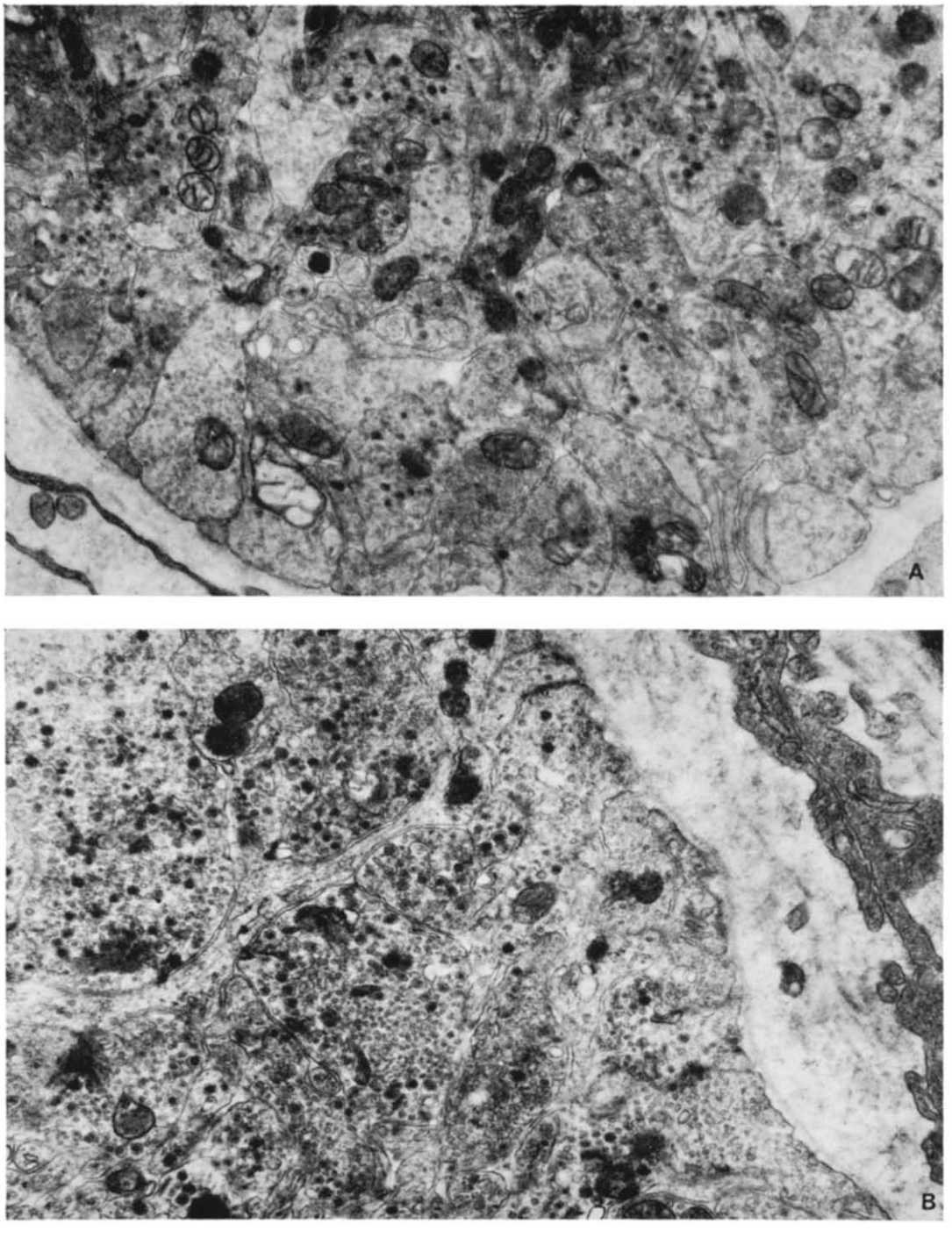

Fig. 1. Perivascular nerve endings in the external zone of the median eminence of the rat. A, General aspect of the zone in the normal animal. B, After nialamide–L-DOPA treatment an increase in the content of granulated vesicles in these endings is clearly noticed. Primary magnification: 25,000 ×.

Numerous axon terminal swellings having clear and granulated vesicles were found around the perivascular space of the median eminence (Fig. 1A), their structural characteristics corresponding to those previously described by several authors[8,10,11,16,18]. The overall denser aspect of the nerve endings after nialamide–L-DOPA treatment (Fig. 1B) was due to an apparent increase in their vesicular content. Granulated vesicles of 800–1200 Å in diameter, increased in number, exhibited a more irregular contour as well as a wider variance of the density of their cores than in the control group. Numerous profiles of dilated tubular structures were also seen in these endings.

TABLE I

GRANULATED VESICLES IN NERVE TERMINALS OF THE PERIVASCULAR ZONE OF THE MEDIAN EMINENCE IN NORMAL AND TREATED RATS*

	Normal	*Treated*
Number of vesicles per 10 sq. μ (mean values $\pm$ SEM)	77.8 $\pm$ 0.632	116.6 $\pm$ 0.690
Difference between mean values	38.8**	
Confidence limits for $P = 0.05$	90.8 — 64.8	130.4 — 102.8

* Animals receiving 400 mg/kg nialamide i.p. for 5–6 h and L-β-3,4-dihydroxyphenylalanine 100 mg/kg s.c. for 30 min. Time before sacrifice is given.
** $P < 0.001$ (Student's t test).

Results of vesicular counts in normal and treated animals are reported in Table I. After nialamide–DOPA treatment the amount of granulated vesicles increased about 49% with respect to control animals. The difference between the mean values is highly significant ($P < 0.001$) as is also indicated by confidence limits. The increase of clear vesicles, although noticeable in absolute values, is below significance thresholds. Some of these vesicles may represent remnants of granulated vesicles.

Although the correspondence between catecholamines and granulated vesicles is firmly established in the adrenal medulla as well as in peripheral sympathetic endings (see *e.g.* refs. 12, 15), the submicroscopic localization of catecholamines in central nervous system is still a matter of controversy[6]. The granulated vesicles observed in median eminence endings correspond in size to the intermediate type of Pellegrino de Iraldi and De Robertis[12]. This type of vesicle was formerly described in the anterior hypothalamus of the rat and interpreted as the possible site of storage of norepinephrine[13]. Further evidence provided by cell fractionation, pharmacological, histochemical and radioautographic methods gave support to this hypothesis (see ref. 14). Similar vesicles have been described in monoamine-containing central neurons and also interpreted as containing catecholamines[1,14]. Recently we have observed granulated vesicles in nerve cell bodies of the arcuate nucleus[7]. These vesicles are therefore characteristic along the catecholaminergic tubero-infundibular system because fibers ending in the external zone of the median eminence originate at least in part in that hypothalamic nucleus[5,9].

The increased amount of granulated vesicles observed in nerve endings of the external layer of the median eminence following an increase in their catecholamine content strongly suggest that these vesicles are involved in the storage or metabolism of monoamines. However, the possibility that these amines may be associated, at least in part, with a morphologically distinct storage site cannot be ruled out. It is also impossible to exclude an increase in granulated vesicles due to an indirect mechanism. Furthermore, the identification of vesicles containing different catecholamines cannot be made with the available techniques. Combined pharmacological and ultrastructural studies will undoubtedly contribute to the clarification of this aspect, but the approach used in this study, resulting in an increase of both norepinephrine and dopamine, does not allow us to state the accurate nature of the vesicles. Further work is also needed to throw some light on the physiological role of catecholamines in this system.

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