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CHANGES IN LARGE DENSE-CORE VESICLES IN SYMPATHETIC NERVE ENDINGS CAUSED BY CERTAIN DRUGS

P. A. Motavkin, V. M. Chertok, and G. G. Bozhko

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An electron-microscopic investigation of the large dense-core vesicles of adrenergic nerve endings in the middle cerebral arteries of vertebrates showed that 60-70 min after injection of iproniazid (3 mg/kg) and dopamine (40 mg/kg) significant changes (compared with the control) were observed in the vesicles, the number of which was increased statistically significantly. The participation of the large dense-core vesicles in the accumulation and conversion of noradrenalin and (or) its precursors is postulated.
KEY WORDS: cerebral vessels; innervation; iproniazid and dopamine.

Numerous investigations have shown that the terminal expansions of the sympathetic nerve fibers contain not only small osmiophilic vesicles, but also large dense-core vesicles (DCV), about 80-110 nm in diameter. The view is held [4, 10] that DCV are formed in the perikaryon of adrenergic neurons, from which they are transported along the axon to the nerve ending. These vesicles can take up noradrenalin or other monoamines [1, 5] and not infrequently they are transformed in the axon terminals of sympathetic nerves into small synaptic vesicles [3, 7] or they are extruded from the nerve ending by a process of exocytosis [10, 11]. Studies of the effect of certain drugs capable of modifying the catecholamine reserves in the tissue depots (6-hydroxydopamine, reserpine, protriptyline) have shown [2, 6, 8, 9] that a few hours after administration of the drugs many modified DCV appear in the adrenergic terminal expansions of the axons and the number of these vesicles increases. This phenomenon has been interpreted as a compensatory reaction to a deficiency of catecholamines in the axon terminals. The appearance of modified vesicles, moreover, has been interpreted either as the result of the direct effect of the drug on the DCV [12] or an an indication of increased activity of the DCV as a result of conversions of noradrenalin in the mobile catecholamine depots [9].

The object of this investigation was to study the responses of DCV in the terminal expansions of sympathetic axons to drugs promoting the accumulation of biogenic amines in the tissue depots.

EXPERIMENTAL METHOD

The middle cerebral arteries of 10 hens and 12 cats were investigated. Five animals from each group were used as controls and the rest received intraperitoneal injections of iproniazid (3 mg/kg), followed 6 h later by dopamine (40 mg/kg). The aminals were killed 60-70 min after the last injection of the drugs. Pieces of the vessels were fixed in 2.5% glutaraldehyde and then stained with osmium in Millonig's mixture, dehydrated in alcohols of increasing concentration, and embedded in Epon 812. The sections were examined in the electron microscope. The results were assessed on the basis of changes in the large vesicles in 100 cross sections of adrenergic axon terminals of the control animals and 125 expansions of axons obtained from experimental tissue samples. The large dense-core vesicles were measured in two mutually perpendicular directions.

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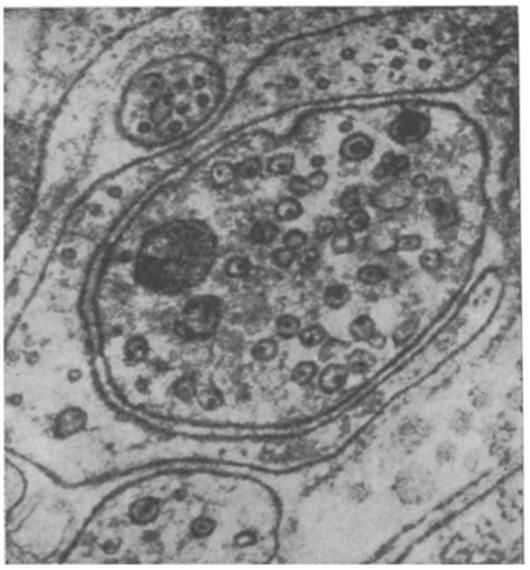

Fig. 1

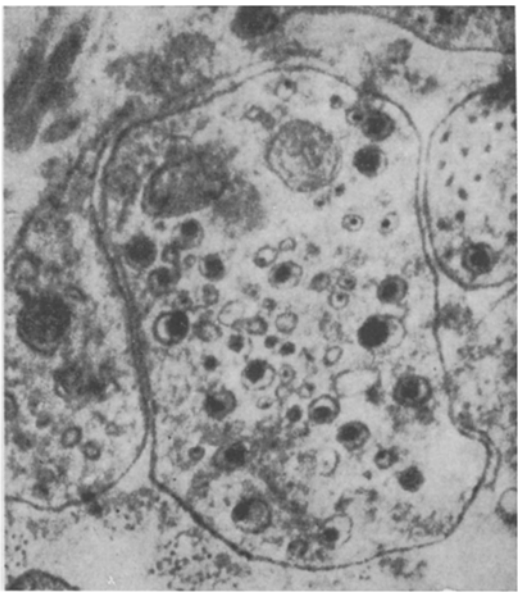

Fig. 2

Fig. 1. Axons with adrenergic vesicles (control; 58,000×).

Fig. 2. Axons with adrenergic vesicles after injection of drugs (46,000×).

EXPERIMENTAL RESULTS

The structural organization of the axon terminals and the distributions of DCV in them were virtually indistinguishable in the two species of animals. A typical cross section of an axon, characteristic of the terminal expansion of adrenergic nerve fibers, is illustrated in Fig. 1. It contains one to three mitochondria, many small vesicles, and substantially fewer DCV.

The DCV in the control sections were spherical formations measuring about 86 ± 11.5 nm in diameter, with a central osmiophilic granule. The contents of the granules are finely dispersed. Between the central substance and the border of the vesicle there was a clearly defined translucent rim. The number of DCV in these axons was as a rule small, namely 1.54 ± 0.18.

If the electron-microscopic pictures of the axon terminals of the control animals are compared with those of the tissue samples obtained after injection of the drugs it can easily be seen that in the second case (Fig. 2) there was a significant increase ($P < 0.01$) in the number of DCV (14.75 ± 3.4). They also were changed in size, which in this case varied around 111.3 ± 9.8 nm, although some vesicles attained a size of 150 nm. These vesicles appeared swollen on the electron micrograph.

The osmiophilic contents of the DCV acquired high electron density and in most cases were displaced toward the border of the vesicles. As a result of the asymmetrical arrangement of the granules, the translucent perigranular rim became irregularly outlined and frequently was absent over some distance.

By the use of drugs promoting the accumulation of biogenic amines, a statistically significant change in the number and a change in the size and structure of the DCV are thus observed in axon terminals of the sympathetic nervous system in the arteries at the base of the vertebrate brain. This suggests that these vesicles participate in the processes of accumulation and conversion of noradrenalin and (or) its precursors.

A similar effect is observed in adrenergic nerves as a result of administration of drugs lowering the monoamine level [2, 6, 8]. The use of these substances, with their opposite action, may evidently be characterized initially by an identical type of response, which is manifested in particular as an increase in the content of biogenic amines in the DCV. Consequently, the results cannot explain, as some workers have done [12], the appearance of modified DCV as a result of the direct action of the drugs on them.

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DEMETHYLATION OF METHINDIONE

V. N. Kiseleva, A. P. Gilev,
V. Ya. Parinov, V. D. Shatts,
and S. K. Germane

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Demethylation of methindione was shown to take place in vivo in rats. The process involves the participation of the microsomal NADPH-dependent electron transport system. In experiments in vivo demethylation of methindione takes place rapidly and is accompanied by the partial loss of its anticonvulsant properties.
KEY WORDS: methindione; demethylation; inactivation; microsomes.

To understand the special pharmacological properties of the new anticonvulsant drug methindione (2-methylamino-2-ethylindanedione-1,3 hydrochloride) [1, 2], information on its metabolism is necessary.

The object of this investigation was to study one of the pathways of methindione metabolism, namely its demethylation, and to estimate the velocity of this process.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 150-230 g. The following preparations of methindione were used: 1) ^{14}C-labeled in the keto group, 2) ^{14}C-labeled in the second position of the indanedione ring, 3) ^{14}C-labeled in the methyl group, and 4) nonradioactive. The preparations were given by mouth. The urine from the experimental animals was collected for 28 h. The level of labeled products was measured with a scintillation counter. To compare the levels of excretion of labeled preparations 1 and 3 with the urine, they were injected in doses of equal radioactivity (14 μCi/kg). Fractionation and determination of methindione and its metabolites were carried out by paper (in a system of n-butanol : glacial acetic acid : water, 4 : 1 : 5), thin-layer (silica gel adsorbent; system of isopropanol : n-butanol : 25% ammonia solution : water, 5 : 10 : 0.3 : 2.5), and gas chromatography, using standards and analytical reagents. In the first case radioactive preparations were used (0.13-0.9 μCi/kg), in the rest unlabeled methindione (300 mg/kg). Methindione and its metabolites were isolated from the urine after its preliminary hydrolysis by β-glucuronidase [9]. The activity of the demethylation enzyme system in the postmitochondrial supernatant and in the microsomes of the liver was determined in vitro from the amount of formaldehyde formed [4]. The microsomal fraction was isolated by the method of Cinti et al. [5]. Protein was determined by Lowry's method [6]. To study the kinetics of the demethylation process the values of K_m and V_{max} were analyzed [3]. The binding of cytochrome P-450 with methindione was recorded on the Specord UV VIS spectrophotometer by the method of Schenkman et al. [7]. In

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