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EFFECT OF SYMPATHOMIMETIC AGENTS AND DOPA ON THE ULTRASTRUCTURE OF THE NERVOUS APPARATUS OF THE HEART

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UDC 612.178.014.46:[615.217.32+615.31:547.583.5

After a single injection of noradrenalin or dopa into albino rats noradrenalin was incorporated into adrenergic axons of the heart and deposited as granules in the small synaptic vesicles measuring about 30 nm in diameter. In this way adrenergic axons could be distinguished from cholinergic. Cholinergic axons were more numerous than adrenergic in the atria. Adrenergic terminals come into very intimate contact with cholinergic terminals and also with capillary endothelial cells and muscle cells of the myocardium. It is postulated that adrenergic fibers may act on heart muscle in three ways: by means of presynaptic inhibition through cholinergic axons, by a humoral mechanism, and directly on the muscle cells of the myocardium.

KEY WORDS: *Innervation of the heart; adrenergic axons; granular synaptic vesicles; noradrenalin synthesis.*

Exogenous noradrenalin is known to be incorporated into adrenergic fibers [7]. Tritiated noradrenalin has been shown to accumulate in unmyelinated axons of the heart containing small synaptic vesicles with a dense core [15]. Vesicles of this sort are seen very rarely in axons of different organs after osmium fixation [6, 9]. They increase in number after injection of labeled noradrenalin into animals [4]. Injection of 5-hydroxydopamine also causes the appearance of granular synaptic vesicles about 40 nm in diameter in the varicose expansions of adrenergic fibers of the heart [5]. What is not yet clear is where the adrenergic synaptic vesicles are formed. It has not been unanimously agreed in which synaptic vesicles noradrenalin is stored or whether it can be deposited as granules in all vesicles of the axon.

The object of this investigation was to study changes in the nervous apparatus of the heart under the influence of noradrenalin, its chemical precursor dopa, and isopropylnoradrenalin (isoproterenol).

EXPERIMENTAL METHOD

Noradrenalin was injected into the caudal vein of 13 male Wistar albino rats in doses of 0.001, 0.03, 0.3, and 0.5 mg/kg. Dopa [β-(3,4-dihydroxyphenyl)-DL-α-alanine; Reanal, Hungary] was injected intraperitoneally into five animals in doses of 20, 50, and 100 mg/kg. Isoproterenol (Izadrin, Spofa, Czechoslovakia) was injected subcutaneously into three animals in a dose of 100 mg/kg. The animals were decapitated 20 or 30 min after the injections. Two animals were given intraperitoneal injections of reserpine in a dose of 2.5 mg/kg 18 and again 2.5 h before the material was taken, and an injection of noradrenalin (0.3 mg/kg) 30 min later. Twelve animals served as the control. Pieces of the atria and of the atrial and ventricular septa were fixed in Caulfield's osmium fixative and embedded in Epon. The atria of four rats were treated by a cytochemical method for the detection of noradrenalin [13].

Laboratory of Cytology, Scientific-Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 11, pp. 1382-1385, November, 1976. Original article submitted April 5, 1976.

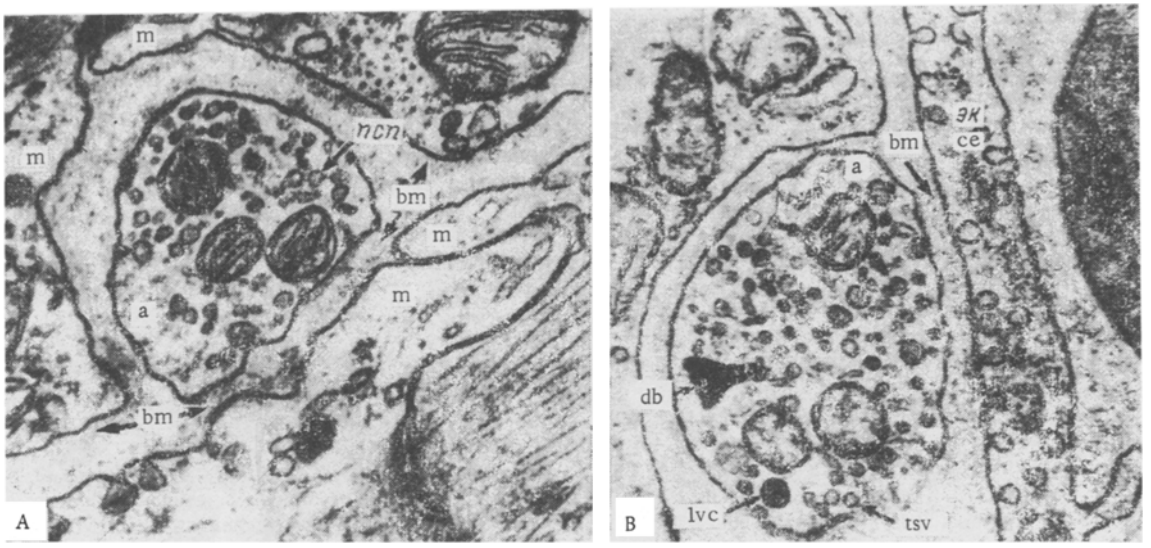

Fig. 1. Axons with small cynaptic vesicles in right atrium of intact rat: A) terminal expansion of axon by four myocardial muscle cells (75,000×); B) axon terminal containing vesicles with tiny granules near capillary endothelium (75,000×). Here and in Fig. 2: a) axon terminal, tsv) translucent synaptic vesicle; svc) small vesicle with dense core; lvc) large vesicle with dense core; db) dense body; ce) capillary endothelium; bm) basement membrane; Sc) Schwann cell; m) myocardial muscle cell.

The fragments were dehydrated and stained with uranyl acetate solution in alcohol. Ultrathin sections were stained with lead nitrate and examined in the Hitachi HU-11B electron microscope.

EXPERIMENTAL RESULTS

In the atrial myocardium of the control animals and also in the atrial and ventricular septa, axons 0.1-0.2 μ thick were found near capillaries and muscle fibers, where they lay either in grooves on the surface of the Schwann cells or freely in the intercellular spaces of the myocardium. Varicose thickenings of the axons were 0.3-1 μ in diameter. The axons contained protofibrils, microtubules, mitochondria, small translucent vesicles, single large vesicles with an electron-dense core, tubules and cisterns of the endoplasmic reticulum, long dense bodies bounded by a membrane, and, infrequently, multivesicular bodies. The thickenings of the axons differed in the fact that translucent synaptic vesicles and mitochondria were particularly numerous in them. Varicose thickenings of the axons lay at a distance of 30 nm or more from the capillary endothelium, the muscle fibers of the myocardium, and the muscle cells of the arterioles. When the terminal expansions of the axons were close to muscle, endothelial, and other cells covered by basement membranes, at a distance of 30-50 nm the basement membranes of the axon and cell fused into one (Fig. 1). Neuromuscular junctions with a single basement membrane in their synaptic space are regarded as the closest of all discovered in the myocardium [10]. In the narrow spaces between muscle fibers terminal thickenings of axons were found for the first time; they occurred at the level of the intercalated discs of two parallel neighboring muscle fibers, and they thus bounded four muscle cells simultaneously (Fig. 1A). Synaptic vesicles and very small dense granules were found in some axons (Fig. 1B).

As early as 20 min after injection of any of the above-mentioned doses of noradrenalin it was incorporated into the adrenergic fibers of the heart and deposited as electron-dense granules measuring 10-15 nm in small synaptic vesicles, mainly in the region of the varicose thickenings of the axons (Fig. 2A). The diameter of the vesicles was about 30 nm (25-40 nm) and the granule was usually situated in the center of the vesicle, less frequently against its membrane. Granules were found in not more than one third of the small synaptic vesicles, in agreement with observations made on other organs [7, 8, 11]. Signs of activation of the endoplasmic reticulum were observed after 30 min in some adrenergic axons: branching and budding of the tubules; budding and detachment of small vesicles sometimes containing dense granules.

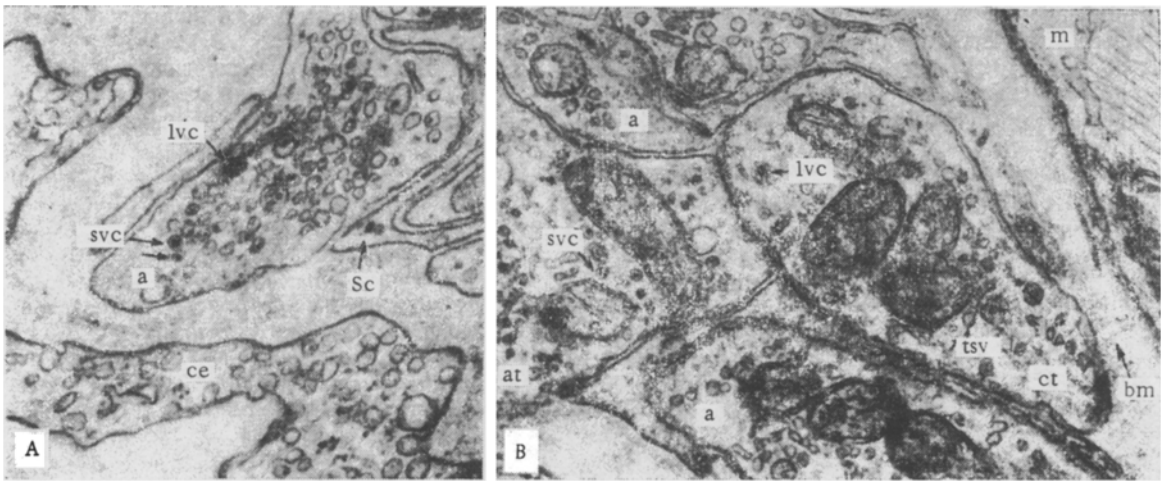

Fig. 2. Deposition of granules containing noradrenalin in small synaptic vesicles of adrenergic axons: A) adrenergic terminal near capillary 20 min after injection of 0.03 mg/kg noradrenalin (75,000×); B) contact of adrenergic terminal (at) with cholinergic terminal (ct) 20 min after injection of 100 mg/kg dopa (50,000×).

Small synaptic vesicles measuring 35-40 nm with a dense core (Fig. 2B) appeared 20 min after injection of dopa in all the doses indicated above in certain axons about 0.2 μ in diameter and, in particular, in their expansions to a diameter of 1 μ. On the surface of the bare adrenergic axons facing the capillary cells pinocytotic vesicles were found, evidence of activation of exchange between capillaries and axons.

After injection of noradrenalin or dopa into the animals there was a statistically significant increase in the proportion of axons containing small granular vesicles up to 21%, from a level of 6% in the control animals. This increase was statistically significant by the chi-square criterion (99.9%). The percentage of these axons increased to 21 (minimum) after injection of noradrenalin in a dose of 0.001 mg/kg and to 23 (maximum) after injection of noradrenalin in a dose of 0.5 mg/kg. Isoproterenol caused no changes in the axons. In animals receiving preliminary injections of reserpine no small granular vesicles were found in the axons.

The fact that the formation of adrenergic synaptic vesicles was observed as early as 20 min after the injection of noradrenalin or dopa confirms the view that small synaptic granular vesicles arise in nerve endings [12], where noradrenalin is synthesized [7]. The so-called large granular vesicles, which in these experiments had a diameter of 60-80 nm and which some workers regard as a feature of adrenergic endings [1, 2, 14], were found in both adrenergic and cholinergic axons. This is in agreement with experimental observations by a number of workers [5, 7].

The saturation techniques and the cytochemical method for detection of noradrenalin used in these experiments showed that there are about four times as many cholinergic axons as adrenergic in the atria. Both types of axons frequently lie together in grooves of the same Schwann cell. Terminal thickenings of adrenergic axons formed the closest contact with cholinergic axons, from which they were separated by a space about 15 nm wide (Fig. 2b). Terminals of both types of axons lie at a distance of 30 nm or more from capillary endothelial cells and myocardial muscle cells, and also close to smooth muscle cells of the arterioles. Near the surface membrane of the myocardial muscle cell terminal expansions of adrenergic and cholinergic axons were found. It is postulated that adrenergic fibers may interact in three ways with heart muscle: by presynaptic inhibition through cholinergic axons, by a humoral mechanism, and directly on the muscle cell.

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ELECTRON-MICROSCOPIC INVESTIGATION OF SATELLITE CELL FORMATION IN SKELETAL MUSCLE DURING PHYSICAL EXERTION

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UDC 612.741.014.2-086.3

The content of condensed chromatin is increased in nuclei of skeletal muscle fibers of rats after repeated physical exertion continued until exhaustion. Some muscle fiber nuclei, together with part of the sarcoplasm, were separated from the muscle fibers. Satellite cells formed from the separated parts of the muscle fibers.

KEY WORDS: *Physical exertion; muscle fibers; satellite cells.*

Much attention has recently been paid to the satellite cells of skeletal muscles. The main reason for this is that these cells are regarded as the principal sources of myoblasts [4, 8, 10]. The role of trophic cells has also been ascribed to them [3].

Satellite cells have been investigated in different phases of ontogeny and under experimental and clinical pathological conditions. Electron-microscopic studies have shown that satellite cells are formed during disturbance of the circulation or cooling of a muscle [9] and after traumatic injury or denervation of muscle [1, 2, 5, 11].

No description of the formation of these cells during physical exertion could be found in the accessible literature, and it was accordingly decided to investigate this problem.

EXPERIMENTAL METHOD

Male Wistar albino rats weighing 200-220 g were used as experimental animals. The physical exertion consisted of swimming in water at a temperature of 30°C carrying a load equivalent to 2-3% of the body weight. The interval between the first and second physical exertions was 24 h, so that the second took place in the phase of increased working capacity.

Pieces of the gracilis muscle were taken during the first and second periods of exertion at a time when the animals were in a state of extreme fatigue, and also 24 and 48 h after the first and second exertions. The material was fixed in 4% formaldehyde solution (prepared from paraformaldehyde) in phosphate buffer at pH 7.4 with the addition of 5% su-

Laboratory of Functional Morphology, All-Union Scientific-Research Institute of Physical Culture, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 11, pp. 1385-1388, November, 1976. Original article submitted April 2, 1976.