DEPENDENCE OF NUMBER OF VESICLES OF VASCULAR SMOOTH-MUSCLE CELLS ON EXTRACELLULAR NORADRENALIN

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UDC 612.731:612.734.16:546.41].014.46:577.175.523]-085.23

KEY WORDS: vesicles of smooth-muscle cells; calcium transport; noradrenalin

Contraction and relaxation of smooth-muscle cells (SMC) are controlled by changes in the intracellular calcium concentration. The latter depends on the entry of calcium into the cell, its release from organelles, and the rate of its elimination from the cell. The mechanisms controlling these processes have not been fully studied, nor do we know the concrete structural elements of the plasma membrane (PM) in which calcium transport is localized. The writers showed previously that an increase in the rate of calcium elimination from vascular SMC is accompanied by an increase in the number of paraplasmalemmal vesicles – specialized regions of PM which probably are responsible for the transfer of calcium ions from the cell into the extracellular space [2] – in these cells.

This paper describes a study of the number of vesicles of arterial SMC during incubation with exogenous noradrenalin (NA), which is known to induce physiological contraction of vascular smooth muscles. Besides normotensive animals, spontaneously hypertensive rats (SHR), in which the mechanisms regulating the intracellular calcium concentration are disturbed [3-5], also were used in the experiments.

EXPERIMENTAL METHOD

Experiments were carried out on inbred male spontaneously hypertensive Kyoto-Wistar rats (SHR) aged 8 and 12 weeks, weighing 180-230 g, and with a blood pressure (BP) of 140-200 mm Hg. Inbred male normotensive Kyoto-Wistar (WKY) rats of the same age, with BP of 80-120 mm Hg, served as the control.

The material was collected under ether anesthesia. Pieces of small mesenteric arteries with an external diameter of 150-200 μ, taken from each animal, and from which the adventitia was removed, were incubated for 10 min at 37°C and shaken with two series of physiological saline, one of which did not contain NA, whereas the other contained NA in a concentration of 10^{-5} M. The composition of the incubation medium (in mM) was: NaCl 140, KCl 4.5, $MgCl_2$ 1, $CaCl_2$ 1.5, HEPES (N-2-hydroxyethylpiperazine-N-2-ethinolsulfonic acid) 5, glucose 5 (pH 7.4). The duration of incubation corresponded to the time required for maximal stimulation of ^{45}Ca release from strips of aorta under the influence of NA [7].

The subsequent processing of the tissue for electron microscopy and counting the number of vesicles was the same as that described previously [2]. No fewer than 30 cells were counted for each animal. The significance of differences between mean values was estimated by Student's t test at the $P < 0.05$ level. Some of the material was used for electron-histochemical demonstration of ATPase activity by the lead method [1] and of calcium deposition by a modified method [10].

EXPERIMENTAL RESULTS

Data on the number of vesicles in arterial SMC depending on the presence or absence of NA in the incubation medium are given in Table 1. The results of counting showed that after incubation in physiological saline before addition of NA (initial state) SMC of SHR aged 8 weeks have fewer vesicles than SMC of the control (WKY) animals, although the differ-

Fourth Main Board, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 10, pp. 479-482, October, 1986. Original article submitted October 2, 1985.

TABLE 1. Number of Vesicles in SMC of Rat Mesenteric Arteries Depending on Presence of NA in Incubation Medium (per micron of PM, M ± m)

Age of rats, weeks	Strain of rats			
	WKY		SHR	
	1	2	3	4
8	3,61±0,125 (3) $P_{1-3}>0,05$	3,41±0,102 (3) $P_{1-2}>0,05$	3,33±0,106 (3) $P_{3-4}<0,001$	3,89±0,125 (3)
12	3,51±0,079 (4) $P_{1-3}<0,001$	3,34±0,095 (4) $P_{1-2}>0,05$	3,05±0,079 (4) $P_{3-4}<0,001$	3,49±0,089 (4)

Legend. 1 and 3) Incubation without NA, 2 and 4) incubation with NA. Number of animals given in parentheses.

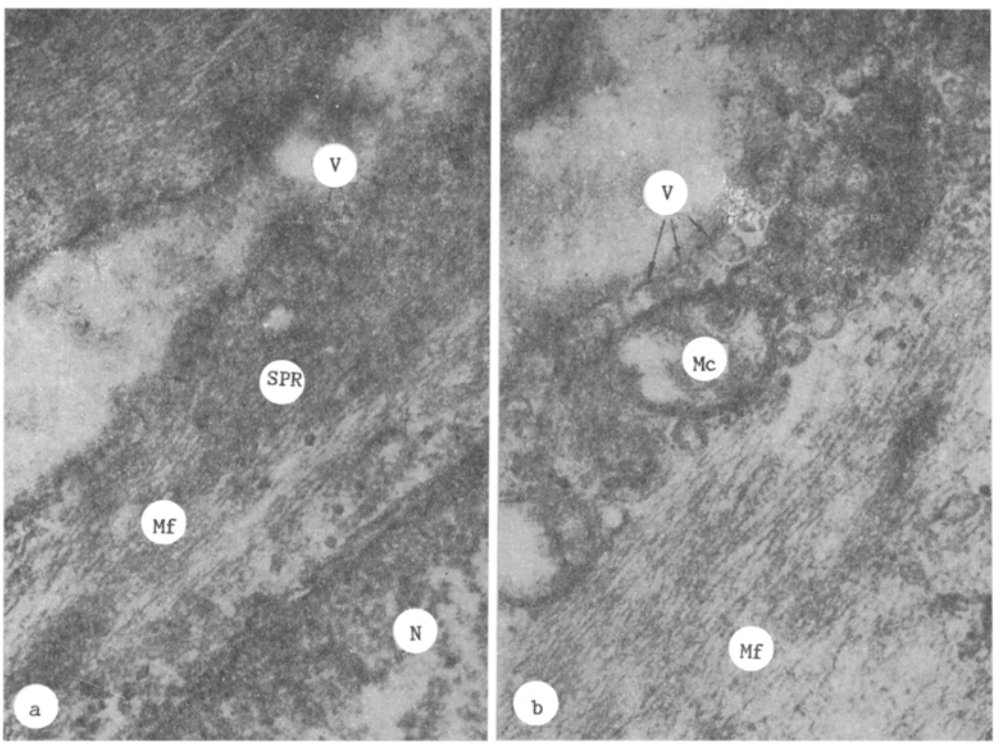

Fig. 1. Fragment of SMC of small mesenteric artery of rat. Example of contact of vesicles with subsarcolemmal elements of SPR (a) and mitochondria (b). V) Vesicles; Mc) mitochondria; Mf) myofilaments; N) nucleus. 74,000×.

ences are not significant. With age the number of vesicles in SMC of WKY rats remained unchanged, whereas their number in SHR decreased with an increase in the duration of hypertension ($P < 0.05$). The result was that SMC in the initial state in SHR aged 12 weeks contain significantly fewer vesicles than in animals with normal BP. The tendency for the number of vesicles in SMC or SHR to fall compared with the control was observed by the writers previously [2]. The stability of appearance of this phenomenon, irrespective of the experimental conditions, is evidence of the insufficiency of PM of vascular SMC of hypertensive animals, expressed as a deficiency of the vasicular apparatus.

Incubation in medium containing NA did not change the number of vesicles in SMC of the normotensive animals, irrespective of age. The number of vesicles in the vascular SMC of SHR after incubation with NA was increased in both age groups. After the action of NA on vascular SMC the intracellular free calcium concentration increased due to its release

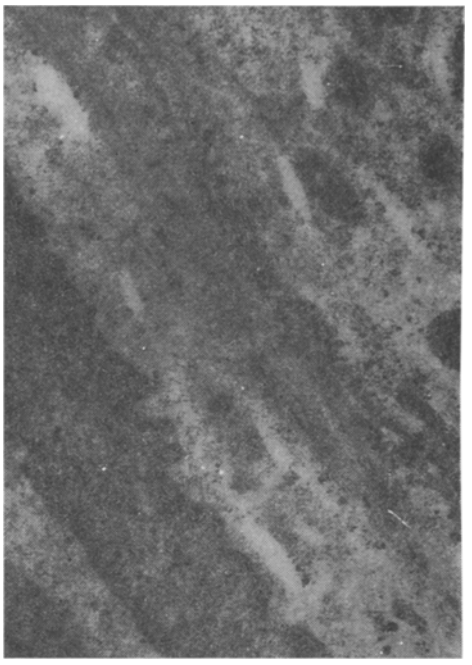

Fig. 2. Deposition of calcium pyroantimonate in SMC of rat artery (22,000×).

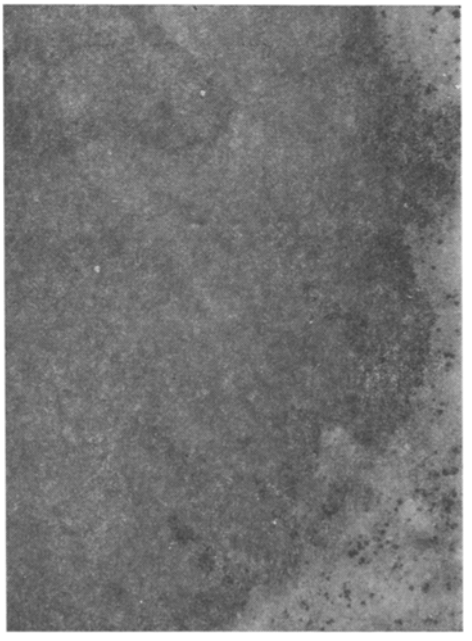

Fig. 3. ATPase activity in vesicles of arterial SMC (18,000×).

from the intracellular space [6, 7, 11]. For the normal calcium distribution between the cell and extracellular medium to be achieved, calcium entering the cell from outside must be expelled through activation of the systems eliminating this ion. The topography of the molecular mechanisms of calcium discharge on PM of vascular SMC is not yet clear. The most likely areas of PM of the vascular SMC through which calcium may be eliminated are the vesicles which are invaginations of PM. This suggestion is supported by several facts.

First, for calcium to be transferred inside SMC any structural specialization of PM can hardly be necessary, for it can penetrate into the cell along the concentration gradient, which is very high (1:10,000). To prevent excessive accumulation of calcium in the cell,

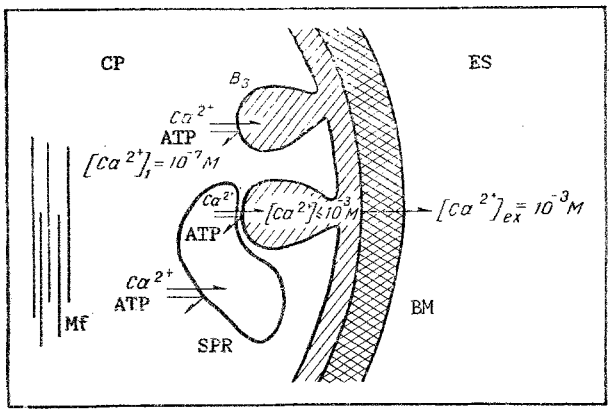

Fig. 4. Scheme of mechanism of Ca^{++} elimination from SMC by means of vesicles. CP) Cytoplasm; ES) extracellular space; BM) basement membrane; Mf) myofilaments.

however, it must be constantly pumped out. Second, vesicles have numerous junctions with the sarcoplasmic reticulum (SPR) and mitochondria (Fig. 1a, b), whose role in intracellular calcium accumulation is not disputed [8, 12]. Third, deposits of calcium pyroantimonate (Fig. 2) were seen on the membrane of the vesicles and inside their lumen, evidence of the ability of these structures to store calcium. Fourth, nonspecific ATPase activity was found in the vesicles (Fig. 3); the smooth areas of PM either possessed no ATPase activity or it was appreciably reduced. It is logical to suggest that the reaction obtained can be attributed at least partially to activity of specific Ca-ATPase – one system of active calcium expulsion (Na-Ca exchange may act as a second system).

The following mechanism of calcium elimination from SMC with the aid of vesicles can be postulated (Fig. 4). Vesicles constitute a unique kind of sluice which facilitates the outflow of calcium from the cell against a high concentration gradient. The internal space of the vesicles can be regarded as a specialized compartment of the extracellular space on the cell surface, whose ionic composition is largely under the control of the cell itself. The material filling the cavity of the SMC vesicles can bind cations, for it possesses polyanionic properties, which are revealed on staining with ruthenium red [2]. The lumen of the vesicles on the cell side is bounded by PM, and on the side of the extracellular space by a basement membrane; for that reason the calcium concentration in it may be intermediate in value between the concentrations of these ions inside and outside the cell. Calcium initially is pumped form the cytoplasm into the lumen of SPR by the work of the Ca pump, the power of which here is high. However, because of the small volume of SPR in the vascular SMC [8] its ability to accumulate calcium ions is limited and for that reason some of the calcium must be expelled from the lumen of SPR into the extracellular space. The latter may perhaps also take place at sites of contact between vesicles and SPR on account of the working of the Ca pump of the vesicles. Vesicles not forming junctions with SPR evidently pump calcium directly out of the cytoplasm. If the calcium concentration in the lumen of the vesicles is lower than its concentration in the greater part of the extracellular space, expulsion of calcium from the cell with the aid of vesicles will require much less expenditure of energy.

In SHR the PM of the aortic SMC is less able to bind calcium than that of normotensive rats, while the calcium-accumulating capacity of the SPR is unchanged [3]. It may be that the smaller number of vesicles in vascular SMC or SHR than of normotensive rats is the structural basis of this disturbance.

The absence of any change in the number of vesicles in WKY rats in response to NA is evidently connected with balance between the processes of calcium release and binding during physiological contraction. Similar data have been obtained for contraction of other, nonvascular, SMC, such as the SMC of taenia coli [9]. In this connection it can be postulated that the increase in the number of vesicles in hypertensive rats in response to incubation with NA is a measure of compensation of the membrane defect characteristic of the primary hypertensions.

Morphological and functional characteristics of vesicles of vascular SMC thus enable them to be regarded as structures facilitating the elimination of calcium ions from the cell into the extracellular space against a high concentration gradient and damage to this apparatus in pathological disturbances of calcium metabolism.

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HEPATOCYTE ULTRASTRUCTURE IN MICE WITH CHRONIC T2 MYCOTOXICOSIS

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UDC 615.918:582.282].015.44:616.36-018.1-076.4

KEY WORDS: trichothecene mycotoxins; T2 toxin; hepatocyte ultrastructure.

T2 toxin is one of the commonest natural contaminants of food products belonging to the trichothecene mycotoxin group [7, 11]. Unlike most mycotoxins, the toxins of this group possess a wide range of toxic action, affecting the central nervous system, immunocompetent and hematopoietic organs, cardiovascular system, and gastrointestinal tract [7, 9, 10]. The principal organ in which most xenobiotics, including T2 toxin, undergo biotransformation and detoxication, is the liver [7]. The writers showed previously that administration of sublethal doses of T2 toxin to rats causes marked destruction of all intracellular organelles of the hepatocytes within 1-3 h, accompanied by disorganization of the liver enzymes [2].

The aim of this investigation was to study the effect of long-term administration of very small doses of T2 toxin on hepatocyte ultrastructure in mice.

EXPERIMENTAL METHOD

Experiments were carried out on male hybrid CBA × C57B1/6 mice receiving the normal balanced animal house diet and water ad lib. Animals of the experimental group received T2 toxin by gastric tube [8] in a dose of 0.33 mg/kg body weight (equivalent to 0.05 LD_{50}) for 1 month and in a dose of 0.45 mg/kg (0.067 LD_{50}) for the next 5 months. Mice of the control group received an equal volume of the solvent, namely 1% aqueous solution of ethanol. The animals were decapitated 24 h after the last injection. The liver tissue for electron microscopy was fixed in 4% paraformaldehyde solution in Hanks' buffer (pH 7.3) for 3 h. After washing to remove the fixative with buffer solution, the preparations were postfixed for 12 h with 1% OsO_4 solution in the same Hanks' buffer for 3 h at 4°C. The fixed prepara-

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(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 10, pp. 482-485, October, 1986. Original article submitted February 14, 1986.