DEPENDENCE OF NUMBER OF VESICLES IN VASCULAR SMOOTH MUSCLE CELLS ON EXTRACELLULAR CALCIUM CONCENTRATION

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Calcium is the principal ion which regulates the contractile function of smooth muscle cells (SMC). Its concentration in SMC depends on the rates of its inflow and outflow, the level of binding of the ion by the inner surface of the plasma membrane (PM), accumulation by membranes of SMC organelles, and the degree of binding by chelating agents of the free cytoplasm.

The total intracellular calcium concentration in SMC is about one order of magnitude lower than the extracellular concentration [7]. This nonequilibrium distribution of calcium between SMC and the extracellular medium is evidently maintained by molecular mechanisms, which actively pump out this cation. By analogy with other types of cells, it is considered that these mechanisms are Na–Ca exchange and, in particular, activity of the ATP-dependent Ca pump [8]. The exact localization and distribution of these systems around the perimeter of PM are not clear. It has been suggested that the areas of PM through which calcium is expelled may be vesicles of SMC, which are invaginations of PM [9, 13, 14]. However, no definite proof that SMC vesicles are related to calcium transport has been obtained.

For the reasons given above, it was decided to use morphometric methods to study dependence of the number of vesicles of arterial SMC on the extracellular calcium concentration. Besides intact animals, rats with genetically determined spontaneous hypertension, in which the Ca-binding capacity of the tissue cell membranes of the internal medium is reduced [3, 5, 6], also were used in the experiments.

EXPERIMENTAL METHOD

Inbred SHR (spontaneously hypertensive rats, Kyoto–Wistar strain) rats aged 8 weeks, weighing 120-140 g, with blood pressure 150-180 mm Hg, were used. Inbred male normotensive Kyoto–Wistar rats of the same age, kept under identical conditions (blood pressure 80-120 mm Hg) served as the control. Each group consisted of six animals. Half of all the rats underwent bilateral adrenalectomy 1 week before the experiment, and instead of drinking water these animals were given 1% NaCl solution. Material was taken under superficial ether anesthesia.

Pieces of small mesenteric arteries from each animal, freed from adventitia, were incubated for 60 min at 37°C with shaking in three version of saline buffer. The composition of the incubation medium, at pH 7.4 (in mM) was as follows: 1) NaCl 140, KCl 5, $MgCl_2$ 2, HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanolsulfonic acid) 5, $CaCl_2$ 1.8, glucose 10; 2) NaCl 6.5, KCl 5, $MgCl_2$ 2, HEPES 5, $CaCl_2$ 50, glucose 10; 3) NaCl 140, KCl 5, $MgCl_2$ 2, HEPES 5, $CaCl_2$ 0.05, glucose 10, EGTA 1 (concentration of free ionized calcium 0.04 μM). The osmolarity of all solutions, verified by means of a semimicro-osmometer (from Knauer, West Germany), was 270-280 milliosmoles/liter. The values of low and high calcium concentrations were chosen so as to cause the maximal shift to the calcium inflow into and outflow from the cell and to bring about changes in the number of vesicles which could escape detection in the usual physiological concentrations of this ion.

During processing of the tissue for electron microscopy, Luft's method of staining with ruthenium red [11] was used to ensure better identification of the vesicles. The material was embedded in a mixture of Epon and Araldite, and unstained sections were examined in the Hita-

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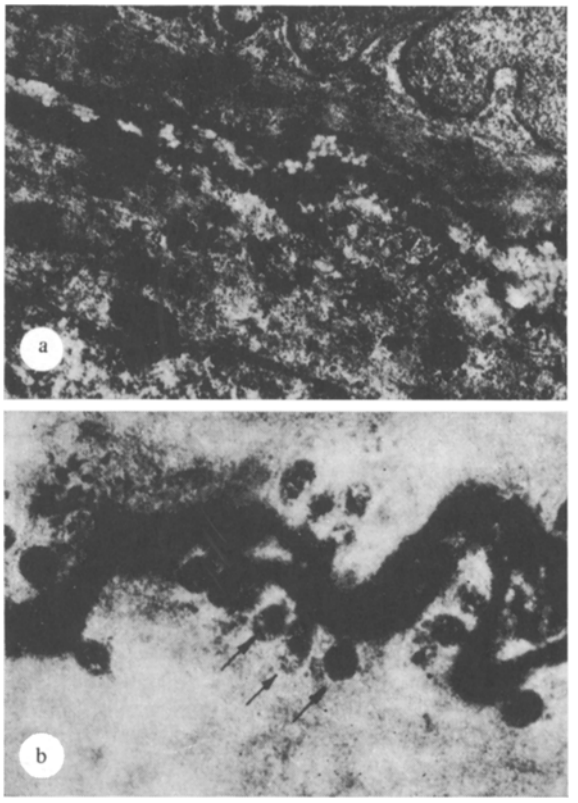

Fig. 1

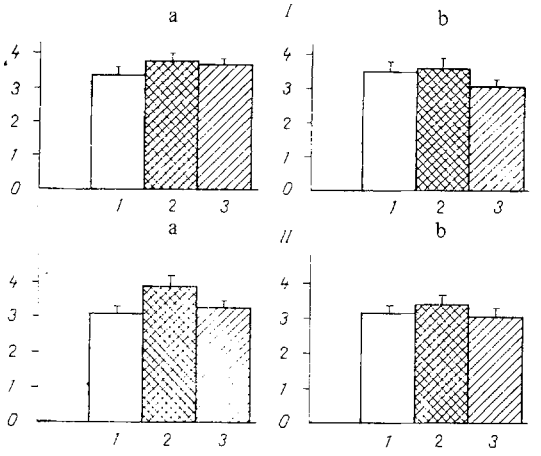

Fig. 2

Fig. 1. Vesicles in SMC of rat mesenteric arteries. Ruthenium red. Magnification: a) 22,000, b) 60,000 ×.

Fig. 2. Number of vesicles in SMC of rat mesenteric arteries during changes in calcium concentration in incubation medium. Abscissa, calcium concentration in incubation medium: 1) 1.8 mM, 2) 50 mM, 3) 0.04 μM; ordinate, number of vesicles/μ length of PM of SMC. I) Normotensive, II) spontaneously hypertensive rats; a) before, b) after adrenalectomy.

chi 11 ES electron microscope. The number of vesicles in SMC in which the section passed through the nucleus was counted on the microscope screen under magnification of 20,000, and divided by the length of PM measured on photographs under the same final magnification.

EXPERIMENTAL RESULTS

The results of counting the number of vesicles in SMC (Fig. 1), depending on changes in the calcium concentration in the incubation medium, are given in Fig. 2. The data showed that with a normal calcium concentration in the incubation solution (1.8 mM) a tendency was observed (not significant) for the number of vesicles in SMC of the spontaneously hypertensive rats to decrease compared with the normotensive control ($P > 0.05$). The same relationship between the number of vesicles in animals of the control and experimental groups was maintained after adrenalectomy, although the operation itself leads to a very small (likewise not statistically significant) increase in this parameter in both cases.

With an increase in the calcium concentration in the incubation medium to 50 mM the number of vesicles in SMC of the vessels rose significantly in both control and experimental groups ($P < 0.05$ and < 0.001, respectively), and this increase was significantly greater in SHR. After adrenalectomy the same high calcium concentration in the incubation medium caused no appreciable increase in the number of vesicles either in the experiment or in the control ($P > 0.05$). A reduction in the calcium concentration in the incubation medium to 0.04 μM caused virtually no change in the number of vesicles in the vascular SMC in animals of both groups ($P > 0.05$). After adrenalectomy the same low extracellular calcium concentration was accompanied by a decrease in the number of vesicles in normotensive animals ($P < 0.01$), whereas in rats with a high blood pressure this parameter was unchanged ($P > 0.05$).

Although the extracellular calcium concentration changed very sharply, the absolute and relative fluctuations in the number of vesicles were on the whole small. This was due largely to the particular features of the morphometric method used, for the number of vesicles was counted by unit length of PM (1 μ). When calculated for the whole surface of SMC *in vitro*, and, in particular, during hypertrophy of the vascular SMC such as is observed during hypertension, these differences in absolute terms would undoubtedly be greater.

The use of radioisotope exchange showed that an increase in the calcium concentration in the incubation medium increases the content of exchangeable calcium in the cells [2] and ought to activate the mechanisms of expulsion of this cation, thus maintaining the normal distribution of calcium between the cell and extracellular medium. The increase in the number of SMC vesicles in response to an increase in the extracellular calcium concentration is evidently the morphological expression of these processes. The free calcium concentration in vascular SMC is largely determined by the Ca-accumulating capacity of PM, which is appreciably reduced in spontaneously hypertensive animals [3]. In that case the greater increase in the number of vesicles observed in SHR in response to an increase in the extracellular calcium concentration can serve as a measure of compensation of the membrane defect observed in spontaneous hypertension.

The small increase in the number of vesicles in SMC of adrenalectomized animals in the presence of a normal extracellular calcium concentration is evidently the result of the operation, which itself increases the intracellular calcium concentration [4]. It is an interesting fact that an excessive calcium concentration in the medium causes a substantially smaller increase in the number of vesicles in adrenalectomized than in intact animals (Fig. 2). This is probably connected with blocking by adrenalectomy of the protein-synthesizing function of the cell, which is responsible for construction of the vesicular regions of PM, since this operation completely removes the controlling influence of glucocorticoids on intracellular protein biosynthesis.

The osmotic concentration of all incubation media used in the present investigation, incidentally, lay within physiological limits, and this factor could not have influenced the number of vesicles [1, 12].

Considering the relationship between SMC vesicles and PM, and their connection with elements of the sarcoplasmic reticulum and mitochondria, which are actively concerned in the intracellular distribution of calcium [9, 14], and also the presence of a Ca-transporting ATPase in them [10, 13], it can be postulated that vesicles are regions of PM that are specialized for transport of Ca^{++} ions. In our opinion statistically significant changes in the number of SMC vesicles in the presence of different extracellular calcium concentrations are one piece of evidence in support of the participation of these organelles in calcium transport through PM. The increase in the number of vesicles in response to a sharp increase in the calcium concentration in the medium is possibly one way of increasing the number of active sites in PM responsible for calcium elimination against the concentration gradient, which is particularly prominent under pathological conditions.

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