Calcium Content of Arteriolar Walls in Normotensive and Hypertensive Rats.* (30773)

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The calcium ion has an essential role in the contraction of arterial smooth muscle. Waugh has demonstrated that a sustained contraction of arterial muscle induced either by epinephrine or by a high extracellular concentration of potassium can be completely abolished if the available calcium ions are chelated by ethylenediaminetetraacetate (EDTA)(1). Moreover, the ability of epinephrine to cause a contraction in potassium-depolarized arterial muscle is abolished by infusion of EDTA and is restored if calcium ions are infused along with the epinephrine (1).

In this system, a sudden brief raising of the calcium concentration in the bath can cause a small contraction of arterial muscle. Such a contraction is enhanced if the muscle has been depolarized by a high concentration of potassium or if the muscle is under the influence of epinephrine(1). Such a contraction is especially enhanced in muscle under the influence of both epinephrine and potassium depolarization(1). It would appear that either of these factors facilitates the entry of calcium into the smooth muscle cell.

The studies of both Waugh(1) and Bohr (2) indicate that calcium is a vital link in the coupling of excitation and contraction in vascular muscle. It also appears to have an effect on membrane excitability in arterial muscle(2). When epinephrine or norepinephrine comes in contact with the cell membrane of arterial smooth muscle, it causes a membrane reaction which leads to a contraction through a basically nonelectrical process. However, the process does increase the influx of calcium ions into the neighborhood of the contractile proteins, either by shifting calcium from binding sites in or near the "membrane" to the interior of the cell, or by increasing the permeability of the cell membrane to extracellular calcium, or by both of these mechanisms operating simultaneously. Epinephrine also increases the permeability of the cell membranes to sodium and potassium and thus induces depolarization of the cell. This depolarization may enhance the entry of calcium coupled to some anion. Once the extra calcium reaches the interior of the cell, it enhances the splitting of ATP by actomyosin(3) and leads to shortening of actomyosin fibrils(4). Calcium may also relate to the action of a "relaxing factor" which binds calcium in arterial muscle, just as it does in skeletal muscle(5).

Thus, the calcium ion plays an integral part in the contraction of isolated segments of arterial smooth muscle. It also has important effects *in vivo*. Haddy(6) infused calcium salts into a dog's limb artery and thereby produced a striking increase in vascular resistance. All the other cations so tested caused vasodilatation. This observation fits well with certain clinical states in man. In the disease known as "idiopathic hypercalcemia of infancy," the afflicted infants have an abnormally high concentration of calcium in the serum, as well as a distinctly elevated arterial pressure. The high calcium levels presumably lead to arterial vasoconstriction with resulting hypertension. Infants with vit D intoxication have the same combination: a high level of calcium in the serum and a high level of arterial pressure. When they cease taking excessive amounts of vit D, the serum calcium returns to normal levels and the arterial pressure becomes normal.

The foregoing studies all suggest that calcium is a basic component of arterial muscle contraction. It therefore seemed pertinent to ascertain its behavior in arterioles which are affected by hypertension.

Methods. Hypertension was produced in male rats of the Harlan strain by placing a clip on one renal artery and removing the contralateral kidney. Six months after this

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TABLE I. Blood Pressure in Both Groups of Rats Before and After Operation.

	Avg blood pressure (mm hg)
14 "normotensive" rats	
Before clip removal	204
After " "	132
16 hypertensive rats	
Before sham clip removal	199
After " " "	192

initial operation, the blood pressure of each operated rat was carefully ascertained during 3 blood presure readings at 3-day intervals, using the microphonic method. The average of these 3 pressure was considered to be the "true" arterial pressure. By means of these averages, the rats were divided into 2 hypertensive groups that were evenly matched for blood pressure. The mean arterial pressures for each of the groups are given in Table I. Both groups of rats were indeed hypertensive when one considers that normal rats of this strain have a mean blood pressure of 120 mm Hg (SD±9).

In one of the groups, the clip was removed from the renal artery of each rat. Each rat in the other group was subjected to a sham clip removal, but the clips were left intact and the animals remained hypertensive. Two weeks after either the operation to remove the clips or the sham operation, final blood pressure readings were obtained and a sample of mesenteric arterioles was dissected out. These final blood pressures are listed in Table I.

The clip removal did effect a sizable drop in arterial pressure, but half of the rats in this group remained mildly hypertensive. The sham clip removal did little to alter the hypertension. We were thus comparing a group of rats with considerable hypertension to another group (the "normotensive" group) with very mild hypertension. We used this method in order to have hypertrophied arterioles in both groups. The pattern of dissection of these arterioles is quite uniform, so that about the same length of arteriole is removed from each rat. Thus, the dry weight of the arteriolar sample is a rough index of arteriolar thickness. The dry weight of the arteriolar samples from each of the 16 hypertensive rats averaged 2.93 mg; the weight of the samples from the 14 "normotensive" rats averaged 3.07 mg. These averages are quite similar, and indicate that the arterioles from both groups at the time of dissection were of approximately equal thickness and had a similar degree of hypertrophy.

The arteriolar samples were dried *in vacuo* at room temperature and then weighed on a microbalance. Each arteriolar sample was then placed in a Vycor vessel and ashed at 485 C for 6 hours to bring about complete ashing. Then two drops of 1 N HCl were added to the ashed material in order to dissolve it. This solution was then completely dried in an oven at 80 C, leaving most of the calcium phosphate salts in the relatively soluble $Ca(H_2PO_4)_2$ form. The dry solids were then dissolved in approximately 1.2 ml of 0.01 N HCl and quantitatively transferred through a microchromatographic column containing Dowex 2 ion exchange resin, according to the principles and technique of Ames and Nesbett for removing phosphorus(7). The total eluate from the chromatographic column was collected in a small polypropylene cup and evaporated to dryness on a hot plate at 80 C. The sample was then dissolved in 0.0001 N HCl, using 200 μliters of diluent per mg of original dry arteriole. Aliquots of this solution were analyzed for calcium using the Singer spectrophotometric method (8) in which calcium is complexed with glyoxal-bis(2-hydroxyanil).

In each "run" an arteriolar sample from a hypertensive rat was compared with a sample from a normotensive rat, as well as with 3 calcium standards. Thus, samples from both groups were always being compared in any given analytical "run." After the ashing procedure, some of the calcium was undoubtedly present as the relatively insoluble sulfate and phosphate salts. The complete analytical procedure was carried out for both of these salts, using the same amount of total calcium that was found in the arteriolar samples. The recovery of tricalcium phosphate or calcium sulfate was just as complete as the recovery of the more soluble calcium chloride.

The outer diameter of arterial vessels which we dissect from the mesentery ordinarily varies between 25 and 140 μ, with most of them between 70 and 130 μ. We are calling them "arterioles" because Maximow's text reserves this term for any arterial vessel with an outer diameter of less than 300 μ.

Results. The calcium content of the arterioles in the 16 hypertensive rats averaged 60.3 mg/100 g dry weight. The calcium content of the arterioles from the 14 "normotensive" rats averaged 53.3. Thus, the calcium content was about 13% higher in the hypertensive arterioles. The P value of the difference between these means was 0.01.

Discussion. In view of the powerful influence that calcium ions have on arterial contraction, the 13% greater calcium content in hypertensive arterioles may be of considerable significance. Since both hypertensive and "normotensive" arterioles were of a comparable degree of thickness, it is unlikely that the difference in calcium content is due to a varying degree of hypertrophy in the two groups.

If the extra amount of calcium in arterioles is located at the site of the actomyosin fibrils, it could readily cause an increase in degree of contraction. Thus the increased vasomotion noted in hypertension(10,11) might well be related to an increased calcium content.

There are two or more general mechanisms by which this could occur in hypertension. First, various causes such as an increased concentration of transmitter substance, a change in transmembrane potential, or a lowered threshold for depolarization could bring about a heightened amount of the membrane phenomena associated with arterial contraction. This increased membrane reaction could then secondarily bring about an increased content of calcium in the interior of the cell. The rise of intracellular calcium would then trigger a greater degree of actomyosin contraction, which could be considered basically a result of increased membrane reaction.

A second possibility might be that the hypertensive diathesis somehow induces a primary rise in calcium content of arterioles without increasing the amount of membrane phenomena. In this situation, a normal amount of membrane reaction might be accompanied by an increased degree of contraction. The increased arterial content of intracellular sodium could be a cause or an effect of the change in calcium(12).

The foregoing speculations may suggest certain possible interrelationships, but the real significance of these observations is yet to be determined. However, calcium does cause muscle protein to contract, and therefore a 13% rise in the calcium content of arterial muscle may partially account for the luminal narrowing which characterizes hypertension.

Summary. Hypertension was produced in rats by narrowing one renal artery and removing the opposite kidney. These rats were then divided into 2 evenly matched groups. The rats in one of the groups were almost cured of hypertension; the rats in the other group remained hypertensive. The amount of calcium in the walls of the mesenteric arterioles was compared in the two groups. The hypertensive group, on the average, had 13% more arteriolar calcium than the "normotensive" group (P=0.01). Since calcium is known to promote the contraction of the muscle protein, actomyosin, the increased calcium content in the hypertensive arterioles may be partially responsible for their narrowed lumens.

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Lipid Metabolism of Two Highly Inbred Strains of Mice.* (30774)

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Reports from this laboratory(1,2,3) have demonstrated differences in the metabolic patterns of 2 highly inbred strains of mice. One of the most marked differences noted was the ease with which the A/Fn strain mouse became obese in response to diets containing up to 50% fat. The I/Fn, when fed the same diets, remained lean(4). This difference was, to a substantial degree, due to the greater food intake of the A strain mouse and particularly to the breakdown in food intake regulation as diets of increasing caloric density were fed. However, pair-feeding of the 2 strains still permitted the A strain to deposit significantly more carcass fat(5).

These observations led us to investigate the metabolic capacities of intact animals and of adipose tissue and liver *in vitro*. The results suggest that the A strain mouse is able to channel more lipid into depots while the I strain is more dependent upon fat oxidation to meet its energy requirements.

Experimental. Male mice of the A/Fn and I/Fn strain, 4-8 months of age, maintained on Purina Laboratory Chow were used throughout this study.

Free fatty acid (FFA) release. Animals were killed by cervical dislocation, the epididymal fat pads removed, weighed and incubated in calcium-free Krebs-Ringer phosphate buffer, pH 7.4, containing 3% bovine serum albumin (Fraction V). The tissue was preincubated for 30 minutes then transferred to fresh buffer for a period of 3 hours at 38°C. Portions of the medium were taken for FFA determination by the method of Dole and Meinertz(6). FFA levels were determined on plasma collected from both strains.

Ketone body formation by liver slices. Liver slices were prepared from the left lateral lobe, weighed and placed in a Warburg flask containing Krebs-Ringer phosphate buffer, pH 7.4, with 3% bovine serum albumin and 93 μg of octanoic acid per 3.0 ml of buffer. This concentration of octanoic acid was shown experimentally to be optimal for ketone body formation by liver slices *in vitro*. The tissue was incubated at 38°C for 3 hours. Ketone bodies were determined by the method of Lyon and Bloom(7) on 1.0 ml portions of the incubation medium.

Glycogen determination. Tissue glycogen was isolated from the left lateral lobe of the liver by digestion in hot 30% KOH followed by precipitation with ethanol. The precipitated glycogen was centrifuged and then dissolved in 1 N H_2SO_4. Glycogen was determined colorimetrically by the method of Fales(8).

Fat oxidation in vivo. Animals of both strains were placed in a metabolism cage suitable for collecting $C^{14}O_2$. Respiratory $C^{14}O_2$ was drawn through the system by suction and trapped in two 25% KOH traps. The temperature in the chamber was maintained at 30°C. Animals were lightly anesthetized with ether, weighed and fed by stomach tube 0.25 ml glyceryl tri (palmitate-1-C^{14}) (New England Nuclear Corp.) dissolved in olive oil. In experiments measuring the effect of carbohydrate on tripalmitin oxidation, the same concentration of fatty acid was administered along with 0.25 ml of a 25% glucose-50% starch solution. The animals were then placed

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