Short communications

The ionic composition of aortic smooth muscle from A.S.-hypertensive rats

R. MASSINGHAM AND S. SHEVDE

Research Division, Pfizer Limited, Sandwich, Kent

The Na^+, K^+ and Ca^{2+} content of plasma and aortic vascular smooth muscle from normotensive and A.S.-hypertensive rats have been compared. There was no significant difference in the plasma concentrations of Na^+, K^+ and Ca^{2+}, or the Na^+, K^+ and water content or ^{3}H-inulin space of aortic tissue in the two groups.

There was a marked increase in the amount of Ca^{2+} in the aortae taken from the hypertensive animals as compared with the normotensive animals ($P<0.01$).

Introduction.—We have recently shown that aortic smooth muscle taken from genetically hypertensive rats (A.S. strain) has a reduced reactivity to both noradrenaline and K^+ (Massingham & Shevde, 1971). We also reported that the resting membrane potential recorded from aortic smooth muscle cells was significantly lower in A.S.-hypertensive rats, probably due to a change in potassium permeability.

In this study we have measured the Na^+, K^+ and Ca^{2+} content of aortae from normotensive and A.S.-hypertensive rats.

Methods.—Rat blood pressures were recorded by the tail-cuff method (Lessin, 1965) on 3 separate days before animals were used in experiments.

Experiments were conducted on female rats with a body weight of 200–300 g. Normotensive rats were of the Wistar strain and the genetically hypertensive rats were from our own colony derived from the New Zealand A.S. strain which were originally bred for transplantation studies at the University of Otago by Dr. N. W. Nisbet and his colleagues. At this time the New Zealand genetically hypertensive rats and A.S. rats are separated by more than 20 generations and are distinct substrains if not different strains (E. L. Phelan, personal communication).

Four hours before the animals were killed a bilateral nephrectomy was carried out under ether anaesthesia and 5 μCi/rat of ^{3}H-inulin (Radiochemical Centre, Amersham) was injected into the lingual vein. From this stage the animals were deprived of food and water. At the end of this 4 h period the animals were again anaesthetized with ether and a blood sample taken by cardiac puncture. Blood samples were rapidly transferred to lithium-heparin coated plastic tubes (Teklab), shaken and plasma samples obtained by centrifugation.

Plasma (0·5 ml) was dissolved in 3 ml of Nuclear Chicago solubilizer (Amersham/Searle) and incubated at 37° C for 30 minutes. After this time 10 ml of a toluene scintillator was added and samples counted in a Unilux-2 scintillation counter. The remainder of the plasma was used for electrolyte determinations.

After the animals were killed the aortae were carefully dissected free of fat from the aortic arch down to the diaphragm and removed. Aortae were blotted between filter papers and cut transversely into two portions which were weighed. That part nearest the arch was used for electrolyte determinations and that nearest the diaphragm for ^{3}H-inulin space measurements.

Electrolyte determinations. — Aortae were placed overnight ($\simeq$16 h) in an oven at 100° C and reweighed to obtain dry tissue weights. They were transferred to stoppered plastic tubes, broken up with a glass rod and 5 ml de-ionized water was added. The tubes were then allowed to stand for 3 days. The fluid was decanted and Na^+ and K^+ measured by flame photometry and Ca^{2+} by atomic absorption spectrophotometry. Lanthanum (0·5% w/v) was added to the calcium samples to reverse PO_4^{3-} and SO_4^{2-} suppression of Ca^{2+} absorption.

^{3}H-inulin determinations.—The aortae were blotted, weighed and dissolved in 3 ml Nuclear Chicago solubilizer at 37–40° C. Toluene scintillator was then added and samples counted in a Unilux-2 scintillation counter, after standing for a week to reduce chemiluminescence.

Results.—The animals used in this study were not age-matched and some hyperten-

TABLE 1. *The body weights, blood pressures, ^3H-inulin space and plasma and aortic electrolyte concentrations of normotensive rats and A.S.-hypertensive rats*

	Normotensive rats	Hypertensive rats
Body weight (g)	214·1±7·3* (12)	245·1±13·1 (10)
Blood pressure (mm Hg)	144·3±3·4 (12)	212·3±3·4 (10)
% wet weight of aorta	66·04±0·60 (12)	66·67±0·50 (10)
^3H inulin space of aorta (l./kg)	0·196±0·015 (6)	0·217±0·020 (6)
Non-inulin space of aorta (l./kg)	0·479±0·012 (6)	0·446±0·007 (6)
Plasma Na^+ (mEq/l.)	131·71±0·51 (8)	129·53±0·88 (8)
Plasma K^+ (mEq/l.)	3·76±0·10 (8)	3·78±0·12 (8)
Plasma Ca^{2+} (mEq/l.)	8·06±0·64 (3)	7·33±0·17 (3)
Plasma Cl^- (mEq/l.)	102·73±0·63 (8)	103·35±0·35 (8)
Aortic Na^+ (mmol/kg wet wt)	93·23±3·32 (12)	96·29±3·16 (10)
Aortic K^+ (mmol/kg wet wt)	33·78±1·74 (12)	32·07±1·70 (10)
Aortic Ca^{2+} (mmol/kg wet wt)	3·11±0·23 (12)	5·12±0·49 (10)

* Figures are means±S.E.M.; figures in parenthesse indicate the number of observations.

sive rats were 9 months older than normotensive animals. Mean body weight of the normotensive group was 214·1±7·3 g and that for the hypertensive group was 245·1±13·1 g ($P<0·1$). Blood pressures in the groups were markedly different being 144·3±3·4 for normotensive and 212·3±3·4 mmHg for hypertensive rats ($P<0·001$). The percent wet weight and ^3H-inulin space of aortae from the two groups of rats were not significantly different (Table 1).

Table 1 shows the values for plasma electrolytes in normotensive and hypertensive rats. There was no significant difference in the Na^+, K^+, Ca^{2+} and Cl^- content of the plasmas. There was no significant difference in the Na^+ and K^+ content of aortic smooth muscle taken from the two groups (Table 1) but the Ca^{2+} content of the hypertensive group was significantly higher than that of normotensive tissues ($P<0·01$).

Discussion.—The values obtained for Na^+, K^+ and water content of rat aortae in this series of experiments are similar to those published by Hagemeijer, Rorive & Schoffeniels (1965) (Na^+ content of approximately 79 mEq/kg wet weight of tissue and a K^+ content of 40 mEq/kg wet weight of tissue).

The amount of water, Na^+, and K^+ in rat aortic smooth muscle is elevated in renal hypertension (Tobian, 1956 and Daniel & Dawkins, 1957), in desoxycorticosterone-hypertension (Daniel & Dawkins, 1957) and in adrenal regeneration hypertension (Tobian & Redleaf, 1958). These changes of Na^+, K^+ and water are not apparent in aortae taken from rats with genetic hypertension as shown by Phelan & Wong (1968), who found that the values for K^+ and water were normal in aortae from genetically hypertensive rats while the Na^+ content was slightly elevated. This increase in Na^+ was very small when compared with that seen in aortae taken from renal hypertensive rats. In the present series this has been confirmed for the A.S. strain except that the Na^+ content of aortae taken from normotensive and A.S.-hypertensive rats was not significantly different. There was however, a marked difference in the total calcium ion content of aortae from the two groups of rats, the hypertensive tissues containing approximately 65% more calcium than the normotensive tissues.

Whether this calcium is intra- or extracellular is not known, but since intracellular calcium is probably only a few percent of the total (Van Breemen & McNaughton, 1970) and since smooth

muscle cells comprise only part of the total aortic weight, it is probable that this increase in calcium ions occurs extracellularly, the calcium perhaps being bound to mucopolysaccharides (Headings, Rondell & Bohr, 1960). Indeed the medial hypertrophy seen in genetically hypertensive rats (Folkow, Hallback, Lundgren & Weiss, 1971) could lead to an increased binding of cations.

This extra calcium, whatever its actual location, does not appear to be available to the smooth muscle cells for contractile purposes since responses to noradrenaline and K^+ are significantly less in A.S.-hypertensive aortae (Massingham & Shevde, 1971). No definite explanation as to why aortic contractility in A.S.-hypertensive rats is reduced is forthcoming but it could be argued that the extra calcium may be able to reduce contractility via an extracellular locus of action perhaps by binding to and thus stabilizing the smooth muscle cell membrane (Daniel, 1965).

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