EFFECT OF INCREASED SODIUM ION ON ARTERIAL SODIUM AND REACTIVITY

G. S. HARRIS AND W. A. PALMER

Department of Pharmacology, University of Melbourne, and Department of Medicine, Austin Hospital, Heidelberg

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SUMMARY

1. Isolated rabbit arterial segments were perfused with buffer containing sodium (155 mmol/l) and vasoconstrictor responses to noradrenaline, histamine and electrical stimulation were measured. When the concentration of sodium in the perfusing medium was raised by 8 mmol/l, the vasoconstrictor responses to all stimuli were significantly increased, though response to electrical stimulation was less enhanced than the responses to the other stimuli.

2. The constrictor response of the isolated perfused rabbit ear to angiotensin was also enhanced by perfusion with buffer of a similarly enriched sodium content.

3. Ileal responses to electrical stimulation and atrial responses to electrical stimuli and noradrenaline were also examined after exposure to similar changes of environmental sodium. No change in responsiveness was demonstrated.

4. Isotope-exchange studies with $^{22}$Na showed that this small increase in sodium concentration of the environmental fluid caused an increased sodium ion content of 20% in arterial wall, 5% in ileum and 11% in atrium.

5. It is suggested that a relationship exists between increased exchangeable sodium ion in arterial wall and arterial reactivity.

Key words: sodium ion, noradrenaline, histamine, angiotensin, arteries.

It has been reported that there is an increased sodium ion content in arteries from animals with experimental hypertension (Tobian & Binnion, 1954) and that arteries from hypertensive animals display an increased reactivity to pressor stimuli (Vick, Ederstrom & Vergeer, 1956; Gordon & Nogueira, 1962). The effect of changing sodium ion concentration on the reactivity of isolated arterial segments has also been studied. A rise in the sodium ion concentration was found to increase the reactivity of isolated arteries from hypertensive animals (Mallov, 1960, 1961). Other studies on arterial segments from normal and hypertensive animals showed that...
a rise in sodium ion concentration decreased reactivity, whereas lowering the sodium ion concentration increased reactivity (Bohr, Brodie & Cheu, 1958; Napodano, Caliva, Lyons, Desimore & Lyons, 1962; Blair-West, Harding & McKenzie, 1968).

Although the above studies appear conflicting, they are not readily comparable, since many of the arterial segment preparations had sustained a severe degree of trauma during preparation. Moreover, the changes in sodium concentration were large (30-40 mmol/l) necessitating replacement of the sodium ion by an inert constituent or resulting in differences in osmolarity.

The increased sodium ion content of arteries from hypertensive animals might be related to the increase in mucopolysaccharide content of arterial wall reported in hypertension (Crane, 1962). The presence of such molecules within the arterial wall (Bunting & Bunting, 1953) having marked cation-binding properties (Dunstone, 1962) suggests that these molecules may modulate ion movement and be involved in maintaining the high concentration of sodium ion reported in arterial wall (Garrahan, Villamil & Zudunaisky, 1965; Friedman, Gustafson & Friedman, 1968; Keatinge, 1968). If these molecules exert ion-exchange properties and thus bind sodium ions, small changes in sodium ion concentration in the surrounding fluid might result in larger changes in the sodium ion content of the arterial wall. Thus large changes in sodium ion concentration might well be toxic and result in a decreased reactivity. This study reports the effect on reactivity of a small increase in the sodium ion concentration of the buffer perfusing isolated arterial segments from normal rabbits.

**MATERIALS AND METHODS**

**Experimental**

South Australian rabbits of either sex weighing 2-3 kg were killed by a blow on the head. The isolated central ear artery or the isolated ear was perfused at 6 ml/min with McEwen's solution (g/l: NaCl, 7.6; KCl, 0.41; CaCl₂, 2H₂O, 0.24; NaH₂PO₄·2H₂O, 0.14; NaHCO₃, 2.1; dextrose, 2.0; sucrose, 4.5) maintained at 37°C and aerated with 95% O₂, 5% CO₂. The perfusion pressure was measured via a mercury manometer writing on a smoked drum, the pressure in the unstimulated arteries being 20-30 mmHg. The vascular reactivity was estimated by constricting the artery and measuring the change in perfusion pressure at constant flow. The constrictor stimulus was either supramaximal electrical stimulation of the periarterial sympathetic nerves (de la Lande & Rand, 1965) at a voltage of 5-20 V (10 Hz, each 0.1 ms for 10 s every 3 min) or repeated single doses of noradrenaline (approx. 5 ng) or histamine (100 ng every 3 min), the dose chosen to give an increase in perfusion pressure of 60-120 mmHg. Each preparation was used with one stimulus and subjected to a single change in sodium ion concentration. When a series of seven isolated whole rabbit ears were studied the conditions of perfusion were the same as for the isolated artery but with this preparation the constrictor agent was Val²-angiotensin II (Hypertensin, Ciba) (5 ng given as a repeated single dose every 5 min). After the preparations were set up and constant responses obtained to repeated constrictor stimuli, five control responses were obtained. The sodium ion concentration of the perfusing medium was then increased by replacing an osmotic equivalent of sodium chloride for the sucrose in McEwen's solution. This increased the sodium ion concentration from 155 mEq/l to 163 mEq/l. The medium containing the increased sodium ion concentration was introduced into the heating coils, after which it was perfused through the artery and mixed with the bathing fluid in the organ bath before it escaped. Since the capacity of the perfusion system was approx.
50 ml it required approx. 15 min for the increase in sodium ion concentration to be completed in the organ bath. Constrictor responses were obtained for 30 min after the introduction of the buffer containing the increased sodium ion concentration.

In another series of arteries the preparation was initially perfused with buffer at the higher sodium ion concentration. Constant responses were obtained and control responses recorded for 15 min. The perfusion buffer was then changed to the lower sodium ion concentration and constrictor responses obtained during the following 30 min.

Individual responses of arteries were found to be variable, which led to the following analysis of the effect of sodium ion concentration on constrictor response. Once constant responses were obtained, five responses were obtained over 15 min and the responses averaged. The responses obtained over the 30 min of perfusion with buffer at a different sodium ion concentration were also averaged. For each individual preparation the five responses of the control period and the ten responses after the change in sodium ion concentration were compared by using an unpaired t test. For each individual preparation the change in constrictor response after the change in sodium ion concentration was expressed as a percentage of the average control response. The percentage change was then averaged for each constrictor agent, allowing comparison between different stimuli. The differences of the average responses for each agent were compared by using an unpaired t test.

To test the effect of increasing the sodium ion concentration on smooth-muscle responses, two other muscle preparations were subjected to the same increase in sodium ion concentration and the effect on muscle reactivity measured. Rabbit ileum was removed and a segment suspended in McEwen's solution at 37°C and aerated with 95% O₂, 5% CO₂. The rhythmic pendular movements were recorded via a frontal writing lever writing on a smoked drum. The amplitude of contraction was measured for a control period in McEwen's solution and after increasing the sodium ion concentration. Rabbit left atria were suspended in briskly aerated McEwen's solution and the atria driven by an electrical pulse of 2 V, 2 Hz, 1 ms. The isometric contractions of the isolated atria were recorded with a strain gauge, the change in tension being recorded on a Beckman Polygraph. The amplitude of responses was again measured over a control period and after an increase in the sodium ion concentration of the bathing fluid.

**Ion-exchange in isolated tissues**

The sodium content of small masses of tissues in vitro was estimated by exchange with ²²Na. Rabbit central ear arteries were incubated in aerated buffer at 37°C for 1 h after dissection to correct any electrolyte shift incurred during preparation. A series of weighed arteries was then incubated singly in McEwen's solution containing ²²Na (20 μCi/100 ml; 315 mCi/mg of Na). At intervals starting at 1 min the artery was blotted on filter paper for 30 s, carefully inserted into a plastic tube and the ²²Na content measured with a Packard Autogamma Spectrometer model 3002. The artery was then replaced in the solution and incubated for a further period before it was again removed and ²²Na content measured as above. This procedure was repeated for a number of time-intervals up to 90 min. Arteries were loaded with ²²Na by incubation in isotope-containing buffer for 120 min. The rate of washout was determined by superfusing and perfusing the artery with ²²Na-free McEwen's buffer (1 ml/min). The wash fluid from the artery was collected at intervals over 90 min and the ²²Na content measured.

A series of five arteries was also incubated for 60 min in McEwen's solution containing ²²Na. The arteries were then removed, blotted on filter paper for 30 s, weighed, and the ²²Na content
measured. The same arteries were then dissolved in 2 ml of conc. HNO₃ and dried by gentle heating. The residue was dissolved in 10 ml of LiCl (15 mmol/l) and the sodium concentration measured with a flame photometer. The sodium concentration in the arterial segments by these two methods was then averaged and compared by using a paired $t$ test.

Analysis of the results obtained in the experiments above showed that the phase of rapid exchange of isotope had a half-life of approx. 1.5 min. An estimate of the change in sodium content of the extracellular compartment of arterial wall after an increase in sodium ion concentration was made by incubating arteries in buffer containing a higher sodium ion concentration for 1.5 min and measuring $^{22}$Na uptake. The experiment was conducted at 37° and 17°. At the lower temperature it was expected that membrane transport of sodium would be significantly decreased, enabling any difference in $^{22}$Na uptake to be attributed to a change in membrane transport. McEwen's solutions with a sodium ion concentration of 155 and 163 mmol/l were prepared and $^{22}$Na was added to give the same specific radioactivity of isotope. In one series of experiments an artery incubating in buffer was blotted dry for 30 s then added to a buffer containing $^{22}$Na for 1.5 min. The artery was again blotted on filter paper and placed in 5 ml of $^{22}$Na-free buffer. The $^{22}$Na content of the artery and buffer solution was then measured over 3 min taking three 1 min counts. The artery was then removed, blotted and added to another 5 ml of $^{22}$Na-free buffer, incubated for 3 min and the process repeated for a third time.

Three 3 min washes in $^{22}$Na-free buffer washed out the $^{22}$Na taken up during the short incubation in $^{22}$Na buffer. After the third wash the artery was blotted and reincubated in $^{22}$Na buffer for 1.5 min and the counting and washing procedures repeated to give five estimates of $^{22}$Na uptake.

The same procedure was then repeated with the same artery and the alternate sodium ion concentration. The uptake of $^{22}$Na was measured in one series at a sodium concentration of 155 mmol/l then at 163 mmol/l and in another series of arteries the order was reversed. This procedure was repeated with rabbit ileum and atria; however, with these tissues the period of washing had to be increased to 10 min to remove all the $^{22}$Na taken up during the short incubation with $^{22}$Na. In all cases at the end of the procedure the tissue was blotted for 30 s on filter paper and weighed. A sample of the $^{22}$Na solutions was also counted for radioactivity in the autogamma spectrometer. Results are expressed as means and standard errors of means.

**RESULTS**

*Effect of increasing sodium ion concentration on constrictor responses*

*Isolated arterial segments.* The constrictor responses of isolated arterial segments in vitro were found to be less variable with sympathetic nerve stimulation than to single doses of noradrenaline or histamine. After perfusion of buffer with an increased sodium ion concentration the constrictor responses were variably increased, maximal constrictor responses being obtained approx. 15 min after the start of perfusion with the increased sodium ion concentration. This period was required for the buffer in the organ bath to reach the sodium ion concentration of the perfusing buffer.

The average of the five responses during the control period was then compared with the average of the ten responses during perfusion with the increased sodium ion concentration. With electrical stimulation twelve of fourteen arteries had a significant increase ($P<0.05$) in
response, the average with the increased sodium ion concentration being $104.9 \pm 1.4\%$ of control responses. When noradrenaline was the constrictor agent seven of eleven arteries had a significant ($P < 0.05$) increase in response, the average during perfusion with the higher sodium ion concentration being $109.8 \pm 2.5\%$ of the control period. Similarly with histamine as the stimulus the responses in nine of twelve arteries were significantly increased ($P < 0.05$), the average being $111.1 \pm 2.1\%$ of the responses in the control period.

In a series of experiments arteries were initially perfused with a sodium ion concentration of 163 mmol/l and the concentration then decreased to 155 mmol/l. The constrictor responses to electrical stimulation and to histamine decreased after the decrease in sodium ion concentration. Sympathetic nerve constrictor responses were decreased significantly ($P < 0.01$) in four out of five arteries during perfusion at the decreased sodium ion concentration, the average being $82.6 \pm 3.8\%$ of the response at the higher sodium ion concentration. Histamine responses were also significantly decreased ($P < 0.01$) in four out of five experiments, the average being $73.5 \pm 4.2\%$ of the response at the higher sodium ion concentration.

**Isolated rabbit ear.** When the average constrictor response to Val$^{1}$-angiotensin II in the control period was compared with the average obtained during perfusion with the increased sodium ion concentration the increase was again greatest about 15 min after the start of the perfusion with the increased sodium ion concentration. The average constrictor response during perfusion with the higher sodium ion concentration was $113 \pm 2.5\%$ of control responses, the increase being significant ($P < 0.01$).

**Rabbit ileum.** The pendular movements of rabbit ileum in a 15 min period before increasing the sodium ion concentration, compared with a 30 min period after, showed no change in amplitude, the average response for nine pieces of ileum in buffer containing an increased sodium ion concentration being $94 \pm 3\%$ of the amplitude of the pendular movements in the control period.

**Rabbit atria.** The isometric contractions of electrically driven rabbit atria were not increased by increasing the sodium ion concentration of the bathing buffer. The average responses after increasing the sodium ion concentration in seven atria were $99 \pm 4\%$ of the average in the control period.

**Effect of sodium ion concentration on $^{22}$Na exchange**

The uptake of $^{22}$Na appeared to be complete after 5 min in $^{22}$Na–McEwen’s solution (Table 1) and use of the $^{22}$Na of five arteries over 60 min uptake to calculate the sodium content of arterial segments resulted in an estimated sodium content of $113.6 \pm 6.9$ µmol/g. When the same arteries were wet ashed and the sodium determined chemically a value of $118.8 \pm 9.6$ µmol/g was obtained. When compared by using a paired $t$ test these values were not significantly different. When five arterial segments loaded with $^{22}$Na were washed out with sodium buffer most of the $^{22}$Na was removed in the first 5 min of the washing procedure, $86 \pm 3.6\%$ being removed in 1.5 min (Table 2).

In a series of nine arteries incubated at 37° for 1.5 min in $^{22}$Na–McEwen’s solution containing 155 mmol of Na/l, the estimated sodium content was $99.2 \pm 3.5$ µmol/g. When these same nine arteries were incubated for 1.5 min in $^{22}$Na–McEwen’s solution with a sodium concentration of 163 mmol/l the estimated sodium content was $118.0 \pm 2.6$ µmol/g, an increase which was highly significant ($P < 0.001$). When the experiment was repeated on a series of nine arteries at 17°, the sodium content after incubation with a sodium concentration of 155 mmol/l was
101.8 ± 2.5 μmol/g and with a sodium concentration of 163 mmol/l, 120.0 ± 3.4 μmol/g, the increase in sodium ion content in this instance being 19% (P < 0.001). The same difference in sodium content was obtained if the arteries were first incubated in the higher sodium concentration and then in the lower. When strips of rabbit atria or rabbit ileum were used in the above experiment a longer period of washing was required between repeated estimations to remove the $^{22}$Na present in the tissue. The difference in sodium content between tissues incubated for 1.5 min in $^{22}$Na buffer at the two sodium concentrations was smaller for rabbit ileum and

| TABLE 1. Rate of uptake of $^{22}$Na by isolated arterial segments |
|---|---|---|---|---|---|---|
| Time (min) after exposure | 1 | 2 | 5 | 10 | 30 | 60 |
| % of final content of $^{22}$Na in artery | 79 ± 5 | 89 ± 4 | 99 ± 3 | 100.5 ± 3 | 98 ± 3 | 100 |
| (mean ± SE) | (n = 7) |

| TABLE 2. Wash out of $^{22}$Na from isolated arterial segments |
|---|---|---|---|---|---|---|---|
| Time (min) | 0.5 | 1.0 | 1.5 | 2.0 | 3.0 | 5.0 | 10.0 |
| Loss of $^{22}$Na from artery (% of the amount originally present) | 64.4 ± 6 | 78.8 ± 5 | 85.8 ± 4 | 90.4 ± 3 | 93.0 ± 2 | 96.7 ± 2 | 98.0 ± 2 |
| (mean ± SE) | (n = 5) |
rabbit atria. At 38° the increase for rabbit atria was 7 ± 2% (n = 5) and for rabbit ileum 3 ± 1% (n = 5) whereas at 17° the increase was 11 ± 3% (n = 5) for rabbit atria and 5 ± 1% (n = 5) for rabbit ileum.

**Weighing of arterial segments**

The small mass of arterial segments used in the perfusion studies necessitated accurate weighing for the determination of sodium content. To determine the error involved in weighing, arterial segments of the mass used for perfusion were removed from buffer and blotted on filter paper for 30 s and weighed, a reading being obtained in 15–30 s. The weight change over the next 2 min was then recorded. A series of five arteries were repeatedly weighed and the standard error of weighing was found to be 1.8% of the mass of the arterial segment. After 1 min on the weighing pan the arterial segments had lost an average of 0.8% and after 2 min 2.7% of the total arterial mass.
DISCUSSION

The previous reports on the effect of changing sodium ion concentration on reactivity of isolated arterial segments have used either preparations such as spirally cut arterial segments which are extensively damaged, or large changes in sodium ion concentration. These studies were undertaken before it had been appreciated to what extent the extracellular mucopolysaccharides act as cation exchangers (Palaty, Gustafson & Friedman, 1969) concentrating extracellular cations.

In a previous study the enzymic depolymerization of the extracellular mucopolysaccharides with testicular hyaluronidase resulted in both a decreased sodium content and reactivity of arterial segments in vitro (Harris & Palmer, 1971). The action of these extracellular polyanions as cation exchangers suggested that a small change in sodium ion concentration of the perfusion buffer might significantly change the sodium content and reactivity of arterial segments. To test this hypothesis the constrictor responses and sodium content of arterial segments in vitro were studied at two sodium concentrations which differed by only 5%, the osmolarity being constant.

Increasing the sodium ion concentration increased the constrictor responses of the isolated arterial segments to the constrictor agents tested. The increase in constrictor responses to histamine and noradrenaline were approximately equal and statistically different from the smaller increase in response observed with electrical stimulation of the periarterial sympathetic nerves. This difference in response might be the result of an increased re-uptake of noradrenaline released from the sympathetic neurone associated with the increase in sodium ion concentration, since noradrenaline uptake into the sympathetic neurone has been reported to be proportional to the concentration of sodium ions (Bogdanski & Brodie, 1969).

In rabbit ileum and atria the same increase in sodium ion concentration in the perfusate did not increase the amplitude of smooth-muscle contractions.

Studies on the distribution of the sodium present in arterial segments have demonstrated that at least two-thirds is extracellular and readily exchangeable (Garrahan et al., 1965; Friedman et al., 1968). The half-time of exchange of the extracellular sodium has been reported to be 42 s at 37°, most of the remaining sodium, which is contained intracellularly, having a half-time of exchange of 5 min. When the temperature was lowered to 17°, inhibiting active transport of cations, the extracellular sodium was exchanged with a half-time of 66 s and the intracellular sodium with a half-time of 42 min (Garrahan et al., 1965).

From the above results it can be estimated that at 37° the exchange of $^{22}$Na that would occur with 5 min incubation would be approx. 85% of the total exchange, whereas in the present study the exchange of $^{22}$Na appeared to be complete in 5 min. In this study when arteries were loaded with $^{22}$Na and washed out with sodium buffer the $^{22}$Na content of arteries was decreased 86% in 1·5 min, which is faster than expected from previously reported results. These results can only be explained if the artery studied has an extracellular sodium content greater than two-thirds of the arterial sodium. The change in sodium content of arteries incubated in a different sodium ion concentration for 1·5 min can best be explained by a change in the extracellular sodium content of the arteries. From the reported studies two half-times of exchange of the extracellular sodium and only one-third of a half-time of intracellular sodium would occur over the 1·5 min period of exchange. Incubation of arteries at 37° and 17° did not result in any significant difference in sodium ion content as measured by $^{22}$Na uptake at either sodium ion concentration. This suggests that the major portion of the exchange had occurred extracellularly.
larly, since the energy-dependent membrane transport of sodium would be inhibited by the
decrease in temperature and, if this had been a major factor, a difference in $^{22}$Na content should have been observed at the different temperatures. The findings described therefore provide further evidence that a large proportion of sodium in artery wall is contained in the extra-
cellular compartment and is presumably bound by the polyanions. This latter suggestion is
supported by the fact that tissues such as ileum or atria which do not contain these polyanions
display a much smaller increase in sodium content at $37^\circ$ when exposed to a high sodium con-
centration. Sodium exchange in these tissues appeared more temperature-dependent than that
in arteries, suggesting a larger intracellular exchange component.

Arterial wall mucopolysaccharides have been shown to be increased in hypertension (Crane,
1962, 1963) and it is suggested that this increase is associated with the increased cation content
of arterial wall in hypertension. In this study increasing the sodium ion concentration by a
small amount resulted in an increase in arterial sodium and reactivity. The mechanism for the
increase in reactivity is not obvious; however, some of these cations are loosely bound and may
still be free to move along a concentration gradient across a cell membrane, increasing the
sodium flux. Another possibility is that the increased sodium decreases calcium binding, in-
creasing free calcium concentrations at the cell membrane.

Since the mucopolysaccharide content of the arterial wall is increased in hypertension, it
seems reasonable to suggest that the increased polyanionic binding capacity might lead to an
increase in arterial sodium content and reactivity, even though the plasma sodium concentra-
tions are not markedly increased.

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