Intracellular Sodium, Membrane Potential, and Contractility of Rat Mesenteric Small Arteries

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SUMMARY. We have investigated effects of altered extracellular sodium, intracellular sodium concentration, and membrane potential on the contractile responses of rat isolated mesenteric small arteries (internal diameter ca. 200 μm), when mounted as ring preparations on an isometric myograph. To avoid possible neural effects, all vessels were denervated in vitro using 6-hydroxydopamine. In unstimulated vessels, exposure to low-Na+ solutions (25 mM sodium, sucrose, or choline-substituted) did not cause any response nor did exposure to ouabain (1 mM) for 1 hour [when intracellular sodium concentration increased to 64 mmol/(liter-cell)]. However, a response was obtained if ouabain-exposed vessels were subjected to low-sodium solutions (ca. 15% of maximal response). The magnitude of the response was dependent on the ratio of intracellular to extracellular sodium and was not inhibitable by the calcium blockers, felodipine (1 nM) or D600 (10 μM). This response could therefore be explained in terms of a Na-Ca exchange mechanism. The responses of activated vessels to ouabain and to low-sodium solutions were also investigated. The responses of vessels to submaximal doses of noradrenaline or potassium were potentiated acutely by ouabain (by 10-30% of the maximal response), even if the extracellular sodium was reduced to 25 mM. In all cases, the potentiation by ouabain was accompanied by a depolarization (3-12 mV). However, only in the case of noradrenaline-activated vessels with normal extracellular sodium was the potentiation accompanied by an increase in intracellular sodium [by ca. 7 mmol/(liter-cell)]. Moreover, the latter response was inhibited by felodipine and D600. The results suggest that Na-Ca exchange mechanisms may be present in these vessels but that they only play a role under extreme conditions; under normal conditions the effect of ouabain on activated vessels seems to be primarily due to its depolarizing effect, and not to its effect on intracellular sodium. (Circ Res 54:740-749, 1984)

THERE has recently been considerable interest in the hypothesis that a raised intracellular sodium ([Na+]i) could cause vasoconstriction and thereby hypertension (Blaustein, 1977; de Wardener and Macgregor, 1980; Swales, 1980). The main experimental basis for this hypothesis is the effect of cardiac glycosides, in particular of ouabain, on the vascular system. On the one hand, glycosides by inhibiting the Na,K-ATPase (Na-K pump), will cause an increase in [Na+] (Ozaki et al., 1978). On the other hand, injection of glycosides produces an increase in peripheral resistance (Hulthén et al., 1983; Robinson et al., 1983), whereas exposure of many isolated vascular preparations (in particular, of aorta) to glycosides induces a contraction (Bohr et al., 1969). The possible importance of this question is emphasized by the finding that hypertension may be associated with a natriuretic plasma factor (Poston et al., 1981; Hamlyn et al., 1982).

To explain why raised [Na+] could lead to the rise in free intracellular calcium concentration ([Ca2+]i), which must be the ultimate cause of the response potentiation, it has been suggested that raised [Na+] by reducing the transplasmalemmal sodium gradient, could reduce calcium extrusion via a Na-Ca exchange mechanism (Blaustein, 1977). Some evidence in support of this mechanism has been obtained from large vessels (Reuter et al., 1973), but there is no evidence available for its presence in the smaller vessels which determine peripheral resistance. It is therefore still not certain that the pressor response to injected ouabain is due to raised [Na+], (Mulvany et al., 1982a, 1982c; Hermensmeyer, 1983), and at least two other mechanisms may be considered. First, ouabain can cause increased transmitter release from the vascular nerve terminals (Gillis and Quest, 1979; Aarhus et al., 1983). Second, inhibition of the vascular Na-K pump will result in membrane depolarization (Hendrickx and Casteels, 1974), which, because of the membrane potential sensitivity of vascular smooth muscle, would also result in potentiation of contractions (Fleming, 1980; Mulvany et al., 1982b).

In the present work, we have investigated the effect of altered sodium metabolism on the responses of isolated small arteries (i.d. ca. 200 μm) from the mesenteric bed of the rat. To avoid possible neural effects, all vessels were denervated in vitro by the method of Aprigliano and Hermensmeyer (1976). The sodium metabolism was adjusted either...
by alterations of the sodium concentration of the bathing solution, or by exposing the vessels to ouabain. In all experiments, the ouabain concentration used was 1 mM, this high concentration being necessary to obtain full inhibition of the Na-K pump in rat vessels (Garay et al., 1978; Aalkjaer and Mulvany, 1983). In some experiments, the results of altered sodium metabolism were investigated by testing the effects of drugs thought to interfere specifically with potential-dependent channels (Bolton, 1979) in the vascular smooth muscle membrane: felodipine (Bostrom et al., 1981; Nyborg and Mulvany, 1984) and methoxyverapamil (D600, Van Breemen and Siegel, 1980).

Methods

Preparation and Myograph

Segments (ca. 2 mm long) of small arteries (internal diameter ca. 200 μm) were taken from the mesenteric bed of Wistar rats and mounted as ring preparations on an isometric myograph (Mulvany and Halpern, 1977). Vessels were set to an internal circumference, L, estimated, on the basis of the resting tension-inner circumference characteristic, to be 90% of the internal circumference which the vessels would have had in situ when relaxed and under a transmural pressure of 100 mm Hg (Mulvany and Warshaw, 1979). L is close to the circumference at which vessels develop their maximum wall tension (Mulvany and Warshaw, 1979). L is taken as the normalized internal diameter.

Solutions

Vessels were dissected in and normally held relaxed in standard saline consisting of (mm): NaCl, 119; KCl, 4.7; NaHCO3, 25; CaCl2, 2.5; KH2PO4, 1.18; MgSO4, 1.17; ethylene-diaminetetraacetic acid (EDTA), 0.026; glucose, 5.5. K+-saline was standard saline in which NaCl was exchanged for KCl on an equimolar basis to give the potassium concentration indicated. Low Na+ solution was taken as the alteration of the sodium concentration of the saline with NaCl and NaHCO3 replaced by 107 mM LiCl (Mulvany and Halpern, 1977). L is used as the vessel length. L is the internal sodium content at the start of the washout. L is then washed out in the various solutions indicated (e.g., standard saline at 37°C). Sodium efflux was determined as the amount of sodium washed out per minute (calculated from the amount detected in the washout aliquots of 1 ml) to give the concentration indicated. In the pharmacology experiments, a suffusion system was used in which solutions were circulated at about 6 ml/min through the chamber (volume 5 ml). In the figures, the myograph records have been calibrated in terms of the wall force measured by the myograph force transducer. For comparison of responses from different vessels, these have been expressed either (1) as active wall tension—that is, the wall force measured at the conclusion of the test period in excess of the wall force of the vessel in standard saline, divided by the wall length (= twice segment length)—or (2) as a fraction of the active wall tension in response to control activating solution, this response being termed ΔT.

Membrane potential was measured with the vessels mounted on the myograph using glass microelectrodes (resistance ca. 100 MΩ when filled with 3.5 KCl) as described previously (Mulvany et al., 1982b). The system was stable enough to permit continuous simultaneous measurements of membrane potential and wall force, even during activations.

Sodium Metabolism

The procedures used are presented in detail elsewhere (Aalkjaer and Mulvany, 1983). In brief, these are as follows.

Intracellular Sodium Determination

Vessels were first mounted on the myograph, and the tunica media volume determined by light microscopy (Mulvany et al., 1978), and from this the cell volume was estimated on the basis that the media cell content is ca. 71% (Mulvany et al., 1978). The vessels then were demounted, denervated as described above, and loaded with 22Na for 30 minutes in 22Na-labeled standard saline. Vessels were then placed in the various test solutions, as described below, these solutions also being labeled with 22Na with the same specific activity. At the times specified, the vessels were transferred to Li+-saline at 0°C to remove the extracellular sodium (Friedman, 1974), and washed through a series of vials containing this medium over 45 minutes. The washout curve after 13 minutes was taken as the efflux of sodium from the intracellular stores. This curve was back-extrapolated to estimate the intracellular sodium content at the start of the washout. [Na+]i was expressed in terms of the intracellular sodium content per cell volume made at the start of the experiments.

Sodium Efflux Rates

Vessels were loaded with 22Na as described above and then washed out in the various solutions indicated (e.g., standard saline at 37°C). Sodium efflux rate was determined as the amount of sodium washed out per minute (calculated from the amount detected in the washout vials) divided by the intracellular sodium content at the time (determined as described above). Efflux rates were determined between 7 and 9 minutes after the start of washout. Ouabain-independent efflux was determined by including
FIGURE 1. Record a: effect of low-Na\(^+\) solution on rat denervated mesenteric small artery. The bars indicate the periods where the solutions were control activating solution (Na-K, 10 \(\mu\)M noradrenaline in K\(^+\)-saline), low-Na\(^+\) solution (25 mM Na\(^+\), sucrose substitution), contained ouabain (1 mM), and/or contained felodipine (1 nM). Elsewhere, solution was standard saline. Vessel internal diameter, \(L\), and response to control activating solutions, \(AT\), were 177 \(\mu\)m and 1.31 N/m, respectively. Record b: dependence of response to low-Na\(^+\) solution on time for which vessel was exposed to ouabain. Points show response of seven vessels which were exposed repetitively to low-Na\(^+\) solution (25 mM, sucrose substitution) for 2 minutes at 12-minute intervals. The dotted characteristic shows how [Na\(^+\)] \(_o\) depends on the time of ouabain exposure in such vessels (from Aalkjær and Mulvany, 1983). Record c: dependence of low-Na\(^+\) responses on sodium concentration of low-Na\(^+\) solution ([Na\(^+\)] \(_o\)). Vessels \((n = 3)\) had been exposed to ouabain for at least 1 hour and were then repetitively exposed to low-Na\(^+\) solutions having decreasing [Na\(^+\)] \(_o\) at 10-minute intervals. Points in records b and c show mean response ± SE.

1 mM ouabain in the washout solutions from time 7 minutes.

Results

Effects of Altered Sodium Gradient on Unstimulated Vessels

Figure 1a demonstrates the effect of altering the transplasmalemmal sodium gradient on the wall force of denervated mesenteric small arteries in the absence of other stimulating agents. Reducing the extracellular sodium concentration to 25 mM (sucrose substitution) had no effect on the wall force under normal conditions. If, however, the vessel was exposed to ouabain (1 mM) for a period (here, 1 hour), and then again subjected to a low-Na\(^+\) solution, a small response was elicited (Table 1). This response could not be inhibited by exposure to felodipine (1 nM) or to D600 (10 \(\mu\)M), even though these concentrations of felodipine and D600 were sufficient to inhibit the tonic response to K-PSS (Table 1). In contrast, if CaCl\(_2\) were omitted from the low-Na\(^+\) solutions, the response to low Na\(^+\) was eliminated (four vessels). Ouabain-exposed vessels also responded to choline-substituted low-Na\(^+\) solutions, but less strongly than to the sucrose substituted low-Na\(^+\) solutions and the response decayed with time [maximum response was 0.08 ± 0.02 AT (8)]. Note that, as seen in previous experiments

Statistics

Values are presented as mean ± SE (number of observations). The significance of changes or differences in mechanical responses and membrane potential was tested using the paired two-tail t-test. Elsewhere, differences were tested using the unpaired two-tail t-test, making allowance for differences in variance between groups where necessary (Sokal and Rohlf, 1969). All statements in the text that values are "greater than," "less than," caused potentiation," etc., indicate that the level of significance of the difference or change was at least 5%.

Nomenclature

\(E_m\): membrane potential (intracellular potential with respect to extracellular potential). \(E_m^c\), \(E_m^i\): equilibrium potentials for Na\(^+\), Ca\(^{2+}\), respectively \((=-(RT/Fz)\ln([X]\_i/[X]\_o))\), where R, T, F, z have their usual meanings and [X] \(_i\) and [X] \(_o\) are the intracellular and extracellular free concentrations of ion X). \(\Delta\mu_X\), \(\Delta\mu_{X^+}\): transplasmalemmal gradients for Na\(^+\) and Ca\(^{2+}\), respectively, defined as \(\Delta\mu_X = E_X-E_m\).

*We made one observation per vessel, unless otherwise indicated.
(Mulvany et al., 1982a, 1982c), ouabain exposure did not in itself produce any response.

The effects of choline-substituted low-Na⁺ solutions and of ouabain on membrane potential, Eᵢm, and on intracellular sodium concentration, [Na]ᵢ, are shown in Table 2. Exposure to low-Na⁺ solution for 2 minutes caused no change in Eᵢm (change in Eᵢm was −1.0 ± 1.1 mV (7)), and although the data suggest that there may have been (as expected) a decrease in [Na]ᵢ, this was not significant. Exposure of vessels to ouabain for 1 hour caused the vessels to depolarize by 6.3 ± 2.3 mV (4) and the [Na]ᵢ to increase by 53.1 mmol/(liter-cell). Low-Na⁺ solution following ouabain exposure caused a repolarization

### Table 1

**Effect of Felodipine and D600**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+ Felodipine</th>
<th>+ D600</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Response to</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K⁺-saline</td>
<td>0.46 ± 0.03 (14)</td>
<td>0.06 ± 0.02 (10)*</td>
<td>0.03 ± 0.01 (4)*</td>
</tr>
<tr>
<td>Low sodium + ouabain</td>
<td>0.21 ± 0.02 (15)</td>
<td>0.21 ± 0.02 (9)</td>
<td>0.26 ± 0.02 (6)</td>
</tr>
<tr>
<td>B Potentiation of NA response by</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ouabain</td>
<td>0.20 ± 0.04 (5)</td>
<td>0.04 ± 0.01 (7)*</td>
<td>0.04 ± 0.01 (4)*</td>
</tr>
<tr>
<td>KCl</td>
<td>0.31 ± 0.02 (5)</td>
<td>0.02 ± 0.01 (7)*</td>
<td></td>
</tr>
</tbody>
</table>

Part A shows response to low-sodium solution (25 mM, sucrose substitution) after exposure to ouabain (1 mM) for 60 minutes or to K⁺-saline (125 mM K⁺); Part B shows the potentiation of a submaximal noradrenaline (NA) dose caused by ouabain (1 mM) or the addition of KCl to give 30 mM K⁺. Values expressed as fraction of responses to control activating solution (mean ± SE). "Control" column shows responses under standard conditions. "+Felodipine" column shows values obtained after up to 30 minutes in felodipine (1 nM). "+D600" shows values obtained after 5 minutes in D600 (10 mM). In part B, the NA dose was adjusted for each vessel to give a response of about 0.3 AT before addition of ouabain or KCl; this required about 0.2 mM NA under control and + felodipine conditions, and about 10 mM under +D600 conditions.

* Significantly different (P < 0.05) from control value.
† Not measured.

### Table 2

**Effect of Ouabain on Wall Tension (AT), Membrane Potential (Eᵢm), and Intracellular Sodium Concentration ([Na]ᵢ) in Rat Mesenteric Small Arteries**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>[Na]ᵢ (mM)</th>
<th>[Ouabain] (mM)</th>
<th>Time (min)</th>
<th>ΔT (ΔTᵢ)</th>
<th>Eᵢm (mV)</th>
<th>[Na]ᵢ [mmol/(liter-cell)]</th>
<th>Δ[Na]ᵢ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>144</td>
<td>0</td>
<td>&gt;60</td>
<td>0</td>
<td>−53 ± 1 (19)</td>
<td>11.0 ± 1.2 (11)</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0</td>
<td>+2</td>
<td>0</td>
<td>−53 ± 2 (7)</td>
<td>9.7 ± 2.7 (3)</td>
<td>71</td>
</tr>
<tr>
<td>None</td>
<td>144</td>
<td>1</td>
<td>60</td>
<td>0</td>
<td>−47 ± 4 (4)</td>
<td>64.1 ± 6.2 (8)</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1</td>
<td>+2</td>
<td>0.11 ± 0.01 (9)</td>
<td>−57 ± 1 (7)</td>
<td>54.9 ± 4.2 (4)</td>
<td>28</td>
</tr>
<tr>
<td>0 2 μM NA</td>
<td>144</td>
<td>0</td>
<td>3</td>
<td>0.53 ± 0.04 (9)</td>
<td>−33 ± 1 (9)</td>
<td>14.9 ± 1.8 (10)</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>144</td>
<td>1</td>
<td>+2</td>
<td>0.72 ± 0.05 (9)</td>
<td>−59 ± 1 (9)</td>
<td>21.8 ± 2.2 (11)</td>
<td>72</td>
</tr>
<tr>
<td>2 μM NA</td>
<td>144</td>
<td>0</td>
<td>2</td>
<td>0.86 ± 0.02 (5)</td>
<td>−31 ± 2 (5)</td>
<td>15.3 ± 0.9 (9)</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0</td>
<td>+2</td>
<td>0.19 ± 0.05 (5)</td>
<td>−41 ± 2 (5)</td>
<td>10.3 ± 1.8 (9)</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1</td>
<td>+2</td>
<td>0.45 ± 0.07 (5)</td>
<td>−29 ± 2 (5)</td>
<td>11.8 ± 0.6 (10)</td>
<td>41</td>
</tr>
<tr>
<td>30 mM K</td>
<td>120</td>
<td>0</td>
<td>3</td>
<td>0.31 ± 0.10 (5)</td>
<td>−32 ± 1 (5)</td>
<td>16.0 ± 1.2 (9)</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>1</td>
<td>+2</td>
<td>0.45 ± 0.11 (5)</td>
<td>−28 ± 1 (5)</td>
<td>16.9 ± 2.5 (12)</td>
<td>73</td>
</tr>
<tr>
<td>30 mM K</td>
<td>120</td>
<td>0</td>
<td>2</td>
<td>0.24 ± 0.06 (5)</td>
<td>−31 ± 1 (5)</td>
<td>16.0 ± 1.2 (9)</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0</td>
<td>+2</td>
<td>0.47 ± 0.08 (5)</td>
<td>−26 ± 2 (5)</td>
<td>9.7 ± 0.7 (12)</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1</td>
<td>+2</td>
<td>0.55 ± 0.08 (5)</td>
<td>−23 ± 2 (5)</td>
<td>9.9 ± 0.9 (9)</td>
<td>40</td>
</tr>
</tbody>
</table>

Vessels were either unstimulated or stimulated, as indicated under "agonist"; extracellular sodium was either normal or low (25 mM, choline substitution), as indicated under [Na]ᵢ; ouabain was present or not, as indicated under "ouabain." Measurements were made after vessels had been in the solution indicated for time indicated under "time"; here "+" indicates that the vessels previously had been subjected to the procedure indicated in the row immediately above. ΔT and Eᵢm were measured simultaneously in vessels mounted on myograph. [Na]ᵢ measured in parallel experiments on unmounted vessels. ΔTᵢ is response to control activating solution (10 μM NA in K⁺-saline). All vessels were denervated. Values show mean ± SE (number of measurements in parentheses). Δ[Na]ᵢ shows sodium electrochemical gradient equal to Eᵢm − Eₛᵢ, where Eₛᵢ is the sodium equilibrium potential.

* When activated with either noradrenaline (NA) or potassium (K⁺): influence of extracellular sodium ([Na]ᵢ).
† Value is significantly different (P < 0.05) from value immediately above it by paired (ΔT, Eᵢm) or unpaired ([Na]ᵢ) t-test.
Circulation Research/Vol. 54, No. 6, June 1984

[\[E_m\] changed by \(-8.2 \pm 1.2\) mV (7)], but, again, although there was a trend toward a decrease in \([\text{Na}]_i\), this was not significant. From these measurements, the electrochemical gradient for sodium was calculated for the various conditions (Table 2).

In further experiments, the dependence of the response to low-\([\text{Na}]+\) solutions on the time of ouabain exposure was investigated (Fig. 1b). This showed that the response was initially zero and increased with time, the maximum response being reached after about 40 minutes. The dependence of this maximum response on \([\text{Na}]_o\) is shown in Figure 1c: the response is seen to be inversely related to \([\text{Na}]_o\) for \([\text{Na}]_o < 70\) mM. These results are therefore consistent with the response to low-\([\text{Na}]+\) solutions being a function of the transplasmalemmal sodium gradient.

**Effect of Ouabain on Activated Vessels**

Figure 2 shows the effect of ouabain on the wall force of mesenteric small arteries when submaximally activated with noradrenaline or with potassium. In both cases, ouabain produced potentiation of the response within a few seconds, whereafter the response remained fairly constant.

As indicated in the introductory portion, in other tissues the potentiating effect of ouabain is attributed to this glycoside inhibiting the Na-K pump. We have therefore examined the effect of ouabain on activated vessels when the Na-K pump should be inhibited by using K\(^+-\)free saline (Thomas, 1972) or by lowering the temperature (Hermsmeyer, 1976). In the absence of potassium, ouabain did not modulate the response to noradrenaline. Note that as seen previously (Mulvany et al., 1982c), K\(^+-\)free saline did not, in itself, cause any response in nonstimulated vessels. Likewise, when the temperature was lowered to 20°C, ouabain had no effect on the response to either noradrenaline or potassium. The results therefore suggest that, also in these small arteries, the potentiating effect of ouabain is due to the ouabain inhibiting the Na-K ATPase.

To investigate whether low temperature and K\(^+-\)free saline do indeed cause inhibition of the Na-K pump in these vessels, we measured sodium efflux rates (Table 3). Under normal conditions, 51% of the sodium efflux rate was ouabain dependent. However, at 20°C and in K\(^+-\)free saline, the sodium efflux rate was unaffected by ouabain and equal to the efflux rate measured in normal solutions in the presence of ouabain. Thus, the low temperature and K\(^+-\)free saline procedures do appear to cause inhibition of the Na-K pump.

The effect of felodipine on the potentiating action of ouabain is shown in Figure 3 and Table 1, which also show the effect of felodipine on the potentiating action of added KCl. In these experiments, vessels were submaximally activated with noradrenaline. Addition of ouabain (1 mM) or KCl (to give a final concentration of 30 mM K\(^+\)) both potentiated the response to noradrenaline by about the same amount. After exposure to felodipine (1 mM) for 30 minutes, the potentiating effects of ouabain and of KCl were almost eliminated. In further experiments, D600 (10 \(\mu\)M) was also found to eliminate the potentiating action of ouabain on the response to noradrenaline (Table 1).

**Ouabain Potentiation of Responses to Noradrenaline and Potassium in Low-Sodium Solutions**

Figure 4 demonstrates the results of experiments in which the potentiating action of ouabain was determined in choline-substituted low-\([\text{Na}]+\) solutions. In normal saline, as seen previously (Fig. 2), submaximal responses to noradrenaline and to po-

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**FIGURE 2.** Effect of low temperature and of K\(^+-\)free saline on the wall force responses of activated rat denervated mesenteric small arteries to ouabain. Records a, b, c and d, e are parts of continuous records in two experiments, the vessels having been held in standard saline for 15-30 minutes between each part. For the times indicated by the bars, solutions contained 0.2 \(\mu\)M noradrenaline (NA), 30 mM K\(^+-\)saline (K), 1 mM ouabain, or were K\(^+-\)free. In records a, b, and d, temperature was 37°C and in records c and e, 20°C. Vessel internal diameters, \(I_b\), and responses, \(\Delta T_w\), to control activating solution (10 \(\mu\)M NA in K\(^+-\)saline) were as follows. Records a-c: 198 \(\mu\)m and 4 N/m, records d and e: 190 \(\mu\)m and 3.06 N/m.
Dependence of Sodium Washout solution

<table>
<thead>
<tr>
<th>Washout solution</th>
<th>Control</th>
<th>+ Ouabain</th>
<th>% Ouabain inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard saline, 37°C</td>
<td>0.131 ± 0.003 (30)</td>
<td>0.065 ± 0.003 (6)*</td>
<td>51</td>
</tr>
<tr>
<td>K⁺-free saline, 37°C</td>
<td>0.075 ± 0.003 (5)</td>
<td>0.072 ± 0.003 (5)</td>
<td>4</td>
</tr>
<tr>
<td>Standard saline, 20°C</td>
<td>0.027 ± 0.002 (6)</td>
<td>0.028 ± 0.003 (9)</td>
<td>-4</td>
</tr>
</tbody>
</table>

Efflux rates (min⁻¹) shows as mean ± se (number of observations).

* Significantly different from control value

Tissue potassium were both potentiated by ouabain [potentiation of response was 0.28 ± 0.02 ΔT; (14) (noradrenaline), 0.17 ± 0.01 ΔT; (12) (potassium)]. Low-Na⁺ solutions reduced the response to noradrenaline even if, as here, the noradrenaline concentration was increased 10-fold. By contrast, the response to potassium was potentiated by low-Na⁺ solutions. However, the low-Na⁺ solutions did not affect the ability of ouabain to potentiate the response either to noradrenaline or to potassium: for both agonists, ouabain potentiated their responses in low-Na⁺ solution as in normal saline solution [0.27 ± 0.03 ΔT; (10) (noradrenaline), 0.09 ± 0.01 ΔT; (12) (potassium)]. Qualitatively similar results were obtained using sucrose-substituted low-Na⁺ solutions.

To measure the effects of these procedures on the sodium electrochemical gradient, we performed repeat experiments in one series to measure membrane potential (and simultaneously measured wall force) and in another series (using unmounted vessels) to measure intracellular sodium concentration (Table 2). Since we found that it was important to maintain the ion concentration of solutions in the electrophysiology experiments, all the low-Na⁺ solutions used in these experiments were choline-substituted, as well as in the experiments where [Na], was measured.

The reduction of the noradrenaline response caused by the 2-minute exposure to low-Na⁺ solutions was associated with a repolarization [change in E₉, was – 10.0 ± 2.5 mV (5)], and a 33% reduction in [Na]. The potentiating action of low-Na⁺ solutions on the potassium response was accompanied by a depolarization of 5.5 ± 1.2 mV (5) and a 39% reduction in [Na]. The potentiating action of ouabain was associated, in all cases, with a depolarization both with noradrenaline stimulation [in normal sodium solution: 4.5 ± 0.5 mV (9); in low-Na⁺ solution: 12.0 ± 0.9 mV (5)] and with potassium stimulation [in normal sodium solution: 3.6 ± 0.3 mV (5); in low-Na⁺ solution: 3.0 ± 0.5 mV (5)]. However, only in the case of the noradrenaline response in normal sodium solutions was the ouabain potentiation accompanied by an increase in [Na], [by 6.5 mmol/(liter-cell)]. The sodium electrochemical gradient after ouabain exposure decreased in all cases, but the extent of the decrease was much smaller in the potassium-activated vessels (4–5 mV) than in the noradrenaline activated vessels (14–16 mV).

Discussion

The aim of this investigation was to determine to what extent the intracellular sodium concentration ([Na]) may be a modulator of tone in rat mesenteric small arteries. Our results suggest that [Na], is not a major determinant of tone in these vessels, but do not exclude the possibility that it may play a minor modulating role.

Consequences of Reduced Transplasmalemmal Sodium Gradient

The indication that raised [Na], can cause raised [Ca], has been demonstrated in dialyzed squid axon, where it was proposed that the mechanism involved was Na-Ca exchange (Baker et al., 1969). Evidence...
Recently, the whole question of Na-Ca exchange as a calcium extrusion mechanism has been questioned (Aaronsen and Van Breemen, 1979). Recently, the whole question of Na-Ca exchange mechanism (Droogmans and Cas- 

cium (K+) wall force responses to rat denervated mesenteric small 

 attempt to support this contention it can, in particular, be shown in Figure 5a, which are obtained on the basis that, if transplasmalemmal Na-Ca exchange is the sole determinant of [Ca]i, then the electrochemical gradient for calcium, $\Delta \mu_{\text{Ca}}$, is given by:

$$\Delta \mu_{\text{Ca}} = r \cdot \Delta \mu_{\text{Na}}$$

where $r$ is the coupling ratio of the exchange process (Mullins, 1977). The expected tension has then been estimated from the calcium dependence of tension development found in chemically skinned vascular smooth muscle (Riegg and Paul, 1983) in the presence of calmodulin. The two dotted characteristics shown in Figure 5a refer to estimates made on the basis of the membrane potential being either $-30 \text{ mV}$ or $-60 \text{ mV}$, as indicated, and the coupling ratio for Na-Ca exchange being 4 (Mullins, 1977). Again, it is evident that for neither case is there any correlation between the estimated relation and the experimental data. Note that if the coupling ratio is less than 4 (Blaustein, 1977), then the calculated characteristics would be even further to the left, and their relation to the experimental data even worse.

It might be argued that our estimates of [Na] and $\Delta \mu_{\text{Na}}$ are inaccurate due to the method we have used to determine [Na]. Indeed, recent evidence has shown, using electron probe analysis, that in rabbit portal vein measurements of [Na] after 30 minutes, washout in lithium saline can seriously underestimate the true [Na] (Junker et al., 1984). However, the same authors showed that under normal conditions this was not the case for guinea pig taenia coli. This suggests that the lithium washout method may be useful for only certain preparations, but for the reasons advanced elsewhere (Aalkjær and Mulvany, 1981), with a hypothesis that the effects of sodium on calcium movement could be explained in terms of a competition between sodium and calcium for transplasmalemmal channels and for intracellular membrane binding sites.

The results of the present investigation suggest that neither the sodium electrochemical gradient, $\Delta \mu_{\text{Na}}$, nor the [Na] are of prime importance in determining the tone of rat mesenteric resistance vessels (Table 2). In particular, it may be noted first that decreasing $\Delta \mu_{\text{Na}}$ to 48 mV and increasing [Na] to 64 mmol/(liter-cell) (by exposing vessels to ouabain for 1 hour) did not result in any force development. Second, in noradrenaline-activated vessels, reduction of $\Delta \mu_{\text{Na}}$ to 26 mV (by reducing [Na] to 25 mM), caused a reduction of force. This lack of correspondence between measured force and sodium metabolism is emphasized by the dotted characteristics shown in Figure 5a, which are obtained on the basis that, if transplasmalemmal Na-Ca exchange is the sole determinant of [Ca]i, then the electrochemical gradient for calcium, $\Delta \mu_{\text{Ca}}$, is given by:

$$\Delta \mu_{\text{Ca}} = r \cdot \Delta \mu_{\text{Na}}$$

FIGURE 4. Effect of extracellular sodium concentration ([Na]o) on the potentiating effect of ouabain on the noradrenaline (NA) and potassium (K+) wall force responses to rat denervated mesenteric small arteries. For the periods indicated by the bars in the top part of the figure, the solutions contained 7 mM ouabain; NA (in the concentrations indicated), K+ (30 mM K+-saline), or low-Na+ (25 mM choline substitution). Records a and b refer to vessel with internal diameter, $l_1 = 189$ $\mu$m; records c and d refer to vessel with $l_1 = 193$ $\mu$m. Between records a and b, and between records c and d, vessels were held in standard saline for 10 minutes. Bars in lower part of figure show average active wall tensions ± se obtained from 10-12 sets of records similar to those shown immediately above them in top part of figure. The wall tensions being measured at the end of the 2 minutes for which the vessels were exposed to each solution.
Confirmation of this must await either electron probe or direct methods for measuring intracellular sodium activity in vascular smooth muscle, e.g., sodium-specific electrodes similar to those now being used in visceral smooth muscle (Aickin and Brading, personal communication). For the present, however, we consider that our results point strongly against a major role for [Na], or ΔμNa as determinants of tone in rat mesenteric small arteries.

On the other hand, our results suggest that ΔμNa does influence tone under extreme conditions. Reduction of ΔμNa to 28 mV [by 1 hour of ouabain exposure followed by exposure to low-Na\(^+\) solution (Table 2)] did produce a mechanical response. The response required the presence of calcium in the extracellular solution, and was dependent both on the time of ouabain exposure and the concentration of [Na],. The response was therefore consistent with it being due to a calcium influx regulated by the transplasmalemmal sodium gradient. Furthermore, the response was associated, as in sheep Purkinje fibres (Bers and Ellis, 1982), with a repolarization, and the response could not be inhibited by felodipine or D600 (Table 1): In other tissues, responses thought to be due to Na-Ca exchange have not been inhibited by calcium antagonists either (Ma and Bose, 1977; Baker, 1978; Chapman and Ellis, 1978; Ozaki and Urakawa, 1981). Therefore, although ΔμNa does not appear to be a major determinant of tone, it does appear that under extreme conditions it may play a role, and it therefore cannot be ruled out that, under more physiological conditions, Na-Ca exchange might also play a minor modulating role.

**Ouabain Potentiation of Active Responses**

As indicated in the introduction, the myogenic effects of ouabain are generally thought to be consequent to this glycoside inhibiting the Na-K pump in the vascular smooth muscle plasmalemma. The results of our present and previous investigations support this hypothesis. First, ouabain had no effect on the active responses when the Na-K pump was inhibited, either by temperature reduction or by exposure to K+-free saline (Fig. 1 b and c; Table 3). This corresponds to a previous finding that the acute depolarizing action of ouabain in resting rat mesenteric small arteries was not seen in the presence of K+-free saline (Mulvany et al., 1982c). Second, the potentiating action of ouabain is mimicked by K+-free saline (Mulvany et al., 1982c). Thus, although the relative insensitivity of rat Na-K ATPase to ouabain (Garay et al., 1978) meant that we had to use high concentrations, it does not seem that ouabain was acting through any mechanism other than Na-K pump inhibition.

Since ouabain, in general, causes both an increase in [Na], and depolarization, many of the arguments in favor of one or the other being responsible for the resulting potentiation have been difficult to resolve (Land and Blaustein, 1980). Our results, how-

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**Figure 5.** Data in Table 2 are plotted to show relationship between developed tension and (a) sodium electrochemical gradient, ΔμNa (where E\(_{\text{Na}}\)) is the sodium equilibrium potential, (b) membrane potential. E\(_{\text{m}}\). Filled symbols refer to measurements in normal sodium solutions; open symbols refer to measurements in low-Na\(^+\) solutions. (triangles, unstimulated vessels; circles, vessels stimulated with noradrenaline; squares, vessels stimulated with potassium). Single-arroved lines show effects seen on changing to low-Na\(^+\) solutions. Double-arroved lines show effects of changing to solution containing ouabain. Diamonds show measurements on unstimulated vessels after exposure to ouabain for 1 hour. Dotted lines show (a) calculated relations between developed tension and ΔμNa on the basis that (1) [Ca], is determined by Na-Ca exchange, with a coupling ratio of 4, according to Equation 3 in the text, (2) that [Ca], is related to tension, according to the experiments of Rüegg and Paul (1983) on skinned vascular smooth muscle in the presence of 4 μM calmodulin, (3) that cells contain 75% water, such that the free intracellular sodium concentration is (1/0.75) times greater than the [Na], values reported in Table 2, (4) that the membrane potential is -60 mV or -30 mV, as indicated, (5) relation between developed tension and E\(_{\text{m}}\), obtained during noradrenaline (NA) and potassium (K) dose-response determinations (Mulvany et al., 1982), as indicated.
ever, point against it being the increased [Na+] or decreased ΔψNa+) that is responsible for the potentiating action of ouabain. First, the ouabain potentiation of the response to potassium with normal sodium solutions and of the responses to both noradrenaline and potassium in low-Na+ solutions were not accompanied by any increase in [Na+] within 2 minutes, although depolarization was seen in all cases. Therefore, increased [Na+] is not a requirement for the potentiating action of ouabain. Second, the ouabain potentiation of the noradrenaline response was inhibited by felodipine and D600. Both these agents are thought to act preferentially on the potential-dependent calcium channels in the plasma membrane (see introduction), but not, as indicated above, on Na-Ca exchange. It would therefore seem that ouabain potentiation is more likely to be due to a potential-dependent increase in calcium influx rather than to a Na-Ca exchange-dependent decrease in calcium efflux.

This conclusion is supported by the data in Table 2 which indicate that, in contrast to the lack of correspondence between developed force and [Na+] or between developed force and ΔψNa+, there is a reasonable correlation between developed force and Em (Fig. 5b). In the noradrenaline- and potassium-activated vessels, the relation between developed force and Em is similar to the relation we have previously seen in these vessels during, respectively, noradrenaline and potassium dose-response determinations (fig. 5b, dotted characteristics). In the unstimulated vessels, the lack of response with 1 hour of ouabain exposure, where vessels depolarized to −47 mV, is consistent with the threshold for potential activation being about −40 mV (Mulvany et al., 1982b).

We thank Jørgen Byg Hansen for his help with some of the experiments and Jørgen Andresen and Kirsten Olesen for excellent technical assistance.

This work was supported by the Danish Medical Research Council. Address for reprints: M.J. Mulvany, Biophysics Institute, Aarhus University, Universitetsparken 185, DK-8000 Aarhus C, Denmark. Received January 24, 1983; accepted for publication March 15, 1984.

References


We thank Jørgen Byg Hansen for his help with some of the experiments and Jørgen Andresen and Kirsten Olesen for excellent technical assistance.

This work was supported by the Danish Medical Research Council. Address for reprints: M.J. Mulvany, Biophysics Institute, Aarhus University, Universitetsparken 185, DK-8000 Aarhus C, Denmark.

Received January 24, 1983; accepted for publication March 15, 1984.

References

INDEX TERMS: Sodium • Na-Ca exchange • Membrane potential • Ouabain • Felodipine • D600 • Resistance vessels
Intracellular sodium, membrane potential, and contractility of rat mesenteric small arteries.
M J Mulvany, C Aalkjaer and T T Petersen

Circ Res. 1984;54:740-749
doi: 10.1161/01.RES.54.6.740

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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