Vascular Smooth Muscle Response to Ouabain

Relation of Tissue Na\(^+\) to the Contractile Response

Laura Stewart, Christine Hamilton, Joanne Ingwall, Shojiro Naomi, Steven Graves, Mitzy Canessa, Gordon Williams, Norman Hollenberg

Smooth muscle responses to Na\(^+\) pump inhibition are thought to reflect two elements: a neurogenic contribution, involving catecholamine release from nerve terminals, and a myogenic response, attributed to relations between pump activity, [Na\(^+\)], and [Ca\(^{2+}\)]. In the present study, we describe the time course and magnitude of cell Na\(^+\) changes, assessed by two methods, atomic absorption and nuclear magnetic resonance spectroscopy during the myogenic contractile response of rabbit aorta strips to ouabain. A threshold concentration of \(3 \times 10^{-7}\) mol/L induced a gradual rise in [Na\(^+\)]. Both methods showed an essentially identical monotonic rise over 4 to 8 hours from a baseline level of 8 to 10 mmol/L water to a peak, which was approximately fivefold higher. The neurogenic (rapid) and myogenic (delayed and gradual) contractile responses were temporally distinct. Ouabain at \(10^{-7}\) mol/L, a concentration 10- to 100-fold lower than the threshold for catecholamine-dependent rapid-onset responses, induced only a delayed and gradual contractile response, which reached a maximum at 6 to 8 hours. With \(10^{-3}\) mol/L ouabain, the delayed response of 1.6±0.2 g peaked at 7.3±1.1 hours and was sustained for 16 hours. The time course was similar to that for change in [Na\(^+\)] but somewhat later. Ouabain at \(10^{-5}\) and \(10^{-4}\) mol/L induced a delayed response that was identical in magnitude but also induced an early rapid contractile response, which was prevented by reserpine or phenolamine pretreatment. These agents did not influence the delayed response. Reduction of the bath [Na\(^+\)] to 25 mmol/L by replacing sodium chloride with choline chloride abolished the delayed contractile response to \(10^{-4}\) mol/L ouabain. In parallel experiments, a fall in \(^{86}\)Rb uptake occurred within 15 minutes after exposure to ouabain; thus, limited diffusion of ouabain was not responsible for the delayed contractile response. The Na\(^+\) content and myogenic response of vascular smooth muscle to Na\(^+\) pump inhibition have a similar time course, gradual in onset and sustained for hours, in accord with models implicating intracellular Na\(^+\) in the vascular smooth muscle myogenic contractile response to Na\(^+\) pump inhibition. (Circ Res. 1993;73:1113-1120.)

**KEY WORDS**: ouabain • intracellular Na\(^+\) • catecholamines • \(\alpha\)-adrenoceptor blockade • phenolamine • reserpine • \(^{86}\)Rb uptake • rabbit aorta

The smooth muscle response to Na\(^+\) pump inhibition is determined by complex interrelations between pump activity, membrane potential, [Na\(^+\)], and possibly [Ca\(^{2+}\)]. Despite the crucial role of changes in [Na\(^+\)], in this model and despite the fall in Na\(^+\) efflux from vascular smooth muscle that has been measured with pump inhibition, little is known about the time course and magnitude of cell Na\(^+\) changes and their relation to the myogenic contractile response. These relations are further complicated by a neurogenic element reflecting catecholamine release from nerve terminals. Our premise in the present study was that shifts in the intracellular milieu due to inhibition of the vascular smooth muscle Na\(^+\) pump would occur very slowly, so that only gradual shifts in vascular tone would occur as a result of Na\(^+\)-linked myogenic mechanisms.

Because of the possible limitations of methods available to us for measuring [Na\(^+\)], we used two methods, atomic absorption studies of ashed tissue and nuclear magnetic resonance (NMR) spectroscopy of intact rabbit aorta.

**Materials and Methods**

The first approach used to estimate intracellular Na\(^+\) content involved atomic absorption and a modification of the method for tissue preparation of Friedman. Twenty to 24 strips were prepared from each rabbit aorta, and at least 4 rabbits were used for each study. These tissues were equilibrated for 2 hours at 37°C in Krebs’ buffer aerated with 95% O\(_2\)-5% CO\(_2\). Ouabain was used at \(10^{-6}\) or \(10^{-5}\) mol/L, with samples taken for analysis at frequent intervals up to 16 hours (see Fig 1). Control incubation medium contained no ouabain. Tissues were removed from the muscle chamber and placed in a 2°C isotonic choline chloride solution (mmol/L: KCl, 4.6; CaCl\(_2\), 2.5; MgSO\(_4\), 1.2; KH\(_2\)PO\(_4\), 1.2; glucose, 10; and choline chloride, 143; pH 7.4) adjusted by choline base (Sigma Chemical Co, St Louis, Mo) for 30 minutes to remove extracellular sodium ion. The 30-minute incubation was selected because preliminary experiments showed a rapid removal of extracel-
lular tissue Na⁺ content over the first 15 minutes of exposure to the cold choline chloride solution, and then the fall was greatly slowed, with approximately first-order kinetics from 30 to 180 minutes. The tissues were blotted gently on filter paper, weighed wet, dried at 120°C for 20 hours, and weighed again. The difference between wet and dry tissue weight was used as the tissue water content. The dried tissues were ashed in a furnace at 400°C overnight. The ashed material was dissolved in 1 mL double-distilled water to liberate sodium ion. After fourfold dilution, the [Na⁺] was determined by atomic absorption.

For the ²³Na NMR studies (our second technique to measure tissue Na⁺ content), the dysprosium (Dy) salt of triethylenetetraminehexa-acetic acid (TTHA), Na₂Dy(TTHA)-3NaCl (10 mmol/L) (prepared from TTHA, Aldrich Chemical Co.) or DyCl₃ (Alpha Chemicals) by the method of Chu et al.,⁷ was added to the buffer, and the NaCl concentration was adjusted so that the total [Na⁺] remained at 145 mmol/L. [CaCl₂] was increased to 3 mmol/L to compensate for the buffering effects of the shift reagent Na₂Dy(TTHA) and EDTA; free [Ca²⁺] was 1.1 mmol/L. The buffer was bubbled with 95% O₂-5% CO₂ to maintain pH at 7.4. To eliminate the contribution of Na⁺ in the buffer to the ²³Na NMR spectrum, the vascular smooth muscle samples were superfused with a Na⁺-free isotonic mannitol solution containing the shift reagent [mol/L]: Dy(TTHA)-3Tris-HCl, 0.01; mannitol, 0.25; and Tris, 0.004; pH 7.4] for 10 minutes before and 5 minutes during spectral acquisition.

A Nicolet wide-bore NT-360-MHz (8.45 T) NMR spectrometer (Nicolet Magnetics Corp., Fremont, Calif) operating at 95.24 MHz for ²³Na was used with a 12-mm sodium/phosphorus dual probe. ²³Na NMR spectra were acquired with 90-second pulses and an interpulse delay of 0.25 seconds with a 5-minute time resolution. The sweep width was ±2000 Hz, and 1000 to 2000 data points were collected. Exponential multiplication of the free induction decay was used for sensitivity enhancement (line broadening of 8 Hz). Resonance areas from ²³Na NMR spectra were calculated with the NMR1 curve-fitting/integration program (NMRi, Syracuse, NY) using a SUN 3/260 data station.

The rabbit aorta strip preparation used in this laboratory to assess contractile responses has been described in detail.⁸ It is based on the original description of Furchgott and Bhardakom.⁹ In brief, four to eight strips from each rabbit aorta were mounted with 4 g of tension in muscle chambers with a 20-mL working volume containing a modified Krebs' medium. The presence of endothelium does not influence contractile responses to ouabain,¹⁰ and it was not removed. The solution was maintained at 37±0.5°C and aerated constantly with a gas mixture containing 95% O₂-5% CO₂. Isometric contractions were measured with force transducers (model FT23D, Grass Instruments) and a polygraph recorder (model 7D, Grass Instruments).

The tissues were allowed to equilibrate for 60 minutes before study. The total interval in Krebs' buffer from removal of the tissue to first exposure to a vasoactive agent was at least 120 minutes, of which less than 30 minutes was at room temperature. Norepinephrine, serotonin, or angiotensin at 10⁻⁷ mol/L was used to assess reactivity, but individual rings were exposed to only one of these agents. Ouabain was used in concentrations ranging from 10⁻⁹ to 10⁻⁴ mol/L. Individual rings were exposed to only a single concentration. The contribution of catecholamines to the contractile response was assessed either through the use of phenolamine mesylate (10⁻⁴ mol/L) or reserpine pretreatment (2 mg/kg for 24 hours before study). The response in each case was monitored for 16 hours.

**Table 1. Effect of Ouabain on Tissue Na⁺ Content**

<table>
<thead>
<tr>
<th>Ouabain Concentration, mol/L</th>
<th>Tissue Na⁺ content, mmol/L H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10⁻⁸</td>
</tr>
<tr>
<td>10.4±0.4</td>
<td>9.6±1.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Measurements were made after 16 hours of continuous exposure to ouabain. Fifteen strips were used for each measurement.

*P<.01 vs control.
To assess further the contribution of changes in tissue Na\textsuperscript{+} content to the contractile response in this preparation, we reduced [Na\textsuperscript{+}] in the bath to 25 mmol/L by replacing NaCl with choline chloride in 12 experiments and measured the contractile responses to ouabain (10\textsuperscript{-5} mol/L).

Vascular smooth muscle Na\textsuperscript{+}-K\textsuperscript{+} pump activity was assessed as \textsuperscript{86}Rb uptake by a modification of the method of Hougen et al.\textsuperscript{11} Tissues were incubated in Krebs' buffer and bubbled with O\textsubscript{2}+CO\textsubscript{2} for 2 hours in the same manner we used for determining intracellular Na\textsuperscript{+} content. Uptake of \textsuperscript{86}Rb was initiated by transferring tissues from the muscle chamber to a 10-mL modified Krebs' bicarbonate solution containing 10 \muCi/mL \textsuperscript{86}Rb (specific activity, 1.28 to 12.8 mCi/mg; New England Nuclear) in the absence or presence of ouabain (10\textsuperscript{-4} or 10\textsuperscript{-5} mol/L). Incubation at 37°C with gentle shaking was stopped by removing tissues from the incubation vessel, followed by serial washing in buffer. Tissues were then blotted with filter paper and weighed. The radioactivity incorporated into the tissues was determined with a gamma well counter (Auto gamma 5000, Packard), and total uptake was calculated as millimoles per milligram wet tissue weight.

Mean values are presented along with a standard error of the mean as the index of dispersion. Differences between strips were assessed by Student's \textit{t} test, and dose-response relations were determined by analysis of variance (ANOVA). For multiple comparisons with a single baseline value, we used Dunnett's \textit{t} test, a form of ANOVA. Levels of \(\alpha<.05\) were considered statistically significant.

Results

Tissue Na\textsuperscript{+} by Atomic Absorption and \textsuperscript{23}Na NMR

The normal vessel wall showed a ratio of dry to wet weight of 0.286±0.027 (\(n=12\)). After 16 hours of incubation with 10\textsuperscript{-5} mol/L ouabain, the dry to wet weight ratio was unchanged (0.287±0.015). Baseline aortic [Na\textsuperscript{+}], measured by atomic absorption was 8.0±0.1 mmol/L and remained at that level during prolonged

![Graph](image)

**Fig 2.** Time course of changes in [Na\textsuperscript{+}] in rabbit aorta measured by nuclear magnetic resonance after exposure to 10\textsuperscript{-5} mol/L ouabain. Note the similarity of the time course to the responses documented in Fig 1. The response to removal of ouabain (washout) is also shown. Each symbol represents a single time point in an individual study.

![Graph](image)

**Fig 3.** Representative \textsuperscript{23}Na nuclear magnetic resonance spectrum from 250 mg rabbit vascular smooth muscle during control superfusion with modified Krebs-Henseleit buffer containing 10 mmol/L Dy(triethylene-tetraminehexaacetic acid)\textsuperscript{3-} (bottom) after 8.25 hours of superfusion with buffer containing both shift reagent and 10\textsuperscript{-5} mol/L ouabain (middle) and after 2.25 hours of washout with buffer containing shift reagent but not ouabain (top). Use of the cationic shift reagent, Dy(triethylene-tetraminehexaacetic acid)\textsuperscript{3-} allows discrimination of intracellular Na\textsuperscript{+} (Na\textsuperscript{+}\textsubscript{i}) and extracellular Na\textsuperscript{+} (Na\textsuperscript{+}\textsubscript{o}). Vascular smooth muscle was flushed with Na\textsuperscript{+}-free mannitol before data acquisition to reduce contribution from Na\textsuperscript{+}\textsubscript{o}.
incubation in control strips (Fig 1). In parallel experiments, for the dose-response assessment, the value was essentially identical (Table 1). Changes in [Na⁺] during ouabain administration were gradual (Fig 1), reaching a peak of 42.2±4.5 mmol/L 4 hours after a dose of 10⁻⁴ mol/L ouabain and 40.1±1.1 mmol/L 16 hours after 10⁻⁶ mol/L ouabain by atomic absorption. The relation between ouabain concentration and tissue Na⁺ content is summarized in Table 1. Changes in cytosolic free Na⁺ content showed a similar pattern by ²³Na NMR measurement after exposure to ouabain. [Na⁺] increased progressively during 8 hours of incubation with 10⁻⁵ mol/L ouabain (Fig 2). The ²³Na signal that corresponds to [Na⁺], increased monotonically, from 8 mmol/L during control perfusion to 50 to 60 mmol/L by 5 hours of ouabain superfusion, and remained at this value for the duration of the ouabain superfusion (total of 8.25 hours). The time to half-maximal [Na⁺] was approximately 3 hours. Since [Na⁺] was maintained at 145 mmol/L, the Na⁺ gradient across the plasma membrane, [Na⁺]/[Na⁺], decreased from approximately 18 to 2.4. When the tissue was superfused with ouabain-free buffer, [Na⁺], decreased rapidly and monotonically to control levels within 2 hours; the time to half-maximal Na⁺ efflux was 45 minutes. Representative tracings of ²³Na NMR signals are shown in Fig 3.

**Contractile Responses**

Control strips showed no change in tone (−0.003±0.005 g). A ouabain concentration of 1×10⁻⁶ mol/L was subthreshold: no contractile response occurred with this ouabain concentration over the 16 hours the tissues were monitored. A small, gradual, late contractile response (0.10±0.01 g) peaked at 6 to 8 hours with 10⁻⁷ mol/L ouabain (Fig 4). A 1×10⁻⁶ mol/L ouabain concentration in all cases elicited a gradual contraction. Tone rose very gradually over approximately 4 to 5 hours and then more rapidly to achieve a peak response of 1.6±0.2 g at 7.3±1.1 hours after ouabain administration (Fig 5). The magnitude of the delayed contractile response did not differ significantly at 10⁻⁶, 10⁻⁵, and 10⁻⁴ mol/L ouabain, but the responses evolved more quickly and peaked earlier with increasing ouabain concentration (Table 2).

A 10⁻⁴ mol/L ouabain concentration also induced a rapid early response in tension (Fig 6), evident in minutes, and reached a peak response within 30 minutes (1.6±0.16 g). Thereafter, a fall and a secondary rise occurred to 1.5±0.1 g, followed by a well-sustained contraction thereafter. The magnitude of the early and delayed responses to 10⁻⁵ mol/L ouabain was essentially identical to the magnitude of responses to 10⁻⁴ mol/L ouabain (Fig 4). After the second peak, there was a variable fall in vascular tone, but the contractile plateau always remained well above baseline.

Pretreatment of aortic strips with 10⁻⁶ mol/L phentolamine sharply attenuated the early aortic contraction induced by 10⁻⁷ and 10⁻⁵ mol/L ouabain. Similar effects were seen with reserpine pretreatment. Phentolamine and reserpine, however, had no effect on the delayed

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**Fig 4.** Graph showing relation between ouabain concentration and contractile response of the rabbit aorta. The early and late responses differ not only in their ouabain concentration requirement and time course (See Figs 5 and 6) but also in their sensitivity to phentolamine or reserpine pretreatment (Fig 6).

**Fig 5.** Graph showing aortic contractions induced by 10⁻⁶ mol/L ouabain given at time 0. Each data point represents mean±SEM of the response of 15 rabbit aorta strips from six rabbits. Phentolamine pretreatment (study not shown) did not influence the response.
TABLE 2. Ouabain Concentration and the Time to the Half-Maximal Rise in [Na+] and Smooth Muscle Concentration

<table>
<thead>
<tr>
<th>Ouabain Concentration, mol/L</th>
<th>10^{-7}</th>
<th>10^{-6}</th>
<th>10^{-5}</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Na+] t_{1/2}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time, min</td>
<td>278±22</td>
<td>171±28</td>
<td>125±14</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>VSM t_{1/2}</td>
<td>329±24</td>
<td>211±16</td>
<td>156±12</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are mean±SEM for time to half-maximal rise in [Na+] ([Na+] t_{1/2}) and time to half-maximal vascular smooth muscle contraction (VSM t_{1/2}) in phentolamine-pretreated rabbit aortic strips (n).

contraction induced by ouabain at any ouabain concentration.

There was a good correlation (r=.99, F=2765, P<.001) between the half-time to maximal rise in tissue Na+ content and the half-time to peak contractile response in phentolamine-treated strips for the three ouabain concentrations studied (Table 2). The measurements of Na+ content and contractile response were made in different strips, so that concordance was assessed for average values. In each case, the rise of Na+ content anticipated the contractile response.

Reduction of bath [Na+] to 25 mmol/L by replacement with choline totally abolished the late contractile response to 10^{-6} mol/L ouabain in each of the 12 experiments. Exposure of the smooth muscle strips to the reduced [Na+] resulted in two minor and one major change in response. The two minor responses were evident early. First, in the 2 hours allowed for equilibration, after application of 4-g tension, strips exposed to the usual 145 mmol/L [Na+] showed the anticipated relaxation, from 3.2±0.06 g to 2.49±0.11 g. The strips exposed to 25 mmol/L Na+ did not relax (3.25±0.08 g to 3.81±0.23 g). Second, the contractile response to 3×10^{-7} mol/L norepinephrine was somewhat reduced by exposure to 25 mmol/L Na+ (3.6±0.16 g versus 2.8±0.13 g, P<.01). The major change involved the response to exposure to ouabain following the 2-hour equilibration (Table 3). The strips bathed in 145 mmol/L Na+ showed the anticipated gradual contractile response that peaked at a 2.7±0.3 g increase, 6 hours after 10^{-6} mol/L ouabain administration, well maintained to 16 hours (1.8±0.13 g). Strips bathed in 25 mmol/L Na+ showed a continued gradual contractile response that peaked at 10 hours (0.8±0.2 g), and addition of ouabain had no additional effect (Table 3).

A fall in total ^86Rb uptake was demonstrable 15 minutes after exposure to ouabain (Fig 7), occurring well before the minimal early contractile response at 10^{-6} mol/L, and again was concentration related. The relation between ouabain concentration and ^86Rb uptake at 2 hours is summarized in Table 4.

Discussion

Vascular smooth muscle responses to Na+ pump inhibition are widely believed to reflect both myogenic and neurogenic components, and both elements participated in the contractile response of rabbit aorta to ouabain in the present study. The catecholamine-dependent response was evident within minutes, required rather high ouabain concentrations, was prevented by α-adrenergic blockade or catecholamine depletion, and occurred well before measurable changes in tissue Na+ content. This response is thought to represent ouabain-induced catecholamine release from neural elements in the vessel wall and probably reflects pump-dependent neural function.\(^5,12,13\) Assessment of this well-known response was not a goal of the present study, and so we did not attempt to ascertain whether higher phentolamine concentrations would abolish the early response entirely.

The second, delayed response was resistant to α-adrenergic blockade, occurred at a ouabain concentration more than an order of magnitude lower than that required for the early response, and displayed a coherent relation in time to the increase in [Na+]. This delayed contractile response was sustained for at least 16 hours after its onset.

The first report on the effectiveness of cardiac glycosides on isolated arterial strips indicated the need for rather high concentrations: 10^{-4} mol/L strophanthidin was required to induce a contraction, and 10^{-6} mol/L was ineffective.\(^14\) Broekhart and Godfraind\(^15\) confirmed this relative insensitivity and reported that the response of rabbit aorta to ouabain at 10^{-5} mol/L was prevented by α-adrenergic blockade. They concluded that ouabain-induced contraction was due to the release of endogenous catecholamines. That observation was quickly

![Graph showing in vitro aortic contractions induced by 10^{-4} mol/L ouabain given at time 0. Pretreatment with phentolamine (10^{-4} mol/L) before the addition of ouabain attenuated the early response but did not modify the late response. Each data point represents mean±SEM of the response of 15 to 18 strips from seven rabbits.](http://circres.ahajournals.org/)

Fig 6.
confirmed and extended: phentolamine, phenoxybenzamine, reserpine pretreatment, and removing the source of catecholamines by stripping the adventitia prevented a contractile response.\textsuperscript{16,18} In all of these reports, the duration of the experiments described was brief, and it is likely that only acute short-term responses were evaluated. Gillis and Quest\textsuperscript{6} reviewed the substantial literature that suggests a role for neuronal Na\textsuperscript{+}, K\textsuperscript{−}-ATPase in both catecholamine uptake and release.

Latency in the smooth muscle response to digitalis glycosides has also been noted,\textsuperscript{1,7,19} but mechanisms responsible for the latency have not been explored. One possibility, that latency reflected very slow diffusion of the bulky and polar ouabain molecule to the smooth muscle cells, is unlikely. Although hours were required for changes in [Na\textsuperscript{+}], and the smooth muscle contraction to evolve and peak, a fall in \textsuperscript{86}Rb uptake, an index of pump activity, was evident in minutes in the present study. Also consistent, labeled ouabain binds quickly in the rabbit aorta.\textsuperscript{20} The substantial delay in the increase in [Na\textsuperscript{+}] probably reflects primarily the rate of Na\textsuperscript{+} leak into the vascular smooth muscle cell.\textsuperscript{21} The sodium pool in vascular smooth muscle that is sensitive to pump inhibition shows very slow exchange.\textsuperscript{22} The rate of rise in [Na\textsuperscript{+}], in the present study showed a time pattern closely paralleling the delayed smooth muscle contractile response. Moreover, in this preparation, as in others,\textsuperscript{18,23} prevention of Na\textsuperscript{+} influx by reducing bath [Na\textsuperscript{+}] to 25 mmol/L prevented the delayed contractile response, although the early response was intact.

The measurements that we have reported as [Na\textsuperscript{+}] by atomic absorption probably underestimate somewhat the change, as no attempt was made to correct for extracellular space with an appropriate tracer. Indeed, there is evidence for multiple extracellular compartments and membrane Na\textsuperscript{+} compartments beyond simple solution in water, which would make such corrections inaccurate.\textsuperscript{22} On the other hand, the measurements provide both a reasonable estimate for normal [Na\textsuperscript{+}], and a predictable and coherent response to pump inhibition that showed excellent accord with the contractile response. An alternative measurement of the changes in vascular smooth muscle Na\textsuperscript{+} content was provided by \textsuperscript{23}Na NMR. By using a paramagnetic shift reagent to alter the characteristic NMR resonance of the extracellular Na\textsuperscript{+}, the unperturbed intracellular Na\textsuperscript{+} NMR signal is proportional to only the cytosolic free Na\textsuperscript{+} content of the vascular smooth muscle. The fact that two methods, wet chemistry and \textsuperscript{23}Na NMR, each using different assumptions, were in good accord with each other and with the time course of the contractile response provides compelling evidence for the temporal sequence.

One observation, however, does not fit with the time course defined in the present study. Changes in [K\textsuperscript{+}], induce rapid shifts in vascular smooth muscle tone, a response thought to reflect an influence on the pump.\textsuperscript{24-27} Without exception, the smooth muscle responses have occurred much more promptly than those defined in the present study, indeed in good agreement with the model of transient and steady-state changes proposed by Brace and Anderson.\textsuperscript{28} No information elicited by the present study makes it possible to reconcile differences in the time course of contractile response when ouabain or potassium is used to influence the pump.

There are two hypotheses concerning the mechanism of the myogenic contractile response, Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange or depolarization.\textsuperscript{29,30} Although Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange has long been recognized in the heart,\textsuperscript{31,32} and evidence for a similar mechanism in vascular smooth muscle has been found,\textsuperscript{33,34} the magnitude of the contribution of Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange to vascular smooth muscle responses has remained in doubt.\textsuperscript{29,30} To the extent that the present study has clarified the time course and magnitude of vascular smooth muscle [Na\textsuperscript{+}] changes following pump inhibition, the way has been cleared for clarification of this mechanism.

Few drugs, if any, have a single specific action, and it is unlikely that ouabain, used in the present study, is an exception to that rule. Other actions, such as depolarization of vascular smooth muscle\textsuperscript{35} or changes in ion conductance,\textsuperscript{36} could have influenced the results. On the other hand, the concordance in the time course and

<table>
<thead>
<tr>
<th>[Na\textsuperscript{+}], mEq/L</th>
<th>Response to 3x10\textsuperscript{-7} g/L Norepinephrine, g</th>
<th>Ouabain Concentration, mol/L</th>
<th>Response After Ouabain Administration, g</th>
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<tbody>
<tr>
<td>145</td>
<td>3.6±0.16</td>
<td>10\textsuperscript{-6}</td>
<td>2.7±0.3</td>
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<tr>
<td>25</td>
<td>2.7±0.2</td>
<td>10\textsuperscript{-6}</td>
<td>0.26±0.2</td>
</tr>
<tr>
<td>25</td>
<td>2.9±0.2</td>
<td>0</td>
<td>0.31±0.2</td>
</tr>
</tbody>
</table>

![Fig 7](http://circres.ahajournals.org/Downloaded from http://circres.ahajournals.org/)

**Fig 7.** Time course of change in total \textsuperscript{86}Rb uptake in the presence and absence of ouabain. Each point represents the mean of 7 to 10 strips for five rabbits. Note that inhibition of rubidium uptake is evident within 15 minutes and, thereafter, shows progressive concentration-related effects of ouabain.
dose relations documented in the present study makes it likely that Na\(^+\) pump inhibition was involved as the dominant contributor to the relations. We have no explanation for the small reduction in vascular tone after the second peak. Possibilities include gradual reduction in the ouabain concentration with its internalization or an unrelated action of the glycoside.

Observations in the present study were made on the rabbit aorta. The time course and magnitude of the contribution of the neurogenic and myogenic elements may vary from one vascular smooth muscle preparation to another, in part depending on the amount of neural tissue present and the number of pump sites. The contribution of catecholamines to the cerebral vascular response, for example, appears to be minimal in the dog and monkey.\(^{18}\) The rabbit aorta does not have an extensive innervation\(^{37}\) and yet shows a striking catecholamine-dependent contribution to the contractile response to ouabain. In the more richly innervated canine saphenous vein, Monteiro\(^{13}\) has recently demonstrated catecholamine release that varies with ouabain concentration and with time after exposure to ouabain.

Leonard\(^{14}\) concluded the original description of the contractile response of isolated vascular smooth muscle to digitalis glycosides on a speculative note, concerning whether there are endogenous substances that might influence arteriolar tone through a similar mechanism. There is still substantial interest in the subject,\(^{29,36}\) but the responsible moiety has remained elusive. The present study has shown that vascular smooth muscle, at least that of the rabbit aorta, is an order of magnitude more sensitive to Na\(^+\) pump inhibition than has been previously recognized. A well-sustained tonic response is a characteristic appropriate to an agent that might contribute to high blood pressure.

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### References

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