Calcium-Dependent Fluxes of Potassium-42 and Chloride-36 during Norepinephrine Activation of Rat Aorta

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SUMMARY. This study was designed to determine whether α-receptor-stimulated monovalent ionic fluxes in rat aorta required calcium, and, if so, whether both extracellular calcium and cellularly stored calcium are active. Calcium removal in the presence of 10 mM magnesium (to maintain membrane stability) inhibited the norepinephrine-stimulated increase in potassium-42 and chloride-36 efflux. However, the norepinephrine-stimulated increase in sodium-24 influx was relatively resistant to calcium depletion. Protocols were designed to measure the time course for the changes in potassium-42 efflux and contraction when calcium was removed or replaced in the presence of norepinephrine. The dose-dependent effect of a calcium antagonist (diltiazem) was also measured. A close correlation (r = 0.94) was found between inhibition of contraction and potassium-42 effluxes which followed the regression: % potassium-42 response = 1.0 X (% contraction) + 1.8%. The slope of 1.0 and intercept near zero suggests the hypothesis that norepinephrine-stimulated potassium-42 efflux and contraction are co-dependent on cellular calcium concentration. This co-dependence held for short phasic responses (~1 minute), as well as longer tonic responses (~25 minutes). It appears that calcium-dependent potassium-42 effluxes can be supported by both the influx of extracellular calcium and release of cellular stores. It is concluded that calcium-dependent potassium channels (and possibly chloride channels) are operative in rat aorta and are an important component of the graded membrane response to norepinephrine. The sodium channels, however, do not appear to share this same calcium dependency. (Circ Res 56: 507-516, 1985)

EXCITATION of arterial smooth muscle by catecholamines is associated with increases in fluxes of ions down their electrochemical gradients (Jones, 1980). Much attention has been given to measures of Ca ++ fluxes in a variety of arteries and species during excitation-contraction coupling (Cauvin et al., 1983; Daniel et al., 1983), and the concept has been developed that α-receptor activation of arterial smooth muscle increases both Ca ++ influx and release from cellular sites (Van Breemen et al., 1980). These preparations generally respond to such stimuli with graded depolarization (Somlyo et al., 1969; Hermsmeyer, 1976); hence, they have been classified as a tonic type of smooth muscle. The timing and magnitude of the changes in K + conductance which accompany α-receptor activation appear to be independent determinants of the tonic response. Large dose-dependent increases in 42K efflux accompanied α-receptor activation in arteries from rats (Jones, 1973) and rabbits (Droogmans, et al., 1977; Martin and Gordon, 1983). The changes reflected alterations in K + permeability since they exceeded the changes in efflux predicted for a simple reduction of the electrochemical driving force (Jones, 1980). Inhibition of K + conductance by tetraethylammonium ion (TEA) resulted in the generation of action potentials in previously tonic arterial smooth muscle (Droogmans et al., 1977; Harder, 1982). These observations indicate that the coupling between conductances which tend to depolarize smooth muscle (Ca ++, Na +, Cl-) and that which hyperpolarizes (K +), is a critical determinant of the type of response.

The coupling between α-receptor occupancy and subsequent changes in ionic events is not well understood. Bolton (1979) suggested that receptors could operate ionic channels directly. Separation of events modulated by receptors from those secondary to changes in membrane potential is not complete. In general, the α-receptor-controlled influx of Ca ++ was additive to that induced by depolarization, and was not as sensitive to blockade by Ca ++ antagonists (Meisheri et al., 1981). It is unclear to what extent α-receptor-related changes in the movements of Na +, K +, and Cl - fall directly under the control of receptors or are secondary events. We reported that cAMP-dependent inhibition of norepinephrine (NE)-induced contraction in rat aorta was closely related to parallel inhibition of α-receptor-induced increases in 42K and 36Cl efflux (Jones et al., 1984). One explanation for this parallel inhibition is that elevated cAMP results in maintenance of cytoplasmic Ca ++ at levels below those needed for contraction and to open Ca ++-dependent ionic channels.
The Ca++ dependence of K+ movements has been observed in a wide variety of tissues. Gardos (1958) reported that the net efflux of K+ from red blood cells was increased by cellular Ca++. This observation has been confirmed in red cells with analyses of unidirectional fluxes of isotopes (Yingst and Hoffman, 1984) and by patch clamp methods (Grygorczyk et al., 1984). Ca++-dependent activation of K+ channels has also been observed in excitatory cells such as nerve (Meech, 1978) and skeletal muscle (Barrett et al., 1982). Relatively few studies of Ca++-dependent K+-conductance have been published for smooth muscle. Recently, Singer and Walsh (1984) have used patch clamp methods to identify a Ca++-dependent K+-conductance in membranes from the stomach muscle of the *Bufo marinus*. Den Hertog and Jones et al., (1984). We removed the endothelial cells from the tissues were mounted on stainless steel holders, followed by a 3-

**Solutions**

The normal physiological solution had the following composition (in mM): Na+, 146.2; K+, 5.0; Mg++, 1.2; Ca++, 2.5; Cl-, 143.9; HCO3-, 13.5; H2PO4-, 1.2; and glucose, 11.4. Solutions were gassed with a 97% O2-3% CO2 mixture to obtain a pH of 7.4. Propranolol (3 μm, Sigma), ethylenediaminetetracetic acid (0.025 mM), and ascorbic acid (1 mM, Sigma) were added to all solutions for β-receptor blockade and to inhibit oxidation of catecholamines. Aqueous stock solutions were prepared for NE (Sigma) and diltiazem, (gift from Marion Laboratories). The 0 Ca++ solutions were prepared with no Ca++, 10 mM Mg++ (as Cl- salt), and 2 mM ethyleneglycol-bis-(β-amino-ethyl)ether)N,N'-tetraacetic acid (EGTA). The corresponding control solutions contained elevated Mg++ (10 mM) and Ca++ = 2.5. In one series, 4.5 mM Ca++ (Cl- salt) was added to the 0 Ca++ solution to compensate for the 2 mM EGTA.

**Isotope Fluxes**

Routine equilibration and washout methods were used (Jones, 1973, 1981). For efflux analyses, open strips were incubated for 3 hours at 37°C in solution containing 42K (20 μCi/ml, University of Missouri Research Reactor) or 4K plus 36Cl (2 μCi/ml, ICN). After a 2-second rinse, each tissue was passed through a series of vigorously gassed tubes containing nonradioactive solution. Solution changes, e.g., drugs, 0 Ca++, temperature, can be made quickly with this method, and diffusional delays can be maintained to a minimum (Jones, 1980). Washout curves were computed (IBM) by sequentially adding the tissue and tube radioactivity (measured by standard gamma and liquid scintillation methods) in reverse order and by normalization in terms of initial activity. Corrections for isotopic decay were also made. The fraction of the isotope exchanged per minute which represents the rate constant, k (per min), was calculated for each washout period and was used to compare the effects of drugs and ionic changes.

The influx of 24Na (University of Missouri Research Reactor) was done on tissues that had been equilibrated 3 hours at 37°C in the normal physiological solution. The strips then were transferred to either 0 Ca++ solution or a high Mg++ (10 mM) physiological solution for either 5 or 20 minutes. Half the strips were exposed to NE for an additional half-minute, or for 5 minutes. The strips then were transferred into the same solution (±Ca++, ±NE) with 24Na added (10 μCi/ml) for a 1-minute period. The influx was terminated by quickly transferring the tissues to the physiological solution chilled to 1°C. After several rinses, the tissues were removed at 10 minutes, blotted, dried for 2 hours at 110°C, then weighed. Previous studies have shown that 10-minute wash at 1°C is sufficient to remove 24Na from the extracellular space without reducing greatly the slowly exchanging (cellular) component (Jones, 1981). The 24Na was released by adding 0.5 ml H2O2 (30% wt/vol) to each tissue, followed by 10-minute treatment in a microwave oven. The sample was extracted into a 0.1 ml solution of HNO3 for 10 minutes, then neutralized, and 10 ml of liquid scintillation cocktail was added. Standards were prepared similarly from the 24Na incubation media, with Na+ concentration verified by flame photometry. The counts were corrected for background and isotopic decay, and the data are presented as μEq Na+/g dry wt per min. The relatively short exposure to 24Na (1 minute) resulted in the exchange of less than 20% of the cellular contents. Because this exchange represents only a 10% average over the period for the influx, back flux corrections were not applied.

**Contraction**

The rings were prepared and mounted according to a standardized procedure (Jones et al., 1984). The rings were stretched to 1.3 times their resting diameter and isometric force was measured in response to agonists added to the bath. The responses during the various protocols were
normalized in terms of the force developed during an initial control response. The response at 1 minute was used to measure the relatively fast, phasic component which was reported to depend mostly on the release of cellular Ca++. (Deth and Van Breemen, 1977). The response after 5 minutes was used to measure the tonic component which required extracellular Ca++ for its maintenance (Deth and Van Breemen, 1977).

Results

Ca++ Removal and Antagonism

Removal of Ca++ (0 Ca++ solution), or addition of diltiazem, a Ca++ antagonist (Triggle and Swamy, 1980) inhibited the contractile response to NE in rat aorta, as shown in Figure 1A. Both the initial fast (phasic) response and the slow (tonic) response were inhibited by prolonged exposure to 0 Ca++ and diltiazem (DZ). Similar treatment also inhibited the NE-induced increase in 42K efflux (Fig. 1B). As in the case of the contractile response (Fig. 1A), inhibition was more complete by 0 Ca++ than by DZ (P < 0.001). Because removal of Ca++ from physiological solution (Mg++ = 1.2 mm) was associated with a major increase in 42K efflux (Jones, 1974), raising the Mg++ was an important adjustment to maintain membrane stability in 0 Ca++. After a preliminary study, Mg++ = 10 mM was chosen because it maintained the resting 42K efflux within 30% of the basal level and had little effect on the maximal 42K response to NE in the presence of Ca++ (Δk = 0.012 ± 0.001/min, n = 12 for both Mg++ = 1.2 and 10 mm). Higher concentrations of Mg++ maintained the resting 42K effluxes closer to basal levels in 0 Ca++, but significantly inhibited contractile and ionic flux responses to NE (data not shown).

Increases in 36Cl effluxes (Fig. 2B) which accompanied 42K efflux during NE stimulation (Fig. 2A) also exhibited Ca++ dependency. About 50% of the initial phasic response (time ≤1 minute) was inhibited by 10 minutes of exposure to 0 Ca++ (Table 1). However, the tonic response at 4.5 minutes was completely inhibited by 0 Ca++ (Table 1). These differential effects may reflect an ability for stored Ca++ released by NE (Deth and Casteels, 1977; Deth and Van Breemen, 1977) to stimulate 42K and 36Cl effluxes for a brief period.

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** Panel A, effects of 0 Ca++ solution and 30 µM diltiazem (DZ) on a typical contractile response of rat aorta to a supermaximal concentration of 3 µM NE. Control responses are from the same ring. The DZ and 0 Ca++ were applied 20 minutes before NE. W indicates washout of NE from controls. The dashed line represents the basal tension before NE. All responses were measured in Mg++ = 10 mM. Panel B, effects of 0 Ca++ and DZ on 42K efflux responses to NE. Means plus SE (representative values) of the rate constants are plotted. After 40 minutes, tissues were placed in either a control solution (●) (Ca++ = 2.5 mM, Mg++ = 10 mM, n = 11 rats), 0 Ca++ solution (○) (EGTA = 2 mM, Mg++ = 10 mM, n = 5 rats), or DZ (■) (Ca++ = 2.5 mM, Mg++ = 10 mM, n = 6 rats). Time is in minutes. Horizontal lines indicate the period of application for the treatments.

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Effect of 0 Ca++ (○) on NE stimulation of 42K and 36Cl efflux. Panel A, 42K efflux plotted as in Figure 1B (n = 6 rats). The 0 Ca++ solution was applied at time = 0, and the composition of the 0 Ca++ and control solution (●) are the same as Figure 1B. Panel B, 36Cl efflux measured simultaneously with the 42K efflux in panel A.

Results are presented as means ±SE, and significance was determined by Student's t-test, with P < 0.05 deemed to be significant. Standard linear regression methods were used in one series.
If the NE-induced increases in ion fluxes are dependent on free intracellular Ca++, then a close relation would be expected between alterations in the efflux response and contraction. Systematic investigations were made to test the closeness of this relation. Protocols were designed to measure (1) the concentration-dependent effect of DZ on NE-stimulated 42K efflux and contraction, (2) the time course for changes in 42K efflux and contraction when Ca++ was replaced in 0 Ca++ + NE, and (3) the time course for changes in NE-stimulated 42K efflux and contraction when Ca++ was removed.

**Diltiazem Concentration**

Diltiazem inhibited the NE-induced changes in both 42K efflux and contraction in a similar manner (Fig. 3). The inhibitory responses were evaluated at 1-minute exposure to NE for the phasic response and at 10 minutes for the tonic response. The percent inhibition at 1 and at 10 minutes was similar for each concentration of DZ. At a DZ concentration of 30 μM, the inhibitory effect on NE-stimulated contraction and on 42K efflux was relatively small compared to the inhibition of KCl-induced changes in 42K efflux and contraction (Fig. 3). This contractile effect is consistent with the report of Triggle and Swamy (1980) that Ca++ antagonists were more potent inhibitors of KCl-induced contractions than of NE contractions in arterial smooth muscle. Similar to the results of Saida and Van Breemen (1983), extremely high concentrations of DZ could produce almost complete inhibition of NE contractions (Fig. 3). This inhibition also extended to NE-induced changes in 42K efflux (Fig. 3).

**Ca++ Replacement**

The addition of Ca++ to 0 Ca++ solution containing NE caused a contraction which approached that developed when NE was added to a Ca++-containing solution (Fig. 4A). The 42K efflux response to replacement of Ca++ in the presence of NE (Fig. 4B) was similar to the contractile response. NE increased 42K efflux transiently in 0 Ca++, followed by a return toward basal rates, in sharp contrast to the response in the presence of Ca++. Ca++ replacement after 15 minutes in 0 Ca++ + NE resulted in an increase in 42K efflux (P < 0.001) to the levels achieved by tissues that had been exposed to NE and Ca++ throughout (Fig. 4B). The response to Ca++ replacement would be expected to be in the opposite direction if its major effect were to stabilize the membrane.

The time courses for the return of the responses after Ca++ replacement appear in Figure 5. The effect on 42K efflux reached a maximum before contraction, but good agreement was noted overall. The return of both 42K efflux and contraction when Ca++ was replaced in the presence of NE indicates that the availability of cellular Ca++, rather than α-receptor activation alone, determined the 42K efflux response.

**Ca++ Removal**

The transient stimulation of 42K efflux by NE in 0 Ca++ is consistent with the hypothesis that [Ca++]_{cell} is elevated by the release of Ca++ from a cellular pool of limited capacity (Deth and Casteels, 1977; Karaki and Weiss, 1979). This hypothesis was tested further by varying the time in 0 Ca++ before expo-
sure to NE. The phasic contractile response (1 minute in NE) was reduced as the time in 0 Ca++ was increased (Fig. 6). Only a small tonic contraction remained after 12.5 minutes in 0 Ca++ + NE (Fig. 6). The initial response of 42K efflux to NE (k at 1 minute) also showed a reduction which depended on the time the tissue was preincubated in 0 Ca++ (Fig. 7). For instance, when the tissue was simultaneously placed in 0 Ca++ plus NE, approximately 90% of the control response occurred, while 40 minutes in 0 Ca++ removed any vestiges of a phasic response to NE (Fig. 7). The 42K efflux returned toward basal levels with continued exposure to 0 Ca++ + NE (Fig. 7). The decrement of the contractile and 42K responses was closely related over a range of times which led to almost complete inhibition (Fig. 8). However, there was a tendency for contraction to decrease more rapidly over the initial period and to retain some long-term residual response in comparison to 42K efflux (Fig. 8).

Correlation of 42K Efflux and Contraction

The hypothesis that both NE-induced changes in 42K efflux and in contraction were dependent on the same factor, [Ca++]_ext, was tested by correlating the average responses derived from the three protocols (Figs. 3, 5 and 8). The correlation was close to 1 and highly significant (P < 0.001). Moreover, the slope of 1.0 and intercept near zero indicated a co-dependence which may reside in a common dependence on [Ca++]_ext. This observation suggests the hypothesis that the established role of [Ca++]_ext as a controller of contraction extends to the activation of 42K efflux during NE stimulation.

Ca++ Dependency of 24Na Influx

The 24Na influx was measured under basal conditions and during NE treatment in the presence
and absence of Ca\textsuperscript{++} (Table 2). The basal \textsuperscript{24}Na influx was increased in the 0 Ca\textsuperscript{++} solution \((P < 0.001\). Ca\textsuperscript{++} depletion for 5 minutes did not greatly inhibit the NE-stimulated \textsuperscript{24}Na influx. At 1.0 minutes in NE, the stimulated influxes were similar in 0 Ca\textsuperscript{++} and controls (+Ca\textsuperscript{++}), whereas 4.5 minutes of additional exposure to 0 Ca\textsuperscript{++} led to only a 30% reduction in NE-stimulated influx, compared to the response in the presence of Ca\textsuperscript{++}. This reduction is in sharp contrast to the attenuation (>80%) of contraction (Fig. 6) and \textsuperscript{42}K efflux (Fig. 7) in 0 Ca\textsuperscript{++} 5 minutes, plus 5 minutes in 0 Ca\textsuperscript{++} + NE. Prolonged exposure to 0 Ca\textsuperscript{++} (20 minute) before NE did not reduce the initial \textsuperscript{24}Na influx (1.0 minutes in NE), but did inhibit the response after an additional 4.5 minutes in 0 Ca\textsuperscript{++} plus NE. Under this latter condition, the contractile response (Fig. 4A) and \textsuperscript{42}K efflux (Fig. 4B) were more than 90% inhibited. The NE-stimulated influx of \textsuperscript{24}Na appears to be resistant to depletion of Ca\textsuperscript{++}, except under conditions of almost complete removal of extracellular and cellular sources for contractile responses.

**Discussion**

**Ca\textsuperscript{++}-Dependent K\textsuperscript{+} Channels**

The results of the three protocols, which were designed to alter cellular Ca\textsuperscript{++}, showed that a close relation exists between force development and \textsuperscript{42}K efflux from rat aorta during \(\alpha\)-receptor stimulation. Since little evidence exists for a cause-and-effect relation between these two events, it is reasonable to conclude that both responses are controlled by a common factor, e.g., [Ca\textsuperscript{++}]\text{inter}i. Force development in skinned arterial smooth muscle was dependent on [Ca\textsuperscript{++}], with half maximal stimulation achieved...
TABLE 2
Influx of Na\(^+\) in Rat Aorta and the Effects of NE (3 \(\mu\)M) and 0 Ca\(^{++}\) Solution Applied at Various Times

<table>
<thead>
<tr>
<th>Time in Basal (-NE)</th>
<th>Time in NE</th>
<th>(\Delta) Na influx (NE)</th>
<th>(P^*)</th>
<th>(P^\dagger)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(min)</td>
<td>((\mu)Eq/g d.w./min)</td>
<td>((\mu)Eq/g d.w./min)</td>
<td>((\mu)Eq/g d.w./min)</td>
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</tr>
<tr>
<td>12</td>
<td>0 (+Ca(^{++}))</td>
<td>1.91</td>
<td>0</td>
<td>1.96</td>
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<tr>
<td></td>
<td></td>
<td>±0.14</td>
<td></td>
<td>±0.13</td>
</tr>
<tr>
<td>7</td>
<td>0 (+Ca(^{++}))</td>
<td>1.96</td>
<td>5.5</td>
<td>2.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.11</td>
<td></td>
<td>±0.12</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>3.04</td>
<td>0.001</td>
<td>3.56</td>
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<tr>
<td></td>
<td></td>
<td>±0.21</td>
<td></td>
<td>±0.24</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>3.10</td>
<td>0.001</td>
<td>3.70</td>
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<tr>
<td></td>
<td></td>
<td>±0.18</td>
<td></td>
<td>±0.21</td>
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<tr>
<td>7</td>
<td>20</td>
<td>3.04</td>
<td>0.005</td>
<td>4.26</td>
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<td></td>
<td></td>
<td>±0.26</td>
<td></td>
<td>±0.45</td>
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<tr>
<td>6</td>
<td>20</td>
<td>3.47</td>
<td>0.001</td>
<td>3.69</td>
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<tr>
<td></td>
<td></td>
<td>±0.03</td>
<td></td>
<td>±0.41</td>
</tr>
</tbody>
</table>

* Comparison between +Ca\(^{++}\) and 0 Ca\(^{++}\) conditions —NE.
† Comparison between —NE and +NE conditions with no change in Ca\(^{++}\).
‡ Comparison between +Ca\(^{++}\) and 0 Ca\(^{++}\) on \(\Delta\) Na influx at equivalent time in NE.

at 0.8 to 6 \(\times\) 10\(^{-7}\) M and maximal responses at 1–3 \(\mu\)M (Ruegg and Paul, 1982; Chatterjee and Murphy, 1983). Studies that employed patch clamp techniques have demonstrated Ca\(^{++}\) dependency of K\(^+\) channels (as well as voltage dependency) in a variety of types of cells. (Miller, 1983; Schwarz and Passow, 1983). At zero or slightly negative membrane potentials, however, [Ca\(^{++}\)] of 1 \(\mu\)M or greater was required to increase the time that K\(^+\) channels were open in red blood cells (Grygorczyzk et al., 1984; Yingst and Hoffman, 1984) and in skeletal muscle (Barrett et al., 1982; Latorre et al., 1982). A unique study on membrane patches from smooth muscle also used high [Ca\(^{++}\)] to increase the open time of K\(^+\) channels (Singer and Walsh, 1984). If our hypothesis is correct, then we would predict that the open time of Ca\(^{++}\)-dependent K\(^+\) channels in arterial smooth muscle would increase with similar [Ca\(^{++}\)] (0.1–3 \(\mu\)M) as contraction in the presence of physiological membrane potentials \(E_m = -25\) to \(-45\) mV in NE (Jones, 1980; Haeusler, 1983). K\(^+\) channels from skeletal muscle required 5 \(\mu\)M Ca\(^{++}\) (or higher) to open at \(E_m = -20\) to \(-40\) mV (Barrett et al., 1982; Latorre et al., 1982). These comparisons are based on skeletal muscle, because the conductances of Ca\(^{++}\)-activated K\(^+\) channels in smooth muscle (Singer and Walsh, 1984) were similar to those in skeletal muscle [200–300 pS (Barrett et al., 1982; Schwarz and Passow, 1983)], in contrast to red blood cells which were 10-fold lower (Schwarz and Passow, 1983; Grygorczyzk et al., 1984). The difference in Ca\(^{++}\) dependence between isolated patches and intact cells may reside in the availability of a cofactor such as calmodulin which would be lost when cellular structures are exposed to experimental solutions (Ruegg and Paul, 1982). This explanation needs experimental verification.

The Ca\(^{++}\) activation and K\(^+\) effluxes during NE stimulation in this study is consistent with observations that increased membrane conductance occurred at constant membrane potential during NE stimulation (Haeusler, 1982, 1983). Treatment with TEA reduced the conductances resulting in depolarization and ability to generate action potentials (Casteels, et al., 1977; Haeusler, 1983). Ca\(^{++}\) activation of K\(^+\) channels (and contractile response) with increasing levels of NE could account for much of the voltage-independent change in conductance. It is also possible that the Ca\(^{++}\) dependency of K\(^+\) channels in the tonic type of smooth muscle requires lower [Ca\(^{++}\)] than the phasic type; hence, regenerative action potentials would be more readily inhibited. A shift to higher [Ca\(^{++}\)] for activation of K\(^+\) channels (at a given membrane potential) would allow a larger inward current to be unopposed by an increase in K\(^+\) conductance, and hence a regenerative response (action potential) would be more likely to occur. Action potentials tend to be conducted in smooth muscle and result in relatively large contractile responses (Golenhofen, 1981) consistent with propulsive motility. Closer coupling of inward Na\(^+\) and Ca\(^{++}\) currents with K\(^+\) efflux would result in graded changes in membrane potential and in contraction (Somlyo et al., 1969; Harder, 1982; Haeusler, 1983). Such graded responses can sustain a continuous opposition to distending forces, e.g., blood pressure, with less expenditure of energy than phasic contractile events (Chatterjee and Murphy, 1983). Much of the above analyses of Ca\(^{++}\)-dependent
K⁺ efflux was based on the assumption that force development quantitatively reflects changes in [Ca++]ₜₖₑₚ and that Ca++ activation of the K⁺ channel (increased probability of being open) occurred quickly with little inactivation (alteration in the probability of being open during constant stimulation). The first assumption is reasonable, although not entirely correct. Hysteresis has been observed between the [Ca++] dependence of contraction induced by increasing [Ca++] and relaxation resulting from reduction of [Ca++] in skinned arterial smooth muscle (Chatterjee and Murphy, 1983). Approximately 10 times lower [Ca++] was required for force maintenance than for force development. This difference may underlie some of the differences in the time courses for changes in ⁴²K efflux and contraction (Figs. 5 and 8). These discrepancies are relatively small, however, in comparison to the correlation derived over the whole range of responses (Fig. 9).

Opening of Ca++-activated channels occurs much more rapidly than the resolution of the ⁴²K efflux method (Barrett et al., 1982). Likewise, inactivation of these channels during constant stimulation was not a dominant feature (Barrett et al., 1982; Latorre et al., 1982). The assumptions that the opening is rapid and stable appear to be reasonable approximations. Some fade occurred in the response of ⁴²K efflux to NE (Jones 1973; Fig. 2a). It is not clear whether this fade reflected slow inactivation of Ca++-activated K⁺ channel or represented a decay of [Ca++]ₜₖₑₚ during force maintenance (Morgan and Morgan, 1982; Kato et al., 1984). Such transients could result from the acute influx and release of Ca⁺⁺ from a pool of limited capacity, followed by α-receptor stimulated influx of Ca⁺⁺ operating alone in the presence of increased Ca⁺⁺ efflux. If it can be confirmed by direct measures than the Ca⁺⁺-activated K⁺ channel has a stable dependence on voltage and [Ca⁺⁺], once the membrane has reached a stable voltage, then the time course for the α-recep-
tor-stimulated ⁴²K efflux may reflect that for [Ca⁺⁺]ₜₖₑₚ.

Our finding of Ca⁺⁺-dependent increases in ⁴²K efflux during NE stimulation are in general agreement with those of Martin and Gordon (1983), with one notable exception. They showed a close relation between NE-induced increases in ⁸⁶Rb efflux and tension development by rabbit aorta. As in our study, these two events were partially inhibited by a Ca⁺⁺ antagonist. It was also noted that the increase in ⁸⁶Rb efflux during Ca⁺⁺ removal could be reversed by elevating Mg²⁺. On the other hand, La⁴⁺ had a differential action on the NE-stimulated contracture and ⁸⁶Rb efflux. A transient contraction occurred, consistent with the release of stored Ca⁺⁺, but no related change occurred in ⁸⁶Rb efflux. The authors’ interpretation, that increased [Ca⁺⁺] from release had no effect on ⁸⁶Rb effluxes, has an alternate explanation. Various types of K⁺ channels can be blocked by divalent and trivalent ions, such as Ba⁺⁺, Cu⁺⁺, Cd⁺⁺, and La⁴⁺ (Takata et al., 1967; Miller, 1983). Treatment with La⁴⁺ may have directly blocked K⁺ channels and, hence, the ⁸⁶Rb response to NE. Despite this point, we feel that it is likely that Ca⁺⁺-dependent K⁺ channels are operative in both rat and rabbit aorta, and are an important factor for the modulation of the membrane potential during NE stimulation.

Ca⁺⁺-Dependent Cl⁻ Efflux

NE also increased ³⁶Cl efflux from vascular smooth muscles with a computed increase in permeability that exceeded that for K⁺ (Table 8, in Jones, 1980). As in the case of ⁴²K, removal of Ca⁺⁺ greatly inhibited the phasic response and abolished the later tonic response (Table 1). Based on the parallelism with the Ca⁺⁺ dependency of ⁴²K efflux, it is speculated that a Ca⁺⁺-dependent Cl⁻ channel is operative in rat aortic smooth muscle. There is additional basis for this speculation, in that a Ca⁺⁺-dependent Cl⁻ current has been observed in at least one cell, the Xenopus oocyte (Barish, 1983). The Cl⁻ equilibrium potential, E_C, in the oocyte was significantly less than the membrane potential (E_M = -24 mV vs. E_m = -60 to -90 mV), a characteristic it shares with arterial smooth muscle which also exhibited a significant difference between E_C and E_m (-30 mV vs. -55 mM, Jones, 1980).

It is unlikely that Cl⁻ and K⁺ share the same channel. The peak Cl⁻ efflux exceeded that for K⁺ by 2-fold (Jones, 1980), and single channel analyses indicated that Ca⁺⁺-dependent K⁺ channels from skeletal muscle were impermeant to Cl⁻ (Latorre et al., 1982). Although little evidence is available concerning Ca⁺⁺ dependency of Cl⁻ fluxes, more detailed investigations of, e.g., single channel behavior, may be a productive endeavor.

NE-Stimulated Na⁺ Influx

The influx of ⁴⁰Na was stimulated by NE, in addition to the stimulation of ⁴²K and ³⁶Cl effluxes. Previously, NE effects were measured on ⁴⁰Na ef-
fluxes from Na+-loaded arteries (Droogmans et al., 1977; Jones, 1980). The percent increases (25-50%) above basal levels were in agreement with the results of this study. The increase in 24Na influx was maintained for at least 5 minutes in NE plus Ca++, which indicated that α-receptor stimulation led to simultaneous increases in 24Na, 42K, and 36Cl fluxes down their electrochemical gradients. Given the limitations of measuring rapid fluxes in tissues strips (Jones, 1980) it was not possible to determine whether increases in 24Na influx occurred before changes in 42K and 36Cl efflux during the initial application of NE. The NE stimulation of 24Na influx was more resistant to Ca++ removal than the 42K and 36Cl effluxes. Only under the extreme condition used (20 minutes in 0 Ca++ plus 5.5 minutes in NE) was the NE-stimulated 24Na influx inhibited. It appears, however, that the increase in 24Na influx falls more directly under the control of the α-receptor than 42K or 36Cl effluxes, which are linked with changes in cellular Ca++.

The basal 24Na influx increased in 0 Ca++ in contrast to the NE-stimulated influx. The basal and NE-stimulated influx of 24Na exceeded the 45Ca influx reported for rat aorta (Godfraind, 1976). The basal 45Ca influx was only one-tenth (molar basis) that for Na+; thus, the elevation in Na+ influx in 0 Ca++ solution was beyond any reduction in Ca++ influx. This elevation may result from increased Na+ permeability (despite the 10 mM Mg++), or perhaps removal of adsorbed Ca++ which had occupied sites for the membrane transport of Na+.

Control of Excitation

The observation that ionic events during α-receptor stimulation were controlled by [Ca++]o seems to support the interpretation of some previous work. Haeusler (1983) proposed that a combination of voltage- and Ca++-dependent processes are operative in arteries during NE stimulation. The NE stimulation of 24Na influx was probably not realistic to separate the responses into independent voltage- and Ca++-regulated events (channels), since it is established that the Ca++-dependent K+ channels in several cell types exhibit a marked voltage dependence (Miller, 1983). The Ca++ sensitivity of the K+ channels (high-conductance type) increased as the inside of the membrane was made more positive (depolarization). Electromechanical coupling and pharmacomechanical coupling (Somlyo and Somlyo, 1970) would therefore be an interactive process during NE stimulation of polarized arterial smooth muscle. It would be difficult to achieve an isolated electromechanical event, since the Ca++ entering during depolarization, e.g., KCl treatment, would itself alter membrane conductance. That is—in addition to controlling Em, it is necessary to control [Ca++]o in order to define membrane channel characteristics. The development of a comprehensive model for excitation of arterial smooth muscle will therefore be even more complex than previously thought. The application of recent improvements in electrophysiological and biochemical techniques to the study of vascular smooth muscle may provide the required definitive approach.

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