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 codependent on cellular calcium concentration. This co-dependence held for short phasic responses (~1 minute), as well as longer tonic responses (~5 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)
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 excitable 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 (1981) observed that the sustained hyperpolarization induced in guinea pig Taeni caeci by \(\alpha\)-receptor activation required extracellular Ca\(^{++}\), as did the increase in K\(^{+}\) efflux. These smooth muscles represent the intestinal, phasic type in which \(\alpha\)-receptor activation tends to inhibit contraction. One study is available on arterial smooth muscle in which a possible Ca\(^{++}\)-dependency was observed during nor- epinephrine (NE) stimulation of \(86\) Rb efflux and contraction (Martin and Gordon, 1983).

It was the purpose of this investigation to determine whether \(\alpha\)-receptor-stimulated \(42\)K efflux required Ca\(^{++}\). If such a dependence existed, it was of further interest to determine whether extracellular Ca\(^{++}\) and/or cellularly sequestered Ca\(^{++}\) are able to activate the \(42\)K efflux. Correlations were made between the Ca\(^{++}\)-dependent changes in \(42\)K efflux and in contractile response to test the hypothesis that a Ca\(^{++}\)-dependent K\(^{+}\) channel is operative in arterial smooth muscle of the tonic type.

A preliminary communication of this study has been made (Jones et al., 1984).

**Methods**

**Animals and Tissues**

Male Sprague-Dawley rats (200–250 g wt) were decapitated, and the thoracic aorta was prepared according to a standardized method described previously (Jones, 1973; Jones et al., 1984). We removed the endothelial cells from the rings (flux studies) by lightly stroking the intimal surface with filter paper while the tissues were in the dissection solution. The tissues were mounted on stainless steel holders, mounted on stainless steel holders, followed by a 3-hour incubation at 37°C before initiation of the protocols.

**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; HCO\(_3\)\(^{-}\), 13.5; H\(_2\)PO\(_4\)\(^{-}\), 1.2; and glucose, 11.4. Solutions were gassed with a 97% O\(_2\)-3% CO\(_2\) mixture to obtain a pH of 7.4. Propranolol (3 \(\mu\)M, Sigma), ethylenediaminetetraacetic acid (0.025 mM), and ascorbic acid (1 mM, Sigma) were added to all solutions for \(\beta\)-receptor blockade and to inhibit oxidation of catecholamines. Aqueous stock solutions were prepared for NE (Sigma) and diltiazem, (gift from Marion Laboratoires). The 0 Ca\(^{++}\) solutions were prepared with no Ca\(^{++}\), 10 mM Mg\(^{++}\) (as Cl\(^{-}\) salt), and 2 mM ethyleneglycol-bis(\(\beta\)-aminoethvl ether)N,N\(^{\prime}\)-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 \(42\)K (20 \(\mu\)Ci/ml, University of Missouri Research Reactor) or \(42\)K plus \(36\)Cl (2 \(\mu\)Ci/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 \(24\)Na (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 \(24\)Na added (10 \(\mu\)Ci/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 the 10-minute wash at 1°C is sufficient to remove \(24\)Na from the extracellular space without reduc- ing greatly the slowly exchanging (cellular) component (Jones, 1981). The \(24\)Na was released by adding 0.5 ml H\(_2\)O\(_2\) (30% wt/vol) to each tissue, followed by 10-minute treatment in a microwave oven. The sample was extracted into a 0.1 M solution of HNO\(_3\) for 10 minutes, then neutralized, and 10 ml of liquid scintillation cocktail were added. Standards were prepared similarly from the \(24\)Na incubation media, with \(Na\) concentration verified by flame photometry. The counts were corrected for back- ground and isotopic decay, and the data are presented as \(\mu\)Eq Na\(^{+}\)/g dry wt per min. The relatively short exposure to \(24\)Na (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).

Statistics

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.

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++ ($\Delta k = 0.012 \pm 0.001/min$, $n = 12$ 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.
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++]_i 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++], 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 dependency on [Ca++]i. This observation suggests the hypothesis that the established role of [Ca++]i 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++ (Table 2). The basal 24Na influx was increased in the 0 Ca++ solution (P < 0.001). Ca++ depletion for 5 minutes did not greatly inhibit the NE-stimulated 24Na influx. At 1.0 minutes in NE, the stimulated influxes were similar in 0 Ca++ and controls (+Ca++), whereas 4.5 minutes of additional exposure to 0 Ca++ led to only a 30% reduction in NE-stimulated influx, compared to the response in the presence of Ca++. This reduction is in sharp contrast to the attenuation (>80%) of contraction (Fig. 6) and 42K efflux (Fig. 7) in 0 Ca++ 5 minutes, plus 5 minutes in 0 Ca++ + NE. Prolonged exposure to 0 Ca++ (20 minute) before NE did not reduce the initial 24Na influx (1.0 minutes in NE), but did inhibit the response after an additional 4.5 minutes in 0 Ca++ plus NE. Under this latter condition, the contractile response (Fig. 4A) and 42K efflux (Fig. 4B) were more than 90% inhibited. The NE-stimulated influx of 24Na appears to be resistant to depletion of Ca++, except under conditions of almost complete removal of extracellular and cellular sources for contractile responses.

Discussion

Ca++-Dependent K+ Channels

The results of the three protocols, which were designed to alter cellular Ca++, showed that a close relation exists between force development and 42K efflux from rat aorta during α-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++]c. Force development in skinned arterial smooth muscle was dependent on [Ca++], with half maximal stimulation achieved...
**TABLE 2**

Influx of $^*{\text{Na}}$ in Rat Aorta and the Effects of NE (3 μM) and 0 Ca** Solution Applied at Various Times

<table>
<thead>
<tr>
<th>Time in Basal (-NE) + NE A 24Na influx (NE)</th>
<th>Time in NE $^*{\text{Na}}$ influx (μEq/g d.w./min)</th>
<th>$\Delta^*{\text{Na}}$ influx (NE) (μEq/g d.w./min)</th>
<th>$P^*$</th>
<th>$P^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Ca++</td>
<td>24Na influx</td>
<td>+ NE</td>
<td>24Na influx</td>
<td>$P^*$</td>
</tr>
<tr>
<td>0 (+Ca**)</td>
<td>1.91 ± 0.14</td>
<td>1.0</td>
<td>2.40 ± 0.13</td>
<td>0.49 ± 0.19</td>
</tr>
<tr>
<td>7 (+Ca**)</td>
<td>1.96 ± 0.11</td>
<td>5.5</td>
<td>2.89 ± 0.12</td>
<td>0.93 ± 0.09</td>
</tr>
<tr>
<td>5 (+Ca**)</td>
<td>3.04 ± 0.21</td>
<td>0.001</td>
<td>1.0</td>
<td>3.56 ± 0.24</td>
</tr>
<tr>
<td>5 (+Ca**)</td>
<td>3.10 ± 0.18</td>
<td>0.001</td>
<td>5.5</td>
<td>3.70 ± 0.21</td>
</tr>
<tr>
<td>20 (+Ca**)</td>
<td>0.005</td>
<td>3.04 ± 0.26</td>
<td>1.0</td>
<td>4.26 ± 0.45</td>
</tr>
<tr>
<td>20 (+Ca**)</td>
<td>0.005</td>
<td>3.47 ± 0.03</td>
<td>5.5</td>
<td>3.69 ± 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 $\Delta^*{\text{Na}}$ influx at equivalent time in NE.

at 0.8 to 6 × 10⁻⁷ M and maximal responses at 1–3 μM (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 μM or greater was required to increase the time that K⁺ channels were open in red blood cells (Grygorczyz 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 μM) as contraction in the presence of physiological membrane potentials [Eₘ = −25 to −45 mV in NE (Jones, 1980; Haeusler, 1983)]. K⁺ channels from skeletal muscle required 5 μM Ca**⁺ (or higher) to open at Eₘ = −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; Grygorsczky 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 (Castells, 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 \([Ca^{++}]_{\text{cell}}\) 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 underline some of the differences in the time courses for changes in \(^{42}\)K 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 \(^{42}\)K 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 \(^{42}\)K 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 \([Ca^{++}]_{\text{cell}}\) 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 \(\alpha\)-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 \(\alpha\)-receptor-stimulated \(^{42}\)K efflux may reflect that for \([Ca^{++}]_{\text{cell}}\).

Our finding of Ca++-dependent increases in \(^{42}\)K 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 \(^{86}\)Rb 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 \(^{86}\)Rb efflux during Ca++ removal could be reversed by elevating Mg++. On the other hand, La+++ had a differential action on the NE-stimulated contracture and \(^{86}\)Rb efflux. A transient contraction occurred, consistent with the release of stored Ca++, but no related change occurred in \(^{86}\)Rb efflux. The authors’ interpretation, that increased \([Ca^{++}]\) from release had no effect on \(^{86}\)Rb effluxes, has an alternate explanation. Various types of K+ channels can be blocked by divergent and trivalent ions, such as Ba++, Cu++, Cd++, and La+++ (Takata et al., 1967; Miller, 1983). Treatment with La+++ may have directly blocked K+ channels and, hence, the \(^{86}\)Rb 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 \(^{36}\)Cl 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 \(^{42}\)K, 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 \(^{42}\)K 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, \(E_{\text{Cl}}\), in the oocyte was significantly less than the membrane potential (\(E_{\text{Cl}} = -24\) mV vs. \(E_m = -60\) to \(-90\) mV), a characteristic it shares with arterial smooth muscle which also exhibited a significant difference between \(E_{\text{Cl}}\) 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 \(^{24}\)Na was stimulated by NE, in addition to the stimulation of \(^{42}\)K and \(^{36}\)Cl effuxes. Previously, NE effects were measured on \(^{24}\)Na ef-
Control of Excitation

The observation that ionic events during a-receptor stimulation were controlled by \([Ca^{++}]_{es}\) alters 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. It is 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 (depolariization). 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 \(E_{m}\), it is necessary to control \([Ca^{++}]_{es}\) 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.

References


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