Effects of Potassium on Isolated Canine Coronary Arteries

Modulation of Adrenergic Responsiveness and Release of Norepinephrine

LEONOR BORDA, RAFAEL SHUCHLEIB, AND PHILIP D. HENRY

SUMMARY We studied effects of changes in the extracellular potassium concentration ([K+]o) on the mechanical responsiveness of canine coronary artery preparations. Four different contractile behaviors were delineated between [K+]o = 0 and 40 mM: (1) at [K+]o < 1 mM, a contracture developed which was augmented by β-adrenergic stimulation and inhibited by propranolol, 1 × 10⁻⁷ M; (2) at [K+]o between 1 and 2.5 mM, arterial tone was minimum and propranolol acted as a potent constrictor. Within this range of [K+]o, arteries exhibited calcium-induced relaxations and verapamil-induced contractions; (3) at [K+]o between 5 and 15 mM there was a steep rise in force which was increased by isoproterenol (β-adrenergic contraction), almost completely blocked by L-propranolol, but not attenuated by α-adrenergic blockade with phenylephrine; (4) at [K+]o > 30 mM, effects of β-adrenergic stimulation and block were very small. Steady increases in force elicited by isoproterenol and by sudden stimulation and blockade became very small. Effects of exogenous and endogenous (tyramine, nerve stimulation) norepinephrine paralleled those of isoproterenol and were blocked by propranolol but not attenuated by phenolamine. In contrast, modulation of the effects of phenylephrine by potassium consisted of monotonically increasing constrictor responses over the whole range of [K+]o tested. Arteries labeled with (3H)-norepinephrine showed substantial changes in (3H)-efflux with relatively small changes in [K+]o. Maximum releases were observed with [K+]o, ranging between 10 and 25 mM. The smallest releases were observed at the highest [K+]o (40 mM). Thus, changes in [K+]o influence arterial tone by modulating α- and β-adrenergic effects and by regulating the release of neurotransmitter from the coronary nerves.

IT IS generally accepted that acute myocardial ischemia and hypoxia result in acute coronary vasodilation. However, effects of prolonged ischemia on coronary arterial tone have been less well defined. In conscious dogs subjected to acute coronary ligation, collateral flow may increase substantially after a few hours of ischemia, suggesting that dynamic factors may limit effective collateral perfusion temporarily. One possible pathophysiological mechanism is that prolonged ischemia may lead to coronary arterial constriction. Among the vasoactive substances that are released from the ischemic myocardium and may potentially increase arterial tone are potassium and norepinephrine. In the present study, we examined the effects of potassium and adrenergic agents on the tone of isolated coronary arteries. Results demonstrate that small changes in the extracellular potassium concentration influence arterial tone by two interacting mechanisms: modulation of the responsiveness of the artery to adrenergic stimuli and regulation of the release of norepinephrine from the vascular nerves.

ANIMALS

Coronary arteries were obtained from mongrel dogs weighing between 16 and 22 kg. Dogs were anesthetized with pentobarbital (25–30 mg/kg, iv) and the beating heart was rapidly removed from the chest. In some experiments dogs were chemically sympathectomized with 6-hydroxydopamine by the method of Gauthier et al. and studied 4–5 days after treatment. Effectiveness of the
sympathectomy was tested in vivo by measuring the blood pressure responses to iv tyramine (0.1 mg/kg).  

CORONARY ARTERIAL PREPARATIONS

After excision of the heart, the aortic root and its attached left anterior descending artery were dissected en bloc, completely cleaned of surrounding (nonarterial) tissue, and immediately immersed in standard buffer containing (mm): NaCl, 118; KCl, 4.0; CaCl₂, 1.5; MgSO₄, 1.2; Na₂HPO₄, 1.2; NaHCO₃, 25; and glucose, 5. Electrolytes in each batch of buffer were measured by standard automated techniques (Technicon). The buffer was equilibrated at 37°C with a 95% O₂-5% CO₂ gas mixture; final pH was approximately 7.38. The potassium concentration in the buffer ([K⁺]₀) was chosen to approximate that of canine plasma. In pilot experiments, arterial blood samples were collected from fasting dogs via implanted left atrial catheters to heparinized tubes and plasma separated immediately, without cooling, by centrifugation (400 g for 2 minutes). Potassium concentration (flame photometry) averaged 3.05 ± 0.03 mm (n = 8).

The intact artery was mounted vertically in a muscle bath with a capacity of 100 ml (Fig. 1). The arterial segment clamped between the mechanical ground and the force transducer was 2.0 cm long (distance between 3.0 and 5.0 cm from the coronary ostium) and ranged between 20 and 26 mg in blotted wet weight. An initial preload of 1000 mg was applied to the artery. Arteries underwent a stress relaxation and tension stabilized after 60 minutes at about 600 mg. After this equilibration, tension was readjusted to 580 mg, the preload that produced maximum increases in tension with an increase in [K⁺]₀ from 4 to 40 mm. To assess the orientation of smooth muscle cells in the walls of large (epicardial) canine coronary arteries, arterial preparations were fixed with a 2.5% gluteraldehyde-2% formalin under a preload of 580 mg. Transverse and longitudinal sections were stained with hematoxylin and eosin and examined by light microscopy. At least 40% of the smooth muscle cells were oriented parallel to the long axis of the vessels, in agreement with previous studies demonstrating a prominent longitudinal muscle layer in the wall of large coronary arteries. As described in Results, the intact artery preparation was used to facilitate the selective electric stimulation of the coronary nerves and to preserve as much as possible the integrity of the contractile cells. Although the light microscopic findings appeared to provide a rationale for the use of this preparation, additional experiments were performed with helical strips (length, 20 mm; width, 1.5 mm; preload, 1.5 g) and arterial rings (length, 5 mm; preload, 1.5 g) from large coronary arteries [outside diameter (O.D.) 0.8-1.2 mm] and with helical strips (length 8 mm; width 0.6 mm; preload 1.0 g) from small intramyocardial arteries (O.D. 0.4-0.7 mm). Isometric force was recorded with a Hewlett-Packard (model FTA-1) or a Konigsberg (model F-5) force transducer, a Brush carrier preamplifier (model 13 4212 02412), and a Brush recorder (model 220). Arteries were stimulated electrically with a Grass stimulator (model S4K) by proximate or remote point stimulation as illustrated in Figure 1. Drugs and electrolytes were added to the bath either as turbulant injections or slow infusions with a Harvard syringe pump (model 975). If not otherwise specified, [K⁺]₀ dose-response curves were obtained by cumulative addition of potassium chloride to potassium-free buffer and refer to steady force (force plateau) at a given constant potassium concentration.

To study the release of norepinephrine, left anterior descending coronary arteries were incubated for 2 hours at 37°C in standard buffer containing (³H)-norepinephrine (3.10⁻¹ m; 10 mCi/liter). The arteries were cut into 3 to 5-mm long segments (rings) and washed for 3 minutes in norepinephrine-free buffer. Four to five rings from different regions of the artery were incubated at 37°C in a vial containing 1 ml of oxygenated buffer. In some experiments, the rings were put under a tension (preload) of 1.5 g, using a modification of the device employed for the experiments on mechanical activity. Arterial rings were incubated in each vial for 20 minutes and subsequently transferred every 20 minutes to a new vial with buffer containing selected [K⁺]₀. At the end of the fourth 20-minute period, the rings were transferred to weighing vials and heated to a constant dry weight in an oven at 75°C. Seven hundred and fifty microliters of buffer were taken from each incubation vial and added to a counting vial containing 10 ml of Aquasol. Radioactivity was measured in a liquid scintillation spectrometer. Results were expressed as dpm (20 min)⁻¹(mg dry wt)⁻¹.

CHEMICALS AND DRUGS

Reagent grade salts, mannitol, L-norepinephrine bitartrate, DL-isoproterenol-HCl, L-phenylephrine-HCl, L-tyramine-HCl, and atropine sulfate were purchased from Sigma Chemical; phentolamine was obtained from Ciba-Geigy. Other drugs were gifts from chemical companies: D- and L-propranolol (Ayerst Laboratory) indomethacin (Merck, Sharp and Dohme), racemic verapamil and D600 (Knoll, West Germany). Tritiated L-(7⁻³H)-norepinephrine (specific activity, 4.5 Ci/mmol) and Aquasol were purchased from New England Nuclear. Frozen aliquots of catecholamine solutions were melted immediately before use. All concentrations are expressed as the final molar concentration present in the bath. De-ionized double distilled water was used throughout.

Figure 1: Intact artery preparation. "Mechanical ground" indicates the stabilization of the strip at a nonmoving site.
FIGURE 2. Relation between $[K^+]_o$ and force in intact artery preparation. $[K^+]_o$ in the bath was raised cumulatively by addition of 1.2 M KCl in the absence (O) and in the presence of $10^{-7}$ M isoproterenol (D). After each increase in $[K^+]_o$, force was allowed to stabilize (force plateau). Vertical bars represent ± 1 SEM ($n = 14$).

STATISTICAL ANALYSIS

The significance of the difference between group means was assessed by the t-test for unpaired samples. Differences between values before and after addition of a drug were evaluated by the t-test for paired samples.

Results

POTASSIUM-FORCE RELATION

The relation between $[K^+]_o$ and steady arterial tension is illustrated in Figure 2. The dose-response curve exhibited an absolute minimum at $[K^+]_o = 2$ mM and relative maxima at $[K^+]_o = 0$ and 40 mM. The rise in tone beginning at $[K^+]_o = 3$ mM, the approximate potassium concentration of canine plasma, reached half-maximum at $[K^+]_o = 12.5$ mM. In the presence of $[K^+]_o = 4$ mM, addition of NaCl, 40 mM, to the buffer increased force by $12 ± 2$ mg (mean ± se) ($n = 12$), and addition of mannitol, 80 mM, had no effect ($n = 12$). Effects of ionic strength or osmolality therefore did not appear to contribute much to the increase in tone produced by $[K^+]_o = 40$ mM.

EFFECTS OF ISOPROTERENOL

Effects of isoproterenol, $1 × 10^{-7}$ M, on the $[K^+]_o$-force relation are illustrated in Figure 2. Isoproterenol produced sustained increases in tension at very low ($<1$ mM) and high (>5 mM) values of $[K^+]_o$. The artery between $[K^+]_o$ of 1 and 2 mM. Arterial tone at $[K^+]_o = 40$ mM was not increased significantly ($P > 0.1$; $n = 16$). In eight arteries, t-propranolol, $10^{-7}$ M, blocked the effects of $10^{-7}$ M norepinephrine completely. Pretreatment with $10^{-6}$ M phenolamine for 20 minutes did not attenuate the responses to $[K^+]_o = 40$ mM.

EFFECTS OF NOREPINEPHRINE

Effects of L-norepinephrine, $10^{-7}$ M, on the $[K^+]_o$-force relation are illustrated in Figure 3. Like isoproterenol, norepinephrine increased arterial tone at very low (<1 mM) and high (>5 mM) values of $[K^+]_o$ and relaxed the artery between $[K^+]_o$ of 1 and 2 mM. Arterial tone at $[K^+]_o = 40$ mM was not increased significantly ($P > 0.1$; $n = 16$). In eight arteries, t-propranolol, $10^{-7}$ M, blocked the effects of $10^{-7}$ M norepinephrine completely. Pretreatment with $10^{-6}$ M phenolamine for 20 minutes did not attenuate the responses at $[K^+]_o = 0$ ($n = 8$), 1.5 ($n = 8$), and 8.5 mM ($n = 8$). Thus, effects of norepinephrine were qualitatively very similar to those of isoproterenol.

To determine whether preincubation in buffers containing $[K^+]_o$ less than 1.5 mM was necessary to induce relaxations with norepinephrine, arteries first were subjected to the standard initial equilibration (60 min; $[K^+]_o = 4$ mM) and subsequently exposed to $[K^+]_o = 1.5$ mM. As soon as force had stabilized at $[K^+]_o = 1.5$ mM (<6 min), the preparations were stimulated with norepinephrine, $10^{-7}$ M. In all arteries, norepinephrine induced a relaxation and the resulting steady force stabilized at $520 ± 30$ mg ($n = 7$; mean ± se), a value that was not statistically different from that found in the cumulative dose-response experiments (Fig. 3). Thus, prior exposure to zero or low $[K^+]_o$ was not required for norepinephrine to elicit relaxation.
EFFECTS OF TYRAMINE

Tyramine, an agent that acts predominantly by releasing catecholamines from the sympathetic nerve endings, exerted effects similar to those of norepinephrine. In six arteries, resting tone at \([K^+]_o = 0, 1.5, \text{ and } 8.5 \text{ mm}\) averaged 628 ± 11, 572 ± 9, and 716 ± 15 mg (mean ± se). At the corresponding \([K^+]_o\), changes in tone induced by tyramine (10⁻⁵ M) were +28 ± 3, −30 ± 4, and +39 ± 4 (mean ± se; \(P < 0.01\) by the t-test for paired samples). After treatment with \(L\)-propranolol, 10⁻⁷ M effects of tyramine were blocked completely at all \([K^+]_o\).

EFFECTS OF ELECTRICAL STIMULATION

Electrical stimulation produced different effects depending upon the electrode placement (Fig. 1). With proximate electrode placement, supramaximal stimulation (10 V; 1 msec; 60 Hz) produced increases in arterial tone at \([K^+]_o = 0, 1.5, \text{ and } 8.5 \text{ mm}\) (Table 1). These electrically induced contractions were not attenuated by \(L\)-propranolol (10⁻⁷ M) or phenolamine (10⁻⁶ M), suggesting that electric stimulation acted to a large extent directly on the smooth muscle (Table 1). Attempts to induce relaxations of arteries preloaded with calcium, the geometry of the electrodes (including field stimulation with 6-cm long platinum wires) and/or applying submaximal stimuli (1.5-8 V; 0.01-1 msec; 1-60 Hz; threshold, 1.5-2.9 V) were unsuccessful. Thus, it was not possible to mimic the effects of exogenous norepinephrine with proximate electrical stimulation. However, if we used remote point stimulation (10 V; 1 msec; 60 Hz) (Fig. 1), contractile responses resembled those elicited by norepinephrine and were blocked readily by propranolol (10⁻⁶ M) (Table 1). The same impulses applied with remote electrodes failed to elicit any mechanical responses (Table 1). In summary, electrical stimulation with remote electrode placement produced norepinephrine-like effects in fresh arteries with intact vascular nerves. With proximate electrodes, electric stimuli evoked contractions which did not depend upon the presence of intact vascular nerves and were not blocked by propranolol.

EFFECTS OF \(L\)-PROPRANOLOL

To evaluate β-adrenergic effects on arterial tone, we studied the \([K^+]_o\) dose-response relation for developed force in the presence of \(L\)-propranolol (Fig. 3). Between \([K^+]_o = 1 \text{ and } 2.5 \text{ mm}\), a narrow range within which arterial tone was minimum, propranolol shifted the curve upward and acted thus as a vasoconstrictor. Conversely, at very low (<1 mm) and high values of \([K^+]_o (>5 \text{ mm})\), propranolol had a relaxing effect and shifted the curve downward. The steep rise in force between \([K^+]_o = 4 \text{ and } 10 \text{ mm}\) was blocked completely by propranolol.

### Table 1. Electrical Stimulation of Isolated Coronary Arteries

<table>
<thead>
<tr>
<th>([K^+]_o, \text{ mm})</th>
<th>Proximate stimulation</th>
<th>Remote stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Intact arteries</strong></td>
<td>−P</td>
<td>+36 ± 3</td>
</tr>
<tr>
<td></td>
<td>+P</td>
<td>+35 ± 3</td>
</tr>
<tr>
<td><strong>6-OHDA-treated arteries</strong></td>
<td>−P</td>
<td>+40 ± 5</td>
</tr>
<tr>
<td></td>
<td>+P</td>
<td>+39 ± 5</td>
</tr>
<tr>
<td><strong>Cold-treated arteries</strong></td>
<td>−P</td>
<td>+42 ± 3</td>
</tr>
<tr>
<td></td>
<td>+P</td>
<td>+39 ± 2</td>
</tr>
</tbody>
</table>

Proximate and remote electrical stimulation (10 V; 1 msec; 60 Hz) in the absence (−P) and in the presence (+P) of \(L\)-propranolol, 10⁻⁷ M, was performed as illustrated in Figure 1. Numbers in parentheses indicate the number of arteries studied in each group. All arteries were equilibrated and preloaded as described in Methods. Values indicate increases or decreases in steady force (mean ± sem) in milligrams. 0 = no response.

* Significantly different from value without propranolol.
† Arteries from dogs treated with 6-hydroxydopamine (6-OHDA) as described in Methods.
‡ Arteries stored at 2°C for 72 hours in standard buffer ([K⁺]₀ = 4 mm, [Ca⁺]₀ = 1.5 mm).
TABLE 2  Stimulation of Isolated Coronary Arteries with Norepinephrine (NE, $10^{-7}$ M) and Tyramine (T, $10^{-6}$ M)

<table>
<thead>
<tr>
<th>[K+]o (mM)</th>
<th>0 mM</th>
<th>1.5 mM</th>
<th>10 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact arteries (9)</td>
<td>NE $+32 \pm 4$</td>
<td>$-46 \pm 4$</td>
<td>$+85 \pm 6$</td>
</tr>
<tr>
<td></td>
<td>T $+28 \pm 3$</td>
<td>$-30 \pm 4$</td>
<td>$+39 \pm 4$</td>
</tr>
<tr>
<td>6-OHDA-treated arteries (7)</td>
<td>NE $+55 \pm 5^*$</td>
<td>$-66 \pm 5^*$</td>
<td>$+125 \pm 8^*$</td>
</tr>
<tr>
<td></td>
<td>T 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cold-treated arteries (9)</td>
<td>NE $+48 \pm 4^*$</td>
<td>$-58 \pm 5^*$</td>
<td>$+118 \pm 7^*$</td>
</tr>
<tr>
<td></td>
<td>T 0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values expressed are as in Table 1. Definitions of t, t, 0, and numbers in parentheses are same as in footnotes to Table 1.

* Significantly different ($P < 0.05$) compared to corresponding value in intact arteries.

Further elevations in $[K^+]_o (>15 \text{ mm})$, however, effects of propranolol became increasingly less prominent. Thus, it appeared that the augmentation in force with increasing $[K^+]_o$ was mediated by at least two mechanisms: a propranolol-sensitive (β-adrenergic) mechanism that was operative in the presence of relatively small increases in $[K^+]_o$ (4-10 mm) and a propranolol-insensitive (non-β-adrenergic) mechanism that became dominant at high $[K^+]_o$.

FORCE TRANSIENTS INDUCED BY RAPID CHANGES IN THE POTASSIUM CONCENTRATION

Potassium has been considered to play a role in mediating exercise-induced and reactive hyperemia in skeletal muscle. The increases in steady force with increases in $[K^+]_o$ demonstrated above may appear to be in contradiction with the concept of potassium-induced vasodilation. However, reports describing potassium as a vasodilator usually have referred to transient effects elicited by abrupt elevations in $[K^+]_o$. Accordingly, we have studied arterial tone as a function of the rate of change in $[K^+]_o (=d[K^+]_o/ dt)$. Figure 4 shows that a sudden increase in $[K^+]_o$ from 3 to 10 mm evoked a biphasic response. During the first 2 minutes, arterial tone was decreased; only secondarily was there a gradual rise in force. This secondary rise in force appeared to reflect the release and extraneural accumulation of catecholamine because it was completely blocked by L-propranolol. On the other hand, the initial transient relaxation was not influenced by the β blocker (Fig. 4). These results confirm that moderate elevations in $[K^+]_o$ may have a direct relaxing effect on arterial smooth muscle.

EFFECTS OF PHENYLEPHRINE

In contrast to norepinephrine, phenylephrine acted as a constrictor between $[K^+]_o$ of 1 and 2 mm. At $[K^+]_o = 1.5$ mm, increases in tension in response to phenylephrine $10^{-7}$, $10^{-6}$, $10^{-5}$, and $10^{-4}$ M averaged 1.0 ± 0.1, 6.1 ± 0.5, 31 ± 2 ($P < 0.01$), and 63 ± 4 mg ($P < 0.01$), respectively ($n = 8$; mean ± SE). With elevations in $[K^+]_o$ above 3 mm, $10^{-7}$ M phenylephrine increased arterial tone monotonically (Fig. 5). Consequently, phenylephrine-induced contractions were strongest at the highest values of $[K^+]_o$ (40 mm), a concentration at which norepinephrine had no significant effect (Fig. 3). Effects of phenylephrine ($10^{-7}$ M) were blocked completely by phentolamine, $10^{-6}$ M ($n = 8$). Phentolamine alone had no effect on resting arterial tone at $[K^+]_o = 4$, 20 and 40 mm ($n = 8$). In eight arteries, L-propranolol ($10^{-7}$ M) had minor, nonsignificant effects ($P > 0.1$) on arterial tone at $[K^+]_o = 4$ and 40 mm (see Fig. 3). At these values of $[K^+]_o$, responses to phenylephrine ($10^{-6}$, $10^{-5}$ M) did not differ significantly ($P > 0.1$) before and after β-blockade. L-propranolol ($10^{-7}$ M) and atropine ($10^{-6}$ M) did not modify the responses to phenylephrine at $[K^+]_o = 4$, 10, and 40 mm (seven arteries).

![Figure 4](http://circres.ahajournals.org/DownloadedFrom)

**Figure 4**  Effect of the rate of potassium admixture on force. Suddenly raising $[K^+]_o$ from 3 to 10 mm (turbulent bolus injection) evoked a biphasic response (panel I). Raising $[K^+]_o$ from 3 to 10 mm slowly over a 6-minute period completely obliterated the transient relaxation (panel II). After pretreatment with $10^{-7}$ M L-propranolol, bolus injection produced a sustained relaxation. The secondary contraction was inhibited (panel III).
POTASSIUM AND CORONARY SMOOTH MUSCLE/Borda et al.

**EFFECTS OF CALCIUM CHLORIDE**

Figure 6 illustrates that increases in the calcium ion concentration ([Ca\(^{2+}\)]\(_{o}\)) from 1.5 to 2.25 mM produced contractions at [K\(^{+}\)]\(_{o}\) of zero and 8.5 mM but resulted in relaxations at 1.5 mM [K\(^{+}\)]\(_{o}\). In 15 arteries, resting tone at [K\(^{+}\)]\(_{o}\) = 0, 1.5, and 8.5 mM averaged 621 ± 10, 568 ± 8, and 722 ± 14 mg (mean ± se). At the corresponding values of [K\(^{+}\)]\(_{o}\), changes in tension produced by raising [Ca\(^{2+}\)]\(_{o}\) from 1.5 to 2.25 mM were +22 ± 2, −18 ± 2, and +29 ± 2 mg (mean ± se; P < 0.01 by t-test for paired samples). Contractions and relaxations induced by increases in [Ca\(^{2+}\)]\(_{o}\) were not blocked by pretreatment with l-propranolol (10\(^{-7}\) M), indicating that calcium acted by a catecholamine-independent mechanism (Fig. 6). As shown above (Fig. 3), arterial tone was minimum at a [K\(^{+}\)]\(_{o}\) of approximately 1.5 mM. The possibility was considered that calcium-induced relaxations were caused by a change in the orientation of the smooth muscle cells when the artery was under a low stress. In six arteries pretreated with propranolol (10\(^{-7}\) M), arterial tone was similar at [K\(^{+}\)]\(_{o}\) = 1.5 and 11.5 mM, averaging 665 ± 18 and 648 ± 20 mg (mean ± se), respectively (see dose-response in the presence of propranolol, Fig. 3). At these [K\(^{+}\)]\(_{o}\), selected to produce matched preloads (same stress and strain), increases in [Ca\(^{2+}\)]\(_{o}\) from 1.5 to 2.25 mM induced increases in tension at [K\(^{+}\)]\(_{o}\) = 11.5 mM (+31 ± 2 mg) and decreases at [K\(^{+}\)]\(_{o}\) = 1.5 mM (−20 ± 2 mg; mean ± se; P < 0.01 by paired t-test). Thus, it did not appear that low resting stress per se was causing the relaxations observed at [K\(^{+}\)]\(_{o}\) = 1.5 mM. Verapamil (10\(^{-7}\) M), a drug that antagonizes the effects of calcium on smooth muscle, exerted effects opposite to those produced by calcium (Fig. 6). In seven arteries, verapamil increased tension at [K\(^{+}\)]\(_{o}\) = 1.5 mM (+16 ± 1 mg; mean ± se; P < 0.01) and decreased it at [K\(^{+}\)]\(_{o}\) = 8.5 mM (−22 ± 2 mg; P < 0.01).

**RELEASE OF NOREPINEPHRINE**

Table 3 shows the results of experiments in which arteries labeled with (\(^{3}\)H)-norepinephrine were exposed to selected values of [K\(^{+}\)]\(_{o}\). Release of tritium during the first 20-minute period, when all arteries were exposed to 4 mM [K\(^{+}\)]\(_{o}\), was similar in different arteries. Subsequent changes in [K\(^{+}\)]\(_{o}\) modified the release rates significantly. Modulation of the release of tritium appeared to parallel the modulation of force between [K\(^{+}\)]\(_{o}\) = 0 and 10 mM. Release rates were maximum at 15 mM, a concentration at which l-propranolol markedly reduced steady force (Fig. 3). On the other hand, 40 mM [K\(^{+}\)]\(_{o}\), a concentration at which l-propranolol exerted little influence on force, resulted in a smaller release of tritium. Arterial rings exposed to 4 mM [K\(^{+}\)]\(_{o}\) under a preload of 1.5 g exhibited the same \(^{3}\)H-efflux as unloaded rings (Table 3), indicating that arterial stress (or tissue pressure) did not influence the release of radioactivity.

**COMPARISON OF DIFFERENT ARTERIAL PREPARATIONS**

The results described above were obtained from intact artery preparations (Fig. 1). To ascertain whether this preparation behaved qualitatively like other vascular preparations, arterial segments from the same artery were used as intact artery, vascular ring, and helical strip. In addition, helical strips were prepared from small, intramyocardial arteries (O.D., 0.4–0.7 mm). These comparative studies showed that the potassium-dependent mod-
ululation of the contractile responsiveness was qualitatively similar in the four preparations (Table 4). Figure 7 illustrates the effects of isoproterenol on a helical strip from a small coronary artery at [K+]o = 8.5 mM. The tracing shows that with each increase in the concentration of the drug, there was a minor, transient relaxation followed by a sustained contraction. The latter was promptly relaxed by L-propranolol. Thus, β-adrenergic contraction was not a unique feature of large, epicardial coronary arteries, but was readily elicited in helical strips from small, intramyocardial arteries.

Discussion

This study demonstrates that [K+]o regulates the tone of isolated canine coronary arteries by modulating arterial responsiveness to vasoactive stimuli and by influencing the release of neurotransmitter from the vascular nerves. The arteries exhibited distinct contractile properties within specific ranges of [K+]o. At very low [K+]o, the arteries exhibited a high resting tone, a response observed in other smooth muscle preparations, in skeleton muscle, and in cardiac muscle. Arterial smooth muscle exposed to K*-free media accumulates intracellular sodium. According to the concept of counterflow exchange of sodium for calcium, a high intracellular sodium diminishes the extrusion of calcium and may result in a net accumulation of intracellular calcium. Accumulation of calcium by this exchange mechanism may explain the development of a contracture at low [K+]o. Low [K+]o contracture was inhibited by L-propranolol (but not by β-propranolol) and enhanced by isoproterenol, indicating that the mechanical response was mediated partly by a β-adrenergic mechanism. Within the range of [K+]o which produced minimum resting tone, the arteries exhibited a remarkable responsiveness. Calcium induced stable relaxations, and verapamil, a potent vasodilator that may block the entry of calcium into muscle cells, elicited sustained contractions. In this range, α-adrenergic stimulation (phenylephrine) induced contractions and β-adrenergic stimulation (isoproterenol, norepinephrine) sustained relaxations. Propranolol acted as a constrictor, presumably by antagonizing endogenous norepinephrine.

The steep rise in tension between [K+]o = 5 and 12 mM was blocked completely by 10^{-7} M L-propranolol and markedly enhanced by isoproterenol. The inhibitory effect of propranolol did not reflect a nonspecific depression of the smooth muscle, as equal concentrations of α-propranolol did not affect the artery and constrictor responses to phenylephrine, which were not attenuated by the β-blockade. Rabbit aortae and feline brain arteries equilibrated in Krebs-Henseleit solution have been reported to contract in response to high concentrations of isoproterenol.

### Table 3  Release of Norepinephrine at Different [K+]o

<table>
<thead>
<tr>
<th>[K+]o, mM</th>
<th>0-20 min</th>
<th>20-40 min</th>
<th>40-60 min</th>
<th>60-80 min</th>
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<tbody>
<tr>
<td>0 (5)</td>
<td>101 ± 5</td>
<td>132 ± 6*</td>
<td>139 ± 6*</td>
<td>140 ± 6*</td>
</tr>
<tr>
<td>1.5 (4)</td>
<td>104 ± 6</td>
<td>94 ± 4</td>
<td>87 ± 5</td>
<td>79 ± 4*</td>
</tr>
<tr>
<td>4 (6)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>41 (6)</td>
<td>101 ± 5</td>
<td>98 ± 4</td>
<td>98 ± 5</td>
<td>96 ± 8</td>
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<tr>
<td>10 (5)</td>
<td>102 ± 5</td>
<td>146 ± 7*</td>
<td>196 ± 8*</td>
<td>201 ± 9*</td>
</tr>
<tr>
<td>15 (5)</td>
<td>98 ± 6</td>
<td>188 ± 8*</td>
<td>202 ± 11*</td>
<td>216 ± 10*</td>
</tr>
<tr>
<td>20 (5)</td>
<td>103 ± 5</td>
<td>101 ± 6</td>
<td>98 ± 5</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>40 (6)</td>
<td>102 ± 4</td>
<td>98 ± 4</td>
<td>79 ± 5*</td>
<td>*68 ± 4</td>
</tr>
</tbody>
</table>

*H-efflux from norepinephrine-labeled arterial rings exposed to selected [K+]o. Release rates at different [K+]o for each time period are expressed as a percent of the releases at (K) o 4 mix (= 100%). Values represent means ± SEM. Differences in the percent changes in the different preparations were not statistically significant (P > 0.05) from 100%.

### Table 4  Comparison of Different Arterial Preparations

<table>
<thead>
<tr>
<th>Potassium concentration (mM)</th>
<th>0</th>
<th>1.5</th>
<th>8.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole artery (n = 6)</td>
<td>NE</td>
<td>+3.3 ± 0.3</td>
<td>-5.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Ca²⁺</td>
<td>+8.3 ± 0.4</td>
<td>-3.4 ± 0.3</td>
</tr>
<tr>
<td>Arterial ring (n = 6)</td>
<td>NE</td>
<td>+3.4 ± 0.3</td>
<td>-5.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Ca²⁺</td>
<td>+8.6 ± 0.4</td>
<td>-3.7 ± 0.2</td>
</tr>
<tr>
<td>Helical strips, large arteries (n = 6)</td>
<td>NE</td>
<td>+3.8 ± 0.4</td>
<td>-5.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Ca²⁺</td>
<td>+8.7 ± 0.5</td>
<td>-3.5 ± 0.2</td>
</tr>
<tr>
<td>Helical strips, small arteries (n = 6)</td>
<td>NE</td>
<td>+4.1 ± 0.4</td>
<td>-5.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Ca²⁺</td>
<td>+8.0 ± 0.8</td>
<td>-3.6 ± 0.4</td>
</tr>
</tbody>
</table>

Values expressed are percent changes of the force before addition of norepinephrine (NE) or calcium (Ca²⁺) and represent means ± SEM. Differences in the percent changes in the different preparations were not statistically significant (P > 0.1).
ever, contractures induced in isolated muscle preparations by isoproterenol (10^{-4} \text{ M}) may be mediated by nonspecific mechanisms and high doses of adrenergic blocking agents required to block such contractures likewise may be nonspecific.\(^{21}\) In the present study, the transition between sustained isoproterenol-induced relaxations and contractions occurred between [K\(^+\)]\(_0\) = 4 and 6 mM, of concentrations slightly exceeding the [K\(^+\)]\(_0\) of normal canine plasma. Within this transitional range of [K\(^+\)]\(_0\), responses to isoproterenol and norepinephrine were small and often indeterminate.

At [K\(^+\)]\(_0\) = 12 mM, the arteries became progressively insensitive to \(\beta\)-adrenergic stimulation and blockade. Within the range of propranolol-insensitive contracture, \(\alpha\)-adrenergic responsiveness was enhanced, indicating a change in the balance between \(\alpha\)- and \(\beta\)-adrenergic responsiveness. At [K\(^+\)]\(_0\) = 40 mM, the concentration producing the strongest responses to phenylephrine, phenotolamine had no effect on arterial tension, demonstrating the absence of endogenous \(\alpha\)-adrenergic tone. The increases in arterial tone with high [K\(^+\)]\(_0\) thus appeared to be mediated by at least two independent mechanisms: a \(\beta\)-adrenergic mechanism between [K\(^+\)]\(_0\) = 5 and 12 mM and a catecholamine-independent mechanism at higher [K\(^+\)]\(_0\).

Effects of propranolol on fresh coronary arteries indicated that the modulatory effects of K\(^+\) on arterial tone were mediated by endogenously released norepinephrine. The \(^3\)H-efflux from arteries labeled with \(^3\)H-norepinephrine increased nonmonotonically with increasing [K\(^+\)]\(_0\), exhibiting relative maxima at [K\(^+\)]\(_0\) = 0 and 15 mM and a minimum at 1.5 mM. High rates of release occurred within the ranges of [K\(^+\)]\(_0\) in which the arteries were sensitive to the constrictor effects of exogenous norepinephrine. High [K\(^+\)]\(_0\) enhanced release only within a relatively narrow concentration range, and above 20 mM [K\(^+\)]\(_0\), release was inhibited. There is considerable evidence that depolarizing stimuli including electrical stimulation of nerves, KCl, and veratridine promote catecholamine release by the same exocytotic mechanism.\(^{22}\) Transient stimulation and subsequent inhibition of catecholamine release evoked by high [K\(^+\)]\(_0\) (72 mM) has been observed in the adrenal medulla.\(^{23}\) During nerve stimulation in the perfused spleen\(^{24}\) and isolated saphenous vein,\(^{25}\) elevation in [K\(^+\)]\(_0\) tends to inhibit the release of norepinephrine. It is possible that the stimulatory (depolarizing) effects of K\(^+\) and electrical stimulation are additive and that inhibition of catecholamine release occurs at lower [K\(^+\)]\(_0\) during electrical stimulation. In support of this hypothesis is the observation of Lorenz et al.\(^{25}\) who demonstrated that a [K\(^+\)]\(_0\) of 15 mM augments the release of norepinephrine from canine saphenous veins during submaximal electric field stimulation, but that further increases in the total depolarizing stimulus by raising [K\(^+\)]\(_0\) above 15 mM and/or by delivering supramaximal electric stimuli inhibit the release of norepinephrine.

In the present study, indirect electrical stimulation with remote electrodes mimicked the effects of exogenous norepinephrine. Contractions evoked at very low and elevated [K\(^+\)]\(_0\) and relaxations elicited at intermediate [K\(^+\)]\(_0\) were blocked by 10^{-7} \text{ M L-propranolol}. The same indirect electric stimuli produced no mechanical responses in arteries from 6-hydroxydopamine-treated dogs or in arteries stored in the cold, interventions known to produce nerve injury.\(^{7,12}\) These arteries were supersensitive to exogenous norepinephrine and responsive to the application of direct electric current, strongly suggesting that the absence of mechanical responses to indirect electric stimulation reflected impaired neural function. Impaired release of endogenous catecholamine in cold-treated arteries also was manifested by a complete absence of increases in resting tone with elevations in [K\(^+\)]\(_0\) up to 10 mM, a response observed in normal arteries after \(\beta\)-adrenergic blockade (see Fig. 3). Absence of increases in tone with elevations in [K\(^+\)]\(_0\) below 10 mM have been observed by Brecht and collaborators\(^{26,27}\) in canine carotid arteries stored in the cold. These authors\(^{28}\) observed similar initial dilator responses to increases in [K\(^+\)]\(_0\) in fresh and cold-treated carotid arteries but did not characterize the sustained effects of potassium on arterial tone. The delayed constrictor response to moderate increases in [K\(^+\)]\(_0\) recently has been observed by a number of authors.\(^{29-31}\) In the present study, abrupt increases in [K\(^+\)]\(_0\) were shown to evoke biphasic responses consisting in initial propranolol-insensitive relaxations and subsequent propranolol-sensitive contractions.

Conflicting reports regarding the effects of catecholamines on isolated coronary arteries may in part be attributed to species differences.\(^{22,35}\) In addition, the results of this study may help resolve some of the contradictory findings. First, at values of [K\(^+\)]\(_0\) between 4 and 6 mM responses to catecholamines may be indeterminate, consisting of small contractions or relaxations (transition between relaxation and contraction). Second, catecholamines may induce biphasic responses. In some studies, it appears that the time course of the responses to adrenergic stimuli was incompletely characterized.\(^{27,28,34}\) Third, storage of coronary arteries, vessels known to be very richly innervated,\(^{35}\) may markedly impair neurally mediated effects and profoundly alter the contractile behavior of the artery.

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References

Effects of potassium on isolated canine coronary arteries. Modulation of adrenergic responsiveness and release of norepinephrine.
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