Hypoxic Contraction of Isolated Canine Coronary Artery 
Mediation by Potassium-Dependent Exocytosis of Norepinephrine

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SUMMARY Effects of hypoxia on arterial tone, efflux of potassium, and efflux of norepinephrine were monitored for isolated canine coronary arteries labeled with radioactive potassium ($^{42}$K) and norepinephrine ($^{3}$HNE). Hypoxia elicited transient relaxation and subsequent sustained contraction accompanied by marked increases in the effuxes of $^{42}$K and $^{3}$HNE. After sympathetic nerve injury with 6-hydroxydopamine or cold storage, arteries responded to hypoxia with sustained relaxation. Sustained relaxation occurred also after pretreatment with L-propranolol, but not with D-propranolol or phentolamine. Inhibition of hypoxic contraction by L-propranolol did not alter $^{42}$K or $^{3}$HNE efflux. Colchicine, an inhibitor of the exocytosis of NE, suppressed hypoxic $^{3}$HNE efflux and contraction, but not $^{42}$K efflux. Proadifen inhibited $^{42}$K and $^{3}$HNE efflux as well as contraction. During proadifen-inhibited $^{42}$K efflux, exogenous K$^+$ augmented overflow of $^{3}$HNE, indicating that proadifen relaxed the hypoxic artery primarily by inhibiting K$^+$-dependent exocytosis of NE. The ratio of NE to dopamine/S-hydroxylase activity was similar in effluents from oxygenated arteries exposed to elevated K$^+$ concentrations and in effluents from hypoxic arteries. Thus, hypoxia evoked exocytotic release of norepinephrine which promoted contraction by a $\beta$-adrenergic mechanism.


IT IS WIDELY held that hypoxia induces relaxation of coronary arterial smooth muscle (Berne, 1974). However, the vasomotor response of isolated coronary arteries to hypoxia has not been completely characterized. Previous studies have focused on alterations in the contractile responsiveness of isolated arteries during established hypoxia, but have not delineated the time course of changes in resting tone induced by hypoxia (Shibata and Briggs, 1967; Detar and Bohr, 1968a; 1972; Gellai et al., 1973; Namm and Zuker, 1973). Detar and others have observed that resting tension of isolated rabbit aortae is not influenced by variations in Po$_2$ between less than 1 mm Hg and 675 mm Hg (Detar and Bohr, 1968b), and that resting tone of isolated rabbit coronary arterial strips remains unchanged during 5-10 minutes of hypoxia (Gellai and Detar, 1974). These observations do not support the concept that hypoxia invariably evokes relaxation of arterial smooth muscle.

In the present study, we have examined the effects of sudden hypoxia on the resting tone of isolated canine coronary arteries. In addition, we have monitored the release of potassium and norepinephrine from the hypoxic artery. As canine coronary arteries contain both $\alpha$- and $\beta$-adrenergic receptors (Zuberbuhler and Bohr, 1965) and as potassium modulates adrenergic effects (Borda et al., 1977), we have considered the possibility that hypoxic coronary vasomotion is mediated in part by the release of these vasoactive agents.

**Methods**
Coronary Arterial Preparation

Dogs of either sex weighing 22-31 kg were anesthetized with pentobarbital (30 mg/kg, iv). The heart was excised and the left anterior descending coronary artery dissected and cleaned of surrounding tissue under a dissecting microscope. Segments of the left anterior descending coronary artery measuring 0.8 to 1.2 mm in diameter were cut into helical strips 20- to 25-mm long and 1.5-mm wide and equilibrated for 60 minutes at 37°C in standard buffer of the following composition (mM): NaCl, 116; KC1, 3.1; CaCl$_2$, 1.5; MgSO$_4$, 1.2; NaH$_2$PO$_4$, 1.2; NaHCO$_3$, 25; dextrose, 5; and disodium ethylenediaminetetraacetate (EDTA), 0.025. The solution was equilibrated with a gas mixture at 15% O$_2$-5% CO$_2$-80% N$_2$. The pH and Po$_2$ measured with an Instrumentation Laboratories gas analyzer model 213 were between 7.38 and 7.42, and 100 and 110 mm Hg, respectively. To label the arteries with radioactive potassium and norepinephrine $^{42}$K (total concentration = 3.1 mmol/liter; initial radioactivity 16...
mCi/liter) and 7-3H-norepinephrine (total concentration = 10^-7 mol/liter; radioactivity 1.5 mCi/liter) were included in the solution. Arteries were mounted for isometric force recording in a tissue bath 0.6 ml in capacity (Fig. 1). The upper end of the artery was attached to a FTA-1A Beckman force transducer mounted on a micropositioner. Signals from the transducer were amplified with a Brush carrier preamplifier and recorded with a Brush 220 recorder. The preload was adjusted to 600 mg, a resting tension found to produce maximum increments in force with increases in the potassium concentration ([K+]o) from 3.1 to 40 mM (Borda et al., 1977). If not otherwise specified, arterial strips were superfused at 37 °C with standard buffer at a constant flow of 1 ml/min with an Ismatec model MP4 roller pump. The effluent was aspirated at the top of the chamber with another roller pump and collected in 1-ml fractions with a fraction collector. Before timed collections were begun, radioactively labeled strips were washed by superfusing them for 30 seconds at a flow rate of 40 ml/min. In some experiments we used arterial strips from dogs chemically sympathectomized with 6-hydroxydopamine by the method of Gauthier et al. (1972). Denervation was assessed on the basis of the in vivo and in vitro responses to tyramine as previously reported (Borda et al., 1977). In other experiments, sympathetic nerve injury was produced by incubating arteries from intact dogs for 72 hours at 2°C (Shibata, 1969; Borda et al., 1977).

**Concentration-Response Curves**

Concentration-response relationships for potassium and catecholamines were obtained by superfusing the artery with buffer containing increasing concentrations of the agonists. With a stopcock at the bottom of the tissue chamber it was possible instantly to change the superfusion from one buffer to another (Fig. 1). Superfusion with each concentration was maintained until arterial tone had stabilized at a new level. One cumulative concentration-response curve was obtained from each preparation.

**Arterial Hypoxia**

To monitor PO2 in the superfusate, the tip of a PO2 catheter-electrode (French size 1.2; IBC-Berkeley) was placed within the tissue chamber and connected to an IBC model 145-MP-A gas analyzer. In pilot experiments, arteries were equilibrated in buffer gassed with 95% O2-5% CO2 (PO2 > 600 mm Hg). The buffer contained either 3.1 mM K+ (eight arteries) or 10 mM K+ (eight arteries). Reequilibration of the 16 arteries with 15% O2-5% CO2-80% N2 (PO2 100-110 mm Hg) resulted in changes in force not exceeding ±3 mg. These findings agree with those of Namm and Zucker (1973) who found that a drop in PO2 from 700 to 100 mm Hg had no effect on the tension of isolated rabbit aortae. Accordingly, in the present study, all control experiments were performed at a physiological arterial PO2 of 100 mm Hg, achieved by equilibrating the buffer with 15% O2-5% CO2-80% N2. Hypoxia was induced by superfusing the artery with buffer preequilibrated with 95% N2-5% CO2. PO2 fell to below 3 mm Hg within 15 seconds and stabilized in eight arteries at 1.8 ± 0.1 mm Hg.

**Efflux of Potassium and Norepinephrine**

The effluent from arteries preequilibrated with 42K and 7-3H-norepinephrine (NE) was collected in 1-ml fractions. One hundred to 500 μl of each fraction were added to 10 ml of Aquasol, and 42K was counted immediately in a scintillation spectrometer with more than 90% efficiency and no tritium contribution. After 10 days, when radioactivity of 42K was negligibly small, the samples were recounted for tritium with 35% efficiency.

To differentiate 3H-NE efflux from total 3H efflux, NE was separated from its derivatives by chromatography. All steps were performed in the cold at 2-4°C. Five-hundred microliter samples from 10 to 30 consecutive 1-ml fractions were pooled in a test tube containing 500 mg neutral alumina oxide (AG 7, Bio-rad) (Anton and Sayre, 1962). To monitor isolation losses, 800 counts/min of 14C-NE were added to each tube. After having been mixed gently for 10 minutes, the tubes were centrifuged for 3 minutes at 2800 g. The sedimented alumina contained 70 ± 1% (SE; n = 9) of the NE. The supernatant fraction was discarded, the alumina resuspended in 10 ml of distilled water, and the tubes centrifuged again. This procedure was repeated three times. Amines were desorbed from the washed alumina by adding 500 μl of 8 N acetic acid.

**Figure 1** Diagrammatic sketch of the superfusion apparatus. During superfusion with one buffer (buffer B), another buffer (buffer A) is circulated past the stopcock at the bottom of the perfusion chamber in preparation for a subsequent superfusion.
After thorough mixing of the suspension, the tubes were centrifuged and the eluate recovered. One hundred to 400 μl of eluate were mixed with 10 μl of 10 mM cold NE and spotted with an automatic spotting (Analytical Instrument Specialties) on 0.5-mm thick preparative silica gel thin layer chromatographic (TLC) plates. After development with n-butanol:1 N H2SO4:glacial acetic acid (60:75:15, vol/vol) spots were visualized with iodine vapor, and scraped off into vials containing 10 ml Aquasol. Radioactivity due to 3H and 14C was measured by scintillation spectrometry with minimum spill of 14C counts into the tritium window. In some experiments, radioactivity in the NE spot was extracted and shown to move as a single spot in two independent two-dimensional TLC systems (Baumann et al., 1971; Fleming and Clark, 1970), validating the chromatographic procedure. Overall recovery based on the recovery of the 14C internal standard ranged between 29 and 32%.

At the end of the experiments, arteries were dried to a constant weight in an oven at 80°C. Efflux of 42K, corrected for decay, and 3H-NE were expressed as dpm (mg dry wt)⁻¹ (min)⁻¹. When mean values were plotted, efflux was expressed as a percent of the initial (maximal) value. For the study of the relation between NE and [K⁺], 7-3H-NE-labeled arteries were superfused for 40 minutes with standard buffer, and subsequently with buffer containing selected potassium concentrations. Mean radioactive efflux between the 80th and 120th minutes of superfusion was plotted vs. the selected [K+]o. Five arteries were studied for each [K+]o. In some experiments, 2–4 ml of effluent were concentrated by lyophilization and used to measure dopamine β-hydroxylase activity (DBH) by the method of Nagatsu et al. (1973). This assay measures the conversion of 14C-tyramine to 14C-octopamine in the presence of an inhibitor of monoamine oxidase activity (JB 516). Octopamine was separated by batch adsorption on Dowex-50-X8, the resin washed with filter samplers (Henry et al., 1975), and octopamine desorbed with 4 N NH₄OH. Octopamine was oxidized to 14C-parahydroxybenzaldehyde with sodium periodate. After acidification of the mixture, parahydroxybenzaldehyde was extracted with ether and counted by scintillation spectrometry. DBH activity exhibited characteristic properties including a pH optimum at pH 5.2, activation by N-ethylmaleimide (NEM) or Cu²⁺ ions, and inhibition by fusaric acid.

Chemicals

DL-isoproterenol, L-norepinephrine, colchicine, 6-hydroxydopamine, ascorbic acid, and NEM were purchased from Sigma. Pheniprazine (JB 516) was obtained from Merrell. L- and D-propranolol, phentolamine, and proadifen (SKF 525A) were gifts from Ayerst, Ciba, and Smith, Kline and French, respectively. Aquasol, 1-7-14C-norepinephrine (specific activity, 50 mCi/mmol), 1-14C-tyramine (specific activity, 50 mCi/mol), and 42K (received with an activity of 0.15 mCi/mg elemental K) were obtained from New England Nuclear. Tritiated norepinephrine (1-7-3H-norepinephrine, specific activity 15 Ci/mmol) was obtained from Amersham. AG-50-8X (H⁺) (mesh 200–400) and neutral alumina (AG 7, mesh 200–400) were obtained from Biorad. Silica gel plates (Polygram Sil G, 0.5-mm thick, Macherey-Nagel) were purchased from Brinkman.

Results

Relations between [K⁺], Adrenergic Stimulation, and Arterial Force under Aerobic Conditions

Figure 2A depicts the relation between [K⁺], and steady force in arteries exposed to a Po2 of 100 mm Hg. Between 0 and 1 mM [K⁺], (zone I) and 6 and 12 mM [K⁺], (zone III) 10⁻⁷ M NE increases arterial force. Between 1 and 4 mM [K⁺], (zone II) NE decreases arterial tone. Effects of NE between 4 and 6 mM [K⁺], are small and indeterminate, reflecting the transition between relaxation and contraction. At all potassium concentrations, L-propranolol (10⁻⁷ M) produces changes in force opposite to those elicited by NE. With marked elevations in [K⁺], (> 20 mM, zone IV) neither NE nor propranolol produces appreciable changes in tone. Figure 2B describes the concomitant changes in 7-3H-NE-efflux. Between 1 and 4 mM [K⁺], the range associated with the lowest resting arterial tone, NE efflux is minimal. Lowering [K⁺], below 1 mM or increasing it above 4 mM augments NE efflux. However, with elevations in [K⁺], above 20 mm, concentrations producing propranolol-insensitive contractures, overflow of NE is suppressed. Results shown in Figure 2 are similar to those previously reported in detail for a different canine coronary artery preparation incubated without superfusion (Borda et al., 1977). In these previous experiments, it was demonstrated that D-propranolol, 10⁻⁷ M, and α blockade with phentolamine, 10⁻⁶ M, did not significantly influence the [K⁺], force relation (Borda et al., 1977).

Figure 2 shows that elevations in [K⁺], above 3 mM result in sustained increases in arterial tone. However, when [K⁺], is raised abruptly, the increase in tone is preceded by transient relaxation. The pre-steady state response to a sudden increase in [K⁺], from 3.1 to 10 mm is illustrated in Figure 3A. After α blockade with 10⁻⁶ M L-propranolol, the constrictor component of the biphasic response is completely inhibited (Fig. 3B). Similarly, in arteries from 6-hydroxydopamine-treated dogs and in arteries stored in the cold, interventions known to produce sympathetic nerve injury, the same increase in [K⁺], induces stable relaxation (Fig. 3, C and D). In contrast, phentolamine, 10⁻⁶ M, and D-propranolol, 10⁻⁶ M, do not modify the biphasic response
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FIGURE 2  A: Relation between $[K^+]_o$ and steady force. O = no drug added; $\Delta =$ addition of $10^{-7}$ M L-propranolol; $\Box =$ addition of $10^{-5}$ M L-norepinephrine. After 40 minutes of superfusion with standard buffer, arteries were superfused with selected $[K^+]_o$ and steady arterial force between the 80th to 120th minutes of superfusion plotted vs. $[K^+]_o$. Data points are mean values from five experiments ± 1 SEM. Beta blockade augments arterial tone between 2 and 5 mM $[K^+]_o$ (zone II) and relaxes the arteries below (zone I) and above this range (zone III). With marked elevations in $[K^+]_o$ (zone IV), $\beta$ blockade has little influence on tone. B: Relation between $[K^+]_o$ and $[^3H]$NE efflux. In each experiment, effluent was collected between the 80th and 120th minutes of superfusion for measurement of $[^3H]$NE as described in Methods. Data points represent mean values ± 1 SEM (n = 5). Thus, it appears that initial relaxation in the intact artery represents a catecholamine-independent or "direct" effect of potassium, and that the subsequent contraction reflects release of NE which stimulates smooth muscle by a propranolol-sensitive mechanism. The responses shown in Figure 3 can be predicted on the basis of the $[K^+]_o$-force relation in the absence and presence of propranolol (Fig. 2A). The concentration-response curves show that an elevation in $[K^+]_o$, from 3.1 to 10 mM increases steady arterial tension in the absence of propranolol, but decreases it significantly ($P < 0.001$, unpaired $t$-test) after $\beta$ blockade (Fig. 2A). Figure 4 describes the concentration-response for isoproterenol at selected $[K^+]_o$ with 3.1 mM $[K^+]_o$ the $\beta$ agonist produces stable, dose-dependent relaxations. With an elevated $[K^+]_o$, of 10 mM, isoproterenol has an opposite effect and augments steady arterial tone. At 5 mM $[K^+]_o$ responses are diminutive and indeterminate, reflecting the transition between $\beta$-adrenergic relaxation and contraction.

Effect of Sudden Hypoxia on Coronary Arterial Tone and Efflux of Potassium and Norepinephrine

In fresh coronary arteries with preserved tyramine responsiveness (Borda et al., 1977) a sudden decrease in $P_O_2$ from 100 to 1-3 mm Hg induces transient relaxation and subsequent sustained con-
FIGURE 4 Effect of isoproterenol on arterial tone at selected \([K^+]_o\). Panels on the left are representative cumulative concentration-response tracings. The diagram on the right shows mean values ± 1 SEM (five experiments for each \([K^+]_o\)).

Contractions (Fig. 5A). The contraction component of the biphasic response is inhibited by L-propranolol, 10^{-7} M (Fig. 5B), treatment with 6-hydroxydopamine (Fig. 5C), and storage in the cold (Fig. 5D). In contrast, phentolamine, 10^{-6} M (Fig. 5E), and D-

propranolol, 10^{-6} M (Fig. 5F), do not suppress the contraction. Thus, contractile responses to sudden hypoxia resemble those elicited by abrupt increases in \([K^+]_o\) (Fig. 3, A–F). Figures 6 and 7 illustrate that

FIGURE 5 Contractile responses to sudden hypoxia. A: control. Interventions B–F were as described in legend to Figure 3. Panels on the left are representative tracings; panels on the right show mean values obtained from five experiments. Vertical bars are 1 SEM.

FIGURE 6 Relationship between arterial tone and effluxes of \(^{42}\)K, total \(^3\)H, and \(^3\)HNE in oxygenated artery. Vertical bars are ± 1 SEM (n = 5). Data points for force, \(^{42}\)K efflux, and \(^3\)HNE efflux at 120, 160, and 180 minutes compared to the data points at 80 minutes were not significantly different (P > 0.05; t-test for paired samples).

FIGURE 7 Effect of sudden hypoxia on the relationship between arterial tone and effluxes of \(^{42}\)K, total \(^3\)H, and \(^3\)HNE. Sequential data points with vertical bars (1 SEM) indicate significantly different values (P < 0.05; paired t-test; n = 5). Data points at 180 and 190 minutes did not differ significantly from the data points at 120 minutes (P > 0.05; paired t-test).
hypoxic arterial contraction is accompanied by marked increases in the efflux of $^{42}\text{K}$ and $^{7-3\text{H}}\text{NE}$. Results presented in Figures 5 to 7 are consistent with the hypothesis that hypoxic coronary contraction is mediated by potassium-dependent release of endogenous NE.

Relations between $[K^+]_o$, Adrenergic Stimulation and Arterial Force under Hypoxic Conditions

Figure 8 illustrates the effect of step-wise increases in the perfusate $[K^+]_o$ during hypoxic contraction. Under control conditions with a $P_{O_2}$ of 100 mm Hg, increases in $[K^+]_o$ between 3.1 and 15 mM produce dose-dependent increases in steady arterial tone, in agreement with Figure 2A. However, after 15 minutes of hypoxia, the same step-wise increases in the perfusate $[K^+]_o$ have no significant effect up to a $[K^+]_o$ of 12 mM. Only with a further increase to 15 mM is there a significant augmentation in tone. One possible explanation for this altered reactivity to exogenous potassium during hypoxia is that cell potassium, accumulating on the outside of the cell membranes, temporarily attenuates the effects of small increases in the perfusate $[K^+]_o$.

As changes in $[K^+]_o$ influence the responsiveness of oxygenated arteries to $\beta$-adrenergic stimuli, we have considered the possibility that $\beta$-adrenergic effects may be altered in hypoxic arteries which release potassium to the interstitial fluid. Figure 9 illustrates that, during hypoxic contraction, stimulation with isoproterenol induces dose-dependent increases in tone. This response to $\beta$ stimulation resembles that seen in the oxygenated artery exposed to elevated $[K^+]_o$ (Fig. 4).

As shown in Figure 2A, $\beta$-adrenergic blockade with L-propranolol alters the $[K^+]_o$-force relation. In particular, augmentations in $[K^+]_o$ between zero and 2 mM in the absence and presence of propranolol produce decreases and increases in arterial tone, respectively (Fig. 2A). If changes in arterial tone induced by hypoxia are mediated partly by the release of endogenous potassium, an artery pre-equilibrated with buffer containing L-propranolol and no added potassium may be expected to respond to sudden hypoxia with an initial increase rather than a decrease in arterial tone. Figure 10 illustrates that hypoxia first evokes a contraction in
a β-blocked artery superfused with potassium-free buffer, then a relaxation, and again a contraction. Reoxygenation induces a reversed contractile sequence, namely relaxation, contraction, and relaxation. The triphasic response to hypoxia may be explained by assuming that gradual accumulation of potassium in the interstitial fluid of the hypoxic artery determines changes in tone that parallel those of the concentration-response curve with propranolol shown in Figure 2A. With reoxygenation, the gradual clearance of potassium produces changes in tone that follow the concentration-response curve in a reversed direction. These observations support the view that contractile responses to hypoxia are determined partly by the release of potassium from hypoxic cells. Furthermore, the findings indicate that hypoxia may determine complex, time-dependent changes in arterial contractility.

**Mechanism of Release of Norepinephrine from the Hypoxic Coronary Artery**

Figure 11 shows that inhibition of hypoxic contraction by β blockade with propranolol does not affect efflux of 42K or NE. This indicates that propranolol has no direct effect on the overflow of neurotransmitter and that arterial relaxation per se does not alter efflux of potassium or NE from the hypoxic artery. Moreover, metabolic effects mediated by β-adrenergic stimulation do not appear to play a major role in promoting 42K efflux. Figure 12 illustrates that colchicine, 10⁻⁵ M, a drug that inhibits exocytotic release of NE but does not affect its release induced by tyramine (Thoa et al., 1975), exerts a potent inhibition on the overflow of NE without influencing efflux of 42K. Furthermore, the inhibition of the release of NE is not overcome by increasing [K⁺]o in the superfusate to 10 mM, a concentration that does not appreciably affect the tone of the hypoxic artery (see Fig. 8). This suggests that hypoxia stimulates the release of NE by an exocytotic (colchicine-sensitive) mechanism. To determine whether colchicine has a direct effect on arterial smooth muscle, arteries from dogs chemically sympathectomized with 6-hydroxydopamine were exposed to 10⁻⁵ M colchicine under aerobic (three arteries) and hypoxic conditions (four arteries). Colchicine produced no change in tone in these denervated arteries. Accordingly, it appears that relaxation of intact, hypoxic arteries by colchicine is caused by the inhibition of the release of NE. Figure 13 illustrates that proadifen (SKF 525A), a drug reported to inhibit efflux of potassium from amphibian skeletal muscle (Henderson and Volle, 1974), suppressed the efflux of both 42K and NE. However, unlike the inhibition of NE release induced by colchicine, an elevation in the superfusate [K⁺]o to 10 mM promptly increases the release of NE, suggesting that potassium-mediated release of NE is not inhibited by proadifen. It appears therefore that proadifen influences NE release predominantly by indirectly decreasing potassium-dependent stimulation of the sympathetic nerves. Although exogenous potassium augments the release of NE, arterial tone increases little, probably reflecting the calcium-antagonistic effect of proadifen on vascular smooth muscle (Kalsner et al., 1970; Henry et al., 1977a).

Potassium and colchicine are known to stimulate...
FIGURE 13 Effect of proadifen (SKF 525A) on the relationship between arterial tone and effluxes of 42K, total 3H, and 3HNE. During proadifen-inhibited efflux of 42K and 3HNE, exogenous K* (10 mM) evokes a prompt increase in 3HNE efflux.

and inhibit exocytosis of NE, respectively (Thoa et al., 1975). To examine further whether hypoxic release of NE involves an exocytotic mechanism, the activity of dopamine β hydroxylase, an enzyme released from the nerve endings during exocytotic discharge of NE (Thoa et al., 1975), was assayed in the effluent of the superfusion experiments described in Figures 6, 7, and 11-13. Figure 14 shows that both potassium and hypoxia promote the release of dopamine β hydroxylase. Furthermore, the relationship between NE and dopamine β-hydroxylase activity is similar in the effluent from oxygenated arteries stimulated with potassium and from hypoxic arteries superfused with or without pharmacological interventions. These similarities suggested to us that the contractile responses to hypoxia were related to the release of potassium from the hypoxic artery. The marked increase in the efflux of 42K triggered by hypoxia is in agreement with this hypothesis. Analysis of the efflux of electrolytes from hypoxic organs poses methodological problems as steady state efflux may never be attained during the progressive changes produced by hypoxia and cell membranes may become permeable to extracellular markers necessary for the estimation of extracellular pools (Henry et al., 1977b). Nevertheless, since potassium is predominantly an intracellular ion and is generally known to be released by hypoxic or metabolically inhibited cells, it appears

Discussion

In the present experiments, sudden hypoxia in isolated canine coronary arteries elicited transient relaxations and subsequent contractions. After sympathetic nerve injury produced by treatment with 6-hydroxydopamine (Gauthier et al., 1972) or storage in the cold (Shibata, 1969), arteries responded to hypoxia with sustained relaxation, indicating that the contractions were neurally mediated. Contractions were inhibited by L-propranolol and enhanced by isoproterenol. In contrast, phentolamine and the inactive D-isomer of propranolol were ineffective in blocking the contractions. Recently, we have demonstrated that adrenergic antagonists and agonists with selectivity for β2 receptors mimicked the effects of L-propranolol and isoproterenol on hypoxic coronary arteries (Henry et al., 1978). On the other hand, cardioselective β blockers and the postsynaptic α blocker prazosin (Davey and Massingham, 1976) in concentrations up to 10^-6 M failed to attenuate the contractions (Henry et al., 1978). Collectively, these findings suggest that hypoxic contraction in isolated canine coronary arteries is mediated by a β-adrenergic mechanism.

As demonstrated in a previous study (Borda et al., 1977), abrupt increases in [K+]o up to 12 mM elicit in oxygenated arteries biphasic relaxation-contraction responses. These responses to potassium have several features in common with those evoked by hypoxia. In both instances, the relaxation is insensitive to adrenergic blockade or denervation, whereas the contraction is effectively suppressed by these interventions. These similarities suggested to us that the contractile responses to hypoxia were related to the release of potassium from the hypoxic artery. The marked increase in the efflux of 42K triggered by hypoxia is in agreement with this hypothesis. Analysis of the efflux of electrolytes from hypoxic organs poses methodological problems as steady state efflux may never be attained during the progressive changes produced by hypoxia and cell membranes may become permeable to extracellular markers necessary for the estimation of extracellular pools (Henry et al., 1977b). Nevertheless, since potassium is predominantly an intracellular ion and is generally known to be released by hypoxic or metabolically inhibited cells, it appears...
very likely that the marked enhancement in $^{42}$K efflux reflected net discharge of potassium from the hypoxic artery (Palaty et al., 1971). In addition to releasing potassium, hypoxic arteries exhibited a decreased reactivity to exogenous potassium. In oxygenated arteries, contractions elicited by elevations in [K+]o, up to 12 mM is mediated by the release of endogenous norepinephrine and is abolished by denervation (Borda et al., 1977). Failure of hypoxic arteries to respond to exogenous potassium may therefore reflect the failure of potassium to stimulate the release of NE. However, when discharge of potassium was inhibited by proadifen, hypoxic nerves were capable of responding to exogenous potassium with enhanced release of NE. Thus, it appears more likely that the unresponsiveness of hypoxic arteries to exogenous potassium reflects the fact that sufficient potassium was released from hypoxic smooth muscle to raise [K+]o in the interstitial fluid to a concentration equaling or exceeding that of the superfusate.

In open-chest animals subjected to coronary ligation, the potassium concentration in blood samples collected from venules in the ischemic zone may exceed 10 mM (Benzing et al., 1971; Downar et al., 1977; Shuchleib et al., 1976). On the basis of the present experiments elevations in [K+]o, in the extracellular fluid of ischemic myocardium may be expected to alter the reactivity of the coronary vasculature to adrenergic stimuli. In dogs, intravenous epinephrine has been found to decrease coronary resistance in the intact heart but to increase it in ischemic myocardium (Grayson et al., 1968). Similarly, in open-chest cats, infusion of norepinephrine has been observed to augment flow in the intact heart but to decrease perfusion in ischemic myocardium (Moore and Parratt, 1973). Stellate stimulation in open-chest dogs has been reported to produce coronary dilation before ischemia, occlusion and contrstion after occlusion (Juhasz-Nagy and Kudasz, 1975). In conscious dogs with acute coronary occlusion, treatment with propranolol has been shown to decrease coronary flow to nonischemic myocardium and to augment perfusion in ischemic zones (Vatner et al., 1977; Barcia et al., 1976). These apparent reversals of adrenergic responsiveness in myocardial ischemia might be related to altered adrenergic effects as described in this study. However, experiments with cut arteries in vitro depend upon arbitrary experimental conditions and the present results may not be directly applicable to physiological phenomena in vivo. Furthermore, results in one species should not be extended to another species (Borda et al., 1977).

In previous reports, isoproterenol in high doses has been observed to induce constrictor responses in vitro in oxygenated rabbit aortas (Furchgott and Bhadrakom, 1952) and feline cerebral arteries (Nielsen and Owman, 1971). High-dose isoproterenol also has been reported to produce increases in blood pressure in dogs (Ludueña, 1962) and cats (Butterworth, 1963) and to elicit venoconstriction in dogs (Kaiser et al., 1964) and man (Eckstein and Hamilton, 1959). The mechanisms mediating these responses to β-adrenergic stimulation have not been completely clarified. However, due to the well-known cytotoxicity of high concentrations of isoproterenol (Rona et al., 1959), it is possible that catecholamine-induced release of potassium may have played a role in these studies.

It is well known that myocardial ischemia and hypoxia promote the release of NE from the heart (Shahab et al., 1969). In an attempt to clarify the mechanism for release of NE induced by hypoxia, the efflux of potassium and/or NE was perturbed with selected inhibitors. Results show that relaxation of the hypoxic artery with β-propranolol had no effect on $^{42}$K efflux. This indicates that maintenance of an elevated arterial tone per se did not promote hypoxic discharge of potassium and that presynaptic β receptors were not involved in regulating overflow of NE. Colchicine, an agent that acts on microtubules and blocks axonal transport and exocytotic discharge of NE (Thoa et al., 1975), inhibited the release of norepinephrine and, like propranolol, relaxed the artery. This effect confirms that hypoxic contraction was mediated by the release of neurotransmitter. Colchicine did not affect $^{42}$K efflux, suggesting that stimulation of arterial smooth muscle by NE was not an important factor in promoting release of potassium. Proadifen, a compound reported to inhibit transmembrane flux of potassium in amphibian skeletal muscle (Henderson and Volle, 1974), inhibited hypoxic efflux of potassium and NE and relaxed the artery. As relaxation and decreased noradrenergic stimulation of the artery did not by themselves decrease potassium efflux, it appears that proadifen inhibited the discharge of potassium by some other mechanism. Effects of proadifen on coronary arterial contractility resemble those of compounds referred to as calcium antagonists (Henry et al., 1977a). However, recent experiments in our laboratory have revealed that other calcium antagonists including verapamil, nifedipine, and diltiazem do not inhibit potassium or NE efflux (unpublished observation). Inhibition of NE overflow by proadifen may reflect a direct effect of the drug on nerves. However, the fact that during inhibited release of endogenous potassium the nerves responded to exogenous potassium with enhanced discharge of NE suggests that proadifen influenced overflow of neurotransmitter indirectly by decreasing the stimulation of the nerves by potassium. Potassium acts on the sympathetic nerve terminals by activating exocytosis of NE (Thoa et al., 1975). A further indication that hypoxic release of NE was mediated by an exocytotic process was provided by the fact that the enzyme dopamine β hydroxylase was released together with the neurotransmitter and that colchicine, an inhibitor of exocytosis, suppressed the release of both NE and the enzyme. Thus, during arterial hypoxia,
overflow of NE appears to involve predominantly an active (exocytotic) process and does not merely reflect a passive leak of neurotransmitter from nerve terminals injured by hypoxia.

In conclusion, the present investigation demonstrates that hypoxia induced in isolated canine coronary arteries cause transient relaxations and sustained contractions. The latter are mediated by potassium-dependent exocytosis of NE which exerted its effect by a β-adrenergic mechanism.

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