A 4-AP-sensitive current is enhanced by chronic carbon monoxide exposure in coronary artery myocytes

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Barbé, Christine, Eric Dubuis, Annie Rochetaing, Paul Kreher, Pierre Bonnet, and Christophe Vandier. A 4-AP-sensitive current is enhanced by chronic carbon monoxide exposure in coronary artery myocytes. Am J Physiol Heart Circ Physiol 282: H2031–H2038, 2002. First published January 24, 2002; 10.1152/ajpheart.00807.2001.—A physiological role of carbon monoxide has been suggested for coronary myocytes; however, direct evidence is lacking. The objective of this study was to test the effect of chronic carbon monoxide exposure on the K⁺ currents of the coronary myocytes. The effect of 3-wk chronic exposure to carbon monoxide was assessed on K⁺ currents in isolated rat left coronary myocytes by the use of the patch-clamp technique in the whole cell configuration. Moreover, membrane potential studies were performed on coronary artery rings using intracellular microelectrodes, and coronary blood flow in isolated heart preparation was recorded. Carbon monoxide did not change the amplitude of global whole cell K⁺ current, but it did increase the component sensitive to 1 mM 4-aminopyridine. Carbon monoxide exposure hyperpolarized coronary artery segments by ~10 mV and, therefore, increased their sensitivity to 4-aminopyridine. This effect was associated with an enhancement of coronary blood flow. We conclude that chronic carbon monoxide increases a 4-aminopyridine-sensitive current in isolated coronary myocytes. This mechanism could, in part, contribute to hyperpolarization and to increased coronary blood flow observed with carbon monoxide exposure.

voltage-gated K⁺ channels; membrane currents; vasodilation; 4-aminopyridine

Carbon monoxide (CO) is an endogenously generated gas that regulates vascular tone. Several lines of investigation provide evidence that CO is a vasodilator acting directly on vascular smooth muscle cells (VSMCs) (27) and that it inhibits smooth muscle cell proliferation (17). In coronary circulation, acute and chronic exogenous CO exposure induced an increase in coronary blood flow (1, 16). Several mechanisms have been suggested to explain this effect, including a direct effect of CO on coronary artery cells (8). Indeed, acute CO induced in vitro an endothelium-independent relaxation of the preconstricted coronary artery (8, 14).

Nevertheless, the cellular mechanism by which acute or chronic CO increases in coronary blood flow is unknown.

Plasma membranes of coronary VSMCs show a dense expression of voltage-gated K⁺ (KV) channels and high-conductance Ca²⁺-activated K⁺ (BKCa) channels. Moreover, the expression levels of these two-gene families are altered in some chronic cardiovascular pathologies, such as arterial hypertension (3). CO can influence the open-state probability of BKCa channels in VSMC membranes (27), and it can activate KV channels in jujenal circular smooth muscle cells (6), thereby regulating the level of resting membrane potential (Em) and contractile force in VSMCs. In the VSMCs of arterial circulation, the hyperpolarizing effect of KV and BKCa channels contributes to the regulation of vascular tone and blood pressure by limiting voltage-dependent Ca²⁺ influx through dihydropyridine-sensitive, L-type Ca²⁺ channels (10).

In the present study, we have examined the contribution of K⁺ current to the regulation of coronary VSMCs and compared the ring Em from control and chronically CO-exposed rats. Subsequently, the action of K⁺ channel blockers was compared on isolated coronary VSMCs from control and chronically CO-exposed rats, and we also tested the effect of these blockers on Em. This study provided the first evidence of an increase in 4-aminopyridine (4-AP)-sensitive current in chronic CO treatment. This gas also induced membrane hyperpolarization and enhancement of the 4-AP effect in membrane cells of coronary artery rings. Furthermore, we found that chronic CO treatment increased the coronary artery blood flow rate, a mechanism probably correlated to the negative Em increase observed in coronary artery rings.

MATERIALS AND METHODS

Exposure to CO. All animal experiments were conducted according to the ethical standards of the Ministè re François

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Another group of rats, named control group (CTR group; n = 6). During the experiments, the CO concentration in the exposure chamber inhaled by an air-CO mixture at 530 parts per million (ppm) for 3 wk. The sudden death of rats was avoided by gradually raising the CO concentration from 300 ppm on the day one to 400 ppm on the second day and to 530 ppm on the third day. This group was named CO group (n = 6). Another group of rats, named control group (CTR group; n = 6), was placed in the same chamber for 3 wk but without CO. During the experiments, the CO concentration in the exposure chamber was continuously monitored (Analyzer Surveyor 5). The CO chamber was opened twice a week for <5 min to change the cages and to replenish them with food and water.

**Isolated heart perfusion.** Rats were anesthetized with intraperitoneal pentobarbital sodium (100 mg/kg). Heparin (1,500 IU/kg) was then injected intravenously. After thoracotomy, hearts were excised, cannulated, and retrogradely perfused through the coronary artery, using the Langendorff method, with a solution at 37°C under a constant perfusion pressure of 70 mmHg. The perfusate medium was a modified Krebs-Henseleit bicarbonate buffer containing (in mM) 118 NaCl, 5.6 KCl, 2.4 CaCl₂, 1.2 MgCl₂, 20 NaHCO₃, 1.2 NaH₂PO₄, and 11 glucose (pH was adjusted to 7.4 with a gas mixture containing 95% O₂-5% CO₂). Perfusate did not recirculate. In each group, hearts were initially perfused with normal solution at a constant perfusion pressure for 40 min. Coronary flow was continuously measured by an electromagnetic blood flow and velocity meter (model 1401; Skalar Medical) placed before the cannula. Moreover, the evolution of coronary flow was stored on a paper polygraph. During the excision of hearts, a direct puncture of blood was performed to evaluate the hematocrit (Hct). At the end of the experiments, hearts were rapidly dissected to separate each ventricle. The degree of hypertrophy was estimated from the ratio of total ventricular mass-to-body mass (heart wt/body wt). This ratio was expressed in milligrams-to-gram. Localization of this hypertrophy (left or right ventricle) was determined by the left ventricular weight-to-right ventricular weight ratio (LVW/RVW).

**Eₙᵣ recordings.** After thoracotomy, the heart was quickly excised and immersed in a cold, physiological Ca²⁺-free saline solution. Physiological saline solution (PSS) contained (in mM) 138.6 NaCl, 5.4 KCl, 1.8 CaCl₂, 1.2 MgCl₂, 0.33 NaH₂PO₄, 10 HEPES, and 11 glucose. The pH was adjusted to 7.4 with NaOH. A segment of the left coronary artery was prepared (500-1,000 μm in diameter). Endothelium was mechanically removed by rubbing the intima. To measure electrical activity, the coronary arterial segment was suspended by two fine stainless steel clips passed through the lumen and maintained in an organ bath at 37°C. One clip was anchored inside the organ bath, and the other clip was connected to a force transducer (model UPI; Pyoden Control). Arterial segments were set at optimal length by equilibration against a passive load of ~15 mN, then the segments underwent a stress-relaxation equilibration period of 80 min (to reach the residual, resting tension). We continuously superfused the ring segments using a peristaltic pump with PSS at the rate of 1 ml/min. Glass capillary microelectrodes were filled with 3 M KCl yielding tip resistances of 40–80 MΩ and connected to a biological high-impedance amplifier (model VF180; Biologic). Transmembrane potentials were recorded with a glass microelectrode mounted on a micromanipulator (Narashige) and monitored under a microscope. Proper impalement was only accepted when a sudden change in voltage was observed on the oscilloscope trace and the potential was maintained for at least 3 min. Measurements were disregarded when the Eₙᵣ slowly decreased, indicating cell damage. Moreover, the electrode tip resistance was monitored before and after impalement to avoid the potential changes caused by electrode artefacts. Change in the Eₙᵣ was displayed on a paper recorder (Linseis), and the data were also stored on a computer.

**Isolation of smooth muscle cells.** The enzymatic isolation of single VSMCs was performed according to published dissociation methods for rat microvessels (9). Left coronary arteries were removed from hearts and cut into small rings. They were incubated for 10 min in a dissociation solution containing (in mM) 145 NaCl, 4 KCl, 10 HEPES, 1 MgCl₂, and 10 glucose (pH was adjusted to 7.4 with NaOH). Papain (5 mg/ml) and dithioerythritol (5 mg/ml) were then added, and the solution was maintained at 37°C for 19 min. The enzyme solution was removed and replaced by a dissociation solution containing 2 mg/ml collagenase and 5 mg/ml trypsin inhibitor (type I-S) at 37°C for 20 min. This solution was removed and replaced by dissociation solution without enzyme. Tissue was then gently agitated at room temperature using a polished wide-bore Pasteur pipette to release the cells. Cells were stored at 4°C and used between 2 and 8 h after isolation. Only long, smooth, optically refractive cells were used for patch-clamp measurements.

**Electrophysiology.** Electrophysiological recordings were obtained using the conventional patch-clamp technique in the whole cell configuration. Cells were placed in a 1 ml volume bath and continuously perfused by gravity at the rate of 4 ml/min from reservoirs containing PSS (see Eₙᵣ recordings). Cell membrane currents were recorded with a patch-clamp amplifier (model EPC-7; List Electronics, Darmstadt, Germany). Patch pipettes were pulled from borosilicate glass capillaries and had a resistance of 3–5 MΩ when filled with pipette solutions. The headstage ground was connected to an Ag-AgCl pellet placed in a side bath filled with the pipette solution linked to the main bath via an agar bridge containing 3 M KCl. Junction potentials between the electrode and the bath were cancelled by using the voltage pipette offset control of the amplifier. Capacitance of the pipette was also cancelled. Intracellular pipette solution contained (in mM) 110 K-aspartate, 20 KCl, 5 HEPES, 2 EGTA, 2 Na₂-creatine phosphate, 1 Na₂-ATP, and 1 MgCl₂ (pH was adjusted to 7.2 using KOH). pCa (−9) was calculated by a computer program developed by Godt and Lindley (7), and the evaluated concentration of K⁺ was 120 mM. Net macroscopic K⁺ currents were generated by stepping the constant holding potential of −60 mV from −90 mV to +60 mV in 10-mV increments (400 ms duration, 5 s intervals). Signals were filtered at 1 kHz and digitized at 5 kHz. Average current amplitudes for the last 50 ms of the pulse were measured. Trials were carried out in triplicate, and an average was calculated in the same cell to estimate current amplitudes expressed in picoamperes per picofarad (pApF) and to normalize differences in the cell membrane area between single vascular myocytes. The tetraethylammonium (TEA)-sensitive current was defined as the difference between the outward current recorded in a drug-free bath solution and the current elicited after cell superfusion of 1 mM TEA. The K⁺ current or 4-AP-sensitive current was defined as the difference between the outward current recorded after cell superfusion with 1 mM TEA and the one obtained after cell superfusion with 1 mM TEA plus 1 mM 4-AP. Voltage-clamp protocols were generated and the data captured with a computer using a Ladmaster TLP-125 interface (Scientific Solutions) and pCLAMP 5.5.1 software.
(Axon Instruments). The analysis was made using Clampfit and Origin 6 (Microcal Software, Northampton, MA).

Chemicals and drugs. 4-AP and TEA were directly dissolved in PSS at the appropriate concentration. All chemicals were from Sigma (St. Quentin Fallavier, France).

Statistics. All results are expressed as means ± SE. In experiments in which comparisons among the different groups were realized, ANOVA was first performed to determine the significance of difference; post hoc analysis was also done using the Student-Newman-Keuls test. The number of experiments (n) refers to the number of cells, rings, or hearts used. Analysis and statistics were performed on isolated cells for patch-clamp experiments, on impaled cells for \( E_m \) measurements, and on isolated heart for perfusion experiments. In all analysis, differences were considered to be statistically significant when \( P < 0.05 \).

RESULTS

Effect of chronic CO on whole cell currents. Membrane capacitance was determined by dividing the integration of capacitive currents by the amplitude of 10 mV voltage steps from \(-60 \) to \(-70 \) mV. Smooth muscle cells isolated from the coronary artery in the CTR group had a membrane capacitance of \( 12.1 \pm 0.9 \) pF \((n = 7)\) not significantly different from that of the CO group \((15.7 \pm 2.0 \) pF, \(n = 7)\).

Figure 1A shows the outward currents recorded on a 12.5-pF cell isolated from CTR group rat. The cell \( E_m \) was held at \(-60 \) mV and stepped in 10-mV increments from \(-90 \) mV to \(+60 \) mV for 400 ms, returning to \(-60 \) mV between steps. Application of 1 mM TEA partially blocked the outward current corresponding to TEA-sensitive channel contribution. Further addition of 1 mM 4-AP had little effect on the remaining outward current, suggesting a small contribution of the 4-AP-sensitive channel in the outward current (Fig. 1A). The current-voltage relationship showed that superfusion of 1 mM TEA resulted in a decrease in the current, whereas the subsequent addition of 1 mM 4-AP to the superfusion solution slightly reduced the residual outward current (Fig. 1B). Figure 2B shows the outward currents recorded on a 17.0-pF cell isolated from a CO group rat. Superfusion of 1 mM TEA partially blocked the outward current, indicating a small contribution of TEA-sensitive channels in this current. Nevertheless, in contrast to the CTR group, the further addition of 1 mM 4-AP mostly blocked the remaining outward current (Fig. 2A). This result suggests that 4-AP-sensitive current constitutes the major part of this outward current. The current-voltage relationship also showed...
that superfusion of 1 mM TEA resulted in a small decrease in the current, whereas the subsequent addition of 1 mM 4-AP to the superfusion solution blocked the residual outward current (Fig. 2).

To take into account the cell membrane area, we divided each current amplitude by the respective cell capacitance. Figure 3 shows the mean current density-voltage relationships in seven CTR group cells and in seven CO group cells. We did not observe any significant modification in the amplitude of the whole cell $K^+$ current density between the two groups (Fig. 3A). Furthermore, near the resting $E_m$ between $-30$ mV and $-40$ mV (corresponding to the $E_m$ where the current density was 0 pA/pF), chronic CO exposure had no effect on currents and no significant differences were observed at $-30$ mV or $-40$ mV. This result suggests that chronic CO exposure did not change 4-AP- and TEA-sensitive currents when cells were held at physiological $E_m$.

Whatever this absence of significant modification, we could not exclude the possibility of a change in the components of whole cell $K^+$ currents. To characterize the $K^+$ current subtypes, we calculated the magnitude of the decrease in the outward current induced by 1 mM TEA by subtracting the global outward current observed in a normal PSS from the outward current recorded in the presence of 1 mM TEA, thereby obtaining the TEA-sensitive current or $I_{TEA}$. In Fig. 3B, the current density-voltage relationship of $I_{TEA}$ is graphically shown in both groups. The density of the current blocked by 1 mM TEA was significantly reduced compared with the density of the control current in both groups. However, this reduction did not significantly differ between CTR and CO groups and for all potentials tested (Fig. 3B). Thus CO inhalation did not change the $I_{TEA}$ density in both groups.

We showed that subsequent addition of 4-AP to the bath induced an additional decrease in the outward current in both types of cells. The magnitude of the outward current inhibited by 1 mM 4-AP was obtained by subtracting the outward current observed in the presence of 1 mM TEA solution from the outward current recorded in the presence of 1 mM TEA plus 1 mM 4-AP, thereby obtaining the 4-AP-sensitive current or $I_{4-AP}$. The current density-voltage relationship of this sensitive current is shown in Fig. 3C. $I_{4-AP}$ was significantly greater in the CO group than in the CTR group for $E_m$ (from $-20$ mV to $+60$ mV; Fig. 3C). Thus CO inhalation increased $I_{4-AP}$ current density in the CO group.

Fig. 2. Effect of TEA and 4-AP on whole cell current-voltage relationships during voltage steps in coronary VSMCs of the CO group. A: typical family of currents recorded in a single smooth muscle cell from the left coronary artery of a rat submitted in vivo to CO in control conditions and after application of TEA and TEA + 4-AP. Whole cell currents were recorded in response to a series of voltage steps from $-90$ to $+60$ mV in 10-mV increments from a holding potential of $-60$ mV. B: current-voltage relationships showing the effect of TEA and 4-AP on the same cell as in A. Note the large effect of 4-AP compared with TEA.
In the presence of TEA and 4-AP, a small residual current persists. To examine the contribution of this current on chronic CO effect, we performed current density-voltage relationship in both groups of cells. Chronic CO had no significant effect on the magnitude of this residual current (data not shown).

**Effect of chronic CO on E\text{m}.** The ring segments of the left coronary artery without endothelium were used to measure \(E\text{m}\) in the coronary artery segments from rats submitted in vivo to CO exposure (CO group) and in those unexposed to CO (CTR group). In the segments from the CO group, \(E\text{m}\) was \(-39.8 \pm 1.6\) mV (\(n = 9\)), and it was \(-30.1 \pm 1.4\) mV (\(n = 9\)) in the CTR group segments (Fig. 4). We concluded that the \(E\text{m}\) of the CO group cells was more polarized compared with that of cells from the CTR group.

This shift of \(E\text{m}\) was accompanied by a modification in 4-AP sensitivity. Indeed, in CTR group cells, application of 5 mM 4-AP produced a small, but not significant, depolarization (\(-3\) mV), contrary to the cells from the CO group (\(-17\) mV depolarization). Note that in the presence of 4-AP, \(E\text{m}\) returned to similar values in both coronary VSMCs of CO and CTR rat.

**Effect of chronic CO on coronary blood flow.** After CO exposure to 530 ppm for 3 wk, the mean body weight did not differ between the CTR group and the CO group (data not shown). CO exposure induced a significant increase in the Hct ratio (Table 1). The heart weight was also increased, and this affected both ventricles (there is an increase in heart weight-to-body weight ratio but not in LVW/RVW ratio) (Table 1).

Because \(E\text{m}\) is more negative after chronic CO exposure, we then predicted that this would induce a vasodilation and then increase coronary blood flow of the CO group. Indeed, we worked under constant pressure, and the flow (corresponding to pressure-to-resistance ratio) is a directed link to arterial coronary resistance. In vivo chronic CO exposure induced a significant ele-


Table 1. Hematological, morphological, and coronary flow changes in isolated perfused hearts after in vivo chronic CO exposure

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<thead>
<tr>
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<th>CTR group</th>
<th>CO group</th>
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<tbody>
<tr>
<td>Hematocrit, %</td>
<td>44.6 ± 0.3</td>
<td>57.8 ± 0.3*</td>
</tr>
<tr>
<td>Heart wt/body wt, mg/g</td>
<td>3.24 ± 0.10</td>
<td>4.04 ± 0.17*</td>
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<tr>
<td>LVW/RVW</td>
<td>3.34 ± 0.09</td>
<td>3.52 ± 0.17</td>
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<tr>
<td>Coronary blood flow, ml-min⁻¹-g⁻¹</td>
<td>12.8 ± 1.1</td>
<td>22.0 ± 3.1*</td>
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Results represent the means ± SE obtained from isolated hearts; n = 6 hearts in each group. CTR, control; CO, carbon monoxide; LVWRV, left ventricle weight-to-right ventricle weight ratio. *P < 0.05, significantly different from CTR group.

Exogenous CO can induce local vascular VSMCs. CO has also been demonstrated to have functional impact on vascular smooth muscles (27). Exogenous CO levels in the air (12, 18) are associated with different results by its competition with oxygen binding to hemoglobin. Nevertheless, a direct action of CO on K⁺ channels is not excluded as observed with BKCa in tail artery smooth muscle cells (27). Moreover, BKCa currents coexist in both populations of cells (CTR and CO groups) but their relative contribution in global current is changed. Before CO treatment, BKCa current component accounts for ~36% and tends to decrease to ~19% after chronic CO treatment. Conversely, the Kv current component increases from ~13 to ~46% after CO exposure. This result suggests that CO treatment could induce a differential expression of K⁺ channels in coronary VSMCs. In this regards, a recent article (24) has already demonstrated a differential expression of Kv and KCa channels in VSMCs after 1-day culture.

Different effects of CO on these K⁺ channels could not be ascribed to different experimental conditions, because for both groups, we used the same experimental conditions. This increase in outward current persists after several minutes of equilibration of intrapipette solution in the cells. A modification of the voltage dependence as well as a direct chemical modification of Kv channels by CO is not excluded as observed with BKCa in tail artery smooth muscle cells (27). More experiments are needed to explore how CO increases Kv current.

Does this increase in 4-AP-sensitive currents have functional impact? In our study, we show that chronic CO exposure induces membrane hyperpolarization of

Fig. 5. Coronary blood flow recorded in the CTR and CO groups. Typical example showing an increase in coronary blood flow in an isolated rat heart excised after 3 wk of exposure to 530 parts/million CO.
coronary rings. This hyperpolarization has already been reported (6, 25, 27) but only during acute CO perfusion and in various preparations, except in coronary VSMCs. VSMCs typically have a stable $E_m$, ranging from $-45$ to $-60$ mV (13). The lower $E_m$ reported in the present study could be due to our own experimental conditions (stretched rings without endothelium were used instead of isolated cells), species, and tissues used. However, variations in $E_m$ observed in this study and in other investigations are similar. Indeed, Rich et al. (23) showed a hyperpolarization of about $10$ mV in rabbit corneal epithelial cells, and a hyperpolarization of about $20$ mV was observed in single VSMCs by Wang (27). Thus these data confirm that the CO effect is endothelium-independent, as suggested in several reports using different techniques and protocols (14, 15, 27).

This hyperpolarization was associated with an increase in 4-AP sensitivity. Indeed, in cells from the CTR group, application of 4-AP produces a small, but not significant, depolarization and significantly depolarized the cells from the CO group. This result obtained in coronary rings suggests that this hyperpolarization and the increase in 4-AP sensitivity are due to an increase in $K_V$ current that regulates $E_m$. Furthermore, $E_m$ returned to similar values in both coronary VSMCs of CO and CTR rat after the addition of 4-AP in the bath solution. Then, 4-AP tends to nullify the effect of chronic CO exposure.

Plasma membranes of coronary VSMCs showed a dense expression of 4-AP-sensitive $K_V$ channels (3, 22), and the hyperpolarizing effect of $K_V$ channels implied that these channels were activated near $E_m$. How then can we explain the hyperpolarization effect of CO (from approximately $-30$ mV to approximately $-40$ mV) observed in coronary rings and the increase in 4-AP sensitivity, when we have no evidence for activation of 4-AP-sensitive $K^+$ currents at resting $E_m$ of isolated coronary VSMCs (between $-30$ mV and $-40$ mV)? The simplest explanation should be that 4-AP-sensitive current is increased by chronic CO at $E_m$ that is more negative in coronary rings than in isolated VSMCs. Indeed, qualitative differences in the physiological responses to natural stimuli could exist between VSMCs and clusters of VSMCs that may arise from electrical contact between VSMCs. For example, nitric oxide induced vasodilation, although cGMP dependent relied on gap junctional communication (11). Furthermore, in whole cell experiments and in contrast to microelectrode experiments, the loss of cytoplasmics constituents, including nucleotides, by rapid diffusion into the pipette may impair intracellular regulatory mechanisms that regulate CO effects on $K^+$ current. For example, it has been demonstrated that 4-AP-sensitive currents of coronary VSMCs were regulated by cyclic nucleotide-dependent kinases (4) and that CO stimulated $K^+$ current by activation of guanylate cyclase, which increased cGMP levels (23). Another possibility is that the hyperpolarization induced by CO is not due to activation of 4-AP-sensitive $K_V$ currents, but how can we explain that 4-AP tended to nullify the effect of chronic CO on $E_m$?

As we were unable to confirm a hyperpolarization induced by chronic CO in isolated VSMCs, the physiological role of this increase in 4-AP-sensitive current amplitude is currently speculative. Nevertheless, the hyperpolarization obtained in coronary rings may contribute to the regulation of vascular tone and blood pressure by limiting voltage-dependent Ca$^{2+}$ influx. We should then observe a dilation and an increase in coronary blood flow in isolated heart preparations from rats exposed to chronic CO. Indeed, our data show that coronary blood flow is increased in rats exposed to CO for 3 wk. This finding is consistent with previous reports showing a vasodilation induced by acute and chronic exposure to CO (1, 14, 16). Furthermore, our data from the CO-exposed rats are in good agreement with previous studies with regard to the increase in the Hct ratio and the cardiomegaly that involves both ventricles (1, 2, 20). Cardiomegaly is a well-known response to chronic CO inhalation (1, 2, 20). Particularly, we demonstrated that under exposure to several concentrations of CO, hypertrophy begins to occur, but, at $530$ ppm CO exposure, hypertrophy is significant after 1-wk exposure and is maintained throughout CO exposure to a similar value (25%). Moreover, CO exposure led to hypertrophy by volume overload (21). Cardiomegaly developed in both ventricles and aorta with the addition of new myocardial constituents (19) and involved an increase in lumen dimensions with some primarily small increase in wall thickness of the ventricles (i.e., eccentric hypertrophy) (21).

In summary, we have demonstrated that chronic exogenous CO increased a 4-AP-sensitive current in isolated VSMCs and induced membrane hyperpolarization and an enhancement of 4-AP’s effect on $E_m$ of coronary artery rings. The causal link between these actions remains to be established, but taken together, these results could, in part, explain the increase in coronary blood flow observed with CO.

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