Heterogeneous Kv1 function and expression in coronary myocytes from right and left ventricles in rats

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Gautier M, Hyvelin J-M, de Crescenzo V, Eder V, Bonnet P. Heterogeneous Kv1 function and expression in coronary myocytes from right and left ventricles in rats. Am J Physiol Heart Circ Physiol 292: H475–H482, 2007. First published May 26, 2006; doi:10.1152/ajpheart.00774.2005.—Coronary blood flow control is not uniform along the vascular tree and particularly between the right coronary artery and the left anterior descending artery. Resting membrane potential that contributes largely to the vascular tone is mainly regulated by K+ channels in coronary myocytes. In the present study, we hypothesized that right coronary artery (RCA) and left coronary artery (LCA) exhibited a cell-specific function of K+ channels. The net outward current was markedly greater in RCA compared with LCA, and this difference was due to a larger 4-aminopyridine (4-AP)-sensitive voltage-gated potassium (Kv) current in RCA cells, whereas the iberiotoxin (IbTx)-sensitive, large conductance Ca2+-dependent potassium (BKCa) current was smaller in RCA cells. To further increase the membrane resting potential of this Kv current, we used 50 nM correolide, which specifically blocked Kv1 family α-subunits. Outward currents generated by ramp depolarization protocols were highly sensitive to correolide in both RCA and LCA cells, suggesting that Kv1 contributed for a large part to the net outward current. 4-AP-induced contractions in isolated RCA, and LCA were greater than IbTx-induced contraction. Furthermore, the 4-AP-induced contraction in RCA was significantly greater than that in LCA, which is in agreement with the electrophysiological data. Finally, the Kv1.2 α-subunit but not the Kv1.5 was detected in both RCA and LCA using primary specific antibody in Western blotting and immunofluorescence assay, and expression of Kv1.2 α-subunit was markedly higher in RCA compared with LCA. In summary, we reported for the first time a heterogeneous function and expression of Kv1 α-subunits in rat coronary myocytes isolated from RCA or LCA.

circulation; potassium channel; perfused coronary; membrane currents

The functional characteristics of the coronary circulation may be described in terms of the relationship between steady-state perfusion pressure and the amount of blood flow. There are functional hemodynamic differences among coronary vascular beds that are linked to pressure differences among the vascular bed. These differences in compressive forces result in almost continuous flow in the right coronary bed, whereas flow in the anterior left descending bed occurs mainly during diastole (24, 30). Furthermore, when aortic pressure rises and the ventricle is loaded, the right coronary bed exhibits reflex increase of blood flow, whereas the anterior left descending coronary artery vasodilates poorly (29). Such differences in the hemodynamic profile between the left (LCA) and right coronary arteries (RCA) may underline differences in the regulation of basal tone between these two vascular beds.

The coronary vascular tone is modulated by several factors, including neural, hormonal, metabolic, and myogenic, which act, in part, on smooth muscle (10, 11, 17). Interestingly, it has been shown that the myogenic response, which contributes significantly to flow regulation (7), is not uniform within the coronary vascular tree (10). In the arterial circulation, it is well known that tone is mainly determined by the membrane potential of vascular myocytes. Under physiological conditions, resting membrane potential is mainly regulated by voltage-gated K+ (Kv) channels, particularly at lower levels of Ca2+, when large conductance Ca2+-dependent K+ (BKCa) are less active (33). Similarly, in the coronary vascular bed, it is assumed that the resting membrane potential under physiological conditions is regulated mainly by Kv with a minor contribution from the BKCa (3, 21, 23, 31). Chemical and mechanical stimuli, such as transmural pressure, can influence the open-state probability of these channels to regulate the level of membrane resting potential (Erest) in arterial myocytes and thereby can alter the coronary vascular tone (9).

Thus it is conceivable that the functional hemodynamic differences between the left and right coronary vascular beds result in different K+ channels expression and consequently in different tone regulation among these arteries. It is noteworthy that different types of K+ channels have been described in coronary myocytes from several animal models (8, 22, 23, 35, 37), supporting that coronary vascular tone may reflect the integrated contribution of a diverse population of K+ channels. Nevertheless, to the best of our knowledge no study has so far compared the contribution of the K+ channels in both LCA and RCA from the same species.

Kv channels may have a heterotetrameric structure. Recently, it has been shown that heteromultimeric Kv1 channels contributed to myogenic control of arterial diameters in rats (27). Moreover, in rat renal microvascularature, K+ current was thought to be heteromultimeric delayed-rectifier Kv1.2 and A-type Kv1.4 subunits that contribute to blood pressure regulation (12), whereas Kv1.2/Kv1.5 heteromultimeric channel was preferentially expressed in rat cerebral myocytes and contribute to Em and diameter of small cerebral arteries (1). Then, in these two vascular types, the Kv1.2 α-subunit has been identified to contribute to the delayed rectifier K+ current (Kur), which strongly regulates Em.

In the present study, we tested the hypothesis that the functional hemodynamic difference between the RCA and the LCA could be linked to differential properties of K+ channels,
and more particularly, the Kv1 channel family, in the membranes of the RCA and LCA myocytes. We assessed the electrophysiological profile RCA and LCA myocytes, and more particularly, the contribution of both IbTx-sensitive BKCa- and 4-aminopyridine (4-AP)-sensitive Kv currents. We confirmed differences in these profiles by performing functional studies, Western blot, and immunohistochemistry.

**MATERIALS AND METHODS**

All animal experiments were conducted according to the ethical standards of the Ministère Français de l’Agriculture for the Care and the Use of Laboratory Animals (authorization no. 006121).

**Tissue preparation.** Adult (10–12 wk old, 300–350 g) male Wistar rats (Charles River Laboratories, L’Arbresle, France) were anesthetized with intraperitoneal pentobarbital sodium (100 mg/kg). After a thoracotomy was performed, hearts were quickly excised and immersed in cold (4°C) physiological saline solution (PSS) containing (in mM) 138.6 NaCl, 5.4 KCl, 1.2 CaCl2, 1.2 MgCl2, 0.33 NaH2PO4, 10 HEPES, and 11 glucose (pH was adjusted to 7.4 with NaOH). The heart was cannulated and retrogradely perfused through the coronary arteries using cold PSS to remove blood from the circulation. Coronary arteries were then carefully dissected in cold PSS. The left anterior descending branch of the main LCA and the right marginal branch of the RCA were removed. Vessels were placed in cold PSS and carefully cleaned of fat and adventitia.

**Isolation of coronary myocytes.** The enzymatic isolation of single coronary myocytes was performed according to published dissociation methods for rat microvessels (6, 18) and modified for coronary arteries (3). Briefly, coronary arteries were cut into small rings that were placed successively in a (Ca2+ free) dissociation solution containing (in mM) 138.6 NaCl, 5.4 KCl, 1.2 CaCl2, 10 HEPES, and 11 glucose (pH was adjusted to 7.4 with NaOH) for 10 min at room temperature and then placed in a second dissociation solution at 37°C containing 1 mg/ml papain and 1 mg/ml dithioerythritol (both from Sigma, St. Louis, MO) for 12 min and in a third dissociation solution at 37°C containing 1.6 mg/ml collagenase (type H, Sigma) and 1.6 mg/ml trypsin inhibitor (type I-S, Sigma) for 9 min. Tissues were then replaced twice in the first dissociation solution for 10 min and were gently agitated using a polished wide-bore Pasteur pipette to release the cells. Cells were stored at 4°C and used on the day of experiment. Only relaxed, long, smooth and optically refractive myocytes were used for patch-clamp experiments.

**Patch-clamp experiments.** Electrophysiological recordings were obtained by using the conventional patch-clamp technique (15) in the whole cell configuration. Myocytes were placed in a 0.5-mL volume bath and continuously superfused by gravity with PSS at the rate of 1 mL/min. Different test solutions were applied to the cell at a rate of 100 μL/min by microcapillaries, and <10 s was needed to completely change the perfusing solution around the cell. Cell membrane currents were recorded with an Axopatch 200B patch-clamp amplifier (Axon Instruments). Patch pipettes were pulled from borosilicate glass capillaries and had a resistance of 5–7 MΩ. Pipette potential and capacitance were electronically compensated. Intracellular pipette solution contained (in mM) 125 glutamic acid, 20 KCl, 1 Na2ATP, 0.37 CaCl2, 1 MgCl2, 10 HEPES, and 1 EGTA (pH was adjusted to 7.2 with KOH). pCa (~7) was calculated by a computer program developed by Godt and Lindley (14), and the evaluated concentration of K+ was 120 mM. The membrane capacitance was determined by dividing integration of capacitative currents by amplitude of 10-mV voltage steps. Membrane resistance was estimated as the slope of the current-voltage curves between ~90 and ~60 mV, where no dynamic currents were activated. Cell membrane currents were recorded with a patch-clamp amplifier (Axopatch 200B, Axon Instruments). Signals were filtered at 1 kHz and digitized at 5 kHz. Peak current elicited at a single membrane potential was defined as the average of the latest 100 ms of the pulse encompassing the maximal current point. Trials were performed in triplicate in the same cell and averaged to estimate peak current amplitude. Currents were normalized to cell capacitance and were expressed as picoamperes per picofarad (pA/pF).

Net macroscopic currents were generated by stepwise 8-mV depolarizing pulses (400 ms duration, 5-s intervals) with a constant holding potential of ~60 mV to +60 mV. Based on their pharmacological and electrophysiological properties, the following K+ current subtypes were identified. Response to K+ channels blockers was recorded after the addition of iberiotoxin (IbTx) for a final concentration of 100 nM in the bath and 4-AP (3 mM) to block BKCa channel type (20) and Kv channel type (32), respectively. Correolide (50 nM), a selective inhibitor of the Kv1.1 gene family (16), was used to examine the molecular basis of Kv current. Both IbTx and 4-AP were provided from Sigma, whereas correolide was a kind gift from Dr. Gregory Kazcorowski and the Merck Research Laboratories.

The IbTx-sensitive current was defined as the difference between outward current recorded in drug-free bath solution and in the presence of 100 nM IbTx. The 4-AP-sensitive current was defined as the difference between outward current recorded in the presence of 100 nM IbTx and that in the presence of 100 nM IbTx plus 3 mM 4-AP.

Voltage-clamp protocols were generated, and the data were captured with a computer using a Digidata 1200 interface (Axon Instruments) and pClamp8 software (Axon Instruments). The analysis was carried out using Clampfit 8.1 and Origin 6.0 software (Microcal Software, Northampton, MA).

**Coronary contractility measurements.** Rats were anesthetized with intraperitoneal pentobarbital sodium (100 mg/kg) and heparinized (100 U/kg). After a thoracotomy was performed, the heart was quickly excised and immersed in cold cardioplegic solution. Myocardium was dissected, under bincular control, to expose the left anterior descending branch of the main LCA or the right marginal branch of the RCA. Basically, a small window (5 × 5 mm) was dissected to expose a small segment of the coronary artery (5 mm length). Thus the vascular segment was carefully cleaned off the myocardium, and a thin dark plastic film was placed under the vessels. The heart was placed into a 3-mL chamber filled with warmed PSS. The pulmonary artery was transected to facilitate coronary venous drainage. Hearts were perfused at constant flow by an aortic cannula delivering warm PSS. The perfusion pressure was set to 60 mmHg and allowed to stabilize over 1 h before the vessel entered into the protocol. Coronary arteries were successively perfused with PSS, PSS plus 4-AP (3 mM), and PSS + IbTx (100 nM). A 30-min rinsing period was allowed between each experimental condition. The diameter of the coronary artery was continuously visualized on a monitor using a CCD black and white camera fitted to a bincular. For each experimental condition, once steady state was achieved, a snapshot of the vessel was taken and stored into a computer for offline analysis.

**Coronary protein preparation and Western blot.** Coronary proteins were prepared as previously published with modifications (38). Briefly, coronary arteries were homogenized with a PowerGen 125 homogenizer (Fisher Scientific) in 1 mL of Tris-buffed saline (10 mM Tris·HCl, pH = 7.4, 1 mM EDTA, 0.3 mM sucrose) containing protease inhibitor mixture (1 μg/ml antipain hydrochloride, 1 μg/ml leupeptin hemisulfate, 1 mM benzamidine hydrochloride hydrate, 1 mM iodoacetamide, 1 mM 1,10-phenanthroline monohydrate, 0.1 mM phenylmethylsulfonyl fluoride, and 0.001 mM peptatin A). Homogenates were clarified by centrifugation at 8,500 rpm at 4°C for 10 min. Protein concentration was determined using Bradford Protein Assay with BSA as standard. Each sample was then analyzed by SDS-7.5% polyacrylamide gel. Resolved proteins were transferred onto nitrocellulose membrane. Primary polyclonal antibodies against Kv1.2 or Kv1.5 (Alomone Labs, Jerusalem, Israel) were used at 1:200 dilution; primary monoclonal antibody against α-smooth muscle actin (Sigma) was used at 1:400. Horseradish peroxidase-conjugated secondary goat anti-rabbit IgG for Kv1.2 and Kv1.5 was used at 1:5,000 dilution, and goat anti-mouse IgG for α-smooth muscle actin was used at 1:10,000. Membranes were incubated overnight at 4°C with primary antibodies.
and 1 h at room temperature with secondary antibodies. The immuno-
reactive bands were detected by Enhanced Chemiluminescence
Western Blotting Detection Kit (ECL, Amersham Biosciences, Little
Chalfont Buckinghamshire, UK). Signal intensity of the immuno-
active Kv bands was normalized to the expression of α-smooth
muscle actin.

Immunofluorescence. Coronary arteries were carefully dissected as
previously described. A small part of thoracic aorta was also collected
and served as positive control. Vascular tissues were embedded in
OCT compound and snap frozen. Thin (10-μm thick) sections were
washed twice for 5 min in solution containing 70% PBS and 30% fetal
bovine serum. Slides were incubated with 1:200 polyclonal anti-
Kv1.2 α-subunit (Alomone) in PBS during 30 min. Slides were then
washed with PBS twice for 5 min and incubated with 1:5,000
secondary labeled goat anti-rabbit IgG antibody with Alexa Fluor 594
fluorescent dye conjugated (Molecular Probes) in PBS during 30 min.
Finally, they were washed with PBS twice for 5 min and allowed to
dry. Slides were cover-slipped in Fluoromount-G. Images were ac-
dquired on a fluorescence microscope (Leica, DMR, Leica Microsys-
tems Wetzlar), where approximate absorption was 590 nm and fluo-
rescence maximal emission was 617 nm for conjugates.

Statistical analysis. Results are expressed as means ± SE. For
electrophysiological data, statistical analyses were made with the
unpaired Student’s t-test or the Mann-Whitney’s test when normality

test failed (Anderson-Darling test). The number of experiments (n)
refers to the number of cells or to the number of Western blots.
Differences were considered significant for P < 0.05. For coronary
contractility measurements, internal coronary artery diameter was
measured using Optimas 6.5 (Media Cybernetics) software. Internal
diameter of coronary artery was measured by two different blind
observers. No statistical differences were observed between the two
observers. The decrease of the diameter induced by each drug 4-AP
and IbTx was expressed as a percentage of the diameter recorded
before the addition of the drug. Comparisons intravascular bed were
made with the paired Student’s t-test, whereas intervascular bed
comparison (i.e., LCA vs. RCA) were made with the unpaired Stu-
dent’s t-test. The number of experiments (n) refers to the number of
coronary arteries tested. All statistical analysis was realized using
Minitab software (Minitab) and SigmaStat 3.0 (Systat Software).

RESULTS

Effect of K+ channel blockers on whole cell currents in LCA and
RCA. Typical effects of IbTx and 4-AP on shown are on the
whole cell current recorded in LCA (Fig. 1A) and RCA (Fig.
1B) myocytes. In these two examples, rapidly activating out-
ward currents were observed at test potentials positive to −20
mV, which showed little inactivation during the 400-ms test pulse
as already described in rabbit, guinea pig, and human coronary myocytes (35). These currents seemed smaller in the
LCA cell compared with the RCA cell. Outward currents
recorded in both LCA and RCA cells exhibited little sensibility
before superfusion with 100 nM IbTx, a specific calcium-dependent
K+ channels inhibitor, whereas a subsequent addition of 3 mM
4-AP, a Kv channel inhibitor, in the superfusion solution
containing 100 nM IbTx blocked a large component of these
currents. This latter component appeared lower in the LCA cell
compared with the RCA cell.

To take into account the cell membrane area, we divided the
mean current amplitude by the respective membrane capaci-
tance (Fig. 1C). The current densities in LCA cells were
markedly and significantly lower (P < 0.05) than in RCA cells
(16.4 ± 1.3 pA/pF, n = 26 and 22.3 ± 1.6 pA/pF, n = 30, at
+60 mV, respectively).

The IbTx-sensitive current density due to the activity of BKCa
was obtained by subtracting the outward current re-
corded in the presence of 100 nM IbTx from the net outward
current recorded in control PSS superfusion (Fig. 1D). This
IbTx-sensitive current density was greater in LCA compared
with RCA cells (9.0 ± 1.5 pA/pF and 4.7 ± 0.7 pA/pF,
respectively, at +60 mV; P < 0.05).

The 4-AP-sensitive current density due to the activity of Kv
was obtained by subtracting the outward current recorded in
the presence of 100 nM IbTx plus 3 mM 4-AP from the
resulting outward current recorded in the presence of 100 nM
IbTx (Fig. 1E). This current density was significantly lower in
LCA (n = 26) than in RCA (n = 30) cells (P < 0.05). At +60
mV, this 4-AP-sensitive current density was 4.3 ± 0.5 pA/pF
in LCA and 13.0 ± 1.2 pA/pF in RCA. Then the net outward
current was lower in LCA than in RCA cells, and this differ-
ence was due to a greater 4-AP-sensitive current density in
RCA cells, whereas the IbTx-sensitive current was lower in
RCA compared with that in LCA cells.

Effect of correolide on whole cell current in LCA and RCA.
To further investigate the nature of the Kv channel involved in
the repolarizing net outward current, we tested the effect of
correolide, a selective blocker of the Kv1 channels family.
Using coronary myocytes preincubated with 100 nM IbTx to
eliminate BKCa current, we tested the effect of 50 nM of
correolide on the remaining current. In the presence of IbTx,
the outward remaining current was higher in RCA than in LCA
myocytes (Fig. 2A), This remaining current was highly sensi-
tive to correolide. The correolide-sensitive Kv-family current
densities averaged 12.6 ± 2.8 pA/pF in LCA cells (n = 6) and
22.0 ± 3.4 pA/pF in RCA cells (n = 7) (Fig. 2B). Finally, after
treatment with correolide, we observed a small residual

current in both LCA and RCA, which was sensitive to 3 mM
4-AP (Fig. 2C). This residual current, after application of
correolide, was equal in the two cell types, which suggests that
the differential Kv current in LCA and RCA cells could be
attributed to a Kv1 current family.

Effect of 4-AP and IbTx on basal tone in LCA and RCA. To
ensure that the difference in the electrophysiological profile has
a functional role in the regulation of basal tone of coronary
bed, we assessed the effect of the K+ channels blockers on
perfused coronary artery. Both coronary arteries LCA and
RCA developed a contractile force in response to K+-rich
solution (KCl 80 mM), as indicated by the decrease in the
internal diameter. The response to 80 mM KCl was similar in
both coronary arteries (Fig. 3).

In the RCA, the addition of 4-AP (3 mM) induced a con-
traction that was about twofold higher than that elicited by
IbTx (100 nM) (P < 0.001), whereas in LCA, the 4-AP-
induced contraction was slightly higher than that elicited by
IbTx (Fig. 3).

Interestingly, the comparison of the RCA to the LCA re-
vealed that the 4-AP induced a greater contraction in the RCA
compared with the LCA, whereas there was no significant
difference in the response induced by IbTx between the two
vascular beds (Fig. 3). These data are in agreement with the
difference in the electrophysiological profile between the RCA
and LCA.

Expression of Kv1.5 and Kv1.2 α-subunits in coronary
arteries. It was reported that the delayed-rectifier K+ current
was due to a heterotetrameric assembly of Kv1.2/Kv1.5 α-sub-
units in LCA.
units in various vascular types, including cerebral (1), mesenteric (25, 38), and coronary (22) in the rat as well as in the rabbit portal vein (19, 34). We tested the polyclonal antibody targeted to the Kv1.5 pore-forming subunit in coronary arteries as well as in the mesenteric artery. Whereas incubation of mesenteric vascular proteins with anti-Kv1.5 revealed two intense immunoreactive bands at 50 and 100 kDa, no signals were detected for coronary arteries (Fig. 4B). On the other hand, we tested the polyclonal antibody targeted to the Kv1.2 pore-forming subunit. Figure 4A showed that incubation of coronary vascular proteins with anti-Kv1.2 antibody revealed one intense immunoreactive band at 75 kDa in both RCA and LCA as previously shown in other vascular tissues as in the mesenteric artery (38) and in the pulmonary artery (36). Kv1.2 immunoreactive bands were normalized to smooth muscle-actin immunoreactive band and indicated that the amount of Kv1.2 protein was significantly lower in LCA (n = 3) than that in RCA (n = 3, P < 0.05; Fig. 4C). In agreement with the Western blot and the functional studies, immunofluorescence on coronary rings showed that LCA expressed a reduced amount of Kv1.2 α-subunit protein compared with the RCA (Fig. 5).

**DISCUSSION**

The present study reveals differences in the repolarizing net outward currents recorded in left and right coronary myocytes of the adult rat, which contribute in a greater role of Kv+ channels in the control of resting tone of the RCA. Specifically, we have identified marked differences in the Kv current between the LCA and RCA. The delayed-rectifier Kv1 current is larger in the RCA. The Kv1.2 pore-forming subunit, but not the Kv1.5, was more expressed in the RCA than in the LCA. Finally, Kv+ channels play a greater role in basal tone of the RCA compared with the LCA.

*Net outward current is greater in RCA than in LCA cells.* Membrane currents elicited by membrane depolarization are outwardly rectifier. These currents displayed fast activation from about −20 mV and slow or no inactivation during the 400-ms pulse (Fig. 1A). Our records are consistent with pre-
Previous studies performed in guinea pigs, humans, rabbits (35), and rats (23). Most studies related to the characterization of membrane currents in coronary smooth muscle cells were performed on cells isolated from the LCA (8, 21, 26, 35, 37). In the present study, macroscopic outward currents were comparatively measured in myocytes from both LCA and RCA, and for the first time, we reported that macroscopic outward current elicited by membrane depolarization were greater in RCA cells than in LCA cells (Fig. 1C).

$K_v$ currents are greater in RCA than in LCA cells. Our data showed that 100 nM IbTx superfusion resulted in a small decrease in the macroscopic outward current, whereas the subsequent addition of 3 mM 4-AP blocked a large part of the residual outward current in both LCA and RCA cells. Our results demonstrated that $K_v$ and $B_{K_Ca}$ currents coexist in both populations of cells (LCA and RCA) and contribute to the majority of the net outward current in coronary myocytes as previously shown (21). We observed that $B_{K_Ca}$ current density was lower in RCA cells compared with LCA and that its contribution in the net outward current is small. On the other hand, the $K_v$ current density is greater in RCA than in LCA cells. Our results demonstrated that this $K_v$ current is highly sensitive to correolide, suggesting that the coronary $K_v$ channel is mainly composed of pore-forming $K_v1\alpha$-subunits (Fig. 2).

Myocardium does not have energetic reserve, thus an adequate oxygen delivery is crucial to ensure a proper functionality of the cardiomyocytes. In this way coronary circulation needs to adapt the blood flow (thus the diameter of coronary) to the variation of myocardial oxygen consumption. Among $K_v$ channels, the $K_v1.2$ and $K_v1.5$ are known to be sensitive to oxygen levels. Also, we specifically looked at their expression in the LCA and RCA. It has been shown that $K_v1.2$ and $K_v1.5$ coassemble to form heteromultimeric delayed rectifier

![Fig. 2: Effect of correolide on outward current-voltage relationship in LCA and RCA.](image)

A: outward current-density $[I_{K(Total)}]$ in both LCA and RCA. B: $K_v$ correolide-sensitive current-density $[I_{K(Corr)}]$ was significantly greater in RCA compared with LCA cells. C: residual 4-AP-sensitive current $[I_{K(4-AP)}]$ was the same for RCA and LCA cells after inhibition of a $K_v1.X$-sensitive current by correolide (50 nM). Each symbol represents the mean $\pm$ SE. *$P < 0.05$ significantly different.

**Fig. 3: Effect of IbTx and 4-AP on basal tone in RCA and LCA.** Left: typical snapshot of a LCA and a RCA artery perfused successively with physiological saline solution (A), KCl (80 mM, B), 4-AP (3 mM, C), and IbTx (100 nM, D). A 30-min rinsing period was allowed among KCl, 4-AP, and IbTx. Right: change in the internal diameter of the RCA and LCA in presence of KCl, 4-AP, or IbTx. Changes in diameter are expressed as percentage of reduction of diameter compared with the diameter of the vessel prior addition of the drug. *, **Significant difference between two experimental conditions within the same vessel ($*P < 0.01$ and $**P < 0.005$, paired Student’s $t$-test). #Significant difference between the RCA and LCA under similar experimental condition ($#P < 0.05$, unpaired Student’s $t$-test).
**K+ current in the rabbit portal vein** (19, 34). Moreover, protein expression of these two Kv1 α-subunits has been reported in various vascular types (1, 2). In the present study, Western blot data provide the first evidence that the pore-forming Kv1.2 α-subunit is expressed in coronary tissues, which is confirmed by immunofluorescence. Kv1.2 is more expressed in the RCA than in the LCA artery, which could explain the electrophysiological heterogeneity between these two vascular sisters (Fig. 4). Surprisingly, we did not detect the pore-forming Kv1.5 α-subunit in rat coronary arteries, whereas our anti-Kv1.5

**Fig. 4. Western blots of vascular tissue homogenates from RCA and LCA (30 µg/lane); molecular mass markers (high-range prestained SDS-PAGE standards, Bio-Rad) are shown on left. A: incubation of proteins with affinity-purified Kv1.2 antibody revealed one intense immunoreactive band at the expected molecular mass (~75 kDa) in the two coronary arteries. B: no signal was apparent in coronary arteries following incubation of proteins with affinity-purified Kv1.5 antibody, whereas two intense immunoreactive bands were revealed in mesenteric artery (MA) tissue. C: normalization of Kv1.2 signal to α-sm-actin signal indicated that the pore-forming Kv1.2 α-subunit is more expressed in RCA (n = 3) than in LCA (n = 3).**

**Fig. 5. Arterial rings (10-µm thick section) of (A) and (B) labeled with anti-Kv1.2 antibody and visualized with Alexa Fluor 594-conjugated secondary antibody. Negative control for RCA and LCA, i.e., staining in absence of the first antibody, are showed in C and D, respectively.**
antibody was able to detect protein of the predicted mass in mesenteric artery samples. Similar negative findings for Kv1.5 expression in vascular tissue have been reported and extensively discussed by Cheong et al. (5). However, Li et al. (22) recently observed Kv1.5 expression in left coronary myocytes. Although our present results differ from those reported by Li et al., in this previous study it is noteworthy that Western blot was performed from myocytes isolated from LCA and incubated in medium culture at 37°C for 24 h. Furthermore, in this previous study, myocytes were isolated from smaller coronary artery (internal diameter 150–200 μm) than that in our present study (300–350 μm). Such differences in the experimental protocol could explain the differences in the results. Finally, protein expression of additional Kv1 subunits, including Kv1.1 (13, 28), Kv1.3 (5, 13, 38), and Kv1.6 (4, 5, 27), has been also reported in vascular tissues, and a complete molecular characterization of the coronary delayed rectifier K⁺ current needs further investigations.

Physiological relevance. At the physiological level of intracellular Ca²⁺ concentration, the Kv channels importantly regulate the resting membrane potential of coronary myocytes (3, 21, 23), which is the major determinant of coronary arterial tone (9), whereas the BKCa channel is enhanced by elevation of intracellular Ca²⁺ and induces membrane hyperpolarization to limit Ca²⁺ entry through voltage-gated Ca²⁺ channels (21). There is much evidence that Kv1 channels contribute to arterial tone and vessel diameter regulation. In rat cerebral small arteries and arterioles as well as in rabbit portal vein, both resting membrane potential and arterial diameter were shown to be sensitive to specific Kv1 blockers that induce depolarization and vasoconstriction (1, 4, 5, 27). In the present study we observed a similar role of the Kv1 channels. Indeed, in pressurized arteries, we clearly showed that 4-AP induced greater contraction than IbTx in both RCA and LCA, suggesting that the Kv1 channels play important role in regulating basal tone. Our finding that 4-AP-induced contraction was greater in RCA than in LCA supported our electrophysiological data (Figs. 1B and 3). The greater contribution of Kv channels in RCA could explain the heterogeneity in autoregulatory mechanisms along the coronary vascular tree (10, 29) and could be linked to the difference in the transmural pressure between the LCA and the RCA. Particularly, the left anterior descending branch appears to be less protected than the RCA and vasodilates poorly when aortic pressure rises and the left ventricle is loaded (29).

In summary, the present study is the first to demonstrate regional electrophysiological heterogeneity at the single cell level in the rat coronary circulation. These differences in repolarizing currents between myocytes from the LCA and the RCA are due to a greater activity and protein expression of Kv1 channels in the RCA.

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DISCLOSURES

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