Heteromultimeric Kv1.2-Kv1.5 Channels Underlie 4-Aminopyridine–Sensitive Delayed Rectifier \(K^+\) Current of Rabbit Vascular Myocytes

Paul M. Kerr, Odile Clément-Chomienne, Kevin S. Thorneloe, Tim T. Chen, Kuniaki Ishii, David P. Sontag, Michael P. Walsh, William C. Cole

Abstract—The molecular identity of vascular delayed rectifier \(K^+\) channels (\(K_{\text{DR}}\)) is poorly characterized. Inhibition by 4-aminopyridine (4-AP) of \(K_{\text{DR}}\) of rabbit portal vein (RPV) myocytes was studied by patch clamp and compared with that of channels composed of Kv1.5 and/or Kv1.2 subunits cloned from the RPV and expressed in mammalian cells. 4-AP block of \(K_{\text{DR}}\) was pulse-frequency dependent, required channel activation, and was associated with a positive shift in voltage dependence of activation. 4-AP caused a voltage-dependent reduction in mean open time of \(K_{\text{DR}}\). Relief of 4-AP block of whole cell currents during washout required channel activation and was unaffected by voltage. Homotetrameric Kv1.5 channels did not exhibit the shift in voltage dependence of activation exhibited by the native channels. In contrast, Kv1.2 channels displayed a shift in voltage dependence of activation, and this characteristic was also evident during 4-AP treatment when Kv1.2 was coexpressed with Kv1.5 or coupled to Kv1.5 in a tandem construct to produce heterotetrameric [Kv1.5/Kv1.2]_{2} channels. \(K_{\text{DR}}\) currents were not sensitive to charybdotoxin, which blocks homotetrameric Kv1.2 channels. The findings of this study (1) indicate that vascular \(K_{\text{DR}}\) are inhibited by 4-AP via an open-state block mechanism and trapping of the drug within the pore on channel closure and (2) provide novel evidence on a comparison of functional characteristics that indicate the dominant form of vascular \(K_{\text{DR}}\) channel complex in RPV involves the heteromultimeric association of Kv1.2 and Kv1.5 subunits. *(Circ Res. 2001;89:1038-1044)*

Key Words: vascular smooth muscle | 4-aminopyridine | Kv1.5 | Kv1.2 | voltage-gated \(K^+\) channel

4-aminopyridine (4-AP) is widely used to selectively inhibit voltage-gated \(K^+\) channels (Kv) and to identify their role(s) in control of vascular smooth muscle (VSM) tone. For example, 4-AP was used to indicate the participation of delayed rectifier \(K^+\) channels (\(K_{\text{DR}}\)) in regulating membrane potential, endothelium-dependent relaxation, and myogenic tone development of VSM (eg, Leblanc et al., Knot and Nelson, and Dong et al.). Despite its widespread use, however, the mechanism of block of vascular \(K_{\text{DR}}\) by 4-AP has not been established conclusively.

The molecular identity of \(K_{\text{DR}}\) of VSM remains to be defined, but transcripts encoding several 4-AP-sensitive Kv1 channel subunits, for example Kv1.2 and Kv1.5, are expressed in VSM (eg, Roberds and Tamkun, Overturf et al., and Yuan et al.) including myocytes of RPV (Clément-Chomienne et al. and see also Thorneloe et al. in this issue of Circulation Research). Binding of 4-AP by Kv occurs from the intracellular face of the membrane, and when applied to the exterior of cells, it must first cross the cell membrane in its non-ionized form. Previous studies show considerable variability in the state-dependence of 4-AP block, eg, during or after activation (open-state block), closed (resting)-state block, or block after inactivation. Identification of the mechanism(s) of 4-AP block has been advanced through the study of recombinant Kv channels. For example, analyses of the effects of 4-AP on Shaker family Kv1 channels, including Kv1.2, Kv1.4 and Kv1.5, as well as Kv2.1 and Kv3.1, indicate that 4-AP binds to these channels in the open state and remains bound (or trapped) after channel closure. In light of the expression in VSM of Kv1 subunits that exhibit open-state block by 4-AP, it would be expected that a similar mechanism should be involved if these subunits contribute to the native channels; however, \(K_{\text{DR}}\) of coronary arterial myocytes were concluded to be suppressed by a closed-state blocking mechanism.

The objectives of this study were 3-fold: (1) to determine the state-dependence of inhibition by 4-AP of RPV \(K_{\text{DR}}\); (2) to test the hypothesis that the features of 4-AP block of the native \(K_{\text{DR}}\) are identical to those of Kv1.5 and Kv1.2, which are the only delayed rectifier-type Kv1 subunits expressed in...
this vessel (see Thorneloe et al\(^9\)); and (3) to assess the relative contribution of homo- and heteromultimeric Kv1.2 and Kv1.5 channels to native K\(_{\text{DR}}\) current of RPV. RPV K\(_{\text{DR}}\) were found to be inhibited by 4-AP while in the open state, but the drug remains trapped in the pore during channel closure. A feature of 4-AP block of K\(_{\text{DR}}\) was identified that was not mimicked by homotetrameric Kv1.5 channels, but was apparent in recordings of Kv1.2 currents, as well as currents due to heteromultimeric association of Kv1.2 and Kv1.5. Finally, native K\(_{\text{DR}}\) current was found to be insensitive to charybotoxin-block of homomultimeric Kv1.2 channels. The findings of this study provide novel insight into the mechanism of inhibition by 4-AP as well as the molecular identity of vascular K\(_{\text{DR}}\) channels.

### Materials and Methods

#### Rabbit Portal Vein Myocyte Isolation

RPV myocytes were isolated\(^2,8,22\) from rabbits (Charles River, Montréal, Quebec, Canada) maintained and killed according to the standards of the Canadian Council on Animal Care.

#### Transfection and Tandem Construct

RPV Kv1.5 (AF056943) and Kv1.2 (AF284420) cDNAs in pcDNA3 were transfected individually, or in combination, into human embryonic kidney 293 (HEK) cells (American Type Culture Collection) along with cDNA encoding green fluorescent protein (GFP).\(^8\) A tandem construct of Kv1.5-Kv1.2 was engineered by adding a linker sequence encoding seven glycines between the 3’ end of Kv1.5 and 5’ end of Kv1.2 and expressed in HEK cells.

#### Electrophysiological Measurements

Whole-cell currents were recorded \(\pm 4\text{-AP (Sigma Chemical)}\) or \(\pm\) charybotoxin (Alomone Laboratories) by whole-cell patch clamp\(^23\) and analyzed as previously described.\(^8,22\) RPV K\(_{\text{DR}}\) open probability \(\pm 4\text{-AP in cell-attached (C-A) patches was determined from amplitude histograms (bin width 0.1 pA) based on identical duration recording periods of 75 to 100 seconds.}\(^24\) Analysis of open dwell time using pClamp software (Axon Instruments) was based on >200 transitions \(\pm 4\text{-AP from 7 C-A patches. Partial transitions of >50% of the unitary amplitude during bursts were considered to be closures. The C-A patch bath and pipette solutions respectively contained (in mmol/L): KCl 140, MgCl\(_2\) 2.3, glucose 10, EGTA 1, and (N-[hydroxethyl] piperazine-N’-[2-ethanesulfonic acid] (HEPES) 10 (pH 7.4) and KCl 5.4, NaCl 140, CaCl\(_2\) 1, MgCl\(_2\) 1, glucose 5.5, and HEPES 10 (pH 7.4; ibotenic acid (200 mmol/L; Alomone Laboratories) was added to block BK\(_{\text{Ca}}\) activity). Data were compared by paired Student’s \(t\) test or repeated measures ANOVA followed by Dunnett’s post hoc test. A level of \(P<0.05\) was considered to be statistically significant.

### Results

#### State Dependence of 4-AP Inhibition of RPV K\(_{\text{DR}}\)

Two voltage clamp protocols were used to evaluate the pulse-dependence of 4-AP inhibition of RPV K\(_{\text{DR}}\) current (Figures 1 and 2). The time course of decline in current amplitude in 4-AP (1 mmol/L) was determined using repeated steps to \(\pm 20\text{ mV at interpulse intervals of 15, 30, and 90 seconds}\) to probe for evidence of open-state block (Figure 1A). Current amplitude declined in a pulse-dependent fashion and required a greater time when longer interpulse intervals were used. A certain degree of pulse-dependent decline and delay to stable inhibition can be expected because 4-AP must first cross the membrane before accumulating in the intracellular compartment.\(^10,11\) However, stable inhibition was achieved after \(\approx 3.25\text{ minutes with a 15-second interpulse interval, but the 4-AP-sensitive current had only declined by 50% and 25\% at this time when the interval was 30 and 90 seconds, respectively (Figure 1B). Additionally, fewer pulses were required to achieve steady-state block when the 4-AP concentration was increased: the inhibition was stable after 22\(\pm\)1.2, 11.4\(\pm\)0.5, and 9.4\(\pm\)1.4 pulses for 0.1, 1, and 10 mmol/L 4-AP, respectively (15-second interpulse interval; \(n>3\) for each concentration).

To assess the extent of closed-state block, cells were treated with 4-AP (0.2 or 1 mmol/L) for 5 to 10 minutes at \(-60\text{ mV prior to the first depolarizing step. Figure 2 shows that a pulse-dependent decline in current amplitude was observed regardless of the length of time of 4-AP (1 mmol/L) treatment and that the initial activation of current during the first depolarizing step in 4-AP was not affected. If the drug interacted with closed K\(_{\text{DR}}\), an immediate stable level of inhibition would have been expected. Also, an increased rate of decay in current amplitude was evident during the first pulse in 4-AP (note the arrow in Figure 2A), consistent with a development of channel block during the pulse. This decay occurred with time constants of 136.1\(\pm\)9.7 and 88.2\(\pm\)9.8 ms in 0.2 and 1 mmol/L 4-AP, respectively (\(n=4\) and 7).

Figure 3 shows representative families of whole-cell K\(_{\text{DR}}\) currents, as well as average data for comparison of the I-V relationship for end-pulse current and steady-state activation.
Figure 2. A, Representative RPV K_{DR} currents evoked by 250-ms steps to +20 mV from −60 mV before (1 and 2) and after (3 and 4–6) 5 minutes treatment with 4-AP (1 mmol/L) at −60 mV. Arrow indicates increased rate of current decay during the 1st pulse (3) in 4-AP. B, Expanded traces from A show a lack of effect on initial activation of current during the 1st pulse (3) but substantial block in the 2nd pulse (3) after 5 minutes of 4-AP treatment at −60 mV. C, Time course of decline in current at 50 ms (dotted line in A) normalized to control amplitude following 5, 7.5, and 10 minutes treatment with 4-AP at −60 mV using the protocol indicated in A.

Based on analysis of normalized tail current amplitude versus voltage ±1 mmol/L 4-AP, 4-AP reduced end-pulse current at all potentials positive to −40 mV and caused a shift of +18.8 ± 1.4 mV in the voltage of half-maximal activation (V_{0.5}), as described for K_{DR} of rabbit coronary arterial myocytes. A similar but smaller shift in V_{0.5} was observed for 0.2 mmol/L 4-AP of ±11.0 mV from −14.6 ± 1.2 to −3.6 ± 1.4 mV (n = 4; P < 0.05).

The concentration-dependence of K_{DR} inhibition by 4-AP was determined. End-pulse current amplitude at +20 mV was reduced to a maximum that was about 30% of that in control conditions, indicating the presence of 4-AP-sensitive and −resistant outward currents in RPV. The concentration required for half-maximal inhibition at +20 mV using the protocols of Figures 1 and 2 were identical at 213 ± 56 and 180 ± 32 μmol/L and not different from the values obtained for RPV Kv1.5 (180 ± 32 and 190 ± 43 μmol/L) (P > 0.05; see online Figure 1 in the online data supplement available at http://www.circresaha.org), but different from the value of 74 μmol/L reported for Kv1.2.

Effect of 4-AP on Mean Open Time of Single RPV K_{DR}

4-AP–sensitive RPV K_{DR} current is due to channels with a conductance of approximately 10 pS in asymmetrical (5.4/140 mmol/L) KCl recording conditions. The effect of 4-AP (0.2 mmol/L) on mean open time of these channels was assessed using C-A patches held at −10 and +30 mV. Patches exhibiting only one channel are rarely obtained, so 4 of the 7 patches analyzed in this study contained multiple channels (bursts involving multiple openings were ignored). Identical effects of 4-AP on mean open time were observed during repeated 500-ms step depolarizations and when the patches were held at a constant voltage, thus the data from these 2 protocols were combined in the analysis. Figure 4 shows representative traces and values for open probability from a single patch, as well as average values of mean open time in 7 patches at −10 and +30 mV ± 4-AP. Unitary current amplitude was not affected (Figure 4A), as previously

Figure 3. A, Representative families of RPV K_{DR} currents before (Control) and after development of stable inhibition by 1 mmol/L 4-AP. B, Average I-V relation for end-pulse current normalized to cell capacitance ±1 mmol/L 4-AP using the protocol of (A), but steps to +40 mV were also added to ensure full K_{DR} activation in 4-AP (n = 7). C, Effect of 4-AP on steady-state activation of K_{DR} current: average values of tail current amplitude at −50 mV normalized to peak amplitude versus command step voltages between −60 and +40 mV ± 4-AP (1 mmol/L; n = 7). The solid lines are Boltzmann functions which best fit each data set determined according to the following equation:

\[ Y = \frac{1}{1 + \exp\left[\frac{V_{0.5} - V}{k}\right]} \]

The voltage of half-maximal activation (V_{0.5}) for each fit is indicated.

Figure 4. A, Representative RPV K_{DR} single channel activity at −10 and +30 mV before (Control) and during 4-AP (200 μmol/L) treatment. Values for open probability at each voltage ± 4-AP are indicated (based on 75-second recording periods). B, Average changes in mean open time for 4-AP–sensitive RPV K_{DR} activity at −10 and +30 mV in absence (open bar) and presence of 4-AP (closed bar) for 7 patches. n values indicate numbers of transitions analyzed in each group. *indicates significant difference in mean open time ± 4-AP (P < 0.01).
Lack of Effect of Voltage on 4-AP Dissociation From RPV Kv$_{D_R}$

Preliminary experiments showed that washout of 4-AP inhibition of Kv$_{D_R}$ required 10 to 15 minutes (ie, 40 to 60 steps at 0.66 Hz; data not shown). A 20-minute period for washout of 4-AP was therefore considered to be sufficient for the intracellular 4-AP concentration to decline to a level that would not affect the channels. To evaluate the voltage dependence of relief of block from a trapped state, cells were treated with 4-AP to achieve stable block and then held at −60 mV for 20 minutes (W1 through W6). Time course of current activation during the first step after 20 minutes washout (W1) at +20 and 0 mV were fitted by biexponential functions with the indicated time constants. Scale bars are of equivalent magnitude for both recordings.

Effect of 4-AP on Cloned Kv of RPV

The state-dependence of 4-AP inhibition of Kv1.2 and Kv1.5 channels was studied previously, but the effect of 4-AP on the voltage dependence of activation was not reported. A pulse-dependent decline in current amplitude and increased rate of current decay during depolarizing steps was observed for homotetrameric Kv1.5 or Kv1.2 channels (Figures 6A and 6B). Identical results were obtained when these subunits were coexpressed (Figure 6C); the coimmunoprecipitation experiments in Thorneloe et al$^9$ illustrate the association of Kv1.2 and Kv1.5 following coexpression in HEK293 cells. However, the subunit composition of the channels is not known for certain in coexpression experiments: mixtures of homotetrameric and heteromultimeric channels with varied numbers of Kv1.2 and Kv1.5 subunits are possible. For this reason, the effect of 4-AP on a tandem construct in which the 2 subunits were linked by a seven glycine repeat sequence, and thereby, heterotetrameric channels with a known 1:1 ratio of Kv1.2 to Kv1.5 subunits was assessed. The tandem construct also exhibited evidence of a pulse-dependent decline in current amplitude (Figure 6D).

The effect of 4-AP on steady-state activation was determined from families of whole-cell currents due to Kv1.5, Kv1.2, coexpression of these subunits, and the tandem construct. Figure 7 shows that the effect of 4-AP (1 mmol/L) on the voltage dependence of activation of these currents was different. Significantly, the voltage dependence of activation of currents due to Kv1.5 channels was not affected by 4-AP (Figure 7A). For comparative purposes, we also studied Kv1.5 expressed in mouse L cells, in which Kv1.5 coassembles with an endogenous Kvβ2.1 subunit,$^{26}$ but an identical lack of change in activation was observed (−15.3±0.5 to −18±1.2 mV; n=3, P>0.05). In contrast, a shift in activation of +22.8±4.1 mV was observed for currents due to homotetrameric Kv1.2 channels in the presence of 4-AP (Figure 7B). Figures 7C and 7D indicate that the shift in activation of Kv1.2 was also evident when this subunit was coexpressed with Kv1.5 to produce heteromultimeric channels: average values were +15.7±5.6 and +12.2±1.0 mV for the coexpression and tandem experiments, respectively. Note that the activation of current due to coexpression of Kv1.2 and Kv1.5 mimics that of Kv1.5, but the tandem activated over a considerably more negative voltage range.
The reason for this is not evident; it is possible that the linkage of the subunits with the polyglycine sequence may enhance the mobility of the voltage sensors of the tandem channels.

Lack of Effect of Charybdotoxin on RPV K\textsubscript{DR}

Charybdotoxin was previously shown to block homomultimeric Kv1.2 channels\textsuperscript{27–29} but not heteromultimeric channels due to coexpression of Kv1.2 with Kv1.5.\textsuperscript{29} Figure 8 shows that charybdotoxin (50 nmol/L) failed to affect the amplitude of K\textsubscript{DR} currents in the presence of iberiotoxin (100 nmol/L). The latter was used to eliminate any residual contamination of the native current recordings by charybdotoxin-sensitive BK\textsubscript{Ca} channels not suppressed by the 10 mmol/L BAPTA-containing pipette solution used in the experiments. The representative data and plots of average end-pulse current in Figure 8 indicate the complete lack of any contribution of charybdotoxin-sensitive Kv channels to whole cell RPV K\textsubscript{DR} current.

Discussion

This study provides novel information concerning the state-dependence of block by 4-AP and molecular identity of vascular K\textsubscript{DR}. We provide the first evidence that vascular K\textsubscript{DR} of RPV are inhibited by 4-AP via an open-state blocking mechanism. The inhibition by 4-AP was associated with a positive shift in the voltage dependence of steady-state activation. Kv1.2 and Kv1.5 cloned from the RPV were used to verify that our approach for determination of the mechanism of 4-AP block of RPV K\textsubscript{DR} would yield data consistent with previous reports concerning the cloned channels.\textsuperscript{14,17–20} We found that RPV Kv1.2 and Kv1.5 displayed evidence of open-state block consistent with previous findings,\textsuperscript{14,17–20} but we made the novel observation that 4-AP has disparate effects on the steady-state activation of homotetrameric channels composed of these subunits. Kv1.2 but not Kv1.5 channels displayed a positive shift in voltage-dependence of activation similar to that observed during 4-AP inhibition of RPV K\textsubscript{DR}. RPV K\textsubscript{DR} current was not affected by charybdotoxin block of Kv1.2 homotetramers\textsuperscript{27–29}; however, we found that heteromultimeric channels containing Kv1.2 and Kv1.5, which are known to be insensitive to charybdotoxin,\textsuperscript{29} did display a shift in activation in 4-AP. These data showing a functional identity of heteromultimeric Kv1.2-Kv1.5 channels and native K\textsubscript{DR} current are consistent with the results of Thorneloe et al.\textsuperscript{9} Direct evidence for the association of Kv1.2 and Kv1.5 in RPV protein extracts was identified by coimmunoprecipitation. When considered together, the data contained in these 2 studies provide compelling evidence that the dominant vascular K\textsubscript{DR} channel complex is a heteromultimer of Kv1.2 and Kv1.5.

The view that vascular K\textsubscript{DR} are inhibited by 4-AP after transition to the open state is supported by the following: (1) the decline in K\textsubscript{DR} amplitude in 4-AP was pulse-dependent and prolonged by increasing the interpulse interval. The time-course of inhibition would not be expected to vary with interpulse interval if 4-AP blocked the channels in the closed state. (2) A stable level of inhibition of K\textsubscript{DR} current was not observed during depolarizing steps applied after prolonged treatment with 4-AP at $-60$ mV. If 4-AP could block the channels in the closed state, then an immediate stable level of inhibition would have been observed. (3) The initial activation of current during the first pulse after 5-minute 4-AP treatment at $-60$ mV was unchanged, but block by 4-AP developed during the pulse. An analysis of the pulse dependence and effect of interpulse interval on inhibition of smooth muscle K\textsubscript{DR} by 4-AP was not performed previously, but a decline in burst duration of K\textsubscript{DR} channels of colonic myocytes consistent with an open channel block mechanism was reported by Koh et al.\textsuperscript{25} Our observation of a decline in mean open time is also consistent with previous reports concerning 4-AP block of Kv1.2, Kv2.1, and Kv3.1.\textsuperscript{12,14} The conclusion that RPV K\textsubscript{DR} are affected by 4-AP after transition to the open state is different, however, from that in a previous study of rabbit coronary arterial K\textsubscript{DR}.\textsuperscript{21} That the inhibition by 4-AP of RPV K\textsubscript{DR} was associated with a positive shift in the voltage dependence of activation is consistent with previous reports for vascular K\textsubscript{DR} of other vessels.\textsuperscript{21,30,31} However, the shift in activation of coronary arterial K\textsubscript{DR} was attributed to unblock of channels inhibited by 4-AP while in the closed state.\textsuperscript{21} Based on the present data, however, this explanation does not appear to be appropriate.
Our findings are not consistent with a closed-state block of vascular Kv$_{\text{DR}}$, but they do imply that the interaction with 4-AP is maintained following channel closure. This is suggested by the lack of relief of 4-AP block of Kv$_{\text{DR}}$ or Kv1.5 during prolonged washout at $-60$ mV. The voltage dependence of unblock was analyzed, but no difference in the time course of increase in current was apparent during pulses to 0 and $+20$ mV. This indicates that the dissociation of 4-AP was unaffected by voltage, similar to that reported for Kv2.1 and Kv3.1 and, therefore, that a voltage-dependent unblock cannot explain the shift in voltage dependence of activation. A recent modeling paper by Armstrong and Loboda suggests that the voltage-dependence of 4-AP action on K$^+$ channels may be attributed to the ability of 4-AP to bias the drug bound–open state to a drug bound–closed state, and therefore, the channels occupied with 4-AP do not open as easily at negative (eg, $-40$ mV) compared with positive potentials (eg, $+50$ mV). Because 4-AP remains bound to the channels after repolarization, the level of current recorded during a series of steps to increasing potentials is determined by (1) the level of block during the previous step and (2) the ability of the channels to open during the subsequent depolarization. A declining level of inhibition occurs when sequential steps are applied to increasingly depolarized potentials because the open state of the activation gate is favored more strongly with depolarization positive to $-40$ mV. The amplitude of tail currents recorded on repolarization will be affected in parallel leading to a shift in activation.

The findings of this study provide evidence of a heteromultimeric association of Kv1.2 and Kv1.5 in vascular Kv$_{\text{DR}}$. The molecular identity of vascular Kv$_{\text{DR}}$ is not known for certain: the expression of subunits from several Kv families, including Kv1.1, Kv2, Kv3, Kv4, and Kv9, as well as Kv$\beta$ subunits has been reported. For example, we identified the presence of Kv1.5 in RPV, and have subsequently identified the expression of Kv1.2 and Kv1.4, as well as Kv$\beta$1.1, Kv$\beta$1.2, Kv$\beta$1.3, Kv$\beta$2.1, and Kv$\beta$2.2 in Thorneloe et al. However, data which correlate the presence of individual Kv subunits with specific biophysical, pharmacological, and/or functional properties of vascular Kv$_{\text{DR}}$ are limited.

This study shows that RPV Kv$_{\text{DR}}$ exhibit a shift in voltage dependence of activation due to 4-AP, as well as a lack of sensitivity to charybdotoxin. Kv1.5 channels are not affected by charybdotoxin, but we found that RPV Kv1.5 did not exhibit the shift in activation displayed by the native channels when expressed alone or in the presence of Kv$\beta$2.1 subunit in L cells. In contrast, block by 4-AP of Kv1.2 channels displayed a positive shift in activation in the presence of 4-AP, but Kv1.2 channels are sensitive to charybdotoxin. These data indicate, therefore, that the properties of RPV Kv$_{\text{DR}}$ cannot be mimicked by homotetrameric Kv1.5 or Kv1.2 channels. Significantly, however, the shift in activation in 4-AP was apparent when RPV Kv1.2 and Kv1.5 were coexpressed or expressed as a tandem construct to yield heteromultimeric channels, which are known to be unaffected by charybdotoxin. Thus, channels due to the heteromultimeric association of Kv1.2 and Kv1.5, but not homotetramers of these subunits, possess pharmacological identity with vascular Kv$_{\text{DR}}$ in terms of their characteristics of 4-AP block and lack of sensitivity to charybdotoxin. Direct evidence that Kv1.2 and Kv1.5 associate to form heteromultimeric Kv channels in RPV was obtained in communoprecipitation experiments using subunit-specific antibodies, as reported in the preceding paper. The presence of a band of appropriate molecular weight for Kv1.5 was identified in immunoblots of RPV protein immunoprecipitated using a Kv1.2 antibody. This finding provides direct evidence that Kv1.2 and Kv1.5 coassemble in RPV, but in isolation, the data do not provide any indication concerning the relative importance of this association to Kv$_{\text{DR}}$ channel formation and whole-cell Kv$_{\text{DR}}$ current. The pharmacological data obtained in this study indicate that heteromultimeric Kv1.2-Kv1.5 channels are the dominant channel complex and that there is a minimal/no contribution of homomultimers of Kv1.2 or Kv1.5 in RPV myocytes. In summary, the findings of this study indicate that 4-AP inhibits native vascular Kv$_{\text{DR}}$ channels via an open-state blocking mechanism that is associated with a shift in voltage dependence of activation. The heteromultimeric interaction of Kv1.2 and Kv1.5 provides a molecular explanation for this shift in activation due to 4-AP and lack of sensitivity of the native channels to charybdotoxin. This feature of 4-AP inhibition is a characteristic that can be exploited as a tool in the determination of subunit composition of vascular Kv$_{\text{DR}}$ of different vessels (eg, coronary artery and pulmonary artery), as well as cardiac myocytes, which are also known to express Kv1.2 and Kv1.5.

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**References**

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Kerr et al. “Heteromultimeric Kv1.2-Kv1.5 underline 4-aminopyridine-sensitive delayed rectifier K⁺ current of rabbit vascular myocytes”

ONLINE DATA SUPPLEMENT

Figure 1: (A) Average end-pulse $K_{DR}$ current amplitude at +20 mV (normalized to control) versus 4-AP concentration assessed using the protocols identified in figure 1 (open circles) and 2 (closed circles) of the paper. Solid lines are best fits to the data which yield the indicated IC₅₀ values (for each data point n is > 5 myocytes). (B) End-pulse current amplitude at +20 mV (normalized to control) versus 4-AP concentration for RPV Kv1.5 (in L cells) assessed with the protocols in figures 1 (open squares) and 2 (closed squares) in the paper. Solid lines are best fits to the data which yielded the indicated IC₅₀ values (for each data point n is > 4 cells).
online figure 1

A

RPV $K_D^{r}$

- ○ Protocol in Figure 1 of Paper
- ■ Protocol in Figure 2 of Paper

IC$_{50}$ 184 ± 49 μM

IC$_{50}$ 213 ± 65 μM

Normalized Current ($I_{4-AP}/I_C$)

[4-AP] (mM) 0.01 0.1 1 10 100

B

RPV Kv1.5

- ■ IC$_{50}$ 190 ± 43 μM
- ○ IC$_{50}$ 180 ± 32 μM

Normalized Current ($I_{4-AP}/I_C$)

[4-AP] (mM) 0.01 0.1 1 10 100