Pharmacological and biophysical isolation of K⁺ currents encoded by ether-à-go-go-related genes in murine hepatic portal vein smooth muscle cells

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Potassium currents encoded by ether-à-go-go-related genes (ERGs) have been studied extensively in neurons and cardiac myocytes, where they are crucial determinants of membrane excitability (see Ref. 22 for review) to the extent that mutations in ERGs lead to hereditary arrhythmias (30). In contrast to the plethora of data on ERG channels in the central nervous system and cardiac myocytes, there is a dearth of information about the physiological importance of these channels in smooth muscle cells that constitute visceral and vascular tissues. ERG expression has been determined in a number of gastrointestinal tissues through a combination of molecular biological and electrophysiological techniques allied to the use of specific ERG channel blockers such as E-4031, MK-499, or cisapride in functional studies. After such studies, ERG channel blockers have been considered to influence cellular excitability in opossum esophagus (1), rat stomach (15), mouse and guinea pig gallbladder (17), and human and equine jejenum (6, 11). In contrast to the view of ERG channels in viscera, only Ohya et al. (16) have investigated ERG expression in a vascular preparation. In that study we showed that myocytes from murine hepatic portal vein (mPV) expressed predominantly mERG1b as well as mERG1a. Protein translation was confirmed by immunocytochemical experiments with an ERG1-specific antibody and also by electrophysiological studies. Similar to the vast proportion of electrophysiological studies on endogenous ERG currents, including the only other study in smooth muscle cells (esophageal myocytes, Ref. 1), the ERG currents in mPV myocytes were characterized by their distinctive “hooked” kinetics and sensitivity to the specific ERG channel blocker E-4031 in cells bathed in an external solution containing 140 mM KCl and the conventional K⁺ channel blockers 4-aminopyridine (4-AP, 5 mM) and tetraethylammonium (TEA, 10 mM). Under these conditions conventional delayed rectifier-type K⁺ channel [e.g., voltage-dependent K⁺ (Kv1.3, Kᵥ3.4) currents, including the only other study in smooth muscle cells] were eliminated and ERG channel currents were enhanced because raised extracellular K⁺ slows the rate of inactivation and increases the channel conductance (31, 26). However, the holding potential (V₉₅) of 0 mV used in the study by Ohya et al. (16) is not relevant to the physiological resting membrane potential (RMP) of these cells (approximately −54 mV; Ref. 32) and the external K⁺ concentration was far from representative of a physiological scenario. Moreover, as 4-AP and TEA inhibit ERG channels to some extent (see, e.g., Ref. 20), the contribution of ERG channel currents to the membrane conductance may have been underestimated significantly.

To assess the functional impact of ERG channels it was necessary to characterize the conductance fully in cells bathed in a solution containing normal physiological K⁺ (~5 mM) and in the absence of either 4-AP or TEA. We therefore used the approach of isolating native ERG currents from other endogenous currents by their distinctive kinetics accompanied by the use of three structurally distinct ERG channel blockers. In addition, studies were undertaken with a novel ERG activator, PD-118057 (33). These studies are the first to characterize ERG channel currents in a smooth muscle cell type under normal ionic conditions and at physiologically relevant membrane potentials, and they revealed that ERG channel currents of significant amplitude were present in most PV myocytes, which appeared to contribute to the resting conductance.
METHODS

Cell preparation. mPV smooth muscle cells were dissociated freshly from 6- to 8-wk-old female BALB/c mice (32). Animals were killed by cervical dislocation, and the PV was removed and bathed in physiologically saline solution (PSS) containing 1 mM Ca\(^{2+}\). Cells were obtained enzymatically by treating the tissue with 100 mM collagenase (type I, Calbiochem), both at 37°C for 6 min. Dispersed cells were kept on ice until required.

Current and voltage recordings. Whole cell currents were recorded at room temperature (20–22°C) with either the conventional ruptured patch configuration or the perforated patch variant. To study the voltage-gated K\(^+\) current in isolation, EGTA (5 mM) and ATP (3 mM) were included in the pipette solution and recordings were made with the ruptured patch technique. This rationale has been used in a number of studies (see, e.g., Refs. 1, 18, 32) to eliminate any contribution from Ca\(^{2+}\)-activated K\(^+\) currents, especially of the large-conductance type (BK\(_{Ca}\)), and ATP-sensitive K\(^+\) (K\(_{ATP}\)) currents to the overall outward conductance. For the perforated patch recordings amphotericin (300 µg/ml) was included in the internal solution and electrical access was monitored by the repetitive application of 10-nM hyperpolarizations until the series resistance was <40 MΩ. V\(_{H}\) in all experiments was −60 mV. Membrane potential recordings were made with the current clamp plus command setting on the amplifier with the perforated patch variant of the whole cell technique. Active responses were evoked by applying 75- to 125-pA current sensitive to the specific ERG channel blocker E-4031 (7), also inhibited currents that were transient in nature. However, external application of TEA and 4-AP had a profound effect on the amplitude of the gross voltage-dependent currents evoked from −60 mV (Fig. 1B). For example, the peak current at +40 mV decreased from 12,528 ± 100 pA to 386 ± 60 pA and the sustained outward current at +40 mV was reduced from 1,029 ± 94 pA to 215 ± 342 pA (n = 11). Moreover, the presence of 4-AP and TEA affected the kinetics of the dofetilide-sensitive currents. As Fig. 1C shows, the current sensitive to 1 µM dofetilide in the absence of 4-AP and TEA was relatively well sustained, in contrast to dofetilide-sensitive currents in the presence of 4-AP and TEA (Fig. 1, C and D), resulting in a steady-state current that was markedly less in the presence of the two K\(^+\) channel.
blocks (Fig. 1D). Consequently, these experiments show that the amplitude and appearance of the ERG channel currents recorded in the presence of TEA and 4-AP by Ohya et al. (16) were a significant underestimate of the contribution of ERG channel currents to the membrane conductance. Therefore, for the remainder of the study ERG channel currents were characterized in the absence of TEA and 4-AP.

Identification of ERG channel currents in PV myocytes. ERG channel currents have distinctive kinetics due to the underlying channel state transitions. Depolarization from a negative $V_{hi}$ causes the channels to transit to an active state that is dominated by a rapid concurrent inactivation. On repolarization the inactivated channels recover and then close relatively slowly (24). These channel properties result in currents with a hooked appearance that were obvious in our previous study in myocytes bathed in high external K$^+$ (Ref. 16), so we aimed to identify whether currents with kinetics consistent with ERG channel activation were present under the ionic conditions and voltage protocols used. Similar to our previous study (32), depolarization from a $V_{th}$ of $-60$ mV generated a time-dependent outward current superimposed on a linear, time-dependent component (Fig. 2), which were followed on repolarization to potentials negative of the $V_{th}$ by inward currents with a hooked appearance. This was manifested as an initial rising phase immediately on repolarization that was followed by the decline of the current to a sustained level (Fig. 2A). The amplitude of the inward current at $-140$ mV was proportional to the extent of depolarization preceding the step to $-140$ mV, and fitting of these data with a Boltzmann function yielded a voltage for half-maximal activation ($V_{0.5}$) of $-5 \pm 2$ mV ($n = 15$, see Fig. 2A, inset) Decay of inward tail currents at different test potentials negative to the $V_{th}$ recorded with a prepulse to $+40$ mV (deactivation protocol) were best fitted with a single exponential (Fig. 2B; see also Fig. 5, C and D). Representative traces in Fig. 2Bi illustrate that current decay became slower at less negative potentials with the mean time constant ($\tau$) for decay increasing from $19 \pm 2$ ms at $-140$ mV to $56 \pm 4$ ms at $-100$ mV ($n = 28$; see also Fig. 5). These values were qualitatively similar to values obtained in PV myocytes bathed in an external solution containing 140 mM K$^+$ (Ref. 16; see also Fig. 5D). These types of kinetics were also similar to those of heterologously expressed ERG channels recorded in symmetrical K$^+$ solutions (see, e.g., Ref. 24). These observations suggested that ERG currents could be recorded in murine PV myocytes even when the cell was bathed in normal external K$^+$.

Effect of selective ERG channel blockers. To confirm that the hooked inward currents at negative potentials were due to the prior activation of ERG channels, the effect of three structurally disparate and selective blockers of ERG channels, namely E-4031, dofetilide, and the peptide toxin BeKM-1, was investigated. Ohya et al. (16) showed that all hooked currents recorded in PV myocytes bathed in 140 mM KCl were abolished by 1 $\mu$M E-4031, and Fig. 3 shows that this agent and 1 $\mu$M dofetilide also abolished the hooked current recorded at $-140$ mV. E-4031 and dofetilide are considered to be selective blockers of ERG channels (25), but they work through similar mechanisms. Consequently, to consolidate our findings we investigated the effect of recombinant BeKM-1, a peptide toxin originally isolated from the Asian scorpion Buthus eupeus, which is a highly selective blocker of ERG channels (9). Moreover, BeKM-1 is preferentially a closed channel blocker (13) and therefore blocks ERG channels by a mechanism completely different from that utilized by E-4031 or dofetilide. Application of BeKM-1 inhibited the hooked inward current at $-140$ mV in a concentration-dependent manner with an IC$_{50}$ of $-8$ nM (Fig. 4). The inhibitory effect of BeKM-1 was relatively slow compared with the effects of E-4031 and dofetilide (see Fig. 4C), but the absolute amplitude of the current blocked by each agent was not significantly different. Thus the amplitude of the current blocked by 1 $\mu$M E-4031, 1 $\mu$M dofetilide, and 100 nM BeKM-1 (i.e., drug-sensitive am-

Fig. 2. Examples of membrane currents in murine portal vein (PV) myocytes. Ai: currents evoked by the activation protocol, in which the cell was held at $-60$ mV and stepped to potentials between $-80$ and $+40$ mV for 200 ms, followed by repolarization to $-140$ mV for 250 ms. Progressive depolarization resulted in larger inward currents at $-140$ mV that displayed a distinctive rising phase followed by decline to a steady-state level, giving a “hooked” appearance. Ai: exploded view of an inward current recorded at $-140$ mV shown in Ai that highlights the characteristic appearance of this current. Inset shows the voltage dependence of activation of the voltage-sensitive current. y-Axis is the amplitude of the conductance (G) at $-140$ mV normalized to the maximal current at $-140$ mV. x-axis shows the prepotential. Data are means $\pm$ SE from 6 cells. Bi: representative currents evoked by membrane depolarization to $+40$ mV followed by repolarization to potentials between $-140$ and $+40$ mV (deactivation protocol). Bii shows the rising and decay phases of the inward currents were voltage dependent. In all experiments the cell was bathed in a solution containing 5 mM KCl.
plitudes) at \(-140\) mV was 127 ± 13.4 (n = 3), 120.7 ± 22.5 (n = 6), and 157 ± 28 (n = 4) pA, respectively. Furthermore, the activation of the dofetilide-sensitive current at \(-140\) mV exhibited a voltage dependence similar to that of the BeKM-1-sensitive currents [mean \(V_{0.5}\) were \(-1 ± 3\) (n = 6) and \(-7 ± 7\) (n = 4) mV, respectively]. These data show that three structurally different and selective blockers of ERG channels inhibit the hooked inward currents recorded at \(-140\) mV after depolarization to \(+40\) mV.

**ERG channel currents recorded with perforated patch configuration.** The aim of this study was to assess the characteristics of ERG channels under more physiological conditions than those used in our previous study (16). As a logical corollary we undertook a series of experiments using the perforated patch variant of the whole cell voltage clamp technique in which there is less disturbance of the intracellular milieu. Under these conditions there was a variable contribution of spontaneous, transient currents at more positive potentials because intracellular \(Ca^{2+}\) concentration was able to rise and activate BKCa. Although these currents contaminated recordings at positive potentials, there was negligible effect on the inward currents at potentials from \(-140\) mV to \(-40\) mV because of the voltage dependence of the BKCa channels. Figure 5 shows examples of dofetilide-sensitive currents at various potentials following a constant prepulse to \(+40\) mV recorded by either ruptured patch (Fig. 5Ai) or perforated patch (Fig. 5Aii) whole cell technique. It is clear that the dofetilide-sensitive currents recorded with the perforated patch technique...
Fig. 5. Dofetilide-sensitive currents recorded with the perforated patch configuration. A: dofetilide-sensitive currents evoked by the deactivation protocol recorded with both ruptured patch (i) and perforated patch (ii) techniques. B: mean amplitude of the dofetilide-sensitive tail current at −140 mV and the dofetilide-sensitive outward current at +40 mV recorded with either the ruptured patch whole cell configuration (filled bars) or the perforated patch whole cell configuration (open bars). ***Significant difference at P < 0.005 for outward currents recorded with the 2 configurations. Data are means ± SE of 5–8 cells. Ci: expanded view of dofetilide-sensitive currents recorded in the ruptured patch configurations at potentials between −140 and −80 mV after activation by depolarization from −60 to +40 mV for 200 ms. Dashed lines represent a monoexponential fit of the current decay. Cii: exploded view of the inward currents shown in Aii to illustrate similar voltage-dependent characteristics of the inward currents in the perforated patch configuration. Di: voltage dependence of the time to peak (TTP) of the dofetilide-sensitive inward currents exemplified in C. TTP was calculated as the time from the apex of the capacity transient to the peak of the inward current. Dii: voltage dependence of the decay of the dofetilide-sensitive currents characterized by a monoexponential fit to the current. These data were recorded in the ruptured and perforated patch configurations. Data from the present study are means of 6–10 cells. Data from Ohya et al. (16), where ERG currents were recorded from murine PV myocytes bathed in an external solution containing 140 mM KCl, are shown for comparison.
had similarly robust inward tail current profiles; however, there was a significant difference in the amplitude of the outward current inhibited by dofetilide (Fig. 5B). Thus at +40 mV the amplitude of the dofetilide-sensitive current was $303.3 \pm 19$ and $29 \pm 13$ pA when recorded with the ruptured and perforated patch configurations, respectively ($n = 6$ and 4; $P < 0.001$). Moreover, the voltage dependence of activation was independent of the recording technique. In addition, kinetics of those currents recorded with the ruptured patch configuration (compare Fig. 5, Ci and Cii; see also Fig. 5D) and the rates of decay recorded in the present study were identical to those presented at the same potentials in PV myocytes bathed in high external K$^+$ by Ohya et al. (Ref. 16; see Fig. 5D). Even though a large degree of similarity is apparent between currents recorded in either configuration, these experiments suggest that some, as yet undefined, intracellular mediator that is dialyzed by the ruptured patch method suppresses outward current flow through ERG channels. This factor(s) effectively increases the degree of inward rectification exhibited by this channel.

**Effects of novel ERG activator PD-118057.** Recently, three chemical agents, RPR-260243, PD-118057, and NS-1643, have been developed that activate human (H)ERG channels selectively (4, 8, 33). As HERG is considerably different from the murine ortholog expressed by PV myocytes, we undertook a series of experiments to determine whether the native ERG channel currents in mPV myocytes are also sensitive to PD-118057. Application of PD-118057 at the concentrations used by Zhou et al. (1–10 μM; Ref. 33) had variable effects on the ERG channel currents (Fig. 6). Application of 1 μM PD-118057 inhibited the tail current at −140 mV by 19 ± 7% in three of six cells and produced a small enhancement in the other three cells (mean increase was 8 ± 2%). In contrast, 3 and 10 μM PD-118057 only enhanced the ERG currents. The enhancement of the tail current at −140 mV produced by 3 μM PD-118057 was variable, ranging from 30% to 411% (mean was 129 ± 52%; $n = 7$), whereas the augmentation produced by 10 μM was more consistent (mean increase was 131 ± 11%; $n = 4$). The effect of PD-118057 on the inward current was relatively slow to reach a stable level, with the mean time to peak (TTP) produced by 3 μM PD-118057 being 15.5 ± 1 min ($n = 6$), and was associated with a marked increase in the outward current (Fig. 6Aii). Consistent with the observations of

![Fig. 6. Effect of PD-118057 on membrane currents.](http://ajpcell.physiology.org/)
Zhou and colleagues (33), there was no apparent change in the voltage dependence of activation (V_{0.5} values and slope were \(-7.6 \pm 4.2\) mV and \(13 \pm 3\) in the absence and \(-2.1 \pm 5.4\) mV and \(10 \pm 3\) in the presence of \(3 \mu M PD-118057\), respectively; \(n = 4\)). Moreover, PD-118057 appeared to have no consistent effect on either the recovery from inactivation or the deactivation of the murine vascular ERG channel (data not shown). Recovery from channel inactivation (i.e., TTP) was reflected by the rising phase of the inward currents, which at \(-120\) mV was \(8.3 \pm 0.6\) ms in the absence of PD-118057 compared with \(8.5 \pm 0.8\) and \(8.2 \pm 0.8\) ms in the presence of \(1\) and \(3 \mu M PD-118057\), respectively (\(n = 6–8\)). For deactivation, \(\tau\) for the decay of the current at \(-120\) mV was \(34 \pm 7\) and \(36.5 \pm 4\) ms in the absence and presence of \(3 \mu M PD-118057\), respectively (\(n = 4\)). However, in contrast to the original study (33), the effects of PD-118057 on native currents in murine PV myocytes were poorly reversed by either \(1 \mu M\) dofetilide or \(100\) nM BeKm-1 (see Fig. 6B). Moreover, the enhanced outward current recorded in the presence of \(10 \mu M \) TEA and \(5 \mu M 4-AP\). Figure 6C shows that in the presence of \(4-AP\) and \(TEA\) \(10 \mu M PD-118057\) still enhanced the tail current recorded at \(-140\) mV (mean increase was from \(94.3 \pm 29.5\) to \(384.0 \pm 131.4\) pA; \(322.8 \pm 145.5\), \(n = 3\)). In addition, \(10 \mu M PD-118057\) converted the transient outward current at \(+40\) mV to a more delayed rectifier profile (see Fig. 6C), with the current at the end of the test step being increased by \(482.7 \pm 156.0\%\) (\(n = 3\)). This enhancement produced by PD-118057 was significantly greater than in the absence of 4-AP and TEA (\(124.2 \pm 10.6\%\); \(n = 5\), data not shown). As 4-AP and TEA at these concentrations block most K\(^{+}\) channels except ERG, these data suggest that the enhanced current in PD-118057 is not due to the de novo activation of a K\(^{+}\) channel. These data show that the ERG channel activator PD-118057 has marked effects on native currents recorded in the absence and presence of high concentrations of 4-AP and TEA.

**Contribution of ERG channels to RMP.** The RMP of single PV myocytes was recorded in current clamp with the perforated patch configuration and external solution containing 2.5 mM CaCl\(_2\) and no other pharmacological agent. Figure 7 shows changes in the RMP on application of dofetilide (Fig. 7A) and PD-118057 (Fig. 7B). One micromolar dofetilide produced a gradual and significant depolarization of the control RMP by \(4.6 \pm 1.6\) mV (\(n = 5\), \(P < 0.05\); Fig. 7A, i and ii). In the absence of dofetilide, an active response as a result of a 2-ms injection of depolarizing current (100 pA) was manifested as a rising waveform after the initial passive depolarization. One micromolar dofetilide did not alter the profile of the active response (Fig. 7Ai). Conversely, application of the novel ERG channel activator PD-118057 at 3 \(\mu M\) produced a gradual but marked hyperpolarization of the control RMP by \(-23.6 \pm 3.6\) mV (\(n = 4\), \(P < 0.01\); Fig. 7B, i and ii). A further interesting observation was the change in profile to the active response with PD-118057. In contrast to dofetilide, no active response was observed in the presence of PD-118057 (Fig. 7Bi) after a current injection of 100 pA. A stronger current injection of 200 pA still did not result in an active response (data not shown). These data show that ERG channels contribute to the RMP and activation of these channels completely suppresses an increase in membrane excitability.

![Fig. 7](http://ajpcell.physiology.org/)
DISCUSSION

Endogenous ERG channel currents have been characterized fully under physiological conditions (~5 mM external K+, negative V_H) in cardiomyocytes and neurons (21, 29, 23). However, the only electrophysiological studies on ERG channels in smooth muscle cells have involved cells bathed in a high-K+ (~140 mM) solution containing the general K+ channel blockers TEA and 4-AP and using a V_H of 0 mV (1, 16). The present study has remedied the situation and represents the first characterization of ERG channels in smooth muscle cells at physiologically relevant potentials. ERG channel currents were identified by their hooked currents at potentials between ~140 and ~40 mV, which reflect the rapid recovery from inactivation developed at depolarized test pulses followed by a slower, voltage-dependent deactivation. Fitting of these kinetics produced values that were similar to those determined for ERG channel currents recorded in the same cell type under symmetrical K+ conditions by Ohya et al. (16). The existence of ERG channel currents in the present study was confirmed by using three structurally distinct and selective blockers of ERG channels, which all inhibited the evoked current by the same extent. E-4031 and dofetilide have been used in a number of different studies on heterologously expressed and native ERG channels including our previous study (16), and at the concentration used (1 μM) they maximally inhibit ERG channels but still retain a high selectivity for this channel. BeKM-1 is a highly selective peptide inhibitor of ERG channels that has not been studied extensively and that acts preferentially on closed channels (9), in contrast to the open channel blockade shown by dofetilide and E-4031. This agent also abolished the hooked inward currents in mPV myocytes with an IC50 of ~8 nM, a value close to that derived for block of heterologously expressed rat ERG1 and ERG2 (respective IC50 values were 19 and 1.5 mM; Ref. 19). Irrespective of the blocker used, the ERG channel current at potentials positive to V_H was well sustained in cells bathed in a normal external solution. However, when the bathing solution was enriched by the addition of 5 mM 4-AP and 10 mM TEA sufficient to block most other K+ channels, the ERG channel current was affected markedly. As a consequence the dofetilide-sensitive current became substantially smaller and very transient in nature, consistent with our previous study (16). The inhibitory effect of 4-AP on heterologously expressed ERG channels has been published previously (20), and a full characterization of the effects of 4-AP on the vascular ERG channel was not the goal of this study. However, this observation validates the importance of investigating endogenous ERG currents in the absence of 4-AP and TEA. Moreover, it also raises the possibility that some of the functional effects of 4-AP in particular may be due to partial inhibition of endogenous ERG channels. This facet has extra relevance following the observation in the present study that selective ERG blockers depolarized the RMP significantly, suggesting that ERG channels contribute to the resting conductance.

ERG currents were also present when currents were recorded with the perforated patch configuration that minimizes intracellular dialysis and therefore approximates the physiological milieu. However, the degree of dofetilide-sensitive outward current recorded with the perforated patch configuration was considerably less than that recorded with the ruptured patch technique, while the amplitudes and kinetics of the inward currents were identical. This suggests that an intracellular mediator is suppressing outward current flow even though the channels are activated to the same degree (as evinced by the amplitude of the hooked inward currents). As greater outward current will have a considerable effect on membrane stability and consequently vascular reactivity, identification of the molecule in question is an important goal. A number of modulators regulate ERG channels positively, including phosphatidylinositol 4,5-bisphosphate (3), PKC (28), and direct binding of cAMP (5). Alternatively, PKA-dependent phosphorylation decreases current amplitude (5, 27), and PKC inhibits heterologously expressed HERG channels by speeding the rate of deactivation (2). Polyamines are also known to mediate the inward rectification of inward rectifier-type K+ currents (14). Future experiments will address the nature of the intracellular regulators dictating the activity of vascular ERG channels.

Until recently there have been no activators of this conductance, although a multitude of agents that block ERG channels have been reported. However, three selective activators of HERG channels, RPR-260243, PD-118057, and NS-1643, have been developed. This type of agent is being considered as possible treatment for acquired or induced long QT syndrome as well as an effective tool to investigate the biophysical and functional properties of ERG channels (4, 8, 33). To date all the data on ERG activators have been derived from either heterologously expressed ERG channels or action potential recordings in cardiomyocytes. The present study is therefore the first to show effects of an ERG activator, PD-118057, outside of the above systems. Our data showed that PD-118057 increased the amplitude of currents in mPV myocytes, and, similar to the report of Zhou et al. (33), this was not accompanied by a change in the voltage dependence of activation or the rate of channel deactivation or recovery from inactivation. Moreover, PD-118057 markedly hyperpolarized the RMP of mPV myocytes under current clamp and abrogated the active response. The observation that an ERG activator hyperpolarizes the RMP is consistent with our observation that ERG channel blockers depolarize the membrane potential, and data suggest that not only do ERG channels contribute to the resting conductance but activation of vascular ERG channels may be an effective mechanism to suppress membrane excitability.

Interestingly, ERG currents enhanced by PD-118057 were poorly inhibited by the ERG channel blocker dofetilide, which was an efficacious inhibitor of ERG currents under control conditions. Dofetilide (1 μM) inhibited the hooked inward currents recorded at ~140 mV by ~80% compared with the complete abolition observed in the absence of PD-118057. The reduced effectiveness of dofetilide in the presence of PD-118057 was even more marked at positive potentials, where the current was not inhibited at all. BeKM-1 was equally as ineffective against currents enhanced by PD-118057. These observations were in stark contrast to those of Zhou et al. (33), who saw complete block of currents in the presence of PD-118057, albeit with 10 μM dofetilide, a concentration considerably higher than the IC50 for block of normal HERG currents (7). The reason for this discrepancy is unknown but does not seem to reflect the de novo activation of another K+ conductance, as PD-118057 enhanced currents in cells bathed in 4-AP and TEA at concentrations sufficient to block other K+ channels including K,1–K,4 and Ca2+-activated K+ channels.
mPV myocytes express mERG1a, which is ~99% homologous to hERG, and mERG1b, which has a 342-amino acid NH2-terminal truncation (10, 12, 16). The mERG1b isoform predominates in the mPV, and heterologous expression of this gene results in K+ currents with characteristics distinct from the classic HERG profile (see Ref 12). In the main these are manifest as a considerably faster rate of deactivation. It is not known how PD-118057 or any ERG channel activator affects mERG1b channels. Moreover, the biophysical and pharmacological properties of ERG channel proteins are modified by association with proteins encoded by members of the KCNE gene family, and the mPV expresses KCNE2 and KCNE3 (16). Consequently, identification of the molecular composition of the native ERG channel in vascular myocytes may reveal the reasons for the complex effects of PD-118057 in the present study.

Overall the data of the present study show that ERG channels are active under quasi-physiological conditions and contribute to the RMP. Moreover, activation of these channels by agents such as PD-118057 may be a novel mechanism to suppress changes in membrane excitability. However, the data of the present study revealed that use of PD-118057 should be undertaken with caution. We only have minimal information as to the molecular composition of the ERG channel in mPV myocytes, and future experiments will focus on determining what proteins constitute the endogenous channel.

REFERENCES