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Effects of the 5-lipoxygenase activating protein inhibitor MK886 on voltage-gated and Ca^{2+}-activated K^+ currents in rat arterial myocytes

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1 The effects on the voltage-gated (I_K) and Ca^{2+} activated ($I_{K,Ca}$) K^+ currents in rat arterial myocytes of the 5-lipoxygenase activating protein (FLAP) inhibitor MK886, and its inactive analogue L583,916 were evaluated.

2 In rat pulmonary arterial myocytes (RPAMs), MK886 caused a concentration-dependent reduction of the I_K, with little obvious change in the kinetics of the current. Half maximal current block was observed at 75 nM MK886.

3 MK886 application led to a concentration-dependent increase in the amplitude of the TEA-sensitive $I_{K,Ca}$ current and single channel activity in RPAMs in whole cell and inside-out configurations, respectively. The threshold concentration for this effect was approximately 300 nM and a maximal 4–5 fold increase was observed at 10 μM MK886. MK886 also increased $I_{K,Ca}$ in rat mesenteric arterial myocytes (RMAMs).

4 L538,916, an analogue of MK886 which does not block FLAP, had no effect on either I_K or $I_{K,Ca}$ at a concentration of 10 μM.

5 Leukotriene C_4 (100 nM) had no effect on either I_K or $I_{K,Ca}$ in RPAMs. MK886 produced its usual increase in $I_{K,Ca}$ and also blocked I_K, in the presence of leukotriene C_4. Similarly, leukotriene E_4 (100 nM) did not alter the amplitude of I_K. Also, the nonselective leukotriene receptor antagonist ICI 198,615 (3 μM) did not affect I_K in RPAMs, and did not affect the response to MK886.

6 Arachidonic acid (10 μM) enhanced $I_{K,Ca}$ in both RPAMs and RMAMs.

7 The results show that MK886 markedly affects both I_K and $I_{K,Ca}$ in a manner similar to that of arachidonic acid and independent of the endogenous production of leukotrienes. It is therefore possible that MK886, which is thought to compete with arachidonic acid for its binding to FLAP, may similarly occupy arachidonic acid binding sites on these K^+ channels, and mimic its effects. Alternatively, MK886 might act via non-selective effects on other arachidonic acid metabolites which could modify K^+ channel function.

Keywords: Leukotrienes; 5-lipoxygenase-activating protein (FLAP); MK886; delayed rectifier K^+ channel; Ca^{2+} activated K^+ channel; rat pulmonary artery; rat mesenteric artery

Introduction

In a previous study (Smirnov & Aaronson, 1996), we demonstrated that arachidonic acid dramatically altered the kinetics of the delayed rectifier K^+ current (I_K) in rat pulmonary arterial myocytes (RPAMs). In the course of this study, it was found that 5,8,11,14-eicosatetraynoic acid (EYTA), a non-selective inhibitor of arachidonic acid metabolism, and nordihydroguaiaretic acid (NDGA) an inhibitor of 5-lipoxygenase (5-LO) and cyclo-oxygenase, decreased the amplitude of I_K. The cyclo-oxygenase inhibitor indomethacin had little effect on I_K. These data were consistent with the possibility that I_K might be modulated by leukotrienes. Although little is known concerning effects of leukotrienes on ion currents in vascular smooth muscle cells, there is evidence that these substances are able to activate the acetylcholine-sensitive K^+ channel in atrial cells (Kurachi *et al*., 1989; Kim *et al*., 1989) and also stimulate a K^+ current in chick cultured skeletal muscle cells (Thomas & Hume, 1993). In addition, epoxyeicosatrienoic acids, metabolites of arachidonic acid produced via the action of cytochrome P450, cause the opening of large conductance $I_{K,Ca}$ channels, and the inhibition of I_K, in vascular myocytes (Hu & Kim, 1993; Smirnov & Aaronson, 1996). Inhibitors of cytochrome P450 have also been shown to cause inhibition of I_K and diverse effects on $I_{K,Ca}$, in rat portal vein myocytes (Edwards *et al*., 1996).

5-Lipoxygenase-activating protein (FLAP) stimulates 5-LO to produce leukotriene A_4, either by recruiting it to the nuclear membrane (Ford-Hutchinson *et al*., 1994), or by transferring arachidonic acid to 5-LO (Hatzelmann *et al*., 1994). Potent and selective inhibitors of FLAP such as MK886 (Rouzer *et al*., 1990) and Bay X 1005 (Hatzelmann *et al*., 1994) therefore provide useful pharmacological probes for determining the role of 5-LO metabolites in cell function. The effects L583,916, an analogue of MK886 which does not inhibit leukotriene synthesis, have also been studied (Miller *et al*., 1990).

Preliminary experiments revealed that MK886 potently inhibited I_K, suggesting that ongoing leukotriene production in these cells might stimulate I_K. We have therefore carried out a more comprehensive characterization of the effect of this compound on both I_K and $I_{K,Ca}$ in myocytes from rat pulmonary and mesenteric artery, including experiments designed to assess the role of leukotrienes in the effects of MK886.

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Methods

The methods used for isolation of cells from rat pulmonary arteries, electrophysiological recordings and the application of external solutions were similar to those described in detail elsewhere (Smirnov & Aaronson, 1994; 1996). Single cells from rat mesenteric arteries were isolated in a similar manner to pulmonary myocytes. However, the length of incubation was set at 30 min.

External physiological salt solution (PSS) contained (in mM): NaCl 130, KCl 5, $MgCl_2$ 1.2, $CaCl_2$ 1.5, HEPES 10 and glucose 10. The pH was adjusted to 7.2 with NaOH. In order to isolated the delayed rectifier K^+ current in RPA myocytes 10 mM tetraethylammonium chloride (TEA) was added to PSS (TEA-PSS, Smirnov & Aaronson, 1994; 1996). In some cases 2 or 10 mM was added to PSS in order to block Ca^{2+}-activated K^+ currents ($I_{K,Ca}$) as indicated in the text. The pipette solution contained (in mM): KCl 110, MgATP 5, HEPES 10, EGTA 10 and $CaCl_2$ 0.5 (giving an estimated free $[Ca^{2+}]$ of 8 nM) and was buffered to pH 7.2 with KOH. In the experiments with RMA myocytes the concentration of $CaCl_2$ was increased to 6 mM (giving a calculated free $[Ca^{2+}]$ of 234 nM) in order to maximize the proportion of $I_{K,Ca}$ in these cells. The composition of the solution for single channel recording was (in mM): KCl 110, Na_2ATP 5, $MgCl_2$ 0.5, HEPES 10, EGTA 10 and $CaCl_2$ 3.8 (calculated free $[Ca^{2+}] = 100$ nM) and was buffered to pH 7.2 with KOH, giving a calculated total potassium concentration of 135 mM. Experiments were performed at room temperature.

General reagents and chemicals were from Sigma. MK886 (3-[1-(*p*-chlorobenzyl)-5-(isopropyl)-3-tert-butylthioindol-2-yl]-2,2-dimethylpropanoic acid) and L583,916 (2-/1-*p*-chlorobenzyl-5-methoxy-2-methylindol-3-yl/propionic acid) were gifts from Dr A.W. Ford-Hutchinson (Merck Frosst, Quebec, Canada). ICI 198,615 ([1-[[2-methoxy-4[[(phenylsulphonyl) amino]carbonyl]-phenyl]methyl]-1H-indazol-6yl]carbamic acid cyclopentyl ester) was from Zeneca Pharma. Data are presented as mean ± s.e.mean and compared for significance by use of Student's *t* test as appropriate; differences were deemed significant at $P < 0.05$. Curve fitting and data analysis were carried out with Origin 4.1 (Microcal Software Inc., U.S.A.).

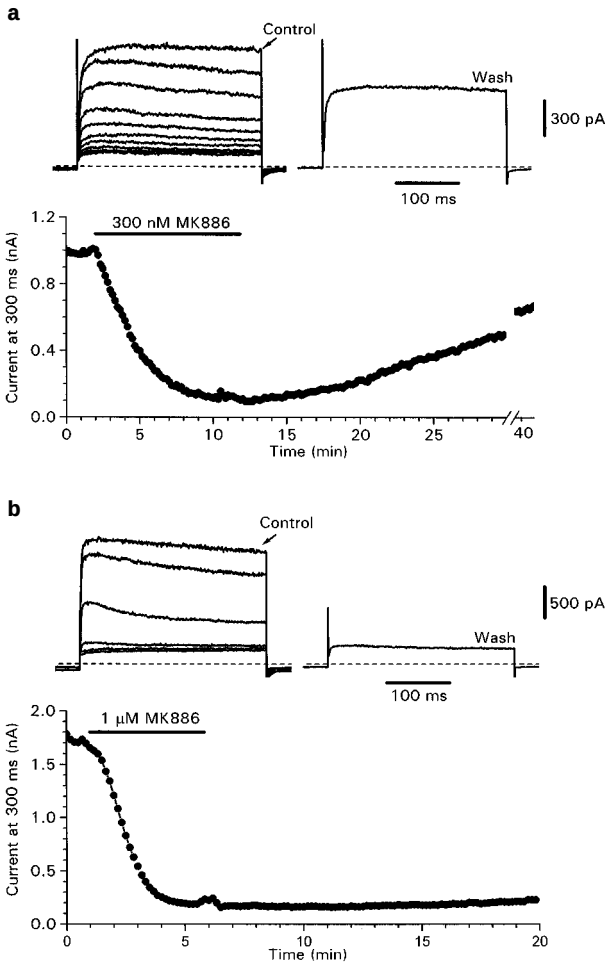

Figure 1 Effect of MK886 on I_K in RPAMs. (a) and (b) The time course of the inhibition of I_K by 300 nM and 1 μM MK886, respectively. Cells were stepped at 0.1 Hz from −60 to +60 mV. Current amplitude was measured at the end of 300 ms. I_K shown on the top left panel represents traces before the drug application (Control) and in the presence of MK886 (shown every min). External solution contained 10 mM TEA. Dashed lines here and in the following figures represent zero current level.

Results

MK886 caused an inhibition of I_K which tended to be partially reversible at submicromolar concentrations, but which became irreversible at higher concentrations (Figure 1a and b). The rate of onset of current inhibition was concentration-dependent (Figures 1 and 2). Figure 2 shows that a stable level of block developed within 2 min in 10 μM MK886, but that the current was still decreasing slowly in the presence of submicromolar concentrations of the drug even after 10 min. No run down of I_K was observed in the absence of MK886 (Figure 2a). The time course of the development of I_K block could be described by a single exponential with a time constant (τ_{MK}) which monotonically decreased from 125 ($n = 1$) and 34 ± 20 ($n = 4$) min at 10 and 30 nM of MK886, respectively, to 43 ± 6 s ($n = 5$) at 10 μM. The dependence of τ_{MK} over the range of concentrations of MK886 is presented in Figure 2b. For a reaction of order n, the initial velocities v at concentrations c are described by the equation:

$$v = k \cdot c^n \quad (1)$$

where $v = 1/\tau_{MK}$ at each concentration (Roberts, 1977). In this case, a plot of $\ln(1/\tau_{MK})$ against $\ln([MK886])$ will have a slope corresponding to the order of the reaction, and an intercept on the ordinate which is equal to the forward rate constant (k) for the binding reaction. Fitting this equation to the data, n was equal to 0.74 and k was equal to 159 s^{-1}.

In order to estimate the potency of the inhibitory effect of MK886 on I_K the relative current amplitude was measured 5 (1 and 10 μM MK886)and 10 (30, 100,and 300 nM MK886) min after the drug was applied. The IC_{50} for the blockade of the current by MK886 was 75 nM (Figure 2d). Figure 3 illustrates that the block of I_K by MK886 was similar over a wide range of test potentials.

The effect of MK886 on the $I_{K,Ca}$ current in RPAMs was studied when the holding potential was set to −20 mV; we have previously shown that this inactivates I_K which would otherwise be prominent in these cells (Smirnov & Aaronson, 1994). Ca^{2+} concentration in the pipette solution was clamped at 8 nM. Figure 4a illustrates that under these conditions, the current elicited at +80 mV was almost completely blocked by 10 mM TEA, indicating that it was due to $I_{K,Ca}$. Removal of TEA and subsequent addition of 10 μM MK886 then caused an approximately 3 fold increase in current amplitude. Application of TEA in the presence of MK886 then completely blocked the enhanced current, confirming that it was $I_{K,Ca}$. The

effect of MK886 on $I_{K,Ca}$ was partially (50–80%) reversed 2–3 min after removal of the drug (data not shown). Figure 4b shows mean results from similar experiments in 3 cells. Figure 4c illustrates the concentration-response relationship for the effect of MK886 on the K^+ current. The drug caused an approximately 4 fold increase at 10 μM.

In order to eliminate the possibility that changes in the intracellular Ca^{2+} concentration were causing the enhancement of $I_{K,Ca}$ by MK886, the activity of single $I_{K,Ca}$ channels were recorded in an inside-out membrane patch from RPAM in symmetrical high K^+ pipette and cytosolic (bath) solutions. The activity of single $I_{K,Ca}$ channels in RPAMs (confirmed by sensitivity to TEA, data not shown) in the presence of low [Ca^{2+}] is small, so the Ca^{2+} concentration in the bath was increased to 100 nM and single channel activity was monitored at 40 mV. Under these conditions the mean $N \times P_o$ measured over 1 min before the addition of the drug was 0.002 ± 0.003 ($n = 3$). After application of 10 μM MK886 to the bath the single channel activity measured over 2 min was significantly increased by approximately 10 times ($N \times P_o = 0.03 \pm 0.009$, $n = 3$, $P < 0.02$) (Figure 4d).

MK886 also stimulated the TEA-sensitive $I_{K,Ca}$ current in rat mesenteric arterial myocytes (RMAMs). The intracellular [Ca^{2+}] was set to 234 nM in order to enhance $I_{K,Ca}$. Under these conditions, RMAMs display a prominent $I_{K,Ca}$ current, with only a minor component of I_K, allowing the response of $I_{K,Ca}$ to MK886 to be measured over a range of potentials with very little interference from I_K. The effect of MK886 on $I_{K,Ca}$ was examined by ramping the membrane potential from -100 to $+50$ mV.

Figure 5a shows an example of the effects of TEA and MK886 observed in RMAMs. In the absence of MK886, the current was almost entirely blocked by 2 mM TEA, confirming that it was mostly due to $I_{K,Ca}$. After TEA was removed and the current had recovered, addition of 10 μM MK886 caused a marked increase in current amplitude. Subsequent addition of TEA in the presence of MK886 then reduced the current markedly, demonstrating that in these cells, as in those of the pulmonary artery, the MK886-stimulated component of current was TEA-sensitive. The mean enhancement of $I_{K,Ca}$ elicited by 10 μM MK886 in a group of mesenteric arterial myocytes is shown in Figure 5b. The increase in current amplitude was accompanied by a leftward shift in the apparent activation threshold.

Although the pipette Ca^{2+} concentration was clamped at 234 nM by the Ca-EGTA buffering system, the possibility that MK886 was altering the intracellular Ca^{2+} concentration, possibly by releasing Ca^{2+} from intracellular stores, could not be entirely excluded. In order to assess this possibility, a pipette solution containing no added Ca^{2+} and 10 mM EGTA and 10 mM BAPTA was used in RMAM cells to reduce intracellular [Ca^{2+}] to very low levels (calculated as 8 nM) and prevent its elevation. Addition of 10 mM caffeine did not affect $I_{K,Ca}$ in RMAMs under this condition (Figure 5c and d). However, 10 μM MK886 produced a significant enhancement of $I_{K,Ca}$. The effect of MK886 was even larger in this low Ca^{2+} pipette solution than in the presence of 234 nM Ca^{2+} ($315 \pm 41\%$, $n = 6$, and $212 \pm 22\%$, $n = 9$, respectively).

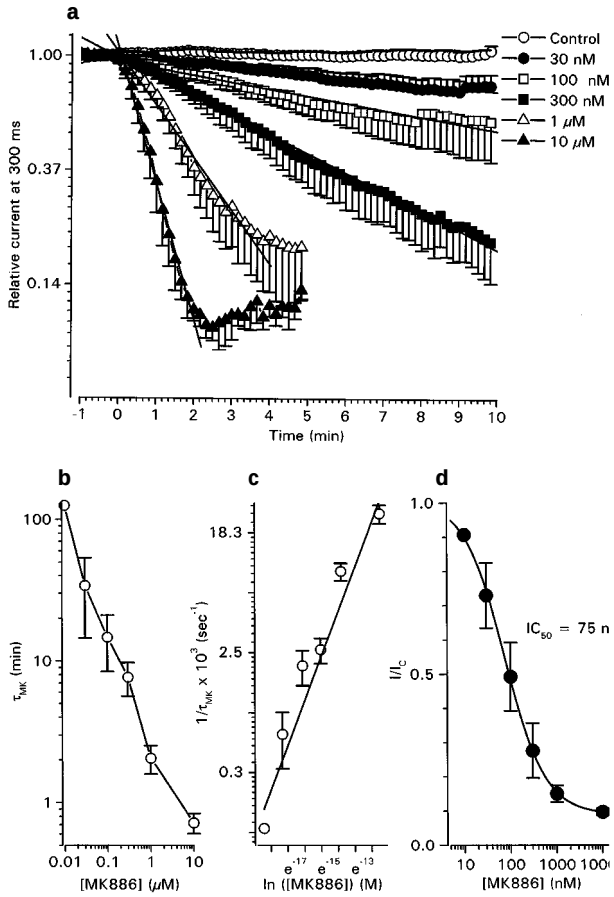

Figure 2 Concentration-dependence of inhibition of I_K by MK886 in RPAMs. (a) Time course of I_K inhibition by various concentrations of MK886 as indicated, plotted on a semilogarithmic scale; drug was applied at time 0. (b) Dependence of the time constant (τ_{MK}) of the development of the block of I_K on the concentration of the drug; τ_{MK} was derived from the fit of the linear range of the experimental results as shown in (a). (c) A plot of $\ln(1/\tau_{MK})$ against $\ln([MK886])$; see text for details. (d) Concentration-dependence of I_K block by MK886. The solid line was drawn according the following equation:

$$I/I_c = \frac{1 - A}{1 + \frac{[MK886]}{IC_{50}}} + A$$

where I/I_C is the ratio of I_K in the presence (I) and absence (I_C) of the drug, IC_{50} (75 nM) is the concentration at which I_K was inhibited by 50%, and A (0.09) is the fraction of unblocked current. Symbols and vertical lines in this and the following figures represent mean and s.e.mean for 4–6 cells studied (except for 10 nM in (b–d), 1 cell studied).

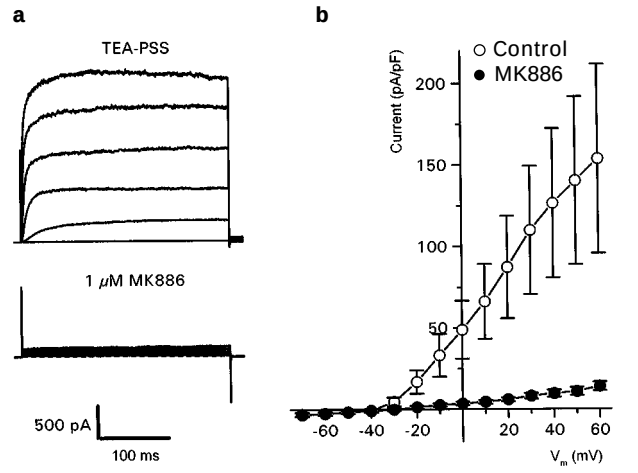

Figure 3 Effect of MK886 on the current-voltage relationship (I-V) for I_K in RPAMs. (a) A family of I_K (from -40 to $+60$ mV in 20 mV increments) in the absence (upper panel) and presence (lower panel) of 1 μM MK886. (b) The average I-V curves for I_K measured at 300 ms in the absence (control) and in the presence of 1 μM MK886 obtained in 4 RPAMs and expressed as current density. External solution contained 10 mM TEA.

Figure 6 shows a comparison of the effects of MK886 and L583,916 (both at concentrations of 10 μM) on I_K in RPAMs (a) and $I_{K,Ca}$ in RMAMs (b). I_K in the RPAMs was measured by means of voltage steps from −60 mV to +60 mV in the presence of 10 mM TEA, which selectively abolishes $I_{K,Ca}$ in these cells (Smirnov *et al.*, 1994). $I_{K,Ca}$ in RMAMs held at −60 mV was elicited by voltage ramps from −100 to +50 mV (in the absence of TEA), and was measured at +50 mV. MK886 inhibited I_K and stimulated $I_{K,Ca}$, whereas L583,916 had no significant effect on either current.

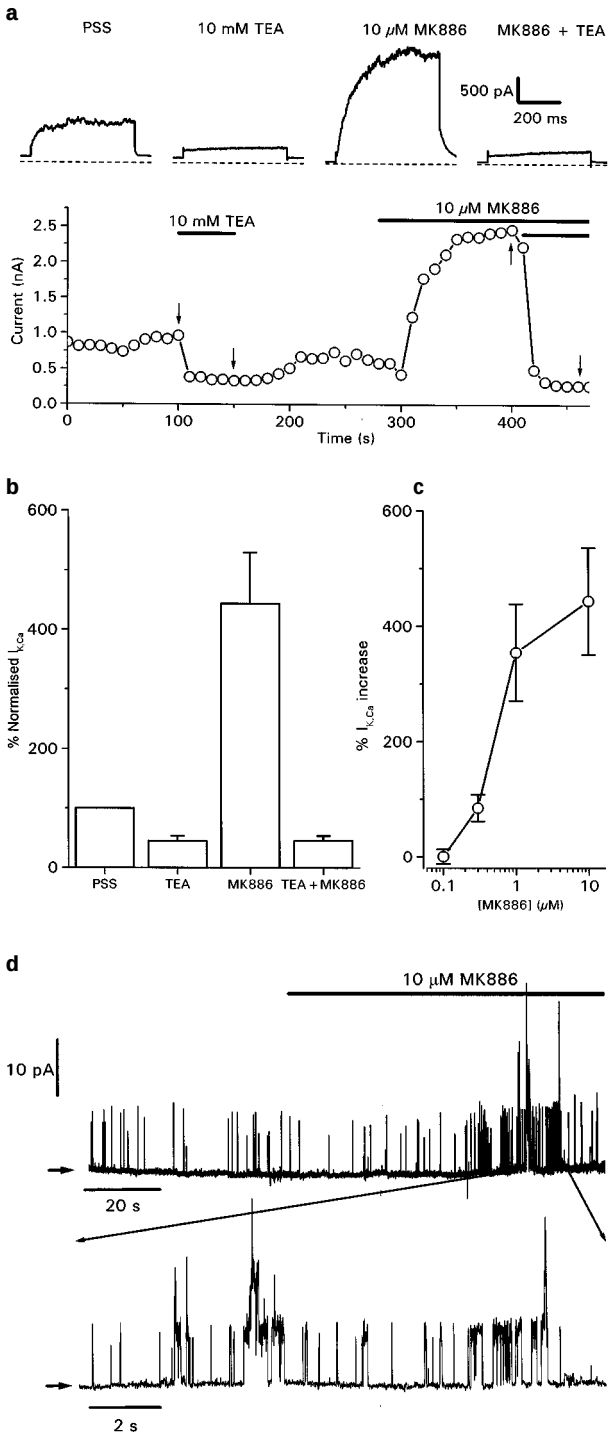

Figure 4 Modulation of $I_{K,Ca}$ by MK886 in RPAMs. (a) Currents (upper panel) and time course of the effect of MK886 and TEA applied at concentrations as indicated. $I_{K,Ca}$ was measured by steps to +80 mV, applied at 0.1 Hz from a holding potential −20 mV. Arrows indicate the position of current traces shown on the top panel. (b) A summary of the effect of 10 μM MK886 and 10 mM TEA on $I_{K,Ca}$ recorded with the protocol shown in (a) ($n=3-7$ cells). (c) The concentration-dependence of potentiation of $I_{K,Ca}$ by MK886 ($n=3-9$ cells). Pipette solution contained 8 nM Ca^{2+} in (a–c). (d) The effect of MK886 on single channel activity of $I_{K,Ca}$ by use of an inside-out configuration in symmetrical high K^+. The drug was introduced to the cytoplasmic side of the cell membrane which was constantly perfused with 100 nM [Ca^{2+}]. The arrow indicates the closed state. Two different time scales are illustrated. The holding potential was +40 mV.

The effect of MK886 on these K^+ currents, together with the lack of effect of its inactive analogue L583,916, suggested that it was possible that an ongoing production of leukotrienes in these cells might somehow be necessary for maintaining I_K. We therefore evaluated the effects of leukotriene C_4 on these K^+ currents. Leukotriene C_4 is the pre-eminently potent and efficacious vasoactive leukotriene in the rat pulmonary artery (Iacopina *et al.*, 1984).

I_K was evaluated by stepping cells from −60 mV to +60 mV in the presence of 10 mM TEA to block $I_{K,Ca}$. Figure 6c shows that leukotriene C_4 had no significant effect on I_K and also did not prevent its inhibition by MK886. In 2 separate experiments where I_K alone was measured, leukotriene E_4 (100 nM) also had no apparent effect on I_K. Figure 6d illustrates that $I_{K,Ca}$ in RPAMs, measured by steps from −20 to +80 mV, was not significantly affected by 100 nM leukotriene C_4 ($n=5$). In addition, the increase in $I_{K,Ca}$ caused by 10 μM MK886 was not significantly altered by the presence of leukotriene C_4 ($n=3$). Additional experiments with the nonselective leukotriene receptor antagonist ICI 198,615 (3 μM) showed that this compound neither significantly affected the amplitude of I_K in RPAMs (5±4% inhibition over a 2 min incubation, $n=7$), nor prevented the inhibition of I_K by 1 μM MK886. I_K was decreased by 85±2% ($n=6$) and 84±4% ($n=4$) in the absence and presence of the leukotriene receptor antagonist, respectively.

The suppression by MK886 of I_K in RPAMs described above shows some similarity to the effect of arachidonic acid (AA) on this current (Smirnov & Aaronson, 1996). In order to determine whether both compounds also share similar effects on $I_{K,Ca}$, we recorded the effect of AA (10 μM) on $I_{K,Ca}$ in both RPAMs and RMAMs, as shown in Figure 7. Figure 7a illustrates that AA increased $I_{K,Ca}$ recorded during steps to +80 mV in RPAMs held at −20 mV. The identity of the current activated by AA was confirmed by use of 10 mM TEA, which selectively blocks $I_{K,Ca}$ in these cells (Smirnov *et al.*, 1994). Similarly, in RMAMs, 10 μM AA increased $I_{K,Ca}$ measured by voltage ramps from −100 to +60 mV (Figure 7b). Again, the current activated by AA was almost completely eliminated by 10 mM TEA. As with MK886, the enhancement of $I_{K,Ca}$ by AA was associated with a leftward shift in $I_{K,Ca}$ activation (Figure 7c).

Discussion

Our results demonstrate that MK886 is a potent blocker of I_K in RPAMs and also stimulates $I_{K,Ca}$ in both RPAMs and RMAMs at micromolar concentrations. The block of I_K by MK886 occurs in a concentration range similar to that over which this compound inhibits FLAP. Half-maximal inhibition of I_K was observed at 75 nM, and this may be somewhat of an overestimate since I_K was still decreasing slowly after 10 min in the presence of the lower concentrations of MK886. In leukocytes stimulated with A23187, leukotriene synthesis was inhibited by MK886 with an IC_{50} of 102 nM (Rouzer *et al.*, 1990). Similarly, MK886 inhibited the binding to FLAP of the affinity probe L-691,831 with an

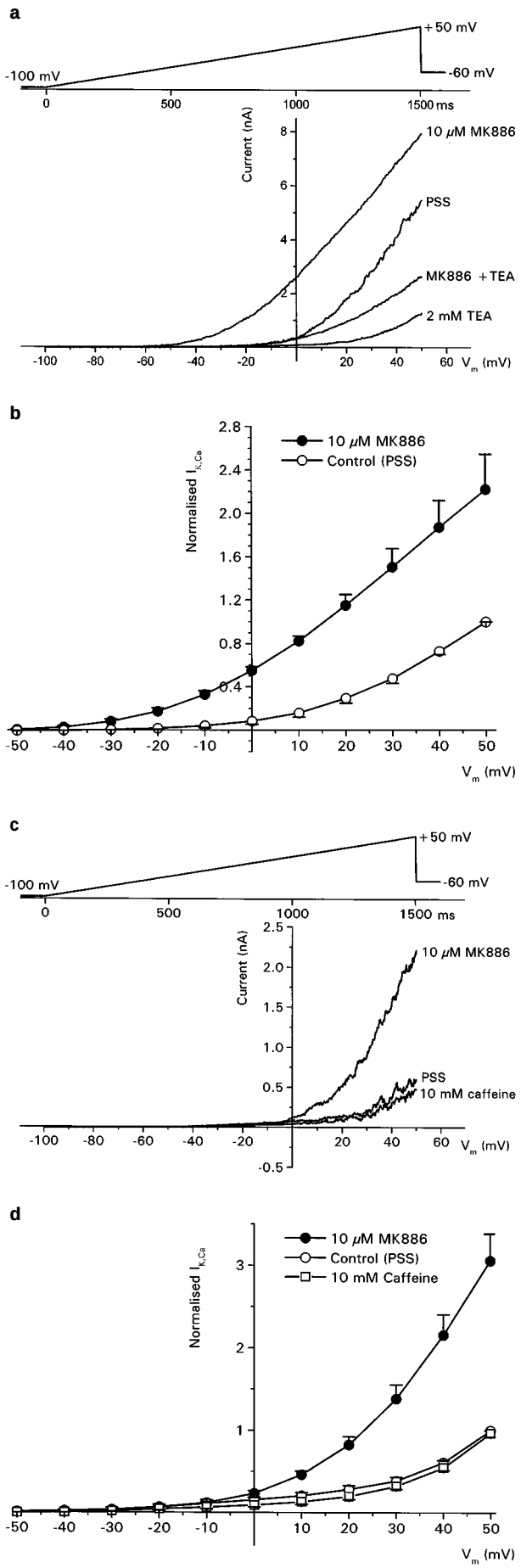

Figure 5 Effect of MK886 (10 μM) on $I_{K,Ca}$ in RMAMs with low and high pipette [Ca^{2+}]. (a) and (b) The whole cell current and mean *I*-V relationships constructed from voltage ramps ($n=9$) for $I_{K,Ca}$ in the absence and presence of MK886 with 234 nM Ca^{2+} in the pipette solution. Inhibition of $I_{K,Ca}$ by 2 mM TEA is also illustrated in (a). (c) and (d) $I_{K,Ca}$ and *I*-V relationships ($n=6$) under control conditions and presence of 10 mM caffeine or MK886 when the pipette solution contained EGTA and BAPTA (10 mM of each) and no added Ca^{2+}. Cells were held at -60 mV and ramped from -100 mV to $+60$ mV as shown in the top panels in (a) and (c).

IC_{50} of approximately 25 nM (Charleson *et al.*, 1992). At a slightly higher concentration, MK886 increased the amplitude of the Ca^{2+} activated K^+ current and also shifted its activation threshold to a more negative potential range. Furthermore, L583,916, an analogue of MK886 which is inactive against FLAP, also did not affect I_K or $I_{K,Ca}$.

These results suggested that ongoing leukotriene production might be exerting tonic effects on these K^+ currents. On the other hand, such a mechanism is inconsistent with findings that the expression of both 5-LO and FLAP is essentially limited to cells of myeloid origin (Ford-Hutchinson *et al.*, 1994; Goetzl *et al.*, 1995). However, since it was not inconceivable that treatment of cells during enzymatic isolation might somehow stimulate the expression of these proteins and thus lead to leukotriene production, experiments were carried out to determine whether leukotrienes were able to modulate these currents or their responses to MK886. However, the nonselective leukotriene receptor antagonist, ICI 198,615, neither inhibited I_K as would be expected if the current was being stimulated by ongoing leukotriene production, nor prevented the inhibition of the current by MK886. Leukotriene C_4 also did not affect either I_K or $I_{K,Ca}$ under the conditions used in these experiments. The responses of these currents to MK886, which should be prevented by leukotriene C_4 if this inhibitor was working by blocking endogenous leukotriene production, were also not different in the presence of leukotriene C_4. Similarly leukotriene E_4 had no effect on I_K. Leukotriene C_4 in particular has been shown to constrict the pulmonary vasculature in the rat (Iacopina *et al.*, 1984; Voelkel

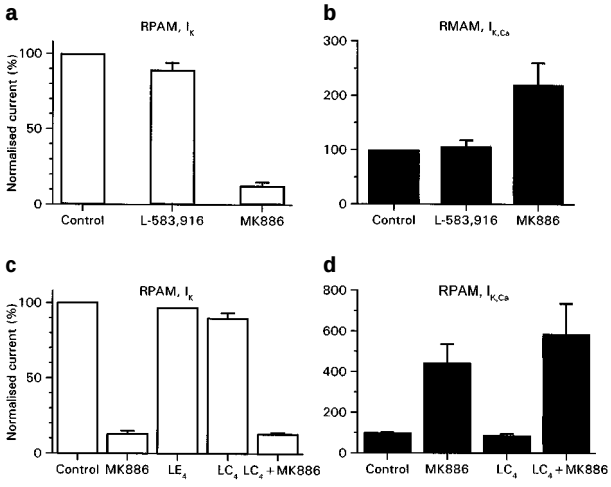

Figure 6 Effect of L-583,916 and leukotrienes C_4 and E_4 on K^+ currents in vascular myocytes. (a) and (b) A comparison of the effects of MK886 and L-583,916 (both at 10 μM) on I_K and $I_{K,Ca}$ in RPAMs and RMAMs as indicated ($n=4-5$ for (a), $n=4-7$ for (b)). (c) The effect of 100 nM leukotrienes C_4 (LC_4, $n=3$) and E_4 (LE_4, $n=2$), and the effect of 10 μM MK886 in the presence of LC_4 on I_K in RPA cells ($n=3$). (d) The effect of 100 nM leukotriene C_4 on $I_{K,Ca}$ in RPAMs and the effect of 10 μM MK886 in the presence and the absence of leukotriene C_4 ($n=3-7$). Further details described in the text. Pipette solution contained 8 nM Ca^{2+} for RPAMs and 234 nM Ca^{2+} for RMAMs.

et al., 1984) and other species (Ohtaka *et al*., 1987; Berkowitz *et al*., 1994).

Taken as a whole, the data therefore suggest that MK886 has effects on I_K and $I_{K,Ca}$ which are independent of any inhibition of leukotriene synthesis. Since increases in intracellular [Ca^{2+}] inhibit the delayed rectifier K^+ current (Post *et al*., 1995) as well as stimulate $I_{K,Ca}$, one possible mechanism for the effect of MK886 was that it was acting to increase the intracellular Ca^{2+} concentration. However, in RPAM cells MK886 significantly enhanced $I_{K,Ca}$ channel opening in inside-out patches which were bathed in a solution with a fixed Ca^{2+} concentration (100 nM, Figure 4d). Furthermore, MK886 was able to increase $I_{K,Ca}$ in RMAMs under conditions when the pipette concentration was clamped at very low Ca^{2+} concentrations (10 mM EGTA and 10 mM BAPTA). The lack of the effect of caffeine (10 mM) on $I_{K,Ca}$ also suggests that the possible release of Ca^{2+} from intracellular stores by MK886 could not be involved in the potentiation of $I_{K,Ca}$ (Figure 5, c–d). An obligatory role for the elevation of intracellular Ca^{2+} in the response to MK886 therefore seemed unlikely.

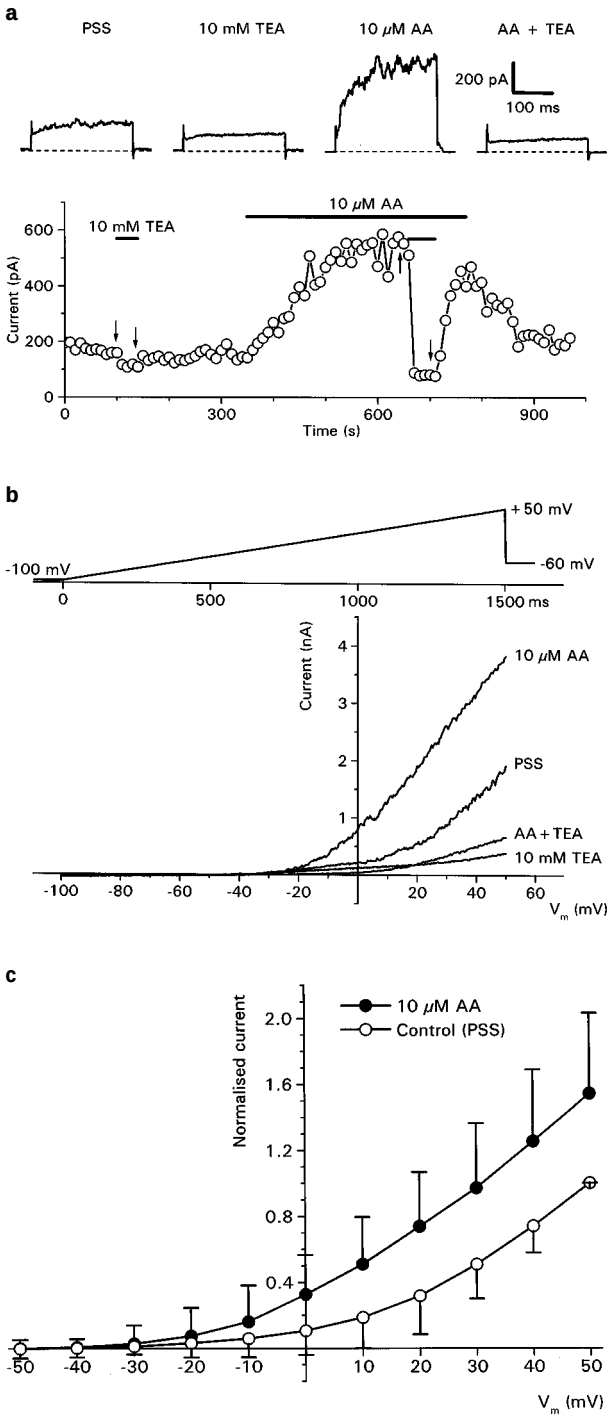

Figure 7 Effect of arachidonic acid (AA) on $I_{K,Ca}$ in vascular myocytes. (a) Currents and time course of the effect of TEA (10 mM) and AA (10 μM) in RPAMs. $I_{K,Ca}$ was elicited by a step potential to +80 mV from a holding potential of −20 mV. Arrows indicate the position of current traces shown on the top panel. (b) The effect of TEA and AA on $I_{K,Ca}$ recorded by means of a ramp protocol (top panel, see also Figure 5) in RMA cell. (c) The *I*-V relationships constructed from the ramps obtained from RMAMs in the absence and presence of 10 μM AA ($n=3$). The pipette solution contained 234 nM [Ca^{2+}].

The inhibition of I_K and the potentiation of $I_{K,Ca}$ by MK886 resembles the effect of arachidonic acid on these currents (see Kirber *et al*., 1992; Smirnov & Aaronson, 1996; also Figure 7), although the response of I_K to arachidonic acid also involves a separate transient protein kinase C-mediated enhancement not observed with MK886 (Smirnov & Aaronson, 1996). It therefore seems possible that MK886 acts at putative arachidonic acid binding sites on these channels, thereby mimicking the effect of this fatty acid. In support of this hypothesis, it has been shown that both MK886 and arachidonic acid compete with a photoaffinity probe analogue of arachidonic acid for binding to FLAP (Mancini *et al*., 1993), suggesting that these substances act at the same binding site on this enzyme. Vickers *et al*. (1992) have localized the binding to FLAP of photoaffinity labels, similar in structure to MK886, to a hydrophilic loop between the putative first and second transmembrane regions of this protein. The amino acid sequence of this arginine-rich region suggests that it forms an amphipathic α helix. It is noteworthy that the proposed voltage-sensor region (the S4 segment) of many voltage-sensitive ion channels, including I_K, is thought to consist of an arginine-rich α helix (Guy & Conti, 1990). We therefore speculate that both arachidonic acid and MK886 exert their similar effects on these channels by interacting with this region. In the case of $I_{K,Ca}$, for example, such binding might also alter the sensitivity of $I_{K,Ca}$ channel to the existing level of free Ca^{2+} and thus enhance the current. However, this speculation requires further experimental support.

It is also possible that the effects of MK886 may be exerted via a leukotriene-independent effect on 5-LO. It has been shown that the interaction of FLAP with 5-LO enables the latter enzyme to metabolize the cytochrome P450-derived substances 12(S) and 15(S)-hydroxeicosatraenoic acid (HETE) (Hill *et al*., 1992). Although the effects of these substances on K^+ channels in vascular smooth muscle remains largely unexplored, the related P450 metabolite 20-HETE has been shown to inhibit the $I_{K,Ca}$ current in cat cerebral arteries (Harder *et al*., 1994). However, further studies are required to elucidate the relationship, if any, between the effects of MK886 on K^+ channels, and its actions on the metabolism of non-leukotriene arachidonic acid metabolites.

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