J. Membrane Biol. 158, 69–75 (1997)

The Journal of
Membrane
Biology

Ca^{2+}-dependent K^+ Channels in Bovine Adrenal Chromaffin Cells are Modulated by Lipoxygenase Metabolites of Arachidonic Acid

W.A. Twitchell, T. L. Peña, S.G. Rane
Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA

Received: 15 July 1997/Revised: 27 January 1997

Abstract. Fatty acids play an important role in a variety of physiological processes including ion channel modulation and catecholamine release. Using patch-clamp techniques we show that arachidonic acid (AA) is converted to lipoxygenase metabolites (LOMs) to potentiate activity of the Ca^{2+} and voltage-dependent, large-conductance K^+ channel (BK) in bovine adrenal medullary chromaffin cells (BAMCCs). AA and LOM potentiation of BK current and recovery from potentiation were unaffected by the nonhydrolyzable ATP analogue AMP-PNP, or by exclusion of nucleotides in excised patch recordings. Also, AA and LOM potentiation of BK channel activity in outside-out patches exposed to strong Ca^{2+} buffering ruled out cytoplasmic messengers or changes in intracellular Ca^{2+} levels as causative factors. Lipoxygenase inhibitor attenuated AA, but not LOM potentiation of BK activity in outside-out patches, indicating that lipoxygenase processing of AA is possible in excised membrane patches, possibly via a membrane associated lipoxygenase. AA and LOM release have been implicated in the mechanics of catecholamine secretion from BAMCCs. By limiting action potential duration and thus voltage-gated Ca^{2+} influx, fatty acid potentiation of BK current may serve an inhibitory feedback function in regulating secretion from BAMCCs.

Key words: BK channel — Arachidonic acid — Lipoxygenase — Bovine adrenal chromaffin cell — Eicosinoid — Secretion

Introduction

In many cells, including bovine adrenal medullary chromaffin cells (BAMCCs), arachidonic acid (AA) and its metabolites are important mediators of both signal transduction events and secretory processes (Axelrod, 1990; Piomelli & Greengard 1990; Koda et al., 1989; Rindlisbacher et al., 1990). Nicotine or K^+ induced release of catecholamines from BAMCCs is associated with the release of AA, possibly via the action of a diacylglycerol lipase or granule associated phospholipase A_2 (Frye & Holtz, 1984; Rindlisbacher, Reist & Zahler 1987; Hildebrandt & Albanesi, 1991). The lipoxygenase inhibitor BW755c inhibited acetylcholine stimulated release of catecholamines from cultured BAMCCs, suggesting that lipoxygenase metabolites of AA may be important paracrine regulators of secretion (Rindlisbacher et al., 1990). One possible site of regulatory control by AA and its metabolites may be at the ion channels which function in excitation-secretion coupling. Although mobilization of fatty acids and their metabolites has been shown to affect ion channel function in other systems (Belardetti et al., 1989; Fraser et al., 1993; Keyser & Alger, 1990; Khurana & Bennett, 1993; Kim & Clapham, 1989; Kim et al., 1995; Kirber et al., 1992; Ordway, Walsh & Singer, 1989; Schmitt & Meves, 1995), their ability to modulate channels in BAMCCs is untested. Therefore, we asked whether AA regulation of catecholamine secretion in BAMCCs could in part be mediated by modulation of ionic currents. Our results suggest that the BK channel, an important contributor to membrane repolarization following action potential generation, is a target for modulation by lipoxygenase metabolites of AA.

Materials and Methods

Materials

Rat tail collagen was obtained from Collaborative Biomedical Products. Dulbecco's modified Eagle's medium, fetal bovine serum, peni-

Correspondence to: S.G. Rane

cillin, and streptomycin were from Gibco Labs. Arachidonic acid, 5-, 12-, and 15-hydroperoxy-eicosatetraenoic acid (HpETE) were obtained from Cayman Chemical, and charybdotoxin was from BACHEM Bioscience. All other compounds were from Sigma Chemical.

Cell Culture

Primary cultures of adult bovine chromaffin cells were obtained via collagenase digestion of fresh bovine adrenal glands as described in Twitchell and Rane (1993). Cultures were maintained on collagen coated 35 mm dishes in DMEM/10% FBS in a 95% air, 5% CO_2 atmosphere at 37°C, and were used for experiments at 1–8 days after plating.

Electrophysiology

For whole-cell and excised patch experiments the bath solution consisted of (in mM): 143 NaCl, 4 KCl, 2 $MgCl_2$, 1 $CaCl_2$ 10 HEPES and 250 μM $CdCl_2$ (to block voltage dependent Ca^{2+} currents, Twitchell & Rane, 1993). The pipette solution contained 150 KCl, 2 $MgCl_2$, 1 ATP, 0.5 GTP, 10 HEPES, 0.7 μM $CaCl_2$ and 1 EGTA (free Ca^{2+} 0.3 μM). The pipette solution used for single-channel recording was identical to this, but both ATP and GTP were omitted. Whole-cell patch pipettes had resistances of 3–5 MΩ, and the pipette solution was allowed to equilibrate with the cell interior for at least 3–5 min before the beginning of data acquisition. Single-channel recording patch pipettes had resistances of 4–7 MΩ, and the pipette solution was allowed to equilibrate with the patch interior for at least 2–4 min before the beginning of data acquisition. Whole-cell and single-channel currents were recorded using an EPC-7 patch-clamp amplifier (List Electronics, USA), filtered at 3 kHz with an 8-pole Bessel filter (Frequency Devices) and digitized at the appropriate frequency. Data acquisition was done with Pulse software (Instrutech, NY and HEKA elektronik, Germany) running on a Macintosh Centris 650 computer. Standard P/N leak subtraction was used on all whole-cell recordings. Analogue compensation was used to attenuate capacitive transients and to determine whole-cell capacitance.

Compound Handling and Application

In all experiments fatty acids were made in a stock solution of either DMSO or EtOH and stored under argon at −30°C. On the day of use compounds were diluted at least 1000-fold into bath solution, sonicated and used immediately. The final concentration of vehicle was 0.1% or less and at these concentrations currents were unaffected by vehicle application. Compounds were applied to cells and excised patches via blunt tipped micropipettes (10–15 μm id). To hasten recovery from compound application, excised membrane patches were routinely manipulated into compound free bath solution. For experiments involving enzyme inhibitors (baicalein for lipoxygenase, indomethacin for cyclooxygenase, proafiden for cytochrome P450, and the nonspecific inhibitor ETYA), cells were treated with the inhibitors for 20 min prior to recording, and the inhibitor was included in the bath, application micropipette and patch pipette solutions.

Data Analysis and Presentation

Data analysis was done offline with Pulse, TAC and TACFit software (Instrutech NY and HEKA elektronik, Germany) running on a Macintosh Centris 650 computer. Single-channel data were collected in 5-sec bins and analyzed offline using MacTac and TacFit software by HEKA. Single-channel activity is expressed as the product of the number of channels (N) times the open probability (P_o), where

$$N \cdot P_o = \Sigma(\text{open time} \cdot \# \text{ channels open})/\text{total time of record.} \quad (1)$$

Increases in whole-cell currents are reported as percent increase over control current. All measurements are given as mean ± SEM. Increases in current during experimental perturbations of signal transduction mechanisms, or applications of agonists, in the presence of antagonists were statistically compared to control increases by a two tailed nonpaired Student's *t*-test at a 0.05 level of significance. Comparison of three or more treatments were statistically compared using a one-way ANOVA at a 0.05 level of significance.

To determine the EC_{50} of the arachidonic acid (AA) effect on whole-cell BK current the following equation was used:

$$I_{AA} = I_{AA(m)}/\{1 + K_{1/2}/[AA])^n\} \quad (2)$$

with I_{AA} denoting the current in the presence of AA, $I_{AA(m)}$ maximum potentiation of BK current by AA, [AA] the AA concentration, $K_{1/2}$ the AA concentration producing half maximal potentiation and n the Hill coefficient.

Results

AA Potentiates BK Current in BAMCCs

The effects of extracellularly applied AA on voltage activated whole-cell Ca^{2+}, Na^+ and K^+ currents in BAMCCs were tested using a whole-cell patch clamp recording conditions previously described (Twitchell & Rane, 1993). AA, in concentrations up to 10 μM, did not affect voltage-dependent Ca^{2+}, Na^+ or voltage-dependent, Ca^{2+}-insensitive K^+ currents in our BAMCCs (*data not shown*). However, 10 μM AA strongly potentiated an outward current which has been previously identified as being due to the large-conductance, Ca^{2+}-sensitive K^+ channel known as BK (Twitchell & Rane, 1993; Marty & Neher, 1985). The AA induced increase in whole-cell BK current amplitude over control was 416 ± 43% (mean ± SEM, $N = 22$ cells, Fig. 1). Onset of BK potentiation occurred within 10 sec of AA application and was maximal after 40 to 70 sec of continued application. The potentiated BK current recovered completely within 4 to 6 min after AA application was stopped. The response of AA was concentration dependent with a $K_{1/2}$ of 3 μM and a Hill coefficient of 1.4 (from Eq. 2). At 0.2 μM AA, the lowest concentration tested, whole-cell BK current was potentiated by 29 ± 3% ($N = 3$ cells). The highest concentration of AA tested, 200 μM, resulted in a mean potentiation of 394 ± 28% ($N = 3$ cells).

AA Potentiates BK Single-Channel Activity in Outside-out Membrane Patches

It is unlikely that AA potentiation of BK current was the result of AA upregulation of voltage activated Ca^{2+} cur-

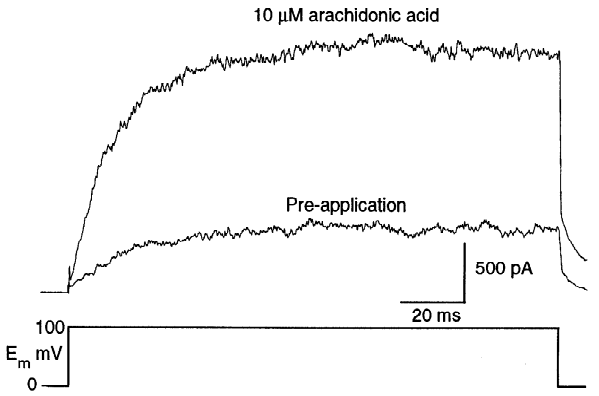

Fig. 1. Arachidonic acid potentiates whole-cell BK current in bovine adrenal chromaffin cells. Whole-cell currents BK currents were evoked by a voltage step to +100 mV from a holding potential of 0 mV, in the presence of 0.3 μM free Ca^{2+} in the patch pipette solution. Application of 10 μM AA to this cell increased peak whole-cell BK current by 310% over pre-application current. 10 μM arachidonic acid caused a mean ± SEM increase in peak current amplitude over pre-application current of 416 ± 43% in 22 cells.

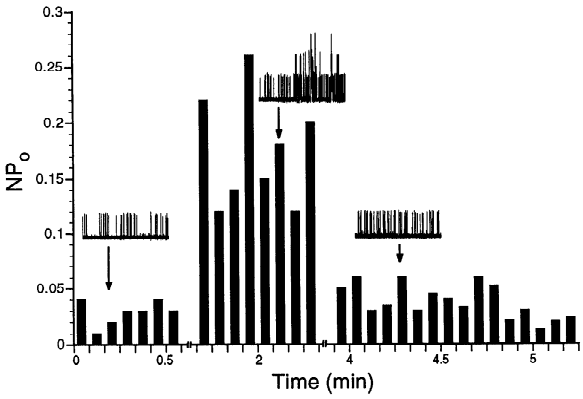

Fig. 2. Arachidonic acid potentiates BK channel activity in outside-out patches. The outside-out membrane patch was held at +20 mV and records were collected in 5-sec bins. Breaks in *x*-axis show suspension of recording while arachidonic acid application pipette was maneuvered close to or away from the patch pipette. Bins represented by closed columns indicate $N \cdot P_o$ in the absence of arachidonic acid. Application of 20 μM arachidonic acid to the extracellular face of the patch, indicated by bins shown as open columns, reversibly increased $N \cdot P_o$. Inset single-channel records show raw data from the indicated bins. Single-channel amplitudes were approximately 11 pA. The composition of the pipette and bath solutions are described in Materials and Methods. This experiment was repeated in 3 patches.

rent, since the presence of 250 μM $CdCl_2$ in the bath solution blocks all current flow through voltage-activated Ca^{2+} channels in BAMCCs (Twitchell & Rane, 1993). Furthermore, no change in BAMCC whole-cell Ca^{2+} currents were observed in response to application of AA. However, AA may increase intracellular Ca^{2+} levels and thus BK activity by releasing Ca^{2+} from intracellular stores. This possibility is difficult to assess in whole-cell experiments, since bulk Ca^{2+} buffering by the whole-cell patch pipette solution may not ensure a constant or predictable Ca^{2+} concentrations at the cell membrane. Therefore, outside-out patch experiments were conducted to minimize the contributions of Ca^{2+}-sequestering organellar systems to AA action on BK. Application of 20 μM AA increased BK single-channel activity in outside-out membrane patches, increasing $N \cdot P_o$ (Eq. 1) from 0.029 ± 0.004 to 0.174 ± 0.018 (N = 3 patches, Fig. 2). The increase in channel activity occurred within 5 to 12 sec of AA application to the membrane patch. Channel activity recovered to normal within 1 min after the removal of AA. To further obviate the possibility that potentiation of BK activity in outside-out patches was due to AA-induced release of Ca^{2+} stores which overwhelmed the 1 mM EGTA buffering system, these experiments were repeated using 5 mM BAPTA to buffer free Ca^{2+} to 0.3 μM. Under these conditions AA was still able to increase BK $N \cdot P_o$ from 0.025 ± 0.004 to 0.21 ± 0.016 (N = 3 patches). The ability of AA to upmodulate BK channel activity in outside-out membrane patches argues against the possibility that AA potentiates BK via mobilization of Ca^{2+} stores. These experiments also suggest that AA potentiation of BK channel activity does not require soluble cytoplasmic signaling components.

AA Modulations of BK Current does not Require ATP or GTP

Receptor-driven modulation of Ca^{2+}-dependent K^+ channels of the BK family has been shown to be mediated by phosphorylation and dephosphorylation (Ewald, Williams & Levitan, 1985; White, Schonbrunn & Armstrong, 1991). The possibility that AA metabolites may affect BK by activating PKC or some other kinase was tested by substituting the nonhydrolyzable ATP analogue adenylylimidodiphosphate (AMP-PNP, 1 mM) for ATP in the patch pipette solution (Seifert et al., 1988; Koda et al., 1989, Keyser et al., 1990). The potentiation of whole-cell BK current by AA in the presence of AMP-PNP (469 ± 32%, N = 8 cells) was not significantly different from the BK response to AA in the presence of ATP (490 ± 80%, N = 6 cells, $t(10) = 0.27$, $P = 0.79$). Conversely, if AA potentiation of BK current was dependent on a dephosphorylation event, then rephosphorylation would be a requisite for recovery from potentiation. In this case recovery from potentiation should have been slowed or inhibited in the presence of the AMP-PNP. Yet in all eight cells tested, BK current recovered within 4 to 6 min after AA application ended, just as for recovery in the presence of ATP. Therefore, it seems that AA acts on BK independently of phosphorylation or dephosphorylation events.

To determine if AA modulated BK current via a G-protein dependent mechanism, the nonhydrolyzable GDP analogue, GDP-β-S, which disrupts GTP depen-

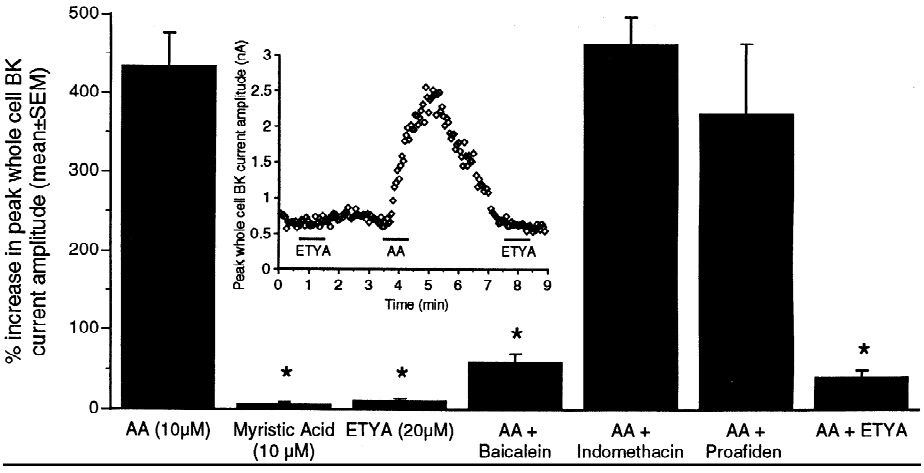

Fig. 3. Experiments with nonmetabolizable fatty acids, and inhibitors of fatty acid metabolic enzymes, suggest that lipoxygenase metabolites underlie arachidonic acid modulation of whole-cell BK current. Whole-cell BK currents were recorded as described in Materials and Methods, while fatty acids, arachidonic acid (AA), myristic acid and eicostateraynoic acid (ETYA), were extracellularly applied via micropipette. Cells were treated with oxygenase inhibitors as described in Materials and Methods. Each column represents the results from at least 4 experiments. Responses that were significantly different ($P < 0.05$) from arachidonic acid alone are marked with asterisks. *Inset:* AA potentiates whole-cell BK current while the nonmetabolizable fatty acid ETYA does not. Peak current is plotted *vs.* time of recording. Fatty acids were applied via micropipette during intervals indicated by horizontal lines. Whole-cell BK current increased by ~300% in response to application of 10 μM AA. This effect was reversible and could not be mimicked by application of the AA analogue eicostateraynoic acid (ETYA).

dent signaling mechanisms in BAMCCs, was used (Twitchell & Rane, 1994). Substitution of 0.5 mM GDP-β-S for GTP in the patch pipette solution did not significantly affect the ability of 2 μM AA to potentiate whole-cell BK current (75 ± 18% potentiation, $N = 3$ cells) when compared to AA potentiation of BK current in the presence of GTP (93 ± 4% potentiation, $N = 8$ cells, $t(9) = 1.45$, $P = 0.18$).

Non-metabolizable Fatty Acids Do Not Modulate BK Current

AA potentiation of a BK channel in rabbit aorta has been shown to result from the direct interaction of AA (and other fatty acids) with the channel, and not from the actions of AA metabolites (Kirber et al., 1992). To determine if AA directly modulates the BK channel in BAMCCs, the effect of eicostateraynoic acid (ETYA) and myristic acid, two fatty acids which mimic AA in directly modulating BK current in other systems, were tested on whole-cell BAMCC BK current. ETYA increases BK channel activity in rabbit aortic smooth muscle, presumably by mimicking the structure of AA (Kirber et al., 1992). Myristic acid also potentiates BK channel activity in smooth muscle, possibly by altering lipid-protein interactions (Petrou et al., 1994). Neither ETYA or myristic acid are substrates for cyclooxygenase, lipoxygenase or cytochrome P450, the major metabolic enzymes for AA released from the plasma membrane (Kirber et al., 1992; Ordway et al., 1991). In BAMCCs, extracellular application of ETYA (20 μM) or myristic acid (10 μM) produced only very small increases in whole-cell BK currents, 11 ± 4% for ETYA ($N = 6$ cells) and 6 ± 3% for myristic acid ($N = 6$ cells, Fig. 3). These results suggest that the mechanism of BK modulation by AA is not due to a nonspecific action common to other fatty acids, but instead is either very specific for AA, or may result from the action of AA metabolites.

To determine if the AA modulation of BK was indirect via its metabolic conversion to oxygenated compounds, ETYA, a substrate inhibitor of AA metabolizing enzymes, was used in an attempt to block AA modulation of BK current. With ETYA (20 μM) in the bath and in the patch pipette solution, 10 μM AA potentiated whole-cell BK current by 38 ± 9% ($N = 4$ cells), a significant reduction compared to the 416 ± 43% potentiation in the absence of ETYA ($N = 6$ cells, $t(8) = 10.7$, $P < 0.05$). This reduced effect of AA on BK current was not the result of occlusion of AA by ETYA induced potentiation, since application of ETYA alone increases BK current by only 11 ± 4% ($N = 6$ cells, Fig. 3 inset).

Hydroperoxy-Eicosatetraenoic Acids Potentiate BK Current

The experiment with ETYA suggested that AA was metabolically processed in order for it to potentiate BK current. To identify the metabolic pathway responsible

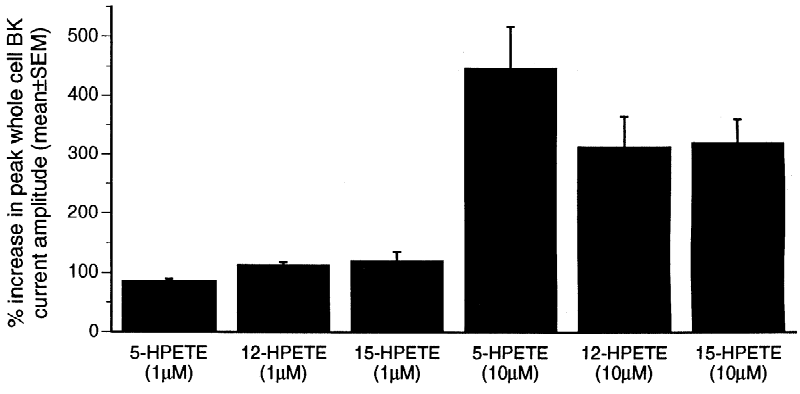

Fig. 4. Lipoxygenase metabolites of arachidonic acid potentiate whole-cell BK current in BAMCCs. Whole-cell BK currents were recorded as described in Materials and Methods, while 5-, 12-, and 15-HpETE were applied via micropipette at 1 μM or 10 μM. Each column represents the results of at least 3 experiments.

for potentiation of BK current by AA, inhibitors of the cytochrome P450, cyclooxygenase and lipoxygenase pathways were used in an attempt to disrupt AA action (Fig. 3). The lipoxygenase inhibitor baicalein (20 μM) significantly reduced the response of cells to 10 μM AA (59 ± 11% increase in presence of baicalein $N = 8$ cells, compared to a 490 ± 77% increase in the absence of baicalein $N = 6$ cells ($t(10) = 6.28$, $P < 0.05$). Inhibitors of the cyclooxygenase and cytochrome P450 pathways, proafiden (20 μM) and indomethacin (20 μM), had no significant effect on AA modulation of BK current (374 ± 89% increase in the presence of proafiden ($t(8) = 0.93$, $P = 0.38$), and 462 ± 35% in the presence of indomethacin ($t(8) = 0.27$, $P = 0.80$), $N = 4$ cells each). These results indicate that AA modulation of BK current is likely via a lipoxygenase metabolite (LOM).

To confirm that AA modulation of BK is primarily due to lipoxygenase processing, the products of the 5-, 12-, and 15-lipoxygenase enzymatic metabolism of AA (5-, 12-, and 15-hydroperoxy-eicostatetraenoic acid (HpETE) respectively) were applied to BAMCCs and their effects on the whole-cell BK current were observed. Application of 10 μM 5-, 12-, or 15-HpETE increased BK current by 448 ± 70% ($N = 4$ cells), 313 ± 52%, ($N = 4$ cells) and 320 ± 40% ($N = 6$ cells), respectively (Fig. 4). Increase in BK current occurred within 3 sec of HpETE application and were maximal within 45 sec. BK currents returned to pre-application levels within 2 min after the HpETE compounds were removed. The potentiation of BK current by HpETE compounds was similar to the effect of maximal concentrations of AA on BK currents (416 ± 43% increase in current over control, $N = 22$ cells). Analysis of variance indicated that all three compounds were equally effective in potentiating BK current ($F(2,10) = 1.88$, $P = 0.20$). BK current was also potentiated by 1 μM concentrations of 5-, 12-, or 15-HpETE (78 ± 5%, $N = 4$ cells, 112 ± 6%, $N = 4$ cells, and 119 ± 17%, $N = 5$ cells respectively, Fig. 4). Again, analysis of variance indicated that the responses to 5-, 12-, and 15-HpETE were not significantly different from one another ($F(2,10) = 2.17$, $P = 0.16$).

Since upmodulation of BK current by AA appeared to require lipoxygenase processing, and AA was effective in outside-out patches, it seemed likely that lipoxygenase activity was present in excised membrane patches to convert AA to LOMs which would then affect BK channel activity. To verify this hypothesis the following experiments were performed. First, all three HpETE compounds (at 1 μM) were shown to upmodulate BK channel activity in outside-out membrane patches in the absence of nucleotides (increases in $N \cdot P_o$ of 0.03 ± 0.01 to 0.81 ± 0.28 for 5-HpETE, $N = 4$ patches; 0.02 ± 0.01 to 0.89 ± 0.21 for 12-HpETE, $N = 3$ patches; and 0.05 ± 0.01 to 1.28 ± 0.20 for 15-HpETE, $N = 5$ patches). The channel potentiated by the LOMs and AA was blocked by the BK channel blocker 100 nM charybdotoxin confirming it as BK (Fig. 5*a*). Finally, to test that AA upmodulation of BK in excised patches required lipoxygenase activity, the lipoxygenase inhibitor baicalein was included in the pipette solution and in drug application solutions. With baicalein in the pipette solution AA upmodulation of BK activity was greatly attenuated, with $N \cdot P_o$ changing from 0.06 ± 0.01 to only 0.09 ± 0.03 in 4 patches (compared with an increase from 0.029 ± 0.004 to 0.174 ± 0.018 without baicalein). As a positive control following washout of AA from these patches, 15-HpETE was applied and its induced increase in $N \cdot P_o$ was shown to be unaffected by the presence of baicalein (0.86 ± 0.28) (Fig. 5*b*). The ability of baicalein to attenuate AA, but not LOM, potentiation of BK activity in excised patches, strongly suggests that lipoxygenase activity is present in these patches to convert AA to LOMs. However, because each of the major HpETE metabolites was equipotent, it is not possible at this time to identify the specific lipoxygenase pathway responsible for AA modulation of BK.

Discussion

Our experiments with AA analogues and inhibitors of the major AA metabolic enzymes suggest that LOMs of AA,

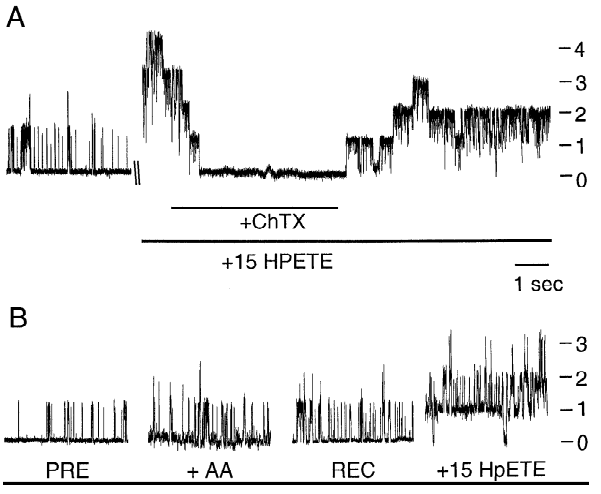

Fig. 5. Lipoxygenase metabolites of arachidonic acid potentiate BK channel activity in outside-out patches. The outside-out membrane patch was held at +20 mV and records were collected in 5-sec bins. Breaks in x-axis show suspension of recording while the application pipette was maneuvered close to or away from the patch pipette. Single-channel amplitudes were approximately 11 pA, and numbers with dashes at right of records show number of open levels. The composition of the pipette and bath solutions are described in Materials and Methods. (*a*) Single-channel records show BK channel activity in the presence of 15-HpETE (1 μM) is abolished by application of the BK channel blocker charybdotoxin (200 nM). This demonstrates not only that the channel potentiated by LOMs is BK, but that BK channels are the only large-conductance channel type present in excised membrane patches under our recording conditions. This experiment was repeated in 3 patches. (*b*) AA action on BK is blocked by the lipoxygenase inhibitor baicalein. Single-channel records show the control (PRE) channel activity, activity during application of 20 μM AA (+AA), and the response of the BK channels to the LOM 15-HpETE (+15 HpETE). Note that the activity in the presence of 15-HpETE was so frequent that there were only brief periods in which no channels in the patch were open. This experiment was repeated in 4 patches.

and not AA itself, potentiate BK current in BAMCCs. This modulation of the BK current does not appear to be the result of ATP- or GTP-dependent processes or from mobilization of intracellular Ca^{2+}. Furthermore, our observation that AA can modulate BK channel activity in outside-out membrane patches suggests that lipoxygenases are present and active in excised membrane patches and can convert AA to LOMs which in turn potentiate BK channel activity. In other systems Ca^{2+}-dependent translocation of lipoxygenases to the plasma membrane has been demonstrated (Rouzer & Kargman, 1988; Hagmann et al., 1993). For this study, the presence of relatively high Ca^{2+} concentrations in the patch pipette solutions may have facilitated Ca^{2+}-dependent translocation and activation of cytosolic lipoxygenases to the plasma membrane prior to patch excision.

Although modulation of BK activity in BAMCCs appears to be a rather direct consequence of LOM mobilization, a more complex pathway has also been described in another model endocrine system, GH_4C_1 cells. In GH_4C_1 cells somatostatin-induced, lipoxygenase dependent upmodulation of BK channel activity has been shown to be mediated by LOM activation of protein phosphatases which act on BK (Duerson et al., 1996); whereas, for BAMCCs nucleotide perturbations did not apparently affect BK modulation by AA or LOMs. Our findings for lipoxygenase activity in excised patches suggests a more direct modulatory pathway for the BAMCC channel, which also differs somewhat from the GH_4C_1 cell BK channel in having a slightly lower single channel conductance (95 *vs.* 120 pS). It seems likely that there are fundamental differences in channel subtypes and modulatory mechanisms in these two systems.

LOMs of AA may play a role in regulating excitation induced catecholamine secretion in BAMCCs by potentiation of BK current. This LOM-induced potentiation of BK current would tend to shorten action potential duration and inhibit Ca^{2+} influx which in turn could inhibit excitation coupled secretion. This effect of LOMs on BK current as well as reports that LOMs appear to be required for catecholamine secretion in BAMCCs (Rindlisbacher et al., 1990) suggests that LOMs may play an important role in regulating secretion in BAMCCs. LOM potentiation of BK current may serve as a negative feedback mechanism to limit Ca^{2+} influx once secretion has begun. BK type channels and lipoxygenases have a widespread distribution in the nervous system, as well as other cell types which express voltage-dependent Ca^{2+} channels. Therefore, LOM modulation of BK may be an important mechanism for controlling Ca^{2+} influx and a number of Ca^{2+}-dependent physiological processes including neurosecretion, synaptic responsiveness, and smooth muscle tone (Rudy, 1988; Piomelli & Greengard, 1990).

We wish to thank DeAnn Black for technical assistance. This work was supported by a grant-in-aid (S.G.R.) and predoctoral fellowship (W.A.T.) from the American Heart Association, Indiana Affiliate, and by a MARC Summer Research fellowship (T.L.P.).

References

Axelrod, J. 1990. Receptor-mediated activation of phospholipase A_2 and arachidonic acid release in signal transduction. *Biochem. Soc. Trans.* **18:**503–507

Belardetti, F., Campbell, W.B., Falck, J.B., Demontis, G., Rosolowsky, M. 1989. Products of heme-catalyzed transformation of the arachidonate derivative 12-HpETE open S-type K^+ channels in Aplysia. *Neuron* **3:**497–505

Duerson, K., White, R.E., Jiang, F., Schonbrunn, A., Armstrong, D.L. 1996. Somatostatin stimulates BK_{Ca} channels in rat pituitary tumor cells through lipoxygenase metabolites of arachidonic acid. *Neuropharmacol.* **35:**949–961

Ewald, D., Williams, A., Levitan, I.B. 1985. Modulation of single Ca^{++}-dependent K^+ channel activity by protein phosphorylation. *Nature* **315:**503–506

Fraser, D.D., Hoehn, K., Weiss, S., MacVicar, B.A. 1993. Arachidonic acid inhibits sodium currents and synaptic transmission in cultured striatal neurons. *Neuron* **11:**633–644

Frye, R.A., Holz, R.W. 1984. The relationship between arachidonic acid release and catecholamine secretion from cultured bovine adrenal chromaffin cells. *J. Neurochem.* **43:**146–150

Hagmann, W., Kagawa, D., Renaud, C., Honn, K.V. 1993. Activity and protein distribution of 12-lipoxygenase in HEL cells: induction of membrane-association by phorbol ester TPA, modulation of activity by glutathione and 13-HpODE, and Ca^{2+}-dependent translocation to membrane. *Prostaglandins* **46:**471–477

Hildebrandt, E., Albanesi, J.P. 1991. Identification of a membrane-bound, glyol-stimulated phospholipase A_2 located in the secretory granules of the adrenal medulla. *Biochem.* **30:**464–472

Keyser, D.O., Alger, B.E. 1990. Arachidonic acid modulates hippocampal calcium current via protein kinase C and oxygen radicals. *Neuron* **5:**545–553

Khurana, G., Bennett, M.R. 1993. Nitric oxide and arachidonic acid modulation of calcium currents in postganglionic neurones of avian cultured ciliary ganglia. *Br. J. Pharmacol.* **109:**480–485

Kim, D., Sladek, C.D., Aguado-Velasco, C., Mathiasen, J.R. 1995. Arachidonic acid activation of a new family of K^+ channels in cultured neuronal cells. *J. Physiol.* **484.3:**643–660

Kim, D., Clapham, D.E. 1989. Potassium channels in cardiac cells activated by arachidonic acid and phospholipids. *Science* **244:**1174–1176

Kirber, M.T., Ordway, R.W., Clapp, L.H., Walsh, J.V., Singer, J.J. 1992. Both membrane stretch and fatty acids directly activate large conductance Ca^{2+}-activated K^+ channels in vascular smooth muscle cells. *FEBS Lett* **297:**24–28

Koda, Y., Wada, A., Yanagihara, N., Uezono, Y., Izumi, F. 1989. *Cis*-unsaturated fatty acids stimulate catecholamine secretion, tyrosine hydroxylase and protein kinase C in adrenal medullary cells. *Neurosci.* **29:**495–502

Marty, A., Neher, E. 1985. Potassium channels in cultured adrenal chromaffin cells. *J. Physiol.* **367:**117–141

Ordway, R.W., Singer, J.J., Walsh, J.V. 1991. Direct regulation of ion channels by fatty acids. *TiNS* **14:**96–100

Ordway, R.W., Walsh, J.V., Singer, J.J. 1989. Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. *Science* **244:**1176–1179

Piomelli, D., Greengard, P. 1990. Lipoxygenase metabolites of arachidonic acid in neuronal transmembrane signaling. *TiPS* **11:**367–373

Petrou, S., Ordway, R.W., Hamilton, J.A., Walsh Jr., J.V., Singer, J.J. 1994. Structural requirements for charged lipid molecules to directly increase or suppress K^+ channel activity in smooth muscle cells. *J. Gen. Physiol.* **103:**471–486

Rindlisbacher, B., Reist, M., Zahler, P. 1987. Diacylglycerol breakdown in plasma membranes of bovine chromaffin cells is a two-step mechanism mediated by a diacylglycerol lipase and a monoacylglycerol lipase. *Biochem. Biophys. Acta* **905:**340–357

Rindlisbacher, B., Sindler, M.A., Galatioto, L.E., Zahler, P. 1990. Arachidonic acid liberated by diacylglycerol lipase is essential for the release mechanism in chromaffin cells from bovine adrenal medulla. *J. Neurochem.* **54:**1247–1252

Rouzer, C.A., Kargman, S. 1988. Translocation of 5-lipoxygenase to the membrane in human leukocytes challenged with ionophore A23187. *J. Bio. Chem.* **263:**10980–10988

Rudy, B. 1988. Diversity and ubiquity of K channels. *Neuroscience* **25:**729–749

Schmitt, H., Meves, H. 1995. Modulation of neuronal calcium channels by arachidonic acid and related substances. *J. Membrane Biol.* **145:**233–244

Seifert, R., Schachtele, C., Rosenthal, W., Schultz, G. 1988. Activation of protein kinase C by *cis*- and *trans*-fatty acids and its potentiation by diacylglycerol. *Biochem. Biophys. Res. Comm.* **154:**20–25

Twitchell, W.A., Rane, S.G. 1993. Opioid peptide modulation of Ca^{2+}-dependent K^+ and voltage-activated Ca^{2+} currents in bovine adrenal chromaffin cells. *Neuron* **10:**701–709

Twitchell, W.A., Rane, S.G. 1994. Nucleotide independent modulation of Ca^{2+}-dependent K^+ channel current by a μ-type opioid receptor. *Mol. Pharmacol.* **46:**793–798

White, R.E., Schonbrunn, A., Armstrong, D.L. 1991. Somatostatin stimulates Ca^{2+}-activated K^+ channels through protein dephosphorylation. *Nature* **351:**570–573