Arachidonic acid inhibits K channels in basolateral membrane of the thick ascending limb

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Gu, Rui-Min, and Wen-Hui Wang. Arachidonic acid inhibits K channels in basolateral membrane of the thick ascending limb. Am J Physiol Renal Physiol 283: F407–F414, 2002. First published March 12, 2002; 10.1152/ajprenal.00002.2002.—We have used the patch-clamp technique to study the effect of arachidonic acid (AA) on the basolateral K channels in the medullary thick ascending limb (mTAL) of rat kidney. An inwardly rectifying 50-pS K channel was identified in cell-attached and inside-out patches in the basolateral membrane of the mTAL. The channel open probability (P0) was 0.51 at the spontaneous cell membrane potential and decreased to 0.25 by 30 mV hyperpolarization. The addition of 5 μM AA decreased channel activity, identified as NP0, from 0.58 to 0.08 in cell-attached patches. The effect of AA on the 50-pS K channel was specific because 10 μM cis-11,14,17-eicosatrienoic acid had no significant effect on channel activity. To determine whether the effect of AA was mediated by AA per se or by its metabolites, we examined the effect of AA on channel activity in the presence of indomethacin, an inhibitor of cyclooxygenase, or N-methylsulfonyl-12,12-dibromodec-11-enamide (DDMS), an inhibitor of cytochrome P-450 monooxygenase. Inhibition of cyclooxygenase increased channel activity from 0.54 to 0.9. However, indomethacin did not abolish the inhibitory effect of AA on the 50-pS K channel. In contrast, inhibition of cytochrome P-450 metabolism not only increased channel activity from 0.49 to 0.83 but also completely abolished the effect of AA. Moreover, addition of DDMS can reverse the inhibitory effect of AA on channel activity. The notion that the effect of AA was mediated by AA per se or by its metabolites is supported by the observation that addition of 100 nM of 20-hydroxyeicosatetraenoic acid, a main metabolite of AA, decreased channel activity from 0.49 to 0.36 but also completely abolished the effect of AA. More-
channel activity by 10.2 ± 0.3 on October 13, 2017 http://ajprenal.physiology.org/ Downloaded from

Fig. 1. The thick ascending limb (TAL) cell showing the major ion channels and transporters that are involved in mediating NaCl transport in the medullary TAL (mTAL) is shown. PD, membrane potential difference; $E_{Cl}$, electrochemical gradient for Cl.

### METHODS

**Preparation of the TAL.** Pathogen-free Sprague-Dawley rats of either sex (90–100 g) were purchased from Taconic Farms (Germantown, NY). The animals were kept on a regular diet and had free access to water. At the time of the experiment, they were fasted for 24 h before use. Rats were killed by cervical dislocation, and the kidneys were removed immediately. Thin coronal sections were cut with a razor blade, and several small bundles of tubules separated from slices of the kidneys were incubated in a solution containing collagenase type 1A (1 mg/ml; Sigma, St. Louis, MO) at 37°C. The isolated tubules were transferred onto a 5-mm cover glass coated with Cell-Tak (Collaborative Research, Bedford, MA) to immobilize the tubule. The cover glass was placed in a chamber mounted on an inverted microscope (Nikon) and superfused with HEPES-buffered NaCl solution composed of (in mM) 140 NaCl, 5 KCl, 1.8 CaCl$_2$, 1.8 MgCl$_2$, and 10 HEPES (pH 7.4). The bath solution was composed of (in mM) 140 NaCl, 5 KCl, 1.8 CaCl$_2$, 1.8 MgCl$_2$, and 10 HEPES (pH 7.4). AA and cis-11,14,17-eicosatrienoic acid were purchased from Nu-Chem Prep (model 56028, Elysian) and dissolved in 100% ethanol. Indomethacin was obtained from Sigma, and N-methylsulfonyl-12,12-dibromodec-11-enamide (DDMS) was synthesized at Dr. J. R. Falck’s laboratory, University of Texas Southwestern Medical Center at Dallas.

**Statistics.** Data are shown as means ± SE. We used paired Student’s t-tests to determine the significance of the difference between the control and experimental periods. Statistical significance was taken as $P < 0.05$.

### RESULTS

We used the method described by Guinamard et al. (14) to patch the basolateral membrane of the mTAL. We observed channel activity in at least 245 patches out of a total of 817 high-resistance seals. Figure 2A is a typical channel trace recorded from a cell-attached patch with 140 mM KCl in the pipette and Ringer solution (5 mM KCl) in the bath. From an inspection of the channel recording, it is apparent that channel activity decreased when cell membrane potential hyperpolarized. Figure 2B shows the relationship between $P_o$ and changes in the cell membrane potential; $P_o$ was 0.51 ± 0.05 at the spontaneous membrane potential and decreased by 50% to 0.25 ± 0.03 by 30-mV hyperpolarization. Therefore, depolarization increases channel activity, whereas hyperpolarization decreases channel activity. Channel activity was completely blocked by 1 mM Ba$^{2+}$ (Fig. 2C), indicating that it is a K channel. The conductance of the K channel was inwardly rectifying, and the current-voltage curve yielded an inward slope conductance of 50 ± 2 pS between −20 and 20 mV (Fig. 2D). Figure 2E illustrates channel open and closed time histograms. The K channel has one open state with a mean open time of 5.8 ± 0.5 ms and two closed states ($\tau_1 = 1.9 ± 0.2$ ms, $\tau_2 = 14.9 ± 1$ ms).

After confirming the presence of a 50-pS K channel in the basolateral membrane of the mTAL, we investigated the effect of AA on the activity of the 50-pS K channel. The reason for studying the effect of AA is because AA has been demonstrated to be an important player in the regulation of the apical K channels and Na-K-2Cl cotransporters in the mTAL (8, 27). Therefore, it is highly possible that AA may also be involved in the regulation of basolateral K channel activity. Figure 3A is a representative recording showing that AA inhibits the basolateral 50-pS K channel in a cell-attached patch. Addition of 5 μM AA decreased channel activity by 86 ± 8%, and $P_o$ fell from 0.58 ± 0.05 to 0.08 ± 0.01 (n = 8). The AA-induced channel inhibition was expected to depolarize the cell membrane potential, which was evidenced by a decrease in the channel current amplitude after AA application. How-
ever, addition of AA did not transiently increase $NP_o$, suggesting that the depolarization-induced increase in channel activity can be observed only when the K channel is in the open state. The effect of AA can be observed in inside-out patches, and AA decreased channel activity from 0.5 ± 0.05 to 0.03 ± 0.02 ($n = 4$; Fig. 3B). Also, the effect of AA is reversible. Figure 3C is a dose-response curve between AA concentrations and channel activity in cell-attached patches, and it shows that $K_i$, a concentration required to decrease channel activity by 50%, is ~3 μM.

To test the specificity of the AA effect, we also examined the effect of *cis*-11,14,17-eicosatrienoic acid, a 20-carbon fatty acid with three double bonds, on the 50-pS K channel in a cell-attached patch. Figure 4 is a channel recording demonstrating that addition of 10 μM *cis*-11,14,17-eicosatrienoic acid did not inhibit the activity of the 50-pS K channel and $NP_o$ was 0.64 ± 0.05 ($n = 7$), which is not different from the control value (0.68 ± 0.05). This suggests that the effect of AA is not mediated by changing lipid fluidity or other nonspecific fatty acid-induced modulation of membrane proteins.

AA can be metabolized by three pathways: cyclooxygenase (COX), lipoxygenase, and cytochrome P-450 monoxygenase (2, 19). It has been shown that both COX and cytochrome P-450 monoxygenase are expressed in the mTAL (6, 9, 20, 24). Therefore, we explored the possibility that the effect of AA was mediated by either COX-dependent or cytochrome P-450-dependent metabolites of AA. Figure 5 is a representative channel recording demonstrating the effect of AA in the presence of indomethacin, an inhibitor of COX. Addition of indomethacin (5 μM) significantly increased channel activity from 0.54 ± 0.05 to 0.90 ± 0.1 ($n = 9$). This indicates that the channel activity was suppressed by endogenous COX-dependent metabolites of AA. However, it is apparent that addition of 5 μM AA can still inhibit the K channel, and $NP_o$ drops from 0.9 ± 0.1 to 0.2 ± 0.03 ($n = 9$). This suggests that it is unlikely that the AA-induced acute inhibition of the 50-pS K channel was mediated by a COX-dependent metabolite of AA.

We next examined the effect of AA on K channel activity in the presence of DDMS (25), an inhibitor of cytochrome P-450-dependent ω-oxidation of AA. Figure 6 is a channel recording illustrating the effect of AA on channel activity in a cell-attached patch in the presence of 5 μM DDMS. Inhibition of the cytochrome P-450-dependent ω-oxidation of AA not only significantly increased channel activity from 0.49 ± 0.04 to 0.83 ± 0.1 but also completely abolished the inhibitory effect of AA, because 5 μM AA did not decrease $NP_o$ (0.89 ± 0.1, $n = 7$). The notion that the effect of AA is mediated by cytochrome P-450-dependent metabolites of AA is further supported by observations that inhibiting the cytochrome P-450 ω-oxidation of AA can completely reverse the AA-induced channel blockade (Fig.
Application of AA reduced $NP_o$ from $0.50 \pm 0.06$ to $0.11 \pm 0.02$, and addition of 5 μM DDMS increased $NP_o$ to $0.8 \pm 0.1$ ($n = 7$). In contrast, inhibition of COX did not restore the AA-induced decrease in channel activity (Fig. 8). From the inspection of Fig. 8, it is clear that addition of 5 μM AA decreased channel activity from $0.51 \pm 0.05$ to $0.12 \pm 0.02$ ($n = 7$) and that application of indomethacin did not abolish the AA-induced decrease in channel activity.

After establishing that the AA-induced acute decrease in channel activity was mediated by cytochrome P-450-dependent metabolites of AA, we examined the effect on the 50-pS K channel of 20-hydroxyeicosatetraenoic acid (HETE), a main product of cytochrome P-450 metabolism of AA in the mTAL (6). Figure 9 is a typical channel recording showing that application of 100 nM 20-HETE reversibly inhibited the activity of the 50-pS K channel in an inside-out patch and that $NP_o$ fell from $0.52 \pm 0.05$ to $0.10 \pm 0.02$ ($n = 5$). The effect of 20-HETE on channel activity was specific, because addition of 100 nM 19-HETE, another metabolite of the cytochrome P-450-dependent pathway (3, 4), had no significant effect on channel activity in inside-out patches (data not shown). Moreover, the effect of 20-HETE could also be observed in cell-attached patches in the presence of DDMS. Figure 10 is a representative recording showing that 20-HETE decreased $NP_o$ from $0.7 \pm 0.1$ to $0.05 \pm 0.02$ in the presence of DDMS ($n = 5$). This indicates that 20-HETE is the most likely candidate to mediate the effect of AA on the basolateral 50-pS K channel.

**DISCUSSION**

The mTAL is responsible for the reabsorption of 25% of filtered Na load and plays a key role in mediating...
urinary concentrating ability (10, 11, 16). Na and Cl enter the cell across the apical membrane through the Na-K-2Cl cotransporters, and Na is then actively transported across the basolateral membrane by Na-K-ATPase, whereas Cl leaves the cell via KCl cotransporters or Cl channels (10, 11). The basolateral K channels serve two cell functions: 1) they are responsible for K recycling across the basolateral membrane; 2) they are responsible for K recycling across the basolateral membrane.

Fig. 5. Effect of AA on the basolateral K channel activity in the presence of indomethacin. Arrows, application of indomethacin and AA. The top trace shows the experimental course. Three parts of the trace, indicated by numbers, are extended to show the fast time resolution. Holding potential was 0 mV.

Fig. 6. Effect of AA on the basolateral K channel activity in a cell-attached patch in the presence of N-methylsulfonyl-12,12-dibromodec-11-enamide (DDMS). The top trace shows the experimental course. Three parts of the trace, indicated by numbers, are extended to show the fast time resolution. Holding potential was 0 mV.

Fig. 7. Depicted is a recording showing that addition of DDMS reversed the AA-induced decrease in channel activity. The experiment was carried out in a cell-attached patch, and the holding potential was 0 mV. The top trace shows the time course of the experiment. Three parts of the trace, indicated by numbers, are extended to demonstrate the fast time resolution.

Fig. 8. Depicted is a recording showing that addition of indomethacin did not reverse the AA-induced decrease in channel activity. The experiment was carried out in a cell-attached patch, and the holding potential was 0 mV. The top trace shows the time course of the experiment. Three parts of the trace, indicated by numbers, are extended to demonstrate the fast time resolution.
and 2) they are involved in generating the cell membrane potential. Although changes in the cell membrane potential are not expected to directly affect the turnover rate of the Na-K-2Cl cotransporters, it is possible that an alteration in the cell membrane potential can indirectly influence the function of the cotransporter. For instance, changes in the cell membrane potential can alter the electrochemical gradient of K across the apical membrane, which can affect K recycling. Because K recycling is essential for maintaining the activity of the Na-K-2Cl cotransporters, changes in cell membrane potential can affect the function of the Na-K-2Cl cotransporters. Moreover, Cl exit across the basolateral membrane via Cl channels is expected to depolarize the basolateral membrane and, accordingly, to diminish the driving force for Cl exit. A decrease in Cl driving force leads to an increase in intracellular Cl concentrations, which have been shown to inhibit the activity of the Na-K-2Cl cotransporters (22). Therefore, activation of the basolateral K channels can hyperpolarize the basolateral membrane and maintain the driving force for Cl diffusion.

Although the importance of the basolateral K channel in the regulation of the transport function in the mTAL is well established, the information regarding the structure and biophysical properties of basolateral K channels is very limited. This is largely because the basolateral membrane is not accessible for patch-clamp studies without removal of the basement membrane. Hurst et al. (17) carried out a patch-clamp study in the collagenase-digested TALs from rabbit kidneys and identified a 41- to 43-pS K channel in cell-attached patches. Recently, Paulais et al. (21) successfully characterized the biophysical properties of the basolateral K channels by using collagenase-treated cTALs from mouse kidneys (21). They have identified an inwardly rectifying K channel with an inward conductance of 50 pS and an outward conductance of 11 pS. This K channel was inhibited by Mg$^{2+}$ and spermine and was sensitive to cell pH (21).

In the present investigation, we confirmed that there is an inwardly rectifying 50-pS K channel in the basolateral membrane of the mTAL from rat kidneys and that the activity of the 50-pS K channel increased by depolarization (22). The depolarization-induced increase in channel activity has physiological significance in maintaining a constant driving force for Cl diffusion, because the K channel activity was expected to increase in response to Cl diffusion across the basolateral membrane. Although it is possible that K channels other than the 50-pS K channel are also present in the basolateral membrane of the mTAL, the 50-pS K channel may be one of the major K channels responsible for the basolateral K conductance in the mTAL. This speculation is supported by the observation that we detected the 50-pS K channel in ~30% of cell-attached patches, and the channel $P_o$ was relatively high (0.5) at the spontaneous cell membrane potential. Thus factors that regulate the 50-pS K channel should have an effect on the basolateral K conductance and cell membrane potential.

In the present study, we have demonstrated that eicosanoids play an important role in regulating the basolateral K channels as follows: 1) inhibiting COX increased the activity of the 50-pS K channel; and 2)
blocking the cytochrome P-450 ω-oxidation of AA could also augment the channel activity. This suggests that COX-dependent metabolites and cytochrome P-450-dependent metabolites of AA are involved in the regulation of the basolateral K channels. The COX-dependent AA metabolites such as PGE₂ have been shown to attenuate the stimulatory effect of vasopressin on Cl reabsorption (7). We have previously demonstrated that PGE₂ at low concentrations (< 1 μM) abolished the vasopressin-induced increase in the activity of the apical 70-pS K channel, whereas PGE₂ at high concentrations decreased the activity of the 70-pS K channel by a PKC-dependent mechanism (18). In addition to PGE₂, the cytochrome P-450-dependent metabolites of AA, such as 20-HETE, have been indicated as potent inhibitors of the Na-K-2Cl cotransporters (9) and the apical 70-pS K channel in the mTAL (27).

Although both COX-dependent and cytochrome P-450-dependent AA metabolites are involved in the regulation of basolateral K channel activity, it is unlikely that the inhibitory effect of AA on channel activity is induced by a COX-dependent metabolite, because indomethacin did not abolish the AA-induced inhibition of channel activity. Our results strongly suggest that the inhibitory effect of AA is mediated by a cytochrome P-450-dependent metabolite of AA. First, inhibition of the cytochrome P-450-dependent ω-oxidation abolished the effect of AA on channel activity. Second, the AA-induced decrease in channel activity was completely reversed by DDMS. In contrast, indomethacin was not able to reverse the AA-induced inhibition. Third, addition of 20-HETE could inhibit the 50-pS K channel even in the presence of DDMS, suggesting that 20-HETE is a mediator for the effect of AA. Therefore, it is most likely that the effect of AA on channel activity under the present experimental conditions is mediated by a cytochrome P-450-dependent metabolite of AA, such as 20-HETE, rather than a COX-dependent metabolite of AA.

Under our experimental conditions, there are two possibilities that can be employed to explain why blocking cytochrome P-450 monoxygenase rather than inhibiting COX can abolish the inhibitory effect of AA on channel activity. One possibility is that AA is preferentially metabolized by the cytochrome P-450-dependent pathway compared with the COX-dependent metabolism, although both COX and cytochrome P-450 monoxygenase are present in the mTAL. Therefore, inhibition of the cytochrome P-450-dependent metabolism of AA abolishes the inhibitory effect of AA. Alternatively, AA cannot be effectively converted to a COX-dependent metabolite that can inhibit channel activity. Thus the inhibitory effect of AA cannot be blocked by indomethacin. The second possibility is that AA is first metabolized by the cytochrome P-450-dependent pathway and that the cytochrome P-450-dependent metabolites of AA are further converted to prostaglandins by COX (5). We need additional experiments to determine the metabolites of AA in the mTALs after addition of exogenous AA to examine why only inhibition of cytochrome P-450 metabolism of AA can block the AA-induced inhibition.

The physiological event in which 20-HETE serves as a mediator to regulate the basolateral K channels is not known. Stimulation of the Ca²⁺-sensing receptor has been shown to increase 20-HETE production (28). Because the Ca²⁺-sensing receptor is located in the basolateral membrane of the mTAL, it is conceivable that the stimulation of the Ca²⁺-sensing receptor may also inhibit the basolateral 50-pS K channel. Moreover, we have previously demonstrated that 20-HETE production increased in the mTAL obtained from rats on a K-deficient diet (12). Therefore, it is possible that basolateral K channel activity differs between mTALs from animals on a normal-K diet and those on a low-K diet. Further experiments are required to test this possibility.

We conclude that an inwardly rectifying 50-pS K channel is expressed in the basolateral membrane of the mTAL and is inhibited by AA and that the effect of AA is mediated by cytochrome P-450-dependent metabolites of AA.

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REFERENCES


