Mefenamic acid bi-directionally modulates the transient outward $K^+$ current in rat cerebellar granule cells

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Abstract

The effect of non-steroidal anti-inflammatory drugs (NSAIDs) on ion channels has been widely studied in several cell models, but less is known about their modulatory mechanisms. In this report, the effect of mefenamic acid on voltage-activated transient outward $K^+$ current ($I_{A}$) in cultured rat cerebellar granule cells was investigated. At a concentration of 5 $\mu$M to 100 $\mu$M, mefenamic acid reversibly inhibited $I_{A}$ in a dose-dependent manner. However, mefenamic acid at a concentration of 1 $\mu$M significantly increased the amplitude of $I_{A}$ to 113±1.5% of the control. At more than 10 $\mu$M, mefenamic acid inhibited the amplitude of $I_{A}$ without any effect on activation or inactivation. In addition, a higher concentration of mefenamic acid induced a significant acceleration of recovery from inactivation with an increase of the peak amplitude elicited by the second test pulse. Intracellular application of mefenamic acid could significantly increase the amplitude of $I_{A}$, but had no effect on the inhibition induced by extracellular mefenamic acid, implying that mefenamic acid may exert its effect from both inside and outside the ion channel. Furthermore, the activation of current induced by intracellular application of mefenamic acid was mimicked by other cyclooxygenase inhibitors and arachidonic acid. Our data demonstrate that mefenamic acid is able to bi-directionally modulate $I_{A}$ channels in neurons at different concentrations and by different methods of application, and two different mechanisms may be involved.

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Keywords: NSAIDs; Mefenamic acid; $I_{A}$; Cyclooxygenase; Arachidonic acid; Granule cell

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) play essential roles as anti-inflammatory, analgesic, and antipyretic drugs. Fenamates (e.g. mefenamic acid, flufenamic acid, niflumic acid) are the NSAIDs most often used clinically to reduce the inflammation and pain associated with arthritis (Dawood, 1993; Alves and Duarte, 2002). In addition, the potential effects of NSAIDs on the central nervous system, such as neuro-protection in Alzheimer’s disease, have instigated several basic and clinical studies (Rich et al., 1995). It is well known that the major mechanism mediating the anti-inflammatory effects of fenamate is inhibition of cyclooxygenase (COX), resulting in decreased arachidonic acid (AA) and prostaglandin formation (Vane, 1996).

The fenamate class of NSAIDs affects various types of membrane channel and their effects on different ion channels have been studied widely. They inhibit non-selective cation channels (Chen and Reith, 1993; Yamada et al., 1996) and Cl$^-$ conductance (Busch et al., 1994). Fenamates also inhibit human neural voltage-gated K$^+$ channels (Lee and Wang, 1999), and effects on ligand-gated ion channels and TRPM2 channels (melastatin-related TRP channels) have been reported (Woodward et al., 1994; Hill et al., 2004). Mefenamic acid has been shown to activate Ca$^{2+}$-independent delayed rectifier K$^+$ currents and large-conductance Ca$^{2+}$-activated K$^+$ channels in the smooth muscle of canine jejunum and pig coronary artery (Ottolia and Toro, 1994). In the trabecular meshwork of the eye, mefenamic acid increases the current through maxi-K channels (Stumpff et al., 2001). In addition, activation and inhibition of kidney CLC chloride channels (CLC-K chloride) by distinct binding sites of niflumic acid and fenamate acid have been reported by Liantonio et al. (2006). Anna et al. reported fenamate-induced enhancement of...
heterologously expressed HERG (human ether-a-go-go-related) currents in *Xenopus* oocytes (Malykhina et al., 2002). However, fenamate NSAIDs were considered to directly block ion channels, and there are few reports concerning the main mechanism that mediates fenamate-induced inhibition of COX.

Primary cerebellar granule cells, an established *in vitro* model for investigating neuronal apoptosis, have been shown to possess two main voltage-activated outward K⁺ currents: rectifier outward K⁺ current (I\(_{\text{K}}\)) and transient outward K⁺ (I\(_{\text{A}}\)) (Mei et al., 2004). They are distinguishable by their activation and inactivation voltage ranges and kinetics, and by their pharmacological sensitivities. Generally, the functional roles of I\(_{\text{A}}\) include influencing cell excitability, action potential firing, controlling spike latency, and repetitive firing (Shibata et al., 2000). Recently, our own data indicated that apoptosis of cerebellar granule neurons induced by incubation with low K⁺ and K⁺-free serum is associated with an increase of I\(_{\text{A}}\) (Hu et al., 2005, 2006). Finding a new I\(_{\text{A}}\) channel modulator or modulation mechanism would be highly useful for further investigations into neuron excitability, neuronal apoptosis, and neuron-protection.

Our recent studies demonstrated that an NSAID with a diphenyl structure, diclofenac, could activate I\(_{\text{A}}\) in rat cerebellar granule cells as a novel voltage-dependent I\(_{\text{A}}\) channel opener (Liu et al., 2005). Diclofenac also inhibited the sodium current (I\(_{\text{Na}}\)) recorded from rat cerebellar granule cells and myoblasts by different inhibitory mechanisms (Lu et al., 2004; Fei et al., 2006). In the present study, we used a whole-cell patch-clamping technique to investigate the effects of mefenamic acid on I\(_{\text{A}}\) channels, and on their steady-state activation and inactivation. The results show that mefenamic acid bi-directionally modulated the I\(_{\text{A}}\) of rat granule neurons at different concentrations and that two mechanisms seem be involved. These results are useful for understanding the effect of NSAIDs on neuron excitability, and neuronal injury and protection.

**Material and methods**

**Cell culture.** Cells were derived from cerebellum of 7–8 day-old Sprague–Dawley rat pups as described previously. Isolated cells then were plated onto 35-mm-diameter petri dishes coated with poly-L-lysine (1 μg/ml) at a density of 10⁶ cells/ml. Cultured cells were incubated at 37 °C with 5% CO₂ in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum, glutamine (5 mM), insulin (5 μg/ml), KCl (25 mM), and 1% antibiotic-antimycotic solution. After culturing for 24 h, cytosine β-D-arabinofuranoside (5 μM) was added to the culture medium to inhibit the proliferation of non-neuronal cells. All experiments were carried using cerebellar granule neurons at 5–7 days in culture.

**Patch-clamp recordings.** Whole-cell currents of granule neurons were recorded using a patch-clamp technique. Prior to I\(_{\text{A}}\) current recording, the culture medium was replaced with a bath solution containing (in mM): NaCl 125, KCl 2.5, HEPES 10, MgCl₂ 1, tetrodotoxin 0.001, TEA 20 (pH adjusted to 7.4 using NaOH). Soft glass recording pipettes were filled with an internal...
I the fact that mefenamic acid was dissolved in 0.01% we simultaneously examined the effect of DMSO on control, suggesting that a lower concentration of mefenamic acid respectively (Fig. 1A). When the membrane potential was held at conditioning potential of then decayed as time progressed. After 1 s intervals at a current (Mei et al., 2004). It is evident in Fig. 1A that 10 μM current that had been previously described as a delayed rectifier voltage-dependent outward K+ currents (42x198)

**Results**

Because rat cerebellar granule neurons display two main voltage-dependent outward K+ currents (IA and IK), we first determined whether mefenamic acid is specific for IA or IK in rat granule neurons. In the experiments, outward K+ currents were evoked by two sequential 200 ms depolarizing pulses to 40 mV at 1 s intervals, from holding potentials of −100 and −40 mV, respectively (Fig. 1A). When the membrane potential was held at −100 mV, the depolarizing voltage pulses elicited a global outward current (IA plus IK) that activated rapidly (5–10 ms) and then decayed as time progressed. After 1 s intervals at a conditioning potential of −40 mV, the same depolarizing step evoked only a slight inactivating or non-inactivating outward K+ current that had been previously described as a delayed rectifier IK current (Mei et al., 2004). It is evident in Fig. 1A that 10 μM mefenamic acid significantly inhibited both the early inactivating IA component and the later current. Mefenamic acid decreased the amplitude of the peak elicited by the first pulse from 1843±235 pA to 1350±200 pA (n=8, P<0.05 by Student’s t-test) and decreased the amplitude of IK from 514±124 pA to 344±73 pA (n=4, P<0.05 by Student’s t-test). The effect of mefenamic acid on the amplitude of IA and IK is illustrated in Fig. 1B. In view of the fact that mefenamic acid was dissolved in 0.01%–1% DMSO, we simultaneously examined the effect of DMSO on IA and IK. As shown in Fig. 1C, neither IA nor IK was affected by 1% DMSO (n=5, P>0.05 by Student’s t-test). In some cases, following prolonged washout of mefenamic acid from the bath solution, the amplitude of IA then recovered to a higher level than that of the control, suggesting that a lower concentration of mefenamic acid could increase IA (Fig. 1D).

We subsequently determined whether mefenamic acid inhibited or activated IA in a concentration-dependent manner. To investigate the effect of mefenamic acid on IA, all of the results described hereafter were obtained using a bath solution containing 20 mM TEA to eliminate IK currents. IA currents were then evoked by 200 ms constant depolarizing pulses, ranging from −100 mV to 40 mV at 10 s intervals. We unexpectedly found that application of mefenamic acid to the bath solution produced a bi-directional modulation of the current amplitude depending on the concentration used (Fig. 2A). At concentrations from 5 μM to 100 μM, mefenamic acid significantly inhibited the amplitude of IA. The inhibitory effect of mefenamic acid on IA occurred very rapidly, reached its maximum effect within 10–20 s, and returned to control levels after 1–2 min of wash-out. Moreover, the inhibition of IA was concentration-dependent. The inhibition of IA produced by mefenamic acid at 5 μM, 10 μM, and 100 μM was 18±2% (n=5), 24±0.9% (n=5), and 64±0.9% (n=4), respectively (P<0.05 by one-way ANOVA). As a result of mefenamic acid...
toxicity, higher concentrations produced an abnormal state in the recorded cells, so we did not test the effect of mefenamic acid on $I_A$ at concentrations greater than 100 $\mu$M. It is of interest that, when the concentration of mefenamic acid was reduced to 1 $\mu$M, $I_A$ increased to 113±1.5% of the control ($n=6$, $P<0.05$). When 500 nM mefenamic acid was used, $I_A$ increased to 100.3±0.03% of the control and there was no significant difference compared with the control ($n=3$, $P>0.05$). Fig. 2A illustrates typical experiments in which mefenamic acid was applied at different concentrations. The sum of the bi-directional modulation of $I_A$ is shown in Fig. 2B.

The effect of mefenamic acid on the activation and inactivation of $I_A$ was studied using the appropriate voltage protocols. In the activation protocol, $I_A$ was evoked by a 200 ms depolarizing pulse from a first pulse potential of $-60$ mV to $+60$ mV in 10 mV steps at 10 s intervals (Figs. 3A and B). When normalized conductance was plotted as a function of the command potential, we obtained the $I_A$ activation curve. The data points were calculated using the equation $g_K = I_K / (V_{in} - V_{rev})$. As shown in Fig. 3C, the activation curve was not significantly shifted by the application of mefenamic acid (10 $\mu$M); the current was half-activated at $-13.4\pm2.0$ mV and $-10.0\pm1.8$ mV in the absence and presence of mefenamic acid, respectively ($n=13$, $P>0.05$), suggesting that mefenamic acid had no effect on the voltage-dependent steady-state activation of $I_A$.

We then studied the effect of mefenamic acid on voltage-dependent steady-state inactivation of $I_A$. Currents were elicited using 1 s conditioning pre-pulses from $-110$ mV to various membrane potentials before a 200 ms test pulse of $+40$ mV (Figs. 4A and B). After normalizing each current peak to the maximal current amplitude obtained from the $-110$ mV pre-pulses as a function of the conditioning pre-pulse potential, we found that mefenamic acid did not affect the steady-state $I_A$ inactivation curve. In four cells studied, the half-maximal inactivation voltage was $-62.2\pm0.4$ mV and $-62.8\pm0.6$ mV in the absence and presence of mefenamic acid (10 $\mu$M), respectively ($n=4$, $P<0.05$). Fig. 4C shows an inactivation curve that has not been shifted by addition of mefenamic acid. These results revealed that mefenamic acid decreased $I_A$ without any effect on steady-state $I_A$ channel activation and inactivation.

The effect of mefenamic acid on $I_A$ channel recovery from inactivation was examined by applying a double-pulse protocol. This comprised two depolarizing pulses to $+40$ mV, separated by intervals of increasing duration (from 5 ms to 95 ms in 10 ms increments), at $-120$ mV. Application of 10 $\mu$M mefenamic acid significantly reduced the amplitude of $I_A$ elicited by the two

![Fig. 3. Externally applied mefenamic acid did not alter the steady-state activation of $I_A$. (A) $I_A$ recordings using an activation voltage protocol in the absence (top) and presence (bottom) of 10 $\mu$M mefenamic acid (MA). The cells were held at $-100$ mV and depolarized in 10 mV steps from $-60$ mV to $60$ mV at 10 s intervals. (B) Voltage-dependent activation curve of $I_A$ obtained in the absence or presence of mefenamic acid. (C) Plot of the normalized conductance as a function of the command potential in the absence or presence of mefenamic acid. The data points were fitted to a Boltzmann function. No significant shift of the voltage-dependent activation curve was observed in the presence of mefenamic acid. The data represent the mean±SEM obtained from six cells.](image-url)
pulses (Fig. 5A). The peak amplitude ($I_{\text{test}}$) elicited by the second pulse ($V_{\text{test}}$) was normalized to that ($I_{\text{control}}$) recorded during the first pulse ($V_{\text{control}}$) and plotted as a function of the inter-pulse duration. The time course of recovery was fitted to a single exponential function: 

$$y = y_0 - A \times \exp \left( \frac{-(x-x_0)}{\tau} \right)$$

(Fig. 5B). Addition of 10 $\mu$M mefenamic acid to the bath solution induced a significant acceleration of recovery from inactivation. The time constants of recovery from inactivation of $I_A$ obtained ($n=6$) were 11.79±3.37 ms and 8.59±2.09 ms in the absence and presence of mefenamic acid, respectively (Fig. 5C, $P<0.05$ by Student’s $t$-test). Moreover, it was noted that, in the presence of mefenamic acid, the amplitude of $I_{\text{test}}$ was significantly greater than that of $I_{\text{control}}$ elicited by the first depolarizing pulse (Fig. 5B).

To investigate the possibility that mefenamic acid acts directly as an open channel blocker, we used a train protocol. In this protocol, mefenamic was applied to the bath solution 2 min before channel opening (i.e. before $I_A$ was elicited by the depolarizing pulse), then the effect of mefenamic on the amplitude of $I_A$ elicited by the first pulse and the flowing pulses was measured. Fig. 6A shows that, after incubation of granule cells in 10 $\mu$M mefenamic acid, the amplitude of the peak current evoked by the first pulse was not reduced; indeed, it was little greater than the control. Thereafter, the current declined with the following pulses to a steady-state level equivalent to 75% of the control. This experiment indicated that blocking cannot occur without prior channel opening. Statistical analysis of these data is shown in Fig. 6B.

To determine at what point mefenamic acid exerts its bi-directional effect on the $I_A$ channel, we investigated whether intracellular application of mefenamic acid could mimic the effect of extracellular mefenamic acid on $I_A$. When 1 $\mu$M mefenamic acid was added to the pipette solution, we found that the amplitude of the current increased with time after establishment of the whole-cell configuration and maximal activation of $I_A$ was reached within 3–5 min; these results were similar to those observed following extracellular application of the lower concentrations of mefenamic acid (Fig. 7A). The percentage of amplitude increase with intracellular 1 $\mu$M mefenamic acid was 145±7% ($n=6$, $P<0.05$ by Student’s $t$-test). We then examined whether intracellular application of mefenamic acid could modify the effect on $I_A$ induced by its extracellular application. As Fig. 7B shows, after the current had reached its maximal amplitude with 1 $\mu$M mefenamic acid in the pipette solution, extracellular application of 10 $\mu$M mefenamic acid could still decrease the amplitude
Fig. 5. Externally applied mefenamic acid modified the recovery of $I_A$ from inactivation. (A) Currents in the absence and presence of mefenamic acid (MA) were evoked by a double pulse. The double-pulse protocol comprised two depolarizing pulses from $-120$ mV to $40$ mV separated by intervals of increasing duration (from 5 to 100 ms in 10 ms increments) at $-120$ mV. (B) Time course of the recovery of $I_A$ from inactivation. The peak current amplitude during the test pulse ($I_{test}$) was normalized to that recorded during the first pulse ($I_{control}$) and plotted as a function of the inter-pulse duration. The best-fit single exponential function was used to derive the time constant for recovery from inactivation. (C) Comparison of the time constants of recovery from inactivation for control and mefenamic acid treatment, respectively ($P<0.05$, $n=6$).

Fig. 6. (A) The same cell was subjected to a train protocol. In this protocol, the current was elicited by a depolarizing pulse as the control (control) and mefenamic was then applied to the bath solution 2 min before channel opening. Thereafter, the effect of mefenamic on the amplitude of $I_A$ elicited by the first and following pulse was measured. Pulses were evoked by 200 ms steps from a $-100$ mV holding potential to $+40$ mV. (B) Statistical analysis of these data is shown. The data were obtained from four independent cells. *$P<0.05$ compared with the MA$^{1\text{st}}$. 

of \( I_A \). In order to exclude the possibility that intracellular solution might be involved in the effect of intracellular application of mefenamic acid, the corresponding control recording was induced by application of normal intracellular solution. Fig. 7C shows that the amplitude of \( I_A \) was stable during the recording time following establishment of the whole-cell configuration. The insets in the graphs show superimposed \( I_A \) traces from the same cell. (C) Time course of the changes in amplitude obtained with normal intracellular solution. The insets in the graphs show superimposed \( I_A \) traces taken from the same cell. (D) Statistical analysis of the effect of internal mefenamic acid alone and that without internal mefenamic acid. The data represent the mean value±SEM obtained from five to seven cells. *\( P<0.05 \) compared with the control group without internal mefenamic acid; °\( P<0.05 \) compared with the control with internal mefenamic acid.

Discussion

Our study shows that mefenamic acid bi-directionally modulated \( I_A \) in rat granule neurons at a low and high

As mefenamic acid, like all NSAIDs, is an inhibitor of COX (Ouellet and Percival, 1995), we investigated whether the ability to activate \( I_A \) is common to all NSAIDs by using three NSAIDs that are members of different chemical groups (flufenamic acid, diclofenac and indomethacin). All three of these NSAIDs mimicked the activation effect on \( I_A \) induced by mefenamic acid. Following internal application of flufenamic acid (Fig. 8A), diclofenac (Fig. 8B), or indomethacin (Fig. 8C) by pipette solution, the amplitude of the current was increased after establishment of the whole-cell configuration and reached its maximum within 2–4 min. The percentage of the amplitude increase with intracellular flufenamic, indomethacin, and diclofenac was 135±7% (\( n=6 \)), 130.2±3.6% (\( n=6 \)), and 124.5±1.3% (\( n=6 \)), respectively (\( P<0.05 \) by Student’s t-test). Statistical analysis of these data is shown in Fig. 8D.

The effects of eicosatetraynoic acid (ETYA) and AA were also investigated to address whether an increase of AA and/or a reduction of its metabolic products was involved. Application of 10 \( \mu \)M ETYA, which blocks the formation of active AA metabolites, in the pipette solution mimicked the effect of mefenamic acid on \( I_A \) (Fig. 9A), and an average increase of amplitude of 23±5% (\( n=5 \)) was observed under these conditions. The effect of AA was similar to that of ETYA, mefenamic acid, and the other NSAIDs (Fig. 9B). In the presence of intracellular AA (10 \( \mu \)M), the amplitude of \( I_A \) was increased by 141±9%. To exclude any effect of DMSO on \( I_A \), an intracellular solution containing 1% DMSO was also tested as the control. 1% DMSO alone did not have any effect on \( I_A \); the amplitude increase was 105±6%, which was not significantly different to that with the normal intracellular solution (\( n=5 \), \( P>0.05 \) by Student’s t-test). Statistical analysis of these data is shown in Fig. 8C.
concentrations and with different methods of application; external application at concentrations above 5 μM inhibited $I_A$, while external or intracellular application of 1 μM mefenamic acid increased $I_A$.

The mechanism by which NSAIDs inhibited ion channels was undefined. As diphenylcarboxylate and its derivates are highly lipophilic molecules, they may modify membrane ion channels via a direct action such as alteration of the permeability of the cell membrane, or via a non-specific lipid effect (Srinivas and Spray, 2003). They may also have indirect effects by reducing and/or increasing intracellular factors such as Ca$^{2+}$ and gene expression (Partridge and Valenzuela, 2000; Malykhina et al., 2002). In our study, blocking of $I_A$ by external mefenamic acid had a rapid onset and was rapidly reversible on washout, suggesting that mefenamic acid is most likely to inhibit $I_A$ through a direct, rapid action at the ion channel. This direct blocking effect on ion channels was similar to that observed in previous studies on rat supraoptic neurons and systems in which mefenamic acid blocked Ca$^{2+}$-dependent non-selective cation channels and human Kv 2.1 subunits stably expressed in the CHO cells (Lee and Wang, 1999; Partridge and Valenzuela, 2000). To our understanding, two general classes of block involve either direct blocking of conductance due to binding within the pore itself or allosteric inhibition due to binding outside the pore. Using a train protocol, we found that prior incubation of mefenamic acid did not reduce the amplitude of $I_A$ elicited by the first depolarizing pulse; thus, the blocking effect on $I_A$ channels occurred only with prior channel opening. It seems likely that mefenamic acid arrives at its binding site within the pore from the extracellular side of the membrane during channel opening. However, additional studies are required to address this issue.

Since mefenamic acid did not shift the state-steady activation and inactivation curve, we presume that this drug causes channel opening without affecting gating. However, extracellular application of mefenamic acid is distinguished by the fact that it significantly accelerated the recovery of $I_A$ channels from inactivation. Moreover, in the presence of mefenamic acid, the recovery current amplitude elicited by second pulse was significantly greater than that elicited by the first depolarizing pulse. This acceleration of recovery from inactivation and the increased peak amplitude evoked subsequent to the pre-pulse have been little reported in any previous studies on NSAIDs. Our results are similar to those of previous studies on 4-aminopyridine in neurons of the rat dorsal root ganglia (Ogata and Tatebayashi, 1993). The results indicate that, in the presence of mefenamic acid,
prior activation of ion channels could lead to use-dependent unblocking of current. This supports our speculation that the inhibitory effect induced by mefenamic acid may occur via direct channel blocking.

It was a surprising finding that lower concentrations of mefenamic acid had an activating effect on $I_A$. This was the opposite of what occurred at higher concentrations, but similar to the findings when the drug was administered internally via a patch pipette. Moreover, intracellular application of mefenamic acid did not alter the inhibitory effect induced by extracellular application of mefenamic acid. It is therefore conceivable that mefenamic acid increased $I_A$ via a mechanism other than channel blocking as occurred with extracellular application. It well known that NSAIDs, including fenamates, are inhibitors of COX (Wu, 1998). We therefore investigated whether $I_A$ activation occurs with other NSAIDs, using three NSAIDs that are members of different chemical groups (diclofenac, flufenamic acid, and indomethacin). All three of these NSAIDs had a stimulatory effect on $I_A$ when they were applied internally by pipette solution. Thus, the increase of $I_A$ with lower concentrations or intracellular application of mefenamic acid may be related to inhibition of COX.

Inhibitors of the COX pathway in the AA cascade can cause accumulation of AA and/or reduce its metabolic products (for a review, see Bazan, 2003). By intracellular application of AA, we investigated whether the activation effect of fenamates on $I_A$ was associated with accumulation of AA or its metabolites. The observation that internal application of AA mimicked the effect of mefenamate acid on $I_A$ is consistent with our hypothesis. To exclude any effect of DMSO, the solvent of AA, on $I_A$, we tested the effect of AA dissolved in 0.1% ethanol, and obtained the same results (data not shown). This indicates that AA can act as a mediator of $I_A$ channels, as reported first in smooth muscle (Ordway et al., 1989). ETYA, which blocks the formation of active AA metabolites, mimicked the effect of AA on $I_A$, indicating that activation with AA was direct and not mediated by oxygenated metabolites, as reported by Danthi et al. (2003) in bovine adrenal zona fasciculata cells. However, whether AA has a direct mediating effect on $I_A$ channels or indirectly activates the downstream signal in granule neurons needs to be further explored.

AA and its metabolites have been shown to modulate many ligand- and voltage-gated ion channels in various systems (Ordway et al., 1991), activating or inactivating ion channels depending on the cell type studied (Takahira et al., 2001; Patel and Honore, 2001; Wei et al., 2004). It is notable that, in rat granule neurons, our own and Holmqvist’s study have revealed that extracellular application of AA markedly inhibited the amplitude of $I_A$ and modulated its kinetics (Wang et al., 2005; Holmqvist et al., 2001). Moreover, molecular cloning showed that the inhibitory effect of AA on $I_A$ occurs by a direct action on the Kv4-KChIP complex (Holmqvist et al., 2001). Here, our results show that, though AA was applied intracellularly, it evoked an opposite stimulatory effect on $I_A$. To date, few investigations have used AA intracellularly, though Kim’s study of excised membrane patches showed that AA applied to the cytoplasmic or extracellular side of the membrane caused opening of three types of channel (Kim et al., 1995). We reason that these conflicting actions of AA may result from differences in its binding location on the cytoplasmic and extracellular sides of the membrane. Further work will be necessary to examine this possibility.

Using cell attachment and a single-channel recording configuration, McCarty et al. investigated blocking of the cystic

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**Fig. 9.** Intracellular application of eicosatetraynoic acid and arachidonic acid mimicked the effect of internal mefenamic acid on $I_A$. (A and B) Time course of the $I_A$ amplitude increases induced by internal application of 10 μM eicosatetraynoic acid (ETYA, A) and 10 μM arachidonic acid (AA, B). The insets in the graphs show superimposed $I_A$ traces taken from the same cells. (C) Statistical analysis of the effect of internal eicosatetraynoic acid, arachidonic acid, and 1% DMSO, which is the solvent of eicosatetraynoic acid and arachidonic acid, on the amplitude of $I_A$. The data represent the mean±SEM obtained from six or seven cells. *P<0.05 compared with the control.
fibrosis transmembrane conductance regulator Cl<sup>−</sup> channel by two closely related arylaminobenzoates, and found that flufenamic acid could cross the membrane from the bath solution and arrive at its binding site from the cytoplasmic side of the channel (McCarty et al., 1993). Considering this finding together with our observations, we can speculate that higher concentrations of mefenamic acid applied externally rapidly blocked the I<sub>K</sub> channels before it passed through the cell membrane into the cytoplasm. In contrast, at lower concentration, mefenamic acid could diffuse across the membrane and access an intracellular site, and by inhibiting COX and increasing AA activate the I<sub>K</sub> channel.

I<sub>K</sub> is an excellent target for any modulatory mechanism influencing cell excitability and action potential firing (Shibata et al., 2000; Kiss et al., 2002). Although the precise mechanism of mefenamate acid-induced bi-directional modulation of I<sub>K</sub> remains unknown, our results may partially account for the unwanted side effects of mefenamate acid, and might be a valuable new avenue for investigation in terms of the therapeutic and/or basic research applications of NSAIDs.

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