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Role of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in Modulating Vascular Smooth Muscle Cells by Activating Large-Conductance Potassium Ion Channels

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1. Introduction

In this chapter we propose to discuss the role of K^+ ion channels in stimulating vasodilatation by altering the membrane potential of vascular smooth muscle cells. We present evidence that the K^+ channels are modulated by a direct action of non-steroidal anti-inflammatory drugs (NSAIDs) to activate the K^+ ion channels.

The primary cellular action of non-steroidal anti-inflammatory drugs (NSAIDs) is thought to be through inhibition of pathways involving cyclo-oxygenase (COX). COX catalyses the conversion of arachidonic acid to prostaglandin endoperoxides (Vane, 1971) which are the precursors of both prostacyclin and thromboxane A_2 (Moncada et al., 1976). Such an action of NSAIDs may be expected to be vasoconstrictive and lead to increased blood pressure, which is a possibility that has been suggested by meta-analyses of clinical studies (Johnson et al., 1994). However, early reports indicated that chronic administration of indomethacin or other NSAIDs had varying effects on blood pressure (Lopez-Ovejero et al., 1978; Ylitalo et el., 1978). For example, whilst indomethacin and naproxen are associated with increases in blood pressure, NSAIDs such as sulindac, aspirin, piroxicam or ibuprofen have negligible effects (Pope et al., 1993). Moreover, in a direct study of the effects of NSAIDs in patients with mild essential hypertension, it was found that ibuprofen increased systolic blood pressure but neither aspirin nor sulindac had any significant effect on systolic or diastolic blood pressure (Minuz et al., 1990). In this chapter we report our investigations of the hypothesis that the variable effect on blood pressure was due to NSAIDs inducing vasodilatation.

Vasodilatation can be mediated by contributions from any one of several independent cellular mechanisms which include release of COX metabolites and nitric oxide (NO), release of an endothelium-derived hyperpolarising factor (EDHF), or activation of ATP-sensitive potassium channels (Feletou & Vanhoutte, 2000; Pinheiro & Malik, 1993). In addition, the particular mechanism underlying the eventual vasodilatation can be related to its initiating chemical mediator. For example, endothelium-dependent vasorelaxation does not appear to be mediated by COX products and is critically dependent on NO (Pinheiro & Malik, 1993), although it may also involve cell hyperpolarisation via the opening of ATP-sensitive potassium channels (Sakuma et al., 1993). Some authors have suggested that the particular type of potassium channel that is activated to produce cell hyperpolarisation may not be confined to the classical ATP-sensitive channel (Seigel et al., 1992). For example, the NO-independent coronary vasodilator effect of bradykinin was found to utilise a Ca^{2+}-activated potassium channel (Fulton et al., 1994).

It has been reported that NSAIDs of the fenamate family, which include mefenamic acid, niflumic acid and flufenamic acid, activated Ca^{2+}-activated potassium channels (Farrugia et al., 1993; Ottolia & Toro, 1994). We examined the possibility, using patch-clamp electrophysiology, that other NSAIDs may also activate Ca^{2+}-activated potassium channels in aortic smooth muscle cells since not all NSAIDs have been shown to cause significant increases in blood pressure. Furthermore, we used the enantiomers of flurbiprofen to separate the COX-mediated effects from those related to potassium channel activation in organ bath experiments where we recorded constrictor responses of the aorta to phenylephrine. It is important to note that R-flurbiprofen has negligible effects on COX pathways compared to S-flurbiprofen, which does inhibit COX pathways (Peskar et al., 1991). Also, R-flurbiprofen does not convert to S-flurbiprofen in biological systems, unlike an enantiomer such as R-ibuprofen. We report that low concentrations of several NSAIDs were found to activate a Ca^{2+}-sensitive and ATP-activated K^+ channel (K_{AC}) in vascular smooth muscle cells, leading to cell hyperpolarisation and vasodilatation. Our results indicate that several NSAIDs may cause vasodilatation which would explain the clinical reports that some NSAIDs have negligible effects, or even reductions, in blood pressure.

2. Experimental procedures and methods

To investigate the effect of NSAIDs, we correlated the results from patch-clamp electrophysiology and physiological organ-bath investigations utilizing rings of vascular tissue. The results from experiments using those techniques were used to test our hypothesis that the variable effect on blood pressure which is reported in the clinical literature is due to the spectrum of potency of NSAIDs to activate K^+ channels (thereby inducing vasodilatation) in synergy with the classical NSAID effect on intracellular pathways that involve cyclo-oxygenase (which usually results in a vasoconstriction).

For the experiments we used the following drugs: acetylcholine, phenylephrine, ATP, ADP, AMP, aspirin, indomethacin, flufenamic acid, niflumic acid, mefenamic acid, pinacidil, TEA, and collagenase were purchased from Sigma. Porcine pancreatic elastase was from Calbiochem-Novabiochem (Sydney). Glibenclamide was from RBI (Natlick MA, U.S.A.). R- and S- isomers of flurbiprofen were a kind donation from the Boots Company (UK) by Dr Ken Williams, St Vincent's Hospital Sydney. Other chemicals used for the intracellular, extracellular, and Krebs bicarbonate solutions were of AR grade.

2.1 Animal studies

This protocol was approved by the Garvan Institute of Medical Research/St Vincent's Hospital Animal Ethics Committee. The study complied with the guidelines published by the Australian National Health and Medical Research Council for the care and conduct of experiments using animals in research, and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2 Preparation of RASM myocytes for patch-clamp electrophysiology

New Zealand white rabbits of either sex, weighing 1 to 2 kg, were anaesthetised with pentobarbital sodium via an ear vein (Nembutal, Boehringer Ingelheim, 45 mg/kg). Heparin (500 Units) was infused at the same time. A short section of the thoracic aorta was dissected and the vessel was washed several times in Hanks Balanced Salt Solution (GIBCO, Life Technologies, Melbourne) and incubated in 1000 U/ml collagenase (Type II, Sigma #C6885) for 30 minutes at 37°C to remove endothelial cells. Strips of media were carefully peeled off using jewellers forceps, diced and incubated in a solution containing 1000 U/ml collagenase (Type II) and 60 U/ml porcine pancreatic elastase (Calbiochem-Novabiochem, Sydney) for 2-3 hours at 37°C with periodic trituration. Either the tissue explants or dispersed cells were seeded in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 100 U/ml penicillin (GIBCO), 100 μg/ml streptomycin (GIBCO), 4 mM fresh L-glutamine (GIBCO) and 10% foetal bovine serum (PA Biologicals, Sydney). The cells (2×10^5/well) at passage 2-3 were plated onto glass coverslips at least 3 days before an experiment and serum-deprived for 24 hours in 4% Monomed (Commonwealth Serum Laboratories, Melbourne) in DMEM. Cultured cells expressed smooth muscle actin and were negative for Factor VIII:RAg, an endothelial cell marker.

2.3 Recording and analysis of K^+ ion channels using patch-clamp electrophysiology

Standard patch-clamp techniques that we have used previously on cardiovascular cells (Martin et al., 1994) were used to record single ion channel activity, at 37°C, in the inside-out, cell-attached and whole-cell configurations from the rabbit aortic smooth muscle (RASM) cells. The channel currents were amplified and filtered at 1kHz (–3dB point) using an Axopatch 1D amplifier (Axon Instruments, Union City, CA, U.S.A.) and sampled on-line by a microcomputer (IBM 486 compatible) using commercial software and associated A/D hardware (pClamp 6.0/Digidata 1200, Axon Instruments and Scientific Solutions Inc., Foster City, CA, U.S.A.). The single-channel open probability was calculated from the areas of Gaussian curves fitted to amplitude histograms compiled from 2 minute channel recordings. We calculated changes in the channel activity following the addition of NSAIDs by dividing the open probability in the presence of the drug by that recorded before the drug was applied. All data are presented as mean±SE with the number of observations in parenthesis (n).

Ion currents are referred to the trans-patch potential (V_m). For cell-attached patches, this was determined from the pipette potential (V_p), cell resting potential (E_m) and the liquid junction potential (E_L) between the bath and pipette solutions. The liquid junction potential (E_L) was calculated using commercial software (Barry, 1994), and was typically around 4mV. Thus

$$V_m = (E_m - V_p) + E_L \quad (1)$$

For inside-out patches, the V_m was only determined by V_p and E_L, with E_m=0 in equation (1), thus

$$V_m = V_p - E_L \quad (2)$$

For recording from inside-out membrane patches an extracellular solution was used in the pipette and for superfusion of the cells during seal formation, which contained (mM): NaCl (130), KCl (4.8), $MgCl_2$ (1.2), NaH_2PO_4 (1.2), N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid] (HEPES) (10), glucose (12.5), $CaCl_2$ (1.0), and Bovine Albumin (0.5mg/ml, fraction V, Sigma, #A7888) (pH=7.4, with NaOH). After a gigaseal was formed this extracellular superfusing solution was replaced with an intracellular solution that contained (mM): KCl (140), $MgCl_2$ (1.2), ethylene glycol-bis (b-amino ethyl ether) tetraacetic acid (EGTA) (5), and HEPES (10) (pH=7.2, with KOH) and an inside-out membrane patch was excised from the cell. The various NSAIDs were added to this intracellular solution.

2.4 Recording and analysis of the contractile properties of arterial rings

Eight 2 mm wide rings were cut from sections of the thoracic aorta of the rabbits. In some experiments the endothelium was removed by gently rotating a wooden swab stick in the lumen of the vessel. Removal of the endothelium was confirmed by the absence of acetylcholine (1μM) induced relaxation in rings that had been pre-constricted with phenylephrine (PE) (1μM). The 8 rings were suspended in individual 10 ml water-jacketed (37°C) organ baths filled with freshly prepared Krebs-Bicarbonate solution, which had the following composition (mM): NaCl (118), KCl (4.7), KH_2PO_4 (1.2), $MgSO_4.7H_2O$ (1.18), glucose (5.0), $NaHCO_3$ (25.0), $CaCl_2.2H_2O$ (2.54). The aortic rings were pre-loaded with a basal tension of 2.00±0.05g. Isometric tension in each ring was measured using a Grass FT03 force transducer (Quincy, MA, USA), the output of which was multiplexed and sampled on-line by a microcomputer (Macintosh IIsi) using commercial software and associated A/D hardware (MACLAB, Analog Digital Instruments, Sydney, Australia).

The design of the organ bath experiments were as follows: *(i)* challenge with a submaximal contractile dose of KCl (40mM), which was used to normalise all subsequent responses to PE in the presence of the NSAID; *(ii)* determine a PE dose-response curve from each vascular ring in order to produce a control response. After suitable washout and re-equilibration periods of 30 minutes, the NSAIDs were added to the organ baths 15 minutes prior to subsequent challenge with PE; *(iii)* time mediated changes in responsiveness of the vascular rings were analysed by repeated challenges/trials with PE during a complete experiment; *(iv)* PE and NSAIDs were added to the organ baths in cumulative concentrations; *(v)* concentrations noted in all figures reflect the final concentration in the organ baths.

Contraction of vascular rings is presented either as tension (g), or a contraction relative to the contraction elicited by KCl (40mM) which was expressed as a percentage (%). Differences between dose-response curves were assessed using 2-way analysis of variance (ANOVA). Further comparisons of individual data-points were tested using unpaired Student's t-tests with the appropriate Bonferroni correction for multiple comparisons (SPSS v10.0, Chicago, Ill).

Separate stock solutions of phenylephrine (10mM), acetylcholine perchlorate (10mM), and KCl (4M) were prepared with deionized water (Milli-Q, Millipore Corporation, Bedford MA, USA). Subsequent dilutions for each drug were made with freshly prepared Krebs bicarbonate solution. R- and S-flurbiprofen were prepared as a 100mM stock with 0.1M Na_2CO_3 solution, and subsequently diluted with freshly prepared Krebs bicarbonate solution. As a control, no change in contraction was recorded after exposing rabbit aortic rings to Na_2CO_3 (0.1M) for 15 minutes.

3. Results from experiments

In summary, the following results of the experiments describe a large-conductance K^+ channel in smooth muscle cells that is activated by intracellular ATP and Ca^{2+}. Furthermore, the K^+ channel is activated by some NSAIDs and pinacidil. We have designated this channel K_{AC}, since it does not have the normal characteristics of the classical ATP-sensitive K^+ channel, and the K_{AC} channels have additional features compared to the previously reported maxi-K channels (Kuriyama et al., 1998). The RASM smooth muscle cells were hyperpolarised by aspirin, an NSAID that potently activated the K_{AC} channel. Both R- and S-flurbiprofen antagonised constrictor responses of the rabbit aorta to PE, suggesting that relaxation occurred via a mechanism other than inhibition of cyclo-oxygenase pathways. Our results allow us to conclude that NSAIDs are potent openers of a Ca^{2+}-activated phosphorylation-dependent potassium channel in vascular smooth muscle cells leading to cell hyperpolarisation and vessel dilatation. The activation of potassium channels is thought to be significant in controlling excitability of vascular smooth muscle cells and regulation of myogenic tone (Brayden & Nelson, 1992), an idea that has been corroborated in coronary arteries (Scornik et al., 1993) and in rabbit aorta (Gelband & McCullough, 1993). The K_{AC} channel that we describe in this paper thus provides a novel target to control excitability of vascular smooth muscle cells and regulate myogenic tone.

3.1 Characteristics of K^+ ion channels in RASM cells

The predominant channel recorded from inside-out membrane patches, with a NaCl-rich extracellular pipette and a KCl-rich intracellular bath solution, had a single-channel conductance of 128±6 pS (n=31) as shown in Figure 1.

Under these conditions the reversal potential of the current-voltage relation was –60 mV (95% confidence interval of -69 mV to -52 mV) indicating that it was permeable to potassium ions (E_K = –85 mV). This was confirmed in experiments with 7 other patches where half of the KCl in the superfusing intracellular (bath) solution was replaced with K-gluconate. As would be expected for a mainly K^+-permeable channel, neither the reversal potential (–47 mV with KCl and –49 mV with K-gluconate) nor the single-channel conductance (140±9 pS with KCl and 146±13 pS with K-gluconate) was altered following this ion substitution. With symmetrical KCl solutions bathing the inside-out patches, the single-channel conductance was 259±18 pS (n=6) and the reversal potential shifted toward zero (+4.3 mV).

The pooled distribution of channel openings from 11 inside-out patches with the pipette filled with the extracellular solution and the bath filled with the intracellular solution and no

applied voltage (trans-patch potential of 0mV) was fitted by a single exponential with a time constant of 2.7±0.0 ms (2,567 events). The average single-channel open probability from these inside-out patches was 0.0125±0.0053 (n=11). The single-channel open probability was increased by 64±21 % (n=4) when the concentration of Ca^{2+} was increased from <0.001 μM to 1 μM at the cytosolic face of the inside-out patches. The open probability of the channel was also increased following the addition of adenosine nucleotides (all 5 mM), with the sequence of potency adenosine 5′-triphosphate (ATP) > adenosine 5′-diphosphate (ADP) > adenosine 5′-monophosphate (AMP) (Figure 2).

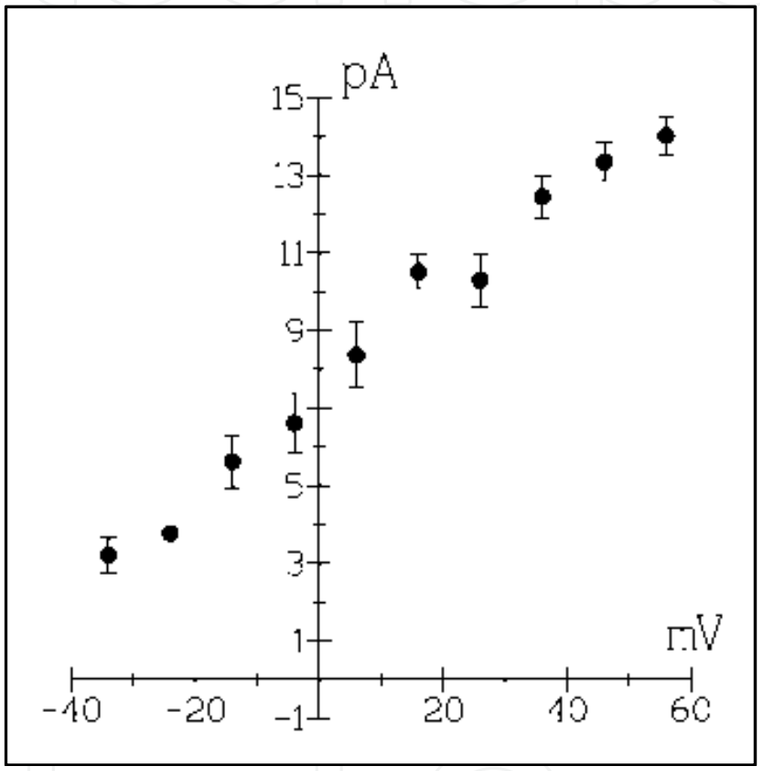


Fig. 1. Current-voltage relation of the predominant K_{AC} channel in rabbit aorta vascular smooth muscle cells recorded from inside-out membrane patches. The pipette solution (extracellular) contained (mM): NaCl (130), KCl (4.8), $MgCl_2$ (1.2), NaH_2PO_4 (1.2), HEPES (10), glucose (12.5), $CaCl_2$ (1.0), and Bovine Albumin (0.5mg/ml) (pH=7.4, with NaOH). The superfusing solution (intracellular) contained (mM): KCl (140), $MgCl_2$ (1.2), EGTA (5), and HEPES (10) (pH=7.2, with KOH). The single-channel conductance with these solutions is 128±6 pS (n=31).

The activation of the 259 pS K^+ channel by ATP and Ca^{2+} led us to use the abbreviation K_{AC} for this channel. The open probability of the K_{AC} channel was unaffected when the ATP-sensitive K^+ channel blocker glibenclamide, in the range from 3 μM to 60 μM, was added to the cytosolic face of inside-out membrane patches. Pinacidil, which is known to activate the classical ATP-sensitive K^+ channel, also increased the open probability of the K_{AC} channel (Figure 3).

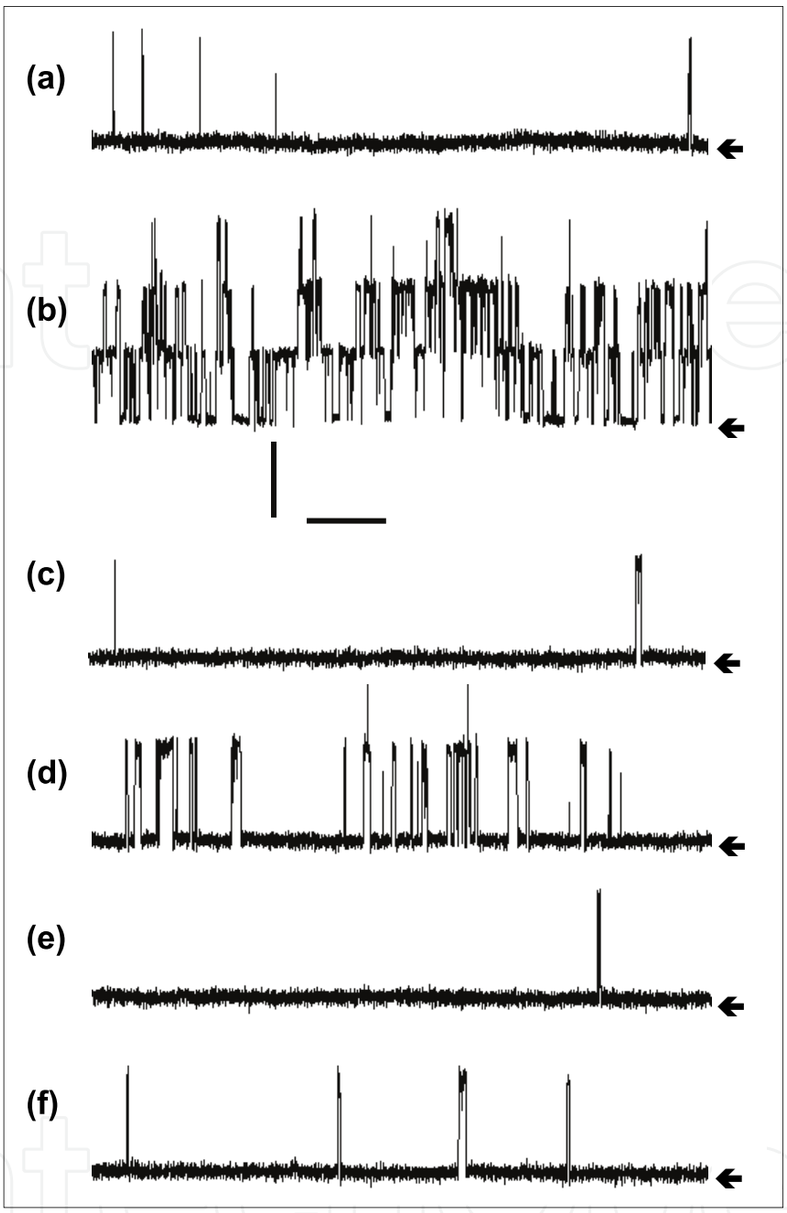


Fig. 2. Activation of the K_{AC} channel in RASM by adenosine nucleotides (all 5mM) applied to the cytoplasmic face of inside-out membrane patches. (a) superfusing solution alone, (b) ATP added to superfusing solution, (c) washout with superfusing solution, (d) ADP added to superfusing solution, (e) washout with superfusing solution, (f) AMP added to superfusing solution. The pipette solution (extracellular) contained (mM): NaCl (130), KCl (4.8), $MgCl_2$ (1.2), NaH_2PO_4 (1.2), HEPES (10), glucose (12.5), $CaCl_2$ (1.0), and Bovine Albumin (0.5mg/ml) (pH=7.4, with NaOH).The superfusing solution (intracellular) contained (mM): KCl (140), $MgCl_2$ (1.2), EGTA (5), and HEPES (10) (pH=7.2, with KOH). The activation of the channel was in the sequence ATP > ADP > AMP. The trans-patch potential was 0 mV and channel openings are upward, with the zero-current (baseline) level indicated with the solid arrow. Scale bars are shown between traces (b) and (c). Vertical bar is 15pA. Horizontal bar is 400 ms.

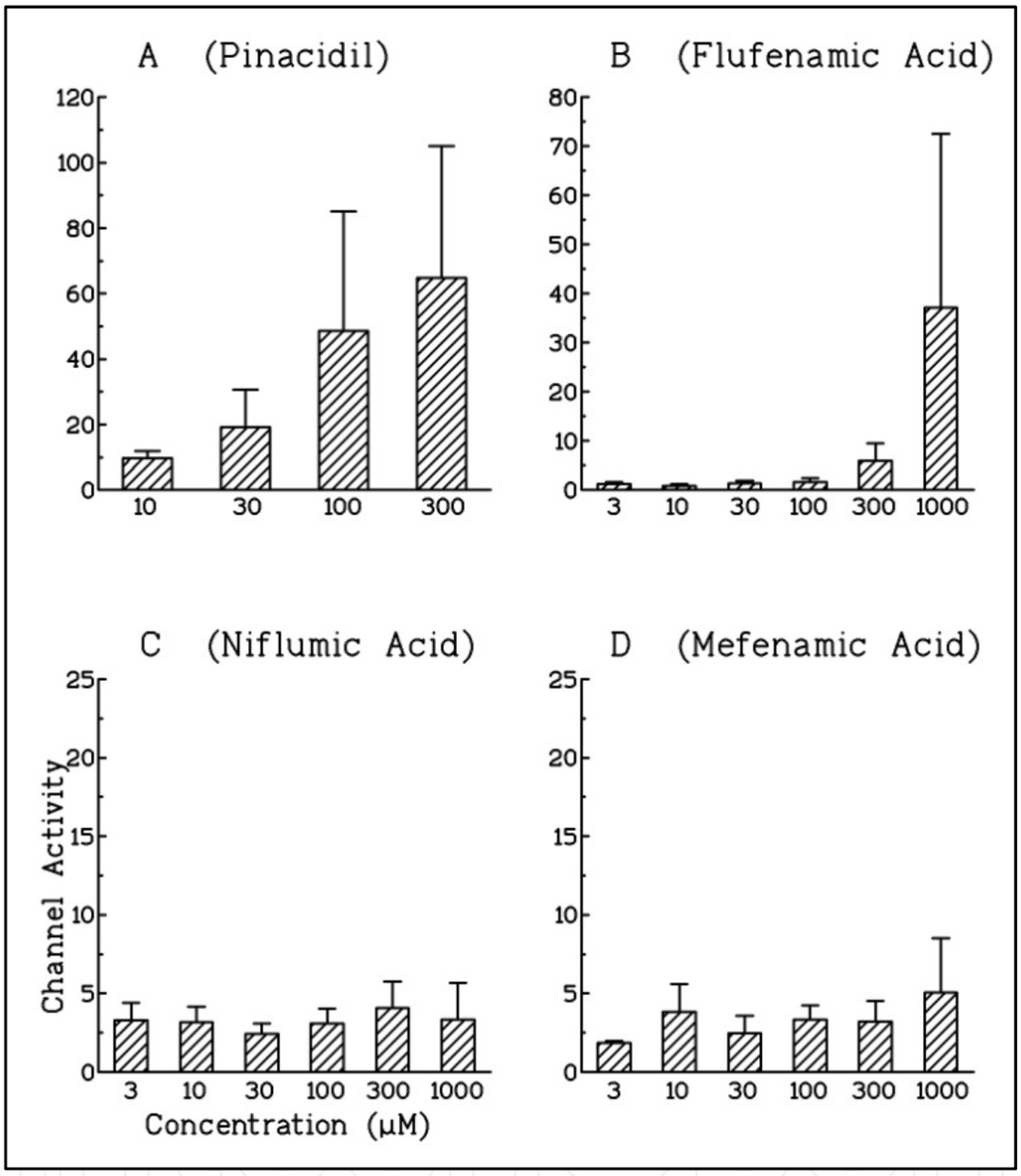


Fig. 3. Effects of (A) pinacidil (n=5), (B) flufenamic acid (n=6), (C) niflumic acid (n=4) and (D) mefenamic acid (n=4) on the open probability of the KAC channel in RASM. In all panels the y-axis represents KAC channel activity, which was calculated by dividing the open probability in the presence of the drug by that recorded before the drug was applied. In all panels the x-axis represents concentration of the drug in μM.

3.2 Effects of NSAIDs on K_{AC} ion channels

There was a dose-dependent increase in the open probability when either aspirin, R-flurbiprofen, S-flurbiprofen, indomethacin or flufenamic acid was added to the cytosolic face of inside-out membrane patches (Figures 3, 4).

Aspirin was the most potent of these NSAIDs, with the order of potency being aspirin > R-flurbiprofen = S-flurbiprofen > indomethacin > flufenamic acid. Those NSAIDs were more potent activators of the KAC channel than pinacidil (Figure 5). The NSAIDs niflumic acid and mefenamic acid were tested, but had no effects on the open probability of the K_{AC} channel.

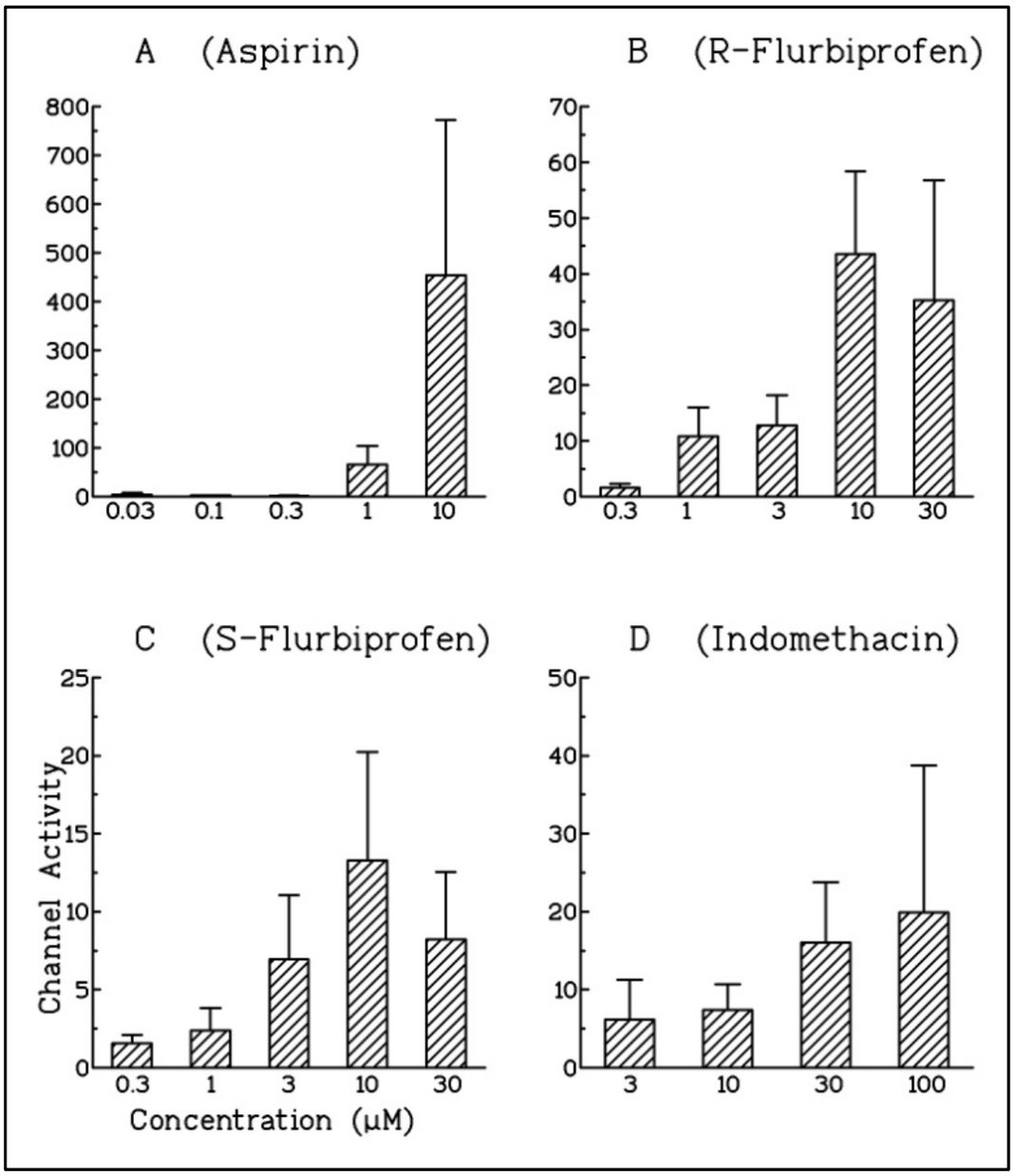


Fig. 4. Effects of (A) aspirin (n=5), (B) R-flurbiprofen (n=6), (C) S-flurbiprofen (n=4) and (D) indomethacin (n=4) on the open probability of the KAC channel in RASM. In all panels the y-axis represents KAC channel activity, which was calculated by dividing the open probability in the presence of the NSAID by that recorded before the NSAID was applied. In all panels the x-axis represents concentration of the drug in μM.

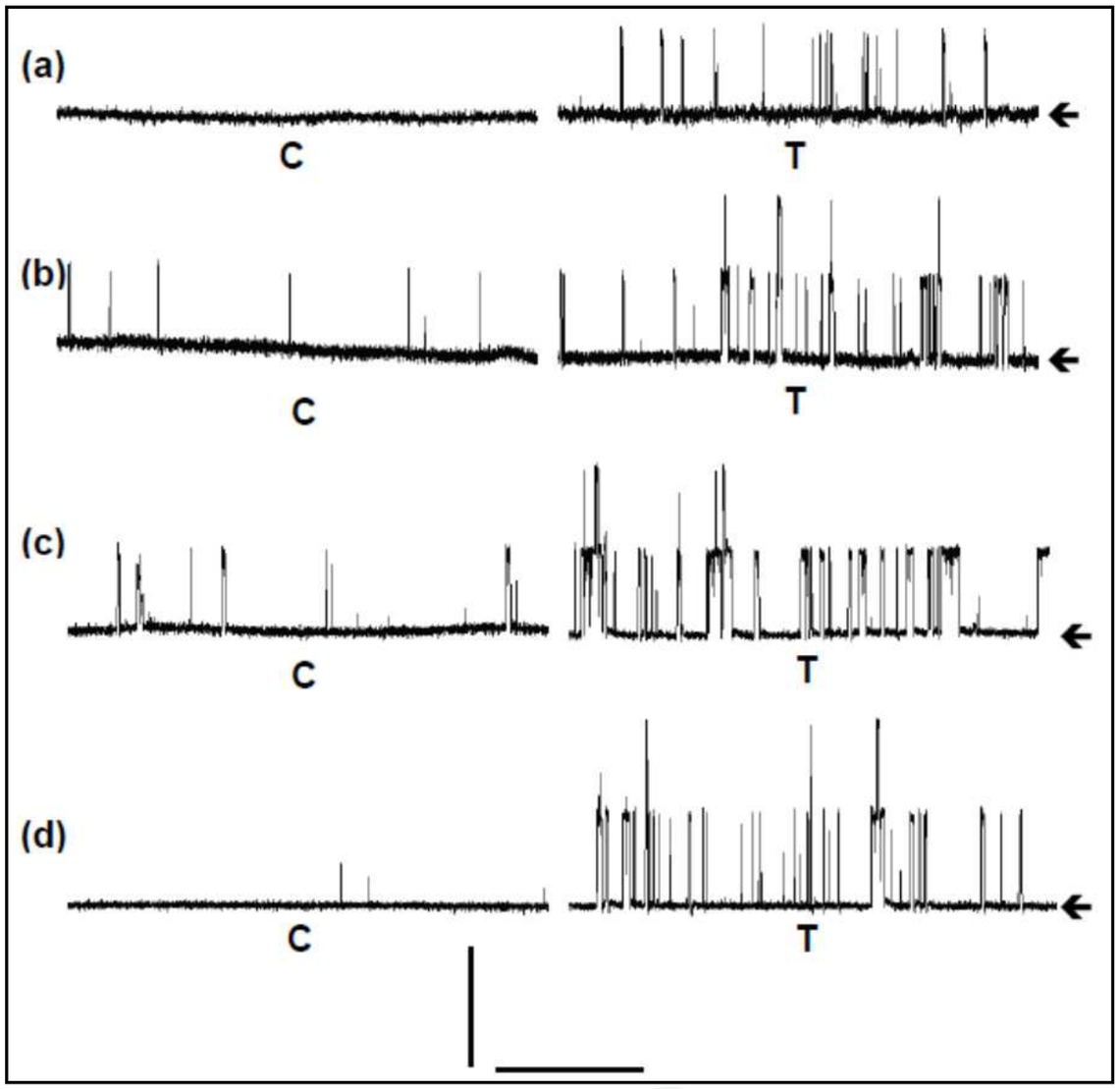


Fig. 5. Activation of the K_{AC} channel in RASM by the NSAIDs (a) aspirin, (b) R-flurbiprofen, (c) S-flurbiprofen, and by (d) pinacidil applied to the cytosolic face of inside-out membrane patches. The trans-patch potential was 0 mV and channel openings are upward, with the zero-current (baseline) level indicated with the solid arrow. In all panels trace C represents the control condition of no drug added to the superfusing solution and trace T represents the recording when the NSAID was added to the superfusing solution. For recording from inside-out membrane patches an extracellular solution was used in the pipette which contained (mM): NaCl (130), KCl (4.8), $MgCl_2$ (1.2), NaH_2PO_4 (1.2), N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid] (HEPES) (10), glucose (12.5), $CaCl_2$ (1.0), and Bovine Albumin (0.5mg/ml, fraction V, Sigma, #A7888) (pH=7.4, with NaOH). The superfusing solution contained (mM): KCl (140), $MgCl_2$ (1.2), ethylene glycol-bis (b-amino ethyl ether) tetraacetic acid (EGTA) (5), and HEPES (10) (pH=7.2, with KOH) and an inside-out membrane patch was excised from the cell. Vertical bar is 15pA. Horizontal bar is 400 ms.

3.3 Effects of NSAIDs on membrane potential

The activation of the KAC channel by the NSAIDs may be expected to hyperpolarise the smooth muscle cells. To test this hypothesis, we recorded the membrane potential while current-clamping (whole-cell mode) the smooth muscle cells used after adding aspirin 1 μM extracellularly. This experiment was repeated on 12 cells that satisfied the criteria of remaining in a stable whole-cell configuration for at least 5 minutes with no depolarising shifts in membrane potential, and the membrane potential was at least –10 mV at the end of this stabilisation period.

The average resting membrane potential for the 12 cells was –19.4±1.9 mV. After 5 minute exposure to aspirin 1 μM in the superfusing solution, the membrane potential was hyperpolarised in the majority (10/12) of these cells. The average hyperpolarisation shift in membrane potential induced by aspirin was by –5.8±1.4 mV (n=12), which was significant when tested with a Wilcoxon Rank-Sum test (p=0.001).

3.4 Effects of NSAIDs on contractile properties of rabbit aorta

The patch-clamp electrophysiology results indicate that the activation of the K_{AC} channel leads to hyperpolarisation of the rabbit aorta smooth muscle cells, which may also lead to relaxation of the intact blood vessel. We tested this hypothesis by measuring the effect of both the R- and S- enantiomers of flurbiprofen (1 mM) on the contraction of rings of rabbit aorta in response to cumulative concentrations of PE. We utilised enantiomers of flurbiprofen in these experiments so that any effects due to K_{AC} channel activation could be distinguished from effects on cyclo-oxygenase pathways. Both enantiomers of flurbiprofen activated the K_{AC} channels. It is important to note that R-flurbiprofen has negligible effects on cyclo-oxygenase pathways compared to S-flurbiprofen, which does inhibit these pathways (Peskar et al., 1991). Also, R-flurbiprofen does not convert to S-flurbiprofen in biological systems, unlike an enantiomer such as R-ibuprofen.

Both R- and S- enantiomers of flurbiprofen shifted the PE dose-response curve to the right and reduced the maximum contraction induced by PE (Figure 6). The EC_{50} values were calculated from non-linear regression of the data in figure 6 using a Gompertz 3-parameter sigmoidal equation (SigmaPlot 2000, Chicago, Ill) and are shown in table 1. The effect of either R- or S- flurbiprofen was to increase the EC_{50} value for PE-induced contraction by approximately 2½ times. The effect of flurbiprofen (R- and S- combined) was to increase significantly the EC_{50} for the PE-induced contraction to 527.0 ± 5.5 nM from the control value of 214.4 ± 9.2 nM ($p<0.05$, $t = 23.976$, Student's t-test).

Ring treatment	R-flurbiprofen		S-flurbiprofen	
	control	treatment	control	treatment
+ endothelium	207.0	517.6 (2.50)	241.5	518.0 (2.14)
– endothelium	200.0	533.0 (2.67)	209.1	539.5 (2.58)

Table 1. The effect of R- or S-flurbiprofen on the phenylephrine EC_{50} derived from rabbit thoracic aortic rings, with (+) or without endothelium (-).Values shown in brackets indicate magnitude of increase from control to treatment EC_{50}. The calculated EC_{50} (nM) is derived from the data shown in figure 6, using non-linear regression of a Gompertz 3-parameter sigmoidal equation (SigmaPlot 6.0).

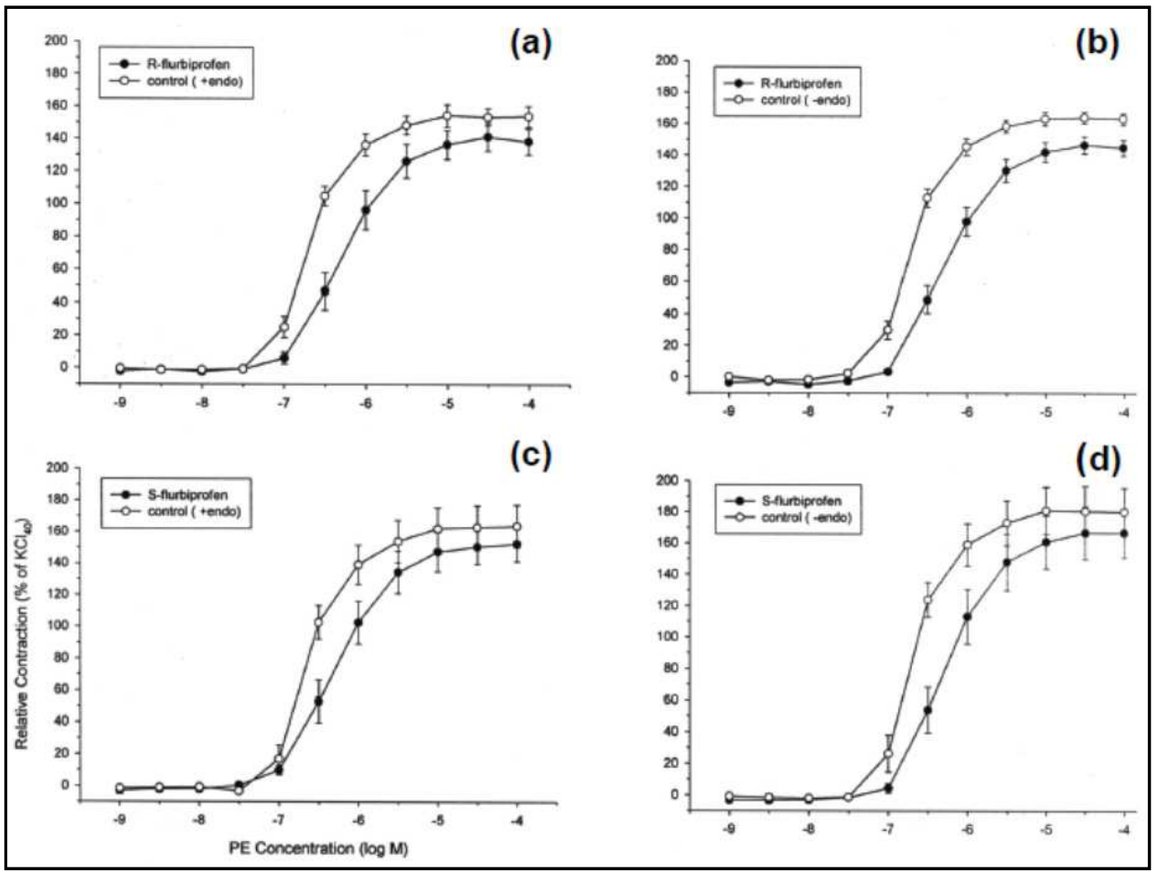


Fig. 6. Effect of enantiomers of flurbiprofen on pre-constricted rings dissected from rabbit aorta. (a) R-flurbiprofen added to rings with intact endothelium, (b) R-flurbiprofen added to rings with no endothelium, (c) S-flurbiprofen added to rings with intact endothelium, and (d) S-flurbiprofen added to rings with no endothelium. Both R- and S-flurbiprofen antagonised the constrictor responses of the aorta to PE. Note that, in comparison to S-flurbiprofen, R-flurbiprofen has a very weak effect on cyclo-oxygenase pathways. The data-points represent mean±SE. In all panels the x-axis represents the PE concentration (log M) and the y-axis represents the relative contraction (%) of the aortic rings relative to the contraction to KCl (40mM).

4. Discussion and conclusion

We describe a large conductance Ca^{2+}-activated K^+ channel (K_{AC}) in RASM that has the unusual property of being activated by ATP applied intracellularly. Investigations of a similar channel in smooth muscle cells of the rat pulmonary artery suggested that phosphorylation is important for its activation (Robertson et al., 1992). A subsequent report from the Kozlowski research group described this type of channel being present in isolated smooth muscle cells from the aorta, mesenteric and basilar arteries of the rat (Hartley & Kozlowsky, 1996). Such large ATP- and Ca^{2+}-activated K^+ channels may represent a link between cellular metabolism and hypoxia (Pinheiro & Malik, 1993), since the glycolysis inhibitor, 2-deoxy-D-glucose, has been shown to inhibit K^+ currents in rat pulmonary arterial smooth muscle cells (Hartley & Kozlowski, 1996).

The novel results that we present are that K_{AC} is activated by several NSAIDs, including R-flurbiprofen, S-flurbiprofen, indomethacin and aspirin. In contrast to the previously published effect of fenamates to activate large conductance Ca^{2+}-activated K^+ channels (Farrugia et al., 1993; Ottolia & Toro, 1994) or to block classical ATP-sensitive K^+ channels (Li et al., 2007), the K_{AC} channels that we report are not sensitive to mefenamic acid, niflumic acid nor flufenamic acid. This insensitivity to fenamates and a sensitivity to intracellular ATP distinguish the K_{AC} channels from the large-conductance Ca^{2+}-activated channels described previously (Farrugia et al;? 1993; Ottolia & Toro, 1994). Activating the K_{AC} channels with aspirin also hyperpolarised the RASM. Furthermore, we report that pinacidil also activated the K_{AC} channels. Pinacidil has been previously thought to only activate classical K_{ATP} channels.

There are some reports of large conductance Ca^{2+}-activated K^+ ("maxi-K") channels as being a target for the fenamate class of NSAIDs. Ottilia & Toro (1994) reported that niflumic acid, flufenamic acid and mefenamic acid rapidly and reversibly activated a large conductance Ca^{2+}-activated K^+ channels. Greenwood & Large (1995) confirmed that those fenamates activated a large conductance Ca^{2+}-activated K^+ channel in rabbit portal vein, and those authors reported that the Ca^{2+}-activated K^+ current was inhibited by TEA but not by glibenclamide. Also, the cardiac delayed rectifier K^+ channel HERG is activated by the fenamates flufenamic and niflumic acids (Malykhina et al., 2002) and flufenamic acid activated the maxi-K channel in the trabecular meshwork of both human and bovine origin (Stumpff et al., 2001). We found that neither niflumic acid, mefenamic acid nor flufenamic acid (but only in very high concentration) could activate the K_{AC} channel. The K_{AC} channels differ from the maxi-K class of channels in at least the lack of a fenamate binding site.

Further insights into the nature of the NSAID binding site on the K_{AC} channels can be obtained from the range of drugs used to activate these channels. K_{AC} was activated by representative NSAIDs from the salicylate (aspirin), propionic acid (flurbiprofen) and indole (indomethacin) families of NSAIDs. The potency of the drugs in activating the K_{AC} channels was aspirin > R-flurbiprofen = S-flurbiprofen > indomethacin > pinacidil > flufenamic acid (very weak effect) > niflumic acid = mefenamic acid (both no effect). The drugs with the most potent activating effect on K_{AC} were generally the more acidic. This implies a different extracellular conformation of the K_{AC} channels compared to the maxi-K channels, such that the nature of the residues available at the NSAID binding site may be different for these two types of large-conductance K^+ channels.

In our organ bath experiments R-flurbiprofen, an NSAID that activates the K_{AC} channels but does not inhibit prostaglandin synthesis, antagonised PE-induced contraction of rings of aorta. In an earlier report, Pallapies et al (1994) found that both R- and S-flurbiprofen relaxed rat aorta that had been pre-contracted with PE. Furthermore, McGrath et al (1990) indicated that flurbiprofen inhibited acetylcholine-induced contractions of rabbit saphenous vein in which the endothelium was intact. In direct measurements of pressure in the perfused vascular bed of isolated rabbit lungs, aspirin was found to inhibit the PE-induced increase in arterial pressure (Delaunois et al., 1994). Our results demonstrate the effect of flurbiprofen was not influenced by the presence of the endothelium, which suggested that flurbiprofen was acting directly on the smooth muscle cells of the aorta rings. The similarity in responses from R- and S-flurbiprofen suggested that the relaxation was due to activation of the K_{AC} channel, rather than through effects on cyclo-oxygenase

pathways. Our results provide evidence that K_{AC} channels provide a mechanism underlying the vasodilatory effect of NSAIDs.

The primary cellular action of non-steroidal anti-inflammatory drugs (NSAIDs) is thought to be inhibition of cyclo-oxygenase pathways, the enzyme that catalyses the conversion of arachidonic acid to prostaglandin endoperoxides (Vane, 1971) which are the precursors of both prostacyclin and thromboxane A_2 (Moncada et al., 1976). Such an action may be expected to be vasoconstrictive and lead to increased blood pressure, a possibility that has been suggested by meta-analyses of clinical studies (Johnson et al., 1994). However, early reports indicated that chronic administration of indomethacin or other NSAIDs had varying effects on blood pressure (Lopez-Ovejero et al., 1978; Ylitalo et al., 1978). On the contrary, whilst indomethacin and naproxen are associated with increases in blood pressure, NSAIDs such as sulindac, aspirin, piroxicam or ibuprofen have negligible effects (Pope et al., 1993). Moreover, in a direct study of the effects of NSAIDs in patients with mild essential hypertension, it was found that ibuprofen increased systolic blood pressure but neither aspirin nor sulindac had any significant effect on systolic or diastolic blood pressure (Minuz et al., 1990). Those clinical effects of different NSAIDs on blood pressure correlate well with our results on the potency of NSAIDs to activate K_{AC}, which induces hyperpolarisation of the smooth muscle cells. Those clinical reports support our hypothesis that the variable effect on blood pressure was due to some NSAIDs, especially non-fenamates, inducing vasodilatation through the mechanism of activating K_{AC}.

The activating effect of NSAIDs on ion channels may be an important therapeutic effect in other parts of the body. Liu et al (2005) reported that diclofenac was able to activate transient outward potassium, I(A), channels in neurons. More recently, it has been reported that diclofenac (1 mg/mL) may exert a "local anaesthetic-like" action by reducing the excitability of muscle nociceptors without involving the opening of K_{ATP} channels (Cairns et al., 2008). The mechanism for such an effect could be hyperpolarisation of the muscle nociceptors by diclofenac activating channels such as those we describe. However, in very high concentrations salicylate (1 mM) applied to rat pyramidal neurons in the auditory cortex was reported to increase the firing rate of neurons and enhance neuronal excitability, with the mechanism apparently to inhibit ion currents including the voltage-gated sodium current, the delayed rectifier potassium current and the L-type voltage-gated calcium current (Liu et al., 2007).

In summary, we describe a large-conductance K^+ channel in smooth muscle cells that is activated by intracellular ATP and Ca^{2+}, and which is activated by some NSAIDs and pinacidil. We have designated this channel K_{AC}, as it does not have the characteristics of the classical ATP-sensitive K^+ channel, and the K_{AC} channels have additional features compared to the previously reported maxi-K channels (Kuriyama et al., 1998). The smooth muscle cells were hyperpolarised by aspirin, an NSAID that potently activated the K_{AC} channel. Both R- and S-flurbiprofen antagonised constrictor responses of the rabbit aorta to PE, suggesting that relaxation occurred via a mechanism other than inhibition of cyclo-oxygenase pathways. We conclude that NSAIDs are potent openers of a Ca^{2+}-activated phosphorylation-dependent potassium channel in vascular smooth muscle cells leading to cell hyperpolarisation and vessel dilatation. The activation of potassium channels is thought to be significant in controlling excitability of vascular smooth muscle cells and regulation of myogenic tone (Brayden & Helson, 1993), an idea that has been corroborated in coronary

arteries (Scornik et al., 1993) and in rabbit aorta (Gelband & McCullough, 1993). The K_{AC} channel that we describe in this paper thus provides a novel target to control excitability of vascular smooth muscle cells and regulate myogenic tone.

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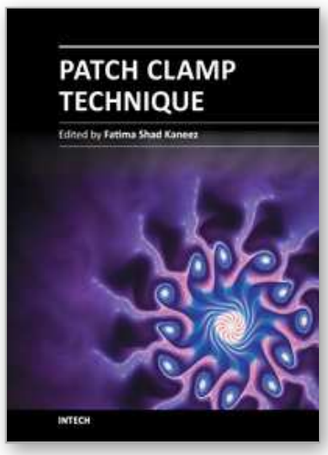
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This book is a stimulating and interesting addition to the collected works on Patch clamp technique. Patch Clamping is an electrophysiological technique, which measures the electric current generated by a living cell, due to the movement of ions through the protein channels present in the cell membrane. The technique was developed by two German scientists, Erwin Neher and Bert Sakmann, who received the Nobel Prize in 1991 in Physiology for this innovative work. Patch clamp technique is used for measuring drug effect against a series of diseases and to find out the mechanism of diseases in animals and plants. It is also most useful in finding out the structure function activities of compounds and drugs, and most leading pharmaceutical companies used this technique for their drugs before bringing them for clinical trial. This book deals with the understanding of endogenous mechanisms of cells and their receptors as well as advantages of using this technique. It covers the basic principles and preparation types and also deals with the latest developments in the traditional patch clamp technique. Some chapters in this book take the technique to a next level of modulation and novel approach. This book will be of good value for students of physiology, neuroscience, cell biology and biophysics.

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