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Flufenamic acid, mefenamic acid and niflumic acid inhibit single nonselective cation channels in the rat exocrine pancreas

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The non-steroidal anti-inflammatory drugs, flufenamic acid, mefenamic acid and niflumic acid, block Ca^{2+}-activated non-selective cation channels in inside-out patches from the basolateral membrane of rat exocrine pancreatic cells. Half-maximal inhibition was about 10 μM for flufenamic acid and mefenamic acid, whereas niflumic acid was less potent (IC_{50} about 50 μM). Indomethacin, aspirin, diltiazem and ibuprofen (100 μM) had not effect. It is concluded that the inhibitory effect of flufenamate, mefenamate and niflumate is dependent on the specific structure, consisting of two phenyl rings linked by an amino bridge.

Mefenamic acid; Flufenamic acid; Niflumic acid; Indomethacin; Non-selective cation channel; Rat exocrine pancreas

1. INTRODUCTION

Recently it was reported that the drug, 3′,5-dichlorodiphenylamine-2-carboxylic acid (DCDPC), blocks non-selective cation channels in the basolateral membrane of rat exocrine pancreatic cells [1]. The authors applied the patch clamp technique to isolated cells and showed that 10 μM of this drug decreased the channel mean open-state probability to about half of its control value, whereas 100 μM of DCDPC blocked the channel completely and reversibly. As the chemical structure of DCDPC is similar to the structure of some non-steroidal anti-inflammatory drugs such as mefenamic acid, flufenamic acid and niflumic acid (Fig. 1), we tested these drugs on nonselective cation channels in cell-excised (inside-out oriented) membrane patches obtained from single cells of the rat exocrine pancreas. We observed inhibition of channel activity by all of these substances. On the other hand, anti-inflammatory substances with different chemical structures, such as indomethacin, aspirin, diltiazem and ibuprofen had no effects on the nonselective cation channel at concentrations of 100 μM.

2. MATERIALS AND METHODS

2.1. *Materials*

Mefenamic acid and niflumic acid were produced in the laboratories of the Hoechst Co. (Frankfurt, FRG). Flufenamic acid, indomethacin, ibuprofen, diltiazem and acetylsalicylic acid (aspirin) were obtained from Sigma (Munich, FRG). The substances were dissolved in dimethylsulfoxide (DMSO, Merck, Darmstadt, FRG, 0.1% of total volume) before addition to the measuring solution. DMSO alone had no effect on the single channel recordings.

2.2. *Methods*

Isolated cells were prepared from rat exocrine pancreati in the laboratory of Professor I. Schulz (Max-Planck-Institute for Biophysics, Frankfurt/Main, FRG) as described previously [2]. The cell-excised (inside-out oriented) mode of the patch clamp technique [3] was applied to the basolateral membrane of isolated acini. Recording of single nonselective cation channels and data analysis was similar as described elsewhere [1,4]. Briefly, patch pipettes were pulled in two steps from borosilicate glass capillaries with a wall thickness of 0.3 mm. Data were recorded with an L/M EPC-7 patch clamp amplifier (List, Darmstadt, FRG) which was remove controlled by an upgrade device [5]. Single channel currents as well as the clamp potential were stored on a video recorder after the signals were digitized with a modified pulse code modulator (PCM 501, Sony, Köln, FRG). The data were analyzed off-line with a computer system (LSI 11/23, Digital Equipment Corporation). After low-pass filtering (4 pole Bessel) with a −3 dB frequency of 0.4 kHz, data were sampled with a sample time of 0.5 ms and were stored on hard disk. The current traces were displayed blockwise on the screen of a digital display module (Hewlett Packard, 1345A). As most patches contained multiples of single channels, the mean open-state probability was calculated in the following way: The baseline level I_o (current where all channels are closed) and the highest occurring current level at a given clamp potential I_{max} were defined under optical control on the display screen. The baseline level was mostly taken from single channel current data where blocker substances were added. The computer evaluated the average current I_{av} and calculated the mean open state probability of each block $P_o^{block} = (I_{av} - I_o)/(I_{max} - I_o)$. Averaging the values for P_o^{block} yielded the mean probability P_o for finding one channel in its open state. The described method to obtain the open-state probability from multiple channel data is fast and independent of the number of active channels in the membrane patch. As in all estimations of P_o from multiple channel data, the assumption has to be made that all channels in the patch have the same individual open-state probability. Previously, evidence was reported that the gating of individual non-selective cation channels is independent of the number of channels in the membrane patch [1].

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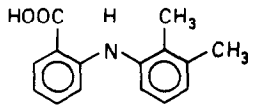
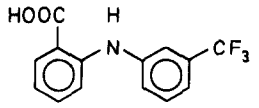
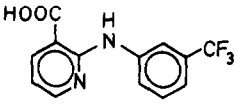


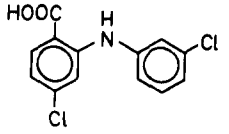
mefenamic acid



flufenamic acid



niflumic acid



DCDPC
3',5-dichlorodiphenylamine-
2-carboxylic acid

Fig. 1. Structural formula of blockers of the Ca^{2+}-activated non-selective cation channel.

In the displayed current traces, single channel currents carried by positive ions moving from the bath into the pipette are depicted as upward (positive) currents. The sign of the clamp potential refers to the bath side with respect to the pipette interior. All experiments were performed at room temperature. The pipette was filled with KCl-solution (in mM): 145 KCl, 1.3 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, pH adjusted to 7.4 with KOH, whereas the bathing medium was NaCl-solution (140 NaCl, 4.7 KCl, 1.3 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, pH adjusted to 7.4 with NaOH). The data are presented as means ± SE of the means.

3. RESULTS

As observed previously, non-selective cations were evoked from rat exocrine pancreatic cells after excision of the membrane patch and after air exposure [1,6]. The non-selective cation channel was the only channel type we observed in this preparation. The single channel conductance was around 27 pS and the number of active channels in a patch varied from 2 up to 10. The channel had similar permeabilities for Na^+ and K^+ ions. The mean open-state probability P_o of one channel was about 0.75 in this series of experiments. Fig. 2a (upper trace) demonstrates a typical experiment under

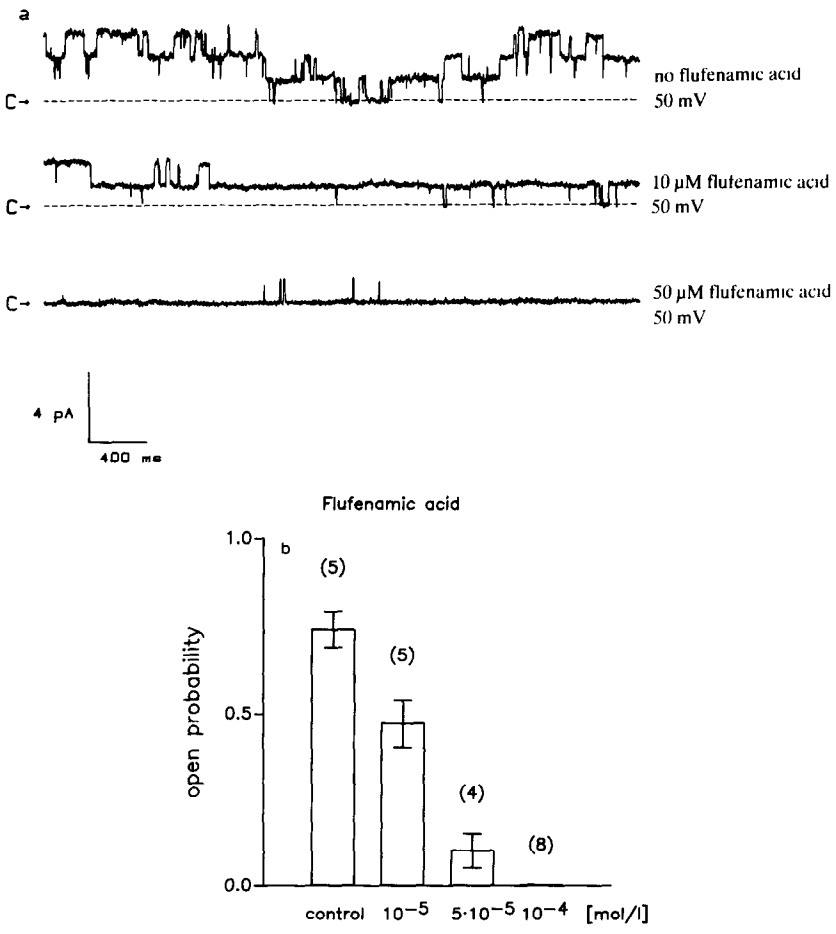


Fig. 2. Blocking effects of flufenamic acid on single non-selective cation channels. (a) Single channel current recordings at a clamp potential of 50 mV. The conditions are given at the right margin of each record. C→ denotes the current, where all channels are closed (baseline). (b) Dependence of the mean open-state probability on the flufenamic acid concentration. The numbers in brackets are the numbers of experiments. The half-maximal inhibitory effect can be estimated to be about 10 μM.

control conditions (KCl-solution in the pipette and NaCl-solution in the bath), where 3 overlapping current levels are discernible. After perfusion of the bath with NaCl-solution plus 10 μM flufenamic acid (second trace in Fig. 2a), the single channel activity decreased significantly, and with 50 μM of flufenamic acid the channels were almost blocked completely. Fig. 2b summarizes the experiments with flufenamic acid, indicating that half-maximal inhibition was reached at a blocker concentration of about 10 μM. The blocking effects were reversible; however it took several minutes to restore channel activity at high blocker concentrations.

Fig. 2 demonstrates the mean open probabilities registered with mefenamic acid (Fig. 2a) and with niflumic acid (Fig. 2b). The half-maximal inhibitory effect is about 10 μM for mefenamic acid and about 50 μM for niflumic acid. The blocking effect of flufenamic acid and mefenamic acid is comparable to that of DCDPC, as reported previously [1].

We also tested some other nonsteroidal drugs with anti-inflammatory action. However, no effect on single channel activity was observed by addition of 100 μM of indomethacin ($n = 3$), diltiazem ($n = 1$), ibuprofen ($n = 2$) or aspirin ($n = 1$) to the bathing solution.

4. DISCUSSION

In the rat and mouse exocrine pancreas the nonselective cation channel is the only one that could be recorded with the patch clamp technique in the basolateral membrane after stimulation of secretion by secretagogues (for review see [7]). However, it remains an unresolved problem how secretion of Na^+ and Cl^- is mediated by these cells. The study of the physiological role of the nonselective cation channel is rendered difficult because no specific blockers were know thus far. It was reported that ATP [8,9] and quinine [1,10] inhibit this channel in the millimolar range. However, it is known that these agents also inhibit K^+ channels [11–14]. Therefore, we were looking for substances which are able to block the nonselective cation channel more specifically and at lower concentrations. Previously it was reported that analogues of diphenylamine-2-carboxylic acid (DPC) are able to inhibit this channel, and that DCDPC was the most potent blocker with an IC_{50} value of about 10 μM [1]. We therefore investigated substances with similar structures as DCDPC and observed that some agents which are known to exert anti-inflammatory effects also act as blockers for the nonselective cation channel. With respect to the specificity of some of these drugs we observed that 10 μM of either DCDPC or mefenamic acid had no effects on Cl^- or on K^+ channels in isolated rat distal colon cells (own unpublished results). On the other hand, it was reported that flufenamic acid inhibits the Cl^- conductance in the basolateral membrane

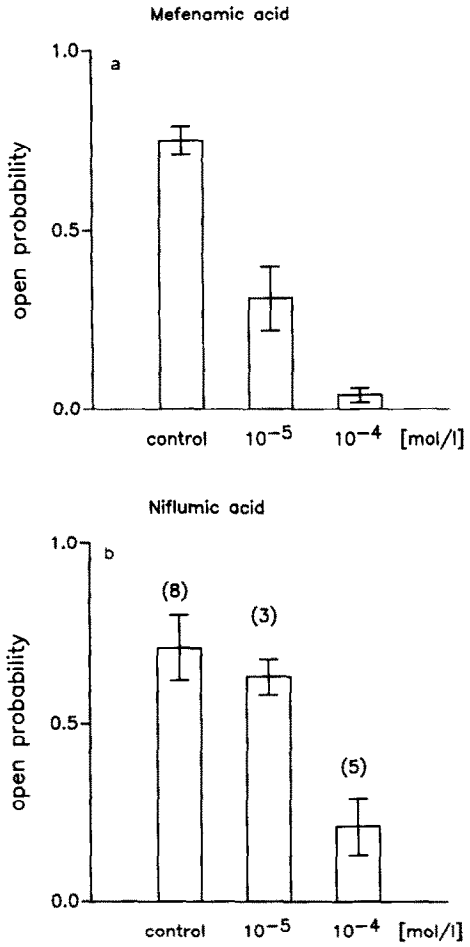


Fig. 3. Summary of the inhibitory effects of mefenamic acid (a) and niflumic acid (b) on the non-selective cation channel. The numbers in brackets are the numbers of experiments. The half-maximal inhibitory effect of mefenamic acid is about 10 μM and that of niflumic acid about 50 μM.

of the thick ascending limb of Henles loop of the rabbit kidney with an IC_{50} value of about 100 μM and that niflumic acid acts on the same preparation with an IC_{50} value of 77 μM [15]. Moreover, it was observed that niflumic acid and flufenamic acid are potent inhibitors of the anion transport in human erythrocytes (IC_{50} values 6×10^{-7} M) ([16,17], reiewed in [18]). It was also reported that flufenamic acid inhibits calcium uptake in isolated mitochondria [19].

The most prominent effects of flufenamate, mefenamate and niflumate are their anti-inflammatory actions, which are thought to be due to inhibition of prostaglandin biosynthesis [20]. On the other hand, it was reported that flufenamic acid failed to decrease prostaglandins in the gastric mucosa [21]. Recently, it was reported that in rat distal colon cells nonselective cation channels could be evoked in cell-attached patches by carbachol, and that these channels were blocked by mefenamic acid [22]. It was also observed, that prostaglandin E2, which is known to stimulate Cl^-

secretion in distal colon cells [23], leads to the activation of nonselective cation channels, which could be inhibited by flufenamate (Siemer and Gögelein, unpublished). Therefore, it is tempting to speculate that part of the anti-inflammatory action of these drugs occurs via the inhibition of nonselective cation channels, which were induced by increased cytosolic prostaglandins.

Other substances with anti-inflammatory actions, such as indomethacin, aspirin, diltiazem and ibuprofen, had no effects on the non-selective cation channel at concentrations of 100 μM. Therefore, it can be concluded that the blocking effects of flufenamic acid, mefenamic acid and niflumic acid are due to their common chemical structure, consisting of two phenyl rings, linked by an amino group. The influence of differences in the side chains has still to be investigated.

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