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Platelet-activating factor activates cardiac G_K via arachidonic acid metabolites

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Platelet-activating factor (PAF), added to the bathing solution, stimulated the cardiac muscarinic K^+ channel (K_{ACh}) in the cell-attached patch (no agonist in the pipette). The PAF-induced K_{ACh} channel activation was blocked by WEB2086, a PAF-receptor inhibitor, indicating that the PAF-receptor mediated the response. PAF-induced activation was prevented by nordihydroguaieretic acid, a lipoxygenase inhibitor, and AA-861, a 5-lipoxygenase inhibitor, but was not affected by indomethacin, a cyclo-oxygenase inhibitor. The PAF-induced K_{ACh} channel activity disappeared upon formation of inside-out patch. In this inside-out patch, intracellular GTP alone induced maximal channel reactivation, which was inhibited by GDP-βS. These results suggest that 5-lipoxygenase metabolites of PAF-released arachidonic acid cause a persistent stimulation of G_K but not the K_{ACh} channel itself, resulting in a receptor-independent activation of the K_{ACh} channel by GTP.

Platelet-activating factor; Arachidonic acid; Lipoxygenase; Cardiac cell; Potassium channel; GTP-binding protein

1. INTRODUCTION

5-Lipoxygenase metabolites of arachidonic acid stimulate the G protein-gated muscarinic K^+ channel (K_{ACh}) in atrial myocytes [1]. Since arachidonic acid is released into the cell in response to chemical and physical stimulation [2,3], arachidonic acid metabolites are possibly common intracellular second messengers to the K_{ACh} channel in a variety of physiological and pathophysiological regulations of cardiac excitation. Platelet-activating factor (PAF) is a phospholipid involved in the pathogenesis of various inflammatory diseases and in the lethality of anaphylactic shock [4,5]. Since it is known that PAF releases arachidonic acid in various tissues [4,5], we hypothesized that PAF may activate the K_{ACh} channel via the lipoxygenase pathway of arachidonic acid. We found that PAF activated the K_{ACh} channel which is blocked by 5-lipooxygenase inhibitors. The PAF-induced activation of the channel clearly depended on intracellular GTP, suggesting that the target of the arachidonic acid metabolites is G_K and not the channel itself. Lipoxygenase metabolite-induced/receptor-independent stimulation of G_K-like proteins ($G_{i/o}$) may underlie PAF-induced cardiac dysfunction [6].

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2. MATERIALS AND METHODS

Single atrial cells were isolated from guinea-pig heart by enzymatic dissociation as described previously [7]. All experiments were performed at 32–36°C. The patch clamp technique was used in the cell-attached and inside-out patch configurations. The pipette solution contained (in mM): KCl 145, $CaCl_2$, 1, $MgCl_2$, 1, HEPES-KOH buffer 5 (pH 7.4), atropine 0.01, and theophylline 0.1. The pipette solution contained high K^+ to increase the single channel conductance of the K_{ACh} channel. The bathing solution was (in mM): NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5, HEPES-NaOH buffer 5 (pH 7.4). The internal solution was (in mM) KCl 140, $MgCl_2$ 0.5, EGTA 5, HEPES-KOH 5 (pH 7.3). PAF, lyso PAF and pertussis toxin (PTX) were purchased from Funakoshi Chemicals (Tokyo, Japan). PAF and lyso-PAF were dissolved in the bathing solution containing 0.1% BSA. Indomethacin, nordihydroguaiaretic acid (NDGA), baicalein, neomycin, 4-bromophenacyl bromide (4-BB) and GTP were purchased from Sigma (St. Louis, MO). GTP-γS and GDP-βS were from Boehringer Mannheim (Germany). WEB2086 and AA-861 were gifts from Boehringer Ingelheim (Germany) and Takeda Ltd. (Osaka, Japan), respectively. Concentrated stock solutions of inhibitors in ethanol were prepared fresh each day and kept at 4°C. The final concentration of ethanol was less than 0.5%, which did not affect the PAF-activation of the K_{ACh} channel.

3. RESULTS

Figure 1 shows the currents recorded from a cell-attached patch from an atrial myocyte before and after exposure to PAF. Under control conditions, channel openings are infrequent in the absence of agonists like acetylcholine (ACh) or adenosine in the pipette [7]. After addition of PAF (5 μg/ml) to the bath, channel openings increased after a delay (0.5–1 min) and reached steady levels within 3–5 min (Fig. 1a). The

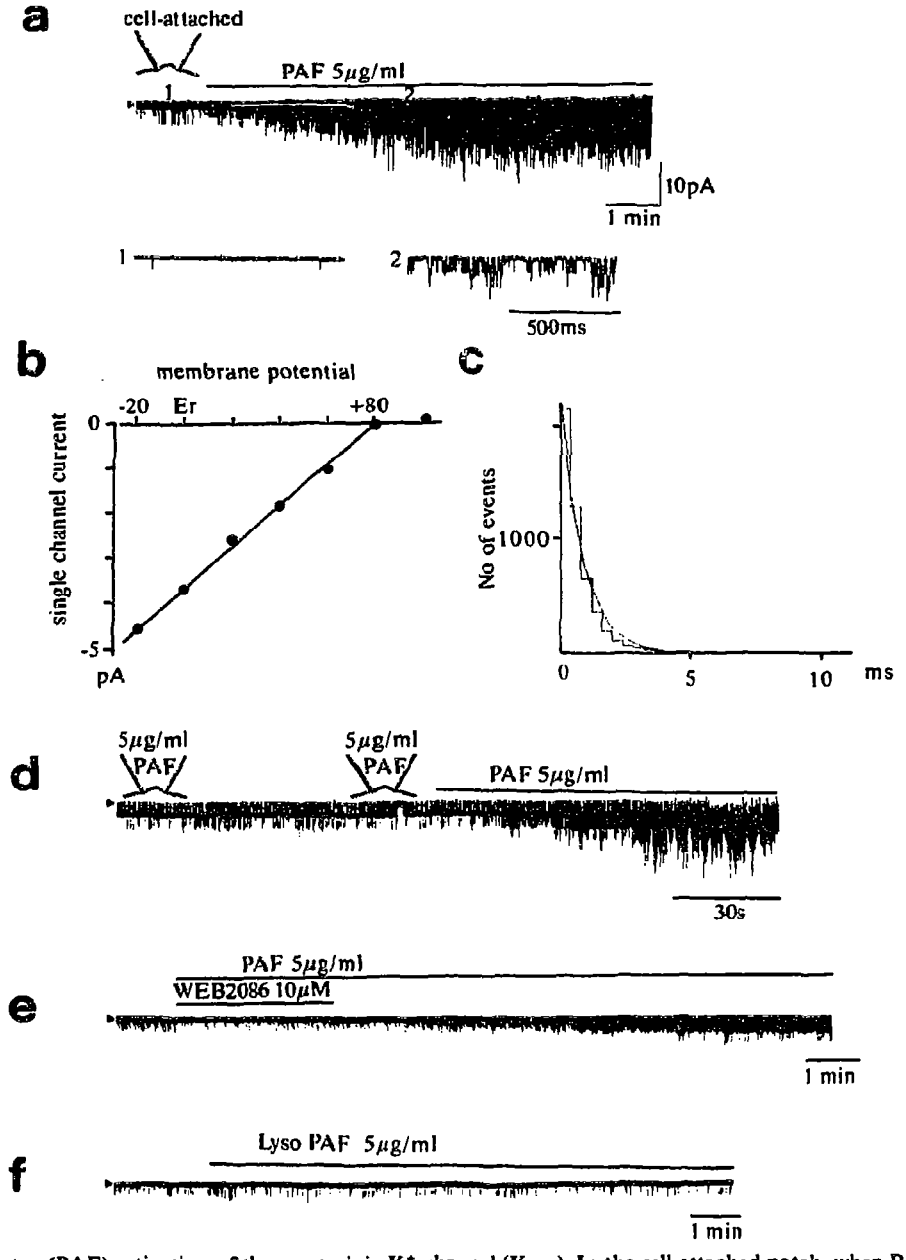

Fig. 1. Platelet-activating factor (PAF) activation of the muscarinic K^+ channel (K_{ACh}). In the cell-attached patch, when PAF applied to the bath activated a K^+ channel (a). The membrane potential of the patch was held at the resting potential (Er) level. The Er of the cell in this case was −−80 mV. The locations of the expanded current traces (1,2) are indicated in the upper trace. Current–voltage relation (b) and open-time histogram at Er (c) of the PAF-induced K^+ channel. The unitary conductance = 46 pS. τ_0 = 0.9 ms. (d) PAF added to the pipette solution did not cause activation. The subsequent application of PAF in the bath caused channel activation. (e) The PAF-receptor antagonist, WEB2086 prevented PAF-activation of the channel. (f) Lyso PAF did not activate the channel.

steady state activity of the channel ($N \cdot P_o$) increased from 0.0006 ($N \cdot P_o$ of the background activity; $N \cdot P_{o,back}$) to 0.1365 in Fig. 1a. In 5 experiments, $N \cdot P_o$ increased to 27.5 ± 8.3 times $N \cdot P_{o,back}$ (means ± SD) with 0.05 μg/ml, 70.3 ± 32.8 with 0.5 μg/ml and 132.6 ± 80.3 with 5 μg/ml of PAF, respectively. Channel openings persisted for more than 10 min after washout of PAF. The PAF-activated K^+ channel showed strong inward-rectification with a conductance of 45–50 pS in symmetrical 150 mM K^+ (Fig. 1b). The open time histogram of the channel could be fitted by a single exponential curve with a time constant of ~1 ms (Fig. 1c). These properties identify this channel as the ACh- or adenosine-activated K_{ACh} channel [7].

PAF, added to the pipette solution, did not affect the K_{ACh} channel (Fig. 1d). Subsequent bath-application of PAF activated the K_{ACh} channel in the same cell-attached patch without affecting i_{K1}, the other cardiac

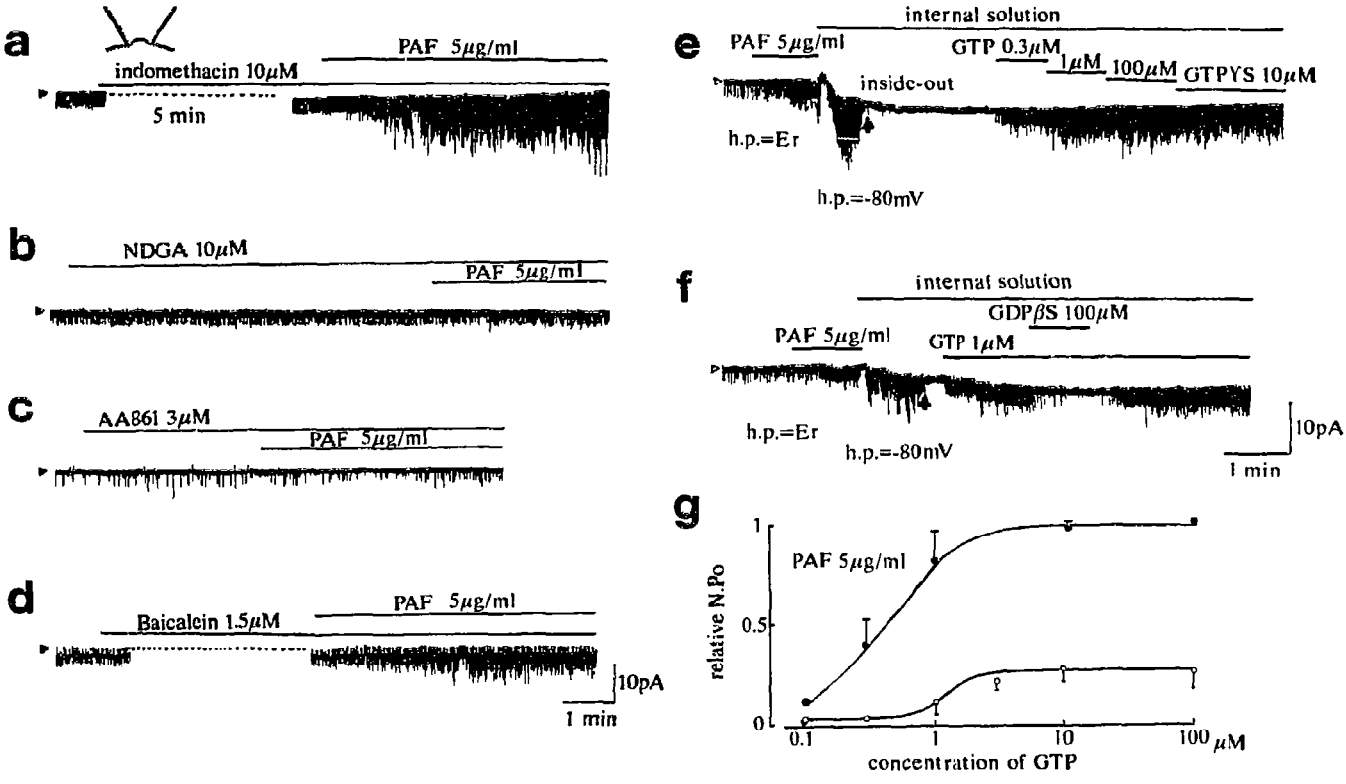

Fig. 2. The effects of inhibitors of the lipoxygenase and cyclooxygenase pathways (a–d) and the GTP-dependence of the PAF-induced channel activation (e–g): (a) Indomethacin enhanced the PAF-activation of the K_{ACh} channel. (b,c) NDGA and AA861 blocked activation of the K_{ACh} channel. (d) Baicalein did not affect activation. These inhibitors (indomethacin, NDGA, AA861 and baicalein) suppressed neither the ACh/adenosine-induced activation of the K_{ACh} channel in the cell-attached patches nor intracellular GTP-γS-induced activation of the channel in the inside-out patches at these concentrations. (e) After activation of the K_{ACh} channel by PAF reached a steady level, the patch was excised in the internal solution, yielding an 'inside-out' patch (arrow). The channel openings disappeared quickly in the inside-out patch but reappeared upon application of GTP (0.3 and 1 μM). In the PAF-pretreated patches, GTP (1 μM) almost fully activated the K_{ACh} channel. (f) The GTP-induced channel openings in the PAF-pretreated patches were inhibited by GDP-βS. (g) Concentration-dependent effect of GTP on the K_{ACh} channel in the PAF-pretreated (●-●) and untreated (○-○) cell membrane. The symbols and bars are the means + SD obtained from three (PAF-treated) and seven (untreated) different patches. The relative $N \cdot P_o$ of the K_{ACh} channel at each concentration of GTP was obtained with reference to the maximum channel activity achieved by 10 μM GTP-γS in each patch.

inward-rectifier K^+ channel observed in this patch (a mean open time of ~80 ms at the resting potential which is ~80 mV from the K^+ equilibrium potential [8]). PAF-induced K_{ACh} channel activation was antagonized by 10 μM WEB2086, a PAF-receptor antagonist (n=5; Fig. 1e) [9], indicating that PAF-receptor mediated theK_{ACh} channel activation. Consistent with these results, lyso-PAF, the inactive form of PAF, failed to stimulate the K_{ACh} channel in cell-attached patches (n=4, Fig. 1f).

Figure 2a–d shows the effects of various inhibitors of arachidonic acid metabolism on the PAF-induced activation of the K_{ACh} channel. Indomethacin (10 μM), a cyclooxygenase inhibitor, failed to affect the PAF-induced activation of the K_{ACh} channel (n=5, Fig. 2a). In contrast, NDGA (5 –10 μM), the lipoxygenase inhibitor, prevented the PAF-induced channel activation (n=10, Fig. 2b). Therefore, lipoxygenase but not cyclooxygenase metabolites of arachidonic acid may be involved in channel activation. In Fig. 2c and d, we examined the effects of AA-861 (3 μM), a 5-lipoxygenase inhibitor [12], and baicalein (1.5 μM), a 12-lipoxygenase inhibitor [13], on PAF-induced channel activation. PAF could not activate the K_{ACh} channel in AA-861-treated cells (n=5), but activated the channel in the baicalein-treated cells (n=5). Similar effects of these inhibitors were observed in arachidonic acid- and α-adrenergic activation of the K_{ACh} channel [1,14]. We conclude, therefore, that the 5-lipoxygenase of arachidonic acid are involved in the PAF-induced activation of the K_{ACh} channel.

In Fig. 2e–g, we examined the GTP-dependence of the PAF-induced activation of the K_{ACh} channel. The PAF-induced K_{ACh} channel activation observed in cell-attached patches disappeared upon formation of inside-out patches (Fig. 2e). Neither ACh nor adenosine were present in the pipette solution. Yet, upon application of low concentrations (0.1–1.0 μM) of intracellular GTP, channel activity reappeared. Maximal channel activity was induced by 1–10 μM GTP in these PAF-treated patches, which was inhibited by GDP-βS (Fig. 2f). Fig-

ure 2g shows the relative activity of the K_{ACh} channel at various concentrations of GTP in both PAF-pretreated and untreated inside-out patches. The GTP-induced activation of the K_{ACh} channel was clearly enhanced by pretreatment of the cells with PAF.

The above observations suggest that the 5-lipoxygenase metabolites of arachidonic acid released by PAF stimulate the basal GDP/GTP exchange of G_K in a receptor-independent manner. Alternately, these metabolites may bind to membrane receptors which are coupled to the K_{ACh} channel. When either PAF or lipoxygenase metabolites of arachidonic acid, e.g. leukotriene A_4 and C_4, were added to the pipette solution, there was no activation of the K_{ACh} channel for up to 10 min (Fig. 1d). This observation rules out the latter hypothesis while supporting the former hypothesis.

To elucidate the coupling between the PAF-receptor and arachidonic acid release, we examined the effects of PTX, 4-BB (a phospholipase A_2 inhibitor) and neomycin (a phospholipase C inhibitor) on the PAF-induced activation of the K_{ACh} channel (Fig. 3a–c). In the PTX-treated atrial cells, ACh or adenosine added to the pipette solution could not activate the K_{ACh} channel in the cell-attached patches (not shown, see [7]), suggesting that PTX ADP-ribosylated G_K and uncoupled the m-ACh and adenosine receptors from G_K in the PTX-treated cells [15,16]. As shown in Fig. 3a, PAF added to the bath solution also failed to activate the K_{ACh} channel in cell-attached patches of the PTX-treated cells. However, since arachidonic acid, applied to the bath, activated the K_{ACh} channel via its lipoxygenase metabolites in the cell-attached patches of the PTX-treated atrial cells [1], the PAF-released arachidonic acid metabolites themselves probably can stimulate the G_K uncoupled from mACh and adenosine receptors. Therefore, inhibition of the PAF-induced activation of the K_{ACh} channel in the PTX-treated cells may indicate that PTX prevents the arachidonic acid release induced by PAF in cardiac atrial cells. Since PTX ADP-ribosylates and uncouples a certain class of G proteins from their receptors [15,16], these results suggest that PTX-sensitive G proteins transduce the signals which cause arachidonic acid release upon stimulation of PAG-receptor in atrial myocytes. Arachidonic acid can be produced from cell membrane phospholipids either by phospholipase A_2 (PLA_2) or by the sequential actions of PLC and diglyceride lipase [2,17]. PAF-activation of the K_{ACh} channel was prevented by 10 μM 4-BB (Fig. 3b, n=5) but not by neomycin (100–300 μM, n=5). These results suggest that PLA_2 is involved in the PAF-induced release of arachidonic acid in cardiac atrial myocytes.

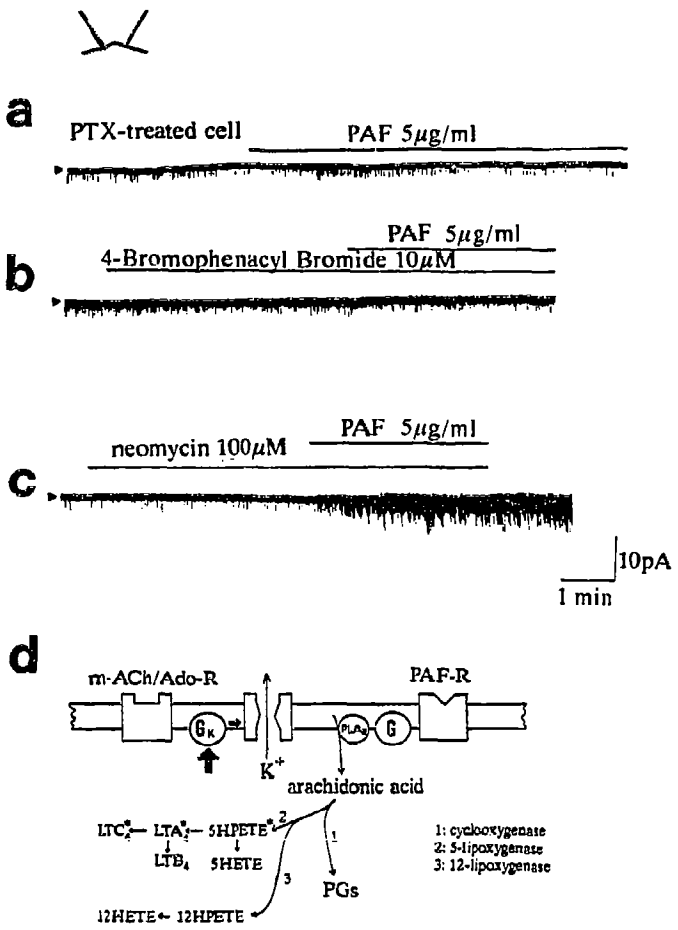

Fig. 3. Signalling from PAF-receptor to arachidonic acid release. (a) PAF could not activate the K_{ACh} channel in the PTX-treated atrial cells. The cells were preincubated in a bathing solution containing PTX (500 ng/ml) for 6 h at 37°C. (b) 4-BB prevented the PAF-induction of the K_{ACh} channel activation. (c) Neomycin did not affect the PAF-induced channel activation. (d) The proposed signalling pathway underlying the PAF-induced activation of the K_{ACh} channel in cardiac atrial myocytes. The asterisks indicate the 5-lipoxygenase substances which activated the K_{ACh} channel in the former study [1].

4. DISCUSSION

The present study indicates that the regulatory mechanisms for the G_K–K_{ACh} channel system by arachidonic acid metabolites play an important role in the action of PAF on cardiac cells [1,10,11]. Based on the present results, the molecular mechanisms underlying PAF-activation of K_{ACh} channel may be summarized as shown in Fig. 3d: PAF binds to the PAF-receptor, which is linked with PLA_2 via PTX-sensitive G proteins. Activation of PLA_2 causes the release of arachidonic acid. The released arachidonic acid is metabolized by 5-lipoxygenase. The 5-lipoxygenase metabolites such as leukotrienes (LTs) cause long-lasting stimulation of the basal turn-over of G_K, resulting in openings of the K_{ACh} channel. Consistent with the present electrophysiological results, Piper and Stewart [18,19] showed that PAF increased the production of LTC_4 in rat and guinea-pig hearts.

Since PAF is produced in ischemic reperfusion and

sensitized hearts [20,21] and PAF-receptor-antagonists prevent the reperfusion- and anaphylaxis-induced cardiac dysfunction and arrhythmia [5,22,23], the arachidonic acid metabolite-induced modulation of the G protein could be involved in the pathogenesis of ischemia and anaphylaxis-induced cardiac damage.

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