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ACTIVATION OF K^+ CURRENT IN MACROPHAGES BY PLATELET ACTIVATING FACTOR

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Puff application of platelet activating factor (10^{-8} M) onto peritoneal macrophages from thioglycollate-stimulated mice induced an outward current at a holding potential of -63 mV. The current was suppressed by an antagonist Y-24180 but not by CV-3988. Charybdotoxin (10^{-6} M) suppressed the current. Reversal potentials were dependent on external K^+ concentrations. The current was not suppressed in Ca^{2+}-free EGTA-containing solution but was completely abolished in BAPTA-AM containing solution. These results suggest that platelet activating factor activates a Ca^{2+}-dependent K^+ channel.

Platelet activating factor (PAF) possesses potent biological activity in leukocytes which is related to pathological process such as anaphylaxis and inflammation (1), including aggregation (2), chemotaxis (3), spreading (4), superoxide anion production (2,4), and release of prostaglandin E and thromboxane B_2 (4) in monocytes or macrophages. Intracellular responses of macrophages to PAF are consumption of glucose (5), break-down of polyphosphoinositides (3), release of Ca^{2+} from intracellular stores and influx of exogenous Ca^{2+} (3,6,7).

Several ionic currents have been demonstrated in macrophages by electrophysiological methods (8-12). There are voltage-gated and Ca^{2+}-gated ionic channels, including 4 K^+-channels, 3 Cl^--channels and a nonselective cation channel. However, the role of those ionic channels in leukocytes remains to be clarified. As actions of PAF in macrophages are well studied, it is very valuable to examine the effects of PAF on ionic channels. The present study was undertaken to analyze the PAF-induced current by means of whole cell patch-clamp method.

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MATERIALS AND METHODS

Cells

Elicited macrophages were produced from C3H/Heslc mice (Shizuoka Dobutsu, Shizuoka, Japan) of either sex (5-10 wk old) by intraperitoneal injection of 3 ml of 3% thioglycollate medium (Nissui. Pharmac. Tokyo, Japan). The cells were plated on 15-mm cover slips in 35-mm culture dishes (Becton Dickinson NJ, U.S.A.) and cultured in medium 199 (Nissui) containing 10% FBS (Boehringer, Mannheim, Germany) and antibiotics (100µg/ml streptomycin and 100 units/ml penicillin G) at 37 °C. The cells were studied 3-20 days after plating.

Electrophysiology

Cover slips with adherent cells were transferred into a recording chamber (0.2 ml). The chamber was superfused at a rate of 0.3 ml/min with normal bath solution containing (in mM); 140 NaCl, 5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, pH 7.4. Pipettes (0.5-1.5 MΩ resistance in 3 M KCl) were filled with high K solution containing (in mM); 145 K-aspartate, 1 $MgCl_2$, 0.1 EGTA, 10 HEPES, pH 7.4. Whole-cell currents were recorded using a List EPC-7 patch-clamp amplifier (Darmstad, Germany) at room temperature (20-23 °C). Access resistance to the cell's interior was 10-20 MΩ. PAF was applied by diffusion from a puff pipette. The puff pipette, filled with 10^{-8} to 10^{-10}M PAF, was placed at 20-40µm from the cell recorded from. The liquid junction potential between the pipette and the bath solution was +12.6 mV.

Materials

PAF was purchased from Avanti Polar Lipids, AL, U.S.A.; CV-3988 from Biomol Res. Lab., Plymouth Meeting, PA, U.S.A.; charybdotoxin (ChTX) from Peptide Inst., Osaka Japan; BAPTA-AM and HEPES from Dojin, Kumamoto, Japan; tetraethylammonium (TEA) from Wako, Osaka, Japan; EGTA and penicillin G from Sigma, St. Louis, MO, U.S.A.; streptomycin from Meiji Seika, Tokyo, Japan. Y-24180 was kindly provided by Yoshitomi Pharmac., Fukuoka, Japan.

RESULTS

PAF induced an outward current (I_o(PAF)) when cells were voltage clamped at -63 mV (Fig. 1A). Two typical responses were observed upon PAF application; one a single outward current (Fig. 3Bc) and the other an oscillating outward current (Fig. 3Ba). Some cases were a mixed type (Fig. 1Aa), i.e. a large outward current followed by an oscillating current. The reason that two different responses were induced by PAF is not yet clear. PAF (10^{-10} M) induced I_o(PAF) in 6 out of 9 cells. At much higher concentrations than $5x10^{-10}$ M, PAF induced I_o(PAF) in 93% of cells (319/341 cells). Repetitive application gradually reduced responses (Fig. 1A) and prolonged application (30-60 sec) induced single response which recovered to baseline level during the application (12 out of 12 cells), suggesting receptor desensitization. To characterize the receptor, specific antagonists were examined (Fig. 1B). Unexpectedly, puff application of CV-3988 (10^{-6} M) alone induced PAF-like action,

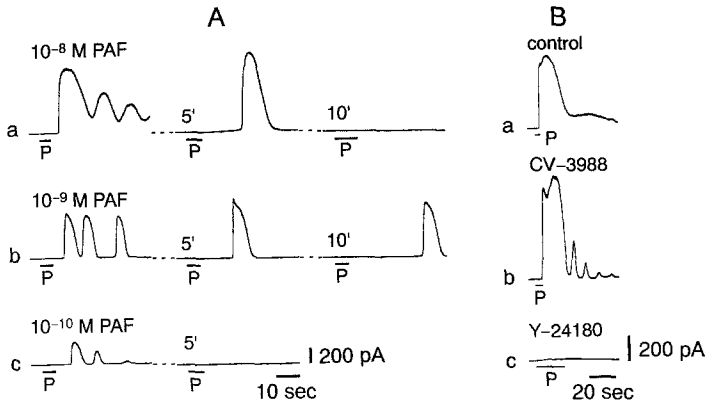

Figure 1. A. Dose response of outward currents to PAF. a. 10^{-8} M PAF was applied at 5 min interval 3 times, the first response oscillated, the second was a single response and the third application failed to induce an outward current. Numbers on second and third traces show the time after first application. In this and subsequent figures, the duration of PAF application is indicated by the bar with "P" below each record. b. 10^{-9} M PAF induced outward current 5 min interval. Note that delays of responses to PAF were gradually prolonged in the second and third applications. c. 10^{-10} M PAF induced smaller amplitude response and second application did not induce any current. B. Effects of antagonists. a. Control outward current by PAF 10^{-8} M. b. PAF induced larger outward current during application of the CV-3988 containing solution. c. During perfusion of Y-24180 containing solution, PAF failed to induce an outward current.

i.e., inducing oscillating outward current (12 out of 23 cells). When another antagonist, Y-24180 (10^{-6} M), was puff applied, no agonist-like effect was observed (11 out of 11 cells). During the perfusion of Y-24180 (10^{-7} M), I_o(PAF) was potently suppressed to 129 ± 126 pA (mean ± S.E., n=10), compared to a control amplitude of 712 ± 130 pA (n=8). On the contrary, CV-3988 (10^{-7} M) had no such effect on I_o(PAF) (735 ± 339 pA, n=10).

To characterize ionic mechanisms of I_o(PAF), the voltage dependency of I_o(PAF) was examined (Fig. 2A). PAF induced an outward current at -53 and -73 mV (n=6). When clamped to -83 mV, the current level did not change (n=5), but at the more hyperpolarized level of -93 mV, the current changed to an inward current (n=10). By extrapolating the voltage dependency of I_o(PAF), the reversal potential of I_o(PAF) in normal solution was estimated to be -82.1 ± 5.3 mV (n=21), indicating that I_o(PAF) was mainly carried by K^+. To confirm this, external K^+ was changed to 2.5, 10, 25 and 50 mM (Fig. 2B). An increase in external K^+ concentrations from 2.5 to 10 mM raised the reversal potential from -84.5 ± 3.4 (n=11) to -50.4 ± 1.5 (n=8). This increase is same value as the theoretical difference of 35 mV, assuming that an internal K^+ concentration is 128 mM (13).

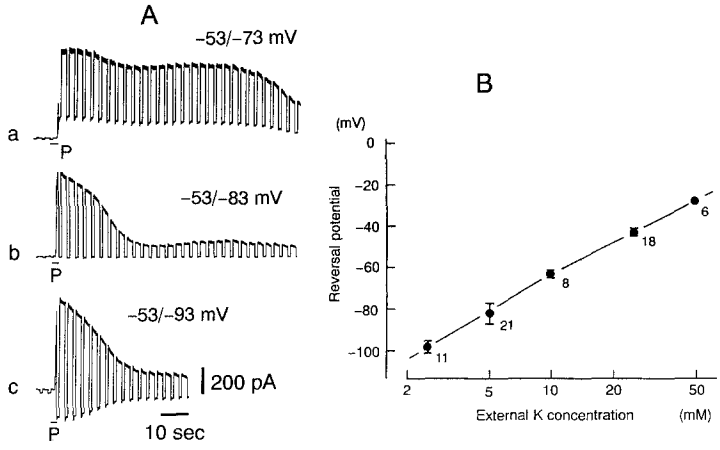

Figure 2. A. The cells were voltage-clamped from -53 mV to -73 (a), -83 (b) and -93 mV (c) by command pulses (1 sec duration, 3 sec interval). a. Current levels at voltage of -53 and -73 mV were outwardly shifted by PAF. b. Current level of -83 mV was not changed after PAF application, but level at -53 mV was greatly outward shifted. c. Current level at -93 mV was inwardly shifted by PAF application. B. Reversal potentials on different K^+ concentrations. Numbers at each point were numbers of experiments. Standard deviation at 50 mM K^+ was smaller than the circle.

Next, the effects of Ca-dependent K^+-channel (K_{Ca}) blockers (TEA, ChTX) were examined on I_o(PAF) (Fig. 3A). TEA (10^{-2} M) accelerated the recovery of I_o(PAF) to baseline (n=7). Application of the bath solution did not have any effect (n=6), so TEA partially blocked I_o(PAF). ChTX (10^{-6} M) markedly suppressed I_o(PAF) returning it to baseline immediately after its application (n=8). The effects of extracellular and intracellular Ca^{2+} levels on I_o(PAF) were examined (Fig. 3B). In Ca^{2+}-free solution containing EGTA (1 mM), PAF induced an I_o(PAF) (399 ± 329 pA, n=14) smaller than that (570 ± 279 pA, n=6) in normal solution. In Ca^{2+}-free solution containing EGTA and BAPTA-AM, which chelates the intracellular Ca^{2+}, PAF did not induce I_o(PAF) (17 out of 17 cells) within 10 min after exchanging the bath solution, compared with the control amplitude of 596 ± 227 pA (n=8).

DISCUSSION

Present results suggest that PAF activates the Ca-dependent K^+ current ($I_{K,Ca}$) in a macrophage. Because of the small effect of Ca^{2+}-free, EGTA solution and sensitivity to BAPTA-AM containing solution, intracellular Ca^{2+} store is important for I_o(PAF). PAF produces inositol 1,4,5-trisphosphate (3), which may

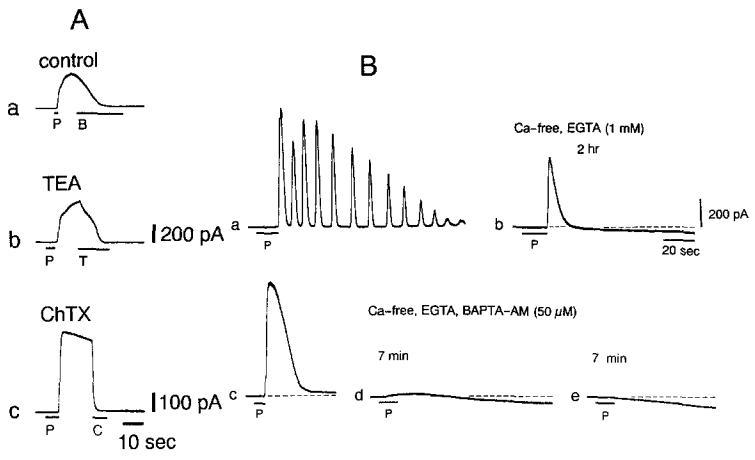

Figure 3. A. Effects of Ca^{2+}-dependent K^+ channel blockers. a. control response to PAF (10^{-8} M). Bar-B shows that application of the bath solution did not produce any effect. b. TEA (10^{-2} M) accelerated the recovery phase, applied at the bar-T. c. Charybdotoxin (10^{-6} M) markedly suppressed outward current, shown in the bar-C. B. External and internal Ca^{2+} dependency of PAF responses. a. Control PAF response. b. Even in Ca-free with EGTA (1 mM) solution, PAF induced an outward current, following an inward shift. Because it is impossible to record repetitive and reproducible responses in single cell, PAF-induced outward current in a and b were recorded from cells in the same culture dish at least in same cultural condition. c. Control response to PAF. d. PAF response was completely suppressed in Ca^{2+}-free with EGTA (1 mM) and BAPTA-AM (50 μM) solution 7 min after perfusion. e. Same condition as d but 7 min after immersing the cell into the chamber. c, d and e were recorded from cells in same culture dish.

induce Ca^{2+} release from storage sites. The increase in intracellular free Ca^{2+} by PAF was observed by using fura-2 or quin-2 (3,6,7). Although none of them reported the oscillation of intracellular Ca^{2+} concentration by PAF, PAF sometimes activated an oscillating outward current in the present study. The mechanisms of oscillating I_O(PAF) remains to be study.

Two K_{Ca} channels, having single channel conductance of 240 ($K_{L,Ca}$) and 36 pS ($K_{i,Ca}$), are known in human macrophages (9). ATP activates K_{Ca} (25 pS) in mouse peritoneal macrophages (10). A single channel conductance of 36 pS, which is the same conductance as the ionomycin-induced Ca^{2+}-activated single channel, was observed by application of PAF in 8 patches of human monocytes (8). Because $K_{L,Ca}$ is active at depolarized voltage (>40 mV) and $K_{i,Ca}$ is independent of voltage, $I_{K,Ca}$ observed here may be a small conductance K_{Ca}. PAF enhances A23187-induced and qunidine-sensitive K^+ efflux measured in macrophages, which is inhibited by the PAF antagonist BN52021 (14). The present study suggests that the K^+ efflux is caused by $I_{K,Ca}$.

When macrophages are placed on a glass surface, the cells exhibit periodic increases of intracellular Ca^{2+} concentrations following cell spreading, which may be related to phagocytosis (15). Such oscillation of Ca^{2+} concentrations are consistent with the present $I_{K,Ca}$ induced by PAF. Because PAF activates aggregation (2), chemotaxis (3), spreading (4), superoxide anion release (2,4), present results suggest that $I_{K,Ca}$ might be related to macrophage activation. Quinine reduces $I_{K,Ca}$ and also inhibits the chemi-luminescence response (parameter of phagocytosis) and leukotriene B_4 release (mediator of asthma), suggesting that $I_{K,Ca}$ is related to the release of inflammation mediators from macrophages (12).

CV-3988, a structural analog, which inhibits aggregation of platelets by PAF (16) and inhibits the specific binding of PAF to platelets (17), had an agonistic action on macrophages. On the other hand, another antagonist (Y-21480) whose structure is related to the anti-anxiety drug (18, 19), etizolam, had no such effect. Because an important role of PAF in various acute allergic and inflammatory reactions has been suggested and various antagonists were reported (1), the present study suggests that the effect of the individual antagonist may depend on target tissues, requiring care when used in treating disorders causally related to PAF.

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