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Platelet-activating factor is a general membrane perturbant

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Platelet-activating factor (PAF) is, at physiological (nanomolar) concentrations, a potent mediator of inflamation and coagulation. At pharmacological (micromolar) concentrations, PAF induces a variety of effects in diverse tissues. Here we show that PAF at micromolar concentrations is a membrane perturbant. Micromolar PAF alters the properties of channels formed by gramicidin A, and at concentrations $\geqslant 4\ \mu M$ disrupts the barrier properties of the host lipid bilayer. PAF thus can act as a detergent and non-specifically alter the behavior of membranes and membrane proteins. This may provide an explanation for some of the effects of PAF seen at high concentrations in vitro.

Platelet-activating factor (PAF) is an alkyl-ether phospholipid: 1-*O*-alkyl-1-2-acetyl-*sn*-glycero-3-phosphocholine [1,2]. It was first described as a 'fluid phase mediator' of the interaction between leukocytes and platelets [3], and it specifically activates, at nanomolar concentrations, pathways involved in coagulation and inflamation [4,5]. At higher concentrations, PAF produces a variety of other effects: 50 nM to approx. 3 μM PAF induces differentiation of cultured neurons [6]; 1 to 10 μM PAF activates macrophages [7,8]; and at concentrations $> 3\ \mu M$, PAF is cytotoxic and causes neuronal death [6]. The molecular and cellular mechanisms underlying these actions are not known. We were struck, however, by the structural similarity between PAF and lysophosphatidylcholine (1-acyl-*sn*-glycero-3-phosphocholine), which like many other detergents induces changes in the molecular behavior of channels formed by the linear gramicidins [9]. Indeed, lysophosphatidylcholines at micromolar concentrations cause lysis of red blood cells and decrease the resistance of planar lipid bilayers [10]. The present study was therefore undertaken to test whether PAF could alter the behavior of lipid bilayers and their resident membrane-spanning channels. If so, it might help to explain some of the effects of PAF at pharmacological concentrations.

Given that we were testing for non-specific (detergent-like) effects we chose the planar lipid bilayer and the channel-forming pentadecapeptide gramicidin A to assay for these PAF effects. Gramicidins form cation-selective channels by combining as head-to-head symmetrical $\beta^{6,3}$-helical dimers that can span lipid bilayers [11]. The sensitivity of these prototypical channels to changes in membrane structure makes them an excellent tool to study general membrane perturbations by hydrophobic and amphipathic molecules [9].

Gramicidin single channel currents (Fig. 1) were recorded using the bilayer punch technique [12]. At 0.2 μM, PAF produced minimal changes in channel behavior. At 1.0 and 2.0 μM, PAF altered all aspects of gramicidin channel behavior: appearance rate, channel duration, mean single channel conductance, and conductance heterogeneity (Fig. 1). The most pronounced effects were the increases in channel appearance rate and duration, which produce an approximately 50-fold increase in channel activity at 1 μM PAF.

The increase in channel duration was quantified in survivor plots in the absence and presence of 1.0 and 2.0 μM PAF (Fig. 2). The survivor plots were fitted by single exponential decays. The average channel duration was 500 ms in the absence of PAF. At 0.2 μM PAF, the channel duration increased slightly (to 640 ms, results not shown). But, at 1 μM PAF, the duration more than doubled (to 1200 ms), and doubled again (to 2100 ms) at 2 μM PAF.

The changes in mean channel conductance and conductance heterogeneity were quantified in current transition amplitude histograms (Fig. 3). In the absence of PAF, 93% of the channels fell within the main peak in the histogram. In the presence of 1 μM PAF, only 82% of the channels were in the main peak, while the remaining channels had current amplitudes that were substantially smaller than those in the main peak. We

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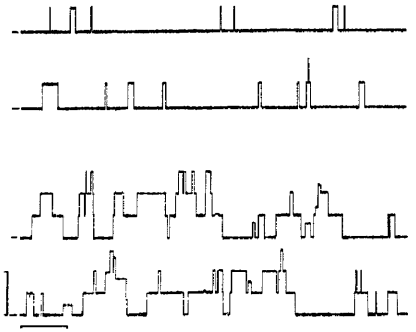

Fig. 1. Gramicidin A channel current traces before and after addition of 1 μM PAF to the aqueous phase. Upper two traces: control, before PAF addition. Lower two traces: after 1.0 μM PAF addition. Current traces were filtered at 50 Hz. The vertical calibration bar represents 2 pA; the horizontal calibration bar, 5 s. Upward current transitions denote channel appearances. PAF addition increases the channel appearance rate and the channel duration. Channel appearance rates (determined as averages over several minutes of recording) rose from approx. 0.2 s^{-1} to approx. 4 s^{-1}. In both upper and lower traces, most channels have quite uniform amplitudes. Note, however, that PAF addition decreases the amplitude of the single-channel current steps, and increases the frequency of 'mini' channels, channels with current amplitudes significantly smaller than standard channels. Experimental: The experimental procedures have been described previously [12]. In brief, gramicidin channels were observed at 100 mV applied potential across bilayers formed from diphytanoylphosphatidylcholine (Avanti Polar Lipids) in *n*-decane (Wiley Organics) separating two Teflon chambers containing unbuffered 1.0 M NaCl. Small aliquots of doubly HPLC-purified gramicidin A (a gift from Dr. R.E. Koeppe II), dissolved in ethanol (concentration 10 nM), were added to the electrolyte solution during vigorous stirring to achieve aqueous concentrations of approx. 10 pM. PAF (L-α-phosphatidylcholine-β-acetyl-γ-*O*-alkyl (Calbiochem) or synthetic 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, a gift from Dr. R. Bittman) dissolved in ethanol (0.2 or 2 mM) was then added to the aqueous phase to achieve nominal concentrations of 0.2, 1.0, 2.0, or 4.0 μM.

refer to these channels as 'mini' channels [9]. For the channels in the main peak, addition of 1 μM PAF reduced the mean current amplitude by 10%. Increasing the PAF concentration to 2 μM caused a further 5% decrease in current amplitude but produced no further increase in mini channel frequency (results not shown).

When the concentration of PAF was increased to 4.0 μM, gramicidin channels could not be seen, because the membrane conductance increased by several orders of magnitude. Consequently, the membrane current was so large that the recording system was saturated at the potential used to record gramicidin single-channel currents. The potential was reduced to determine the mechanism(s) underlying the increase in membrane conductance. Two types of conductance were observed: a noisy

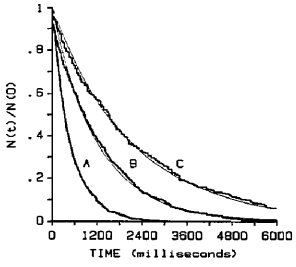

Fig. 2. Normalized survivor plots of gramicidin A channel durations before and after addition of PAF. The curves denote the fit of a single exponential decay to the results: $N(t) = N(0) \cdot \exp(-t/\tau)$ denotes the number of channels remaining open at time t and τ the average single-channel duration. $N(0)$ and τ were estimated using a maximum likelihood estimator [22]. (A) Control, before PAF addition: 667 channels from the main peak of current transition histograms from five measurements on a single day (see Fig. 3); $\tau = 500$ ms, $N(0) = 700$. (B) after addition of 1 μM PAF: 527 channels from the main peak of current transition histograms from four measurements on the same day as (A); $\tau = 1200$ ms, $N(0) = 580$. (C) Results after addition of 2 μM PAF; 350 channels from the main peak of current transition histograms from four measurements on the same day as (A) and (B); $\tau = 2100$ ms, $N(0) = 370$. Experimental: Channel durations were measured in current traces where no more than five channels were conducting simultaneously. When two or more channels appeared simultaneously (e.g. Fig. 1b), disappearance events were randomly assigned to appearance events [12].

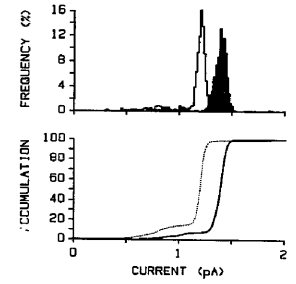

Fig. 3. Current transition histograms (top) and distribution functions (bottom) of gramicidin A channels before and after addition of 1 μM PAF. The 'filled' histogram and solid distribution curve are based upon 1010 events from four measurements on the same day. The main peak contains 934 (93%) events, the average single-channel current was 1.38 ± 0.05 pA (mean $\pm$ S.D.). The average current in the individual measurements varied from 1.32 to 1.43 pA. The 'empty' histogram and the stippled distribution function are based upon 931 events from four measurements on the same day after the addition of 1 μM PAF. The main peak contains 762 (82%) events, where the average single-channel current was 1.19 ± 0.04 pA. The average current in the individual measurements varied from 1.18 to 1.20 pA.

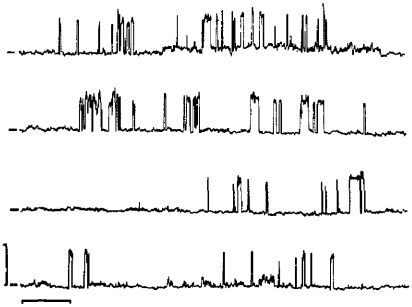

Fig. 4. PAF-induced currents. PAF was added to a nominal concentration of 4 μM in 1.0 M NaCl. The traces illustrate currents recorded at 8 mV applied potential. "Channel" appearances are upward. The traces were filtered at 80 Hz and represent 33 s of continuous recording from the same membrane. The vertical calibration bar represents 20 pA; the horizontal calibration bar, 1 s.

background 'leak'; and, superimposed on this leak, were large channel-like events (Fig. 4). Both conductance types were observed in the presence and absence of gramicidin and were therefore induced solely by PAF. In the absence of gramicidin, 2 μM PAF increased the membrane conductance $<$ 2-fold (note also that the baseline current noise in Fig. 1 was not affected by the addition of PAF), while increasing [PAF] to 4 μM increased the 'leak' conductance 40-fold. PAF-induced channels were likewise never seen at 2 μM PAF. At 4 μM PAF (and 1.0 M NaCl) the conductance of the channel-like events was remarkably reproducible. In three separate experiments, the conductance varied between 4 and 6 nS. Similar events were observed in 0.1 M NaCl and KCl, but their behavior was much less reproducible. Addition of 10 mM $CaCl_2$ did not significantly alter the PAF-induced currents.

The large increase in membrane conductance was most likely a result of the formation of large membrane defects. To test whether this could be the case, we determined the selectivity of the PAF-induced membrane conductance by measuring its reversal potential in asymmetric salt solutions. With 0.5 M KCl in the *cis* solution and 0.1 M KCl in the *trans* solution, the reversal potential was approx. -25 mV (*cis-trans*) in two separate determinations, and the K^+/Cl^- mobility ratio was calculated to be approx. 4 using the Henderson equation [13]. In similar experiments with NaCl, the reversal potential was approx. -11 mV, and the Na^+/Cl^- mobility ratio was approx. 2.

The ability of PAF to alter the behavior of gramicidin channels is most likely a general detergent property. At similar concentrations, lysophosphatidylcholine and a variety of synthetic detergents with markedly different structures have very similar effects on gramicidin channel appearance rates and duration [9]. The ability of detergents to alter the conductance of the standard channels varies with the structure of the detergent polar group [9], but the conductance decrease induced by PAF is similar to that seen with lysophosphatidylcholine.

The induction of mini channels by PAF is also similar to that seen with other detergents. The gramicidin mini channels represent subtle conformational substates of the gramicidin dimer that probably occur during peptide folding/insertion [9,14]. The exact conformational changes that alter the gramicidin channel conductance are unknown. But the ability of most detergents (including PAF) to induce gramicidin mini channels suggests that the change occurs at the channel entrance. It may be useful therefore to think of the mini channel as a partially denatured gramicidin channel [9]. We also note that other channels, e.g. the nicotinic acetylcholine-gated channel [15], can be desensitized by detergents at concentrations similar to those that induce gramicidin A mini channels [9].

PAF binds with high affinity to specific receptors in plasma membranes [16,17] *. As would be expected for an amphipathic molecule, there is also a low-affinity/high-capacity binding component, which presumably reflects the adsorption of PAF at the membrane/solution interface. The PAF effects on gramicidin channel behavior are presumably consequences of this nonspecific binding (although the induction of mini channels most likely results from association of detergent with peptide [9]). 'Specific' PAF effects that are seen in vitro at PAF concentrations $>$ 0.1 μM may also result from similar mechanisms, involving changes in membrane behavior and subtle disruption of protein–lipid interactions. Importantly, while the aqueous PAF concentration is low (micromolar), the membrane concentration of PAF is high. The detergent/lipid ratio in the membrane can be estimated from the aqueous detergent concentration and the detergent's critical micellar concentration (CMC) [18,9]. The CMC for PAF is 3 μM [19]; the PAF/lipid ratios are therefore approx. 0.3 at 1 μM, and approx. 1 at 4 μM! At such membrane concentrations PAF increases the 'fluidity' of lipid bilayers [20], which might account for some of the changes in gramicidin channel behavior. Similar membrane-modifying effects of PAF may be involved in PAF-

* The reported estimates for K_d for specific PAF binding to platelets differ by three orders of magnitude. The larger of these values [16] appears to be the result of a curve-fit error, because recalculation of K_d from the binding curve in their Fig. 1 (accounting for bound PAF) gives an estimate of approx. 50 pM, in good agreement with the findings of Kloprogge and Akkerman [17].

synthesizing neutrophils were PAF concentrations approach 1 mole percent in phagolysosomes [8]. In addition, the effects of PAF on lipid–protein interactions as demonstrated with the gramicidin channel may also be important for understanding the cell physiological actions of PAF.

When the PAF concentration reaches some critical level (which is close to the critical micellar concentration), PAF induces the formation of large holes in the membrane. These channel-like events have conductances and cation/anion selectivities that are similar to other detergent-induced channels [21] *. Lysolipids likewise induce large increases in membrane conductance and are lytic at similar concentrations to those used here [10]. The modest cation selectivity of the PAF-induced conductance, in fact, provides a likely mechanism by which PAF (and other detergents) can exert their cytotoxic effects, because disruptions of the cell membrane's barrier properties would disrupt cell volume regulation and lead to cell lysis and death. Before such extremes are reached, however, the intracellular Na^+, and Ca^{2+} concentrations would increase, and perturb cell metabolism, which, perhaps, accounts for some of PAF's cell activating effects in addition to the cytotoxic effect. These results thus highlight the importance of considering (non-specific) physico-chemical ligand effects when attempting to understand a ligand's mechanism of action.

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* The cation/anion mobility ratio for the Triton-induced conductance was reported to be 31 [21]. Given the quoted reversal potential, −48 mV, a mobility ratio of 9 is calculated using the Henderson equation, similar to the ratio for the PAF channels.

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