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INVOLVEMENT OF K^+ MOVEMENTS IN THE MEMBRANE SIGNAL INDUCED BY PAF-ACETHER

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Abstract—We investigated the effects of PAF-acether and its specific antagonist BN 52021 on Na^+ and K^+ transport systems in human red cells and mouse macrophages.

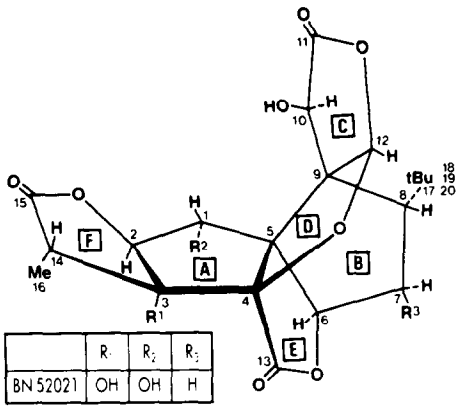
PAF-acether and BN 52021 exhibited specific and opposite effects on a Cs^+-stimulated, K^+ efflux in human red cells. PAF-acether increased and BN 52021 decreased the apparent dissociation constant for external Cs^+ without significant effects on the maximal rate of K^+ translocation.

In mouse macrophages, PAF-acether stimulated a quinidine-sensitive K^+ efflux. In the presence of the Ca^{2+}-ionophore A 23187, PAF-acether and BN 52021 showed opposite effects (stimulation and inhibition respectively).

For most cells, membrane potential is dependent on K^+-permeability. In addition, opening of potential-dependent Ca^{2+} channels appears to be associated with cell activation in several models. We thus propose that the specific interaction of PAF-acether with a K^+:K^+ exchange increases Ca^{2+} uptake through transitory changes in membrane potential. This in turn may lead to a more permanent membrane hyperpolarization through to opening of Ca^{2+} dependent, K^+ channels.

Platelet activating factor (PAF, PAF-acether, AGEPC)‡ is an etherphospholipid (1-*O*-alkyl-2(*R*)-acetyl-glyceryl-3-phosphoryl-choline) generated by stimulation of rabbit basophils [1], human polymorphonuclears (PMNLs) [2, 3], vascular endothelial cells [4], human macrophages [5] and eosinophils [6].

In these recent years, multiple biological effects of PAF-acether have been described (for review see ref. 7). A better understanding of these phenomena was facilitated by the discovery of specific PAF-acether antagonists like BN 52021 (Fig. 1).



	R	R_2	R_3
BN 52021	OH	OH	H

Fig. 1. Structure of BN 52021, a PAF-acether antagonist isolated from *Ginkgo biloba* L.

‡ Abbreviations used: $[Ca^{2+}]_i$-dep. P_k, calcium dependent K^+ permeability; $[Cs^+]_0$-dep. P_k, Cs^+-dependent K^+ efflux; OBR, ouabain- and bumetanide-resistant; Pct, phagocrit, fractional cell volume of macrophage suspension; BN 52021, PAF-acether antagonist (see structure in Fig. 1), PAF-acether, 1-*O*-alkyl-2(*R*)-acetyl-glyceryl-3-phosphorylcholine.

One main effect of PAF-acether is a marked hypotension and increase in vascular permeability [7], suggesting some kind of interaction with membrane ion transport. Indeed, PAF-acether increases Ca^{2+}-uptake in human [8] and rabbit [9] platelets and in guinea-pig papillary muscle [10]. However, PAF-acether does not act as a Ca^{2+} ionophore [11].

In spite of the above evidence, almost none is known concerning the interaction of PAF with membrane ion transport systems. On the other hand, the human red cell has been extensively used as a model for transport studies (see Fig. 2). In addition, we recently developed methods for measuring ion fluxes in mouse macrophages [12]. We thus decided to investigate the effect of PAF-acether and BN 52021 on different ion transport systems in human red cells and mouse macrophages.

METHODS

Preparation of human red cells. Twenty ml of venous blood collected in heparinized tubes were

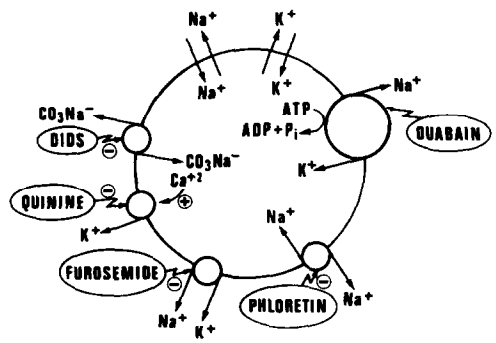


Fig. 2. Na^+ and K^+ transport pathways in human red cells.

centrifuged at 1750 *g* for 10 min and the plasma and buffy coat were aspirated. The red-cell pellet was used immediately or stored at 4° for no more than 2 days in a preserving solution containing (nM): 140 KCl, 10 NaCl, 1 $MgCl_2$ and 2.5 Na^+ phosphate (pH 7.2 at 4°).

Preparation of mouse macrophages. Peritoneal mouse macrophages were obtained according to a previously published method [12]. Briefly, 2–5 ml of sterile thioglycollate medium (Institut Pasteur, Paris) were injected into the peritoneal cavity of female mice 5–8 weeks old, of the 57 BL/5 (H-2^b) and DBA/2 (H-2^d) inbred strains. Elicited cells were collected 3–5 days later by washing the peritoneal cavity with Hank's balanced salt solution. The number of cells collected per mouse was 10–20 × 10^6 and of these more than 80% presented the morphological aspect of macrophages. A pool of 5–10 mice was used in each experiment. The collected suspension was immediately centrifuged at 1000 *g* for 1 min at 4° and the supernatant was removed. Macrophages were then rapidly washed once with cold 110 mM $MgCl_2$ or 150 mM NaCl and resuspended in the different efflux media (see later). The resulting suspension contained between 2 and 5% of cells (v/v measured like an hematocrit). This fraction was called Phagocrit (Pct).

Measurement of ion fluxes. Na^+ and K^+ fluxes were measured in fresh macrophages and human red cells according to previously published methods [12, 13]. Briefly, (i) Na^+-pump activity was taken as the ouabain-sensitive component of total Na^+ efflux in a Mg^{2+}, sucrose medium containing 2 mM K^+, (ii) Na^+, K^+-cotransport fluxes were taken as the ouabain-resistant net Na^+ and K^+ effluxes inhibited by loop diuretics (furosemide, bumetanide, etc.), (iii) the ouabain- and bumetanide-resistant (OBR) Na^+ effluxes stimulated by Li^+ were equated to Na^+:Li^+ exchange, (iv) (OBR)-K^+ efflux was measured in Mg^{2+} sucrose medium and in Cs^+/Na^+ medium. The Mg^{2+}-sucrose medium contained (mM): 75 $MgCl_2$, 85 sucrose, 10 MOPS–Tris (pH 7.4 at 37°) and 10 glucose. The Cs^+/Na^+ medium had the following composition (mM): 10 CsCl, 140 NaCl, 10 MOPS–Tris (pH 7.4 at 37°) 0.1 or 1 ouabain, 0.02 bumetanide and 10 glucose. (v) Calcium-dependent K permeability ($[Ca^{2+}]_i$-dep. P_k) was equated to the K^+ efflux stimulated by the Ca^{2+} ionophore A 23187 and inhibited by 0.2 mM quinidine.

The effects of PAF-acether and BN 52021. PAF-acether (1-*O*-alkyl-2(*R*)-acetyl-glycero-3-phosphorylcholine) was purchased from Bachem (Bubendorf, Switzerland) and stored at −80° in a 0.5% bovine serum albumin solution. BN 52021 (9H-1, 7a-(Epoxy-methano)-1*H*,6a*H*-cyclopenta[*c*]-furo[2,3-*b*]furo-[3′,2′ : 3,4]cyclopenta[1,2-*d*]furan-5,9,12-(4*H*)-trione,3-tert-butylhexahydro-4,7*b*-11-trihydroxy-8-methyl) was produced by IHB-IPSEN Institute for Therapeutic Research (Le Plessis, France). To study the effect of the above agents on Na^+ and K^+ transport in human red cells and mouse macrophages, the compounds were dissolved in a minimal amount of water, ethanol or dimethylsulphoxide, provided that the final concentration of these solvents had no effect *per se* on Na^+ and K^+ transport.

RESULTS

Human red cells

Interaction of PAF-acether and BN 52021 with sodium transport systems. Table 1 shows the effect of high concentrations of PAF-acether and BN 52021 on the Na^+, K^+-pump, Na^+, K^+-cotransport and Na^+, Li^+-countertransport systems. It can be seen that none of these Na^+ transport systems was affected by PAF-acether or BN 52021. A similar lack of effect was observed at concentrations one or two orders of magnitude lower (data not shown).

Effects of PAF-acether and BN 52021 on K^+ transport. Figure 3 shows the effect of BN 52021 on (OBR)- K^+ efflux. It can be seen that low concentrations of this agent stimulate K^+ efflux in Cs^+/Na^+ medium but not in Mg^{2+} sucrose medium.

Figure 4 shows that BN 52021 has no effect on $[Ca^{2+}]_i$-dep. P_K. Conversely, Fig. 5 shows that it stimulates a Cs^+-dependent K^+ efflux ($[Cs^+]_0$-dep. P_k).

Similarly to BN 52021, PAF-acether has no effect on $[Ca^{2+}]_i$-dep. P_K (data not shown). However, it is able to inhibit $[Cs^+]_0$-dep. P_K (Fig. 5). The simultaneous addition of PAF-acether and BN 52021 resulted in a neutralization of the effects (Fig. 5).

Kinetic analysis. Figure 6 shows a Hanes plot of the data of Fig. 5. The intercept with the *x*-axis represents the apparent dissociation constant for $[Cs^+]_0$ (K_{Cs}). This is increased by PAF-acether and decreased by BN 52021. The reciprocal of the slope is the maximal rate of $[Cs^+]_0$-dep. P_k. This was not modified by PAF-acether or by BN 52021.

Mouse macrophages

Table 2 shows the effects of PAF-acether on K^+

Table 1. Lack of effect of PAF-acether and BN 52021 on Na^+ transport systems in human red blood cells

Condition	Na^+, K^+-pump	Na^+, K^+ cotransport	Na^+, Li^+ countertransport
Control	2 310 ± 20	105 ± 10	110 ± 15
10^{-6} M PAF-acether	2 020 ± 80	110 ± 40	110 ± 30
Control	1 970 ± 40	120 ± 10	140 ± 20
8.5×10^{-5} BN 52021	1 950 ± 20	140 ± 10	105 ± 30

Fluxes are given as mean ± range in μmol (l. cells × hr)$^{-1}$. Control values correspond to erythrocytes from two different blood donors. Similar results were obtained in two other experiments.

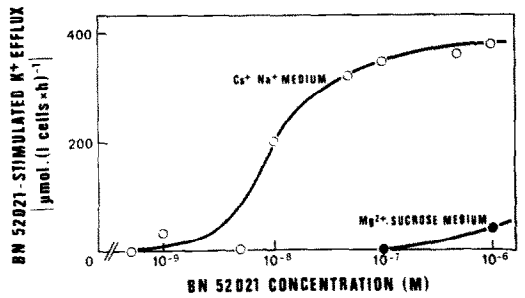


Fig. 3. Stimulation of basal K^+ efflux by BN 52021 in human red cells incubated in Cs^+/Na^+ medium containing 5 μM A23187. Lack of effect in Mg^{2+} sucrose medium.

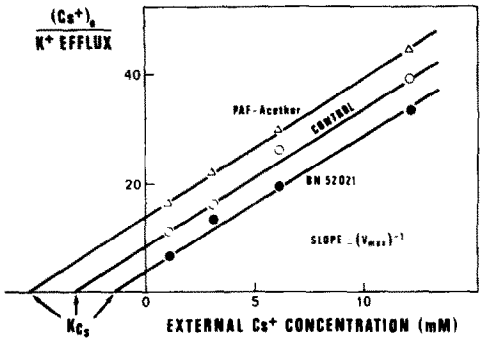


Fig. 6. Hanes plot of Fig. 5.

efflux in mouse macrophages. It can be seen that PAF-acether was able to stimulate K^+ efflux and that this effect was inhibited by quinidine.

Figure 7 shows the effect of PAF-acether and BN 52021 on K^+ efflux stimulated by the Ca^{2+} ionophore A 23187. These agents had opposite effects, BN 52021 inhibited and PAF-acether stimulated K^+ efflux.

Figure 8 shows in dose–response curve the effect of BN 52021 alone on K^+ efflux. It can be seen that 10^{-6} M of BN 52021 concentration inhibits about 1/3 of total K^+ efflux. Increases in BN 52021 concentrations up to 10^{-4} M result in a reduction of K^+ efflux to 1/3 of its basal value (Fig. 8).

Table 2. Stimulation of Ca^{2+}-dependent, K^+-efflux in mouse macrophages by 10^{-7} M PAF-acether.

Condition	PAF-stimulated, K^+-efflux $(hr)^{-1}$
Control	0.19 ± 0.04 (4)
2×10^{-4} M quinidine	−0.01 ± 0.03 (4)

Values are given as mean ± SD. The number of experiments is indicated in brackets.

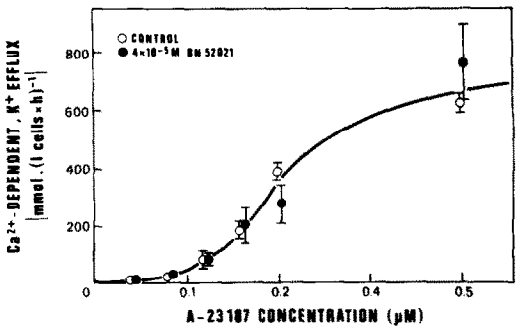


Fig. 4. Lack of effect of BN 52021 on Ca^{2+}-dependent, K^+ permeability in human red blood cells. A similar lack of effect was obtained at lower BN 52021 concentrations.

DISCUSSION

One main result of our study is that PAF-acether inhibits a $[Cs^+]_0$ stimulated K^+ efflux ($[Cs^+]_0$ dep. P_k). This effect appears to be rather specific because PAF-acether was not able to modify Na^+ transport through the Na^+-pump, the Na^+, K^+-cotransport or the Na^+, Li^+-counter transport systems.

It has been previously reported that human red cell membrane contains a $K^+:K^+$ exchange mechanism, independent of the $Na^+:K^+$-pump (see for instance ref. 14). On the other hand Cs^+ is a K^+ analogue for most (but not all) ion transport systems (see for instance refs. 15 and 16). Thus, it appears likely that $[Cs^+]_0$ dep. P_k corresponds to a K^+/Cs^+ exchange. This is resistant to bumetanide and therefore independent of the Na^+, K^+-cotransport system.

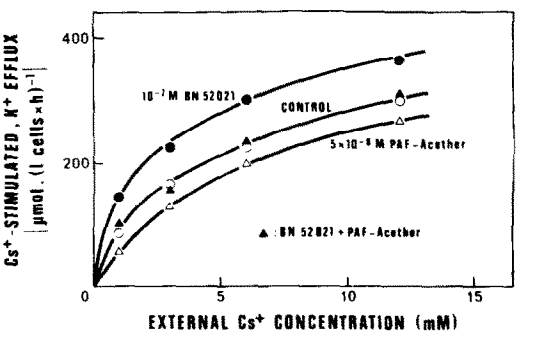


Fig. 5. Antagonistic effects of BN 52021 and PAF-acether on a Cs^+-dependent K^+efflux in human red blood cells.

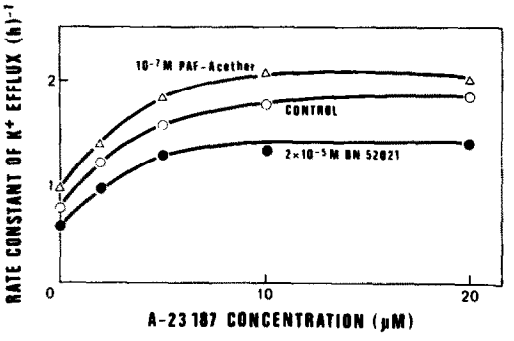


Fig. 7. Antagonistic effects of BN 52021 and PAF-acether on Ca^{2+} dependent, K^+ efflux in mouse macrophages. Similar results were obtained in two further experiments.

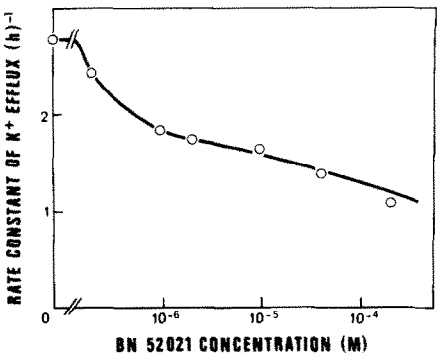


Fig. 8. Inhibition of K^+ efflux by BN 52021 in mouse macrophages. Similar results were obtained in two further experiments.

A kinetic analysis of the effects of PAF-acether on K^+ fluxes indicates that it decreases the apparent affinity for external Cs^+ without affecting the maximal rate of cation translocation.

BN 52021 showed opposite effects, i.e. it increased the apparent affinity for external Cs^+. Our results thus indicate that PAF-acether and BN 52021 have opposite and specific effects on the external sites of a K^+/K^+ exchange.

Human red cells exhibit a basal K^+ efflux of about 1 mmol(1.cell × hr)$^{-1}$ [13]. In addition, if the stoichiometry of K^+/K^+ exchange in erythrocytes is close to one : to : one, it cannot catalyze net K^+ movements. It appears likely therefore that BN 52021 and PAF-acether do not significantly modify erythrocyte K^+ contents.

In mouse macrophages, PAF-acether stimulates a K^+ efflux. This effect is inhibited by quinidine, suggesting that it is due to the opening of Ca^{2+}-dependent K^+-channels. Experiments in red cells indicate that PAF-acether (and BN 52021) has(ve) no direct effects on this channel. On the other hand PAF-acether enhances Ca uptake in human and rabbit platelets [8, 9] and in guinea-pig papillary muscle [10]. Our results therefore suggest that PAF-acether increases Ca^{2+} uptake in mouse macrophages thus opening Ca^{2+}-dependent, K^+ channels through an increase in cytosolic free Ca^{2+} content. BN 52021 may inhibit such effects at the PAF-acether receptor site.

Membrane potential in mouse macrophages is mainly dependent on K^+ permeability. In addition, voltage-dependent Ca^{2+} channels have been described in a variety of cells including macrophages (for review see ref. 17). On the other hand, PAF-acether stimulates O_2^- production in guinea-pig macrophages (an effect specifically inhibited by BN 52021 [18]). Thus it is tempt to speculate that PAF-acether induces cell depolarization by a direct interaction with a K^+/K^+ exchange mechanism (Fig. 9).

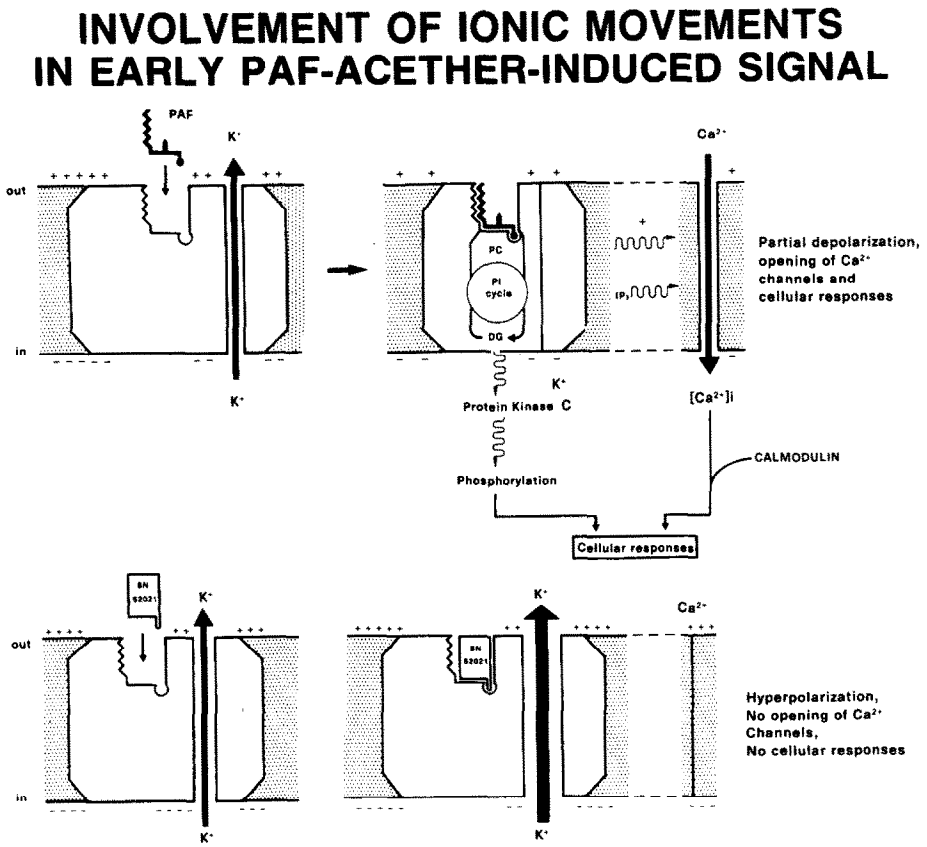


Fig. 9. A model for the interaction of PAF-acether and BN 52021 with ion transport systems in cell membranes.

This depolarization triggers the opening of voltage dependent Ca^{2+}-channels leading to an increase in $[Ca^{2+}]_i$ and cell activation. Unfortunately, our experimental protocol does not allow to distinguish if the changes in K^+ transport are the cause or the consequence of cell activation.

It is interesting to note that ouabain appears to induce vascular contraction by a mechanism similar to the one in Fig. 9, i.e. ouabain interacts with the K^+ sites of the Na^+, K^+-pump, thus inducing slight cell depolarization by partial opening of voltage-dependent Ca^{2+} channels [19].

It has been reported that PAF-acether stimulates the phosphatidyl inositol (PI) cycle [20]. On the other hand, one product of PI metabolism—inositol triphosphate (IP_3)—is able to increase cytosolic free calcium concentration [21]. This in turn may open Ca^{2+}-dependent K^+ channels as we observed here (Fig. 9). An alternative explanation of our results could be therefore that $K^+ : K^+$ exchange is linked to PI cycle.

Interestingly, BN 52021 acts on the same external cation sites of the K^+/K^+ exchange mechanism. This indicates that it can not only antagonize the effects of PAF-acether but also have effects by itself.

In conclusion, the specific interaction of PAF-acether with a membrane K^+ transport system may be a primary event in the stimulation of Ca^{2+} uptake.

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