THE CATION-SENSITIVE PHOSPHATASES OF THE LEUCOCYTE CELL MEMBRANE

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Leucocidin induces in polymorphonuclear leucocytes changes that mimic those in excitable or secreting tissues. Thus the permeability to cations increases, proteins of the cytoplasmic granules are secreted and calcium is accumulated in vesicles. The earliest response of the cell appears to be the increased cation permeability. The leucocyte differs from other mammalian cells in having a high sodium content (Elsbach and Schwartz, 1959) and in regulating the electrolyte balance by controlling the potassium content. Woodin and Wieneke (1968) suggested that leucocidin induces a structural change in the potassium pump, and, as tetraethylammonium ions inhibited the potassium efflux, that the changes induced by leucocidin might be relevant to membrane depolarisation in peripheral nerve.

The present paper shows that the peculiarities of electrolyte control in the leucocyte correlate with the properties of the cation-sensitive phosphatases and that leucocidin alters the potassium-sensitive component. The study was made possible because fragments of the leucocyte cell membrane can be unambiguously identified in cell homogenates (Woodin and Wieneke, 1966a).

Materials and Methods

Leucocytes were obtained from peritoneal exudates of rabbits. The cell surface membrane was prepared by the method of Wieneke and Woodin (1967), except that the membranes were finally centrifuged to give a pellet and suspended in 0.32 M sucrose solution. Ox brain microscomes were prepared by the method of Gibbs, Roddy and Titus (1965). Membrane preparations were treated with sodium iodide as described by Israel and Titus (1967), and with deoxycholate as described by Woodin and Wieneke (1966b). The two

components of leucocidin were the crystalline products (Woodin, 1960). The anti-leucocidin antibody was the international standard preparation. The phosphatase activities were determined by the following methods: ATPase (Woodin and Wieneke, 1966b), acetyl phosphatase (Israel and Titus, 1967), p-nitrophenyl phosphatase, by determining the p-nitrophenol produced by incubating 10 μg membrane protein in 0.2 ml of 5 mM p-nitrophenyl phosphate, 5 mM $MgSO_4$, 100 mM tris chloride buffer, pH 7.2, for 20 minutes at 37°. Carbamyl phosphatase was determined from the orthophosphate produced from carbamyl phosphate and 80 μg membrane protein under the conditions used for the p-nitrophenyl phosphatase assay.

Results

Adenosine triphosphatase. The properties of the ATPase of the leucocyte cell membrane resembled those of the impure membrane fraction studied by Woodin and Wieneke (1966b). The dependence of the activity on the calcium or magnesium concentration was similar and the activity was not stimulated by either sodium or potassium and was not changed by strophanthin G (10^{-3} M) or treatment with leucocidin (5 μg of each component/mg of membrane protein). Treatment with deoxycholate together with M NaCl, or M KCl or M tris chloride did not change the activity. Treatment with NaI decreased the activity but did not induce stimulation by cations (Table 1), or sensitivity to leucocidin or strophanthin G.

The potassium-sensitive phosphatases. When assayed immediately after

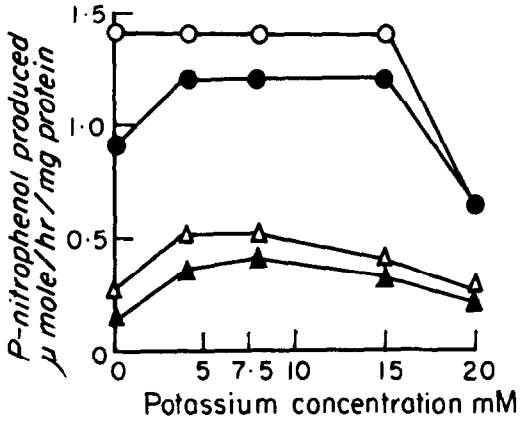
Table 1. ATPase activity of the leucocyte cell membrane

	Orthophosphate produced, μmoles/hour/mg membrane protein									
	Na^+ conc.				K^+ conc.			Na^+ and K^+ conc.		
	0	10	60	150	10	60	150	10	60	150
Normal leucocyte membranes	5.7	5.7	5.2	4.7	5.2	4.8	4.5	5.0	4.4	3.6
NaI-treated leucocyte membranes	3.6	3.6	3.3	3.0	3.4	3.0	2.9	3.1	2.7	2.4

preparing the membranes the p-nitrophenyl phosphatase activity was stimulated only 10% or in some cases not at all, by potassium (5-20 mM) but if the membranes were maintained at 0° in 0.32 M sucrose mM-EDTA solution for several days the p-nitrophenyl phosphatase activity was stimulated 50% by low concentrations of potassium. Washing the membranes in mM EDTA, mM tris chloride buffer, pH 7.2, or treating them with NaI had similar effects to ageing (Fig. 1). Strophanthin G (10^{-4} M) prevented the stimulation of sensitised membranes by potassium. NaCl or LiCl (10-40 mM) inhibited the phosphatase as did potassium at concentrations greater than 20 mM.

The p-nitrophenyl phosphatase activity of leucocyte membranes, but not

Fig. 1. The dependence of the p-nitrophenyl phosphatase activity of leucocyte membranes on potassium



Leucocyte membranes, treated with NaI (Δ) washed in mM EDTA, mM tris buffer pH 7.2 (●), aged at 2° in 0.32 M sucrose, mM EDTA solution (▲), or untreated (O), were tested for p-nitrophenyl phosphatase activity.

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ox brain microsomes, was stimulated by leucocidin (Table 2). The stimulation was greatest when the membranes were capable of being activated by potassium, and when the potassium concentration was high enough to partially inhibit the phosphatase, the effect of leucocidin treatment was small. If the leucocidin was neutralised with antibody before testing the phosphatase activity the stimulation was still observed, showing that it results from a structural change to the membrane and not from participation of leucocidin itself in the phospha-

Table 2. Hydrolysis of p-nitrophenyl phosphate by leucocyte cell membranes

Material	p-Nitrophenol produced, μmole/hour/mg protein					
	No leucocidin treatment			Treated with leucocidin		
	No K	7.5 mM K	20 mM K	No K	7.5 mM K	20 mM K
Leucocyte membranes	1.8	2.0	1.3	2.2	2.8	1.4
Leucocyte membranes treated with mM tris buffer, pH 7.2	1.6	2.2	1.0	2.7	2.7	1.2
Leucocyte membranes treated with NaI	0.67	0.97	0.8	1.1	1.5	0.95
Ox brain microsomes	0.56	—	2.5	0.56	—	2.5

Membranes, treated with NaI, washed with mM EDTA, mM tris buffer, pH 7.2, or untreated, were maintained at room temperature for 15 minutes alone or with 1 μg of each component of leucocidin/mg membrane protein. They were then tested for p-nitrophenyl phosphatase activity.

tase reaction. Strophanthin G and leucocidin act independently of each other. The former prevented the stimulation of activity by potassium in leucocidin-treated membranes while the latter produced an increase in activity (independent of potassium concentration) in the presence of strophanthin G.

Acetyl phosphate was also hydrolysed by leucocyte membranes. The rate of hydrolysis by membranes 48 hours old (0.6-1.2 μmole/hour/mg membrane protein) was stimulated 30-50% by potassium (5-10 mM), and treating the membranes with leucocidin increased the activity by 50% in the presence or absence of potassium. These effects of potassium and leucocidin were also found when carbamyl phosphate was the substrate. Carbamyl phosphate was hydrolysed in the absence of potassium at 0.3 μmole/hour/mg membrane protein.

Discussion

The peculiarities of the regulation of the cation balance in leucocytes described by Elsbach and Schwartz (1959) correlate with the properties of the cation-sensitive phosphatases of the leucocyte membrane. When leucocytes are cooled to 0° they accumulate sodium and on subsequent warming this is not extruded. It is possible that the sodium pump of the leucocyte is defective and

the ATPase of the leucocyte cell membrane has little in common with that of other mammalian cells (Glynn, 1968). On the other hand the potassium that leaks from leucocytes on cooling is regained when they are re-warmed and the potassium-sensitive phosphatase of the leucocyte cell membrane resembles that of other cells (Glyn, 1968). These correlations provide further evidence that the cation-sensitive phosphatases are indeed responsible for the regulation of the electrolyte content of cells.

It has been shown in the present work that leucocidin changes the activity of the potassium-sensitive phosphatase of the leucocyte cell membrane. This observation supports the suggestion of Woodin (1968) and Woodin and Wieneke (1968) that leucocidin produces a steric change in a component of the potassium pump. The study of the interaction of leucocidin with the isolated leucocyte cell membrane and with purified phospholipids led Woodin (1968) and Woodin and Wieneke (1968) to suggest that the primary action of leucocidin is to change the conformation of triphosphoinositide in the leucocyte cell membrane. If this is true triphosphoinositide should be a component of the potassium pump, in the leucocyte at least. It has not been possible at present to provide independent evidence for this. A major obstacle is the difficulty of purifying the membrane-bound phosphatases.

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