CHEMICAL AND PHYSICO-CHEMICAL ALTERATIONS OF HUMAN ERYTHROCYTES
BY PERIODATE, SUCCINIC ANHYDRIDE, 2-PHENYLETHANOL, AND 1,1-DIMETHYL-
PHENYLETHANOL: EFFECTS ON MEMBRANE PERMEABILITY AND ON METABOLISM
OF INTACT CELLS AND HEMOLYSATES

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Received 18 March 1974

1. Introduction

In two previous reports we described membrane and metabolic effects on Ehrlich ascites tumour cells brought about by the amphipathic compounds 2-phenylethanol (PEA) and 1,1-dimethylphenylethanol (DMPEA) [1] and by the specific chemical reagents periodate and succinic anhydride [2]. We arrived at the conclusion that the metabolic effects of all four compounds, although by quite different mechanisms, may be attributed to primary alterations of the cell membrane. To support this assumption, studies with cell-free lysates which retain metabolic activity would be of great value. Since similar difficulties as those encountered with ascites tumour cells in preparing metabolically active lysates [1,2] did not arise with erythrocytes, this was one of the reasons to extend our studies to red cells [3]. Although the metabolic pathways of glucose in erythrocytes differ from those in ascites tumour cells, a comparison of the metabolic effects of the compounds on intact cells on the one hand and hemolysates on the other, may yield valuable information as to the involvement of the cell membrane. Under the experimental conditions employed by us (methylene blue catalysis, pH 7.2 or 7.5) glucose is utilized almost exclusively via the oxidative pentose phosphate pathway [4,5].

For more details and references on the properties of the four compounds employed see [1] and [2].

2. Materials and methods

Erythrocyte suspensions were obtained from freshly drawn venous blood by washing five times with saline solution at 4°C and removing the buffy coat with a water-jet pump.

All incubations with intact cells were performed in buffered suspensions with a hematocrit of 50. Stromal-free hemolysates were prepared according to Roigas et al. [4], i.e. hypotonic hemolysis, dialysis of hemolysates with subsequent addition of definite amounts of nucleotides and cofactors, resembling intracellular conditions.

$O_2$-consumption was determined by conventional Warburg manometry and with a combined polarographic–titrimetric method [6]. $K^+$-efflux from intact cells was measured with an atomic absorption spectrophotometer. Since higher concentrations of the phenylethanol compounds are not readily soluble, sonication was employed for the preparation of stable emulsions [1]. Periodate was applied as sodium salt and was of analytical grade as well as succinic anhydride. PEA and DMPEA were ≥ 99% pure as controlled by gas–liquid chromatography.

3. Results and discussion

As can be seen from fig. 1, the amphipathic alco-
Fig. 1. Methylene-blue catalyzed $O_2$-consumption of intact erythrocytes (---) and hemolysate (-----) after treatment with 2-phenylethanol (PEA) and 1,1-dimethylphenylethanol (DMPEA). Incubation 15 min at 37°C, pH 7.2; addition of 30 mM glucose after incubation. $n$ = 14

Alcohols are strong inhibitors of methylene-blue-catalyzed oxygen consumption of intact erythrocytes whereas stroma-free hemolysates are not affected at all. At higher concentrations, a slight stimulation is even observed. This clearly supports the view that these compounds exert their action primarily by adsorption to the cell membrane by virtue of their amphipathic nature. Previous studies with ascites tumour cells, especially determination of adsorption isotherms and measurement of $^{14}$C-glucose uptake [1], had led to the assumption that the adsorption of the alcohols to the cell membrane would impair the uptake of metabolic substrate and allow for some leakage of potassium. The nature of this impairment ('membrane crowding', conformational changes or physical chemical interactions with molecules involved in transport or diffusion) is not yet clear. Higher concentrations of the surface-active agents which exceed the critical micellar concentration probably lead to micelle formation within the membrane causing membrane breakdown with subsequent free diffusion of electrolytes and macromolecules.

From fig. 3 it can be seen that substantial K$^+$-loss from intact cells occurs only at those concentrations which greatly reduce metabolic activity. Thus metabolic inhibition of erythrocytes may also be related to potassium losses, unlike with ascites tumour cells [1]. It should be expected that DMPEA, with its bulkier hydrophobic moiety, is more effective. In fact, all dose–response curves are shifted to the left with respect to the parent compound (figs. 1 and 3). This is in good agreement with our previous findings, especially with the different octanol/water partition coefficients of the two compounds [1]. Figs. 2 and 3 show the effects of periodate oxidation and succinylation. It can be seen that the hemolysates are less affected although they permit unimpaired access of the compounds to critical molecules involved in metabolism. The latter fact, of course, explains why there

Fig. 2. Effect of sodium periodate (NaIO$_4$) and succinic anhydride (succ.) on methylene-blue catalyzed $O_2$-consumption of intact erythrocytes (---) and hemolysate (-----). Incubation 15 min at 37°C, pH 7.2; addition of 30 mM glucose after incubation. $n$ = 14

Fig. 3. K$^+$-efflux from red cells after treatment with 2-phenylethanol (PEA), 1,1-dimethylphenylethanol (DMPEA), sodium periodate (NaIO$_4$), and succinic anhydride (succ.). Incubation 15 min at 37°C. $n$ = 12

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is some metabolic inhibition of the hemolysates, even at low concentrations: direct interactions of periodate and succinic anhydride occur with critical nucleotides and enzymes, respectively. This explains the finding that addition of higher concentrations of NADP⁺ and ATP shifts the dose-response curve for periodate to the right (not shown on the graph).

It may be concluded that periodate and succinic anhydride act by chemical modification of membrane carbohydrate and protein moieties, respectively, which are important for transport and/or regulatory functions. A striking difference is found between the two reagents as to K⁺-leakage from intact erythrocytes: succinic anhydride produces remarkably little potassium efflux, even at high concentrations, whereas periodate brings about considerable K⁺-losses which increase gradually with rising concentrations. Thus it may be assumed that periodate affects membrane carbohydrate moieties of glycoproteins or glycolipids responsible for ion distribution. Succinylation, on the other hand, would primarily modify a membrane protein moiety associated with metabolic regulation. A comparison between ascites tumour cells and erythrocytes [1,2] reveals that the latter are more resistant towards all compounds, with one exception: periodate, which affects erythrocytes to a much greater extent than tumour cells. Concomitantly, K⁺-efflux from erythrocytes is much larger than from EAT cells [2]. On the other hand, production of K⁺-leakage and inhibition of glucose utilization seem to be two separate actions of periodate because the bulk of intracellular potassium leaks out at concentrations which highly exceed those needed for complete metabolic block, but which do not yet cause hemolysis.

Significant involvement of SH groups in the periodate oxidation experiments could largely be excluded for the concentrations employed: in vitro studies with SH-group-sensitive enzymes as well as the metabolic studies with hemolysates and lyophilized yeast [7] indicate that sulfhydryl oxidation plays only a subordinate role.

Acknowledgement

The skilfull technical assistance of Miss A. Sauer is gratefully acknowledged.

References