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Pyruvate prevents the ATP depletion caused by formaldehyde or calcium-chelator esters in the human red cell

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Formaldehyde released during hydrolysis of calcium-chelator esters incorporated into cells blocks glycolysis in the human erythrocyte (Tiffert, T., García-Sancho, J. and Lew, V.L. (1984) Biochim. Biophys. Acta 773, 143–156). This blockade is due to the inhibition of glyceraldehyde-3-phosphate dehydrogenase by NAD^+ depletion caused by enzymatic oxidation of formaldehyde coupled to NADH production. The addition of pyruvate to the incubation medium prevents or reverts ATP depletion.

Calcium chelators which can be incorporated into the cells without disruption have become useful tools for the study of intracellular Ca^{2+} levels and its variations in relation to different physiologycal processes [1]. Loading of the chelator into the cells involves the hydrolysis of the hydrophobic tetraacetoxymethyl ester derivative by cell esterases with the production of formaldehyde as a by-product [2]. This formaldehyde causes ATP depletion in human red cells, the half-maximal effect being reached with about 90 μmol/l of cells of the calcium chelator [3]. This is a serious shortcoming for the use of these compounds for studies where ATP-sensitive processes are involved. In this paper the action mechanism of formaldehyde has been investigated, and an straightforward procedure to prevent ATP depletion is described. A preliminary report of some of the results shown here has been presented elsewhere [4].

In order to identify the glycolytic step blocked by formaldehyde, the levels of several metabolic intermediates were compared in control and formaldehyde-treated cells (Table I). Formaldehyde treatment increased fructose diphosphate, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate and decreased 3-phosphoglycerate levels. These results suggest a blockade of glyceraldehyde-3-phosphate dehydrogenase. However, the activity of this enzyme prepared either from control or from formaldehyde-treated cells was the same (Table I), and the addition of formaldehyde (up to 5 mM) to the enzymatic assay medium did not significantly modify the activity (data not shown).

A 'functional' blockade of glyceraldehyde-3-phosphate dehydrogenase could take place if formaldehyde depleted the cells of NAD^+. No direct interaction of formaldehyde with either NAD^+ or NADH could be evidenced, however. In order to test a possible enzymatic reduction of NAD^+ by formaldehyde, subtrate-deprived erythrocytes were incubated with pyruvate and the lactate production was measured (Fig. 1). Formaldehyde accelerated lactate production by more than 10 times (from 0.15 to 2.65 mmol/l cells per hour). This result suggests that formaldehyde is able to reduce NAD^+, making possible its cycling for pyruvate reduction. Either glyceraldehyde-3-phosphate dehydrogenase itself, which is able to oxidize slowly several aldehydes [7], or most probably acetaldehyde dehydrogenase [8] could be the enzyme involved.

TABLE I

EFFECTS OF FORMALDEHYDE (4 mmol/l CELLS) ON THE CELL LEVELS OF SEVERAL METABOLIC INTERMEDIATES AND ON GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPD) ACTIVITY

Cells were incubated 60 min at 37°C in a high-K^+ medium (solution A of Ref. 4) containing 10 mM glucose and with or without formaldehyde. At the end of this incubation period, aliquots of the cell suspension were processed for the determination of metabolic intermediates or enzyme activity [5] or for ATP [6]. Each value is the mean ± S.E. of four determinations.

	Control	Formaldehyde-treated
Metabolite (μmol/l cells)		
Fructose diphosphate	< 1 [a]	283 ± 22
Dihydroxyacetone phosphate	4.2 ± 1.6	540 ± 26
Glyceraldehyde-3-phosphate	< 2 [a]	16.2 ± 3.6
3-Phosphoglycerate	10.4 ± 0.4	< 2 [a]
ATP	1339 ± 39	322 ± 6
GAPD activity (μmol/l cells per min at 25°C)	52 ± 2	56 ± 3

[a] Below the sensitivity limit of the method.

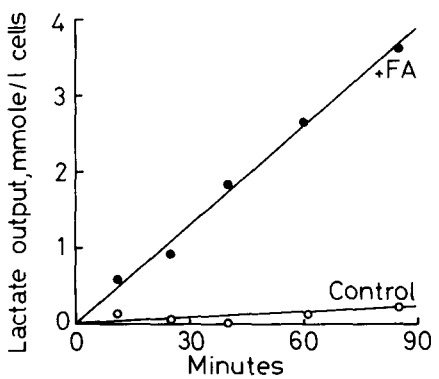

Fig. 1. Effects of formaldehyde (3 mM) on lactate production by substrate-deprived human red cells. Cells were first incubated without substrate in a high-K^+ medium (Medium A of Ref. 5) during 2 h at 37°C. Then they were washed and resuspended at 30% haematocrit in the same medium, and pyruvate (3 mM) was added at $t = 0$. At different times 0.1-ml aliquots of the cell suspension were removed and mixed with 0.2 ml of ice-cold 1 M perchloric acid. Lactate was determined spectrophotometrically in these extracts using a commercial kit (Sigma London, Procedure No. 826-UV). FA, formaldehyde.

If the glycolitic blockade by formaldehyde is due to NAD^+ reduction, then pyruvate added to the medium should be able to reoxidize the generated NADH and prevent the blockade. This prediction was confirmed using either formaldehyde or a calcium-chelator ester as the ATP-depleting agent (Table II). In another experiment a 90-min incubation with pyruvate and inosine (10 mM each) restaured ATP to near normal levels in cells previously depleted by formaldehyde treatment (data not shown). These results support the use of pyruvate in addition to substrates during loading with calcium-chelator esters to avoid ATP depletion.

The results shown here allow also an explanation to the relative insensitivity to ATP depletion by calcium-chelator esters reported previously in cells other than erythrocytes [9–11], since in respiring cells the generated NADH can be reoxidized in mitochondria. On the other hand, the lowering of ATP observed in human platelets on loading with the calcium-chelator ester Quin-2-AM [1] in the millimolar range was not prevented by pyruvate (Sanchez, A., unpublished observation). That indicates that the mechanism of the ATP depletion is different in this case.

On the basis of the results reported here for-

TABLE II

EFFECTS OF PYRUVATE ON THE ATP DEPLETION INDUCED BY FORMALDEHYDE OR Benz-2-AM

Cells were incubated at 10% haematocrit during 30 or 60 min at 37°C in a high-K^+ medium (Medium A or Ref. 5) containing 10 mM inosine and other additions as stated in the table. FA, formaldehyde; Pyr, pyruvate. Benz-2-tetracetoxymethyl ester [1] was a generous gift of Dr. V.L. Lew, Physiological Laboratory, Cambridge. ATP is given in μmol/l cells and was determined by a luciferin-luciferase procedure [6].

Expt. No.	Condition	30 min	60 min
1	Control	1439	1378
	FA (2 mmol/l cells)	544	445
	Pyr (10 mM)	1365	1412
	FA + Pyr	1378	1411
2	Control	903	904
	Benz-2-AM (1 mmol/l cells)	287	84
	Pyr (10 mM)	966	1022
	Benz-2-AM + Pyr	931	1008

maldehyde could be used as an alternative to other known procedures [12] to modify the NADH to NAD^+ ratio in human erythrocytes, or as a reversible ATP-depleting agent.

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