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EFFECT OF OUABAIN ON THE Ca^{2+}-DEPENDENT INCREASE IN K^+ PERMEABILITY IN DEPLETED GUINEA-PIG RED CELLS

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SUMMARY

1. The hypothesis that the inhibitory action of ouabain on the Ca^{2+}-dependent increase in K^+ permeability observed in depleted human red cells is mediated by changes in the intracellular level of ATP was tested by measuring simultaneously the ouabain sensitive K^+ loss and the concentration of ATP in depleted guinea-pig red cells in the presence and absence of external Ca^{2+}.

2. The results showed that ouabain has opposite effects on the K^+ loss. Thus, in the presence of external K^+, when the forward running of the Na^+ pump contributes to the breakdown of ATP, ouabain reduces K^+ loss, as observed in human red cells; in a K^+ free, high Na^+ medium when the pump running backwards induces a net synthesis of ATP (which can be detected in guinea-pig red cells but not in human red cells), ouabain increases K^+ loss.

3. The results suggest that the action of ouabain is mediated through the changes in the intracellular level of ATP induced by the forward or reverse operation of the Na^+ pump.

Recently, BLUM AND HOFFMAN[1] reported that ouabain, known to be a specific inhibitor of the Na^+ pump, inhibits a substantial proportion of the Ca^{2+}-dependent increase in K^+ permeability observed in depleted human erythrocytes[2,3] in the presence of external K^+. They concluded "that most, if not all, of the increased K^+ outflux results from an increased turnover of at least part of the same system which serves as the Na^+–K^+ pump in normal red cells".

If the Ca^{2+}-dependent change in K^+ permeability is sensitive to small variations in the intracellular level of ATP as suggested before[4], an alternative explanation of BLUM AND HOFFMAN's[1] results can be proposed based on the contribution of Na^+ pump activity to the intracellular ATP changes when the cells are being depleted. In the presence of external K^+, when the pump runs forward, ouabain should reduce the rate of ATP hydrolysis, whereas in a K^+-free high-Na^+ medium, when the pump would be expected to run backwards, ouabain should reduce the rate of formation of ATP. If the Ca^{2+}-induced K^+ leak depends on ATP depletion, ouabain should there-

Abbreviation: EGTA, ethyleneglycol-bis-(*p*-aminoethylether)-*N*,*N*′-tetraacetic acid.

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fore decrease the leak in K^+-containing media but increase it in a high-Na^+, K^+-free medium. This communication describes experiments designed to test these predictions.

Preliminary experiments had shown that (i) while ouabain sensitive differences in the intracellular concentration of ATP in the presence of external K^+ are observed both in human and guinea pig red cells, in a K^+-free high-Na^+ medium they can be detected only in guinea pig red cells[5]. (ii) Guinea pig red cells behave in a generally similar way to human red cells[6] in showing a Ca^{2+}-dependent increase in K^+ permeability when they are being depleted. In view of these results, guinea-pig red cells were the obvious choice for the present experiments.

Red cells from at least two guinea pigs were washed, pooled and preincubated in a substrate-free medium containing $^{42}K^+$ for 4 h at 37° and overnight at 5°. After the preincubation the cells were loaded with inorganic phosphate by incubating them for about 20 min in a phosphate–citrate medium[7]. The cells were washed thoroughly in a K^+-free, high-Na^+ medium and resuspended at a haematocrit of about 5 % in more of the same solution to which iodoacetamide, inosine and Tris–EGTA had been added. Ca^{2+}, K^+ and ouabain were added as indicated in Table I. At the end of a 30- or 60-min incubation period the cell suspensions were cooled and centrifuged for 3 min at 2500 × *g*. The $^{42}K^+$ activity was measured in the supernatant and ATP was determined in the cells (see legend of Fig. 1).

TABLE I

EFFECT OF OUABAIN AND Ca^{2+} ON THE $^{42}K^+$ LOSS FROM STARVED GUINEA-PIG RED CELLS IN THE PRESENCE OF IODOACETAMIDE AND INOSINE

Guinea-pig red cells pretreated as described in the text were incubated for 30 or 60 min at 37° in a medium containing 150 mM NaCl, 10 mM Tris–HCl (pH 7.4 at 37°), 5 mM iodoacetamide, 5 mM inosine and 0.6 mM Tris–EGTA. When present, the concentration of ouabain was $5 \cdot 10^{-5}$ g/ml, that of KCl, 10 mM, and that of $CaCl_2$, 0.75 mM. $^{42}K^+$ loss is expressed as the percent of the total $^{42}K^+$ activity contained in the cells at the beginning of the final incubation that was present in the supernatant at the end of the 30- or 60-min incubation period. Each figure is the mean of three estimates ± S.E.

Addition to the medium		*Incubation time (min)*	$^{42}K^+$ *loss (% initial activity in cells)*		
Ca^{2+}	K^+		*Without ouabain (A)*	*With ouabain (B)*	*Difference (A −B)*
Absent	Absent	30	2.17 ± 0.07	1.36 ± 0.04	0.81 ± 0.08
		60	3.80 ± 0.03	2.46 ± 0.06	1.34 ± 0.05
Present	Absent	30	4.91 ± 0.11	5.46 ± 0.13	−0.55 ± 0.17
		60	8.52 ± 0.13	10.80 ± 0.08	−2.28 ± 0.15
Absent	Present	30	1.78 ± 0.03	1.55 ± 0.04	0.23 ± 0.05
		60	3.44 ± 0.04	2.82 ± 0.04	0.62 ± 0.05
Present	Present	30	6.26 ± 0.13	5.99 ± 0.19	0.27 ± 0.23
		60	13.06 ± 0.16	11.00 ± 0.16	2.06 ± 0.22

Two identical experiments were performed with similar results. The results of one of these experiments are reported in Fig. 1 and Table I.

Fig. 1 shows that, as expected and in agreement with earlier results[5], ouabain leads to a higher level of ATP when the cells are incubated in a K^+-containing medium,

and to a lower level of ATP when the cells are incubated in a K^+-free, high-Na^+ medium.

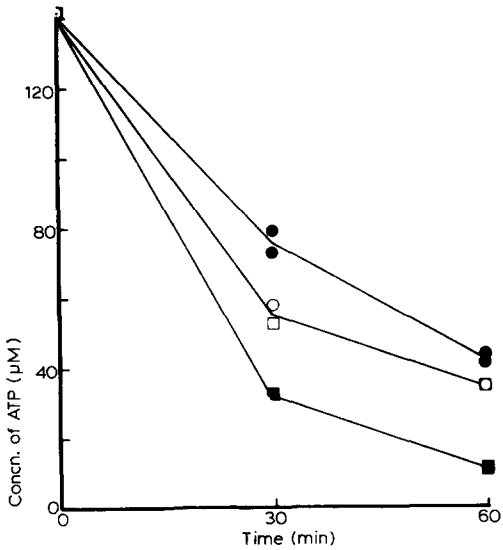

Fig. 1. The change in ATP concentration with time in the same cells from which the $^{42}K^+$ loss is reported in Table I (see legend of table). ○—○, cells incubated in a K^+-free medium; □—□, 10 mM K^+ present in the medium. Full symbols, ouabain-free medium; empty symbols, ouabain present. The cells were lysed in a fixed volume of distilled water and the protein precipitated with trichloroacetic acid (final concentration 5 %), The trichloroacetic acid was extracted with diethyl ether and the diethyl ether was blown off with a gentle stream of air. The remaining supernatant was diluted in a solution containing 20 mM glycylglycine buffer (pH 7.4), 5 mM sodium phosphate buffer (pH 7.4) and 25 mM $MgCl_2$. ATP was determined in this solution using a firefly extract method. (G. L. SLAYMAN, personal communication *via* I. M. GLYNN). When the sample containing ATP was suddenly mixed with the firefly extract, the recorded intensity of the initial flash of light was proportional to the concentration of ATP within the final concentration range used (0.05–0.5 μM). The presence of external Ca^{2+} had no effect on the intracellular ATP levels. Accumulation of K^+ lost from the cells in the nominally K^+-free medium allowed some running forwards of the pump and decreased slightly the ouabain sensitive difference in the concentration of ATP during the second 0.5 h.

Table I shows the effects of ouabain on K^+ efflux. In the absence of external Ca^{2+}, the ouabain-sensitive K^+ loss appears with its normal characteristics in guinea-pig red cells as reported before[8]. In the presence of Ca^{2+} in excess of EGTA, there is a substantial increase in the K^+ loss under all conditions. But the significant finding is that the effects of ouabain on the loss of potassium are, as predicted, in opposite directions in the K^+-free and K^+-containing media. In the presence of K^+, ouabain reduces K^+ loss as described by BLUM AND HOFFMAN[1]; in the K^+-free medium ouabain increases K^+ loss. The effects are accentuated during the second 0.5 h when ATP depletion has proceeded further.

The fact that ouabain can either inhibit or stimulate the Ca^{2+} induced K^+ loss from depleted cells according to whether the high-Na^+ external medium contains K^+ or not, strongly suggests an indirect action of ouabain on the mechanism responsible for the change in K^+ permeability. The present results support the hypothesis that the effects of ouabain on the Ca^{2+}-dependent increase in K^+ permeability observed in

depleted red cells are mediated by the changes in the intracellular concentration of ATP induced by the forward or reverse running of the Na^+ pump.

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