IN VITRO EFFECTS OF D(+) AND L(-) OZOLINONE ON SODIUM AND POTASSIUM FLUXES IN HUMAN ERYTHROCYTES

C. GUARENA, R. BOERO, C. ROSATI, G. FORNERIS, G. BELTRAME, F. QUARELLO, G. PICCOLI

Cattedra di Nefrologia Medica dell'Università di Torino (Prof. A. Vercellone).
Divisioni di Nefrologia e Dialisi dell'Ospedale Maggiore di S. Giovanni Battista. Torino. Italy.

INTRODUCTION

Several observations suggest that diuretic drugs may decrease renal Na reabsorption by direct inhibition of Na transport carrier in different segments of the nephron [1,2]. Ouabain inhibits basolateral sodium reabsorption, which is catalyzed by the Na,K pump, loop diuretics, like furosemide and bumetanide, cause natriuresis by inhibition of the Na,K,Cl cotransport system, which is located in the luminal side of Henle's loop cells.

Human erythrocytes (RBC) have been used for studying the cellular action of various diuretic drugs [3,4]: sodium movements across human RBC membranes are mediated by several transport systems, which are qualitatively similar to those present in renal tubular cells [4]. The ouabain-sensitive Na,K ATPase is the main mechanism maintaining the Na and K electrochemical gradients across RBC membranes against the passive Na and K diffusion. At least two additional transport systems, ouabain-insensitive, have been described in human RBC: a furosemide- (and bumetanide-) sensitive one to one Na,K cotransport system, which catalyzes a simultaneous efflux of both Na and K and a phloretin-sensitive Na,Na exchange, which can be detected by using Li as a tracer and is thus called Na, Li countertransport [4].

Etozolin is a diuretic and antihypertensive drug recently available in Italy (Elkapin, Knoll S.p.A.). The drug is a racemic mixture of two enantiomers; after oral administration both undergo liver metabolism in d+ and l-ozolinone, which are clinically active. Only the l-enantiomers show diuretic and natriuretic properties, while both optical isomers have vasodilating activity [5].

We thus decided to investigate whether the diuretic and vascular effects of d+ and l-ozolinone involve the Na and K transport systems, as studied in human RBC, to elucidate the underlying molecular mechanism.

METHODS

Simultaneous measurements of ouabain sensitive (Na,K pump), bumetanide-sensitive (Na,K cotransport), Li-stimulated (Na, Li countertransport), outward Na fluxes and ouabain- and bumetanide-resistant (passive permeability) Na and K efflux, were made in fresh erythrocytes by the method of Garay et al. [4], as previously reported [6]. D+ and l-ozolinone, dissolved in dimethylsulfoxid, were added to the incubation media in order to obtain the following final concentrations: 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M.

RESULTS

Neither d+ nor l-ozolinone exert any significant effect on Na efflux mediated by Na,K pump, Na, Li countertransport and passive membrane leak (data not shown). The effects on Na,K cotransport and passive K permeability are reported in Tab. I and in Figg. 1,2.

<table>
<thead>
<tr>
<th>Drug Conc. (M)</th>
<th>Bumetanide-sensitive Na efflux (μmol/l RBC/h)</th>
<th>Ouabain- and bumetanide-resistant K efflux (μmol/l RBC/h)</th>
<th>l-ozolinone</th>
<th>d-ozolinone</th>
<th>l-ozolinone</th>
<th>d-ozolinone</th>
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<td>190±27</td>
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Tab.1 Effects of d+ and l-ozolinone on Na,K cotransport and passive K permeability in human RBC. Values are expressed as mean±SEM of five experiments.

Fig. 1. Inhibition by d+ and l-ozolinone of Na efflux mediated by Na,K cotransport in human RBC.

Fig. 2. Effects of d+ and l-ozolinone on passive K efflux in human RBC.