Estimates of clearance and volume of distribution, assuming that the absolute bioavailability of indobufen is 1, give mean values of 1.2 L/hr and 15.1 L for the *R*-enantiomer and of 2.3 L/hr and 20.1 L for the *S*-enantiomer.

The urinary excretion of total *S*-indobufen and of total *R*-indobufen after administration of the racemate was essentially the same. No difference was observed either in the urinary excretion of total *S*-indobufen after administration of the racemate or of the *S*-enantiomer.

The results obtained in this study provide some clarification of the most important aspects of the disposition of the indobufen enantiomers in man. First, after administration of racemic indobufen, the plasma levels of the *S* form were markedly lower than those of the *R* form. Pharmacokinetic analysis suggests that this difference is due to faster elimination of the *S*-enantiomer. After administration of the *S*-enantiomer no detectable levels of the *R*-enantiomer were found either in plasma or in urine, showing that no chiral inversion of *S*-indobufen to its *R*-antipode occurs in man.

Plasma levels, urinary excretion and pharmacokinetic parameters of *S*-indobufen were the same after administration of the racemate or of the *S*-enantiomer when this was given at half of the dose, showing that in man the pharmacokinetic behaviour of the *S*-enantiomer is not affected by the presence of the *R*-enantiomer and that *R*-indobufen is not converted metabolically to the *S*-enantiomer. In man, the urinary excretion of total indobufen (free + glucuronide) is very important and this is in sharp contrast with data obtained in the rat [5]. In conclusion, as the pharmacokinetic behaviour of *S*-indobufen, which is the active enantiomer, in man showed some differences as compared with the *R*-enantiomer, any future work attempting to establish plasma concentration–response relationships should be done using the *S*-enantiomer values.

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Effect of ethanol on the Na^+- and the Na^+,K^+-ATPase activities of basolateral plasma membranes of kidney proximal tubular cells

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Abstract—The Na^+- and the Na^+,K^+-ATPase activities of basolateral plasma membranes from rat kidney proximal tubular cells were affected differentially by ethanol. Moreover, at concentrations of ethanol that can be reached *in vivo* in the blood plasma (50 mM) there was a significant effect on the Na^+-ATPase activity and practically no effect on the Na^+,K^+-ATPase activity.

Two active mechanisms of Na^+ extrusion (pumps) have been shown to be present in proximal tubular cells of mammalian kidney: the Na^+,K^+-pump, which extrudes Na^+ in exchange for K^+, and the Na^+-pump, which extrudes Na^+ accompanied by Cl^- and water [1, 2]. The first system is totally inhibited by ouabain, is partially inhibited by ethacrynic acid and is insensitive to furosemide. The second system is insensitive to ouabain and is totally inhibited by ethacrynic acid and furosemide [3]. Similarly, two Na^+-stimulated ATPases have been described to be present in basolateral plasma membranes of cells from this tissue: the Na^+,K^+-ATPase, which is stimulated by Na^+ and K^+, is inhibited by ouabain, is partially inhibited by ethacrynic acid and is insensitive to furosemide; and the Na^+-ATPase, which is stimulated by Na^+, is insensitive to ouabain and is totally inhibited by ethacrynic acid and furosemide [4–6].

Ethanol has been shown to increase the fluidity of biological membranes [7, 8] and also to inhibit the activity of several enzymes associated with them, particularly, the Ca^{2+}-ATPase [9] and the Na^+,K^+-ATPase [10–13].

The present work was undertaken to compare the effect of ethanol, *in vitro*, on the Na^+-ATPase and the Na^+,K^+-ATPase activities of basolateral plasma membranes from rat kidney proximal tubular cells.

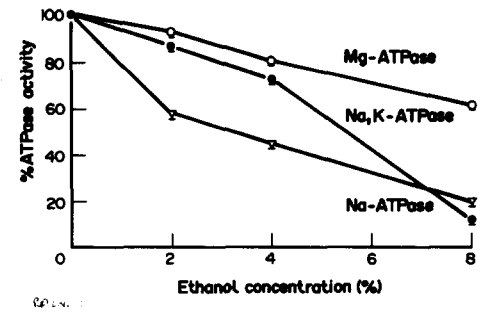

Fig. 1. Mg^{2+}-, Na^+- and Na^+,K^+-ATPase activities (expressed as percentages) of basolateral plasma membranes from rat kidney proximal tubular cells as a function of the ethanol concentration of the incubation medium. The 100% ATPase activities were [nmol $P_i \cdot (mg\ protein)^{-1} \cdot min^{-1}$]: 410 ± 15, 119 ± 6 and 547 ± 18 for the Mg^{2+}-, Na^+- and Na^+,K^+-ATPase, respectively. Values are means ± SEM (for six separate membrane preparations).

Materials and Methods

Healthy male rats (3 months old) of the Sprague–Dawley strain were anesthetized with ether and immediately killed by decapitation. The kidneys were removed, decapsulated, and collected in a medium containing 250 mM sucrose/20 mM Tris–HCl (pH 7.2)/0.5 mM dithiothreitol/0.2 mM phenylmethylsulfonyl fluoride (sucrose/Tris medium), at 4°. The outermost slices of the kidney cortex (rich in proximal tubules) were homogenized and centrifuged at 4° according to the method described elsewhere [14, 15], to prepare the basolateral plasma membrane enriched fractions. The final pellet was resuspended in the sucrose/Tris medium, frozen, and kept at −20° until used.

The Mg^{2+}-, the Na^+- and the Na^+,K^+-ATPase activities were determined as already described [16, 17]. Before the ATPase assays, the membranes were treated with sodium dodecyl sulfate (SDS, Bio-Rad Laboratories, Richmond, CA), using optimal ratios of SDS/protein, in order to avoid vesicles [17]. All the assays of the Na^+-ATPase activity were carried out in a K^+-free medium in the presence of 7 mM ouabain (Sigma Chemical Co., St. Louis, MO). The ATPase activity was expressed as nanomoles of P_i per milligram of protein per minute. The three enzyme activities for all the tested concentrations of ethanol were determined for six separate preparations which were assayed in quadruplicate at each point. Each preparation consisted of pooled membranes from the kidneys of six rats. Protein was determined by the Coomassie blue dye binding assay (Bio-Rad Laboratories, Richmond, CA) [18]. Data are expressed as the mean ± SEM of the determinations. Differences between the results were analyzed according to Student's *t*-test and to the analysis of variance from multiple comparisons, following the Fisher's LSD test. Significance was accepted at $P < 0.05$.

Results and Discussion

There is enough evidence showing that the Na^+- and the Na^+,K^+-ATPases are functionally two different entities [for a review see Ref. 19]; since both mechanisms are responsible for the active extrusion of Na^+ from the cells, it is important to compare the effects of ethanol on the two systems. Figure 1 shows the effect of the presence of relatively high quantities of ethanol in the incubation medium on the Mg^{2+}-, Na^+- and Na^+,K^+-ATPase activities of the basolateral plasma membrane fractions from rat kidney proximal tubular cells. Notice that ethanol produced differential effects on the activity of the three systems. The Mg^{2+}-ATPase was affected poorly by ethanol, even at very high concentrations (30% inhibition at 8% ethanol). On the other hand, the Na^+- and the Na^+,K^+-ATPase activities were inhibited strongly at 8% ethanol (80 and 90%, respectively). However, the degree of inhibition of these two systems was totally different at lower concentrations of ethanol. At 2% ethanol, the Na^+,K^+-ATPase activity

Table 1. Effect of 50 mM ethanol on the Mg^{2+}-, Na^+- and Na^+,K^+-ATPase activities of basolateral plasma membranes from rat kidney proximal tubular cells

Incubation medium	ATPase activity [nmol $P_i \cdot (mg\ protein)^{-1} \cdot min^{-1}$]		
	Mg^{2+}-ATPase	Na^+-ATPase	Na^+,K^+-ATPase
Control	416 ± 19	114 ± 5	534 ± 21
+ 50 mM Ethanol	410 ± 14	91 ± 3	519 ± 19
Ethanol effect	−6 ± 24*	−23 ± 6†	−15 ± 28*

Values are means ± SEM (for six separate membrane preparations). The SEM for the Na^+- and the Na^+,K^+-ATPases were calculated for paired data.

* Not significant.

† $P < 0.01$.

was only inhibited by about 10%, while the Na^+-ATPase activity was inhibited by about 40%. At 2, 4 and 8% ethanol concentrations, the Na^+-ATPase was inhibited differently than the Mg^{2+}- and the Na^+,K^+-ATPases ($P < 0.001$ in all cases, except for the Na^+,K^+-ATPase at 8% ethanol with a $P < 0.05$). The Na^+,K^+-ATPase was also inhibited differently than the Mg^{2+}-ATPase at 4 and 8% ethanol concentrations ($P < 0.05$ and $P < 0.001$, respectively). At 2% ethanol, the two ATPase activities were not different. Finally, the three curves, Mg^{2+}-, Na^+-, and Na^+,K^+-ATPase activities as a function of the ethanol concentrations tested, were different with a $P < 0.001$.

The maximal concentration of ethanol found in the blood plasma of rats after a strong ingestion of this alcohol is 50 mM [20]. At this concentration, ethanol has practically no effect on the activity of rat brain Na^+,K^+-ATPase [21–23]. We studied the effect of this concentration of ethanol (eight times lower than the lowest one that we have tried) on the ATPase activities of the kidney membranes. The results are shown in Table 1. As shown, the Mg^{2+}- and the Na^+,K^+-ATPase activities were not inhibited significantly, while the Na^+-ATPase activity was inhibited by approximately 20%. This result might be very important, since it suggests that the Na^+-ATPase could be inhibited to some extent *in vivo*, after heavy ingestion of alcohol. If it is considered that Na^+-ATPase participates in the active regulation of the cell volume, its inhibition (even partially) could result in an increased cell volume with all the involved consequences. However, more work must be done on this aspect before any conclusions are reached.

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