It has previously been postulated that the loop diuretics may act by competing with chloride for binding sites on the sodium-potassium-chloride cotransport system. This theory is supported by the preliminary observation that a change in the chloride concentration of the incubation medium alters the inhibitory potency of piretanide as measured in the studies of chloride dependent sodium uptake by rectal gland plasma membrane vesicles. An increase in the chloride concentration of the incubation medium results in a decrease in the apparent affinity of the membranes for piretanide. These preliminary data demonstrate a trend which may provide additional evidence that chloride and the loop diuretics interact at the same site on the rectal gland plasma membrane. This work was supported by NIH Grant AM 27441, NIH T32GM to the Medical Scientist Training Program, Albert Einstein College of Medicine and the Max Planck Society, Germany.

PLASMA MEMBRANE PREPARATION FROM FROG KIDNEY

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Clearance studies, micropuncture studies and determination of urea tissue concentrations in kidney slices of the frog, Rana catesbeiana, have led to the assumption that in the proximal tubule urea is actively transported across the basal-lateral membrane. The exit step is supposed to be passive (Schmidt-Nielsen, B. and Shrauger, C.R. Amer. J. Physiol. 205:483-488, 1963, Long, W.S. Amer. J. Physiol. 224:482-490, 1973). In order to get more information about the mechanism of urea transport we attempted as a first step to isolate functionally active brush border and basal-lateral plasma membrane vesicles from proximal tubules of frog kidney. For this purpose the dorsal halves of the kidneys were dissected from freshly sacrificed animals and stored for one day at -20°C until used. About 2.5 g of renal tissue were thawed at room temperature and used for membrane preparations. The preparation for brush border membranes followed the protocol of Booth and Kenny with the modification that 20 mM Mg^{2+} was used in the precipitation steps (Booth, A.G. and Kenny, A.J. Biochem. J. 142:575-581, 1974). For the preparation of basal-lateral plasma membranes differential centrifugation in a sucrose medium was employed as described for rat kidney (Heidrich, H.-G. et al. J. Cell Biol. 54:233-245, 1972). The enzyme activities and the protein content were determined as described previously (Kinne, R. et al. Pflügers Arch. 329:191-206, 1971). Uptake of radioactively labeled D-glucose by the membrane vesicles was studied by a rapid filtration technique (Kinne, R. et al. J. Membrane Biol. 21:375-395, 1975).

Table 1 shows the activities of the marker enzyme for brush border membranes, alkaline phosphatase, and of the marker enzyme for basal-lateral membranes, Na^+-K^+-ATPase, in the starting homogenate and in the final membrane preparations.

Using the Mg^{2+}-precipitation technique a membrane fraction was obtained which is highly enriched in alkaline phosphatase and only slightly enriched in Na^+-K^+-ATPase. Such enzyme enrichment pattern is typical for a brush border membrane fraction, although the cross-contamination with basal-lateral plasma membranes is usually somewhat lower. The differential centrifugation membrane preparation yields a membrane fraction enriched mainly in Na^+-K^+-ATPase. The relatively high cross-contamination with alkaline phosphatase indicates that basal-lateral plasma membranes and brush border membranes have not yet been separated sufficiently. Thus, a further purification step has to be considered in future experiments.

As shown in Table 2, the brush border membrane fraction isolated from frog kidney exhibits the typical transport activity of brush border membrane vesicles. Uptake of D-glucose is markedly stimulated in the presence of sodium as compared to potassium. Furthermore, in the presence of a sodium gradient across the membrane, D-glucose is transiently accumulated inside the vesicles ("overshoot"-phenomenon). Finally, phlorizin strongly inhibits the sugar uptake.

Table 1. Comparison of enrichment of marker enzyme activities found in the two membrane preparations from frog kidney using two different isolation procedures.

Isolation procedure	Alkaline Phosphatase enrichment	yield	Na^+-K^+-ATPase enrichment	yield
Mg^{2+}-precipitation	14.2	19.6%	1.9	7.6%
Differential centrifugation	3.0	16.4%	7.3	9.5%

Mean values of three experiments are given. Enrichment represents the ratio between the specific activity found in the membrane fraction and the specific activity in the homogenate. The latter were 7.6 µmoles/hr/mg protein for alkaline phosphatase and 1.9 µmoles/hr/mg protein for the Na^+-K^+-ATPase. The total recovery of both enzymes was 85%, a value identical with the recovery of protein.

Table 2. D-Glucose transport of isolated brush border membrane vesicles from frog kidney.

	15"	1'	1'45	2'30	60'
+ 75 mM NaCl	1,584	2,651	2,425	2,235	1,168
+ 75 mM KCl	137	299	390	398	1,218
+ 75 mM NaCl and 0.1 mM Phlorizin	264	468	650	562	1,252

One transport experiment with triplicate determinations at a concentration of 0.5 mM D-glucose is shown. The transport activity is expressed as pmoles/mg protein. The uptake studies were carried out at 15°C with membrane vesicles preloaded with 100 mM mannitol, 20 mM Tris-HEPES, pH 7.4. The membrane vesicles were suspended in a medium of the same composition and the additions indicated in the Table.

These experiments show that functionally active brush border membrane vesicles can be isolated from frog kidney. This preparation might be useful for the study of urea transport.