DRIVING FORCES FOR URATE UPTAKE INTO HEPATOPANCREATIC BASOLATERAL MEMBRANE VESICLES FROM THE AMERICAN LOBSTER (HOMARUS AMERICANUS)

Anne Nies[1], Evamaria Kinne-Saffran[2], Rolf K.H. Kinne[2]
and Manfred K. Grieshaber[1]
[1]Department of Zoology, Universität Düsseldorf,
4000 Düsseldorf, FRG
[2]Max-Planck-Institut für Systemphysiologie, 4600 Dortmund, FRG

In crustaceans urate is degraded in an oxygen-dependent process catalyzed by uricase. This enzyme is located in the peroxisomes of the hepatopancreas (Sharma, M.L. & Neveau, M.C., Comp. Biochem. Physiol. 40B: 863-870, 1969; Noguchi et al., J. Biol. Chem. 254: 5272-5275, 1979). Under hypoxic conditions in vitro urate is released from the hepatopancreas into the incubation medium (Grieshaber, unpublished results) whereas, in vivo, accumulation of urate in the hemolymph is observed (Czytrich et al., Proc. German Zool. Soc. 80: 207, 1987). However, during normoxic recovery hemolymph urate levels decrease within minutes to normoxic levels. In a previous study (Nies et al., Bull. MDIL 31: 92-94, 1992) the existence of an urate transport mechanism mediating the movement of urate across basolateral membrane vesicles of lobster hepatopancreas could be demonstrated. The kinetic characteristics and the relative specificity of this uptake mechanism were analysed. In the present study it was attempted to examine the driving forces for urate uptake into lobster hepatopancreatic basolateral membrane vesicles.

Preparation of the hepatopancreatic basolateral membrane vesicles (BLMV) from the American lobster (Homarus americanus) and uptake experiments were carried out as described previously (Nies et al., Bull. MDIL 31: 92-94, 1992). [2-^{14}C]urate was purchased from the American Radiolabeled Chemicals Inc. (St. Louis, MO, USA; 52.5 mCi/mmol, 1.94 GBq/mmol). Data are given as means and standard errors of at least three experiments. Means were considered to be statistically different when $p<0.05$ using Student's t-test.

Uptake of urate by BLMV was investigated in the presence of an initial inwardly directed cation gradient of either 100 mM NaCl or 100 mM KCl. Uptake was not significantly different ($p>0.05$) using a sodium or potassium chloride gradient. So, there is no evidence for a sodium-urate cotransport system.

Urate uptake by BLMV was also examined in the absence and in the presence of a positive vesicle interior (membrane potential) induced by pretreating the vesicles with the potassium ionophore valinomycin (Figure 1). After 1 min of incubation uptake is significantly ($p<0.05$) higher in the vesicles pretreated with valinomycin indicating that urate uptake by lobster BLMV can be enhanced by a positive potential difference across the vesicular membrane.

As recently described, urate uptake by pig basolateral membrane vesicles of the kidney was mediated by a 2-oxoglutarate/urate exchange mechanism (Werner, D. & Roch-Ramel, F., Am. J. Physiol. 261: F265-F272, 1991). A similar mechanism was supposed to exist in the basolateral membrane of lobster hepatopancreas. This hypothesis was investigated with the following experimental setup: Urate uptake was measured in vesicles preloaded with 2-oxoglutarate or other organic acids under conditions when no membrane potential is present ("voltage clamp

conditions"; Figure 2). Urate uptake was significantly ($p<0.05$) trans-stimulated by 1mM 2-oxoglutarate or 1mM L-lactate. Glutarate (1 mM) and butyrate (1 mM) did not show a trans-stimulating effect.

In the present study it was demonstrated that urate uptake by BLMV is either stimulated by a positive vesicle interior or trans-stimulated by 2-oxoglutarate. These results indicate the existence of a potential sensitive uptake component, its nature has not yet been defined, and a 2-oxoglutarate/urate exchange mechanism. In vivo the exchange mechanism is probably the only urate uptake mechanism as a positive diffusion potential is not achieved in the cell under physiological conditions. Further studies are necessary to elucidate whether the basolateral membrane of lobster hepatopancreas includes a sodium/2-oxoglutarate uptake system which in conjunction with the exchange mechanism could operate in urate uptake against an electrochemical potential difference.

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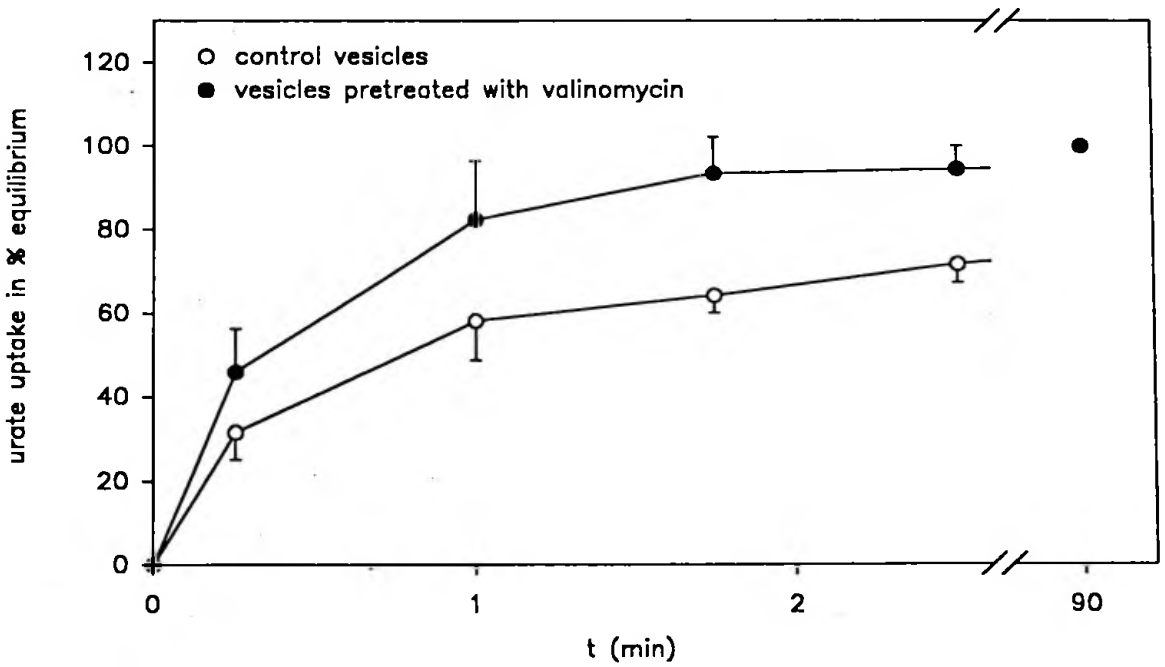

Figure 1. Time course of urate uptake by hepatopancreatic basolateral membrane vesicles in the absence and in the presence of valinomycin. Vesicles were loaded with 100 mM mannitol, 10 mM Tris-HEPES, pH 7.6 and were untreated or pretreated for 20 min with 2.3-4.4 µg valinomycin/mg protein. Uptake was measured in media containing 50 mM mannitol, 10 mM Tris-HEPES, pH 7.6, 50 mM K_2SO_4 and 0.77 mM urate. Open circles (n=4) represent the uptake into untreated vesicles, filled circles (n=4) uptake into pretreated vesicles with a positive vesicle interior.

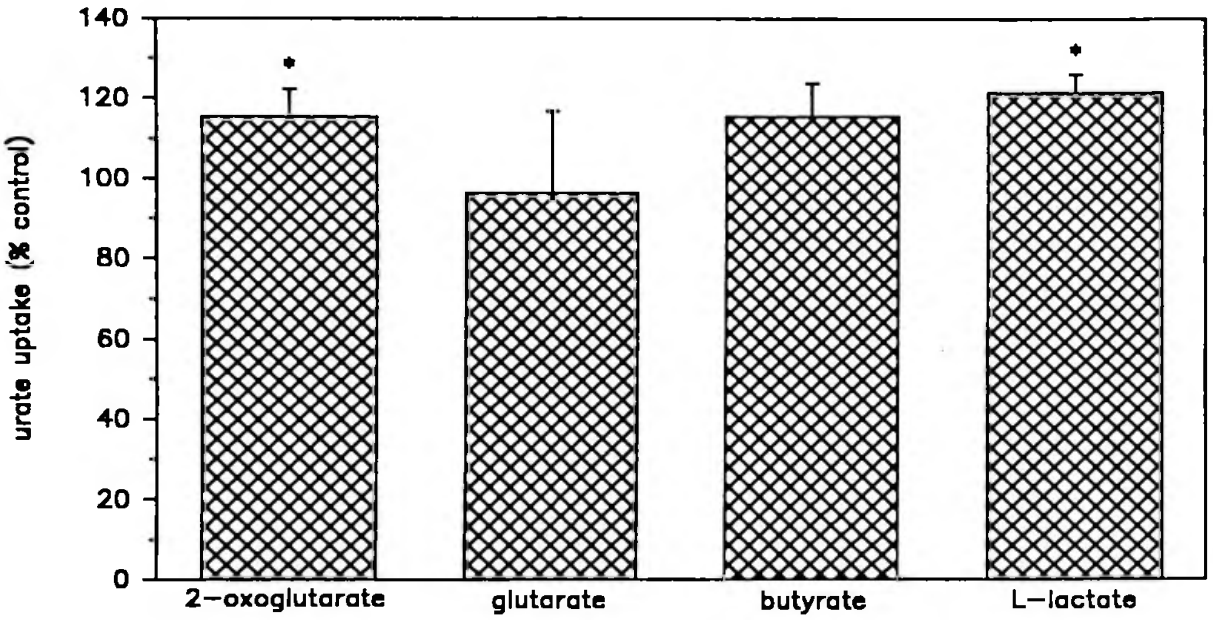

Figure 2. Trans-stimulating effect of different organic acids on 0.77 mM urate uptake by hepatopancreatic BLMV. Vesicles were preloaded for 90 min with 100 mM mannitol, 10 mM Tris-HEPES pH 7.6 and 1 mM of the indicated substances. 20 min before initiation of uptake 34 mM potassium gluconate (final concentration) and 2.3-4.4 µg valinomycin/mg protein were added to the incubating vesicles. Vesicles were diluted 20fold in the uptake medium containing 100 mM mannitol, 10 mM Tris-HEPES pH 7.6, 100 mM NaCl and 34 mM potassium gluconate. Uptake values are given in % control (untreated vesicles = 100%). Data are means ± SE of three experiments. *$p<0.05$.