STOICHIOMETRY OF SODIUM-D-GLUCOSE COTRANSPORT IN RENAL BRUSH BORDER MEMBRANES OF THE DOGFISH (SQUALUS ACANTHIAS) AND THE LITTLE SKATE (RAJA ERINACEA)

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Previously we have demonstrated the presence of sodium-D-glucose cotransport in isolated renal brush border membrane vesicles from hagfish, dogfish, and flounder [Kinne, Issues Biomed. 15: 69-94, 1991]. These studies were now extended to the skate in order to provide functional information on glucose transport in both species in parallel to investigations at the mRNA and DNA level.

Brush border membranes were isolated from the caudal two thirds of the dogfish kidney and whole kidneys of the skate as described previously [Bevan et al., J. Comp. Physiol. B 159: 339-347, 1989]. Transport measurements were performed by a rapid filtration method at 15°C. To determine the stoichiometry of the sodium-D-glucose cotransport two methods were employed. One is based on the analysis of the number of sodium ions interacting with the transport system when measuring the sodium dependence of the initial rate of D-glucose uptake ("activation method"). The other determines those sodium gradients which, at a given ratio of intravesicular to extravesicular D-glucose, prevent the efflux of the sugar from the vesicles ("thermodynamical analysis") [for further information see Turner, Ann. N.Y. Acad. Sci. 456: 10-25, 1985].

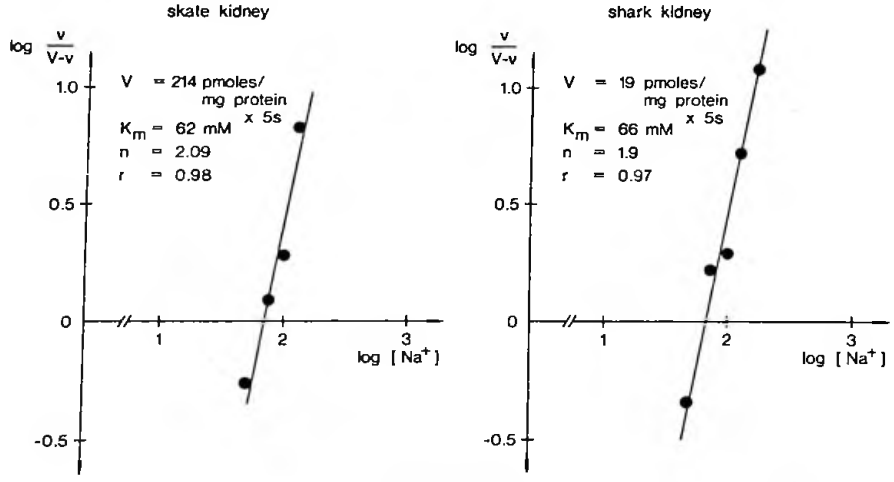

Figure 1. Stoichiometry of the sodium-D-glucose transporter in skate and shark kidney brush border membranes (activation method). Uptake of D-glucose (0.1 mM) was measured for 5 s in media containing various concentrations of NaCl. NaCl was replaced isoosmotically by choline chloride. The vesicles were electrically short-circuited by the presence of 100 mM KSCN at both inside and outside and contained 15 μg valinomycin/mg protein. Sodium-dependent D-glucose transport was calculated as the difference between the uptake in the presence and absence of sodium. The data points represent mean values of 3 experiments performed as duplicates. The standard deviation of the mean values was ± 9% or less.

In figure 1 the sodium dependencies of D-glucose uptake into renal brush border vesicles isolated from shark kidney and skate kidney are compared. It can be seen in the Hill plot that both sodium-D-glucose cotransport systems exhibit an almost identical sodium dependence with a halfsaturation between 62 and 66 mM and a slope of 1.9 and 2.09, respectively. These data suggest that both transport systems interact with two sodium ions and accordingly should exhibit a 2:1 stoichiometry during transport.

This hypothesis was investigated further by preloading brush border membrane vesicles with D-glucose and sodium and measuring efflux of D-glucose into media containing different sodium concentrations. The results of these studies are compiled in table 1.

Table 1.
Changes in intravesicular D-glucose content
after dilution into media varying in sodium concentration

D-glucose gradient glu_i/glu_o	sodium gradient Na_i/Na_o	stoichiometry at which no flux should occur	glucose content after 4 s: shark kidney brush border	glucose content after 4 s: skate kidney brush border	p
5/1	1/2.5	1.75	92.8±4.0%	96.1±4.3%	n.s.
5/1	1/5	1	95.4±3.9%	109.1±5.1%	<0.02
5/1	1/10	0.7	104.7±5.4%	128.3±8.5%	<0.02

The intravesicular glucose concentration was 0.1 mM, the intravesicular NaCl concentration 20 mM. NaCl in the efflux media was replaced isoosmotically by choline chloride. The vesicles were electrically short-circuited by 100 mM KSCN and 15 μg valinomycin/mg protein. Values are given as % of the initial content and represent means ± SD of 3 experiments performed in triplicate. The column stoichiometry denotes that stoichiometry at which according to the driving forces employed in this experimental condition no net movement of glucose should occur. Loss of D-glucose suggests a lower stoichiometry, uptake of D-glucose a higher stoichiometry. p-values calculated according to Student's t-test apply to the differences between shark and skate kidney.

It is obvious that under these experimental conditions the brush border membranes of the two species behave differently. In shark kidney brush border membranes efflux of D-glucose still occurs when the D-glucose and the sodium gradient compensate each other - as predicted for a system with a 1:1 stoichiometry - and is only stopped by an even higher inwardly-directed sodium gradient. This result suggests that the stoichiometry of the shark kidney sodium-D-glucose cotransport is between 0.7 and 1 when the energetics of transport are investi-

gated. On the opposite, in skate brush border membranes the transition point between efflux and uptake of D-glucose is clearly above a stoichiometry of one, in agreement with the result obtained from investigating the sodium dependence of transport.

These studies indicate that stoichiometries determined by kinetic or thermodynamical methods do not always yield similar results. One possible explanation for this discrepancy is the presence of internal leak pathways, i.e. in which D-glucose transport can occur without the coupling to sodium [Centelles et al., Biochim. Biophys. Acta 1065: 239-249, 1991]. The apparent stoichiometry as determined thermodynamically would decrease when the relative contribution of such leak pathway increases. From the studies reported above it could be hypothesized that the skate sodium-D-glucose transport system has less internal leak pathways than the transport system in the shark. Since, thus far, in these two species no differences between the transport systems have been dectected at the molecular level [Shetlar et al., MDIBL Bull. 30: 35-37, 1991], this might suggest that different degrees of coupling can occur within the transport molecule or between the transport molecules in the tetrameric configuration which has been found to represent the minimum functional unit for sodium-coupled D-glucose transport across mammalian membranes [Lin et al., Biochim. Biophys. Acta 777: 201-208, 1984].

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by grants of the Max-Planck Society to R.K. and E.K.-S.