THE RENAL SODIUM-D-GLUCOSE COTRANSPORTER IN THE SKATE (RAJA ERINACEA) AND SHARK (SQUALUS ACANTHIAS): INTERACTION WITH INHIBITORS

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Although closely related in evolution the two elasmobranch species shark and skate seem to differ in their renal D-glucose transport system. Recent studies on renal brush border membrane vesicles revealed an apparent K_m for D-glucose of 0.9 ± 0.1 mM in the shark, whereas in the skate the K_m was significantly lower 0.16 ± 0.03 mM (Kinne et al., unpublished). Investigating the transport stoichiometry thermodynamically in the shark, a value close to 1 sodium/1 glucose translocated was observed whereas in the skate the stoichiometry was significantly higher, approaching 2. The aim of the present work was to further elucidate the differences of the renal sodium-D-glucose cotransporter in the two species using natural and synthetic inhibitors.

Brush border membrane vesicles (BBMV) were isolated from the kidney of male or female shark or skate by a differential calcium precipitation method described earlier (Kinne-Saffran et al., Bull. MDIBL 24:61-63; 1984; Bevan et al., J. Comp. Physiol. 159: 339, 1989). The inhibitory potency of various glucose analogues was determined by measuring the initial (5 sec) uptake rate of ^{3}H-D-glucose into the BBMV in the presence of inhibitors using the rapid filtration technique (Hopfer et al., J. Biol. Chem. 248:25-32, 1973). Data presented refer to mean values ± SE of three independent experiments usually performed in duplicate or triplicate and are corrected for sodium independent uptake. Inhibitors employed were either products of Sigma (Taufkirchen, Germany) or synthesized in our laboratory (H. Kipp et al., unpublished results). Identity and purity of the synthesized inhibitors were proven by ^{1}H (500 Mhz) and ^{13}C (127.5 MHz) NMR spectroscopy on a Bruker AM 500.

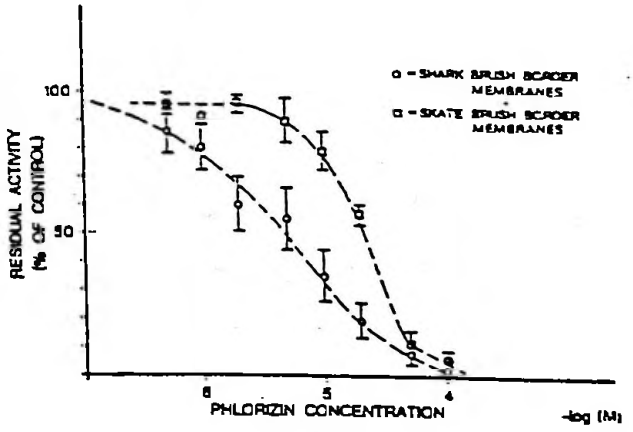

Fig. 1: Dose response to phlorizin

Phlorizin is a specific inhibitor of sodium-D-glucose cotransport systems. We therefore investigated its interaction with the two transport systems present in skate and shark BBMV. Measuring the inhibitory effect of phlorizin on D-glucose transport in dose response experiments showed the shark system to be more sensitive to phlorizin than the skate system (Fig. 1). From that experiment an I_{50}

of 5 µM was obtained for the shark and 23 µM for the skate system. Arbutine, which exhibits some structural similarity to phlorizin (Fig. 2), also showed a stronger inhibiton in shark BBMV. An I_{50} of 16 mM for the shark and 66 mM for the skate was obtained (Fig. 3). High affinity inhibitors for the renal sodium-D-glucose cotransporter are known to interact with multiple binding sites on the transporter, one being identical with the D-glucose binding site. Taking the significantly higher D-glucose affinity for the skate transport system into account it is therefore remarkable that phlorizin and arbutine are more effective inhibitors in the shark, since an opposite behaviour was to be expected. Measuring the effect of phlorizin at different substrate concentrations and evaluating the obtained data according to the method of Dixon showed a different pattern. In the skate typical competitive kinetics were found (from three such experiments a K_i of 5.2 ± 0.4µM was calculated), in contrast the shark seems to exhibit a more complex, non linear relationship and neither the inhibition type nor a distinct K_i could be determined. These data suggest that the D-glucose and the aglucon moiety of phlorizin contribute to a different extent to the inhibition observed in skate and shark BBMV. The phenolic aglucon part of phlorizin and arbutine seems to interact more tightly with the shark transporter, which explains why no competitive inhibition kinetics were found.

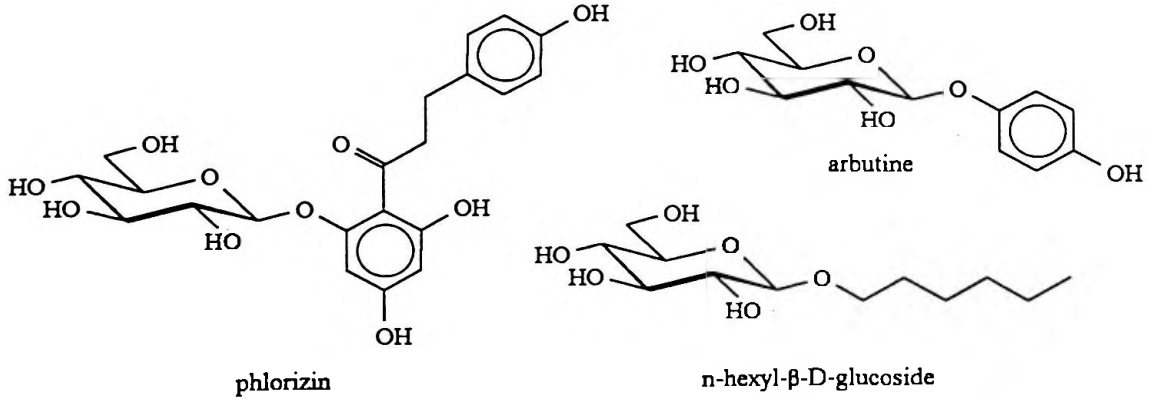

Fig. 2: Structures of inhibitors

In addition the inhibitory effect of alkylglucosides on D-glucose uptake into BBMV from skate and shark kidney was tested. The degree of inhibition was found to be strongly dependent on length, anomeric configuration and on flexibility of the alkyl sidechain. The effect of increasing the length of the n-alkyl sidechain is shown in Fig. 4. Maximal inhibition of sodium-D-glucose cotransport was achieved with n-nonyl-β-D-glucoside; inhibition of 5 sec 0.1 mM D-glucose uptake in the presence of 0.1 mM n-nonyl-β-D-glucoside: skate BBMV 92 %, shark BBMV 80 %. The local maximum in the inhibition pattern observed in

both species using n-hexyl-β-D-glucoside as inhibitor can be explained by the presence of two additional hydrophobic sites. The one next to the glucose binding site exhibits optimal interaction with a hexyl residue. A further elongation of the sidechain leads to a transient net loss of affinity until an optimal interaction with both hydrophobic sites is achieved. Very long sidechains (> 10 carbon atoms) seem to be too bulky, assuming a randomly coiled conformation of the sidechain, to fit into the binding pocket, in consequence no effect of these compounds was observed. Axially orientated n-alkyl sidechains in α-alkylglucosides were less effective inhibitors than the corresponding equatorially orientated β-anomers. Saturated hexyl-β-D-glucoside showed a significantly higher inhibition than the corresponding 3-hexenyl compounds. Moreover the cis-isomer was a significantly more effective inhibitor than the trans-isomer in both skate and shark BBMV. Thus the interaction of alkylglucosides with the sodium-D-glucose cotransporter is favoured by equatorial orientation and high flexibility of the sidechain. These compounds were in general more effective inhibitors in the skate suggesting that the interaction with the D-glucose binding site is the predominant feature.

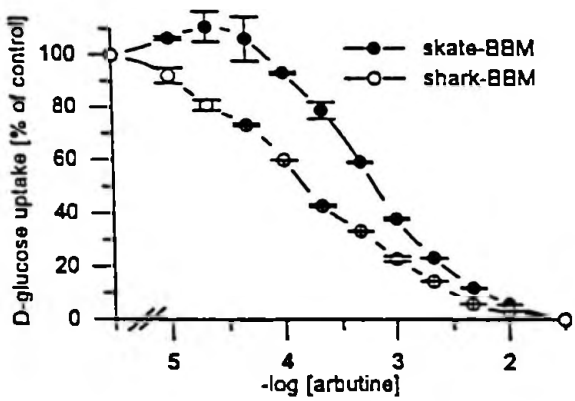

Fig. 3: Dose response to arbutine

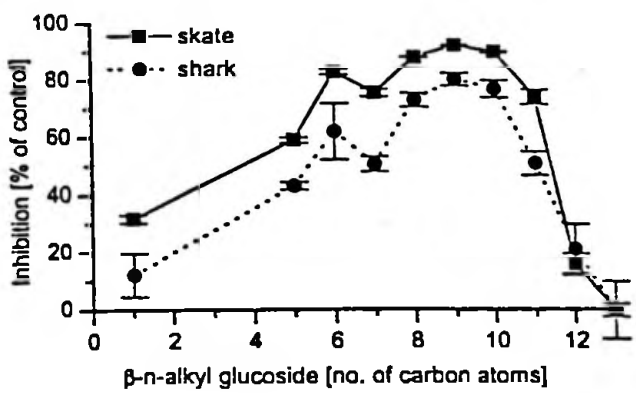

Fig. 4: Effect of β-alkylglucosides

Supported by EPSCOR grant