EFFECTS OF DIET ON NA^+-D-GLUCOSE COTRANSPORTER MRNA EXPRESSION IN INTESTINE AND KIDNEY OF THE WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS)

Robert Shetlar[1] and Kristin Simokat[2]
[1]Max-Planck-Institut für Systemphysiologie, Rheinlanddamm 201, W-4600 Dortmund 1, FRG
[2]Wesleyan Station, 222 Church Street Box 5192, Middletown, CT 06459

These studies were initiated in order to study the effects of diet on the expression of Na^+-D-glucose cotransporter mRNA in the flounder kidney and intestine (i.e. dietary or nutritional adaptation). It has been shown in mammals and in some marine and freshwater fishes that dietary content of sugars and amino acids plays a role in the regulation of intestinal transport of these substances [Titus et al., Am.J.Physiol. 261:F1568-F1574, 1991, Karasov and Diamond, Am.J.Physiol. 245:G443-G462. 1983, Buddington et al., J.Physiol. 393:261-281, 1987, and Ferraris and Diamond, Am.J.Physiol. 262:G1069-G1073, 1992]. In the kidney, however, the story is less clear and to date only the effect of dietary phosphate on renal phosphate transport has been studied in any depth [Stoll et al., Biochem.J. 180:465-470, 1979 and Kempson et al., Kidney Int. 18:36-47, 1980]. With regard to the intestine it has been shown that an increase in dietary glucose causes an increase in the magnitude of glucose reabsorption through the Na^+-dependent mechanism, presumably through the synthesis of new transporters owing to the observed increase in V_{max} and unchanged K_m [Karasov and Diamond, Am.J.Physiol. 245:G443-G462, 1983]. Preliminary evidence from the flounder suggests that the renal Na^+-D-glucose cotransporter is influenced by the nutritional state of the animal, but in an opposite manner to that seen in the intestine.

When freshly caught flounder (intestines and stomach still contained partially digested food, i.e. fed flounder) were used to isolate renal mRNA [Stallcup and Washington, J.Biol.Chem. 258:2802, 1983] and perform northern blot analysis [Thomas, Proc.Natl.Acad. Sci.,USA, 77:5201,1980]using ^{32}P-labeled cDNA for the rabbit renal Na^+-glucose cotransporter as a probe [Morrison et al., Biochim. Biophys. Acta 1089:121, 1991], little or no signal was seen. This suggests that under these conditions the mRNA coding for the cotransporter was not present in quantities large enough to detect by this method. The opposite was found using mRNA isolated from the intestines of freshly caught flounder (see above for definition of freshly caught). On the other hand, isolation and northern analysis of mRNA from the kidneys of flounder not fed for at least 4 weeks indicated that the Na^+-D-glucose cotransporter mRNA was present in quantities large enough to detect. Analysis of intestinal mRNA from these un-fed animals suggested that lack of a dietary source of glucose led to a decrease in mRNA coding for the Na^+-D-glucose cotransporter. Table 1 summarizes these results.

These results might be explained as follows. In the case of fresh flounder dietary glucose is present, especially in the intestine, and the intestinal system is therefore induced to produce transport protein to deal with the increased load of sugar (it is not yet clear if this induction is a direct effect or a secondary effect of glucose i.e. hormonal or secondary messenger). Reabsorption of sugar through the kidney would be of lesser importance because the animal is getting all it needs through the intestine, therefore the signal would be difficult if not impossible to detect in renal

mRNA samples. In the case of fish which were un-fed for 4 weeks no glucose would be present in the intestines because no food had been consumed. It then follows from the above arguments based on studies in mammals that the intestinal system is down regulated under these conditions, thus to detect a signal in mRNA isolated from such animals may be difficult if not impossible. The reason that a signal was observed in renal mRNA samples from these animals may be a result of the animals last attempts to retrieve what little sugar was available from the forming urine. Clearly more work needs to be done to determine the exact mechanisms which are at work here. The results from such experiments should give an insight into the workings of the regulatory mechanisms for what is thought to be the same transport protein from 2 different tissues.

Table 1. Effect of nutritional state on the Na^+-D-glucose cotransporter mRNA in flounder kidney and intestine.

	fed	un-fed
kidney	+/-	+++
intestine	+++	+/-

+/- = weak or no signal observed with cDNA probe from rabbit kidney Na^+-D-glucose cotransporter in northern blot analysis; +++ = strong signal observed.

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