At the ventrolateral extreme of the tectum the cellular laminae of the periventricular zone separate to form a diffusely laminated nucleus, the torus semicircularis, that protrudes as a small bulge into the mesocoel . The torus semicircularis is particularly apparent in species with a large mesocoellike the spiny dogfish and other primitive squalomorph sharks. In skates and other batoids hypertrophy of the midbrain roof has greatly reduced the size of the mesocoel and we can recognize no distinct torus in these animals. We have shown here that in the dogfish the laminated structure that has been recognized as the torus semicircularis (Smeets et al., Cent. Nerv. Syst. Cartilag. Fishes, Springer, 1983; Northcutt, Amer. Soc. Zool. 17(2): 411, 1977), is not the main target of ascending electrosensory information. Instead electrosensory activity is associated with the lateral mesencephalic nucleus (LMN) just lateral to the torus. An apparently homologous LMN is the major mesencephalic electrosensory nucleus in the skate. Although it has not been previously recognized as such, the LMN appears to be a non-laminated subdivision of the torus semicircularis in both sharks and skates.

As stated above, we cannot recognize a laminar torus in skates but the LMN is a complex structure with a lateral electrosensory portion and separate medial subdivisions that appear to be associated with lateralis mechanoreception and audition. Subdivisions of LMN are not readily apparent in the dogfish and the ascending projections of mechanosensory and auditory fibers are unknown in this species. It is possible that the laminar torus of the dogfish is the target of ascending mechanosensory and/or auditory fibers. However, further studies are required to determine this. This work was supported in part by NIH grants to DB and RGN.

FURTHER STUDIES ON THE CELLULAR IONIC COMPOSITION OF THE DOGFISH (SQUALUS ACANTHIAS) RECTAL GLAND

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Our previous studies on the solutes and water in the cells of the dogfish rectal gland (cf. Kleinzeller et al., Bull. MDIBL 18-22, 1978-1982) indicated that the apparent concentrations of bulk cell cations [Na^+] plus [K^+], are of the order of 215 mM, i.e., markedly lower than in the plasma or incubation medium (290 mM). The possibility was now explored whether the missing cation(s) might be organic, e.g., amines and/or related substances.

1. The apparent intracellular pH (pH_i). pH_i lower than in the cellular environment (pH_o) might represent a sink for the entry into, and cellular accumulation of organic cations. The cell pH was determined on the basis of the distribution of dimethyloxazolidine dione (DMO) between the cells (DMO_i) and the incubation medium (DMO_o) (Waddell and Butler, J. Clin. Invest. 38:720-729, 1959). Slices were incubated aerobically (1% CO_2 in O_2) at 15°C for 60 min in saline containing 0.1 mM [^{14}C]-DMO (0.04 μCi/ml) (pH 7.60) when a steady state of DMO distribution has been reached, and the probe was extracted from the tissue. The activity in the tissue extract and the medium after incubation and the final pH_o were determined. In a separate group of slices, the extracellular (polyethylene glycol, PEG) space and tissue water were measured. From these data, $[DMO]_i$ was assessed and pH_i was calculated. Mean values of 4 experiments (17 analyses $\pm$ S.E.M.): pH_o = 7.49 - 7.63; $[DMO]_i/[DMO]_o$ = 3.53 $\pm$ 0.48; pH_i = 8.17 $\pm$ 0.06. This last value is consistent with data computed from the distribution of HCO_3^- between the plasma and the rectal gland (Swenson et al., Bull. MDIBL 22:76-78, 1982) assuming a pCO_2 of 0.2 mM H_2CO_3 (Dr. Maren, personal communication). Further independent confirmation of a cell pH more alkaline than that of the plasma will be sought.

2. Tissue NH_4^+, volatile amines (probably trimethylamine, TMA), and trimethylamine oxide (TMAO): These nitrogenous compounds were determined by Conway microtitration (see Cohen et al., Am. J. Physiol., 194:229-235, 1958) in perchloric acid extracts of the dogfish plasma, fresh rectal gland, and slices of the tissue incubated aerobically for 60 min at 15°C in elasmobranch Ringer devoid of TMAO. Values, in μmol/g tissue wet wt. $\pm$ S.E.M. are given

in Table 1. Considerable amounts of NH_4^+ were found in the tissue; the values of "TMA" were below 1 mM. Preliminary experiments showed that TMA entered the cells by a saturable mechanism. The plasma level of TMAO was

Table 1.--Ammonia, Volatile Organic Amines ("TMA") and Trimethylamine Oxide (TMAO) in the Dogfish Plasma and Rectal Gland

	NH_4^+ μmol/g W.W.	"TMA" μmol/g W.W.	TMAO μmol/g W.W.
Plasma	--	0.22 ± 0.07	71 ± 5.2
Fresh Tissue	7.6 ± 1.3	0.59 ± 0.16	50 ± 7.2
Slices	10.1 ± 1.6	0.18 ± 0.11	34 ± 4.5

Nitrogenous compounds, in μmol per gram tissue or ml plasma are the mean values of 5 fish (20 analyses), ± SEM. Slices from a portion of the tissue were incubated aerobically (1% CO_2 in air) at 15°C for 60 min in a balanced elasmobranch saline devoid of TMAO. Mass spectrographic analysis by Dr. G.C. Kahn (Dept. of Pathol., University of Pennsylvania) revealed major amounts of TMA and dimethylamine in extracts of rectal glands. These results require a further evaluation of existing analytical procedures for tissue ammonia and TMA, but do not affect the above data for TMAO.

in the range of values given by Cohen et al. From the data on tissue TMAO (fresh gland and incubated slices) the apparent tissue and cell TMAO concentrations were assessed after appropriate corrections for tissue dry wt. and extracellular space. Within the limits of experimental error, $TMAO_i$ was identical with that in the plasma, indicating that a) in vivo the tissue TMAO equilibrates with the plasma; b) in vitro the cell membrane appears to be either impermeable to TMAO, or this compound is held in the cells by a specific process. Preliminary experiments indicate the latter possibility.

The data suggest that NH_4^+ and TMA do not contribute significantly to the perceived cation deficit in the cells of the rectal gland. While the 70 mM intracellular TMAO may play an osmotic role, it cannot serve as a cellular cation in view of its low pK (4.5 at 25° C) and the high intracellular pH. This conclusion raised the question whether the presence of an intracellular osmotic agent can affect the cellular ionic distribution.

3. Intracellular osmotic agents and the ionic distribution: The steady state of the simplest possible pump-and-leak (double Donnan) system is defined by the following simplified equations cf. Kleinzeller, Biochim. Biophys. Acta 48:41-50, 1960. In the double Donnan system, the osmotic pressure gradient across the membrane is assumed to approach 0. $[Na^+]$, $[K^+]$, and $[Cl^-]$ denote molal concentrations; A: intracellular non-diffusible anion with n charges/molecule ($n >> 1$); B^+: sum of bulk cations, e.g. $[Na^+] + [K^+]$; π: osmotic pressure; ψ: E_m; subscripts i and o denote the intra- and extracellular compartments, respectively.

$$[Na^+]_i + [K^+]_i = n[A^{n-}]_i + [Cl^-]_i \quad (1)$$

$$[Na^+]_o + [K^+]_o = [Cl^-]_o \quad (2)$$

$$\psi = RT/zF \times \ln [K^+]_i/[K^+]_o = - RT/zF \times \ln [Cl^-]_o/[Cl^-]_i \quad (3)$$

and

$$\Delta\pi/RT = (\pi_i - \pi_o)/RT = ([B^+]_i + [A^{n-}]_i + [Cl^-]_i) - ([B^+]_o + [Cl^-]_o) \quad (4)$$

It can be easily shown that at $\Delta\pi/RT > 0$, and/or $\psi \neq 0$, $[B^+]_i > [B^+]_o$. By replacing in eq. (4) the value of $[B^+]_i$ according to (1), and $[B^+]_o$ from eq. (2), and by rearranging, we get

$$\Delta\pi/RT = 2[Cl^-]_i + (n+1)[A^{n-}]_i - 2[Cl^-]_o \quad (5)$$

Since the membrane is assumed to be readily permeable to water, at the steady state $\Delta\pi$ cannot have negative values, and hence $2[Cl^-]_i + (n+1)[A^{n-}] > 2[Cl^-]_o$, and therefore $[B^+]_i > [B^+]_o$.

How does the presence of a non-dissociated, effectively impermeable intracellular solute S_i affect the above conditions?

Equations (1), (2) and (3) will remain unchanged, since they relate to the conditions of electroneutrality in compartments i and o, and to the transmembrane potential. However,

$$\Delta\pi'/RT = (\pi'_i - \pi'_o)/RT = ([B'^+]_i + [A'^{n-}]_i + [S]_i) - ([B^+]_o + [Cl^-]_o)$$

also, $\pi'_i = \pi'_o$. Hence

$$\Delta\pi'/RT = 2[Cl^-]_i + (n+1)[A'^{n-}]_i + [S]_i - 2[Cl^-]_o \qquad (5a)$$

and $[B^+]_i < [B^+]_o$. Clearly, $[B^+]_i$ will decrease with increasing $[S]_i$. The presence of major amounts of an intracellular osmotic agent such as TMAO will therefore have a tendency to decrease $[B^+]_i$ in the cells of the dogfish rectal gland below $[B^+]_o$ and hence decrease the originally perceived cationic deficit. It can also be shown that the presence of Si decreases ψ. This work was supported in part by the Whitehall Foundation.

SPECIFICITY OF THE SUGAR-INDUCED CHANGES IN SHORT-CIRCUIT CURRENT IN THE INTESTINAL MUCOSA OF THE WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS)

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Winter flounder (Pseudopleuronectus americanus) enterocytes have been reported to actively secrete the sugars D-galactose (Gal) and its 2-deoxy-derivative (2dGal). (Naftalin & Kleinzeller, Am. J. Physiol. 240:G392, 1981.) The addition of 2dGal to the serosal surface of an in vitro short-circuited intestinal preparation increases the mucosally directed tissue current. (Kleinzeller, et al., J. Physiol., 326:19P, 1982.) It was thus of interest to determine the specificity of this effect of sugars on the short-circuit current.

Stripped sheets of intestinal mucosa were mounted in a modified Ussing chamber and bathed on both sides with identical saline (in mM: Na^+, 143; Cl^-, 129; K^+, 6; Ca^{2+}, 1; Mg^{2+}, 1; HCO_3^-, 14; acetate, 6; SO_4^{2-}, 1; HPO_4^{2-}, 1; pH 7.8) held at 15°C and gassed with 1% CO_2: 99% O_2. The addition of 20 mM Gal to the serosal surface of the intestinal mucosa under short-circuited conditions (equimolar mannitol added to mucosal solution to maintain osmotic balance) produced a change in short-circuit current (I_{SC}) similar in magnitude and direction to that seen with the same concentration of 2dGal (mean $\Delta I_{SC} \pm$ S.E.M.: Gal - 7.9 ± 1.3 μA cm^{-2} (n = 13); 2dGal - 8.2 ± μA cm^{-2} (n = 42)). In contrast to the insignificant effect of D-glucose (Glc) on the tissue's electrical parameters if presented to the mucosal surface under standard conditions (Thompson & Kleinzeller, Bull. MDIBL 22:54, 1982), serosal Glc (6 mM) elicits a large increase in the tissue I_{SC} (15.2 ± 1.7 μA cm^{-2} (n = 19) - almost 2-fold greater than that of Gal or 2dGal). This effect was evident even in tissues previously stimulated with 2dGal. The glucose induced ΔI_{SC} is a saturable function of glucose concentration (K_m - 1.7 ± 0.4 mM; V_{max} - 25.0 ± 1.7 μA cm^{-2} ($\bar{X}$ ± S.E.M.)). The non-metabolizable 3-O-methyl-D-glucose (3-O-MG), which accumulated intracellularly to the same extent as Glc when present serosally (mean (n = 3) nmol/μl H_2O_i ± S.D.:3-O-MG - 0.57 ± 0.03; free Glc -0.44 ± 0.10) affected the tissue I_{SC} less than Gal or 2dGal.

The above data suggest that the observed effects of some sugars, particularly of Glc vs. 3-O-MG, on the I_{SC} reflect their effects on cell metabolism rather than the involvement of some ionic flux associated with the sugar transport across the basolateral cell membrane. These tentative conclusions are consistent with the following preliminary observations:

a) metabolic substrates (6 mM L-lactate, pyruvate or β-OH-butyrate) also produced a significant ΔI_{SC} when presented to the basolateral cell membrane.