

In My View. . . .

A Miss for NIS?

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THIS DISCOVERY AND CHARACTERIZATION of the sodium iodide symporter (NIS) by Carrasco and colleagues (1), and the many subsequent papers based on this important finding are, for the most part, fully consistent with what has been learned in the past 50 years from physiologic iodide transport studies and clinical material. One problem that has not been resolved, however, involves the anion selectivity differences between NIS and transport studies. The inward currents produced by high concentrations of various anions in *Xenopus* oocytes expressing NIS define a selectivity series: $I^- > ClO_3^- > SCN^- > NO_3^- > Br^- > F^-$. No current is produced in the presence of the tetrahedral anions ClO_4^- and ReO_4^- (Table 1 in Eskandari et al. [2]). By contrast, physiologic anion transport in thyroid tissue defines a selectivity series: $ClO_4^- \sim TcO_4^- > ReO_4^- > SCN^- > I^- \gg NO_3^-, Br^-$ (3). Partial selectivity series consistent with this sequence have been published by many other groups (4,5). In biologic systems ion selectivity is best described by the Eisenman theory in which the balance of coulombic forces and hydration enthalpy of the ion and sequestering molecule determines selectivity (6). Reichenberg (7) has pointed out that desolvation of the anion is likely to be partial, and that the degree of loss of the hydration shell is an important determinant in the selectivity. This formulation has led to the classification of seven sets of anion selectivity in different biological systems (8). Thyroid gland selectivity fits series 1, whereas NIS selectivity does not precisely fit any of the seven anion sets. It must be pointed out that certain anions are difficult to place because they are rapidly metabolized, e.g., SCN^- (to sulfate), although whether or not this is sufficient to account for the low accumulation of thiocyanate remains to be determined. Moreover, saliva of smokers is rich in thiocyanate, substantially higher than serum thiocyanate levels (9), suggesting a concentrating mechanism. IO_3^- is also rapidly reduced to iodide and ClO_3^- is also rapidly reduced (see below).

How can this significant discrepancy be explained? Because iodide and its incorporation into thyroglobulin is the defining characteristic of thyroid tissue, the selectivity issue is of more than academic interest. The chief problem areas stem from the high activity of ClO_3^- and Br^- in the NIS system but not in the thyroid, and the absence of any inward

current from the tetrahedral anions, ClO_4^- , ReO_4^- and, by implication, TcO_4^- in NIS-expressing oocytes, although they are readily accumulated in iodide-concentrating tissues. Some details regarding these discrepancies are listed below.

Bromide

Although small concentration gradients for Br^- in the thyroid are sometimes reported, many other reports are negative (10–12). Furthermore, large concentrations of bromide are required to inhibit thyroid iodide accumulation and these may be toxic or nonthyroidal effects. Thus, bromide is very poorly concentrated by thyroid tissue if at all, and these results are not consistent with the substantial inward current seen in oocytes expressing NIS (2).

Chlorate

ClO_3^- is readily reduced in both acid and basic solutions and it bleaches the pigments present in thyroid slices in neutral pH conditions (3). Perchlorate, however, is stable at neutral pH. It should be pointed out that iodate, with less oxidizing power than chlorate, is rapidly reduced to iodide in thyroid slices (13). It is likely, therefore, that chlorate undergoes a fate similar to iodate and would not long survive in thyroid tissue. This lability may also explain the poor antithyroid activity of chlorate (14). The rather short-term electrical measurements using NIS may well not experience chlorate reduction, hence there is perhaps no conflict between the two systems.

Perchlorate

The body-centered tetrahedral oxyanions of concern here, in which the central atom is heptavalent (+7), are perchlorate, ClO_4^- , perrhenate, ReO_4^- , and pertechnetate, TcO_4^- . The X-O bond lengths are 1.49 Å, 1.97 Å, and 1.75 Å, respectively (15). Perchlorate is the smallest and perrhenate the largest of these anions, although the differences are less for the hydrated oxyanions. The first two are stable in biologic experiments (10,16), whereas pertechnetate is somewhat less

so, forming products of a lower valence state (see below). Because of the low specific activity of ^{36}Cl , it has been difficult to study the necessary low concentrations of radioactive perchlorate. However, several studies have succeeded in showing ClO_4^- accumulation by the thyroid. In the first, using low specific activities, accumulation was only moderate (17). In the other two studies, perchlorate, produced by a different method, led to thyroidal accumulation comparable to that of iodide (16,18).

Essentially no $^{36}\text{Cl}^-$ or other radioactive products were found. It has been argued by Carrasco et al. (20) that the perchlorate used above must have been contaminated with radioactive chlorate. This is remotely possible in the first method because it used electrolytic oxidation of chloride, although conditions can be adjusted to minimize this. In the second preparation (16,18) perchlorate was produced by a proprietary method on a porous metal catalyst in which chlorate is not a byproduct, according to the manufacturer. It seems unlikely therefore, that chlorate accumulation is the basis of the ^{36}Cl from perchlorate found in the latter studies. The lower stability of chlorate would also argue against such an interpretation. There is thus a major difference between the two types of transport measurement.

Another oxyanion that failed to elicit an inward current is the larger perrhenate, ReO_4^- (partial molal ionic volume $48.7 \text{ cm}^3/\text{mol}$ vs. $44.0 \text{ cm}^3/\text{mol}$ for perchlorate [3]). This stable anion is readily transported into the thyroid, its uptake is blocked by iodide, and in turn, it blocks iodide transport. The K_m and K_i values are fully interchangeable. It is also a potent goitrogen (20) and induces hypothyroidism (21). The recent interest in the use of $^{188}\text{ReO}_4^-$ for prevention of restenosis after coronary angioplasty has shown excellent concentration of this anion in the thyroid and stomach by imaging techniques. Iodide or perchlorate readily blocked such uptake (22,23). Perrhenate is thus efficiently accumulated by iodide-concentrating tissues.

Finally, it is obvious that pertechnetate, $^{99m}\text{TcO}_4^-$, is concentrated in iodide concentrating tissues because it has been a favorite imaging tool for many years because of its favorable radiation properties. The unidirectional clearances of iodide and pertechnetate are similar in euthyroid patients, but may be higher in hyperthyroidism and certain congenital goiters (24). The metastable isotope ($t_{1/2} = 6.0$ hours) decays to ^{99}Tc ($t_{1/2} = 2.12 \times 10^5$ y), thus permitting relatively easy chemical work to check K_m values etc. Although pertechnetate is closer in chemical reactivity to perrhenate than to permanganate, a fraction is converted to lower oxidation states and no longer behaves like the anion, clouding the interpretation of long-term experiments (25,26). Nevertheless, it is clear that the anion is well transported as anion into the thyroid.

Although inward transport does not necessarily imply accumulation of anions (e.g., high efflux rates), the reverse, i.e., accumulation without transport, is difficult to envision. We are, therefore, left with a paradox: the tetrahedral anions are readily transported into the thyroid and other iodide-concentrating tissues but they fail to elicit an inward current such as iodide in oocytes or Chinese hamster ovary (CHO) cells expressing NIS or FRTL-5 cells (27). How can this be reconciled? The following are some possibilities that can be examined experimentally:

1. The transport of tetrahedral anions is electrically silent. Simultaneous measurement of current and transport of, e.g., $^{188}\text{ReO}_4^-$ might provide an answer.
2. Nonpolarized cells transfected with NIS may not provide a complete model for the polarized epithelia of iodide-concentrating tissues. The fact that isolated nonpolarized thyroid cells behave like transfected oocytes (27) lends some credence to this suggestion. For example, could the tetrahedral anions fail to dissociate from the symporter in nonpolarized cells? Does this require an additional factor? Moreover, polarized cells have a second extracellular compartment, and thus two different pathways for efflux. Transfection of NIS into polarized epithelia would help to answer this question.
3. The high concentrations of anions that are used for electrophysiologic studies may use additional entry mechanisms. Can the sensitivity of these methods be increased to study transport at or below the K_m ?
4. Could a different system transport the tetrahedral anions as has been suggested for pertechnetate transport in parotid acinar cells by the $\text{Na}/\text{K}/\text{Cl}$ system? (28). Note that the coexistence of two transporters in the parotid gland has not been considered in this study.

One is forced to conclude, therefore, that although NIS is the most important component of iodide transport, it may not provide the complete answer.

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