Kinetics of the iodide trapping mechanism in normal and pathological human thyroid slices

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Abstract. Kinetics of the iodide trapping mechanism in thyroid slices was studied in human and animal tissues. Slices were incubated with several medium iodide concentrations, ranging from $5 \times 10^{-8}$ M to $2 \times 10^{-4}$ M, in order to calculate in the steady state the following kinetic parameters of the iodide transport: Km, maximal capacity (C) and diffusion factor (D). Results indicated that the Km was similar in magnitude ($10^{-8}$ M) in all cases where trapping activity was present, while maximal capacity (C) values showed significant differences between those pathologies in which trapping activity was hyperstimulated (dishormonogenetic goitre, Graves' disease, toxic adenoma) and those where thyroid tissues presented focal or total alterations on its structure (non-toxic nodular goitre, Hashimoto's thyroiditis, thyroid cancer) or where thyroid tissues were not sufficiently stimulated by TSH (extranodular tissue of toxic adenoma). 'Warm' nodules were not significantly different from normal human thyroid. These results suggest that the scattered trapping values observed in the different thyroid pathologies correspond to quantitative differences between them rather than to qualitative alterations in the thyroid iodide pump.

Iodide is actively accumulated in the thyroid gland by an energy dependent process in which ion-pair formation may be important for anions of a certain partial molal volume (Wolff 1964). Kinetic studies of the iodide trapping mechanism have previously been reported (Wolff 1964, 1968; Fayet & Hovsee- pian 1977) but in vitro studies of this type have never been performed in human thyroids.

Wollman & Scow (1954) demonstrated that, in the steady state, different models may explain active iodide concentration by the thyroid, although all follow the same general equation:

$$\frac{I_T}{I_M} = \frac{C}{K_m + I_M} + D \text{ (eq. 1)}$$

In this equation $I_T$ and $I_M$ represent the total thyroid iodide content and the iodide concentration in the incubation medium, respectively; $K_m$, C and D are constants whose interpretation depends on the model utilized, although they correlate with the capacity (C) and quality (Km) of the iodide pump, and with the passive diffusion (D) of the iodide from the incubation medium into the thyroid cell.

The present study deals mainly with the measurement of C, Km and D in human thyroid slices obtained from normal and pathological glands; some animal tissues were utilized for comparison.

Materials and Methods

Thyroid specimens
Pathological thyroid specimens were obtained from patients who underwent thyroidectomy for non-toxic nodular goitre (12 cases: 8 patients with 'cold' nodules and 3 with 'warm' nodules for kinetic studies and 1 case for time course studies), dishormonogenetic goitre with iodide organification defect (4 cases), Graves' disease (6 cases: 5 patients for kinetic studies and 1 case for time course studies), toxic adenoma - 'hot' nodules - (8 cases, in

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5 of them extranodular tissue was also assayed), Hashimoto's thyroiditis (1 case) and papillary carcinoma (1 case). The normal portions removed with single 'cold' nodules from euthyroid patients were taken as normal thyroids; this was corroborated by the histological examination of part of the material. Animal thyroids were obtained at a nearby slaughterhouse.

### Tissue preparation

Surgically excised glands were immediately examined by the pathologist, who divided the tissue in two pieces, one for histological studies and the other for these experiments. Thyroid specimens were immersed in a standard medium at 4°C which contained: 130.8 mM NaCl, 1.0 mM CaCl₂, 1.33 mM MgSO₄, 4 mM KCl, 10 mM D-glucose, 1 mM methimazole and 8.6 mM Na₂HPO₄. HCl was added to obtain a final pH of 7.4. Finally, slices of 0.5 mm thickness were prepared with a Stadie-Riggs microtome.

### Incubation of thyroid slices

Slices of 50 to 150 mg wet weight from each piece of thyroid were rinsed for 5 min in the standard medium at 37°C and then incubated in separate flasks containing 3 ml of the standard medium plus different concentrations of KI, ranging from $5 \times 10^{-6}$ M to $2 \times 10^{-4}$ M. Finally, at least 3 slices for each KI concentration and no less than 6 different concentrations of KI were utilized for each thyroid under study. Each flask contained about 0.2 to 0.5 µCi of carrier and reductor free $^{131}$I. In each experiment some slices were incubated only with $^{131}$I and $10^{-2}$ M KClO₄ in the standard medium to block active transport of iodide in order to measure the passive diffusion (D). All slices were incubated at 37°C for 2 h to be sure that they would reach the steady state, with air as the gaseous phase and continuous shaking at 60 cycles/min. After incubation, slices were rinsed, blotted, weighed and their $^{131}$I radioactivity measured in a well scintillation gamma counter. The $^{131}$I was also counted in 1 ml of the incubation medium. All final results were expressed in terms of wet weight.

### Time course studies

Three thyroid specimens including a Graves' disease, a multinodular goitre and the normal extranodular tissue from a follicular adenoma were studied at different times, from 5 to 150 min, in order to obtain the data of the time course of the thyroidal iodide transport. Medium iodide concentration in all these experiments was $10^{-6}$ M.

### Kinetics

In the steady state, the actively concentrated thyroidal iodide (Iₜ) is equal to the total thyroid iodide concentration (Iₜ) minus the thyroidal iodide which enters solely by diffusion (D × Iₘ), where D is the diffusion factor and Iₘ the iodide concentration of the incubation medium.

![Fig. 1.](image)

Lineweaver-Burk plot of a thyroid with Graves' disease showing the inverse of the iodide concentration actively trapped by each thyroid slice ($1/Iₜ$), as a function of the inverse of the iodide concentration in the incubation medium ($1/Iₘ$). Intrathyroidal iodide is expressed as nmol/g of fresh tissue and the medium iodide as nmol/ml. Each circle (●) represents one thyroid slice.
When \( I_M \) is expressed as nmol/ml, \( I_T \) is expressed as nmol/g of tissue, then:

\[
I_T = I_M - (D \times I_M) \quad \text{(eq. 2)}
\]

Considering that slices incubated with KClO₄ have \( I_T \) equal to zero,

\[
D = \frac{I_T}{I_M} \quad \text{(eq. 3)}
\]

then, starting from equation 1, 2 and 3, in the steady state:

\[
I_T = \frac{C \times I_M}{K_m + I_M}
\]

By Lineweaver-Burk plots we calculated the maximal capacity (C) and the Km of the different thyroidal iodide pumps (Fig. 1). Plots always have a correlation coefficient higher than 0.7, significant at \( P < 0.01 \) according to Snedecor tables (Snedecor 1956).

![Graph](image)

**Fig. 2.**
Time course studies of iodide transport in thyroid slices. Graves’ disease (▲), multinodular goitre (●) and normal extranodular tissue from a follicular adenoma (■). Each point represents the mean value of three determinations. Incubations with KClO₄ show the diffusion values in the different cases.

When \( I_M \) is expressed as nmol/ml, \( I_T \) is expressed as nmol/g of tissue, then:

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![Graph](image)

**Fig. 3.**
Maximal capacity (C) values of normal and pathological thyroid glands, expressed as mg of I' per 100 g of fresh tissue. Bars indicated mean values ± SD. On the top, statistical \( P \) values for each group tested against the control (normal human tissue) were obtained by Student’s \( t \)-test (*\( P < 0.001 \); **\( P < 0.01 \); N.S., \( P > 0.05 \)).

A) Normal thyroid glands: NT, human thyroid (\( n = 5 \)); BT, bovine thyroid (\( n = 2 \)) and PT, porcine thyroid (\( n = 2 \)). B) Thyroid slices with hyperfunctioning trapping activity: DG, dishormonogenetic goitre (\( n = 4 \)); GD, Graves’ disease (\( n = 5 \)) and TA, toxic adenoma (\( n = 8 \)), compared with normal human tissue. C) Pathological thyroid glands with decreased iodide trapping: CN, ‘cold’ nodules (\( n = 8 \)); ET, extranodular tissue from toxic adenoma (\( n = 5 \)); HT, Hashimoto’s thyroiditis (\( n = 1 \)) and CA, papillary carcinoma (\( n = 1 \)), compared with normal human thyroid. D) ‘Warm’ nodules (WN) from non-toxic nodular goitre compared with normal human slices.
Table 1.
Km values in normal and pathological thyroid glands.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>n</th>
<th>Km ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal human thyroid</td>
<td>5</td>
<td>3.12 ± 0.98 × 10⁻⁵ M</td>
</tr>
<tr>
<td>Non toxic nodular goitre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) 'warm' nodules</td>
<td>3</td>
<td>1.96 ± 0.77 × 10⁻⁵ M</td>
</tr>
<tr>
<td>b) 'cold' nodules</td>
<td>8(4)*</td>
<td>2.22 ± 1.25 × 10⁻⁵ M</td>
</tr>
<tr>
<td>Dishormonogenetic goitre</td>
<td>4</td>
<td>1.77 ± 0.71 × 10⁻⁵ M</td>
</tr>
<tr>
<td>Graves' disease</td>
<td>5</td>
<td>3.34 ± 0.55 × 10⁻⁵ M</td>
</tr>
<tr>
<td>Toxic adenoma</td>
<td>8</td>
<td>2.48 ± 0.86 × 10⁻⁵ M</td>
</tr>
<tr>
<td>Extranodular tissue of toxic adenoma</td>
<td>5</td>
<td>2.10 ± 0.75 × 10⁻⁵ M</td>
</tr>
<tr>
<td>Hashimoto's thyroiditis</td>
<td>1</td>
<td>1.80 × 10⁻⁵ M</td>
</tr>
<tr>
<td>Papillary carcinoma</td>
<td>1**</td>
<td></td>
</tr>
<tr>
<td>Bovine thyroid</td>
<td>2</td>
<td>3.90 × 10⁻⁵ M</td>
</tr>
<tr>
<td>Porcine thyroid</td>
<td>2</td>
<td>3.10 × 10⁻⁵ M</td>
</tr>
</tbody>
</table>

* Km in 'cold' nodules was calculated in the cases (n = 4) where a measureable trapping activity was present.

** Km was not measured because there was no trapping activity.

Results

The time course studies (Fig. 2) showed that the steady state was reached at 120 min, with a marked fall of the T/M values at 150 min in the case of the Graves' disease slices.

Table 1 shows the Km values, which were similar in magnitude (10⁻⁵ M) in those glands, where active transport was present. Spread values are most probably the result of experimental handling rather than an actual difference between the groups. For this reason we have calculated an average Km value for normal and pathological human glands of 2.35 × 10⁻⁵ M. Animal tissues were not statistically different from normal human thyroids (Table 1).

On the other hand, normal human thyroid slices had a maximal capacity (C) of 1.16 ± 0.2 mg I⁻¹/100 g of fresh tissue (Fig. 3). One group of the pathological specimens, comprised by Graves' disease (8 ± 2.8), toxic adenoma (4.08 ± 2.74) and dishormonogenetic goitre (2.79 ± 0.72), showed an elevated maximal capacity (Fig. 3B). In contrast iodide concentrating capacity was decreased or even absent (Fig. 3C) in non-toxic nodular goitre (0.03 ± 0.05), Hashimoto's thyroiditis (0.05), extranodular tissue from toxic adenoma (0.53 ± 0.20) and papillary thyroid carcinoma (non-detectable). Maximal capacity of 'warm' nodules from nodular goitres (0.75 ± 0.27) showed no significant difference from normal human glands (Fig. 3D).

Normal bovine and porcine thyroid slices showed C values several times higher than normal human tissues (Fig. 3A).

Passive diffusion of iodide measured on pathological tissue was similar to control values obtained.

Table 2.
Passive diffusion of iodide (D) values in human and animal thyroid glands.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>n</th>
<th>D factor ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal human thyroid</td>
<td>5</td>
<td>0.79 ± 0.22</td>
</tr>
<tr>
<td>Non toxic nodular goitre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) 'warm' nodules</td>
<td>3</td>
<td>0.75 ± 0.12</td>
</tr>
<tr>
<td>b) 'cold' nodules</td>
<td>8</td>
<td>0.78 ± 0.14</td>
</tr>
<tr>
<td>Dishormonogenetic goitre</td>
<td>4</td>
<td>1.02 ± 0.30</td>
</tr>
<tr>
<td>Graves' disease</td>
<td>5</td>
<td>0.67 ± 0.12</td>
</tr>
<tr>
<td>Toxic adenoma</td>
<td>8</td>
<td>0.75 ± 0.30</td>
</tr>
<tr>
<td>Extranodular tissue of toxic adenoma</td>
<td>5</td>
<td>0.73 ± 0.13</td>
</tr>
<tr>
<td>Hashimoto's thyroiditis</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Papillary carcinoma</td>
<td>1</td>
<td>0.85</td>
</tr>
<tr>
<td>Bovine thyroid</td>
<td>2</td>
<td>0.85</td>
</tr>
<tr>
<td>Porcine thyroid</td>
<td>2</td>
<td>0.67</td>
</tr>
</tbody>
</table>
both with animal and normal human thyroids (Table 2). The passive diffusion measurements in dishormonogenetic goitre (X = 1.02) and Hashimoto's thyroiditis (1.03) were slightly higher.

Discussion

Although the ability to accumulate iodide is not unique to the thyroid (Wolff 1964) it is in this organ that trapping activity plays a fundamental role. Previous studies on thyroid transport were mostly performed in animal tissues (Wolff 1968; Fayet & Hovsepian 1977; Endo et al. 1981) and there are a few reports in which human thyroid slices were used to investigate the iodide pump (Shumacker et al. 1958; DeGroot 1970; Field et al. 1973). However, apart from our preliminary report (Niepomniszcze et al. 1975) and a recent study on a congenital goitre with a trapping defect (Saito et al. 1981), none of these studies have measured the Km and the maximal capacity of human glands. Since active transport of iodide follows Michaelis-Menten kinetics, Bagchi & Fawcett (1973) have developed a model for iodide transport in which Km is equivalent to (1 + K1/[Na+])K2, where K1 and K2 are the dissociation constants of the carrier: Na+ and the carrier: Na+-I- complexes, respectively. When the extracellular concentration of Na+ remains fixed, changes in the Km would be due to the changes in the K1 or K2. Because those latter constants depend on the properties of the carrier, it is possible to correlate the Km with the quality of the iodide pump (Niepomniszcze et al. 1975).

According to our results the Km values of the pathological thyroids were not significantly different from those of normal human thyroids, were obtained in the animal glands and possibly represent a common characteristic of the trapping in the different species (Wolff 1964; Fayet & Hovsepian 1977). Since this type of thyroid pathology showed no significant difference in the magnitude of the Km, it can be assumed that a hypothetical alteration in the quality of the thyroidal iodide carrier is not the cause of the abnormal trapping in these thyroid diseases.

On the other hand, our studies showed significant differences in the maximal capacities (C) of normal and pathological glands. In those diseases where the thyroid is hyperstimulated by TSH (dishormonogenetic goitre) or by abnormal stimulators (Graves' disease) as well as in the hyperfunctional adenomata, the capacity of the iodide pump is elevated. Conversely, where thyroid tissue showed focal or total alterations in its structure (Hashimoto's thyroiditis, non-functional nodular goitre) the maximal capacity was low or even undetectable (thyroid cancer). Extraneural tissues from glands with toxic andenoma showed a diminished maximal capacity compared to the control group.

Since maximal capacity may be related to the TSH concentration (Wolff 1964), and the total amount of carrier in a membrane is regulated by serum TSH (Knopp et al. 1970), it is possible that the high values found in dishormonogenetic goitre and Graves' disease represent an increased amount of membrane carrier induced by high levels of TSH or the presence of thyroid-stimulating immunoglobulins. However, in our system, since we are working in the steady state, there are some other factors that can be responsible for the variations of maximal capacity, such as the intrathyroidal iodide pool and the magnitude of the iodide efflux. Bagchi & Fawcett (1973), studying the influx of iodide at initial velocity, have postulated that Vmax is equivalent to the total carrier.

Previous reports (Schumacker et al. 1958; DeGroot 1970) have shown that T/M (tissue/medium) ratios for iodide are higher in the animal thyroid than in the human one. We have also observed the same phenomenon when C values of animal and normal glands were compared.

Field et al. (1973) studied the trapping of iodide in non-functioning thyroid nodules from human thyroid glands and found that this tissue was unable to concentrate the iodide in vitro. 'Cold' nodules were studied by DeGroot (1970) with similar conclusions. Our findings agree with these studies and suggest a quantitative alteration of the iodide pump as the cause of the diminished trapping activity.

Time course studies have shown that the steady state was reached at 2 h incubation, but in Graves' disease an important fall of the T/M value was observed at 150 min, reflecting, perhaps, intrinsic alterations in these slices produced by the long period of incubations.

In conclusion, we have reported for the first time kinetic studies of the iodide pump performed in slices of pathological thyroid specimens. Quantitative differences between these disease states have been shown, although the quality of the iodide pump seemed to be undamaged in all the thyroids under study.
References


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