Thyroxine effect on intestinal Cl⁻/HCO₃⁻ exchange in hypo- and hyperthyroid rats

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Abstract
Changes in transepithelial water and electrolyte transport as causative or contributing factors of the diarrhoea and constipation found associated with changes in thyroid physiology were studied. Albino Wistar rats were pharmacologically made either hypothyroid or hyperthyroid. After sacrifice, the small intestine was mounted in Ussing chambers in order to measure in vitro ion net fluxes under short-circuit conditions. Hypothyroid animals showed an increase in intestinal transit time, Cl⁻ absorption (mainly due to an increment in its mucosal to serosal component) and residual ion flux (which is believed to represent HCO₃⁻ secretion) when compared with euthyroid animals. The hyperthyroid animals showed a decrease in Cl⁻ mucosal to serosal transport. Furthermore, a significant correlation was found between serum 1-thyroxine (T₄) levels and both net Cl⁻ transport (r = -0.74, P<0.00001) and residual ion flux (r = -0.55, P<0.005).

These results indicate that the effect of T₄ is firstly to inhibit Cl⁻/HCO₃⁻ anion exchange thereby influencing transepithelial flux transport and secondly to affect intestinal motility. Such inhibition was not found when T₄ was acutely added to rat ileum, suggesting that the effect on electrolyte transport probably requires protein synthesis. In conclusion, the phenomenon observed in vitro could explain the clinical manifestations of constipation and diarrhoea in hypo- and hyper-thyroidism respectively. Journal of Endocrinology (1996) 151, 431-437

Introduction
It is generally accepted that thyroid hormones exert an effect on the digestive tract. However, divergent opinions concerning the kind of action that thyroxine (T₄) exerts on gastrointestinal function have been reported. Diarrhoea and steatorrhoea associated with hypothyroidism have been frequently described (Middleton & Thompson 1969, Thomas et al. 1973) and also constipation associated with hypothyroidism (Middleton 1971). Although modification of intestinal transit time (ITT) has usually been considered to be the most important factor responsible for diarrhoea and constipation (Shafer et al. 1984), convincing results from experiments with hyperthyroid patients have suggested the possible involvement of secretory/absorptive processes (Culp & Pizik 1986).

The conflicting data on the influence of thyroid hormones on gastrointestinal function have prompted us to investigate whether changes in ion and water transport across the intestinal mucosa are involved in the genesis of constipation and diarrhoea in hypo- and hyper-thyroid states respectively. We have obtained compelling data suggesting an inhibitory effect of T₄ on Cl⁻/HCO₃⁻ anion exchange. This effect may be responsible for the gastrointestinal symptoms of hypo- and hyper-thyroid patients.

Materials and Methods

Treatment protocol
Adult albino Wistar rats weighing 150–200 g were used for all experiments. The animals were divided into four groups (eight rats in each group). Group A was composed of untreated animals and served as a control. Group B animals were made hypothyroid by the oral administration of 1% Tapazole (Eli Lilly, Indianapolis, IN, USA) for 4 weeks in the drinking water. To ensure that any changes seen in group B animals were due to the hypothyroid state and not to the Tapazole itself, rats in group C were also given Tapazole on the same schedule as those in group B but were supplemented orally, in their drinking water, with T₄ (sodium salt; Bracco, Milan, Italy) to make them
euthyroid (approximately 2 μg/100 g body weight per day calculated on the basis of the daily water intake). Group D rats were made hyperthyroid by treating them with T₄ (6 μg/100 g body weight per day for 4 weeks). All drinking solutions were prepared fresh daily. In order to avoid light-induced degradation of T₄, the drinking bottles were covered by aluminium foil sheets. All animals were housed in single cages and each rat received food and water ad libitum. All animals were weighed at the beginning of the study and again at the end of the 4 weeks of treatment. All experiments were carried out under the rules of the local committee for animal experiments.

**Intestinal transit time**

ITT was measured by calculating the time elapsed between the oral administration of 1 ml carmine red (1% in saline solution) and the first appearance of coloured faeces. ITT was determined in each animal before and at the end of the 4 weeks of treatment.

**Experimental preparation**

At the end of the period of treatment, the rats were weighed, ITT was determined and the animals were subsequently sacrificed by chloroform inhalation. Blood (3–4 ml) was obtained by cardiac puncture from each animal and the serum was kept frozen at −80 °C until all sera were simultaneously assayed for their T₄ concentration by solid phase RIA (Biochem-Immunosystem, Milan, Italy).

A portion of the distal ileum (20 cm long) was excised, opened along the mesenteric border, and rinsed free of intestinal contents with cold Ringer’s solution of the following composition (in mM): NaCl (53); KCl (5); Na₂SO₄ (30.5); mannitol (30.5); Na₂HPO₄ (6.19); Na₄HPO₄ (0.3); CaCl₂ (1.25); MgCl₂ (1.1); NaHCO₃ (25). The Cl⁻ concentration was maintained at 62.5 mM to reduce the diffusion flux of the ion, thereby allowing an accurate assessment of its net flux and of its possible variations.

**Short-circuit current (Iₛ) and ion flux measurements**

Four to eight pieces of small intestinal segments were mounted in Lucite Ussing chambers (0.33 cm² opening) and bathed in Ringer’s solution (at 37 °C) through which a gas mixture of O₂ (95%) and CO₂ (5%) was allowed to gently bubble. Glucose (10 μmol/ml) was added to the serosal (s) side and an equimolar amount of mannitol was added to the mucosal (m) side. Transepithelial electrical potential difference, total tissue electrical conductance (Gₛ) and Iₛ were determined as previously described (Guandalini et al. 1987).

At 20 min after the addition of the isotopes, oppositely directed unidirectional transepithelial fluxes of Na⁺Cl⁻ and Na⁺³⁶Cl⁻ (Amersham International, Amersham, Bucks, UK) from mucosa to serosa (m→s) and from serosa to mucosa (s→m) were measured in tissues matched on the basis of Gₛ under Iₛ conditions, as previously described (Field et al. 1971). Net fluxes for Na⁺ and Cl⁻ were calculated as the difference between the two opposing unidirectional fluxes, i.e. Jₘ⁻ and Jₛ⁻. The residual ion flux (Jₚₙₑₙ) corresponds to the net ion flux not accounted for by transepithelial net fluxes of Na⁺ and Cl⁻. The Jₚₙₑₙ is calculated as Jₚₙₑₙ=Jₛ⁻−(Jₘ⁺−Jₚₙₑₙ−Jₛ⁻) and is generally believed to represent HCO₃⁻ secretion (Dietz & Field 1973).

**Cyclic nucleotides**

At the end of ion flux measurement, three pieces of mucosa from each chamber were immediately placed in ice-cold 5% trichloroacetate containing 4 mCi of [¹⁴C]cAMP (0.07 pmol) or [¹⁴C]cGMP (0.1 pmol) as recovery markers and homogenized with a motor-driven pestle. The [¹⁴C]cAMP (60 mCi/mmole) and the [¹⁴C]cGMP (52 mCi/mmole) were obtained from Amersham International. The amount of cyclic nucleotide recovered was determined as previously reported (Guandalini et al. 1984). Lyophilized cAMP and cGMP antibodies and [³H]-labelled cAMP and cGMP (Harper & Brooker 1975) were also obtained from Amersham. The trichloroacetate precipitates were dissolved in 1 ml NaOH and assayed for protein by the method of Lowry et al. (1951). All the other reagents used were of analytical grade.

**Statistical analysis**

Data analysis was performed with a computer-assisted package (Epistar, Tracy L Gustafson, Round Rock, TX, USA). ANOVA was used for comparison of means. Values are reported as means ± s.d. or, where indicated, means ± s.e.m. Statistical significance was set at P<0.05.

**Results**

**Serum T₄ concentrations, body weight and ITT**

The animals treated with Tapazole alone (group B) had a mean serum T₄ concentration (2.5 ± 0.6 μg/dl) significantly different from that observed in the animals of group D (8.4 ± 0.7 μg/dl; P<0.001). The mean serum T₄ concentrations in group A (untreated) animals and those designed to be euthyroid (group C; Tapazole+T₄) did not differ from each other (4.9 ± 1.3 and 5.2 ± 2.2 μg/dl respectively) but both were significantly different from the mean concentration in the group B (P<0.001) and group D rats (P<0.005).

ITT at the start of the study did not differ among the four groups of animals (group A=620 ± 45 min;
group B = 605 ± 50 min; group C = 615 ± 55 min; group D = 615 ± 60 min).

Because a number of animals from each group died during the 4-week course of treatment and because the T4 concentration in group C animals varied from hypo- to hyper-thyroid concentrations, these animals were re-grouped with the other animals on the basis of whether their T4 concentration was within 1 s.d. from the mean of group B or group D rats. All subsequent results will be expressed on the basis of the final T4 concentration in animals which will be designated as pertaining to the hypothyroid (2.5 ± 0.4 pg/dl; n=8), euthyroid (4.9 ± 1.1 pg/dl; n=12) or hyperthyroid (8.2 ± 1.2 pg/dl; n=7; P<0.001) group.

Although the body weights of the animals were similar at the beginning of the study, the mean of the euthyroid group increased by 86 ± 13 g by the end of the study which was significantly greater than the increment of 44 ± 14 g in the hyperthyroid (P<0.001) and 51 ± 10 g in the hypothyroid (P<0.001) animals (Table 1).

Despite the difference in body weight gain between the eu-, hypo- and hyper-thyroid animals, no differences were observed in the microscopic appearance of the villi of the small intestine in any of the groups (data not shown).

Although no apparent differences in ITT were observed when the animals were studied according to the original four groups (A-D), a significant difference was observed in ITT when the animals were studied according to their thyroid functional state (Table 2). The hypothyroid rats had a mean change in ITT of 111 ± 25 min which was significantly different from the change observed in either the euthyroid (−45 ± 30 min, P<0.0001) or hyperthyroid (−51 ± 52 min, P<0.0001) groups.

**Basal transepithelial ion fluxes**

In the euthyroid rats a significant net absorptive flux of Na+ and a small (not significant) secretory flux of Cl− was observed. The hypothyroid animals exhibited a significant increase in net Cl− absorptive transport (+2.5 ± 0.35 μEq/cm² per h) with respect to control group animals (P<0.0001), which was mainly related to an increment in its m−s flux component. Moreover, an increase in residual ion flux (JR−/Na+), which represents a sizeable shift toward secretion of HCO3− (+1.8 ± 0.5 μEq/cm² per h, P<0.01) was observed. Both unidirectional Na+ fluxes were unaffected. The data are collectively presented in Table 3.

**Relationship between T4 concentration and ion flux**

According to the results obtained in both hypothyroid and hyperthyroid animals, it was speculated that T4 influences transepithelial transport of both Cl− and HCO3−. Serum T4 concentrations were correlated with net Cl− and HCO3− transport by plotting the results obtained in all animals, regardless of their thyroid state. Figure 1 shows the correlation between T4 levels and net Cl− flux. The data clearly demonstrate that low T4 levels are associated with an enhancement of Cl− absorption while high T4 levels are associated with Cl− secretion (r = −0.74; P<0.0001).

An inverse correlation was also found between net HCO3− flux (as judged by JR−/Na+) and the T4 concentration (Fig. 2; r = −0.55, P<0.05), with high levels of T4 inducing HCO3− absorption and vice versa. Figure 3 shows the correlation between residual ion flux and net Cl− flux (r = 0.80, P<0.00001). These findings suggest that net transepithelial Cl− flux is counterbalanced by net transepithelial flux of HCO3− in the opposite direction.

In *vitro* effect of T4 on ion fluxes

To evaluate whether T4 had a direct and immediate action on anion exchange, T4 at a concentration of 9 μg/dl was added to the serosal side of ileal segments of euthyroid animals. This high dose was chosen to reproduce the T4 concentration found in hyperthyroid rats. The results presented in Table 4 clearly indicate that the acute *in vitro* addition of T4 does not affect ion transport.

<table>
<thead>
<tr>
<th>Thyroid functional state</th>
<th>n</th>
<th>ITT before treatment (min)</th>
<th>ITT after treatment (min)</th>
<th>ITT gain (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroid</td>
<td>8</td>
<td>533 ± 32</td>
<td>644 ± 20</td>
<td>+111 ± 25</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>12</td>
<td>635 ± 41</td>
<td>590 ± 25</td>
<td>−45 ± 30*</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>7</td>
<td>636 ± 56</td>
<td>585 ± 68</td>
<td>−51 ± 52*</td>
</tr>
</tbody>
</table>

*P<0.0001 with respect to the hypothyroid group.
Table 3 Transepithelial ion fluxes across the ileal mucosa in hypo-, eu- and hyperthyroid rats. Values are expressed as means ± S.E.M. Ion fluxes are in μEq/cm² per h.

<table>
<thead>
<tr>
<th>Functional state</th>
<th>n</th>
<th>( J_{Na}^{m-s} )</th>
<th>( J_{Na}^{e-m} )</th>
<th>( J_{Na}^{net} )</th>
<th>( J_{Cl}^{m-s} )</th>
<th>( J_{Cl}^{e-m} )</th>
<th>( J_{Cl}^{net} )</th>
<th>( I_{sc} )</th>
<th>( I_{net}^{R} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroid</td>
<td>8</td>
<td>16.8 ± 0.99</td>
<td>14.62 ± 1.29</td>
<td>+2.18 ± 0.43</td>
<td>8.9 ± 0.3*</td>
<td>6.4 ± 0.53</td>
<td>+2.5 ± 0.35*</td>
<td>-1.44 ± 0.22*</td>
<td>+1.8 ± 0.50**</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>12</td>
<td>17.3 ± 0.30</td>
<td>14.5 ± 0.40</td>
<td>+2.8 ± 0.60</td>
<td>6.6 ± 0.5</td>
<td>7.9 ± 0.16</td>
<td>-1.3 ± 0.33</td>
<td>+1.9 ± 0.25</td>
<td>-2.2 ± 0.45</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>7</td>
<td>18.7 ± 1.28</td>
<td>16.2 ± 1.45</td>
<td>+2.5 ± 0.60</td>
<td>3.33 ± 0.6**</td>
<td>6.77 ± 1.06</td>
<td>-3.44 ± 1.10</td>
<td>+0.9 ± 0.09</td>
<td>-5.0 ± 0.7***</td>
</tr>
</tbody>
</table>

*P<0.0001, **P<0.01, ***P<0.05 with respect to the euthyroid group.
Cyclic nucleotide measurement

To establish whether the observed effect of \( T_4 \) on ion transport was mediated by one of the recognized intra¬
cellular modulators of ion transport, tissue concentrations of cAMP and cGMP were examined in the eu-, hypo¬
and hyper-thyroid rats. No difference in content of either cyclic nucleotide was observed among the different groups (Fig. 4).

Discussion

It has long been recognized that thyroid hormones affect the structure and function of the gastrointestinal tract (Shier 1933, Verbruycke 1936, Tinker 1936, Brown et al. 1941, Carriere 1963, Neporent & Spesivteva 1963, Miller et al. 1978, Anand et al. 1981, Bozzani et al. 1985), leading to pronounced gastrointestinal symptoms. In particular, it is well known that hyperthyroidism is associated with diarrhoea (both steatorrhea and secretory diarrhoea have been described) (Thomas et al. 1973, Culp & Piziak 1986), while hypothyroidism is associated with constipation. Although these observations have been well established, the pathophysiological mechanisms underlying these changes are still unclear.

The most commonly held hypothesis for these changes involves intestinal motor activity. Studies on ITT using barium meals (Shier 1933, Shafer et al. 1984, Bozzani et al. 1985) or the pulmonary excretion of \( H_2 \) after ingestion of a non-absorbable carbohydrate such as lactulose (Bronk & Parson 1965, Emvo et al. 1986) had in fact provided evidence for accelerated intestinal transit in hyper¬
thyroidism and decreased intestinal motility in hypo¬
thyroidism. In keeping with the literature, the present results indicate that ITT, as measured using carmine red, was longest in the hypothyroid animals. However, the rate of transit of the luminal contents through the intestine is

Figure 1 Correlation between the serum levels of \( T_4 \) and net Cl\(^-\) flux in the rat ileum. Values above the 0 line represent net absorption and values below represent net secretion.

Figure 2 Correlation between the serum levels of \( T_4 \) and the residual net ion flux in the ileum. The residual net ion flux is thought to represent net transepithelial HCO\(_3^-\) flux. Values above the 0 line represent net secretion, and those below net absorption.
not only dependent on gastrointestinal motor activity but also on the bulk of the luminal contents, which, in turn, depend on intestinal absorptive/secretory processes. In fact, secretory changes, brought about by increasing the fluid into the lumen, are known to induce a faster intestinal transit. Thus, changes in transit time and motility may occur as a consequence of changes in rates of absorption and/or secretion, and therefore one cause does not exclude the other. Interestingly, the ITT of the euthyroid animals was shorter than before treatment and similar to that found in the hypothyroid animals. Since there was definite evidence of increased secretion in hypothyroid animals compared with the euthyroid ones, the reduced ITT in the latter cannot be due to the same mechanism. Possible aspecific stress (hormone)-related conditions in the euthyroid rats may have been contributing factors.

Previous studies in both animal (Fink 1944, Ligumsky et al. 1980, Johnson 1981) and human (Culp & Piziak 1986) models have, in fact, suggested that alterations of the intestinal transport processes might be the primary cause of the intestinal symptoms in both hyper- and hypo-thyroidism. The results of the present investigation clearly show that a reduction in the serum thyroid hormone concentration has a pro-absorptive effect on net \( \text{Cl}^- \) transport, while increasing \( J_{\text{net}}^R \) by a similar magnitude. Furthermore, when the serum T4 levels were plotted against both net \( \text{Cl}^- \) and \( J_{\text{net}}^R \) (Figs 1 and 2), a strong correlation was seen among these three parameters, suggesting that the levels of T4 affect anion exchange over a wide range, with low levels promoting the exchange and high levels inhibiting it.

It is not a new finding that the inhibition of \( \text{Cl}^- / \text{HCO}_3^- \) exchange results in diarrhoea; in fact, the complete lack of such a mechanism is known to cause the

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**Table 4** Lack of immediate effect of T4 on transepithelial ion fluxes across ileal mucosa. Values are means ± S.E.M. Ion fluxes are in \( \mu \text{Eq/cm}^2 \) per h and \( G_0 \) in mhos. T4 was added to the serosal side of ileum 10 min after completion of baseline ion flux measurement.

<table>
<thead>
<tr>
<th></th>
<th>( J_{\text{Na}}^{\text{m-s}} )</th>
<th>( J_{\text{Na}}^{\text{s-m}} )</th>
<th>( J_{\text{Na}}^{\text{net}} )</th>
<th>( J_{\text{Cl}}^{\text{m-s}} )</th>
<th>( J_{\text{Cl}}^{\text{s-m}} )</th>
<th>( J_{\text{Cl}}^{\text{net}} )</th>
<th>( I_{\text{sc}} )</th>
<th>( J_{\text{net}}^R )</th>
<th>( G_0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>19.4 ± 1.3</td>
<td>14.5 ± 1.5</td>
<td>4.9 ± 1.7</td>
<td>9.3 ± 0.9</td>
<td>7.3 ± 0.9</td>
<td>20.0 ± 0.9</td>
<td>1.2 ± 0.1</td>
<td>-1.7 ± 2.0</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>T4 (9 μg/dl)</td>
<td>22.8 ± 3.1</td>
<td>18.4 ± 2.3</td>
<td>3.4 ± 2.2</td>
<td>9.9 ± 1.6</td>
<td>7.8 ± 1.0</td>
<td>21.1 ± 1.0</td>
<td>0.8 ± 0.2</td>
<td>-0.3 ± 1.1</td>
<td>4.3 ± 0.4</td>
</tr>
</tbody>
</table>

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**Figure 3** Correlation between the net flux of residual ion (assumed to represent \( \text{HCO}_3^- \)) and the net flux of \( \text{Cl}^- \) in the rat ileum. Values above the 0 line represent net absorption for \( \text{Cl}^- \) and net secretion for \( \text{HCO}_3^- \); values below represent net secretion for \( \text{Cl}^- \) and net absorption for \( \text{HCO}_3^- \).

**Figure 4** Concentrations of cyclic nucleotides in the ileal mucosa of euthyroid (solid bar), hypothyroid (hatched bar) and hyperthyroid (stippled bar) rats. Values are means ± S.E.M. No statistically significant difference was noted among the three groups.
proliferation of watery diarrhoea typical of congenital Cl⁻-losing diarrhoea (Evans & Stanbury 1965).

Finally, the lack of a direct immediate effect of T₄, added in vitro to the tissue at a similar concentration to that found in the hyperthyroidy animals, suggests that the biological effect of the hormone is the end result of a series of modifications possibly induced by T₄ in a similar way to that already shown for other metabolic functions, i.e. requiring protein synthesis. In the light of this, it is not surprising that the known intracellular mediators of ion secretion, i.e. cAMP and cGMP, are not involved.

It has been previously reported that hyperthyroidy rats have decreased Na⁺, K⁺-ATPase and diminished rates of absorption of water, Na⁺ and glucose (Berant et al. 1993). These findings are not in keeping with the present results. These observations were obtained in vivo, and discrepancies have previously been documented between in vitro and in vivo experiments on the effects of thyroid hormones on intestinal function (Brönk & Parson 1965, Levin & Syme 1971, Kowaleski & Kolodej 1977, Rahje et al. 1977). Furthermore, it should be noted that the diminished water absorption in hyperthyroidism reported by Berant et al. (1993) is in conflict with the clinical evidence of constipation in hyperthyroidy patients.

References


Evans JH & Stanbury SW 1965 Congenital chloridorrhoea or so-called congenital alkalosis with diarrhoea. Gut 6 29–38.


Received 17 April 1996
Revised manuscript received 10 July 1996
Accepted 23 July 1996