EFFECT OF MAGNESIUM STATUS ON THYROID ACTIVITY AND IODIDE METABOLISM

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SUMMARY

The close positive correlation between the magnesium concentration in serum and the activity of the thyroid gland, as indicated by the ratio of (protein-bound $^{125}$I):(total serum $^{125}$I) 24 h after injection of a tracer dose of $^{125}$I iodide, was investigated in young male rats. Dietary deficiency or loading with magnesium salts had no effect on the concentration of cyclic AMP within the thyroid gland or the release of thyroid hormone from glands incubated in vitro.

Accumulation of radioactive iodide by thyroid glands in vivo was stimulated by magnesium loading and inhibited by magnesium deficiency, but there was no selective effect on the synthesis of iodinated tyrosines or thyronines within the gland. As similar differences in radioactive iodide uptake were observed in other soft tissues, this appears to be part of a general influence of magnesium status on iodide transport, rather than a specific action on the thyroid gland.

INTRODUCTION

The magnesium status of the rat has been found to influence thyroid activity, a close positive correlation between the magnesium concentration in serum and the ratio of (protein-bound iodine):(total serum iodine) being observed in rats subjected to specific dietary deficiency or loading with magnesium salts (Humphray & Heaton, 1972). This relationship is due perhaps to a direct action of magnesium on the thyroid gland, or it may be secondary to other metabolic effects produced by alterations in magnesium metabolism.

The availability of magnesium may directly affect thyroid activity in several ways. It has been established that the action of thyrotrophin on the thyroid gland is mediated by the formation of cyclic AMP (adenosine 3':5'-cyclic monophosphate) (Schell-Frederick & Dumont, 1970; Robison, Butcher & Sutherland, 1971), and since magnesium activates the membrane-bound adenylate cyclase of the thyroid in vitro (Patsan & Katzen, 1967; Burke, 1970; Wolff & Jones, 1971), its availability could modify the response of the gland to the pituitary hormone. Other possibilities are that magnesium could influence the uptake of iodide by the thyroid gland, the synthesis of thyroid hormone within the gland, or the release of hormone from the gland. This paper reports a series of experiments that were undertaken to investigate these possibilities.
MATERIALS AND METHODS

Three main experiments were performed using male Wistar albino rats that were allocated randomly to magnesium-deficient, control or magnesium-loaded groups in each experiment. The animals weighed initially about 80, 45 and 100 g in Expts 1, 2 and 3 respectively, and the period of feeding varied from 11-13 days in Expts 1 and 2 to 21 days in Expt 3, to produce a comparable degree of magnesium deficiency. In any one experiment all the rats received an amount of the appropriate diet equal to that consumed by the magnesium-deficient animals in the same experiment and all were fed automatically (Loveless, Williams & Heaton, 1972) to prevent differences in feeding pattern. The synthetic diets were prepared as described previously (Humphray & Heaton, 1972); the magnesium concentrations in the deficient, control and loaded diets were 0-3, 75 and 380 mg/100 g diet, respectively. Distilled water was provided ad libitum.

Measurement of thyroid activity and radioactive iodide uptake by tissues

Carrier-free $^{125}$I was obtained as sodium iodide from the Radiochemical Centre, Amersham. In Expts 1 and 2 each animal was injected i.p. with 10 μCi $^{125}$I contained in 1 ml 0-9% NaCl solution; in Expt 3 this was increased to 100 μCi $^{125}$I/rat. The animals were killed by exsanguination from the heart under ether anaesthesia 24 h after the injection, and the activity of total serum $^{125}$I and protein-bound $^{125}$I (separated by precipitating serum proteins with 10% trichloroacetic acid and washing the precipitate twice with distilled water) was measured with a Nuclear Enterprises well-type scintillation counter. In Expts 2 and 3, the thyroid glands and various other tissues were removed immediately after death and their $^{125}$I activity was counted in the same way. Corrections were made for background and isotopic decay.

Release of iodine by thyroid glands incubated in vitro

Thyroid glands were removed from the rats in Expt 2 immediately after death and incubated using a modification of the procedure developed by Brown & Munro (1967) for the assay of thyrotrophin with mouse thyroids. The gland, attached to a fragment of trachea, was excised from each rat and incubated for 18 h in 3 ml of medium contained in a 25 ml stoppered conical flask. The flasks were shaken vigorously (180 cycles/min) while immersed in a water bath at 35 °C. The basic incubation medium consisted of Earle’s solution to which was added 500 mg glucose, 500 mg bovine serum albumin (fraction V powder, Sigma Chemical Co. Ltd), 14 mg potassium perchlorate, 5 mg penicillin G, 5 mg streptomycin sulphate and 2 mg phenol red/100 ml solution. The complete medium was sterilized by passing through a membrane filter (Oxoid Ltd) and it was equilibrated with 95% oxygen: 5% carbon dioxide before use. At the end of the incubation period the glands were washed free from medium and the $^{125}$I activity was measured in both the glands and the incubation medium.
Separation of iodinated components in thyroid tissue

Thyroid glands from rats in Expt 3 were hydrolysed by proteolytic digestion as described by Tong & Chaikoff (1958), and the iodinated tyrosines and thyronines in the hydrolysate were separated by thin-layer chromatography according to the method of Ensor & Kendall-Taylor (1970). The positions of iodinated amino acids and iodide were determined by spraying with 0-25 % ninhydrin and 0-1 % palladium chloride solutions, respectively; the material in each spot was identified by comparison with the mobility of pure compounds in the same system. The location of radioactivity was checked by scanning the plates with a thin-layer scanner (Panax model RTLS 1) and the spots were carefully scraped into test-tubes for measurement of their $^{125}$I activity. Aliquots of the extract from each gland were chromatographed in duplicate and good agreement was obtained.

Analytical methods

The thyroid glands of rats in Expt 1 were excised immediately after death and frozen in liquid nitrogen. Cyclic AMP was extracted and assayed by the method of Brown, Albano, Ekins, Sgherzi & Tampion (1971), which uses a specific binding-protein obtained from bovine adrenal cortex. Magnesium was determined in serum deproteinized with 10 % trichloroacetic acid by atomic absorption flame photometry; the solutions for measurement contained HCl (0-1 mol/l) to prevent interference from other constituents of the sample. The statistical significance of differences was assessed by Student’s $t$-test.

RESULTS

Effect of magnesium status on cyclic AMP concentration within the thyroid gland

Experiment 1 was similar to that reported previously (Humphray & Heaton, 1972), except that automatic feeding apparatus was used, and the effects of magnesium deficiency and loading on serum magnesium concentration and thyroid activity (expressed as the ratio of radioactivity in protein-bound $^{125}$I to total radioactivity in serum) were similar to those observed before (Table 1). The concentrations of cyclic AMP within the thyroid glands and the weights of the glands were, however, almost identical for rats in all three groups.

Table 1. Serum magnesium concentrations, uptake of $^{125}$I by serum proteins expressed as the ratio (protein-bound $^{125}$I): (total serum $^{125}$I), thyroid gland weights and cyclic AMP concentrations in magnesium-deficient and magnesium-loaded rats (means ± S.E.M.)

<table>
<thead>
<tr>
<th>State of animals</th>
<th>Serum Mg (mg/100 ml)</th>
<th>PB $^{125}$I: total serum $^{125}$I</th>
<th>Thyroid wt (mg/rat)</th>
<th>Cyclic AMP concen. in thyroid (pmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>2-69 ± 0-13</td>
<td>0-115 ± 0-017</td>
<td>12-5 ± 0-5</td>
<td>2-34 ± 0-09</td>
</tr>
<tr>
<td>Mg-deficient (10)</td>
<td>0-70 ± 0-06***</td>
<td>0-056 ± 0-004***</td>
<td>12-6 ± 0-6</td>
<td>2-40 ± 0-20</td>
</tr>
<tr>
<td>Mg-loaded (8)</td>
<td>3-17 ± 0-12***</td>
<td>0-174 ± 0-013***</td>
<td>12-4 ± 0-8</td>
<td>2-48 ± 0-14</td>
</tr>
</tbody>
</table>

Significance of difference from controls: ***$P < 0-001$.

Number of rats in parentheses.
The variations in (protein-bound $^{125}$I):(total serum $^{125}$I) ratio in this experiment were due mainly to differences between groups in total serum $^{125}$I activity, which was significantly raised in magnesium-deficient animals and lowered in magnesium-loaded rats, suggesting that the availability of magnesium may influence removal of iodide from the blood.

*Release of thyroid hormone from the thyroid glands of magnesium-deficient and magnesium-loaded rats incubated in vitro*

The release of $^{125}$I from thyroid glands of rats that had been pre-labelled by injection of $[^{125}$I]iodide 24 h previously was used as an index of thyroid hormone liberation in Expt 2. Small rats were used in this investigation because their glands gave a better response to stimulation by thyrotrophin than did glands from larger animals. In order to obtain a good response to the pituitary hormone it was also important to ensure adequate oxygenation of the tissue by rapid shaking and to avoid any physical damage to the glands.

Thyroid glands from magnesium-deficient rats were incubated in magnesium-free medium since a preliminary experiment had shown that this did not affect the release of $^{125}$I, and glands from control and magnesium-loaded rats were incubated in media containing the normal concentration of magnesium (2 mg/100 ml). The release of $^{125}$I was measured with glands from half the rats in each group incubated...
Magnesium status and thyroid activity

in basic medium, glands from the other rats were stimulated by adding to the incubation medium 0·005 i.u. thyrotrophin (Thytopar, Armour Pharmaceutical Co. Ltd)/ml.

Thyrotrophin increased the release of $^{125}$I from about 13 to 36% of that originally present in the glands of all three groups of rats during the 18 h incubation period (Fig. 1), but the only significant difference between the groups was a small increase in the thyrotrophin-stimulated release of radioactivity from the glands of magnesium-loaded rats compared with control animals ($P < 0.01$).

Uptake of $^{125}$I by thyroid glands and other tissues

The results of Expt 1 suggested that magnesium status may influence removal of iodide from the blood, and the three groups of rats in Expt 2 were, therefore, placed immediately after the injection of $^{125}$I in stainless steel metabolism cages equipped

Table 2. Urinary and faecal excretion of $^{125}$I by magnesium-deficient and magnesium-loaded rats during the 24-h period after injection of $[^{125}$I]iodide (means ± S.E.M.)

<table>
<thead>
<tr>
<th>State of animals</th>
<th>Activity excreted (% of $^{125}$I administered) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
</tr>
<tr>
<td>Control (6)</td>
<td>62.7 ± 2.1</td>
</tr>
<tr>
<td>Mg-deficient (7)</td>
<td>67.0 ± 2.8</td>
</tr>
<tr>
<td>Mg-loaded (6)</td>
<td>63.6 ± 1.0</td>
</tr>
</tbody>
</table>

Significance of difference from controls: ***$P < 0.001$.
Number of rats in parentheses.

Table 3. Accumulation of $^{125}$I by tissues of magnesium-deficient and magnesium-loaded rats when fasting (means ± S.E.M.)

<table>
<thead>
<tr>
<th>State of animals</th>
<th>Thyroid</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.486 ± 0.149</td>
<td>0.564 ± 0.062</td>
<td>0.545 ± 0.037</td>
</tr>
<tr>
<td>Mg-deficient</td>
<td>2.170 ± 0.305</td>
<td>0.418 ± 0.030*</td>
<td>0.523 ± 0.023</td>
</tr>
<tr>
<td>Mg-loaded</td>
<td>2.492 ± 0.324**</td>
<td>0.478 ± 0.027</td>
<td>0.664 ± 0.035*</td>
</tr>
<tr>
<td>Thigh muscle</td>
<td>Gastrointestinal tract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.258 ± 0.026</td>
<td>0.716 ± 0.045</td>
<td></td>
</tr>
<tr>
<td>Mg-deficient</td>
<td>0.327 ± 0.016*</td>
<td>0.598 ± 0.040**</td>
<td></td>
</tr>
<tr>
<td>Mg-loaded</td>
<td>0.358 ± 0.035**</td>
<td>0.696 ± 0.041</td>
<td></td>
</tr>
</tbody>
</table>

Significance of difference from controls: * $P < 0.05$; ** $P < 0.01$.
Ten rats in each group.

with urinary/faecal separators. Urine and faeces were collected for 24 h before death during which time the animals received no food. Approximately two-thirds of the $^{125}$I injected was excreted in the urine by all rats during this period and the excretion tended to be increased in magnesium-deficient animals (Table 2), although this was of marginal statistical significance ($0.1 > P > 0.05$). Relatively little activity was removed in the faeces, but the amount excreted was increased between two and three times in both groups of experimental animals.

The thyroid gland, liver, both kidneys, thigh muscle and complete gastrointestinal
tract were removed from each animal immediately after death and counted for $^{125}$I activity. The activity in each tissue was related to that in the serum of the same animal, because when stock rats were injected with different amounts of $^{125}$I, and killed 24 h later, the uptake of activity by the thyroid gland was directly proportional to the $^{125}$I in serum. Magnesium deficiency reduced the incorporation of activity into the liver and gastrointestinal tract, and loading with magnesium salts increased the accumulation by the thyroid and kidney (Table 3); the uptake of $^{125}$I by thigh muscle was apparently increased in both groups of experimental animals.

Table 4. Accumulation of $^{125}$I by tissues of magnesium-deficient and magnesium-loaded rats in the post-absorptive state (means ± S.E.M.)

<table>
<thead>
<tr>
<th>State of animals</th>
<th>Thyroid</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lung</th>
<th>Thigh muscle</th>
<th>Heart</th>
<th>Femur</th>
<th>Stomach</th>
<th>Small intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>4.375 ± 0.678</td>
<td>0.319 ± 0.12</td>
<td>0.475 ± 0.029</td>
<td>0.656 ± 0.033</td>
<td>0.523 ± 0.036</td>
<td>0.303 ± 0.035</td>
<td>0.340 ± 0.027</td>
<td>0.293 ± 0.018</td>
<td></td>
</tr>
<tr>
<td>Mg-deficient (9)</td>
<td>2.174 ± 0.389*</td>
<td>0.320 ± 0.13</td>
<td>0.357 ± 0.011*</td>
<td>0.299 ± 0.020***</td>
<td>0.236 ± 0.024</td>
<td>0.293 ± 0.018</td>
<td>0.296 ± 0.014***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg-loaded (8)</td>
<td>11.16 ± 2.12**</td>
<td>3.572 ± 0.011</td>
<td>1.701 ± 0.177</td>
<td>3.457 ± 0.279</td>
<td>1.936 ± 0.236</td>
<td>2.816 ± 0.204***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.344 ± 0.009</td>
<td>3.915 ± 0.287</td>
<td>0.403 ± 0.014**</td>
<td>3.115 ± 0.116*</td>
<td>2.816 ± 0.204***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance of difference from controls: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
Significance of difference between deficient and loaded rats: † $P < 0.05$.
Number of rats in parentheses.

The effect of magnesium status on iodide uptake by tissues was investigated in greater detail in Expt 3, when the rats were injected with a larger amount (100 μCi/ rat) of $^{125}$I. A preliminary study with stock rats of approximately the same size as the experimental animals, showed that accumulation of iodide by tissues varied considerably. Twenty-four hours after the injection of isotope, heart and thigh muscle had a tissue:serum ratio of about 0.2; in liver, spleen, submaxillary gland, large intestine and femur the ratio was about 0.3; kidney and lung had a ratio close to 0.5; while small intestine and stomach had ratios of 1.3 and 3.3, respectively. Organs from all these four categories were therefore taken for study in Expt 3. This time the rats were fed until shortly before death, the liver and kidneys were perfused through the aorta with cold isotonic saline to remove blood and the lumen of the stomach and small intestine was washed out before counting.

Magnesium status had a particularly marked effect on iodide uptake by the thyroid gland, magnesium deficiency inhibiting, and loading enhancing the accumulation of $^{125}$I (Table 4). Its effect on other tissues was usually consistent with this, and with the observations in Expt 2. Thus magnesium-loading significantly increased the iodide uptake by liver and small intestine, and a similar tendency was
observed in lung, although it decreased $^{125}$I uptake in the stomach. The effect of magnesium deficiency was not so well defined as in the previous experiment; it tended to inhibit $^{125}$I uptake in lung, but increased it in thigh muscle and femur.

**Influence of magnesium status on the synthesis of iodotyrosines and iodothyronines**

After the total $^{125}$I activity in the thyroid glands of rats in Expt 3 had been measured, the glands were hydrolysed and the products separated by thin-layer chromatography. The activity in the spots containing the four main iodinated tyrosines and thyronines amounted to between 95 and 96 % of the total radioactivity recovered from the plates (Table 5).

**Table 5. Distribution of $^{125}$I in hydrolysates of thyroid glands from magnesium-deficient and magnesium-loaded rats (means ± S.E.M.)**

<table>
<thead>
<tr>
<th>State of animals</th>
<th>Monoiodotyrosine</th>
<th>Di-iodotyrosine</th>
<th>Tri-iodothyronine</th>
<th>Thyroxine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>27.0 ± 1.0</td>
<td>56.6 ± 0.9</td>
<td>1.7 ± 0.2</td>
<td>10.2 ± 0.5</td>
</tr>
<tr>
<td>Mg-deficient (9)</td>
<td>27.1 ± 0.9</td>
<td>56.5 ± 1.0</td>
<td>1.7 ± 0.1</td>
<td>10.3 ± 0.6</td>
</tr>
<tr>
<td>Mg-loaded (8)</td>
<td>28.7 ± 1.0</td>
<td>55.0 ± 1.0</td>
<td>1.7 ± 0.2</td>
<td>9.4 ± 0.5</td>
</tr>
</tbody>
</table>

Number of rats in parentheses.

The magnesium concentrations in serum and the indices of thyroid activity in these rats were very similar to those of corresponding animals in Expt 1 (Table 1). Despite this, and the variations in iodide uptake by the thyroid glands (Table 4), the distribution of $^{125}$I among the iodinated components of the glands was virtually identical in animals of all three groups (Table 5). The ratios of activity in diiodotyrosine:monoiodotyrosine and in iodinated thyronines:iodinated tyrosines were very similar, indicating that magnesium status does not affect either the iodination process or the coupling of iodinated tyrosines to form iodothyronines.

**DISCUSSION**

The results of these three experiments all confirm the previous observation that the availability of magnesium affects thyroid activity in the rat, as judged by the ratio of protein-bound radioactivity to total radioactivity in the serum 24 h after the injection of $[^{125}]$Iodide (Humphray & Heaton, 1972), but the absence of a difference in thyroid weight suggests that it is not a gross effect. They do not, however, support the concept that magnesium may produce this effect by influencing the adenylate cyclase system. Magnesium has been shown to activate thyroid adenylate cyclase in tissue homogenates (Pastan & Katzen, 1967; Burke, 1970) and isolated enzyme preparations (Wolff & Jones, 1971), but it also stimulates phosphodiesterase activity in vitro (Cheung, 1971). The response of any cyclic AMP-mediated system will depend on the balance of adenylate cyclase and phosphodiesterase activities, and measurement of cyclic AMP levels is, therefore, likely to be a more useful criterion of activity than measurement of either enzyme alone.
No differences in cyclic AMP concentration within the thyroid were observed in Expt 1 (Table 1), despite considerable variation in thyroid activity between different groups of rats. This does not exclude the possibility that magnesium status could influence the rate of cyclic AMP turnover within the gland, which cannot be measured at present, but it indicates that physiological variations in the availability of magnesium have no selective action on either the formation or destruction of the cyclic nucleotide in vivo.

Similarly, there was no convincing evidence that magnesium status modifies the release of thyroid hormone from thyroid glands incubated in vitro. The basic rate of hormone release was similar with glands from magnesium-deficient, control and magnesium-loaded rats (see Fig. 1). Stimulation by a physiological dose of thyrotrophin caused a slightly greater increase in hormone release from glands of magnesium-loaded animals than from glands of control animals, although Williams (1972) found that elevated levels of magnesium in the incubation medium had no such effect with mouse thyroids. However, as the response did not differ significantly from that obtained with glands from magnesium-deficient animals, it is of very doubtful physiological significance. These similarities in the behaviour of the glands were in marked contrast to the variations in thyroid activity observed in the same rats before death.

Although magnesium deficiency and loading produced similar changes in the ratio of protein-bound radioactivity to total serum radioactivity in all three experiments, these were due principally to variations in serum iodide activity between animals of different groups in Expts 1 and 3, and mainly to variations in serum protein-bound iodine in Expt 2. The reason for this difference is not known, but the very young rats in Expt 2 were fasted after injection of $^{125}$I, whereas the older animals used in the other experiments were fed automatically until just before death. As the uptake of $^{125}$I by the thyroid glands of stock rats was proportional to the concentration of $[^{125}$I]iodide in serum, these observations suggest that magnesium status was influencing both the accumulation of iodide by the thyroid gland and its removal from the blood in other ways.

Examination of individual organs showed that this was particularly marked with the thyroid gland, where the accumulation of iodide, relative to its concentration in serum, was significantly inhibited by magnesium deficiency in Expt 3 and enhanced by magnesium loading in both Expts 2 and 3 (Tables 3 and 4). Similar effects were, however, observed with other tissues, especially in magnesium-loaded animals, where iodide uptake was increased in kidney and thigh muscle during Expt 2 and in liver and small intestine during Expt 3. Conversely magnesium deficiency reduced the uptake by the liver and complete gastrointestinal tract in Expt 2 and tended to reduce the accumulation by lung in Expt 3, although its effects were not so consistent as those observed with magnesium loading. Stomach and small intestine, which concentrated iodide very effectively, both contain an iodide-trapping mechanism similar to that in the thyroid gland (Pastan, 1957; Brown-Grant, 1961), but nothing appears to be known about the mechanism of iodide uptake by other organs.

Although the magnesium status of the rats made a substantial difference to the amount of $^{125}$I accumulated by their thyroid glands (Table 4), it did not affect the subsequent metabolism of the tracer. The distribution of $^{125}$I among iodinated
tyrosines and thyronines was very similar to that reported in the rat by Tong & Chaikoff (1958) and Sofianides, Meloni, Alger & Canary (1966), but the proportion of the total radioactivity in the gland associated with each of the four main iodinated components was remarkably constant in all three groups of animals (Table 5).

The results of these experiments indicate that the major effect of magnesium status on thyroid activity is to modify the accumulation of iodide by the gland. Various types of stress have been found to reduce the uptake of iodide by the thyroid and to lower the concentration of protein-bound iodine in the serum of rats (Van Middlesworth & Berry, 1951; Bondy & Hagewood, 1952) and other experimental animals (Pitt-Rivers & Tata, 1959). These changes are similar to those observed in magnesium-deficient rats, which are in a state of stress, but as magnesium loading produced converse changes, they are likely to be due to a more selective action of magnesium. Moreover, since similar effects were also observed in other tissues, this appears to be part of a general influence of magnesium status on iodide transport rather than a specific effect on the thyroid gland.

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REFERENCES


