STUDIES OF THE IN VITRO SENSITIVITY OF IODOTHYRONINE SYNTHESIS TO METHIMAZOLE IN NORMAL HUMAN THYROID CELLS

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SUMMARY

The comparative effects of methimazole (MMI) on resting and thyrotropin (TSH) - stimulated human thyroid cell cultures were investigated in terms of the release of iodoprotein and newly - synthesised iodothyronine hormones into the culture medium during a 48h period of incubation.

Iodoprotein recovery was increased after TSH, but both basal and TSH - enhanced iodoprotein release were depressed by MMI. TSH increased the release of tri-iodothyronine (T3) and thyroxine (T4), and although the TSH - enhanced T3 and T4 levels were depressed after MMI, (i) the basal levels found in control cultures were not attained, and (ii) T3 was more susceptible than T4 to MMI suppression, at high TSH levels.

These findings indicate a retention of the in vivo thyroidal sensitivity to MMI, under basal conditions and moderate TSH stimulation in vitro. The system may therefore facilitate further investigation into the mode of MMI suppression of peroxidase systems involved in iodothyronine hormone synthesis within the intact human thyroid cell.

INTRODUCTION

It is a well - documented observation that the elevated serum iodothyronine levels associated with hyperthyroidism are rapidly reduced to within the normal range by treatment of the patient with the thionamide drug 1-methyl-2-mercaptoimidazole (methimazole, MMI) [1]. This compound is rapidly and specifically taken up by the thyroid [2] and inhibits the iodide peroxidase system(s) which normally oxidizes iodide and couples iodotyrosines to produce the iodothyronine hormones tri-iodothyronine (T3) and thyroxine (T4) [3 - 5]. Despite this knowledge of the site, and mechanism of intracellular action of MMI however, little information is available concerning the resultant overall responses to MMI of iodothyronine hormone output from functionally - intact cells from the normal human thyroid.

The experiments reported in the present communication were therefore undertaken to study further the response of thyroid hormone release to MMI, under both resting and hyper - TSH stimulated conditions, using a previously developed cell culture technique [6], which permits close monitoring of in vitro thyroid cell metabolism.

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METHODS

Cell culture

Human thyroid tissue was obtained immediately post-operatively from patients undergoing surgery for the removal of non - iodide trapping nodules. The wide margin of histologically normal tissue surrounding each nodule was removed, and rinsed in ice - cold Hank's balanced salt solution (Gibco:Biocult Limited, Paisley, Scotland). The tissue was then aseptically chopped into 2mm cubes, and disaggregated in 0,25% (w/v) trypsin in Hank's solution (Gibco:Biocult) as described previously [7]. After 2 h of trypsin treatment at 37°C, the suspension of cells was filtered through sterile gauze which had been pre-rinsed in Hank's solution. The suspension of cells was then centrifuged at 600 x g. The resulting pale upper layer of cells was then removed from the dark brown button of red blood cells, and resuspended in 0.25% (w/v) soybean trypsin inhibitor (Sigma Chemical Company, Kingston, Surrey, England) for 30 minutes at 37°C, followed by recentrifugation at 600 x g. The thyroid cell pellet was then resuspended in Eagle's Basal Medium (Gibco:Biocult), 200µg/ml gentamicin (Flow Laboratories Limited, Irvine, Ayrshire, Scotland), 20 mmol/l HEPES buffer (Gibco: Biocult) and 0.1µmol/l sodium iodide, trace - labelled with 125-I (the Radiochemical Centre, Amersham, Bucks, England). Foetal calf serum was then added to a final level of 5% (v/v). The final volume of each culture was 5.0ml. Six groups of cultures were initiated: group (i) received no addition; group (ii) received 3mmol/l MMI (Sigma); group (iii) received 10mu/ml TSH; group (iv) received 10mu/ml TSH + 3mmol/l MMI; group (v) received 100mu/ml TSH; group (vi) received 100mu/ml TSH + 3mmol/l MMI. Cultures were incubated at 37°C for 48h in multidish cell culture plates (6 wells/ plate, Gibco:Biocult).

Post - incubation treatment of cultures

After the incubation period, the cell culture plates were placed on crushed ice and duplicate 500µl aliquots of medium aseptically removed. These medium samples were frozen to -20°C for subsequent analysis.

Fractionation of ^{125}I-labelled iodothyronines in medium

Five hundred microlitre aliquots of each medium sample were applied to Sephadex G - 10 columns (Pharmacia, Uppsala, Sweden) and fractionated according to the method of Thomopoulos [8] as modified by ourselves [7]. Components of the medium were eluted from the column in two stages; stage (i) fractions 1 - 100, using 500mmol/l Tris - maleic acid, pH 6.0; and stage (ii) fractions 101 - 140, using 50mmol/l Tris - HCl, pH 9.0. Column dimensions were 0.9 x 30cm. An eluant flow rate of 4ml/h was employed, and eight - minute fractions were collected directly into polystyrene gamma - counting tubes. Each fraction was then counted for 125-I activity in a well - type gamma counter.

RESULTS

The fractionation procedure used in this study effectively separates secreted T3 and T4 from free iodide, iodopeptides, monoiodotyrosine (MIT), and di-iodotyrosine (DIT) [9]. All iodinated compounds except T3 and T4 are eluted in the first stage of the fractionation, whilst T3 and T4 are eluted during the second stage, T4 emerging before T3 (Fig.1).

In Fig.2, the results of a representative single series of fractionations are presented, for the media of cells differentially supplemented with 10 or 100 mu/ml of TSH and 3mmol/l MMI. The results of four such series of experiments are combined and expressed in terms of the results shown by untreated control cultures, in Table 1.

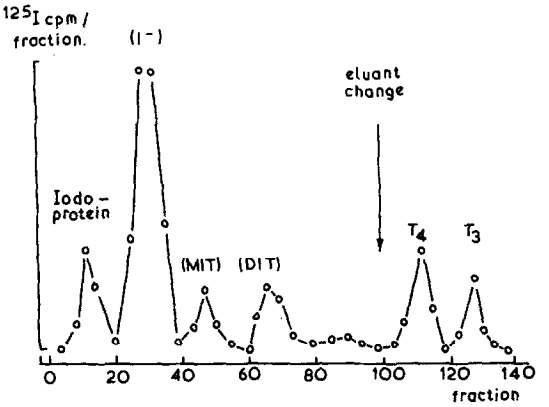

Figure 1. Representative Sephadex G – 10 fractionation of a medium aliquot from a human thyroid cell culture, supplemented with 100mu/ml of bovine thyrotropin (TSH) and 0.1μmol/l sodium iodide, trace – labelled with 125-I. Column dimensions were 0.9 x 30cm. Elution was performed as described under "Methods". (I^- = inorganic iodide; MIT = mono-iodotyrosine; DIT = di-iodotyrosine; T4 = thyroxine; T3 = tri-iodothyronine).

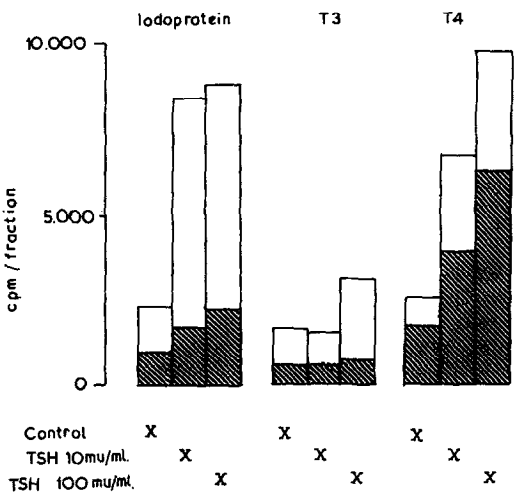

Figure 2. Accumulation of 125-I labelled iodoprotein, tri-iodothyronine (T3) and thyroxine (T4) in culture medium, during culture of isolated human thyroid follicular cells, differentially supplemented with 10mu/ml or 100mu/ml of bovine thyrotropin (TSH) and 3mmol/l methimazole (MMI). All cultures were incubated with 0.1μmol/l sodium iodide and 125-I. Medium samples were fractionated on Sephadex G-10 after 48h of culture. The shaded portion of each bar = amount of 125-I labelled component recovered from cultures supplemented with 3mmol/l methimazole.

Table 1.

Thyrotropin (TSH) and Methimazole (MMI) - induced changes in the levels of 125-I - labelled iodoprotein, thyroxine (T4) and tri-iodothyronine (T3) recovered on Sephadex G- 10 fractionation of culture medium used to support normal human thyroid follicular cells. Cells were exposed to 125-I for 48h, and differentially treated with 10 or 100 mu/ml of bovine TSH and 3mmol/l MMI. Results are the per centages of the recoveries obtained from medium used to support untreated control cells otherwise cultured under identical conditions (Mean percentages of the Control values ± S.D., n = 4).

	Percentage of levels found in medium from control cultures		
Treatment	Iodoprotein	T4	T3
Control	(100)	(100)	(100)
MMI	35.01 ± 4.1	67.28 ± 9.3	49.7 ± 5.8
TSH (10mu/ml)	352.8 ± 41.3	271.8 ± 31.6	137.1 ± 12.3
TSH (10mu/ml) + MMI	67.5 ± 9.3	155.6 ± 11.3	50.3 ± 4.2
TSH (100mu/ml)	361.2 ± 40.8	397.8 ± 41.4	292.2 ± 31.6
TSH (100mu/ml) + MMI	89.1 ± 11.6	252.3 ± 26.8	62.6 ± 9.3

The release of ^{125}I-labelled protein into the medium was significantly ($p < 0.01$) enhanced in response to TSH at a level of either 10 or 100mu/ml, although both these elevated levels and the basal levels released by control cultures were reduced in the presence of MMI.

In the absence of MMI, T3 levels in the medium were only significantly elevated in the presence of high initial TSH levels (100mu/ml) ; lower levels of TSH (10mu/ml) were apparently without effect on T3 release, although T4 release was significantly increased ($p<0.01$) at both levels of TSH employed (Table 1). MMI treatment reduced both the basal and TSH - stimulated levels of T3, and although control T4 levels were also decreased upon exposure of cells to MMI alone, TSH - stimulated T4 levels were still in excess of the control values, even in the presence of MMI (Table 1).

DISCUSSION

The primary objective of the present series of investigations was to study the effect of the thionamide compound methimazole on the *in vitro* release of iodothyronine hormones from isolated cells derived from the normal human thyroid. Although a great deal of information is available in the literature with regard to the site and mechanism of primary action of MMI within the thyroid [3 - 5] these latter studies were performed predominantly on non - intact cells or on the isolated, solubilised thyroidal

peroxidase system itself. Furthermore, despite the *in vivo* effect of MMI in decreasing serum T3 and T4 in hyperthyroidism [1], the susceptibility of isolated normal thyroid cells to MMI suppression, in terms of iodothyronine release, has received little attention, either in non-stimulated cells, or in hyper-TSH stimulated cells where intracellular iodide supply [6 ,10], iodide oxidation [11] and thyroidal peroxidase synthesis [5, 12] are all subject to TSH stimulation, resulting in an enhanced output of the iodothyronine hormones. Therefore an investigation of the possible MMI - induced modification of this overall response to TSH was also included in the present study.

As in previous investigations [13, 14], normal human thyroid cell cultures exposed to TSH showed enhanced levels of iodoprotein within the medium, after 48h of culture. This is unlikely to reflect an increased level of protein synthesis (Bidey, S.P. and Marsden, P., unpublished), and is most likely the result of an increased ^{125}I labelling of protein in the presence of TSH. This TSH - enhanced incorporation of iodide was diminished in the presence of MMI (Table 1) although the resultant levels of labelled protein obtained after MMI and TSH were still in excess of those found in MMI - treated non - TSH supplemented cells. This finding suggests either that, in the conditions used for this investigation, the final MMI concentrations acheived within the cells at the level of the peroxidase system may be insufficient to totally "inactivate" the high intracellular iodide levels obtained following TSH treatment [6] or that the MMI - bound peroxidase may be rapidly replaced through increased peroxidase synthesis in the presence of TSH [5]. It may well be that both of these mechanisms may be involved in overcoming the inhibition by MMI in cells simultaneously exposed to TSH and MMI. However, further studies using higher MMI levels are required in order to establish whether TSH stimulation of iodoprotein formation is totally suppressible by MMI treatment.

In agreement with the pattern of response of medium iodoprotein levels to MMI suppression, in both basal conditions, and conditions of TSH stimulation, the recoveries of 125-I labelled T3 and T4 in the culture medium were reduced by MMI in both basal and TSH - stimulated conditions, despite the probable post - secretory degradation of both iodothyronines *in vitro* [15]. The results in Table 1 suggest, however, that whereas T3 levels were very sensitive to MMI at high levels of TSH, T4 levels were less subject to suppression by MMI with increasing TSH levels. From this unexpected finding, one may perhaps speculate as to the identity and relative sensitivity to MMI of the peroxidase systems involved in the coupling of two DIT residues (giving T4) and one each of MIT and DIT (giving T3). At the present time we know of no reports to suggest the existence of more than a single thyroidal peroxidase system, although in the light of this apparent differential sensitivity of TSH - stimulated T3 and T4 release to MMI, the existence of more than one peroxidase system, or alternatively a differential effect of MMI on two distinct forms of

activation of a single peroxidase enzyme species, is worthy of investigation.

This present investigation therefore demonstrates that treatment *in vitro* of both normal, and hyper - TSH stimulated thyroid cells with MMI results in a diminished iodothyronine release into the culture medium, which is analogous to the observation *in vivo* of decreased serum T3 and T4 levels in hyperthyroid patients after treatment with MMI. Having established that such *in vitro* preparations retain their characteristic *in vivo* sensitivity to MMI, in addition to their potential TSH response, this system should facilitate further investigations into the sensitivity, specificity and susceptibility to modification by MMI of the thyroidal peroxidase systems involved in the various stages of iodothyronine synthesis, the total suppression of which results in a cessation of the release of iodothyronines *in vivo*.

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REFERENCES

1. Greer, M.A., Kendall, J.E. and Smith, M. (1964) in "The Thyroid Gland" (Pitt - Rivers, T. and Trotter, W.R., eds.) Vol.1., p357 - 389, Butterworths, London.
2. Marchant, B. and Alexander, W.D. (1972) Endocrinology,91, 747 - 756.
3. Richards, J.B. and Ingbar, S.H. (1959) Endocrinology, 65, 198 - 207.
4. Morris, D.R. and Hager, L.P. (1966) Journal of Biological Chemistry, 241, 3582 - 3589.
5. Nagasaka, A. and Hidaka, H. (1976) Journal of Clinical Endocrinology and Metabolism, 43, 152 - 158.
6. Bidey, S.P., Marsden, P., Anderson, J., McKerron, C.G. and Berry, H. (1977) Journal of Endocrinology, 72, 87 - 96.
7. Bidey, S.P., Marsden, P., McKerron, C.G. and Anderson, J. (1976) Biochemical and Biophysical Research Communications, 70, 418 - 424.
8. Thomopoulos, P. (1975) Analytical Biochemistry, 65, 600 - 605.
9. Bidey, S.P., Marsden, P., Berry, H., Anderson, J. and McKerron, C.G. (1976) Biochemical and Biophysical Research Communications, 73, 1042 - 1048.
10. Knopp, J., Stolc, V. and Tong, W. (1970) Journal of Biological Chemistry, 245, 4403 - 4408.
11. Dickson, J.A. (1966) Endocrinology, 79, 721 - 731.
12. Ahn, C.S. and Rosenberg, I.N. (1970) Endocrinology, 86, 396 - 405.
13. Lissitzky, S., Fayet, G., Giraud, A., Verrier, B. and Torresani, J. (1971) European Journal of Biochemistry, 24, 88 - 99.
14. Bidey, S.P., Marsden, P., McKerron, C.G. and Anderson, J. (1976) Clinical Science and Molecular Medicine, 50, 29P - 30P (abstract).
15. Bidey, S.P., Anderson, J., Marsden, P. and McKerron, C.G. (1976) Biochemical and Biophysical Research Communications, 72, 67 - 73.