CLINICAL RESEARCH

Blocking activity to action of thyroid stimulating hormone in serum from patients with primary hypothyroidism

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Abstract

Spontaneous primary hypothyroidism in adults is usually associated with autoimmune thyroiditis. The hypothesis that hypothyroidism may result from the presence in serum of a factor that blocks stimulation of the thyroid by thyroid stimulating hormone was examined. Serum samples were collected from 28 patients with recently diagnosed primary hypothyroidism. After removal of endogenous thyroid stimulating hormone the effect of the serum on secretion of triiodothyronine induced by thyroid stimulating hormone or thyroid stimulating antibodies was examined in thyroid slices incubated in vitro. Serum samples from six of the patients demonstrated significant blocking of the stimulation by bovine thyroid stimulating hormone. Inhibition of the stimulatory action of thyroid stimulating antibodies was also exhibited by serum samples with blocking activity.

It is concluded that in some patients with primary hypothyroidism a serum factor, which is probably an IgG, exists that can block the thyroid response to thyroid stimulating hormone and thyroid stimulating antibodies; it may represent an important mechanism in the pathogenesis of hypothyroidism.

Introduction

Most cases of spontaneous primary hypothyroidism in adults are considered to be due to chronic autoimmune thyroiditis, in which there is a progressive destruction of the thyroid gland such that, ultimately, insufficient functional thyroid tissue remains to maintain normal serum concentrations of the thyroid hormones triiodothyronine and thyroxine despite maximal pituitary drive by thyroid stimulating hormone. The inability of the thyroid gland in primary myxoedema to respond to the stimulatory action of the excess thyroid stimulating hormone has generally been attributed to the extent of the glandular destruction. However, the presence in serum from such patients of antibodies that block stimulation of the thyroid by thyroid stimulating hormone is another possible pathogenic mechanism. In 1980 Matsuura *et al* reported transient neonatal hypothyroidism in two siblings whose serum IgG inhibited the stimulation of thyroidal adenylate cyclase by thyroid stimulating hormone.[1] Endo *et al* reported that serum from an adult with autoimmune hypothyroidism contained IgG with similar inhibitory properties.[2] We studied serum samples from adults with primary hypothyroidism for the ability to block the effect of thyroid stimulating hormone, using a biological assay that measures the secretion of triiodothyronine from slices of thyroid incubated in vitro.[3]

Patients and methods

Serum samples were collected from 28 patients with biochemically confirmed primary hypothyroidism (low serum thyroxine and high thyroid stimulating hormone concentrations) at, or near, the time of diagnosis. The group comprised four men and 24 women with a mean age of 52·3 years (table I). All but three patients had a high titre of microsomal antibodies; 10 patients had appreciable titres of antithyroglobulin antibodies; and goitre was present in 13 patients. Serum was also collected from 15 normal subjects, who served as controls. Serum was stored at −40°C until assay.

REMOVAL OF ENDOGENOUS THYROID STIMULATING HORMONE

Endogenous thyroid stimulating hormone, which is present in high concentrations in the serum of patients with primary hypothyroidism, produces detectable stimulation in the thyroid slice bioassay (limit of detection about 25 mU/l) and thus would interfere with the detection of the hormone blocking activity. The hormone was therefore removed from all serum samples by a solid phase affinity technique before examination in the bioassay. Antiserum to human thyroid stimulating hormone (raised in the guinea pig) was coupled to

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TABLE I—*Details of 28 patients with primary hypothyroidism. (Thyrotrophin binding inhibitory immunoglobulin (TBII) results are for the solubilised thyroid stimulating hormone (TSH) receptor assay)*

Case No	Sex	Age (years)	Goitre	Microsomal antibodies	Thyroglobulin antibodies	TSH blocking activity	TBII index
1	M	29	−	>1/1600	ND	+	−3·3
2	F	46	−	>1/1600	ND	+	−4·4
3	F	81	−	1/800	1/640	+	−11·3
4	F	61	+	>1/1600	1/1250	+	−14·1
5	F	69	+	>1/1600	ND	+	−2·6
6	M	38	−	>1/1600	1/4000	+	−1·2
7	F	73	+	>1/1600	ND	−	1·9
8	F	37	+	>1/1600	ND	−	
9	F	44	+	>1/1600	ND	−	−6·1
10	F	62	+	>1/1600	ND	−	−6·4
11	F	70	+	>1/1600	1/3200	−	3·0
12	F	51	+	>1/1600	>1/10 000	−	−2·9
13	F	38	+	>1/1600	1/1250	−	1·6
14	F	57	+	>1/1600	>1/10 000	−	−0·7
15	F	34	+	ND	ND	−	−7·8
16	F	52	+	ND	ND	−	−6·5
17	M	50	+	ND	ND	−	−2·4
18	F	65	−	>1/1600	>1/10 000	−	−14·0
19	F	60	−	>1/1600	1/400	−	0·5
20	F	27	−	1/800	>1/10 000	−	
21	F	33	−	>1/1600	ND	−	1·8
22	F	59	−	1/1600	ND	−	−15·0
23	F	45	−	1/1600	ND	−	−7·0
24	F	44	−	>1/1600	ND	−	−0·7
25	F	71	−	>1/1600	ND	−	−3·7
26	F	56	−	1/1600	ND	−	−7·9
27	F	45	−	>1/1600	ND	−	−7·4
28	M	68	−	>1/1600	ND	−	−10·4

ND = Not detectable.

TABLE II—*Mean (SEM) concentrations of free triiodothyronine (pmol/l) released from thyroid slices by bovine thyroid stimulating hormone (0·2 U/l) in the presence of pooled normal serum and hypothyroid serum from the six patients who showed blocking activity*

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Pooled normal serum	173 (22)	176 (13)	143 (33)	143 (33)	138 (23)	187 (16)
Hypothyroid serum	45 (19)	96 (18)	26 (31)	49 (25)	61 (21)	128 (9)

Significance: $p \leq 0{\cdot}05$ in all cases.
Conversion: SI to traditional units—Triiodothyronine: 1 pmol/l ≈ 0·65 pg/ml.

cyanogen bromide activated cellulose as described by Wide.[4] Aliquots of hypothyroid serum (900 μl) were mixed with 100 μl of a suspension of this cellulose coupled to antithyroid stimulating hormone antibody in 0·1M HEPES (pH 7·5) for 48 hours at 4°C. After centrifugation (1500 *g* for 15 minutes) the supernatant was removed and stored at −40°C until assay. The efficiency of the extraction procedure was checked for each sample by measuring the concentration of thyroid stimulating hormone in the supernatant by radioimmunoassay; this was never greater than 8 mU/l.

ASSAY FOR BLOCKING ACTIVITY

Having been heated at 56°C for 30 minutes after removal of endogenous thyroid stimulating hormone, the serum samples were assayed for biological activity by measuring their effect on the response to bovine thyroid stimulating hormone (0·2 U/l) of secretion of free triiodothyronine from slices of porcine thyroid incubated in vitro, as previously described.[3] Two slices (1 × 1 × 0·5 mm) of porcine thyroid tissue were placed with 100 μl of serum on a Visking dialysis membrane in a diffusion pot with two compartments containing 5 ml buffer (HEPES/gelatin/Earles balanced salts; pH 7·4). The pots were incubated at 37°C for five hours. The tissue was then washed with 200 μl buffer and removed. The pots were sealed and incubated for a further 18 hours at 37°C in a shaking waterbath to allow free thyroid hormones, released by the thyroid tissue in response to stimulation, to dialyse across the membranes. Free triiodothyronine in the dialysate was measured by radioimmunoassay.

Pooled normal serum obtained from 15 healthy members of the laboratory staff was similarly prepared and included in each assay as a control. Assays included five replicates for each serum sample and were repeated on at least two occasions. Blocking activity was considered to be demonstrated if the amount of free triiodothyronine released by bovine thyroid stimulating hormone in the test serum was significantly lower than that released when the bovine thyroid stimulating hormone was incubated in pooled normal serum. To establish the nature of the blocking activity antiserum to human IgG (Fc) (nephelometric grade from Seward Laboratory) was included in some later experiments. Aliquots of serum free of thyroid stimulating hormone (750 μl) were incubated with 500 μl anti-IgG for one hour before addition of bovine thyroid stimulating hormone (to give a final concentration of 0·2 U/l). The serum samples were then assayed as before for the presence of blocking activity; serum samples preincubated with buffer in the same proportions were included as controls.

ASSAY FOR THYROTROPHIN BINDING INHIBITORY IMMUNOGLOBULINS

Immunoglobulin concentrates were prepared from serum samples by precipitation with macrogol (polyethylene glycol) 4000, as described by Shewring and Rees Smith.[5] The immunoglobulin preparations were assayed for their ability to inhibit binding of bovine thyroid stimulating hormone labelled with iodine-125 (^{125}I) to thyroid stimulating hormone receptors solubilised from porcine thyroid tissue with Triton X-100, and to particulate porcine thyroid membranes prepared by differential centrifugation of tissue homogenate in hypotonic medium.[6] Solubilised porcine thyroid plasma membranes (50 μl (80 μg protein)) in 10 mM trometamol (TRIS) and hydrochloric acid (pH 7·4) were incubated with 50 μl immunoglobulin concentrate (10 g/l) and 50 μl ^{125}I bovine thyroid stimulating hormone (4000 counts/min) for 30 minutes at 37°C in a shaking waterbath. The reaction was stopped by adding 500 μl bovine IgG (2 g/l in 10 mM trometamol and hydrochloric acid (pH 7·4)) and 650 μl of 30% (w/v) macrogel 4000 in 1 M sodium chloride to each tube. After mixing the samples were centrifuged at 4000 *g* for 20 minutes at 4°C, the supernatant aspirated, and radioactivity in the pellet counted. To investigate binding of serum IgG to particulate thyroid membranes 50 μl immunoglobulin concentrate (10 g/l) was incubated with 50 μl thyroid membranes (80 μg protein) in 10 mM trometamol and hydrochloric acid and 50 mM sodium chloride (pH 7·4) containing bovine serum albumin 1 g/l for 30 minutes at 37°C in a shaking waterbath. ^{125}I bovine thyroid stimulating hormone (50 μl; 4000 counts/min) was added to each tube and incubation continued for a further 60 minutes at 37°C in a shaking waterbath. After the addition of 1 ml ice cold buffer the tubes were centrifuged at 17 000 *g* for 20 minutes, the supernatant aspirated, and the pellet counted for radioactivity.

All samples were assayed in triplicate. Results were expressed as the index for thyrotrophin binding inhibitory immunoglobulins: index = 100 × (1 − (^{125}I thyroid stimulating hormone specifically bound in the presence of test sample/^{125}I thyroid stimulating hormone specifically bound in the presence of pooled normal serum)). Thyrotrophin binding inhibitory immunoglobulins are considered to be present when the index is greater than two standard deviations above the mean value from 77 normal subjects (>16·7).

THYROID STIMULATING HORMONE AND THYROID AUTOANTIBODIES

Thyroid stimulating hormone was measured in a radioimmunoassay using a second antibody method. Thyroid autoantibodies were detected by tanned red cell haemagglutination using commercially available kits (Fujizoki Pharmaceutical, Tokyo, Japan).

STATISTICAL ANALYSIS

Data were assessed for significance using the Mann-Whitney U test.

Results

Serum from six of the 28 patients with hypothyroidism consistently inhibited the stimulatory effect of bovine thyroid stimulating hormone 0·2 U/l on release of free triiodothyronine from porcine thyroid tissue in vitro (table II). None of the serum samples from the 15 normal subjects demonstrated any such blocking activity. Three of the serum samples that blocked bovine thyroid stimulating hormone were similarly examined for their ability to inhibit the action of human thyroid stimulating hormone 0·2 U/l (Medical Research Council standard A); inhibition of release of triiodothyronine occurred in all three cases (table III).

Two of the serum samples with blocking activity were examined for their effect on the stimulatory action of Graves' serum containing thyroid stimulating antibodies on porcine thyroid tissue. Both

significantly reduced the response to these antibodies (table IV). Eight of the serum samples without blocking activity were similarly

TABLE III—*Mean (SEM) concentrations of free triiodothyronine (pmol/l) released from thyroid slices by human thyroid stimulating hormone (0·2 U/l) in the presence of pooled normal serum and hypothyroid serum showing blocking activity*

	Case 3	Case 4	Case 6
Pooled normal serum	424·4 (30·9)	52·6 (10·8)	241·1 (43·8)
Hypothyroid serum	315·4 (37·4)	6·7 (11·4)	71·1 (22·8)

Significance: $p \leq 0.05$ in all cases.
Conversion: SI to traditional units—Triiodothyronine: 1 pmol/l ≈ 0·65 pg/ml.

TABLE IV—*Mean (SEM) concentrations of free triiodothyronine (pmol/l) released by thyroid slices in response to thyroid stimulating antibodies in the presence of pooled normal serum and hypothyroid serum showing blocking activity*

	Case 5	Case 6
Pooled normal serum	119 (13)	119 (13)
Hypothyroid serum	35 (25)	66 (6)

Significance: $p \leq 0.05$ in both cases.
Conversion: SI to traditional units—Triiodothyronine: 1 pmol/l ≈ 0·65 pg/ml.

assessed for their ability to affect the stimulatory action of thyroid stimulating antibodies on porcine thyroid tissue; no inhibition was observed.

Incubation of serum with antiserum to human IgG reduced the blocking activity in both of the samples for which this was examined, indicating the immunoglobulin nature of the blocking factor. To determine whether the blocking activity was due to binding of serum samples (150 μl) that had been treated previously with anti-thyroid stimulating hormone antibody coupled to cellulose were incubated with 50 μl ^{125}I bovine thyroid stimulating hormone (20 000 counts/min) for five hours at 37°C. Any ^{125}I bovine thyroid stimulating hormone bound to immunoglobulins present was precipitated by 200 μl of 30% (w/v) macrogol 6000, centrifuged, and the pellet counted. There was no significant difference in the amount of ^{125}I bovine thyroid stimulating hormone present in the precipitates from samples with blocking activity in the thyroid slice bioassay compared with those without such blocking activity.

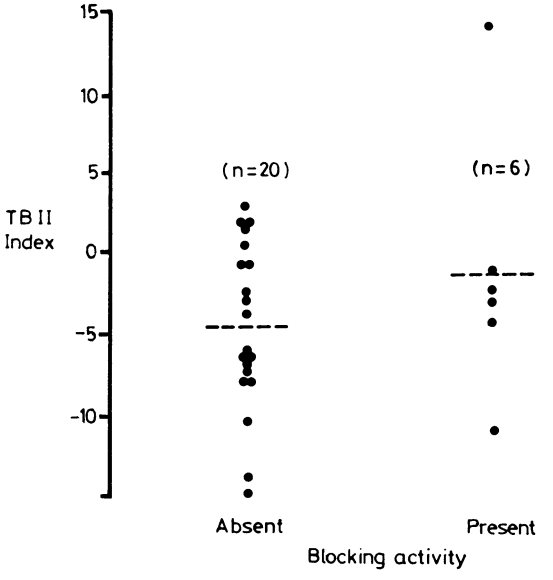
Binding of immunoglobulin to solubilised thyroid stimulating hormone receptors could not be detected in the six samples with blocking activity or in 20 without this property (table I, figure). Similarly, thyrotrophin binding inhibitory immunoglobulin was not detected when particulate thyroid membranes were used.

There were no clinical features that distinguished the patients whose serum contained blocking activity from those whose serum did not (table I). All six patients with blocking activity in their serum (two men, four women) had a high titre of thyroid microsomal antibodies, and three had detectable antithyroglobulin antibodies; two had a goitre at presentation. Of the 22 without blocking activity, 19 had demonstrable microsomal antibodies and seven had antithyroglobulin antibodies; 11 had a goitre.

Discussion

This study showed that serum from some patients with primary hypothyroidism contains a factor that inhibits stimulation of the thyroid by thyroid stimulating hormone and thyroid stimulating antibodies. The degree of inhibition of thyroid hormone secretion at the concentration of thyroid stimulating hormone used in this study suggests that this in vitro phenomenon is relevant clinically and that the blocking activity probably has an important role in the pathogenesis of primary hypothyroidism.

The blocking effect observed could be explained by interference at the level of the thyroid stimulating hormone receptor or a postreceptor mechanism, or by binding of the hormone to a factor present in the serum, preventing it from interacting with the hormone receptor. Specific binding of thyroid stimulating hormone by human serum globulins has been described,[7 8] but this possibility was excluded in this study. Furthermore, this mechanism of blocking would not explain the inhibition of the stimulatory effect of thyroid stimulating antibodies that we found. From these results we might postulate that there is inhibition of binding of thyroid stimulating hormone to its receptor due to the presence of serum factors blocking the interaction between the hormone and the receptor. None of the patients reported on here, however, had demonstrable thyrotrophin binding inhibitory immunoglobulin activity. This discrepancy between the receptor binding data and the effect on



Index of thyrotrophin binding inhibitory immunoglobulins (TBII) in immunoglobulin concentrates prepared from hypothyroid serum and assayed with solubilised porcine thyroid stimulating hormone receptors. Normal range < 16·7. Horizontal lines indicate mean values in each group.

release of triiodothyronine stimulated by thyroid stimulating hormone might suggest either that the interference occurs at a membrane site remote from, but influencing, the hormone receptor or that postreceptor mechanisms play a part. Further work is planned to clarify the site of blockade of the action of thyroid stimulating hormone. The nature of the factor that blocks the hormone's action has not been fully defined, but preliminary studies using anti-IgG indicate that it is almost certainly an IgG. Studies to confirm this are in progress.

No correlation has been found between the presence or absence of goitre and the occurrence of blocking activity in the serum. This implies that the blocking activity shown in the thyroid slice bioassay is distinct from the antibodies blocking thyroid growth that have been reported to be present in the serum of patients with primary hypothyroidism.[9] Likewise, there is no correlation between the presence of blocking activity and a high titre of thyroid autoantibodies as measured by haemagglutination.

There have been previous reports of antibodies that block the action of thyroid stimulating hormone in primary hypothyroidism. Using immunoglobulin G preparations, inhibition of stimulation by thyroid stimulating hormone of adenylate cyclase activity in human thyroid membrane preparations was demonstrated in serum from a patient with hypothyroidism secondary to Hashimoto's thyroiditis[2] and from a mother with hypothyroidism and her newborn baby.[1] While producing monoclonal antibodies to the thyroid stimulating hormone receptor, Yavin *et al* reported the development of antibodies that inhibited increases in the cyclic adenosine monophosphate content of thyroid cells induced by thyroid stimulating hormone.[10] Recently Konishi *et al* reported spontaneously occurring IgGs

in serum from 11 out of 18 patients with primary hypothyroidism that blocked the increase in cyclic adenosine monophosphate stimulated by bovine thyroid stimulating hormone in cultured thyroid adenoma cells.[11] Our findings may be of greater clinical relevance as whole serum, not just the IgG fraction, was examined and secretion of thyroid hormone by thyroid tissue was measured; this is the first report of a direct inhibitory effect on such secretion.

The clinical importance of this blocking effect found *in vitro* needs to be assessed further, but it is probably an important factor contributing to the pathogenesis of some cases of primary hypothyroidism.

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Bovine thyroid stimulating hormone (Thytropar) was purchased from Armour Pharmaceutical, ^{125}I triiodothyronine from Amersham International, Buckinghamshire, HEPES from Flow Laboratories, and bovine IgG from Sigma, London. All other chemicals were of analytical grade.

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IgA deficiency during treatment of infantile hypothyroidism with thyroxine

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Abstract

Serum IgA concentrations in five children with infantile hypothyroidism fell soon after the start of treatment with thyroxine. In one child the IgA concentration fell appreciably (to <0·01 g/l) and remained reduced; in the four others it returned to normal. IgM and IgG concentrations were roughly normal throughout.

The deficiency in IgA concentrations may have been due to stimulation by thyroxine treatment of a T cell suppressor system that, in the original hypothyroid state, was less than normally active.

Introduction

Selective IgA deficiency occurs in about one in 500 of the general population and is sometimes familial. It is the commonest immunodeficiency state, and known causes include treatment with various drugs, including phenytoin, penicillamine, sodium aurothiomalate, and sulphasalazine.[1] I report on five children with hypothyroidism in whom IgA concentrations fell during the first few months of treatment with thyroxine.

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Patients and methods

Five girls aged from 11 months to 4 years 9 months were diagnosed as having infantile hypothyroidism on the basis of clinical findings together with low serum thyroxine concentrations and raised thyroid stimulating hormone concentrations in the blood. Serum immunoglobulin concentrations were measured by single radial immunodiffusion (Partigen plates, Hoechst). Blood was taken from each child at the time of diagnosis and again whenever thyroid function tests were monitored during treatment with thyroxine sodium.

Results

In all five girls serum IgA concentrations fell after treatment with thyroxine was started (figure). In four of them IgA concentrations fell below the range of normal for healthy British children.[2] In one child the serum IgA concentration fell to less than 0·01 g/l and remained reduced. IgA concentrations returned to normal in the other four and then increased with age in the usual way. Concentrations of IgG and IgM generally fluctuated within the normal range during treatment; one child, however, had a marginally low IgG concentration at seven months, and three children developed marginally raised IgM concentrations between 18 months and three years after the start of treatment.

The child who developed long lasting IgA deficiency subsequently did not show any evidence of recurrent infection or allergic disease. Four years after the start of treatment with thyroxine her serum gave a negative result when tested for thyroid microsomal and colloid antibodies.

Discussion

Physical and intellectual development are delayed in children with infantile hypothyroidism. The development of their