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STUDY ON THE MECHANISM OF THE STIMULATION OF ADENYLATE CYCLASE BY GRAVES' IgG IN SOLUBILIZED THYROID MEMBRANES.

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We examined whether the detergent solubilized thyroid adenylate cyclase is stimulated by TSH or Graves' IgG in vitro. Solubilization of TSH receptor adenylate cyclase was performed by incubating crude porcine thyroid membranes (10,000 g pellets) in 25 mM Tris,50 mM NaCl,1% Lubrol PX pH 7.4 for 16 h at 4°C. Solubilized complex protein was concentrated and used for experiments. Adenylate cyclase activity was measured by Orgiazzi's method. cAMP was assayed by RIA. IgG was prepared by affinity column chromatography with Protein A Sepharose.

Results 1)TSH Stimulated solubilized adenylate cyclase in a dose dependent manner (0.04 - 4 mU/ml). 2)Six of 14 TBII positive G-IgGs increased adenylate cyclase activity. 3)The binding of I-125-bTSH was found to the solubilized protein complex. 4)Adenylate cyclase responsiveness to NaF was observed.

Our results suggest that solubilized porcine thyroid TSH receptor-adenylate cyclase has TSH receptor,transducer and catalytic unit. However,responsiveness of adenylate cyclase to some G-IgGs of patients with untreated Graves' disease may be lacking.

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CHANGES IN TSI AND TBII ACTIVITIES IN IgG OF PATIENTS WITH GRAVES' DISEASE BY ADDING BLOCKING IgG OF A PRIMARY MYXEDEMA PATIENT.

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Two methods are available, at present, to assess abnormal IgGs in sera of Graves' patients, thyroid stimulating IgG(TSI) and TSH-binding inhibiting IgG(TBII). Both activities are not always paralleled with each other in individual IgG. However, no evidence has been reported that the IgG contains both activities in its molecule or IgG is a mixture of IgGs with varying TSI and TBII. The present study was performed to examine changes in TSI and TBII activities in IgGs of untreated Graves' patients(G-IgG) by adding graded doses of a blocking IgG(B-IgG) from a patient with nongoitrous myxedema.

Methods: Mixtures of IgG which were prepared by adding varying doses(1,2,4mg)B-IgG to 10 mg G-IgG of each patient with a final volume of 1ml and changes in TSI and TBII activities were assessed. TSI was measured by porcine thyroid cell assay and TBII by Smith's method.

Results:(1)Decrease in TSI activity and increase in TBII value were observed in mixed IgG and (2)IgGs with varying TSI and TBII activities were obtained by mixing G-IgGs with B-IgG.

Conclusion: Graves' IgGs might be mixture of IgGs with varying TSI and TBII activities.

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PORCINE THYROID MEMBRANE-BINDING ANTIBODIES IN PATIENTS WITH AUTOIMMUNE THYROID DISEASE.

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Abnormal IgG(G-IgG) present in the sera of patients with Graves' disease is postulated to be antibodies to components of the thyroid cell membrane,including TSH receptor. Heterogeneity,such as thyroid stimulating or inhibiting activity has been demonstrated among G-IgG's. We conducted to test the membrane binding IgG in patients with thyroid disease by using 10,000 x g pellets of porcine thyroid membranes. Membrane protein(500μg) was incubated with 1 mg IgG and ^{125}I-Protein A in 1 ml 0.025M Tris,10^{-3}M MMI and 10^{-5}M NaI at 4°C for 16 h. After that tubes were centrifuged at 10,000 g for 15 min followed by two times washing. The sediment was counted for ^{125}I. Of 20 untreated patients with Graves' disease,16 (80%) showed greater binding than upper normal limits. Nine of 13 treated patients with antithyroid drug became normal value. Six of 10 patients with Hashimoto's thyroiditis or subacute thyroiditis were observed in normal range. The binding was not correlated with TSI or TBII in individual patients. No relationship was found between TGHA or MCHA and the binding. These results suggest that the porcine thyroid binding IgG,although detected in patients with Hashimoto's disease,might contain IgG which plays a significant role in Graves' disease.

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EVALUATION OF TSH-RECEPTOR ANTIBODIES AS PROGNOSTIC MARKERS AFTER CESSATION OF ANTITHYROID DRUG TREATMENT IN PATIENTS WITH GRAVES' DISEASE.

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Clinical usefulness of TSAb(thyroid stimulating antibodies) and TBII(TSH-binding inhibitor immunoglobulins) measurements for predicting prognosis of Graves' disease after cessation of antithyroid drug treatment was evaluated, and compared with that of T3 suppression test and the assessment of goiter size. Incidence of TSAb, TBII, T3 suppressibility and large goiter (transeverse diameter:>4.36 cm in female; >4.74 cm in male), determined at the time of discontinuation of treatment, was 87.5 % (n=14), 56.3 %(9), 78.6 %(11) and 81.3 %(13) in 16 relapsed patients, and 56.5 %(13), 24.1 %(5), 35.7 %(8) and 26.1 %(6) in 23 remitted patients. Both TSAb and TBII activities remarkably increased at the time of relapse in the 16 patients. Among 34 patients remaining in remission, TSAb were detected in 23(67.6 %), most of whom had normal serum TSH levels determined by an ultrasensitive immunoradiometric assay. It is suggested that impaired response of the thyroid to TSAb probably due to destructive changes and/or shrinkage is involved in the cause of clinical remission in most of the patients. Remission was found to be predictable in all patients with any two of those indices such as negative TSAb, positive T3 suppressibility and small goiter.