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THYROID STIMULATING ACTIVITY OF RABBIT ANTIBODIES TOWARD THE HUMAN THYROTROPIN RECEPTOR PEPTIDE

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SUMMARY We have produced antibodies against a peptide corresponding to the unique N-terminal segment (amino acid residues 29-57) in the extracellular domain of the human thyrotropin (TSH) receptor by immunizing it to rabbits, and evaluated for their thyroid stimulating antibody (TSAb), thyroid stimulation blocking antibody (TSBAb) and TSH-binding inhibitor immunoglobulin (TBII) activities. Antibody raised in rabbit B showed significant TSAb activity but not TSBAb activity. In contrast, antibody raised in rabbit A lacked TSAb activity but possessed TSBAb activity. None of these antibodies had TBII activity. These results indicate that TSH receptor antibody can successfully mimic the action of TSH and also suggest that the N-terminal region of TSH receptor is substantially associated with both TSAb and TSBAb activities, but not parallel to TBII activity.

Graves' disease is considered to be caused by TSH receptor autoantibodies (1). Therefore, determination of the sites on TSH receptor that interact with TSH or thyroid stimulating immunoglobulin (TSI) is extremely important for the study on the pathogenesis of Graves' disease.

Recently, human TSH receptor-encoding cDNA was cloned and its deduced amino acid sequence has been reported (2,3). The primary structure of the TSH receptor was very similar to luteinizing hormone (LH) receptor (4). But TSH receptor has two unique insertions not present in LH receptor, 8 amino acids tract (amino acid residues 38-45) near N-

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terminus and 50 amino acids tract (amino acid residues 317-366) near the transmembrane region (2,3).

Previously, we developed antibodies toward peptides corresponding to the TSH receptor unique insertion of 50 amino acids, and found that these antibodies had TSBAb and TBII activities. But none of the antibodies showed TSAb activity (5).

In the present study, we therefore focused on N-terminal region of TSH receptor including the unique insertion of 8 amino acids and developed antibodies toward the region in order to evaluate their biological activities.

MATERIALS AND METHODS

Peptide synthesis and its antibody production: A peptide (CECHQEEDFRVTCKDIQRIPSLPPSTQTL) corresponding to N terminal region of human TSH receptor (amino acid residues 29-57) (2) was synthesized by Automatic Peptide Synthesizer (Pharmacia, BioLynx), as previously reported (5). The purified peptide (10 mg) was conjugated to bovine serum albumin (2 mg, Sigma Chemical Co.) by disulfide bond formation. Antisera to this TSH receptor peptide were raised in rabbits by serial injections of emulsion of the conjugate in Freund's complete adjuvant. The presence of anti-peptide antibodies in the serum was detected as follows: 500 ng of the peptide was applied onto nitrocellulose sheets, incubated with the rabbit antiserum (1:200) in Tris buffered saline containing 2% of gelatin for 2 h at room temperature. After washing in Tris buffered saline containing 0.3% of Tween 20, the sheets were further exposed for 1 h to peroxidase-labeled anti-rabbit IgG (Jackson Immunoresearch Lab., 1:400). The sheets were washed as above, and the peroxidase activity was demonstrated with diaminobenzidine/H_2O_2.

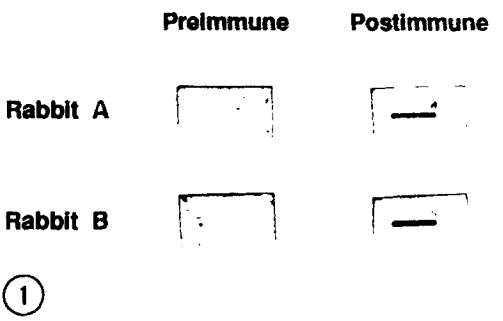
TSAb/TSBAb/TBII: TSAb and TSBAb activities were measured using FRTL-5 cells (6) as previously reported (5). cAMP measurement by radioimmunoassay was performed in triplicate determinations. TSBAb activity was defined as the ability to inhibit 100 μU/ml bTSH-induced cAMP increase. TBII activity was assayed using a commercial kit (TRAb kit, Baxter).

RESULTS AND DISCUSSION

We immunized two rabbits (rabbit A and B) with a TSH receptor peptide (amino acid residues 29-57), termed N peptide, which includes highly conserved domain among the

different species of TSH receptor but contains TSH receptor unique segments of 8 amino acids (2,3,4). After 2 months, both of the rabbits produced anti-N peptide antisera with nearly the same titer (Fig. 1). These antibodies showed no cross-reactivity with other TSH receptor peptides previously synthesized by us (5) (data not shown).

Table 1 summarizes TSAb, TSBAb and TBII activities of anti-N peptide antibodies from rabbit A and B. The antibody from rabbit B showed 189% of TSAb activity when FRTL-5 cells, rat thyroid cells, were used for the assay, and this TSAb activity was dose-dependent (Fig. 2). To confirm this, we also measured TSAb activity using pig thyroid cells instead of FRTL-5 cells, and obtained essentially the same result as was observed in the study using FRTL-5 cells. On the other hand, the antibody from rabbit B did not show any TSBAb and TBII activities.



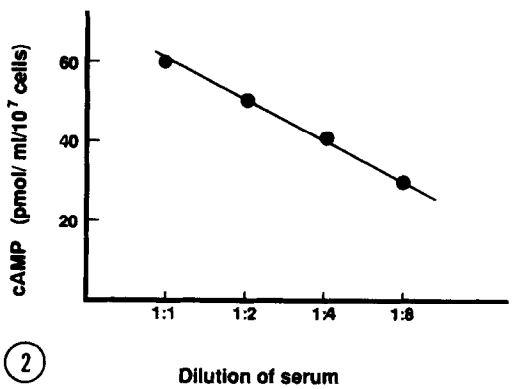


Figure 1. Immunoblotting of TSH receptor peptide 29-57 with rabbit antibodies. The peptide was incubated with preimmune and postimmune sera from rabbit A and rabbit B, and specific reaction was obtained in both of postimmune sera.

Figure 2. Dose-response curve of TSAb activity of antibody from rabbit B. Antiserum from rabbit B was diluted with the control sera and then measured for the TSAb activities.

Table 1. TSAb, TSBAb and TBII activities of rabbit antibodies toward hTSH receptor peptide

	TSAb activities (%)[a]		TSBAb[b] activity (%)	TBII[c] activities (%)
	FRTL-5 cells	Pig thyroid cells		
control	100 ± 5.6[d]	100[e]	0 ± 5.6	0 ± 1.6
antibody from rabbit A	104 ± 7.3	129	66 ± 10.5*	2.9 ± 0.7
antibody from rabbit B	189 ± 12.0*	186	1>	1>

(a) TSAb activity (%) was calculated as follows:

$$\left(\frac{\text{cAMP increase in the presence of test IgG}}{\text{cAMP increase in the presence of normal control IgG}}\right) \times 100.$$

(b) TSBAb activity (%) was calculated as follows:

$$\left(1 - \frac{\text{cAMP increase in the presence of test IgG and bTSH 100 μU/ml}}{\text{cAMP increase in the presence of normal control IgG and bTSH 100 μU/ml}}\right) \times 100.$$

(c) TBII activity was calculated as follows:

$$\left(1 - \frac{\text{labeled TSH specifically bound in the presence of test serum}}{\text{labeled TSH specifically bound in the presence of normal control serum}}\right) \times 100.$$

(d) Mean ± S.E.M. of triplicate determinations.

(e) Mean of duplicate determinations.

* $p<0.01$ vs. control.

In contrast, the antibody from rabbit A had not TSAb activity, but, interestingly, it was strongly positive for TSBAb activity (66 %). The antibody from rabbit A was also negative for TBII activity (Table 1).

It is controversial about the candidates for TSH binding sites or TSI interacting domains of TSH receptor. Since comparison of TSH receptor with LH receptor revealed that TSH receptor has two unique insertions, 8 amino acids tract (amino acid residues 38-45) near N-terminus and 50 amino

acids tract (amino acid residues 317-366) near the transmembrane region (2,3), these regions were thought to be potential TSH binding sites (2). Subsequently, using site-directed mutagenesis of the TSH receptor cDNA, Wadsworth et al. demonstrated that deletion of the latter tract had no effect on TSH and TSI activities but deletion of the former tract abolished these activities (7). Recently, using TSH-LH receptor chimeras, Nagayama et al. revealed the importance for TSH binding of the mid-region of the TSH receptor (amino acid residues 171-260) (8).

On the other hand, another useful approach to the determination of domains responsible for signal transduction of TSH or TSI is the application of synthetic peptides of TSH receptor and the evaluation of their antibodies. In the foregoing studies, we reported that antibodies against TSH receptor unique domain of 50 amino acids tract such as P2 and P4 (P2: amino acid residues 372-397, P4: amino acid residues 341-358) had TSBAb and TBII activities (5), but none of the antibodies showed TSAb activity.

The present study demonstrated that anti-TSH receptor peptide (amino acid residues 29-57) antibody successfully mimicked the action of TSH. Our data as well as recent findings by Murakami and Mori that Graves' IgG significantly bound radiolabeled synthetic TSH receptor peptide near N-terminus suggest that these regions of TSH receptor is substantially associated with TSAb activity.

It is of interest that another antibody against the same peptide showed only TSBAb activity. These results suggest that immunogenic sites for TSAb and TSBAb are closely interrelated but different, and further that these activities might not be parallel to TBII activity.

Studies are now in progress in our laboratory to determine these epitopes by preparing monoclonal antibodies to N peptide.

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