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RECOGNITION OF RAT LIVER AND KIDNEY NUCLEAR T_3 RECEPTORS BY AN ANTIBODY AGAINST C-ERB A PEPTIDE

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It has been reported that c-erb A encodes nuclear T_3 receptors (NT_3R). Based on the sequence of c-erb A cDNA, we synthesized a polypeptide consisting of 15 amino acids, the sequence of which has high homology between c-erb A α_1 and β. The antibody against this c-erb A peptide not only immunoprecipitated rat liver and kidney NT_3R but also inhibited T_3 binding to NT_3R. In a displacement study, the inhibition of [^{125}I]T_3-binding by the antibody was parallel to that by T_3 in terms of the concentration of the competitor added in the incubation mixture. Scatchard analysis revealed that the antibody decreased the value for the association constant in a dose dependent manner. The antibody did not bind T_3 itself. The results show that the antibody against c-erb A peptide recognizes rat liver and kidney NT_3R and that the sequence encoding this peptide, the closest carboxyl-terminal of c-erb A may be critical or at least closely related to the hormone binding.

Recently the proto-oncogene of v-erb A was isolated and it is considered that the c-erb A protein is identical to nuclear T_3 receptors (NT_3R). Until now, c-erb A has been cloned from various tissue cDNA libraries including human placenta (1), chicken embryo (2), rat brain (3), human testis (4), human kidney (5), rat GH_3 cells (6) and rat liver (7). Unexpectedly multiform c-erb A cDNAs have been found but it is not clear whether every c-erb A gene expresses functional receptor proteins or whether different types of the c-erb A may have different tissue or species distribution. From the analogy with the steroid hormone receptors, the cysteine-rich region is considered a DNA binding domain and a carboxyl-terminal region may be the hormone binding domain. In the present study, we have raised the antibody against a synthetic polypeptide

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encoded by the putative hormone binding domain of c-erb A and studied the interaction between anti c-erb A peptide and NT_3R.

MATERIALS AND METHODS

Production of antibody against synthetic c-erb A peptide

Based on the amino acid sequence of c-erb A reported by Weinberger et al. (1), a synthetic polypeptide consisting of 15 amino acids from proline (442nd amino acid in the sequence) to asparagine (456th) was synthesized by standard solid-phase methods and purified by HPLC. The cDNA sequence encoding this polypeptide is included in the putative hormone binding region and has high homology between α_1 (3) and β forms (Only one out of 15 amino acids is different). The polypeptide was coupled to bovine serum albumin or hemocyanin using carbodiimide. The conjugates were emulsified with Freund's complete adjuvant and were given to rabbits through multiple intradermal injections. The titer of antiserum was assayed by measuring the binding of synthetic polypeptide radiolabeled by chloramine T. Two rabbits were found as having the antibody after several booster injections. One was against the conjugate with BSA (designated 4BII-IgG) and another against the conjugate with hemocyanin (4H-IgG). IgG was prepared with Staphylococcus aureus Protein A-agarose (Affi Gel Protein A, Bio-Rad Laboratories, Richmond, CA).

Immunoprecipitation of NT_3R with antibody

From rat liver and kidney, NT_3R were prepared as previously described (8). Radiolabeled NT_3R were prepared by incubating NT_3R with $[^{125}I]T_3$ at 20℃ for 3 h and removing the free form of $[^{125}I]T_3$ with anion exchanger resin. Aliquots of $[^{125}I]T_3$-NT_3R complex were incubated with IgGs obtained from immunized or nonimmunized rabbit sera at 4℃ overnight. The immune complex between $[^{125}I]T_3$-NT_3R and IgG was precipitated by Staplylococcus aureus cells (RANSORBIN cell, Calbiochem Co., La Jolla, CA).

T_3-binding to NT_3R

Rat liver or kidney NT_3R (100 μg protein) was preincubated with 30 μg of IgG at 20 ℃ for 1 h, then $[^{125}I]T_3$ was added and the mixture was incubated for another 2 h at 20℃. Bound and free $[^{125}I]T_3$ were separated by 2 % resin suspension. In a displacement study, NT_3R was incubated with $[^{125}I]T_3$ at 20℃ for 2 h first, then IgG or a competitor was added and the mixture was incubated at 20 ℃ for 1 h. T_3-binding to rat liver NT_3R (150 μg protein) in the presence of various amounts of 4BII-IgG (0 ~ 40 μg) was analyzed by Scatchard plots.

RESULTS

Immunoprecipitation of the $[^{125}I]T_3$-NT_3R complex by the antibody

When the mixture of rat liver NT_3R radiolabeled with $[^{125}I]T_3$ and IgG was immunoprecipitated by adding Staphylococcus aureus cells, 4BII-and 4H-IgG, but not other IgGs or control nonimmunized IgGs immunoprecipitated the receptors. The ability of 4BII- and 4H-IgG to immunoprecipitate NT_3R increased with the number of booster injections (Fig. 1). 4BII-IgG (30 μg) from serum obtained after the 6th booster precipitated about 50% of $[^{125}I]T_3$-NT_3R complex. Under the same condition less than 17% of the radiolabeled receptor was precipitated

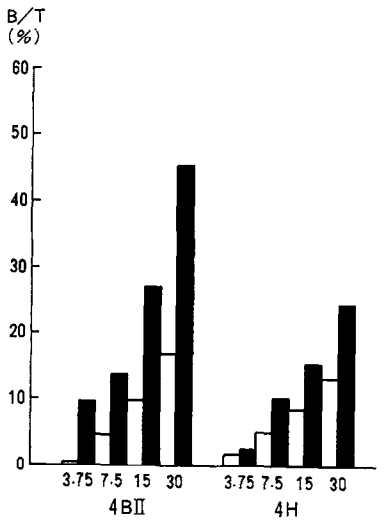


Fig 1. Immunoprecipitation of the complex between [^{125}I]T_3-NT_3R and IgG using Staplylococcus aureus cells. The effects of various amounts of 4BII- and 4H-IgG (3.75 - 30 μg) from serum obtained after the 1st (□) and 6th booster (■) were compared. B/T: radioactivity in immunoprecipitates/total radioactivity.

by 4BII-IgG from serum drawn after the first booster. The immune complex between NT_3R and 4BII- or 4H-IgG was also precipitated by goat anti-rabbit IgG serum purchased from two different suppliers (Sigma, St. Louis, MO; Tago, Burlingame, CA). None of IgGs including 4BII- and 4H-IgG bound T_3 itself significantly.

A tracer amount of [^{125}I]4BII-IgG radioiodinated by the chloramine T method was mixed with 30 μg non-labeled 4BII-IgG or normal IgG and incubated with rat liver NT_3R (150 μg protein) at 4°C overnight. The immune complex was precipitated using different concentrations of polyethylene glycol (PEG). As shown in Table 1, 7% PEG, which did not precipitate free IgG, precipitated 21% of [^{125}I]4BII-IgG incubated with NT_3R in the presence of 30 μg 4BII-IgG. In the presence of 30 μg normal IgG, however, only 4.7% of [^{125}I]4BII-IgG was precipitated. The data suggest the formation of immune complex of NT_3R with 4BII-IgG, but not with normal IgG.

4BII-IgG recognized rat kidney NT_3R as well as rat liver NT_3R. Fig. 2 shows that 20 μg 4BII-IgG immunoprecipitated both NT_3Rs similarly. The antibody, however, did not recognize rat liver cytosol T_3 binding proteins, TBG or reverse T_3 binding proteins in rat liver nuclear extracts (data not shown).

Table 1
Immunoprecipitation of rat liver NT_3R and 4BII- or normal IgG in the presence of a tracer dose of [^{125}I]4BII-IgG with various concentrations of PEG

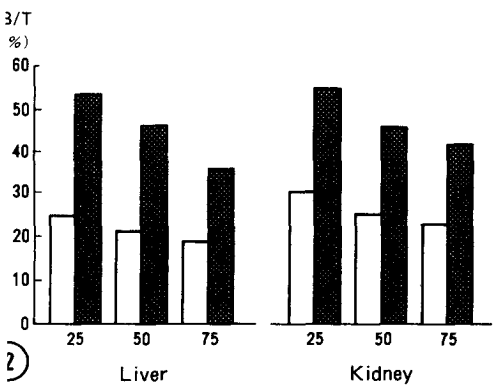
	PEG	5.5%	7.0%	8.5%	20.0%
group A*		21.6%**	21.5%	20.9%	92.1%
B		3.0%	4.7%	7.4%	92.0%
C		2.1%	2.3%	4.8%	90.0%

* NT_3R (150 μg protein) + a tracer amount of [^{125}I]4BII-IgG + 30 μg 4BII-IgG (group A) or normal IgG (group B). Group C: [^{125}I]4BII-IgG + 30 μg 4BII-IgG only (no NT_3R)

** Radioactivity of precipitates is expressed as % of total radioactivity in the incubation mixture. Mean of two determinations.

Inhibition of T_3-binding to NT_3R by 4BII-IgG

Preincubation of rat liver NT_3R (100 μg protein) with 30 μg 4BII-IgG decreased T_3-binding significantly, to 55% of the initial binding. The same amount of 4H-IgG did not inhibit T_3-binding with various amounts of NT_3R (12.5 μg - 100 μg protein). In a displacement study 4BII-IgG or T_3 was added to the incubation mixture 2 h after NT_3R had been bound with [^{125}I]T_3. The displacement of [^{125}I]T_3 from NT_3R by 4BII-IgG paralleled that by T_3 in terms



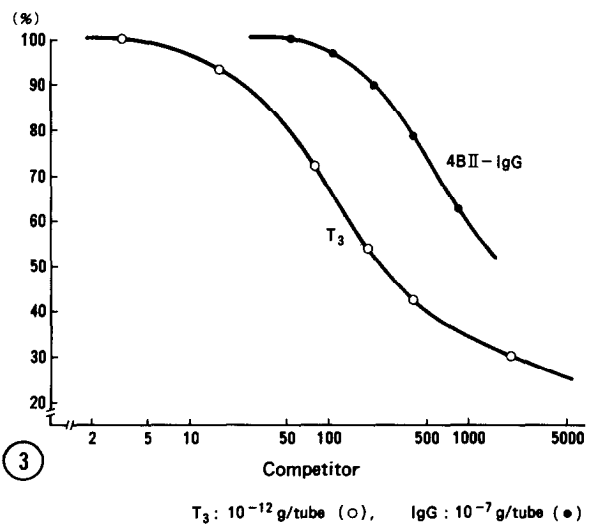


Fig 2. Immunoprecipitation of rat liver and kidney NT_3R. Various amounts of rat liver or kidney NT_3R (25 - 75 μg) were incubated with 20 μg 4BII-IgG (■) or normal rabbit IgG (□) and immunoprecipitated by Staplylococcus aureus cells.

Fig 3. Displacement of [^{125}I]T_3 from rat liver NT_3R by 4BII-IgG or non-labeled T_3.

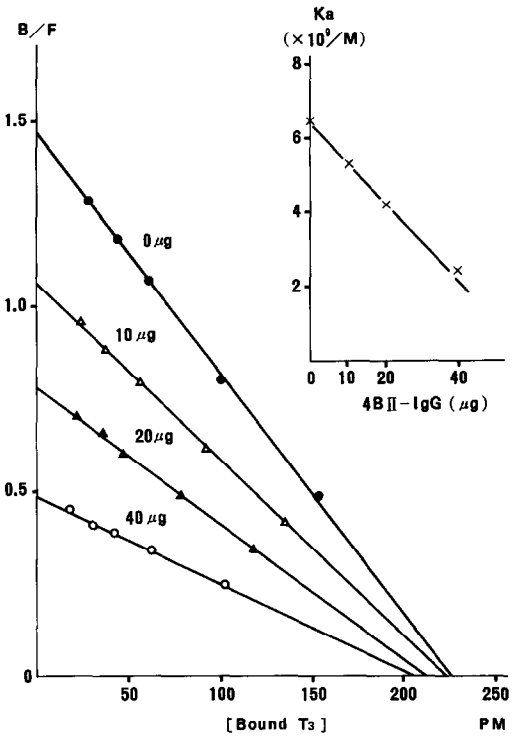


Fig 4. T_3-binding to rat liver NT_3R in the presence of various amounts of 4BII-IgG (0 - 40 μg) was analyzed by Scatchard plots. B/F: bound form of $[^{125}I]T_3$/free $[^{125}I]T_3$. The values for Ka were plotted against the concentrations of the antibody used.

of the concentration of the competitor added (Fig. 3). Scatchard analysis revealed that 4BII-IgG decreased the value for the association constant (Ka) in a dose dependent manner (Fig. 4).

DISCUSSION

The c-erb A protein has been found to have characteristics strongly suggestive of NT_3R. By the analogy of steroid hormone receptors, the hormone binding site is assumed to exist in the carboxyl-terminal portion, although no conclusive data have yet been obtained. We have raised the antibody directed against the c-erb A peptide, the sequence of which is closest to the carboxyl-terminal and has high homology between c-erb A β and α_1. The antibody, 4BII-IgG, not only immunoprecipitated NT_3R but also inhibited T_3-binding. The interaction of 4BII-IgG with NT_3R is specific, since 4BII-IgG did not immunoprecipitate cytosol T_3 binding proteins, TBG and nuclear reverse T_3 binding

proteins and no interference with the hormone bindings to these proteins was seen (Tagami, Nakamura, Imura, manuscript in preparation). Very recently Freake et al.(9) studied the interaction between NT_3R and five different v-erb A related antibodies and reported that only one antibody which was directed against v-erb A epitope from 231st to 311st amino acid immunoprecipitated chick liver and brain NT_3R. They considered that the reactive peptide sequences lie within the approximately 90 amino acids closest to the carboxyl terminal. Interestingly this amino acid sequence of v-erb A includes the sequence encoding the synthetic c-erb A peptide we used to generate the antibody. We assume that the amino acid sequence from 442nd to 456th in c-erb A which is conserved between α and β, may be critical or at least closely related to the T_3-binding domain.

The antibody was directed against c-erb A β of human placenta and yet recognized rat liver and kidney NT_3R similarly. In the liver which is a very good target organ for T_3, no definitive detection of c-erb A mRNA was reported at first (1, 3), but recent studies have shown that both α and β forms exist in the liver (7, 10). It is quite interesting to see whether the antibody recognizes NT_3R from different tissues or species similarly. This question is currently under investigation.

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