Activation of adrenal adenyl cyclase by anti-thyroid plasma membrane antibodies

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Abstract. We have previously shown that IgG isolated from rabbit anti-bovine thyroid plasma membrane (anti-BTPM) antiserum exhibits properties similar to thyroid stimulating antibodies (TSAb) in that it activates thyroid adenyl cyclase. In this study, the organ non-specificity of this reaction was investigated. It was observed that anti-BTPM IgG stimulated not only adenyl cyclase of bovine thyroid but also that of the adrenal. The stimulatory activities on the thyroid and adrenal adenyl cyclase were abolished by absorption of the IgG with bovine adrenal plasma membrane (BAPM). These results indicate that anti-BTPM antibodies, similar to TSAb, exert both thyroidal and extra-thyroidal effects. Thus anti-BTPM antibodies may be directed against antigenic determinants that are common to both thyroid and adrenal plasma membranes.

Like the anti-BTPM IgG, anti-BAPM IgG also activated both thyroid and adrenal adenyl cyclase. However, when IgG of the anti-BAPM antiserum was absorbed with thyroid plasma membranes, only the thyroid, but not the adrenal stimulating activity was abolished. It was concluded that the anti-BAPM antiserum contained antibodies directed against membrane antigens specific for the adrenal as well as common antigens shared by the thyroid.

The thyroid stimulating antibodies (TSAb) of Graves' disease have been shown to stimulate thyroid (Adams & Purves 1956; McKenzie 1958) as well as extrathyroidal tissues, e.g., fat cells (Kabir-Taylor & Munro 1971), adrenal (El Kabir et al. 1971; Dandona & El Kabir 1980), testis (Trokoudes et al. 1979) and possibly the pituitary (Dandona et al. 1975), in several species. Moreover, its activity has been demonstrated to be absorbed by both thyroid (Kriss et al. 1964; El Kabir et al. 1966; Benhamou-Glynn et al. 1969; Dandona & El Kabir 1980) and non-thyroidal tissues, such as kidney (Kriss et al. 1964; El Kabir et al. 1966; Benhamou-Glynn et al. 1969) adrenal (Kriss et al. 1964; Dandona & El Kabir 1980), testis, brain (Kriss et al. 1964), fresh skeletal muscle, gastric mucosa (El Kabir et al. 1966) and liver (Kriss et al. 1964; El Kabir et al. 1966).

We have previously shown that IgG purified from rabbit anti-bovine thyroid plasma membrane antiserum exhibits a property similar to TSAb in that it stimulated adenyl cyclase activity of the bovine thyroid plasma membrane (Ong et al. 1976). If the purified IgG is the experimental counterpart of TSAb, then it must be capable of exerting extra-thyroidal effects as does TSAb. In order to test this hypothesis, we have performed experiments to define the tissue specificity of these immunoglobulins by a) comparing the action of these immunoglobulins on the thyroid, prior to and after absorption with bovine adrenal plasma membrane preparations, and b) studying the effect of these immunoglobulins on the adenyl cyclase activity of the bovine adrenal gland. This paper reports the results of these experiments and provides evidence for the extra-thyroidal effects of thyroid-stimulating immunoglobulins.
Materials and Methods

Preparation of bovine thyroid plasma membrane (BTPM)
The preparation of BTPM, which sediments at the 35% phase of a sucrose density gradient ultracentrifugation, was carried out as previously described (Ong et al. 1976), except that in the second and third homogenization steps, a motor-driven teflon glass homogenizer was used instead of a Dounce homogenizer.

Preparation of bovine adrenal plasma membrane (BAPM)
Bovine adrenal glands were trimmed free of fat and the outer capsule and plasma membranes were prepared by a procedure similar to that described for the preparation of the BTPM. However, in the first sucrose density gradient ultracentrifugation, the runs were carried out for 1 h at 93,000 x g.

Immunization of rabbits
Rabbits were immunized and bled as described previously (Ong et al. 1976). Bovine thyroid and adrenal plasma membrane fractions sedimenting at the 35% phase of a sucrose density gradient ultracentrifugation were used as antigens for the immunization of separate rabbits to obtain the anti-BTPM and anti-BAPM antisera, respectively.

Absorption studies
Purified IgG (10 mg/ml) isolated from anti-BTPM antiserum was mixed with packed BAPM preparation in a ratio of 1:2 (V/V). The BAPM preparation used for this experiment was the adrenal plasma membrane fraction sedimenting at the 35% phase of a sucrose density gradient ultracentrifugation. The mixture was incubated at 45 min at 37°C and left overnight at 4°C. Following incubation, the mixture was centrifuged for 2 h at 4°C; the supernatant was then tested for its effect on the thyroid and adrenal adenyl cyclase. As a control, the same procedure was carried out concurrently using IgG isolated from the serum which was obtained from each rabbit prior to immunization.

The absorption of anti-BAPM IgG with BTPM was carried out as described above for the absorption of anti-BTPM IgG.

Isolation of IgG from the normal rabbit sera (NRS) and rabbit antisera (AS), enzyme assays, and protein determinations were carried out as previously described (Ong et al. 1976).

Porcine adrenocorticotropic hormone (ACTH), mol.
Characterization of bovine adrenal plasma membrane (BAPM) fractions by various enzyme markers

By comparing the various enzyme activities in the four plasma membrane fractions (Fig. 1), it is quite obvious that the 30 and 35% plasma membrane fractions were relatively higher in 5'-nucleotidase and Na⁺K⁺-dependent ATPase (plasma membrane marker enzymes) activities than other fractions but their succinate cytochrome c-reductase (mitochondrial marker enzyme) and DPNH diaphorase (microsomal marker enzyme) activities were much lower than those present in the 40 and 45% plasma membrane fractions. These results suggested that the 30 and 35% plasma membrane fractions were more enriched in plasma membranes and were less contaminated with microsomes and mitochondria.

Comparison of ACTH- and NaF-stimulated adenyl cyclase activities in the four BAPM fractions

It can be seen from Fig. 2 that the different BAPM preparations (panels A = 30%, B = 35%, C = 40% and D = 45%) possessed approximately equal amounts of basal adenyl cyclase activity. The adenyl cyclase activity in each of the membrane fractions was responsive to stimulation by both adrenocorticotropic hormone (ACTH) and NaF. Whereas stimulation of adenyl cyclase in the different BAPM preparations by ACTH was about 45 to 60% higher (P < 0.01) than the various basal activities, stimulation by NaF markedly exceeded those observed with the hormone by 4- to 11-fold.

Effects of rabbit anti-BTPM IgG and anti-BAPM IgG on adenyl cyclase activities in thyroid and adrenal plasma membranes

IgG of rabbit anti-BTPM antiserum significantly stimulated adenyl cyclase of both thyroid and adrenal plasma membranes (Fig. 3). Such stimulatory activity on the thyroid and adrenal plasma membrane adenyl cyclase could be abolished after absorption of the IgG with adrenal plasma membranes (Fig. 3). Similar to the adenyl cyclase stimulating capacity of the IgG of anti-BTPM, IgG of anti-BAPM antiserum was also capable of activating the adenyl cyclase of the thyroid and adrenal plasma membranes (Fig. 4). However, absorption of the IgG with thyroid plasma membranes could only abolish the thyroid but not the adrenal stimulating activity (Fig. 4). These results suggested that IgG from each of the two antisera (anti-BTPM and anti-BAPM) not only stimulated adenyl cyclase of the homologous plasma membrane but also the adenyl cyclase of the heterologous plasma membrane to the extent of triggering the production of cyclic AMP.
The 5'-nucleotidase and the Na⁺K⁺-dependent ATPase have been shown to be localized in the plasma membranes of rat liver (Ray 1970; Emmelot & Bos 1966), bovine thyroid (Ong et al. 1976; Wolff & Jones 1971) and a variety of other tissues (Morgan et al. 1971; Ebel et al. 1971; Meldolesi et al. 1971). Hence, the occurrence of elevated activities of these enzymes in the isolated adrenal plasma membrane preparations reported in this study, especially the 30 and 35% plasma membrane fractions, indicated that the membrane preparations were enriched in plasma membranes.

The presence of succinate cytochrome c-reductase and DPNH diaphorase activities in our adrenal plasma membrane preparations suggested contamination of the plasma membrane preparations with mitochondrial and microsomal elements. This is not surprising since complete separation of the different subcellular organelles is impossible by current techniques of differential centrifugation.

Adenyl cyclase appears to be ubiquitous in living organisms. In the rat liver, it is associated with the plasma membrane (Marinetti et al. 1969; Ray 1970) to the exclusion of other subcellular components (Sutherland et al. 1962). In the bovine thyroid (Wolff & Jones 1971; Ong et al. 1976) and the rat adipose tissue (McKeel & Jarett 1970), the adenyl cyclase is also reported to be localized in the plasma membranes. Moreover, a report by Finn et al. (1972) shows that adenyl cyclase can also be detected in the bovine adrenal plasma membranes. Our present study supports this observation.

The adenyl cyclase activity of our adrenal plasma membranes could be stimulated by ACTH (Fig. 2). This finding is in keeping with similar observations made by other investigators using various adrenal preparations (Kelley & Koritz 1971; Finn et al. 1972, 1975). In addition to demonstrating that adenyl cyclase activities can be stimulated by ACTH, we were also able to show that NaF was a better stimulator of adenyl cyclase in the adrenal plasma membranes (Fig. 2). Our observations confirm previous reports that NaF is much more potent than hormone in the activation of adenyl cyclase activities in a variety of tissues (Wolff & Jones 1971; Kelley & Koritz 1971; Finn et al. 1972; Melson et al. 1970).

In the present study we observed that the IgG of the anti-BTPM antiserum not only stimulated the adenyl cyclase of the thyroid but also that of the

Discussion

Fig 3.
Activation of thyroid and adrenal adenyl cyclase by IgG of anti-BTPM antiserum. Various volumes of purified IgG (10 mg/ml) of anti-BTPM antiserum (designated AS-IgG) were used for stimulation of adenyl cyclase of bovine thyroid plasma membranes (upper panel) and adrenal plasma membranes (lower panel). The thyroid and adrenal plasma membrane fractions used in these experiments were isolated at the 35% phase of a sucrose density gradient ultracentrifugation. The stimulatory activities of the As-IgG before (● --- ●) and after (● - ●) absorption with bovine thyroid plasma membranes are shown in each panel. Purified normal IgG from the same rat before immunization (designated NRS-IgG) was added, either directly or after absorption with adrenal plasma membranes, to each tube to serve as internal control. Each point represents the mean ± SEM of triplicate determinations. The P values (Student's t-test) are in comparison to the mean stimulation of adenyl cyclase activities of control samples in the presence of NRS-IgG alone. Similar results were obtained in 2 other experiments using a different IgG preparation in each. Absorption conditions are described in the text.
adrenal (Fig. 3). This finding is in accordance with observations reported by other investigators on the ability of TSAb to stimulate thyroid (Adams & Purves 1956; McKenzie 1958), adrenal (El Kabir et al. 1971; Dandona & El Kabir 1980), as well as other non-thyroidal tissues (Kendall-Taylor & Munro 1971; Dandona et al. 1975; Trokoudes et al. 1979). Thus, both anti-BTPM antibodies and TSAb can exert thyroidal and extra-thyroidal effects. Our results indicate that anti-BTPM antibodies were directed against common antigens shared by the thyroid and adrenal plasma membranes.

The absorption of thyroid stimulating antibodies with adrenal plasma membranes resulted in a complete loss of thyroid and adrenal stimulating activities. It is of interest to note that Kriss et al. (1964) demonstrated a significant loss of TSAb potency upon absorption of TSAb-containing plasma with canine adrenals. In a more recent study, the absorption of TSAb-IgG with human adrenals was found to cause a minimal reduction of thyroid and adrenal stimulating activities in the mouse (Dandona & El Kabir 1980). In essence, results of our present study and that of others indicate that the activity of thyroid stimulating antibodies can be removed or reduced by the absorption with antigens of non-thyroidal organs. This simply reaffirms the presence of common antigens shared by different organs of the same (this study) and different (Kriss et al. 1964; Dandona & El Kabir 1980) species.

Similar to the IgG of the anti-BTPM antiserum, the IgG of the anti-BAPM antiserum was also found to stimulate both the adrenal and the thyroid adenyl cyclase (Fig. 4). However, unlike the IgG of the anti-BTPM antiserum, whose thyroid and adrenal stimulating activities were abolished by absorption with adrenal plasma membranes (Fig. 3), absorption of the IgG of the anti-BAPM antiserum with thyroid plasma membranes only abolished its thyroid (Fig. 4, upper panel) but not its adrenal stimulating activity (Fig. 4, lower panel).

**Fig. 4.** Effect of IgG of anti-BTPM antiserum on thyroid and adrenal adenyl cyclase activities. Various volumes of purified IgG (10 mg/ml) of anti-BTPM antiserum (designated AS-IgG) were used for stimulation of adenyl cyclase in bovine thyroid plasma membranes (upper panel) and adrenal plasma membranes (lower panel). The thyroid and adrenal plasma membrane fractions used in these experiments were isolated at the 35% phase of a sucrose density gradient ultracentrifugation. The stimulatory activities of the As-IgG before (●—●) and after (○—○) absorption with bovine thyroid plasma membranes are shown in each panel. Purified normal IgG from the same rabbit before immunization (designated NRS-IgG) was added, either directly or after absorption with thyroid plasma membranes, to each tube to serve as internal control. Each point represents the mean ± SEM of triplicate determinations. The P values (Student's t-test) are in comparison to the mean stimulation of adenyl cyclase activities of control samples in the presence of NRS-IgG alone. Similar results were obtained in 2 other experiments each using a different IgG preparation. Absorption conditions are described in the text.
These data, therefore, indicate that the anti-BAPM antiserum contained antibodies directed against antigens specific for the adrenal as well as common antigens shared by the thyroid.

Schwyzer et al. (1971) and Seelig et al. (1971) presented evidence to show that of the 39 amino acids of ACTH, the sequence of amino acids involved in the activation of its adrenal receptor is in the region of 4 to 10 of the ACTH molecule; amino acids in the region 11 to 24 are not involved in activation but rather provide affinity of the ACTH for the receptor. Applying this analogy to our findings, it is suggested that adenyl cyclase of the adrenal gland and thyroid gland is stimulated by both anti-thyroid and anti-adrenal antibodies because the antigen-binding sites of these antibodies have a similar conformation in that region of the molecule which is involved in the activation of the adenyl cyclase. The corollary that follows is that there may be shared antigen determinants on the binding sites in thyroid and adrenal plasma membranes for thyroid stimulating immunoglobulins. These findings suggest a mechanism to explain the results of other investigators (El Kabir et al. 1971; Dandona & El Kabir 1980) on the stimulatory effects of TSAb on the mouse adrenal glands.

It is generally believed that, in humans, the actions of the thyroid stimulating antibodies on the thyroid leads to hyperthyroidism. Although many of the metabolic effects of hyperthyroidism have been attributed to the action of triiodothyronine (T₃) and thyroxine (T₄), some doubt has been expressed (Pittman 1971) whether these hormones are entirely responsible for the alterations produced in adrenal metabolism (Jao & Koritz 1962; Linquette et al. 1976). In view of the evidence which we have presented in this study it would be of interest to determine, to what extent some of the metabolic manifestations of Graves' disease can be related to the extra-thyroidal action of these immunoglobulins. We feel that we have developed an experimental model which may be used to help clarify some of these problems.

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References


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