THE EFFECTS OF TRITON ON THE BINDING OF TSH TO THE THYROID AND TESTIS

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Received 24 August 1979

1. Introduction

The binding of labelled TSH to thyroid membranes has been shown to be increased in the presence of propranolol [1,2] and recently we have observed that several other compounds including low doses of the detergent Triton X-100 have similar effects. The mechanism of their action is not known at present but a greater knowledge of the events involved could well lead to an increased understanding of the nature of the TSH receptor and its interaction with TSH. Consequently, we have undertaken an investigation of this process using Triton X-100 and TSH binding to soluble and particulate thyroid preparations. In addition, the effects of Triton on the testicular TSH binding sites described [3,4] have been studied and our results indicate that Triton increases TSH binding to testis as well as to thyroid particulate fractions. However Triton did not influence TSH binding to thyroid membranes solubilised with the detergent Lubrol.

2. Methods

Crude membranes were prepared from homogenates of porcine thyroid tissue or guinea pig testis by differential centrifugation at 800 × *g* for 5 min and 15 000 × *g* for 15 min as in [3]. The crude membranes were extracted by gentle homogenisation in a solution (2 ml/g equiv. membranes) of 0.1% Lubrol in Tris–NaCl (50 mM NaCl; 10 mM Tris–HCl (pH 7.4)) followed by centrifugation at 100 000 × *g* for 2 h. The sediment was re-extracted with Lubrol, re-sedimented and the supernatant (0.1 mg protein/ml) used for TSH binding studies. Analysis on calibrated columns of Sepharose 6B showed that the complex formed between this Lubrol extract and TSH had mol. wt ~200 000 [5].

Highly purified bovine TSH (30 units/mg, a generous gift from Dr J. G. Pierce) was labelled with ^{125}I to spec. act. ~50 μCi/μg using the lactoperoxidase method and purified by gel filtration on Sephadex G-100 followed by absorption to and elution from thyroid membranes [6]. Human chorionic gonadotrophin (10 000 units/mg, a generous gift from the National Pituitary Agency) was also labelled to spec. act. ~50 μCi/μg using the lactoperoxidase method and purified by Sephadex G-100 chromatography [3].

Binding studies were carried out as follows: Labelled hormone (~10^4 cpm of TSH or hCG in 100 μl of Tris–NaCl containing 1 mg/ml BSA) was added to 100 μl Triton X-100 or similar test substance diluted in the same buffer with or without unlabelled hormone. A suspension of membranes (100 μl, 20 mg equiv.) or detergent-solubilised membranes was added and the mixture incubated for 90 min at 37°C. The amount of label bound was then determined by centrifugation (in the case of particulate fractions) or precipitation with human IgG (0.5 mg) and polyethylene glycol (final conc. 15%) in the case of solubilised membrane preparations [5]. Labelled TSH binding in the presence of 10 mU unlabelled TSH was taken as non-specific binding.

3. Results

Several substances including thiomersal, Lubrol, methyl hydroxybenzoate and Triton X-100 caused an

Table 1
% ^{125}I-labelled TSH bound to thyroid membranes[a]

Test substance[b]	Unlabelled hormone only	Labelled hormone + 0.1 units of unlabelled TSH
Buffer	24.5	2.8
Lubrol (0.002%)	29.2	2.8
Thiomersal (0.2 mM)	34.5	3.4
Propranolol (3 mM)	50.8	1.8
Triton X-100 (0.016%)	63.3	3.0

[a] Values are means of closely agreeing triplicate determinations
[b] Concentration of test material giving a maximal effect is shown

increase in the binding of TSH to thyroid membranes (table 1). Triton showed the greatest effect and consequently was studied in more detail. Scatchard analysis [7] of TSH binding to porcine thyroid membranes gave linear plots in agreement with [5,8] and conventional interpretation of these data suggested that the reaction's association constant was 3.1 ± 1.1 X 10^9 M^{-1} with a TSH binding capacity of 1.7 ± 0.4 μU/mg equiv. (means ± SEM; n = 4). Increasing concentrations of Triton appeared to cause an increase in both binding capacity and affinity of the TSH–thyroid membrane interaction. In a series of 4 separate experiments, the association constant was increased 1.31 ± 0.13-times and the binding capacity 1.24 ± 0.05-times in the presence of 0.01% Triton. In the presence of 0.016% Triton the affinity and capacity were increased 1.6 ± 0.21-times and 1.4 ± 0.07-times, respectively. In contrast to its effect on TSH binding to thyroid particulate fraction, Triton did not significantly influence the interaction between TSH and Lubrol solubilised thyroid membranes (fig.1).

Triton also increased TSH binding to testis membranes but higher concentrations were required (maximum effect at 0.08%) than with the thyroid (maximum effect at 0.016%). Binding of hCG to the testis however was not increased by Triton but was progressively reduced in the presence of Triton at > 0.006% (fig.2).

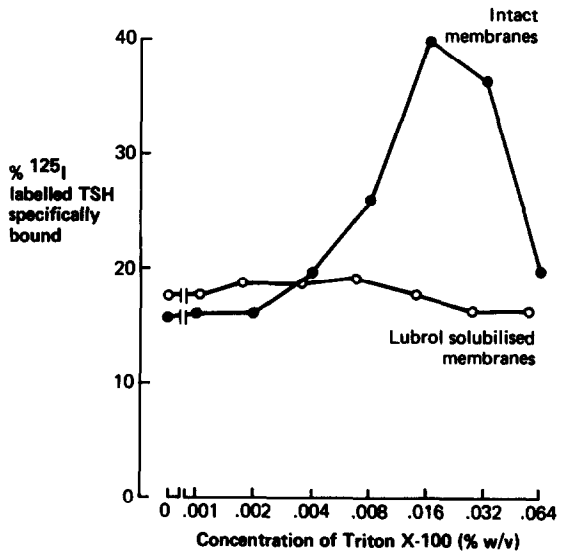

Fig.1. Effect of Triton on TSH binding to thyroid membranes and Lubrol-solubilised membranes. Data are typical of several separate experiments.

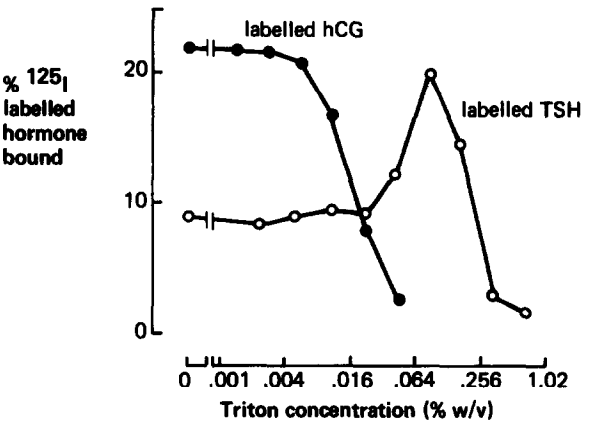

Fig.2. Effect of Triton on TSH and hCG binding to guinea pig testis. Data are typical of several separate experiments.

4. Discussion

Our investigations indicated that the binding of labelled TSH to thyroid membranes was markedly increased by Triton X-100 with a maximum effect at 0.016%. This concentration of Triton was considerably lower than that capable of solubilising TSH binding sites (0.5%) [5]. The Triton-induced increase in TSH binding appears to be due to a progressive increase in both the affinity and binding capacity of the system whereas previous studies with propranolol have suggested that the drug only increased binding affinity [1,2].

The binding of labelled TSH to Lubrol solubilised membranes was not influenced by Triton. This indicated that Triton was unable to affect TSH binding when TSH receptors were dispersed in Lubrol micelles and suggested that Triton did not interact directly with the TSH binding site or TSH itself. It appeared therefore that the Triton-induced increase in TSH binding to thyroid membranes was mediated by an indirect action of Triton. The mechanism of this process was not clear but possibly Triton-induced changes in membrane lipids were involved and these resulted in:

(i) Exposure of more binding sites;
(ii) Conformational changes in the binding sites which gave rise to greater affinity for hormone.

Triton also increased TSH binding to testis membranes. Higher concentrations of the detergent were required than with the thyroid and this possibly reflected differences in membrane structure between the two tissues. The effects of Triton on TSH binding to both thyroid and testis were reduced at higher Triton concentrations and this could have resulted from more extensive membrane disruption. However in marked contrast to the effect of Triton on TSH binding to the testis, hCG binding was reduced in the presence of Triton at >0.006%. Previous studies have suggested that the testicular binding sites for hCG and TSH are different [3,4] entities and our data with Triton emphasise this distinction.

References

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