Specific epidermal growth factor receptors on porcine thyroid cell membranes

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Specific saturable receptors for epidermal growth factor (EGF) of high affinity (K_a 1.7×10^9 M^{-1}) have been demonstrated on porcine thyroid membranes. Optimal conditions for EGF binding have been determined. TSH and other peptide hormones do not inhibit the binding of ^{125}I-EGF and EGF does not inhibit ^{125}I-TSH binding to thyroid membranes. The results suggest that EGF may be involved in the regulation of thyroid follicular cell growth and function.

Thyroid *Epidermal growth factor* *Receptor* *Thyrotrophin*

1. INTRODUCTION

Epidermal growth factor (EGF) is a polypeptide molecule that has potent mitogenic effects on cells of various origins [1,2], and the presence of specific, high affinity, saturable receptors has been demonstrated in many cell types [3].

Previous reports on the effect of EGF on thyroid cells have been conflicting. Some groups have reported an effect of EGF on thyroid cell proliferation in culture [4–6], others have reported no such effect [7–9]. Most relevant to the clarification of this issue is the demonstration of EGF receptors on thyroid cell membranes. A previous study has failed to detect EGF receptors on bovine thyroid membranes [10].

Here, we demonstrate the presence of specific, high affinity, saturable EGF receptors on porcine thyroid cell membranes.

2. MATERIALS AND METHODS

2.1. *Iodination of EGF*

EGF (Bethesda Res. Labs., Cambridge) extracted from male mouse submaxillary glands, was iodinated using Na^{125}I carrier-free (Amersham), by the following procedure. EGF (10 μg) was dissolved in 10 μl phosphate buffer (0.1 M, pH 7.4), 5 μl (500 μCi) of Na^{125}I, 10 μl (1.8 IU) of lactoperoxidase and 10 μl of 1 mM H_2O_2 were added. The iodination mixture was incubated for 20 min at room temperature and the reaction then stopped by the addition of 200 μl of ice-cold 0.1 M phosphate buffer containing bovine serum albumin (2%).

The iodination mixture was applied to a Sephadex G-50 column (30 × 1.2 cm) and eluted with phosphate buffer 0.1 M (pH 7.4), containing NaCl (50 mM) and BSA (0.1%). The peak of activity corresponding to 2.2 × breakthrough volume was applied to an Amberlite IRA 400 ion-exchange column (15 × 1.2 cm) and the resulting peak pooled and stored at −70°C. By this procedure ^{125}I-EGF of spec. act. 28–40 Ci/g was obtained.

2.2. *Preparation of thyroid membranes*

Porcine thyroid tissue was collected fresh from a local abattoir and maintained on ice during the preparation. Fat and connective tissue was removed and discarded, cubes of tissue were homogenised in 8–10 vol. Tris (10 mM) buffer on a polytron tissue blender. The homogenates were centrifuged at 800 × g for 10 min at 4°C, the pellet resuspended in 5 vol. Tris buffer and glass–glass homogenised. The suspension was centrifuged at 10000 × g

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for 20 min at 4°C and the pellet resuspended in 10 mM Tris buffer + NaCl 50 mM + BSA 1% (pH 7.4) to give the appropriate concentration.

The protein concentration of the membrane preparation was estimated by the Lowry method [11]. Typically, 1 g tissue would produce 5–10 mg of protein in the membrane preparation.

2.3. *Binding assay for EGF*

Thyroid membrane preparations were diluted in Tris–NaCl–BSA buffer such that a 100 μl aliquot/tube gave a final membrane protein concentration of 1 mg/ml. A 200 μl aliquot/tube of serially diluted 'cold' EGF and 100 μl of ^{125}I-EGF dilution (20 pg, 5000 cpm) was added to each tube. Tubes were incubated for 1 h at 26°C in a shaking water bath, the reaction terminated by addition of 1 ml of ice-cold Tris–NaCl–BSA buffer and bound and free moieties separated by centrifugation at 10000 × *g* for 20 min at 4°C. The supernatant was aspirated and the radioactivity in the pellet counted.

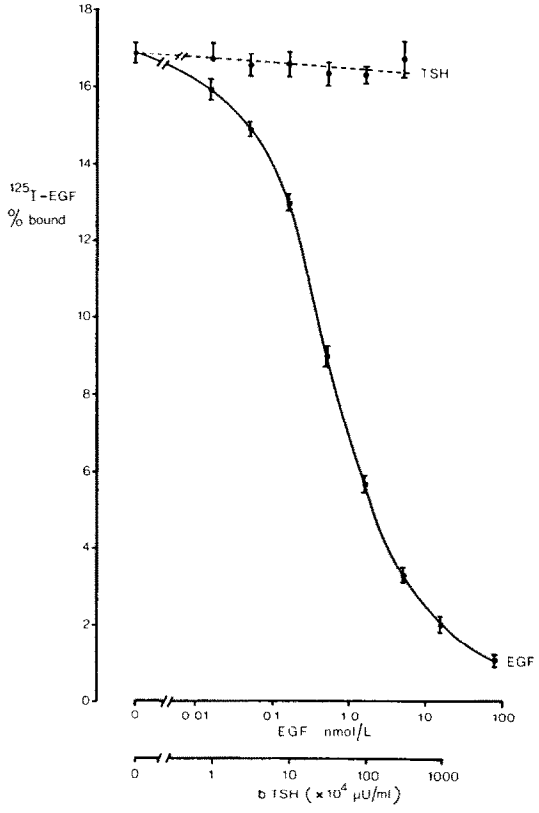

Fig.1. Inhibition of binding of ^{125}I-EGF to porcine thyroid membranes by: (a) unlabelled EGF; (b) bovine TSH (mean ± SEM, n = 3).

3. RESULTS

3.1. *Inhibition of ^{125}I-EGF binding to porcine thyroid membranes by unlabelled EGF*

The inhibition of ^{125}I-EGF binding to porcine thyroid membranes by unlabelled EGF has been demonstrated (fig.1). The lower limit of detection of EGF was ~8 pmol/l (50 pg/ml). No further decrease in ^{125}I-EGF binding was detected above 82 nmol/l unlabelled EGF concentration which was consequently used to estimate non-specific binding.

Fig.2 illustrates the effect of thyroid membrane protein concentration on the binding of ^{125}I-EGF; a membrane protein concentration of 1 mg/ml was selected and used for all subsequent experiments.

3.2. *Effect of incubation temperature on the time course of ^{125}I-EGF binding*

Fig.3 illustrates the effect of incubation

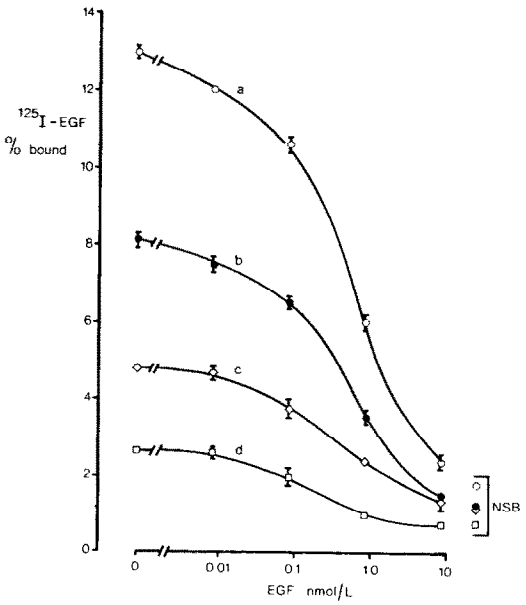

Fig.2. Effect of membrane protein concentration on the binding of ^{125}I-EGF to thyroid membranes. Final membrane protein concentration: (a) 1.0 mg/ml; (b) 0.5 mg/ml; (c) 0.25 mg/ml; (d) 0.125 mg/ml. Values not corrected for non-specific binding (mean ± SEM, n = 3).

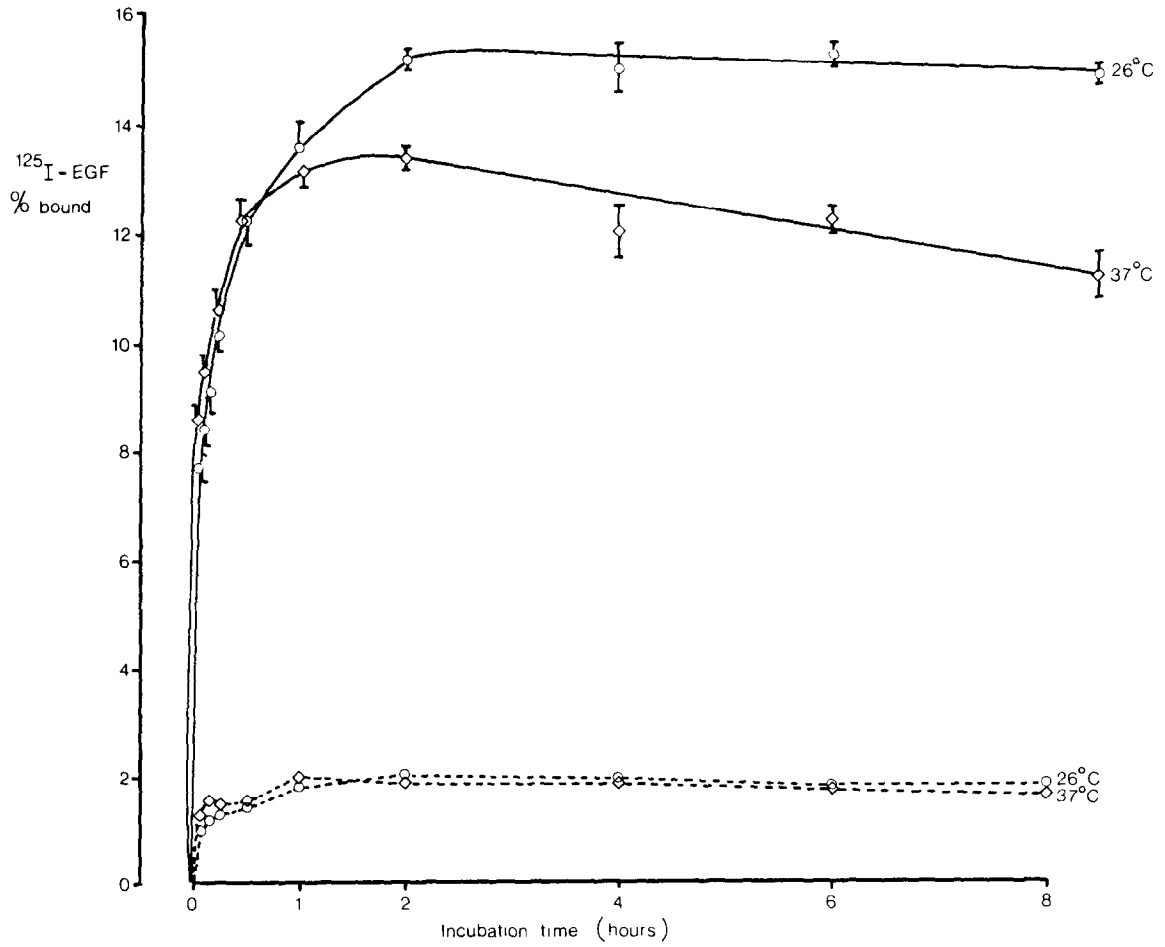

Fig.3. Effect of incubation temperature on the time course of ^{125}I-EGF binding to porcine thyroid membranes: (——) total binding; (– – –) non-specific binding (mean ± SEM, n = 3).

temperatures of 26°C and 37°C on the total and non-specific binding of ^{125}I-EGF to porcine thyroid membranes. Some degradation of EGF-receptor binding was noted after 1 h incubation at 37°C but not at 26°C. No significant temperature- or time-dependent effect on non-specific binding was noted.

3.3. *Scatchard analysis of EGF binding to porcine thyroid membranes*

Scatchard analysis of EGF binding (fig.4) identified receptor sites of high affinity (K_a 1.7 × 10^9 M^{-1}) and low capacity (2.5 × 10^{-10} M/mg membrane protein). At higher concentrations of ligand the participation of lower affinity binding sites is indicated. This experiment was repeated a further 4 times and similar values obtained.

3.4. *Specificity of EGF binding to porcine thyroid membranes*

The specificity of EGF binding to porcine thyroid membranes was investigated by measuring the inhibition of ^{125}I-EGF binding caused by cross-reacting hormones. Fig.1 illustrates the lack of cross-reactivity of bovine TSH (Thytropar, Armour Pharmaceuticals) at concentrations that inhibit all specific ^{125}I-TSH binding (see fig.5). Table 1 demonstrates the absence of ^{125}I-EGF binding inhibition resulting from incubation with various other peptide hormones.

Fig.5 illustrates the lack of cross-reactivity of EGF with porcine TSH receptors, as measured by the inhibition of binding of ^{125}I-TSH (40 pg, spec. act. 77.3 Ci/g) to porcine thyroid membranes (0.6 mg membrane protein/ml).

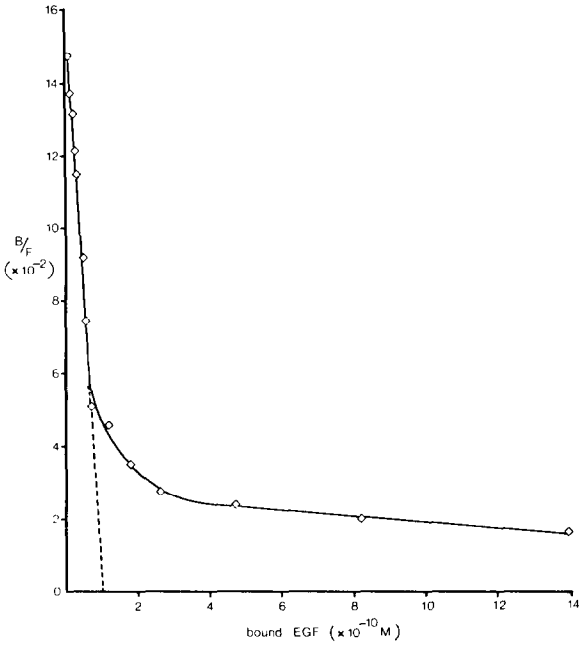

Fig.4. Scatchard analysis of EGF binding to porcine thyroid membranes.

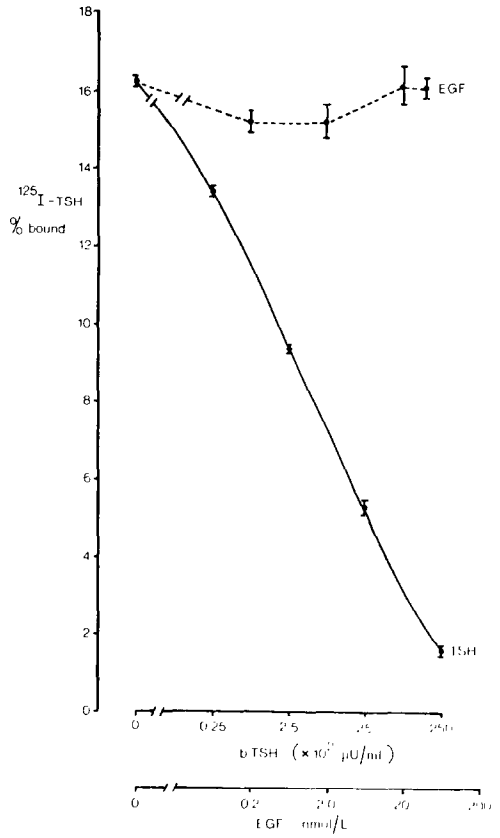

Fig.5. Inhibition of binding of ^{125}I-TSH to porcine thyroid membranes by: (a) unlabelled TSH; (b) EGF (mean ± SEM, n = 3).

4. DISCUSSION

This study clearly demonstrates the presence on porcine thyroid cell membranes of receptors for epidermal growth factor. These receptors are specific, saturable and of high affinity (K_a 1.7 × 10^9 M^{-1}). High concentrations of bovine TSH did not inhibit the binding of ^{125}I-EGF to thyroid membranes, nor did EGF inhibit ^{125}I-TSH binding. The binding affinity of porcine thyroid EGF receptors is similar to values quoted for EGF receptors on other cell types (review [3]).

Epidermal growth factor has been shown both to be a potent mitogen to ovine thyroid cells in culture [5] and to control the proliferation and the expression of differentiation of canine thyroid cells in culture [6]. However, other studies have failed to demonstrate an effect by EGF on the proliferation of rat [7,9] or bovine [7] thyroid cells. The reason for these discrepancies is not clear but might involve differences in responsiveness of the experimental species used. EGF is a physiologically occurring plasma hormone [12] which has also been extracted from thyroid tissue [13]. The demonstration of EGF receptors on thyroid cell membranes indicates that thyroid tissue may be a

Table 1

Effect of various hormones on the binding of ^{125}I-EGF to porcine thyroid membranes

Hormone	Conc.	^{125}I-EGF % bound (mean ± SEM)
None	–	16.5 ± 0.3
EGF	82 nmol/l	12 ± 0.1
hTSH	54 nmol/l	16.5 ± 0.4
LH	17 nmol/l	15.8 ± 0.3
FSH	17 nmol/l	15.8 ± 0.4
ACTH	6 μmol/l	15.7 ± 0.3
Glucagon	70 μmol/l	15.7 ± 0.1
Insulin	100 nmol/l	15.6 ± 0.5
Somatostatin	3 μmol/l	17.5 ± 0.8

site of hormonal action of EGF. Further evidence for a relationship between EGF and the thyroid gland is the T_4 stimulation of EGF synthesis by neonatal mouse submaxillary glands [14].

This study suggests that EGF may have a specific role in the regulation of thyroid follicular cell growth and function in normal and pathological situations.

ACKNOWLEDGEMENTS

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