

## EFFECT OF CATECHOLAMINES ON IODIDE TRANSPORT IN ISOLATED THYROID CELLS

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### 1. Introduction

Numerous studies have suggested that the sympathetic adrenergic system can have a direct effect on the secretion of thyroid hormone in mouse and man [1–3] through the release of norepinephrine by intra-thyroidal nerve endings. Both thyroid alpha- and beta-adrenergic receptors have been implicated in this effect [4–6]. In freshly isolated calf thyroid cells, catecholamines were shown to greatly stimulate the accumulation and organic binding of iodine [7,8]. Since this effect was blocked by phentolamine but not by propranolol, it was concluded that alpha-adrenergic receptors were involved. In addition methimazole but not perchlorate prevented epinephrine-stimulated iodine accumulation in these cells lending support to the view that epinephrine acted principally by stimulating iodide organification rather than iodide transport. Since iodide transport is the first and probably the rate-limiting step in thyroid iodine metabolism and thyroid hormone synthesis and is located in the plasma membrane as well as the catecholamine receptors, it was of interest to specify the possible action of catecholamines on iodide transport.

In this study, we used primo-cultured porcine thyroid cells to study the effect of catecholamines on iodide influx and efflux in blocked cells.

**Abbreviations:** IPNE, isoproterenol; E, epinephrine; NE, norepinephrine; TSH, thyroid-stimulating hormone or thyrotropin

### 2. Materials and methods

#### 2.1. Isolated thyroid cells

Thyroid cells were obtained from porcine glands by the discontinuous trypsinization procedure as in [9]. Isolated cells were seeded in 75 cm<sup>2</sup> Falcon plastic bottles at a concentration of  $2 \times 10^6$  cells/ml in Eagle minimum essential medium enriched with 10% calf serum, penicillin (200 U/ml) and streptomycin sulfate (50 µg/ml) and where indicated 0.4 mM dibutyryl cyclic AMP or 5 mU/ml TSH. Flasks containing 20 ml cell suspension were incubated at 37°C in 95% air/5% CO<sub>2</sub> for 48 h. In the presence of dibutyryl cyclic AMP or TSH, follicles develop within the cell layer whereas a typical monolayer is obtained in their absence.

#### 2.2. Conditions of study of iodide uptake and efflux

This was done as in [10]. Briefly, after 2 days incubation, the medium was discarded and the cell layer was washed with spinner salt solution. The latter, 10 ml without Mg<sup>2+</sup> but supplemented with 3 mM EGTA, was added. After 15 min incubation at 37°C in air, the cells were partially detached from the support with the aid of gentle scraping. After washing, the cells were suspended in Earle's solution buffered with 20 mM Hepes, at pH 7.0, containing 1 µM NaI to obtain a concentration of  $3.3 \times 10^6$  cells/ml. Cell suspension, 0.3 ml, was supplemented with 2 mM methylmercaptoimidazole and 1 µM NaI traced with 1 µCi Na<sup>125</sup>I in final vol. 0.5 ml in buffer and was incubated in air at 35°C for 30 min. Efflux of iodide in the presence or absence of effectors was studied by post-incubation of the tubes for 5 min in the same

conditions. The reaction was stopped by addition of 4 ml cold medium containing  $1 \mu\text{M}$   $\text{Na}^{127}\text{I}$ . The cells were collected by centrifugation, washed once and counted for radioactivity in a well-type scintillation spectrometer. All assays were done in triplicate. The results are expressed as mean  $\pm$  SE. Typical results on at least two independent experiments are described.

Cell content in cyclic AMP was determined by addition of perchloric acid (1 N final conc.) and sonication. After centrifugation, cyclic AMP content in the supernatant was estimated by radioimmunoassay as in [11].

### 2.3. Materials

Trypsin was obtained from GIBCO (Paisley, Scotland), dibutyryl cyclic AMP from Boehringer (Mannheim, FRG), thyrotropin (1 U/mg) from Miles (Slough, England) and L-epinephrine bitartrate, L-norepinephrine bitartrate, L-isoproterenol bitartrate and DL-propranolol. HCl from Sigma (St Louis, MO). Phentolamine was the gift from CIBA (Horsham, England). All other reagents were of the best quality commercially available.

### 3. Results

Cells cultured in the presence of dibutyryl cyclic AMP for 48 h and then incubated in suspension in the presence of iodide ( $1 \mu\text{M}$ ) actively concentrate iodide [10]. In the conditions utilized, equilibrium is reached in 30 min. As previously shown for thyrotropin, dibutyryl cyclic AMP and prostaglandin  $\text{E}_1$ , catecholamines also provoke a rapid efflux of intracellular iodide. This effect is shown in fig.1 for epinephrine and isoproterenol. Maximum discharge is obtained in less than 5 min so that dose-effect experiments were performed at this time. At maximum concentration, isoproterenol (0.1 mM), the most potent agonist tested, provoked a 50–60% efflux of intracellular iodide never reaching the effect (80% efflux) obtained with maximum TSH dosage.

Dose-response curves for IPNE, E and NE are shown in fig.2. Half-maximum responses were obtained for about 5 nM IPNE, 50 nM E and 500 nM NE indicating a potency ratio of 10 : 1 : 0.1 for IPNE : E : NE. This ordering suggests stimulation of iodide efflux via beta-adrenergic receptors. This con-

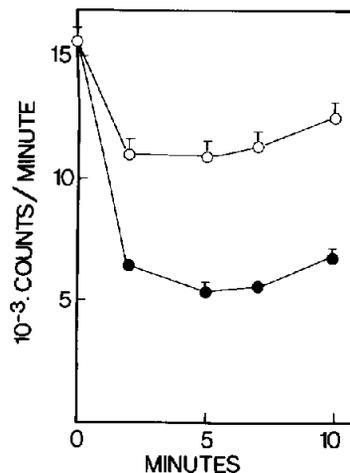


Fig.1. Stimulation of iodide efflux by epinephrine ( $\circ$ )  $1 \times 10^{-6}$  M and isoproterenol ( $\bullet$ )  $1 \times 10^{-6}$  M in isolated porcine thyroid cells cultured for 2 days in the presence of 0.4 mM dibutyryl cyclic AMP. Conditions, see section 2. Values are mean  $\pm$  SE of assays in triplicate.

clusion was strengthened by study of the inhibition of catecholamine effect by propranolol, a potent beta-adrenergic antagonist which at a 10-times lower concentration than used for NE- and E-stimulation cancelled its effect (table 1). Phentolamine ( $5 \mu\text{M}$ ), an

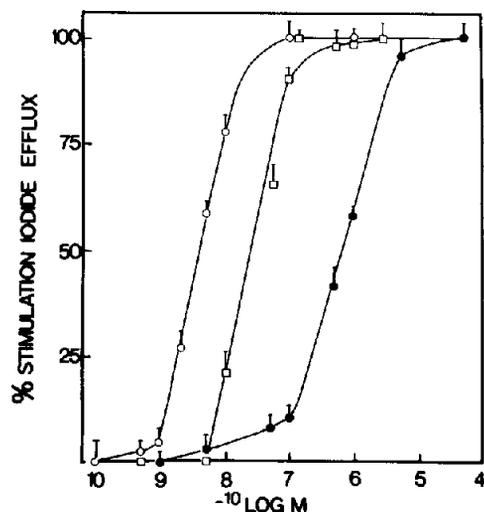


Fig.2. Concentration dependence of stimulation of iodide efflux by isoproterenol ( $\circ$ ), epinephrine ( $\square$ ) and norepinephrine ( $\bullet$ ).

Table 1  
Effect of catecholamines and adrenergic-blocking drugs on the efflux of intracellular iodide in isolated porcine thyroid cells cultured for 2 days in the presence of dibutyryl cyclic AMP

Addition	Iodide in cells (cpm)	Iodide in cells in the presence or absence of catecholamines	
		+ Phentolamine $5 \times 10^{-6}$ M (cpm)	+ Propranolol (M) (cpm)
None (control)	24 373 $\pm$ 447	25 310 $\pm$ 181	$5 \times 10^{-6}$ 23 925 $\pm$ 185
Norepinephrine $5 \times 10^{-6}$ M	14 287 $\pm$ 455	13 600 $\pm$ 70	$5 \times 10^{-7}$ 24 121 $\pm$ 1702
Epinephrine $5 \times 10^{-7}$ M	11 329 $\pm$ 898	10 842 $\pm$ 601	$5 \times 10^{-8}$ 19 968 $\pm$ 50
Isoproterenol $5 \times 10^{-8}$ M	14 075 $\pm$ 240	14 013 $\pm$ 432	$5 \times 10^{-9}$ $5 \times 10^{-8}$ 17 730 $\pm$ 833 24 025 $\pm$ 80

Values are mean  $\pm$  S.E. of each experiment analysed in triplicate

alpha-adrenergic blocking agent, was without effect. Propranolol and phentolamine were shown to have by themselves no effect on iodide efflux.

The beta-adrenergic receptors appear to be coupled to membrane-associated adenylate cyclase in all tissues [12] as in the thyroid [13]. To assess this relation in isolated porcine thyroid cells we have verified that the effect of catecholamines on iodide efflux was accompanied by the activation of adenylate cyclase as measured by the intracellular cyclic AMP level. The kinetics of cyclic AMP content in cells stimulated by IPNE ( $5 \mu\text{M}$ ) is shown in fig.3. Doubling of the cyclic AMP content is observed at 2 min, time at which an almost maximum effect on iodide efflux is observed. A return to basal level is obtained after 10 min for both parameters.

Cells cultured in the presence of TSH for 48 h instead of dibutyryl cyclic AMP also reassociate into follicles and concentrate iodide [3]. However induction of intracellular iodide efflux by catecholamines was strikingly reduced and attained in 3 independent experiments only 10–20% of the effect obtained with cells cultured in the presence of dibutyryl cyclic AMP.

Cells cultured for 48 h in the absence of stimulator do not form follicles. They show aggregates and begin to develop as a monolayer. They continue to concen-

trate iodide at a slow rate, this property disappearing after 3 days of culture in the absence of stimulator. In some experiments, after the cells have concentrated iodide for 30 min no efflux induced by catecholamines (NE and E) was noticed but instead a stimulation of

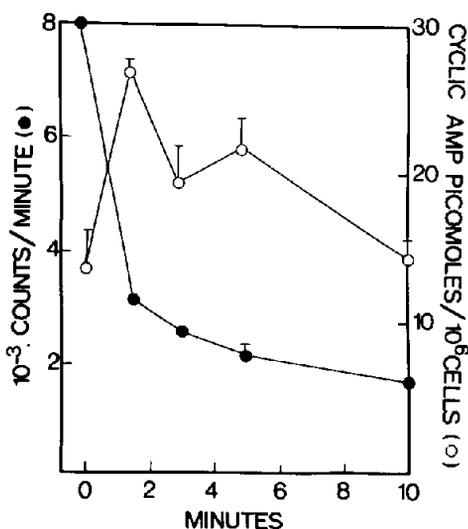


Fig.3. Relation between isoproterenol-stimulated efflux of iodide and accumulation of cyclic AMP in isolated porcine thyroid cells.

Table 2  
Effect of norepinephrine and adrenergic blocking drugs on the uptake of iodide by isolated porcine thyroid cells cultured for 2 days in the absence of stimulator

Addition	Iodide in cells (cpm)
None	29 385 ± 308
Norepinephrine 1 × 10 <sup>-5</sup> M	37 113 ± 314 <sup>a</sup>
+ phentolamine, 1 × 10 <sup>-5</sup> M	25 866 ± 197
+ propranolol, 1 × 10 <sup>-5</sup> M	36 564 ± 475

<sup>a</sup>  $p < 0.05$  compared to assay without norepinephrine

Values are mean ± SE of each experiment analysed in triplicate

influx was observed. This effect was small (about 25%) but significant (table 2). It was inhibited by phentolamine but not by propranolol.

#### 4. Discussion

Isolated porcine thyroid cells cultured in the presence of dibutyryl cyclic AMP (or TSH) show the morphological and metabolic properties of gland follicles *in vivo* [14]. In cells cultured for 2 days in the presence of dibutyryl cyclic AMP, TSH and prostaglandin E<sub>1</sub> which exert a positive control of thyroid function were shown to rapidly stimulate the efflux of intracellular iodide [10]. In the present studies, it is demonstrated that in such cells, catecholamines have the same effect on iodide efflux via a beta-type adrenergic receptor as shown by:

- (i) A potency ratio of 10 : 1 : 0.1 for IPNE : E : NE.
- (ii) Inhibition of the effect by propranolol and not by phentolamine.

In addition a correlation was found between the time-course of catecholamine-stimulated iodide efflux and increased levels of intracellular cyclic AMP. These results are in contrast with the alpha-type effects

shown by catecholamines on the stimulation of accumulation and organic binding of iodide in freshly isolated not stimulated calf thyroid cells [7,8]. However, it was noticed occasionally that in porcine cells cultured in the same conditions but in the absence of dibutyryl cyclic AMP or TSH, catecholamines were unable to stimulate iodide efflux but enhanced iodide uptake via an alpha-type adrenergic receptor. If these observations are confirmed they would indicate that the nature of the adrenergic receptor and its alpha-beta interconversion would depend upon the metabolic state of thyroid cells as shown for other tissues [15,16] and as already suggested for the thyroid itself [6].

In contrast to cells cultured in the presence of dibutyryl cyclic AMP, cells cultured in the presence of TSH showed only a very small stimulation of iodide efflux by catecholamines, an effect also mediated via a beta-type receptor. This is in agreement with previous observations that, in mice, catecholamines stimulate endocytosis of thyroglobulin and the release of thyroid hormone only when endogenous TSH secretion is blocked [5].

Our observations suggest that the role of catecholamines in the positive control of iodide transport in thyroid cells is subordinated to that exerted by TSH and might take its full expression in conditions where thyroid regulation by thyrotropin is depressed.

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