INHIBITION OF THYROID ADENYLATE CYCLASE BY IODIDE

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1. Introduction

Iodine has a generally inhibitory effect on the thyroid gland. Excess iodide has been shown to inhibit hormone synthesis and release in man and animals [1]. Increased iodide intake leads to depression of iodide concentration activity [2]. Since the effect of iodide was blocked by drugs which inhibited intrathyroidal iodination processes, it was postulated that the administrated iodide was incorporated into a substance which acted as an inhibitor of the iodide transport mechanism [3].

We have recently demonstrated [4,5] that iodide inhibited the stimulatory effect of TSH on cyclic AMP accumulation in dog thyroid slices in vitro. Sherwin and Tong [6] have also reported such an effect in bovine isolated thyroid cells. This report presents evidence that horse thyroid adenylate cyclase activity from slices preincubated with iodide is inhibited. This inhibition can be presented by methimazole.

2. Materials and methods

Horse thyroid was collected at a local slaughter house just after killing, trimmed of fat tissue and sliced with a Stadie-Riggs microtome. The slices (± 2 g) were incubated for 90 min at 37°C in 100 ml of Krebs–Ringer bicarbonate buffer enriched with 8 mM glucose 0.5 g/l bovine serum albumin and KI (0.1 mM). When TSH was used, the hormone (0.5 mU/ml) was added after 60 min preincubation and caffeine (1 mM) after 80 min. Methimazole was used at 1 mM concentration. cAMP level and adenylate cyclase activity were measured. For cAMP assay, two slices (40–60 mg wet weight) were dropped into 1 ml of boiling de-ionized water, homogenized and centrifuged. The supernatant was lyophilized and resolubilized with water (10 μl/mg wet weight). Aliquots (2 × 10 and 2 × 20 μl) were taken for cAMP determination according to Gilman [7]. For adenylate cyclase assay, the slices were rapidly washed with ice cold Tris–HCl 50 mM (pH 7.4), 0.25 M sucrose, chopped with scissors, homogenized (2 g/15 ml) with the Tris sucrose medium through a Teflon glass motor driven homogenizer (5 strokes) at reduced speed and filtered through a double layer of gaze. The whole homogenate (10 mg of protein/ml) was kept at 0°C and immediately assayed for cyclase. Fractionation of the homogenates proceeded through a centrifugation of 30 min at 12 500 rev/min (25 000 × *g*) at 4°C with HB 4 rotor on a RC-2B Sorvall centrifuge. The pellet was rehomogenized and resuspended in 30 ml of Tris–sucrose. This washing procedure was repeated twice.

Adenylate cyclase (EC 4.6.1.1) was measured as follows: 100 μl of whole homogenate, or resuspended pellet, (± 1 mg of protein) was added to 100 μl of the assay mixture containing at final concentration Tris–HCl 50 mM (pH 7.4), 2 mM ATP (Boehringer), 5 mM $MgCl_2$, 1 mM 3-isobutyl-1-methylxanthine (Aldrich) 2 mM cAMP, 10 mM creatine phosphate, 100 μg of muscle creatine phosphokinase, 0.1% bovine serum albumin, $2 \cdot 10^6$ cpm (^{32}P) [α-^{32}P]ATP (Amersham). The incubation was performed at 30°C for 20 min. Incubation was ended adding 100 μl of ice-cold solution of 50 mM ATP, 20 mM cAMP, 20 000 cpm [^3H]cAMP. The 300 μl medium was centrifuged at

Abbreviations: TSH: Thyrotropin; cAMP: Adenosine 3′:5′-monophosphate; MMI: Methimazole

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10 000 × *g* for 5 min and the supernatant passed through a neutral alumina oxyde column to separate cAMP from ATP as previously described [8]. The fraction containing cAMP was counted for radioactivity with a Packard scintillation counter. cAMP recovery was around 50% and ATP contamination did not exceed $5 \cdot 10^{-3}$%. Proteins were measured according to Lowry [9] using bovine serum albumin as standard.

Cyclic nucleotide phosphodiesterase (EC 3.1.4.17) activity was measured by an adaptation of Thompson and Appleman method [8,9]. cAMP 1 μM with [^{3}H]cAMP was incubated for 10 min at 30°C with thyroid homogenates (50 μg protein) in a medium (100 μl) containing Tris–HCl 40 mM (pH 8), and $MgCl_2$ 5 mM. Assays were performed within 25 min of the end of the slices incubation.

3. Results

TSH (0.5 mU/ml) markedly increased the concentration of cAMP in horse thyroid slices. Preincubation of the slices during 60 min with iodide (0.1 mM) markedly inhibited the TSH effect on cAMP accumulation. The inhibitory action of iodide was completely suppressed when methimazole (1 mM) was added with iodide (fig.1) [4,5]. Homogenates or plasma membranes enriched fractions from horse thyroid posses an adenylate cyclase sensitive to TSH, PGE_1 NaF and GppNHp [8]. Preincubation of the slices with iodide inhibited significantly ($p < 0.035$) the adenylate cyclase activity measured in whole homogenates (fig.2). Iodide preincubation inhibited not only the TSH stimulated cyclase activity but also basal NaF and the persistent GppNHp stimulated activity (figs. 2 and 3). Such a non-selective decrease in active suggests that the inhibition bears at the level of the catalytic unit. The inhibitory effect of iodide on adenylate cyclase activity was suppressed by pretreatment of the slices with methimazole. These data eliminate an inhibitory action of iodide per se and implicate and oxidation step in the inhibitory mechanism, perhaps of the enzyme itself. The presence of TSH during incubation of the slices was not necessary for the inhibition of cyclase (fig.3).

Rapoport et al. [11], working with long term (48 h) cultured thyroid cells, which had lost the iodide effect,

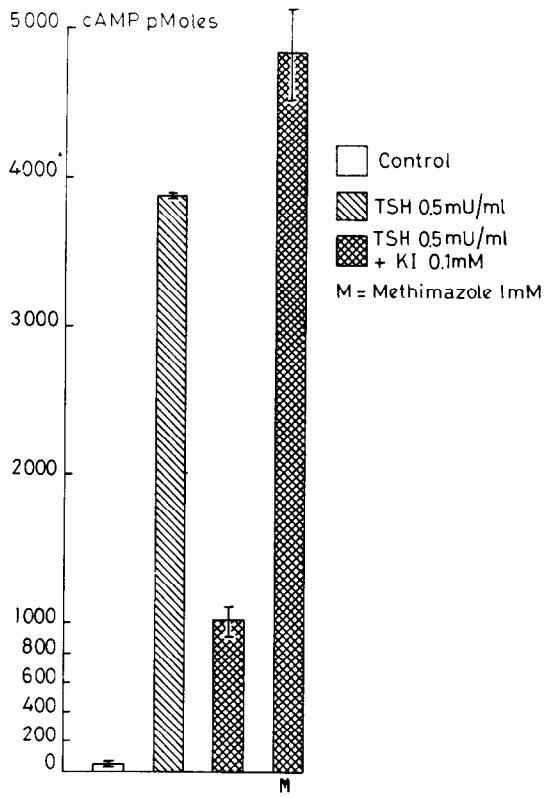

Fig.1. Effect of iodide on cAMP content of horse thyroid slices (60 mg wet wt) incubated 90 min at 37°C in KRB buffer. cAMP, pmol/100 mg wet weight; TSH 30 min; KI, 90 min; MMI, 90 min. One typical experiment among three. Each value is the mean of duplicate results in this experiment.

could restore it by adding an H_2O_2 generating system in the culture medium. This further suggests that the organification of iodide is necessary. In order to reveal a possible soluble iodinated intermediate, homogenate was fractionated by centrifugation. The cyclase activity of the washed pellets alone was measured. Most of the cyclase activity was concentrated in the pellet and very little activity remained in the supernatant [8]. Discarding the supernatant, and washing the pellet with the Tris–sucrose medium, showed a marked amplification of the inhibitory effect observed in homogenates (fig.3). Addition of supernatant to the pellet, to reconstitute homogenates, slightly but not significantly diminished the rate of inhibition. Addition of supernatant from slices pretreated with KI alone to

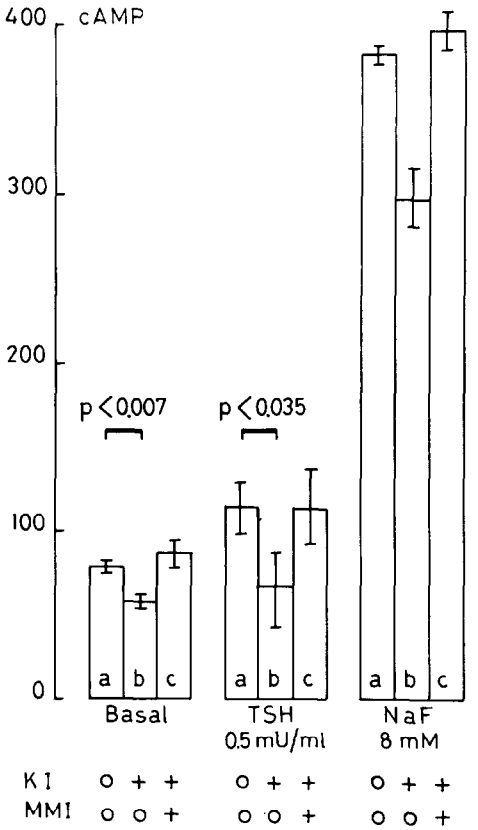

Fig.2. Effect of iodide on intact horse thyroid slices: homogenate adenylate cyclase activity. (a) Refers to homogenate from slices preincubated with TSH 0.5 mU/ml 30 min, caffeine 1 mM 10 min and without KI. (b) Refers to homogenate from slices preincubated as slices (a) but with KI 0.1 mM 90 min. (c) Refers to homogenate from slices preincubated as slices (b) but with MMI 1 mM 90 min. Each homogenate was assayed for cyclase under basal conditions, with TSH 0.5 mU/ml and with NaF 8 mM. Each value is the mean of triplicates. The values are expressed in pmol/mg prot/20 min at 30°C.

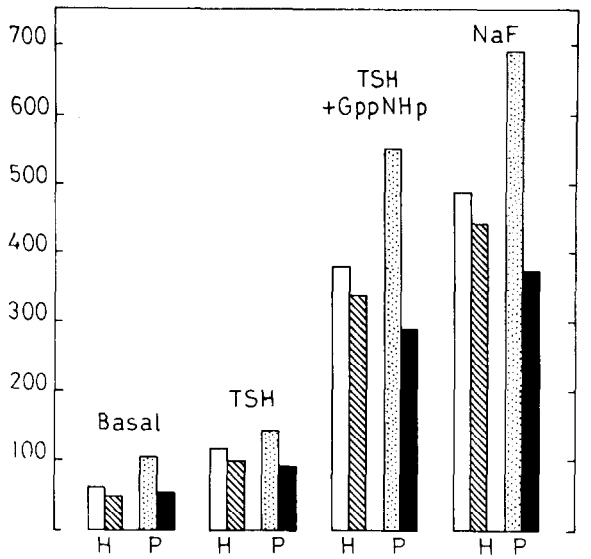

Fig.3. Effect of iodide on horse thyroid slices. Adenylate cyclase activity in homogenate and 25 000 × *g* pellet. A: Homogenate from slices preincubated 90 min with KI 10.1 mM,MMI 1 mM and without TSH and caffeine. B: Homogenate from slices preincubated 90 min. with KI alone. : 25 000 × *g* washed pellet prepared from homogenate A. : 25 000 × *g* washed pellet prepared from homogenate B. TSH, 0.5 mU/ml; GppNHp, 0.1 mM; NaF, 8 mM. Means of triplicate results. Results are expressed in pmoles/mg prot./20 min at 30°C. For the clarity of the fig. standard deviations were not drawn, they never exceeded 8%.

pellets from slices pretreated with KI and methimazole or without KI did not significantly modify the cyclase activity. Thus a direct inhibition, rather than a soluble intermediary inhibitor, could explain the effect on the particulate fration.

In vivo studies with high iodine diet and low iodine diet hypophysectomized rats, have shown an inhibition of the thyroid adenylate cyclase when the former were injected with 0.5 U TSH i.v. No inhibition of the basal and NaF stimulated cyclase activity was detected [12,13]. Using low iodine diet mice injected with NaI, Hashizume et al. have shown an inhibition of the in vitro TSH stimulated cyclase activity [14]. In neither study was the effect of MMI investigated which makes the nature of the studied effect uncertain with regard to a possible effect on cAMP degradation in our system; phosphodiesterase activity (pmoles cAMP hydrolyzed/min/mg protein) was not significantly different in the homogenates of control slices and of slices preincubated with iodide (25.4 ± 2.6 versus 27.8 ± 3.4).

Measurement of the accumulation and disposal rate of cAMP in TSH-stimulated dog thyroid slices have already suggested that iodide, through an oxidized intermediate, depresses cAMP levels by decreasing the rate of synthesis [15].

The present studies demonstrates directly such an effect on adenylate cyclase, presumably at the level

of the catalytic unit, and further suggests the absence of phosphodiesterase activation. Whether formation of an iodinated intermediate after iodide oxidation or only iodide oxidation is required for this effect is still unknown.

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