CALCIUM REQUIREMENTS IN THE ACTION OF THYROTROPIN ON THE THYROID

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1. Introduction

Douglas and coworkers [1] have shown that the induction of secretion in cells of the adrenal medulla is caused by an increase in the intracellular level of calcium. This increase appears to be secondary to a burst of calcium penetration in the cell. Because of its obvious similarity with the stimulus contraction coupling in muscle, this phenomenon has been called the stimulus secretion coupling. This concept has been extended to other cells in which secretion is thought to involve the exocytosis of material contained in intracellular vesicles [1]. Thyroid secretion is believed to involve the endocytosis of thyroglobulin by follicular cells and its digestion in secondary lysosomes with a consequent release of thyroid hormones [2]. Although in this case secretion corresponds to endocytosis and not to exocytosis, the hypothesis has been proposed that this phenomenon may also be triggered by Ca^{2+} [3]. In these experiments the role of Ca^{2+} in the stimulations by thyrotropin of glucose oxidation, iodide binding to proteins, colloid endocytosis and secretion in dog thyroid slices has been investigated.

Abbreviations:

$BE^{131}I$	: butanol extractable ^{131}I iodine (iodine and thyroid hormones)
cAMP	: cyclic 3',5'-adenosine monophosphate
DBcAMP	: N^6-2-O'-dibutyryl cyclic adenosine monophosphate
TSH	: thyrotropin

2. Methods

Dogs (± 15 kg) were administered 150 μCi of carrier free ^{131}I by subcutaneous injection, then for 3 days received 150 mg of thyroid powder (Thyranon, Organon, Oss, Nederland) per day in their food. On day 4, thyroid lobes were resected and thyroid slices prepared. The slices were incubated in Krebs-Ringer bicarbonate supplemented with glucose 8 mM, at 37°, in an atmosphere of carbogen or in Krebs-Ringer phosphate supplemented with glucose 8 mM, at 37° in an atmosphere of oxygen [4, 5]. The incubation medium was supplemented with 1-^{14}C-glucose 0.5 μCi/ml for the measurement if glucose carbon 1 oxidation, and of intracellular colloid droplet formation, with ^{125}I iodide 10 μM (specific activity 50 mCi/mole) for the measurement of ^{125}I iodide binding to proteins, and with methimazole 2 mM and $NaClO_4$ 1 mM for the measurement of thyroid secretion. We have previously described the methods used for the measurement of glucose oxidation [4, 5], ^{125}I iodide binding to proteins [6], intracellular colloid droplet formation [6] and ^{131}I iodine secretion [7].

The experimental protocol generally involved the preparation and 1 hr preincubation of the slices in a medium containing no Ca^{2+} but 1 mM EDTA Mg^{2+} and an incubation either in a similar medium or in a medium containing Ca^{2+} 1.45 mM but no EDTA. Bovine TSH (Thytropar) and DBcAMP were obtained from Armour (Kankakee, USA) and Boehringer (Mannheim, Germany) respectively.

3. Results and discussion

As described previously, TSH markedly enhanced the oxidation of glucose carbon 1 and the organification of iodide in our dog thyroid slices [6] (fig. 1). In the absence of Ca^{2+}, the effects of low concentrations of TSH were abolished, the action of higher concentrations was greatly inhibited (fig. 1). Inhibition by the absence of Ca^{2+} had been previously observed for the action of TSH on iodide organification [8] and glucose oxidation [5, 9]. The fact that increasing the hormone concentration partially relieves the inhibition might explain other negative experiments [10].

The action of TSH on iodide organification and, at least for low concentrations, on glucose oxidation appears to be mediated by intracellular cAMP [11]. Inhibition of these effects in the absence of Ca^{2+} might therefore bear on cAMP accumulation or on cAMP intracellular action. The fact that the stimulations of glucose oxidation and iodide organification by DBcAMP were also inhibited in the absence of Ca^{2+} suggests that it is at the level of cAMP action that Ca^{2+} is required.

The stimulations by TSH of intracellular colloid droplet formation and of $BE^{131}I$ release in dog thyroid slices is not inhibited in the presence of EDTA and in the absence of Ca^{2+} (fig. 2).Rather, incubation in such a medium slightly but consistently increased the $BE^{131}I$ release in control slices; the release of $NBE^{131}I$ was also increased in many but not all experiments. $BaCl_2$ 2 mM per se had no marked effect on iodide organification, glucose oxidation, intracellular colloid droplet formation or $BE^{131}I$ release in Ca^{2+} depleted thyroid slices.

A Ca^{2+} requirement for the cAMP mediated stimulation of iodide organification and glucose oxidation has been demonstrated, whereas no such requirement has been demonstrated for the stimulation of colloid endocytosis or secretion. Extrafollicular Ca^{2+} is not required for thyroid secretion. There

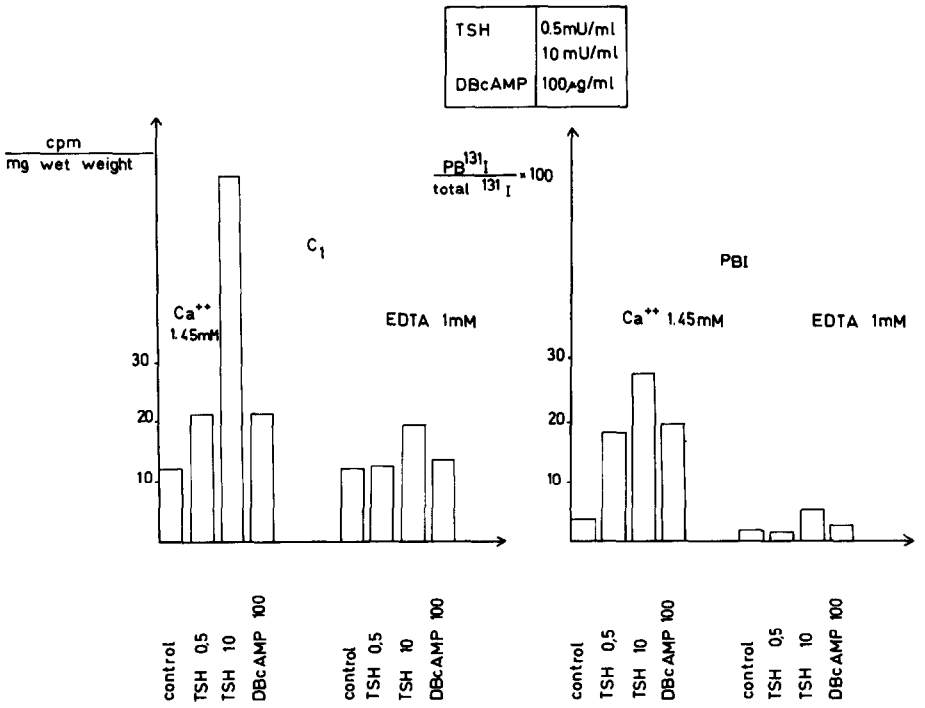

Fig. 1. Stimulation by TSH and DBcAMP of glucose carbon 1 oxidation and iodide binding to proteins in Ca^{2+}-depleted dog thyroid slices in vitro. Effects of EDTA and absence of Ca^{2+}. Results of 2 closely agreeing duplicates of representative experiments. Ca^{2+} = medium containing Ca^{2+} 1.45 mM. EDTA = medium containing EDTA Mg^{2+} 1 mM but no Ca^{2+}. T 0.5 = TSH 0.5 mU/ml; T 10 = TSH 10 mU/ml; DBcAMP = 100 µg/ml.

COLLOID DROPLETS		
	Ca^{++} 1.45mM	EDTA 1mM
control	0	0
TSH 0.5mU/ml	++	++
TSH 1.25mU/ml	++	++

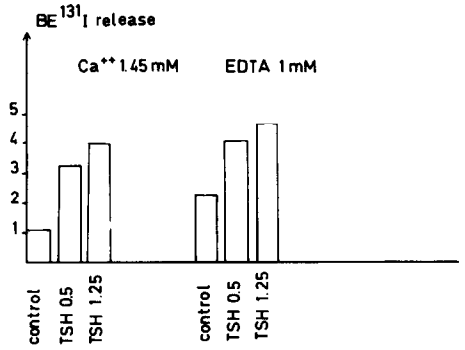

Fig. 2. Stimulation by TSH of colloid endocytosis and thyroid secretion in Ca^{2+}-depleted dog thyroid slices in vitro. Effects of EDTA and Ca^{2+} absence. Results of 2 closely agreeing duplicates of representative experiments. $BE^{131}I$ release: expressed in percent of ^{131}I content of the slices. Ca^{2+} = medium containing Ca^{2+} 1.45 mM; EDTA = medium containing EDTA Mg^{2+} 1 mM but no Ca^{2+}; T 0.5 = TSH 0.5 mU/ml; T 1.25 = TSH 1.25 mU/ml.

is therefore no support for the hypothesis that the stimulation secretion concept of Douglas [1] applies to thyroid secretion. However this hypothesis is not completely ruled out as there is no proof that all thyroid Ca^{2+} had been removed by EDTA and enough Ca^{2+} may still be available, for instance in the follicle lumen.

The complete dissociation between the cAMP mediated stimulation of colloid endocytosis and thyroid secretion on the one hand and activation of glucose oxidation and iodide organification on the other hand, further supports the conclusion that in these conditions Ca^{2+} depletion did not interfere with cAMP accumulation, i.e. with the activation of thyroid adenyl cyclase, but with the action of cAMP on glucose and iodide metabolism. Further this dissociation supports our conclusion [6] that contrary to a previous hypothesis [12, 13], the stimulation of glucose oxidation is not required for colloid endocytosis or thyroid secretion.

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