EFFECT OF IONOPHORE A23187 ON THYROID SECRETION

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1. Introduction

Douglas [1] has 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]. In the thyroid, TSH increases the release of calcium from an intracellular compartment [2]. Moreover, the presence of calcium in the medium is required for the activation by TSH or DBcAMP of glucose oxidation [3–5], iodide binding to proteins [4], and ^{32}P-incorporation into phospholipids [5] in dog thyroid slices. On the other hand, calcium depletion in the slices and in the incubation medium does not inhibit the TSH induced intracellular accumulation of colloid droplets [6] in the thyroid hormone secretion [4] or cAMP accumulation [6,7] in such slices. To elucidate the role of calcium in the metabolism of thyroid cells and in the activation of this metabolism by TSH, we have investigated the effect of increasing intracellular concentrations of calcium using the new divalent cations ionophore A23187 [8].

2. Materials and 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°C in an atmosphere of carbogen [3,9]. The incubation medium was supplemented with 1-[^{14}C]glucose 0.5 μCi/ml for the measurement of glucose carbon 1 oxidation, with ^{131}I iodide 40 μM (specific activity 1.25 Ci/mole) for the measurement of ^{131}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 [3,9], ^{131}I iodide binding to proteins [10], ^{131}I iodine secretion [11] and of cAMP content [7,12].

The experimental protocol generally involved a first preincubation of 1 hr with ionophore A23187 10^{-6} M or 10^{-5} M and EGTA 2 mM in a buffer containing no calcium, to deplete the cells of calcium and three successive washings at room temperature in calcium-free buffer. The slices were then incubated for 30 min with various calcium concentrations and ionophore A23187 10^{-6} M or 10^{-5} M for equilibration. Finally, they were incubated in fresh medium with the same calcium and ionophore concentration and the tracer and chemicals required for the measurement of the metabolic para-

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† *Abbreviations:* cAMP: adenosine 3′,5′-cyclic monophosphate. TSH: thyroid stimulating hormone. DBcAMP: N^6-2′-*O*-dibutyryl cyclic adenosine 3′,5′-monosphosphate. A23187: ionophore A23187.

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meter under study. This third incubation in the presence or absence of TSH lasted 1 hr when cAMP, glucose oxidation and iodide binding to proteins were measured and 2 hr when secretion was investigated.

Ionophore A23187 was a gift from Lily Company (Indianapolis, USA). Bovine TSH (Thytropar) was purchased from Armour (Kankakee, USA).

3. Results

TSH increased cyclic 3′,5′-AMP accumulation in dog thyroid slices. In the presence of ionophore, calcium depletion does not much modify basal cyclic 3′,5′-AMP levels (fig. 1). On the other hand, at high concentrations, i.e., above 10^{-4} M, calcium inhibited the accumulation of cyclic 3′,5′-AMP in TSH stimulated slices.

TSH enhanced secretion by dog thyroid slices in vitro, as measured by the release of $BE^{131}I$. As already shown [4] pretreatment of slices with EGTA induces a leakage of colloid out of the slices which is evidenced by the release of non butanol extractable ^{131}I from 10 to 20% to 60–70%. This effect is presumably due to follicular disruption [13]. A small but consistent increase in $BE^{131}I$ release by the slices (fig. 2) may perhaps be attributed to the same cause. In the presence of ionophore, none of these two releases were modified by increasing calcium concentrations. On the other hand, under these conditions, calcium at concentrations higher than 10^{-4} M inhibited the TSH induced secretion.

In slices depleted of calcium, the oxidation of glucose carbon 1 was not decreased, but the activation of this metabolism by TSH was abolished. In the presence of ionophore, the inhibition of glucose oxidation in the absence of calcium was almost complete (fig. 3). On the other hand, calcium by itself greatly enhanced this variable at concentrations of 10^{-4} M or higher; the action of TSH being no longer apparent.

Depletion of calcium greatly decreased the binding of iodide to proteins in dog thyroid slices and abolish-

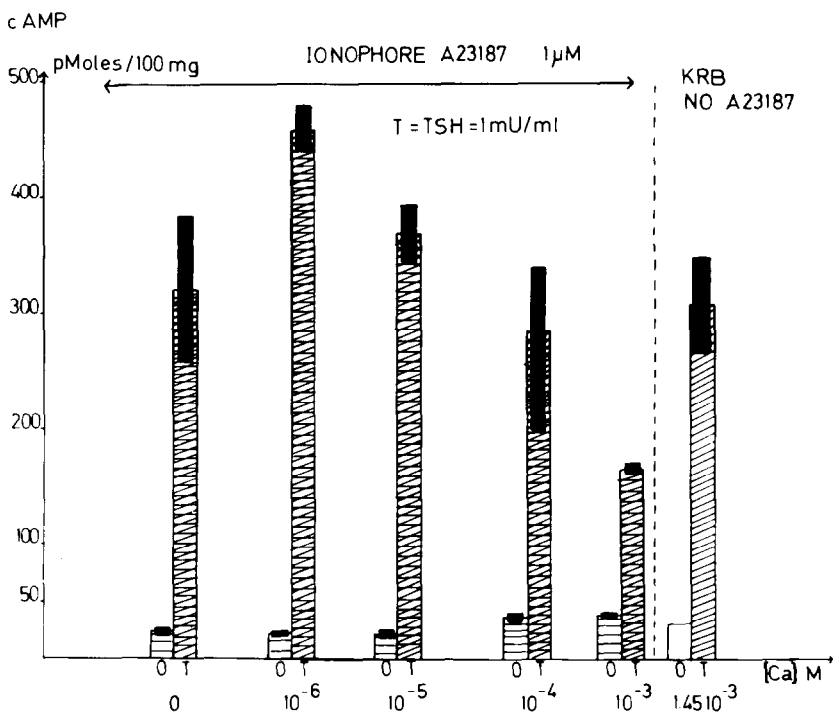

Fig. 1. Effect of calcium on cAMP accumulation in dog thyroid slices treated with ionophore A23187 1 μM. Results of one typical experiment expressed as means ± ranges of the duplicates or triplicates. O: control. T: TSH : 1 mU/ml. [Ca] : concentration of calcium in the incubation medium. KRB: Krebs Ringer bicarbonate buffer.

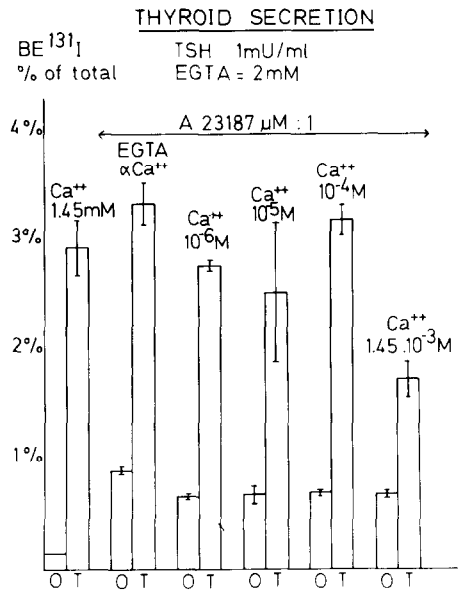

Fig. 2. Effect of calcium on secretion by dog thyroid slices treated with ionophore A231871 μM. Secretion is measured by the release of butanol extractable ^{131}I in percent of total ^{131}I of the slices. Results of one typical experiment are expressed as means ± ranges of duplicates or triplicates. O: control. T: TSH 1 mU/ml. αCa^{++}: medium without calcium.

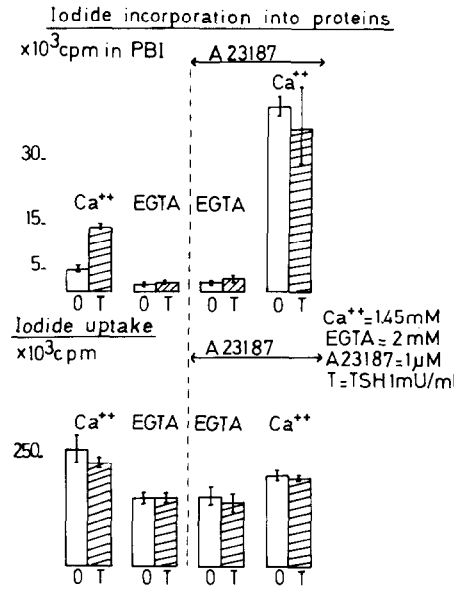

Fig. 4. Effect of calcium on the iodide uptake and incorporation into proteins by dog thyroid slices treated with ionophore A23187 1 μM. Results of one typical experiment are expressed as means ± ranges of duplicates or triplicates. EGTA: medium without calcium, with EGTA 2 mM. PBI: protein bound iodine ^{131}I ($\times 10^3$ cpm/100 mg wet weight). Iodide uptake ($\times 10^3$ cpm/100 mg wet weight of tissue).

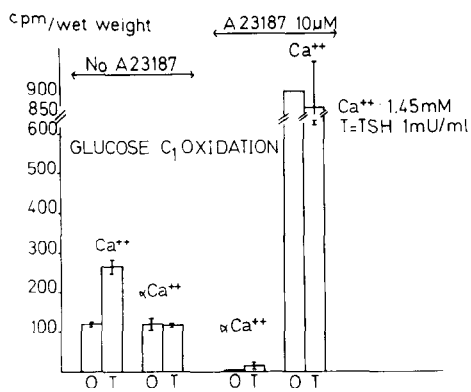

Fig. 3. Effect of calcium on the oxidation of [1-^{14}C] glucose by dog thyroid slices treated with ionophore A23187 10 μM. Results of one typical experiment are expressed as means ± ranges of duplicates or triplicates. O: control. T: TSH 1 mU/ml. αCa^{++}: incubation medium without calcium. cpm/wet weight : cpm per mg $BaCO_3$ and 100 mg wet weight.

ed the TSH effect on this variable. In the presence of ionophore, calcium depletion had the same effect (fig. 4). With ionophore, calcium at concentrations higher than 10^{-4} M greatly enhanced this binding, up to the same level in control and TSH activated slices. Calcium depletion in the presence or absence of ionophore decreased to some extent the uptake of iodide in the slices which is a known consequence of follicular disruption [13]. Under the conditions of the experiment [10], the step of iodide binding by itself is limiting the overall incorporation of the anion into proteins. Therefore, the limited decrease in iodide trapping does not influence the level of protein iodination. The absence of effect of ionophore per se on iodide trapping makes a general toxic effect of this compound on the slices unlikely [14].

4. Discussion

With regard to the requirement of extracellular calcium two types of TSH effects have been demonstrated:

1) Effects which take place normally in calcium depleted thyroid slices and incubation media, such as the enhancement of cAMP accumulation and of secretion [6,7].

2) Effects which are suppressed in such slices, e.g., the activation of glucose carbon 1 oxidation, of the binding of iodide to proteins and of the incorporation of ^{32}P into phospholipids [3–5].

This suggests that calcium may mediate some but not all TSH effects in thyroid. However, modifications of the level of extracellular calcium may have little influence on intracellular stores of the ion; therefore the absence of an extracellular calcium requirement in a hormonal action is not sufficient to exclude a role of calcium in this action. This is even more so in the thyroid in which an intracellular pool of sequestrated calcium has been demonstrated, which releases the ion under TSH stimulation [2]. In order to investigate the role of calcium in thyroid metabolism and in the activation of this metabolism by TSH a more direct approach was necessary. The new divalent cation ionophore A23187, which permeates membranes to calcium, allows the modulation of intracellular calcium concentration by extracellular calcium [8]. It should however be pointed out that in the presence of ionophore, the intracellular calcium concentration is not likely to be equal to the extracellular concentration as the normal pumping mechanisms of the cell, for extrusion at the plasma membrane, and for uptake by the mitochondria may still operate.

Using this approach, it has been shown that the effects of TSH which require the presence of calcium in the incubation medium, i.e., the activation of glucose oxidation and of iodide binding to proteins are mimicked by a raise in the extracellular calcium level. Moreover, in the presence of ionophore, there is a direct relation between the activity of these metabolism and the extracellular calcium concentration. On the other hand, the effects of TSH which are little modified by the absence of calcium in the medium, i.e., the enhancement of cAMP accumulation and of secretion are not mimicked by a raise of intracellular calcium and in fact these effects are inhibited by high calcium concentrations. The activating and inhibiting actions of calcium take place in the same range of extracellular concentration. Thus calcium may be the intracellular modulator of two metabolisms in the thyroids: the hexose monophosphate pathway and the binding of iodide to proteins; it does not appear to be involved in the activation by TSH of cAMP accumulation and of secretion. Further investigation to determine whether calcium acts directly or through other signals on these metabolisms are in progress.

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