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Effects of Calcium Ionophore (A-23187) on Glucose Oxidation and Iodide Transport in Dog Thyroid Slices

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Synopsis

A calcium ionophore (A-23187, 20μg/ml) stimulated ^{14}C-1-glucose oxidation in dog thyroid slices to an extent equivalent to that obtained by the optimal concentration of dibutyryl cyclic AMP (1mM). Furthermore, the ionophore augmented the stimulation by dibutyryl cyclic AMP much more than the simple additive effect. The ionophore also enhanced the effect of TSH, but to a lesser extent.

Under conditions where organic binding was blocked, T/M ratio of radioiodine concentration was lowered in slices by the ionophore; the findings similar to those obtained with TSH and dibutyryl cyclic AMP.

The ionophore exhibited a slightly depressive effect on the basal cyclic AMP level. The elevation by TSH of cyclic AMP levels was also slightly depressed by the ionophore, but statistically insignificant in most cases.

These results indicate that calcium ion may play an important role in the TSH regulation of iodide transport and glucose metabolism in the thyroid, in some cases by augmenting the effects of cyclic AMP.

Thyroid stimulating hormone (TSH) appears to regulate some of thyroid gland functions through activation of the adenylate cyclase-cyclic AMP system (Field, 1975). On the other hand, the importance of calcium for the exertion of TSH effects in the thyroid has repeatedly been demonstrated. Calcium was required for the *in vitro* TSH stimulation of iodine incorporation from the intrathyroidal iodide pool into thyroglobulin (Kondo and Ui, 1963). Zor *et al.* (1968) reported that basal glucose oxidation was decreased in thyroid slices incubated in the absence of Ca^{++} and that the TSH stimulation of CO_2 production and ^{32}P incorporation into phospholipid was abolished in a Ca^{++}-free buffer. Since the effects of dibutyryl cyclic AMP (dbcAMP) on these parameters were also reduced in the absence of Ca^{++}, it was suggested that Ca^{++} was essential for the action of cyclic AMP rather than for its formation (Zor *et al.*, 1968). This was supported by the previous work in which it was reported that Ca^{++} was not essential for the TSH stimulation of adenylate cyclase-cyclic AMP system (Yamashita *et al.*, 1971).

With the advent of calcium ionophores which increase the permeability of cell membranes to calcium, several groups could

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Abbreviations:
TSH=thyroid stimulating hormone; cyclic AMP=adenosine 3′, 5′-cyclic monophosphate; dbcAMP=N^6-2′-O-dibutyryl cyclic adenosine 3′, 5′-monophosphate; cyclic GMP=guanosine 3′, 5′-cyclic monophosphate

show that the addition of calcium ionophores caused a stimulation of the functions of exocrine and endocrine glands (Eimerl *et al.*, 1974; Nakazato and Douglas, 1974; Prince *et al.*, 1973), and suggested that the induced influx of Ca^{++} functioned as a mediator of the above metabolic responses.

The present studies were undertaken to elucidate the role of Ca^{++} in the TSH effect on ^{14}C-1-glucose oxidation, iodide transport and cyclic AMP content in dog thyroid slices. A calcium ionophore was employed for this purpose.

While these studies were in progress, Van Sande *et al.* (1975) reported that calcium in the presence of ionophore A-23187 increased cyclic GMP levels and activated the oxidation of ^{14}C-1-glucose and the binding of iodide to proteins in dog thyroid slices. However, they have not examined the effect of the ionophore on TSH or dbcAMP stimulation of glucose oxidation and on the iodide transport.

Materials and Methods

Dog thyroid gland were obtained, sliced and incubated for determination of ^{14}C-1-glucose oxidation and cyclic AMP content as described previously (Yamashita *et al.*, 1970; Oka *et al.*, 1973).

Iodide transport was examined by the method as reported previously (Field *et al.*, 1973). In short, thyroid slices were incubated initially for 1 hour in Krebs-Ringer phosphate buffer (pH 7.4) in an atmosphere of air at 37°C in a Dubnoff metabolic shaker. Approximately 60 mg of slices were then incubated for 30 minutes in 2 m*l* of the same buffer containing 1 mg/m*l* glucose, 0.5 mg/m*l* alumin, 0.25 mg/m*l* methimazole and 1 μg/m*l* I^- as KI with 0.05 μCi/m*l* ^{131}I. The thyroid/medium concentration ratio for radioiodide (T/M) was calculated as counts per gram of tissue over counts per m*l* of medium.

A calcium ionophere (A-23187) was dissolved by first adding 0.25 m*l* of acetone per 1 mg and then adding 0.75 m*l* of ethanol and was added to the incubation medium in appropriate flasks. The same amounts of acetone and ethanol were added to the control slices.

Calcium ionophore (A-23187) was kindly supplied by Dr. Robert L. Hamill of the Eli Lilly Co. Bovine TSH was obtained from Armour Pharmaceutical Co. ^{14}C-1-glucose (2.4 mCi/nmole) was purchased from Amersham-Searls Co. and ^{3}H-cyclic AMP (14.2 Ci/nmole) from Schwarz Bioresearch Co.

Results

As shown in Figure 1, dibutyryl cyclic AMP (1 mM) stimulated ^{14}C-1-glucose oxidation as reported previously (Zor *et al.*, 1968). The calcium ionophore, although it had no effect in the lower concentration (2 μg/m*l*), produced an increase in ^{14}C-1-glucose oxidation (+35%) in the higher concentration (20 μg/m*l*). The extent of this stimulation is similar to that obtained by the optimal concentration of dbcAMP. When the slices were incubated in the buffer containing both the calcium ionophore and dbcAMP, glucose oxidation was enhanced much more than that explicable by an additive effect. A submaximal dose of TSH (10 mu/m*l*) increased glucose oxidatlon by about 50%. The ionophore enhanced the TSH stimulation, but to a lesser extent than in the case of dbcAMP stimulation. Calcium ionophore

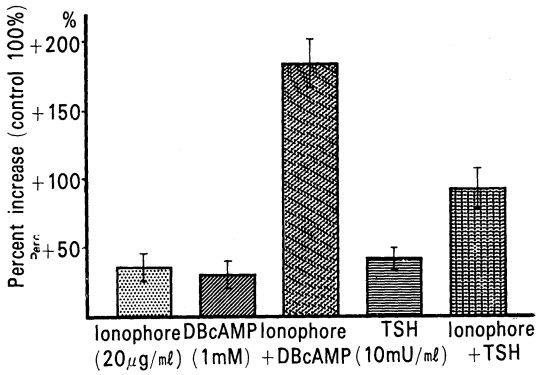

Figure 1. Effects of ionophore A-23187, dibutyryl cyclic AMP and TSH on ^{14}C-1-glucose oxidation in dog thyroid slices. Glucose oxidation was determined during 45 minutes of incubation with or without reagents. The range of basal glucose oxidation was $5\times10^{3}\sim10^{4}$ cpm/g tissue. The results are averages±S.E.M. of four independent experiments as expressed by the percentage over the control values. Each increase of glucose oxidation by reagents alone or in combination was significant ($P<0.01$).

did not influence the effect of a higher dose of TSH (100 mu/m*l*).

Under conditions where organic binding was blocked, the T/M radioiodine concentration ratio was lowered not only by TSH or dbcAMP but also by the calcium ionophore (Table 1). In this aspect of tissue response, however, the TSH or dbcAMP action was not influenced by the additional ionophore.

The data in Fig. 2 indicate that the calcium ionophore caused a slight depression in the basal cyclic AMP level. This fall in concentration was significant ($P < 0.05$). The elevation by TSH of cyclic AMP levels was slightly depressed by the ionophore, but statistically not significant in most cases.

Table 1. Effects of ionophore A-23187, TSH and dibutyryl cyclic AMP on the radioiodine T/M ratio.

Addition	T/M ratio
none	2.40
IP (20 μg/m*l*)	2.01
TSH (33 mu/m*l*)	1.87
IP+TSH	1.88
db cyclic AMP (1 mM)	1.83
IP+db cyclic AMP	1.83

T/M ratio means the thyroid/medium concentration ratio for radioiodide calculated as counts per gram of tissue over counts per m*l* of medium. Results are representative of three independent experiments with high reproducibility and averages of duplicate determinations. IP=ionophore A-23187

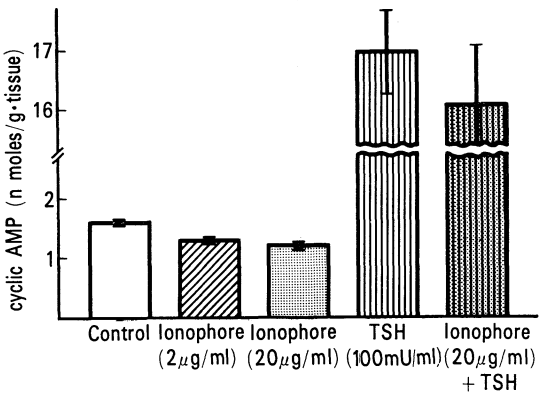

Figure 2. Effects of ionophore A-23187 and TSH on cyclic AMP levels in dog thyroid slices Cyclic AMP was assayed following 20 minutes incubation in the presence of 10 mM theophylline and with or without reagents. Results are representative of three independent experiments and averages±S.E.M. of triplicate determinations.

Discussion

The present data indicate that the calcium ionophore by itself enhanced the ^{14}C-1-glucose oxidation as reported by Van Sande *et al.* (1975) and furthermore it was demonstrated that the ionophore augmented the effect of dbcAMP and TSH on this metabolic parameter. Since the ionophore is expected to induce an increase in the net influx of Ca^{++} from the medium into the cell interior along the concentration gradient (Prince *et al.*, 1973), the avove results suggest, in the first place, that the rise in the intracellular Ca^{++} leads, even in the absence of increase in cyclic AMP production, to the stimulation of ^{14}C-1-glucose oxidation and secondly, that Ca^{++} potentiates the effect of cyclic AMP on this metabolic response. In view of the model that the action of many polypeptide hormones on the cell membrane of target cells not only induces stimulation of the membrane bound adenylate cyclase, but also causes an increase in the influx of Ca^{++} (Rasmussen, 1974), the deduction that cooperation of Ca^{++} and cyclic AMP produces the maximal effect on ^{14}C-1-glucose oxidation appears to have a considerable significance in interpretation of the mode of hormon action. If one assumes that TSH causes an increase in the Ca^{++} influx as well, then the fact that the stimulation was more pronounced by TSH than by dbcAMP and the fact that potentiation by the calcium ionophore was less marked in the TSH effect than in the dbcAMP effect are explained quite satisfactorily,

The T/M ratio of radioiodine concentra-

tion was suppressed in the slices by either TSH, dbcAMP (Ahn and Rosenberg, 1970 and Table 1) or the calcium ionophore (Table 1). In this aspect, however, the effect of either TSH or dbcAMP was not potentiated by the calcium ionophore in the conditions employed in the present study. Although it may be premature to draw any definite conclusions from this experiment, one possibility is that the mode of development of the hormone effect is different in the iodide transport from in the ^{14}C-1-glucose oxidation. Van Sande *et al.* (1975) reported that calcium in the presence of ionophore caused an increased incorporation of iodide into protein in thyroid slices. Our data and their results suggest that calcium ion as well as cyclic AMP plays an important role in the TSH effects on iodine metabolism in the thyroid.

The present data indicate that the calcium ionophore caused a slight but significant decline in tissue cyclic AMP levels. Similar observations were described in fly salivary glands (Prince *et al.*, 1973). In contrast, calcium in the presence of ionophore markedly enhanced cyclic GMP levels (Van Sande *et al.*, 1975; Yamashita *et al.*, 1975).

Thus, all of the above results and considerations are in line with the concept that intracellular Ca^{++} along with cyclic nucleotides plays a very critical role in the exertion of TSH effect on various metabolic processes in the thyroid.

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