CONTROL AND ROLE OF CYCLIC 3',5'-GUANOSINE MONOPHOSPHATE IN THE THYROID

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SUMMARY

Carbamylcholine, NaF, KCl and calcium in the presence of ionophore A23187 enhanced cyclic GMP accumulation and activated the oxidation of glucose carbon 1 and the binding of iodide to proteins in thyroid slices. These agents decreased cAMP accumulation and secretion in TSH-stimulated slices. None of these treatments increased cyclic GMP accumulation in calcium depleted slices and media. The various effects of carbamylcholine were also abolished in the absence of calcium. The data are consistent with the hypothesis of Schultz et al. that intracellular calcium modulates cyclic GMP levels. They also show that in a undirectional system (submitted only to positive signals) the regulators cyclic AMP and Ca^{++} and/or cyclic GMP may have both similar and opposite effects.

In dog thyroid slices the level of cyclic GMP is increased in the presence of acetylcholine and fluoride, but is not modified by TSH (1,2). A guanylate cyclase activity has been demonstrated in rat thyroid (3). Cyclic GMP at high concentrations activates aminoacid incorporation into proteins in bovine thyroid acellular systems (4). In vivo, cyclic GMP enhances protein synthesis and growth in rat thyroid (5). Apart from this scanty information, little is known about

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TSH : thyroid stimulating hormone.
$BE^{131}I$: butanol extractable ^{131}I iodine.
$NBE^{131}I$: non butanol extractable ^{131}I iodine.
KRB : Krebs Ringer bicarbonate buffer.
KRB KCl : KRB in which all Na^+ has been replaced by K^+.
αCa^{++} : KRB with EGTA 2mM but without Ca^{++}.

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the regulation of the cyclic GMP level and the role of cyclic GMP in this tissue. Recent experiments have suggested that in rat ductus deferens cyclic GMP levels could be regulated by cytosol calcium levels (6). With regard to the role of cyclic GMP, the prevalent hypothesis is that in tissues responding to positive and negative signals (bidirectional control) cyclic AMP and cyclic GMP may have opposite effects, while in tissues submitted to positive signals (unidirectional control) cyclic AMP and cyclic GMP would act similarly (7). The purpose of this work was to evaluate the validity of such a general hypothesis in a well differentiated and studied tissue (8).

MATERIALS AND METHODS

Slices from thyroids of dog pretreated with thyroid extract (100 mg/day for 3 days, Thyranon, Organon, Oss, Nederland), were prepared and incubated at 37°C under an atmosphere of O_2-CO_2 (95:5, V/V) in 2ml of KRB enriched with 8mM glucose and 0.5g/l bovine serum albumin. For cyclic AMP assay, caffeine 1mM was added 10 min before the end of the incubation (9,10). The cyclic GMP was purified and assayed according to F. Murad (11) with slight modifications to improve the sensitivity of the assay. Ten µg of bovine serum albumin was added per assay tube (12) and the incubation of cold and ^3H cyclic GMP with lobster kinase preparation was stopped by $(NH_4)_2SO_4$ precipitation (13). The filtration was performed on Millipore filters (Cat. N° AAWP 02500 0.80µ). The cyclic AMP as well as cyclic GMP assay was routinely performed in a total volume of 50µl with 1pMole of cyclic ^3H AMP and 0.5pMole of cyclic ^3H GMP. The limit of detection was 0.05pMole of cyclic GMP. The incubation medium was supplemented with [1-^{14}C] glucose 0.5µC/ml for the measurement of glucose carbon 1 oxidation (14) and with ^{131}I iodide 40µM (specific activity 1.25C/Mole) for the measurement of ^{131}I iodide binding to proteins (15). For secretion dogs (± 15Kg) were administered 150µC carrier free ^{131}I 3 days before the experiment (16). The medium was supplemented with methimazole 2mM and $NaClO_4$ 1mM (16). After a preincubation of 1 hour, the incubation in the presence of the stimulating agents was carried out for 20 min for cyclic GMP measurement, 1 hour for cyclic AMP, glucose oxidation and iodide binding to proteins determination, and 2 hours in secretion experiments.

When the effect of ionophore A23187 was studied, the experimental protocol generally involved a first preincubation of 1 hour with ionophore A23187 10^{-6}M or 10^{-5}M and EGTA 2mM in a buffer containing

no Ca^{++} in order to deplete the cells of calcium, and three washings in calcium free buffer. The slices were then incubated for 30 min with various calcium concentrations and ionophore A23187 to allow equilibration. Finally, they were incubated in an identical fresh medium for the metabolic determination. In the evaluation of KCl action, a KRB was prepared in which all Na^+ had been replaced by K^+ (i.e., 155meq/l). For each experiment, the activity of TSH and the reactivity of the tissue was checked by measuring the activation of iodide binding to proteins in control slices. Results are always expressed as means ± range of at least 2 duplicates in one typical experiment.

Ionophore A23187 was a gift from the Eli Lilly Company (Indianapolis, USA). Bovine TSH (Thytropar) was purchased from Armour (Kankakee, USA), Carbachol from K and K (Plain View, New York, USA), cyclic AMP and cyclic GMP from Boehringer Pharma (Mannheim, Germany), cyclic ^3H AMP and cyclic ^3H GMP from the Radioachemical Centre (Amersham, United Kingdom).

RESULTS AND DISCUSSION

As reported previously (1), cyclic GMP level in control dog thyroid slices varied from 10 to 40pMoles/g wet tissue, and this level was greatly enhanced by NaF 10mM and carbamylcholine 10^{-6} to 10^{-4}M. The

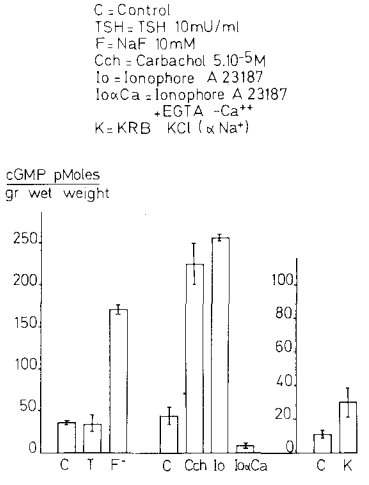


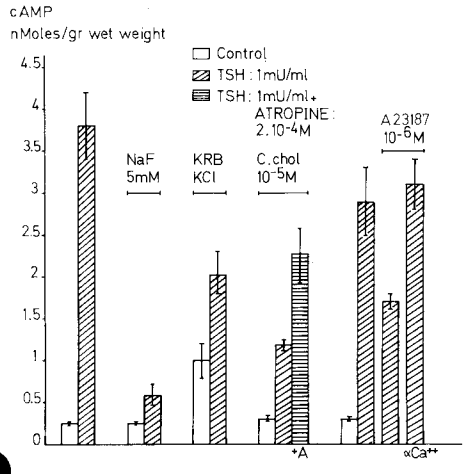
FIGURE 1.

Effect of TSH, NaF, carbamylcholine, Ca^{++} and KCl on cyclic GMP accumulation in dog thyroid slices.

effect of carbamycholine was potentiated by caffeine 1mM and inhibited by atropine 0.2mM. TSH 10mU/ml, i.e., at a concentration which maximally activates all metabolisms in this system (8), did not markedly modify cyclic GMP levels (Fig. 1). KCl which enhances several aspects of thyroid metabolism (17) markedly increased cyclic GMP accumulation. In the presence of ionophore A23187, which permeabilizes membranes to divalent cations such as Ca^{++} and Mg^{++} (18), extracellular calcium 1.45mM also markedly enhanced cyclic GMP levels. In such experiments, the level of intracytoplasmic calcium is not necessarily equal to medium level, as active transport of Ca^{++} out of the cell or into the mitochondria may persist.

As shown in Fig. 2, all the agents which enhanced cyclic GMP accumulation markedly inhibited the enhancement of cyclic AMP accumulation in TSH treated slices. Carbamylcholine, sodium fluoride and calcium in the presence of ionophore A23187 did not markedly modify cyclic AMP levels in control slices while KCl increased this level. These effects of carbamylcholine (19) and fluoride (20,21) have been previously described.

Agents which enhance cyclic GMP accumulation could therefore be



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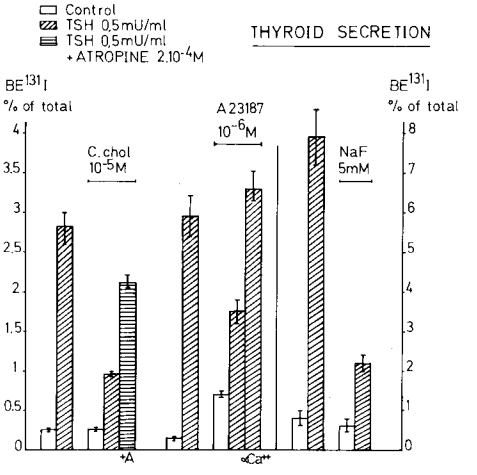

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FIGURE 2.

Effect of TSH, NaF, KCl, carbamylcholine and Ca^{++} on cyclic AMP accumulation in dog thyroid slices. A = atropine 0.2mM.

FIGURE 3.

Effect of TSH, carbamylcholine, Ca^{++}, NaF on dog thyroid secretion. A = atropine 0.2mM.

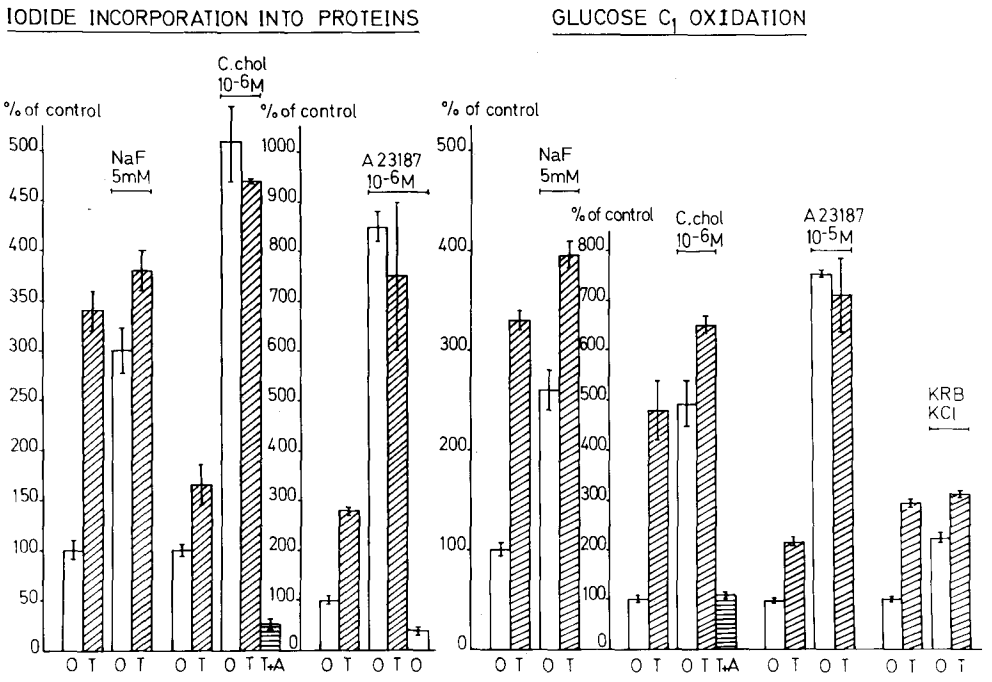

FIGURE 4.

Effect of NaF, carbamylcholine, Ca^{++} and KCl on iodide incorporation into proteins and glucose carbon 1 oxidation.
T = TSH 1mU/ml
T + A = TSH 1mU/ml + atropine 0.2mM

expected to inhibit cyclic AMP mediated TSH effects. Carbamylcholine, NaF, calcium in the presence of ionophore A23187 and KCl all inhibited the stimulation by TSH of thyroid secretion (Fig. 3). The action of carbamylcholine was partly relieved by atropine. Carbamylcholine, NaF and KCl barely affected the very low basal secretion of control slices. In non-stimulated slices preincubated with EGTA and without Ca^{++}, a higher $BE^{131}I$ and $NBE^{131}I$ release was observed whatever the incubation medium, which suggests follicular disruption and non physiological thyroglobulin proteolysis (22) induced by calcium depletion.

All the agents which enhance cyclic GMP accumulation in dog thyroid slices also enhanced glucose carbon 1 oxidation by these slices (Fig. 4). This effect had already been demonstrated for NaF (23) and KCl (17). The effect of carbamylcholine was inhibited by atropine. Carbamylcholine, NaF and calcium in the presence of ionophore also activated the binding of iodide to proteins (Fig. 4). The effect of carbamylcholine was inhibited by atropine. The activation bears on the binding of iodide itself as under the conditions of these experi-

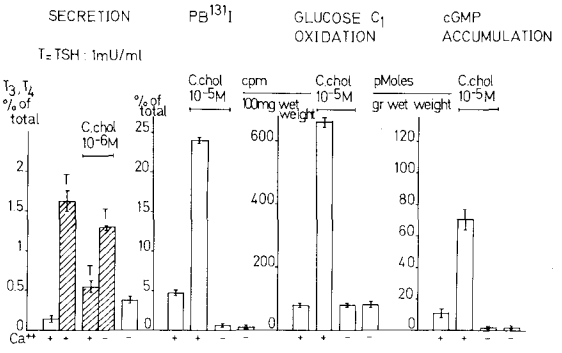

FIGURE 5.

Effects of carbamylcholine on secretion. ^{131}I iodide incorporation into proteins, glucose carbon 1 oxidation and cyclic GMP accumulation in slices incubated with or without Ca^{++} (1.45mM) in the medium.

ments this step is limiting (15). Moreover, the 3 agents decreased to some extent the iodide uptake. KCl which markedly inhibits iodide uptake (24) has not been studied in this regard.

KCl and carbamylcholine, which enhance cyclic GMP accumulation in dog thyroid slices have been shown in other tissues to affect the penetration or the distribution of calcium (25). Moreover, calcium, when allowed to penetrate the thyroid cell by ionophore A23187, has the same effect as these agents. In view of the hypothesis (6) that intracellular calcium may regulate cyclic GMP levels, it was of interest to investigate whether carbamylcholine and the other agents would exert their effects in calcium depleted slices and incubation media. Fig. 5 demonstrates that in such slices cyclic GMP levels were at the limit of detection and were not enhanced by carbamylcholine, KCl or NaF. Moreover, carbamylcholine did not inhibit TSH induced secretion or enhance glucose carbon 1 oxidation or iodide binding to proteins. This therefore suggests that the enhancement of cyclic GMP levels and the concomitant metabolic effects may be secondary to increased intracellular calcium levels originating from the extracellular medium.

The fact that calcium in the presence of ionophore A23187 greatly enhanced cyclic GMP accumulation and that agents which also enhanced cyclic GMP levels (KCl, carbamylcholine, NaF) have no effect in calcium depleted thyroid slices thus support the hypothesis of Schultz et al. (6) that intracellular calcium levels may regulate cyclic GMP accumulation. In the presence of ionophore, calcium also elicits other metabolic effects : an inhibition of cyclic AMP accumulation

and secretion in TSH stimulated thyroid slices and an activation of glucose carbon 1 oxidation and of the binding of iodide to proteins in control slices. The agents which enhanced cyclic GMP accumulation had similar effects on the slices. These data are therefore consistent with the hypothesis that intracellular calcium modulates cyclic GMP level which itself elicits various metabolic effects. However, it should be pointed out that there is no argument to link increased cyclic GMP levels to the other effects. Therefore a scheme according to which calcium would independently induce all carbamylcholine effects is equally plausible. Moreover, in such schemes, the causal links may not be direct and some prostaglandins may play a role at any step of the causal sequences (19,26).

It has been proposed that in systems which only respond to one kind of signal (unidirectional) cyclic AMP and cyclic GMP would act in parallel whereas in systems responding to opposite signals (bidirectional) the nucleotides would have opposite effects (7). The present data show, that contrary to this hypothesis, in a typically unidirectional system, the thyroid, cyclic AMP on the one hand, and cyclic GMP and/or Ca^{++} on the other, hand may have both similar and opposite effects.

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