DISSOCIATION OF CYCLIC 3',5'-GUANOSINE MONOPHOSPHATE. ACCUMULATION AND SECRETORY INHIBITION IN THE ACTION OF CARBAMYLCHOLINE ON THYROID

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1. Introduction

A calcium cyclic GMP regulatory system has been demonstrated in thyroid [1]. Acetylcholine through calcium activates cyclic GMP accumulation [1,2]. It also depresses the TSH induced cyclic AMP accumulation and thyroid secretion [1]. NaF, KCl and Ca^{2+} in the presence of ionophore A23187 have similar effects [1,2].

As in other systems [3,4] the role of cyclic GMP in the regulation of thyroid metabolism remains unclear. Specifically, cyclic GMP accumulation could be the necessary link in acetylcholine action or a side effect of the rise in cytosol Ca^{2+}. The purpose of the present communication is to show that cyclic GMP accumulation can be dissociated from the inhibition of secretion by acetylcholine which suggests that this nucleotide is not the mediator of this effect.

2. Materials and methods

Slices from dog thyroids pretreated with thyroid extract (100 mg/day for 1 day, Thyranon, Organon, Oss, The Netherlands) were prepared and incubated at 37°C under an atmosphere of $O_2 - CO_2$ (95:5, v/v) in Krebs-Ringer bicarbonate buffer enriched with 8 mM glucose and 0.5 g/l bovine serum albumin. For cyclic GMP assay, caffeine 1 mM was added 10 min before the end of the incubation [5,6]. After boiling, homogeneization and centrifugation, the supernatants were placed on 0.5 × 5 cm AG 1 × 8 column (formate form) to separate cyclic AMP and cyclic GMP. The cyclic GMP was assayed according to Murad [7] with slight modifications to increase the sensitivity of the assay [1]. For secretion, dogs (± 15 kg) were administered 150 μCi carrier–free ^{131}I 3 days before the experiment [8]. The medium was supplemented with methimazole 2 mM and $NaClO_4$ 1 mM [8]. After a preincubation of 1 h, the slices were incubated in the presence of the tested agents for 20 min for cyclic GMP measurements and 4 h in secretion experiments. Results are always expressed as means ± range of at least duplicates in one typical experiment. Each type of experiment was at least performed on three different animals.

Bovine TSH (thyrotropar) was purchased from Armour (Kankakee, USA), Carbachol from K and K (Plain View, New York, USA), $MnCl_2$ from Merck (Darmstadt, Germany). Cyclic GMP from Boehringer Pharma (Mannheim, Germany), cyclic [^{3}H]GMP from the Radiochemical Centre (Amersham, United Kingdom) and AG 1 × 8 from Bio-Rad.

3. Results and discussion

As reported previously [2], cyclic GMP level in control dog thyroid slices varied from 10 to 40 pmol/g wet tissue and this level was greatly enhanced by NaF 10 mM, carbamylcholine 10^{-6} to 10^{-4} M, KCl and Ca^{2+} in the presence of Ionophore A23187 [1]. Carbamylcholine, NaF, Ca^{2+} in the presence of

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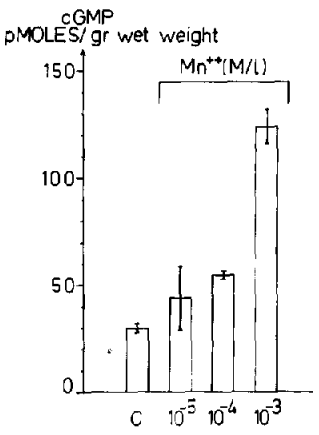

Fig.1. Effect of Mn^{2+} on cyclic GMP accumulation C, Control.

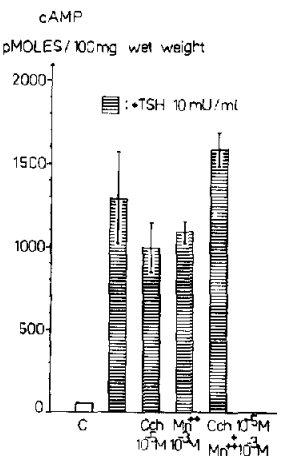

Fig.3. Effect of TSH, carbamylcholine (Cch) and Mn^{2+} on cyclic AMP accumulation. C, Control.

Ionophore A23187 all inhibited the stimulation of thyroid secretion in TSH treated slices.

All results shown were obtained in slices incubated in a medium without added Ca^{2+} (i.e. about 10^{-5} M). Mn^{2+} has been shown to be an activator of the guanylate cyclase [9,10] and is a competitor ion of Ca^{2+} in many systems. Mn^{2+}, 10^{-5} to 10^{-3} M enhanced cyclic GMP accumulation (fig.1) and increased the effect of carbamylcholine on cyclic GMP accumulation (fig.2A).

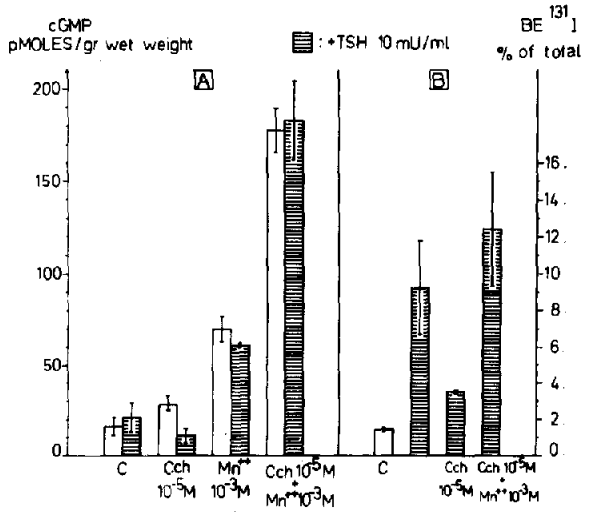

Fig.2. (A) Effect of TSH, carbamylcholine (Cch) and Mn^{2+} on cyclic GMP accumulation (B) Effect of TSH, carbamylcholine (Cch) and Mn^{2+} on thyroid secretion. C, Control.

TSH 10 mU/ml, at a concentration which maximally activates all metabolism in this system [11], inhibited the enhancement of cyclic GMP accumulation in the carbamylcholine treated slices, but did not modify the cyclic GMP levels obtained with Mn^{2+} (fig.2A). Under such conditions, carbamylcholine and Mn^{2+} did not modify the cyclic AMP accumulation in TSH treated slices (fig.3). In slices incubated in the same conditions, TSH 10 mU/ml stimulated thyroid secretion, carbamylcholine inhibited this stimulation and Mn^{2+} relieved the inhibition by carbamylcholine (fig.2B). These results show a clear cut dissociation between accumulation of cyclic GMP and inhibition of TSH induced secretion in thyroid slices. In slices incubated with carbamylcholine and TSH, thyroid secretion was inhibited although after 5 to 180 min there was no change in cyclic GMP levels (fig.4A). The small increase in cyclic GMP observed at 5 min cannot be considered as responsible for the continuous secretory inhibition that was observed (fig.4C). This suggests that a cyclic GMP rise is not necessary for the inhibition by acetylcholine of thyroid secretion. In slices incubated with TSH, carbamylcholine and Mn^{2+} cyclic GMP concentrations were maximal from 5 to 120 min (fig.4B) while at the same time carbamylcholine inhibition of TSH stimulated thyroid secretion was completely relieved (fig.4C). This suggests that a cyclic GMP rise is not sufficient to inhibit thyroid secretion.

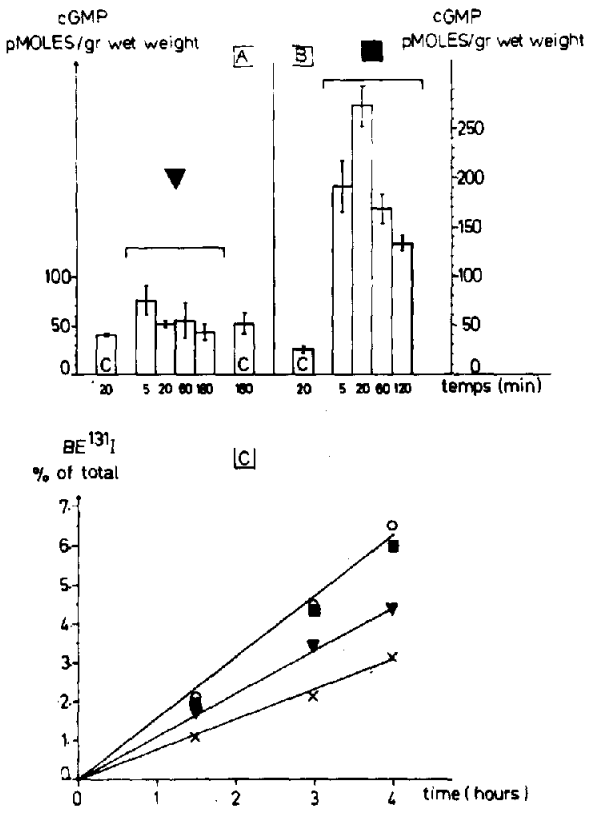

Fig.4. (A – B) Kinetics of accumulation of cyclic GMP in presence of TSH and carbamylcholine (Cch) from 5 to 180 min (A, ▼), or in presence of TSH, carbamylcholine (Cch) and Mn^{2+} from 5 to 120 min (B, ■). (C) Kinetics of thyroid secretion from 1 to 4 h. X or C, Control. (o–o) + TSH 10 mU/ml. (▼–▼) + TSH 10 mU/ml + carbamylcholine 10^{-5} M. (■–■) + TSH 10 mU/ml + carbamylcholine 10^{-5} M + Mn^{2+} 10^{-3} M.

Our hypothesis is that increased intracytoplasmic ionized Ca^{2+} content resulting from the carbamylcholine action could be sufficient to block thyroid secretion, while cyclic GMP, at least in the presence of Mn^{2+}, would lack any effect on TSH-induced hormone secretion.

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