CHOLINERGIC CONTROL BY ENDOGENOUS PROSTAGLANDINS OF cAMP ACCUMULATION UNDER TSH STIMULATION IN THE THYROID

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Received 10 July 1974

1. Introduction

Recently, Goldberg et al. [1] have studied the relation of cyclic GMP with regulatory processes in various systems. According to the concept of Yin-Yang, they hypothesized that the intracellular concentration of cyclic AMP and of cyclic GMP are correlated and that these concentrations vary inversely in numerous regulatory events. This hypothesis implies that the system under study is modulated by two antagonistic effectors.

Many of the biological effects of thyroid stimulating hormone (TSH) on the thyroid appear to be mediated by an increase of the synthesis of cyclic AMP (cAMP)[2].

On the contrary cyclic AMP is not implicated in the ^{32}P incorporation into phospholipids evoked either by TSH [3,4] or by acetylcholine. This latter result is supported by the fact that acetylcholine does not increase cyclic AMP concentrations in thyroid slices [5]. Moreover, cyclic GMP levels are significantly increased by acetylcholine [6].The present studies demonstrate that acetylcholine antagonizes the increase of cyclic AMP promoted by TSH and that prostaglandins Fα are implicated in this process.

2. Materials and methods

Pig thyroid slices (about 100 mg) were preincubated for 30 min at 37°C in 2 ml of Krebs–Ringer phosphate buffer (KRP) with glucose (1 mg/ml) in presence or absence of dibutyryl cyclic GMP (5×10^{-5} or 10^{-4} M) or indomethacin (10 μg/ml).

Then the slices were transferred into 2 ml of the same buffer containing theophyllin 10^{-2} M) and, according to the assays, various effectors: TSH (20 mU/ml), acetylcholine chloride (5.5×10^{-5} M) and eserine sulfate (3×10^{-4} M), atropine sulfate (10^{-5} M), indomethacin (10 μg/ml), $PGF_2\alpha$ (3×10^{-5} or 6×10^{-5} M), DBcGMP (5×10^{-5} or 10^{-4} M). After incubation (15 min), the slices were homogenized in 2 ml of trichloracetic acid (TCA) 5% and the cAMP content was assayed according to the method of Gilman [7] as previously described [8]. All determinations were performed in triplicate. The comparisons between assays and standards were assessed by the t-test of Student [9].

One single gland was used for all assays of each experiment. The experiments were repeated several times with identical, significant results.

N^2 -2′-*O*-Dibutyryl-3′,5′-cyclic GMP (DBc-GMP) was purchased from Boehringer (Mannheim GmbH lot No. 70 14312), Eserine sulfate from Sigma, atropine sulfate from Merck, acetylcholine chloride from Lematte et Boinot laboratories, Paris. Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) was a gift from Dr Pike (Upjohn Co.). Thyreostimulin (TSH) was a gift from Endocrinology Study Section (NIH, Bethesda). Indomethacin was a gift from Merck, Sharp and Dohme Laboratories.

3. Results

Fig. 1 shows that acetylcholine decreases cyclic AMP accumulation induced by TSH in pig thyroid slices. The control levels of endogenous cyclic AMP were not altered by acetylcholine. The inhibitory effect of acetylcholine on TSH-induced cyclic AMP accumulation is reversed

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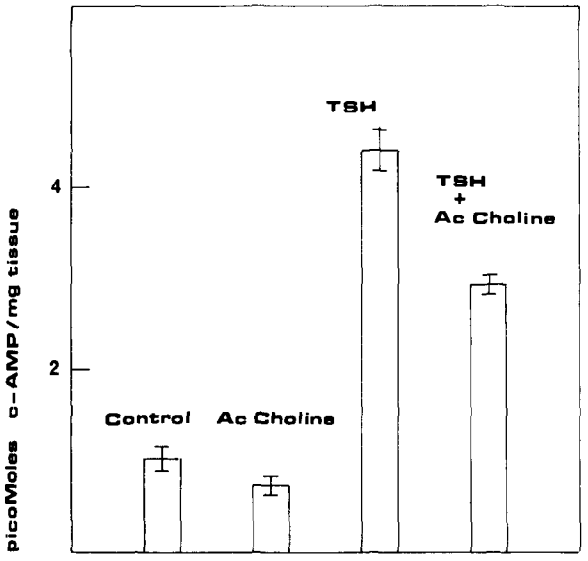

Fig. 1. Action of acetylcholine on the stimulation of cAMP production by TSH. TSH = 10 mU/ml. Ac. Choline: acetylcholine 5.5 X 10^{-5} M + eserine 3 X 10^{-4} M TSH + Ac. Choline $P < 0.01$ vs. TSH TSH $P < 0.001$ vs. control. Ac. Choline N.S. vs. control.

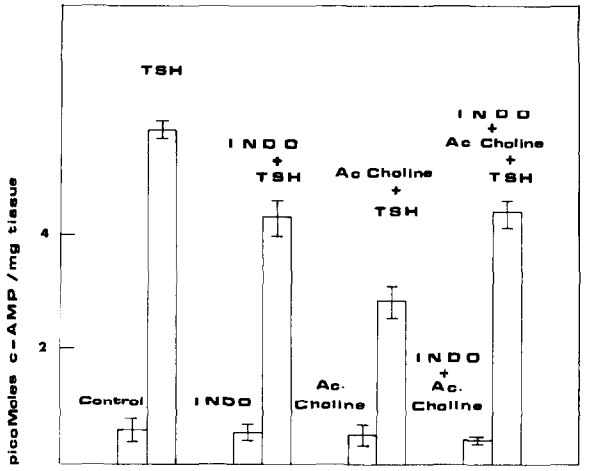

Fig. 3. Inhibition by indomethacin of the action of acetylcholine on the accumulation of cAMP. TSH = 20 mU/ml Ac. Choline: acetylcholine 5.5 X 10^{-5} M + eserine 3 X 10^{-4} M Indomethacin 10 μg/ml (during the preincubation and incubation) TSH $p < 0.001$ vs. control INDO N.S. vs. control Ac. Choline N.S. vs. control INDO + Ac. Choline N.S. vs. control INDO + TSH $p < 0.05$ vs. TSH Ac. Choline + TSH $p < 0.01$ vs. TSH INDO + TSH + Ac. Choline $p < 0.05$ vs. TSH INDO + Ac. Choline + TSH N.S. vs. INDO + TSH.

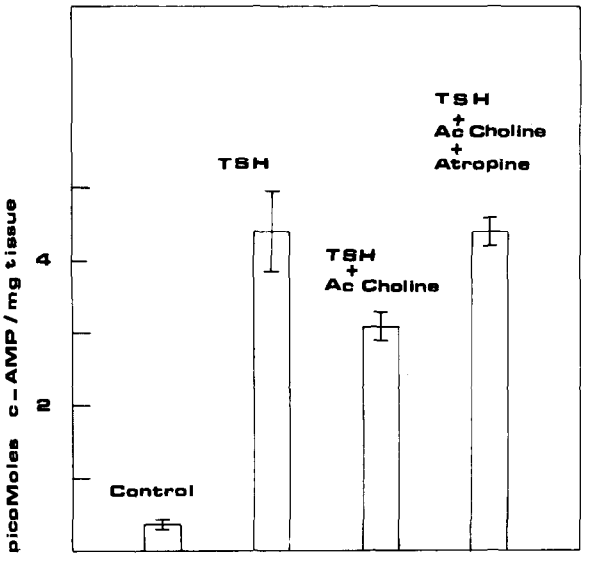

Fig. 2. Inhibition by atropine of the decrease of the accumulation of cAMP induced by acetylcholine. TSH = 20 mU/ml Ac. Choline: acetylcholine 5.5 X 10^{-5} M + eserine 3 X 10^{-4} M atropine 10^{-5} M TSH + Ac. Choline $p < 0.05$ vs. TSH TSH $p < 0.001$ vs. control TSH + Ac. Choline + atropine N.S. vs. TSH.

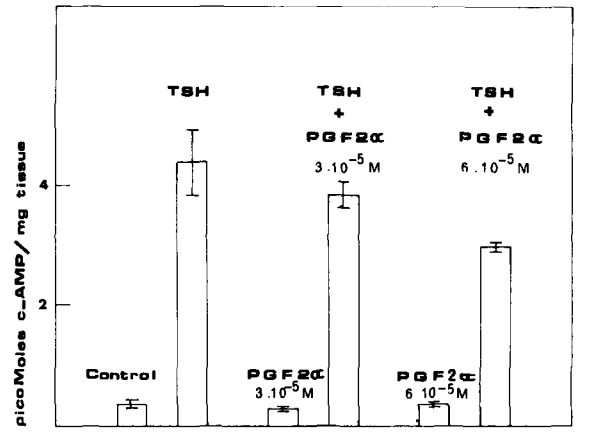

Fig. 4. Action of $PGF_2\alpha$ on the accumulation of cAMP stimulated by TSH. TSH = 20 mU/ml $PGF_2\alpha$ 3 X 10^{-5} M or 6 X 10^{-5} M TSH $p < 0.001$ vs. control TSH + $PFG_2\alpha$ 3 X 10^{-5} M N.S. vs. TSH TSH + $PGF_2\alpha$ 6 X 10^{-5} M $p < 0.01$ vs. TSH.

by atropine, indicating the specificity of the acetylcholine action (fig. 2).

Fig. 3 shows that this effect of acetylcholine is completely inhibited by the tissue preincubation in the presence of indomethacin. This inhibitor of prostaglandin synthetase [9], decreases by about 25% the cyclic AMP accumulation induced by TSH. The same value is obtained in the assay containing TSH + acetylcholine + indomethacin. $PGF_2\alpha$, without effect on basal cyclic AMP levels in the thyroïd, decreases in a dose dependent manner the accumulation of the cyclic nucleotide promoted by TSH (fig. 4).

At the two levels tested (5×10^{-5} M and 10^{-4} M) dibutyryl cyclic GMP (DBc-GMP) is without effect either on the basal or on the TSH stimulated accumulation of cyclic AMP.

4. Discussion

The effect of acetycholine which we describe here, is similar to those studied in the heart [11, 12], in the intestinal smooth muscle [12], in the brain [12], in estrogen treated rat uterus [13], in myocardial tissue stimulated by isoproterenol or glucagon [14] or in rat lung slices [15]. The control levels of endogenous cyclic AMP were not altered by acetylcholine, but the TSH induced rise in cyclic AMP was decreased. The suppression of this effect by indomethacin, an inhibitor of prostaglandin synthetase, shows the implication of endogenous prostaglandins in the inhibitory role of acetylcholine. We have shown previously [8] that the TSH stimulation of the thyroïd adenyl cyclase is amplified by PGE_2 synthesized from phospholipidic arachidonate. Two hypotheses can be made about the mode of action of the prostaglandins mediating the acetylcholine action: a dose related biphasic effect of PGE as suggested by Burke's results [16] or an antagonistic effect of PGFα, which has been claimed to be associated with the cyclic GMP generation [17].

Results reported in fig. 4 support the second explanation. PGFα, devoid of action on the basal level of cAMP, as reported by several authors [18–20], mimics the action of acetylcholine. We believe that the role of acetylcholine would be to modify the balance between endogenous PGE_2 and $PGF_2\alpha$, thus increasing the concentration of the latter.

We do not know how to interpret the failure of dibutyryl cyclic GMP in reproducing the acetylcholine effect. Perhaps this is due to the fact that the cyclic GMP derivative does not easily enter the cells. An alternative explanation would be that the enhancement of cyclic GMP, concentration, consecutive to acetylcholine action [6], is not directly related to a depression of cyclic AMP. Therefore cyclic GMP would be an antagonist of the cyclic AMP action and would not decrease its concentration.

Nevertheless the preliminary results reported here strongly support Goldberg's concept of the antagonism between cyclic AMP and cyclic GMP, but various parameters have not yet been tested, for example: the ability of TSH to suppress cholinergically stimulated cyclic GMP levels.

Experiments are in progress to establish the relationship between cyclic GMP and prostaglandins under acetylcholine stimulation.

Acknowledgements

This work was supported by a grant from CNRS (ATP No. 429.912 and ERA 401).

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