THE ROLE OF CYCLIC GMP IN THE ACTION OF CARBAMYLCHOLINE ON THE CYCLIC AMP LEVEL IN THE DOG THYROID

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1. Introduction

Acetylcholine decreases the level of cyclic AMP in thyroid slices stimulated by thyreostimulin. This effect described by us in pig tissue [1] has also been studied in dog and horse glands by Dumont and his collaborators [2,3]. Their results show that the cholinergic effect is due to the stimulation of a phosphodiesterase activity which hydrolyses cyclic AMP [3]. As carbamylcholine stimulates the cyclic GMP accumulation in thyroid tissue [4], a link between the two observations has been suggested. This hypothesis is supported by the fact that low concentrations of cyclic GMP stimulate the hydrolysis of cyclic AMP by a crude cell free preparation of horse thyroid phosphodiesterase [5].

The results reported here show that prostaglandin $F_2\alpha$, which produces an elevation of cyclic GMP concentration in dog thyroid slices, does not reproduce the carbamylcholine effect neither on the cyclic AMP level, nor on its in situ hydrolysis rate.

2. Materials and methods

Dog thyroid slices (about 100 mg) were routinely preincubated for 30 min at 37°C in 2 ml of Krebs-Ringer phosphate buffer (KRP) with glucose (1 mg/ml). Then the slices were transferred into 2 ml of the same buffer containing theophyllin (10^{-3} M) and various effectors according to the assays: TSH (15 mU/ml), carbamylcholine (10^{-5} M), $PGF_2\alpha$ (10 μg/ml). After incubation, the slices were transferred into 1.5 ml perchloric acid (1 M) and homogenized. The cyclic nucleotides content was assayed by the radioimmunological method of Cailla et al. [6,7], except that bound and free ligand were separated by filtration on Millipore filters (HAWP 0.45 μm). To study the time course of cAMP disposal, we used incubation conditions similar to those described by Van Sande et al. [8]. Preincubated slices were incubated in the presence of TSH 25 mU/ml and theophyllin 10^{-3} M. After 15 min, trypsin was added (0.01% final concentration) and incubation was followed for 4 min. Then the slices were washed twice for 2 min in a medium containing a trypsin inhibitor. Finally they were incubated in fresh medium containing carbamylcholine or $PGF_2\alpha$. At the end of the incubation, slices were dropped into perchloric acid and cyclic nucleotides assayed as described.

The results are the means of duplicate determinations of separate experiments expressed with standard errors.

3. Results

In slices previously stimulated by TSH and theophyllin, we have studied the effect of carbamylcholine and $PGF_2\alpha$ on cGMP accumulation and cAMP disposal.

Figure 1 compares the time course of cyclic GMP level variations induced by carbamylcholine (a) and $PGF_2\alpha$ (b). The same maximal level is attained at 2 min with the two stimulators, though a certain lag time is observed with $PGF_2\alpha$. The elevation is transient in both experiments, and the level returns to

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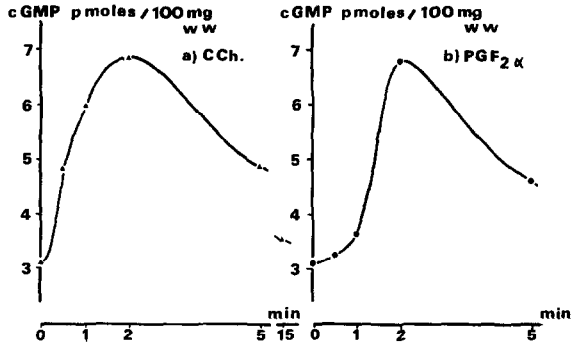

Fig.1. Time course of cGMP variations in dog thyroid slices in which the cAMP level was previously elevated by TSH (25 mU/ml). (a), in the presence of carbamylcholine (10^{-5} M); (b), in the presence of $PGF_2\alpha$ (10 μg/ml M).

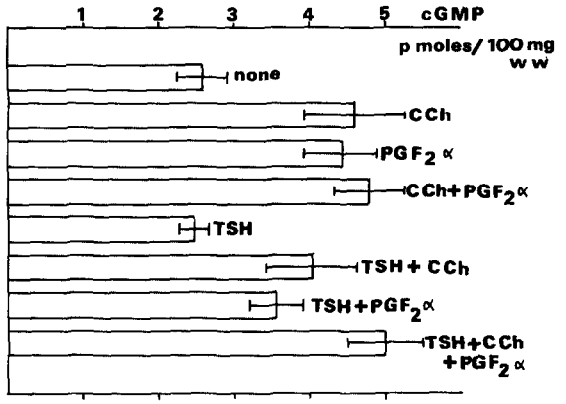

Fig.3. Action of carbamylcholine (10^{-5} M) and/or $PGF_2\alpha$ (10 μg/ml) on the cGMP accumulation in dog thyroid slices in the presence or absence of TSH (15 mU/ml). Theophyllin 10^{-3} M is present during the 15-min incubation.

basal after 15 min. In the absence of any stimulator, the basal level of cyclic GMP remains constant during the period of incubation (not shown).

Figure 2 shows the degradation rate of cyclic AMP which returns to basal level after removal of the TSH stimulation. As previously reported [3], carbamylcholine, increases the rate of hydrolysis, whereas $PGF_2\alpha$ is without effect.

Figures 3 and 4 depict the result of experiments performed with the addition of 10^{-3} M theophylline in order to decrease the degradation rate of cyclic nucleotides and to allow the comparison of the levels of cyclic AMP and cyclic GMP; these levels do not culminate at the same time when phosphodiesterase activities are not inhibited. After 15 min incubation with theophylline, we observe again the increased level of cyclic GMP in the presence of carbamylcholine or $PGF_2\alpha$. When present together, the two effectors do not produce additive effects. As previously

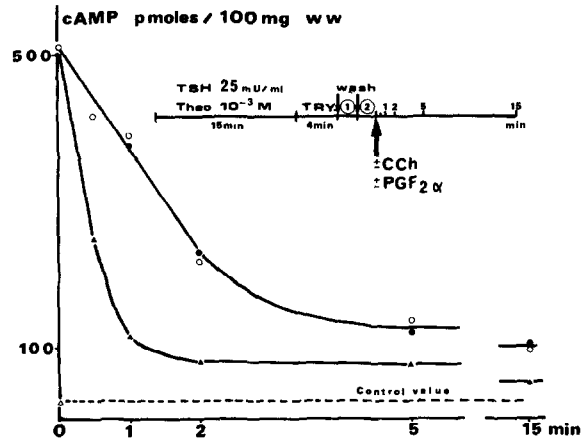

Fig.2. Time course of cAMP disposal in dog thyroid slices where its level was previously elevated by TSH (25 mU/ml). Effect of carbamylcholine (10^{-5} M) and $PGF_2\alpha$ (10 μg/ml). (o——o), none; (▲——▲), carbamylcholine; (●——●), $PGF_2\alpha$.

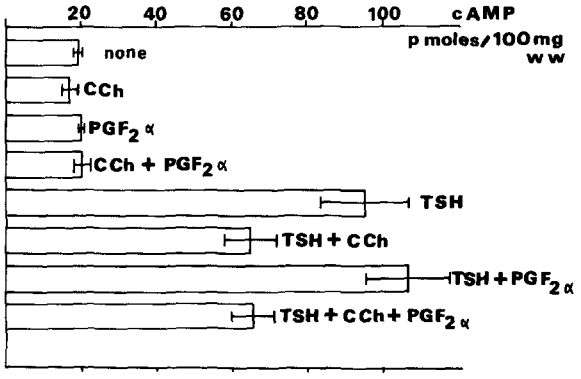

Fig.4. Action of carbamylcholine (10^{-5} M) and/or $PGF_2\alpha$ (10 μg/ml) on the stimulation by TSH (15 mU/ml) of the cAMP accumulation in dog thyroid slices. Theophyllin 10^{-3} M is present during the 15-min incubation.

reported [2,4], TSH is without effect on cyclic GMP levels. Slight decreases are observed when TSH is added to carbamylcholine and particularly to $PGF_2\alpha$. However TSH is devoided of action when the two stimulators are present together.

Cyclic AMP levels, which are not affected by carbamylcholine or $PGF_2\alpha$ alone or together, are stimulated five times by TSH. The TSH stimulation is partially antagonized (40%) by carbamylcholine and rather, enhanced by $PGF_2\alpha$. Moreover, $PGF_2\alpha$ does not modify the inhibitory effect of carbamylcholine.

4. Discussion

The decrease of the stimulated level of cyclic AMP, observed in dog thyroid tissue submitted to the simultaneous action of TSH and carbamylcholine, seems unequivocally consecutive to the increased degradation rate of the cyclic nucleotide [3]. It was tentatively proposed that cyclic GMP was the mediator of this cholinergic effect [5], though its elevation by Mn^{2+} is not accompanied by a decrease of the cyclic AMP level [9]. The same absolute rise of cyclic GMP content with carbamylcholine and $PGF_2\alpha$, joined to the absence of additivity, seems to be an indication of identical mode of action of the agonists on the same guanylate cyclase. Except for this similarity, other effects are completely different: $PGF_2\alpha$ has no effect at all on the cyclic AMP level increased by TSH, nor on its degradation rate.

Cyclic GMP alone is not directly responsible for the decrease of cyclic AMP level and free Ca^{2+} levels must be considered.

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