MECHANISM OF TSH ACTION. STUDIES WITH DIBUTYRYL CYCLIC AMP AND DIBUTYRYL CYCLIC GMP

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Received 19 January 1971

1. Introduction

It is currently thought that cyclic AMP in an intracellular mediator of many of the effects of TSH on the thyroid gland [1]. This hypothesis is primarily supported by the observations that TSH increases the levels of cyclic AMP in thyroid tissue [2, 3], cyclic AMP stimulates 1-^{14}C-glucose oxidation in beef thyroid homogenates [4], and dibutyryl cyclic AMP, a cyclic AMP analogue, mimics some of the effects of TSH in the thyroid gland [1, 5, 6]. In all these studies, however, the maximal response produced by dibutyryl cyclic AMP is in general less than that produced by TSH itself [7] and, in some species, qualitative differences between the effects of TSH and dibutyryl cyclic AMP also exist [1].

The failure of dibutyryl cyclic AMP to completely mimic TSH action, suggests that some of the TSH effects may be unrelated to the cyclic nucleotide. However, other nucleotides, such as cyclic GMP, have also been reported to be involved in the intracellular mediation of hormonal responses [8, 9]. In order to clarify this possibility, it was considered interesting to investigate what relationship, if any, exists between TSH, dibutyryl cyclic AMP and dibutyryl cyclic GMP by comparing the ability of the two latter nucleotides to produce some TSH-like effects in hog-thyroid slices.

2. Materials and methods

Dibutyryl cyclic AMP and dibutyryl cyclic GMP were kindly and generously supplied from Boehringer, Mannheim GmbH (Mannheim). Thyroid stimulating hormone (TSH) (2 U/mg) was a gift from the Endocrine Study Section, National Institutes of Health, Bethesda (USA). The sources of the other materials used have been previously described [10]. Glucose oxidation, phospholipid synthesis, iodide accumulation and colloid droplet formation were measured as previously described [11, 12]. RNA and protein synthesis were measured as described elsewhere [13]. In all studies, fresh thyroid slices were used. Thyroid glands, obtained from the local abbatoir, were sliced with a Stadie-Riggs microtome, washed in KRB buffer and incubated under the experimental conditions indicated in the tables.

3. Results and discussion

Since the thyroid response to TSH and dibutyryl cyclic AMP vary widely from animal to animal, the effects of TSH, dibutyryl cyclic AMP and dibutyryl cyclic GMP were tested on the same gland in each experiment.

Table 1 shows that dibutyryl cyclic AMP, like TSH, stimulates the incorporation of ^{32}Pi into total phospholipids, while dibutyryl cyclic GMP has only a slight effect. The effect of the cyclic nucleotides is not due to the presence of the butyrate group in the molecule since butyrate alone has no effect. When dibutyryl cyclic GMP was tested on 1-^{14}C-glucose oxidation, and ^{131}I uptake, it failed to stimulate these two metabolic activities in thyroid tissue at all concentrations used. On the other hand, TSH and dibutyryl cyclic AMP are both active in this respect. In contrast, TSH, dibutyryl cyclic AMP and dibutyryl

Table 1
Effects of TSH, dibutyryl cyclic AMP and dibutyryl cyclic GMP on phospholipid synthesis, glucose oxidation, iodide accumulation and ^{3}H-leucine incorporation into total proteins, in hog-thyroid slices.

Compound	Concn. (μM)	^{32}P incorporation into phospholipids (cpm/mg/hr)	$^{14}CO_2$ produced (cpm/mg/hr)	^{131}I uptake (cpm/g/min)	^{3}H-leucine incorporated into total proteins (cpm/mg/hr)
None	–	45±2	20±1	13,000	2,000
DBcAMP	100	50±1	28±1	15,000	3,700
DBcAMP	200	57±3	25±2	–	2,900
DBcAMP	500	63±5	24±2	18,000	2,700
DBcGMP	50	44±2	20±2	13,000	3,000
DBcGMP	100	46±3	21±1	14,000	4,600
DBcGMP	200	48±3	20±2	13,500	4,300
DBcGMP	500	49±2	22±2	13,000	2,700
Butyrate	200	45±1	20±2	13,100	–
TSH	100 mU/ml	113±7	39±3	18,000	3,140

For studies on phospholipid synthesis, each slice was incubated 2 hr in 1 ml of KRB buffer containing 1 mg of albumin, 1 mg of glucose and 0.5 μCi of ^{32}Pi. For studies on glucose oxidation each slice was incubated 1 hr in 1 ml of KRB buffer, containing 1 mg of albumin, 1 mg of glucose and 0.3 μCi of 1-^{14}C-glucose. In the iodide accumulation studies, the slices were individually incubated in 3 ml of KRB buffer containing 3 mg of albumin, 3 mg of glucose and, where indicated, TSH, dibutyryl cyclic AMP and dibutyryl cyclic GMP. All these compounds were added at the start of the incubation, whereas ^{131}I was added 20 min before the incubation was terminated. For the experiments on the ^{3}H-leucine incorporation into total proteins, the slices were individually incubated for 1 hr in 1 ml of Eagle's medium without stable leucine but in the presence of radioactive leucine (10 μCi). All experiments were repeated at least five times; typical results are reported, each value represents the average obtained from four slices ±S.E.

cyclic GMP are all active in stimulating the incorporation of ^{3}H-leucine into total thyroid proteins. Among these compounds, dibutyryl cyclic GMP, whose optimal activation occurs at a concentration of 100–200 μM is the most effective. Higher concentrations of this nucleotide abolish the stimulatory effect.

It has been previously reported that dibutyryl cyclic AMP, like TSH, is able to stimulate RNA synthesis [14]. We have now compared the effect of dibutyryl cyclic GMP to that of TSH and dibutyryl cyclic AMP on the time-course of incorporation of ^{32}Pi into RNA. As illustrated in table 2, it is evident that the stimula-

Table 2
Effects of TSH, dibutyryl cyclic and dibutyryl cyclic GMP on RNA synthesis and colloid droplet formation in hog-thyroid slices.

Compound	Conc. (μM)	^{32}Pi incorporation into RNA (cpm/mg tissue)		
		30 min	60 min	120 min
None	–	1,700	3,900	6,300
DBcAMP	500	1,770	4,500	10,000
DBcGMP	500	1,900	4,100	7,600
TSH	100 mU/ml	2,400	5,300	11,000

For experiments on RNA synthesis, each slice was incubated in 1 ml of KRB buffer, containing 1 mg of albumin , 1 mg of glucose and 0.5 μCi of ^{32}Pi.

tion by dibutyryl cyclic GMP is only slight, as compared to that of dibutyryl cyclic AMP, which is almost as effective as TSH. Furthermore, while TSH is active at an earlier time, both nucleotides need a longer incubation period. This delayed effect is probably due to their slower intracellular penetration. However, the effects of both dibutyryl cyclic AMP and dibutyryl cyclic GMP, as was observed in the case of ^{32}Pi incorporation into phospholipids (see table 1), show their maximum effect at a concentration of 500 μM. Below this concentration, the degree of stimulation is much lower, while at higher concentrations the incorporation is not influenced any further.

A more specific response to the in vitro addition of TSH appears to be the stimulation of the formation of intracellular colloid droplets [14]. Again, when tested for this effect, dibutyryl cyclic GMP shows a different behaviour as compared to dibutyryl cyclic AMP and TSH. In fact, we have observed that these latter compounds stimulate droplet formation, whereas this morphological effect is absent when dibutyryl cyclic GMP is added to the tissue.

The above results, while further supporting the hypothesis that cyclic AMP is the intracellular mediator of TSH action, suggest, on the other hand, that cyclic GMP might not have the same role. Nevertheless, it is possible that cyclic GMP is involved in the control of some metabolic activity of thyroid gland such as protein synthesis.

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