The Effect of Dibutyryl Cyclic 3',5'-AMP on the Thyroid

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It has been proposed that some polypeptide hormones exert their action on target tissues by stimulating the formation of cyclic 3',5'-AMP (Sutherland et al., 1965). In the case of thyroid gland, thyroid-stimulating hormone (TSH) has been reported to increase the levels of cyclic 3',5'-AMP in thyroid homogenates (Klainer et al., 1962) and slices (Gilman and Rall, 1966). If cyclic 3',5'-AMP is the mediator of the TSH response, then it should be able to produce the same metabolic responses as TSH when incubated with thyroid tissue.

Two very prominent metabolic responses to TSH in thyroid slices are an increase in the oxidation of glucose-1-^{14}C to $^{14}CO_2$ (Field et al., 1962) and an increase in the incorporation of $^{32}P_i$ into phospholipids (Morton and Schwartz, 1953). It has been previously found that cyclic 3',5'-AMP failed to increase $^{14}CO_2$ production (Field et al., 1960). Cyclic 3',5'-AMP also does not increase the incorporation of $^{32}P_i$ into phospholipids (Table 1). This failure may be due to both the inability of this phosphorylated compound to enter the thyroid cell and to its rapid enzymatic hydrolysis. Posternak, Sutherland and Henion (1962) have reported the synthesis of N^6-2'-0-dibuytyryl-3'5'-adenosine monophosphate (DBC). This biologically active derivative of cyclic 3',5'-AMP enters cells more readily than the parent compound and is resistant to enzymatic hydrolysis. For example, DBC increases lipolysis when added to adipose tissue (Butcher and Sutherland, 1965) while cyclic 3',5'-AMP does not (Vaughn, 1960). Therefore, DBC[1] has been tested for its ability to stimulate (a) the oxidation of glucose-1-^{14}C to $^{14}CO_2$ and (b) the incorporation of $^{32}P_i$ into phospholipid in thyroid slices. The data of Table 1 show that DBC, like TSH, stimulates both the oxidation of glucose-1-^{14}C to $^{14}CO_2$ and the incorporation of $^{32}P_i$ into phospholipid.

TABLE 1

The Effect of Dibutyryl Cyclic 3',5'-AMP on $^{14}CO_2$ Production and $^{32}P_i$ Incorporation of Thyroid Slices

Compound	Concentration	$^{14}CO_2$ Produced* (cpm/mg/ hr)	$^{32}P_i$ Incorporation** (cpm/mg/3 hr)
---	---	61 ± 2.6	199 ± 10.1
DBC	50 µg/ml	82 ± 3.8	---
DBC	125 µg/ml	118 ± 9.3	298 ± 5.9
DBC	250 µg/ml	122 ± 9.0	---
DBC	375 µg/ml	123 ± 2.6	---
cyclic 3',5'-AMP	250 µg/ml	62 ± 2.2	180 ± 8.6
TSH	20 mU/ml	154 ± 2.9	---
TSH	100 mU/ml	---	376 ± 2.2

Legend to Table 1. The production $^{14}CO_2$ was measured as previously described (Field et al., 1960). Dog thyroid slices weighing 20 mg were incubated for 1 hr at 37° in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.35) containing 5 mg of albumin, 1 mg of glucose and 500,000 cpm of glucose-1-^{14}C. The $^{14}CO_2$ was collected in hyamine and counted in a liquid scintillation counter. The incorporation of $^{32}P_i$ into phospholipids was measured by the method of Kogl and van Deenen (1961). Beef thyroid slices weighing 100 mg were incubated at 37° for 3 hours in 1 ml of Krebs-Ringer bicarbonate buffer containing 2.3 x 10^6cpm of $^{32}P_i$. The gas phase in all incubations was 95% O_2:5% CO_2.

**Each value is the average from 3 slices ± S.E. of the mean.
*Each value is the average from 5 slices ± S.E. of the mean.

[1] DBC was a generous gift of Dr. Th. Posternak, Geneva, Switzerland

In a separate study it has been found that DBC cause the formation of intracellular colloid droplets in dog thyroid slices.[2] These results are compatible with the hypothesis that cyclic 3',5'-AMP, under the control of TSH, regulates the metabolic activity of the thyroid cell.

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[2] Pastan, I. H. and Wollman, S. H., Unpublished results.