MECHANISM OF TSH ACTION: EFFECTS OF DIBUTYRYL CYCLIC AMP ON RNA SYNTHESIS IN ISOLATED THYROID CELLS[†]

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SUMMARY

Continuous incubation of isolated thyroid cells for 1 hr with ^{14}C-adenine or ^{14}C-uridine in the presence of either 0.2 U/ml TSH or 2 mM DBC enhanced the incorporation of the labels into RNA by about 30% and 50% respectively. The time-course of the responses to both TSH and DBC was almost identical and relatively short-lived. No significant increase in the total uptake of the ^{14}C labels by the cells was found, indicating that TSH and DBC stimulated the net synthesis of RNA. These observations suggest a TSH control of DNA-dependent RNA synthesis which is mediated by cyclic AMP.

Recent evidence suggests that TSH regulates the function of the thyroid gland via the adenyl cyclase-cyclic AMP mechanism postulated by Sutherland (1). TSH rapidly increased the cyclic AMP levels in bovine thyroid slices (2) and in isolated thyroid cells (3), and also stimulated adenyl cyclase activity in thyroid homogenates (4). Furthermore, dibutyryl cyclic 3', 5'-AMP (DBC) has been shown to reproduce many actions of TSH, both in isolated thyroid cells (3) and in thyroid slices (5, 6, 7, 8).

TSH has been observed to enhance the synthesis of RNA in thyroid slices (8, 9, 10, 11, 12). Lindsay et al., (13) have shown that TSH stimulates the conversion of orotic acid to pyrimidine nucleotides with a resultant increase in the synthesis of RNA. Kerkof and Tata investigated, both *in vivo* (14) and *in vitro* (8), the actions of TSH on thyroidal RNA synthesis and found

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that TSH accelerated the incorporation of ^{32}P into RNA. While a TSH-like action of DBC was also observed, the increase in RNA synthesis apparently resulted from an enhanced uptake of ^{32}P by the tissue.

Wilson et al., (3, 15) reported that in isolated cell-preparations, the stimulatory actions of both TSH and DBC on iodide transport were inhibited in an almost identical manner by puromycin, cycloheximide and actinomycin D. Assuming that the actions of the antibiotics are due to their well-known inhibitory effects, these observations suggest that TSH probably acts to stimulate synthesis of cyclic AMP which, in turn, induces protein formation at the level of DNA-dependent RNA synthesis. To test this possibility the effects of TSH and DBC on the rate of incorporation of ^{14}C-labelled precursors into the RNA of isolated thyroid cells were investigated.

Methods and Materials

Bovine thyroid cells were isolated by a modified trypsinization procedure previously described (16). The cells were collected in batches instead of by the continuous flow technique.

Approximately 0.2 ml isolated thyroid cells were suspended in 10 ml cold Eagle's Minimum Essential Medium (MEM) pH 7.4, containing 100 units of penicillin, 35 - 150 µg streptomycin/ml, 5 µc ^{14}C-adenine or ^{14}C-uridine, and various concentrations of carrier depending on the label used. When the incubation time was less than 30 min 10 µc of the ^{14}C-label was used. TSH (0.2 U/ml) and DBC (2 mM) were added to the appropriate cell suspensions just before commencement of incubation. The cells were incubated at 37° with gentle shaking in an atmosphere of 95% oxygen - 5% carbon dioxide for periods ranging from 15 min to 4 hr.

After incubation the cells were sedimented at 850 x g, washed twice in MEM, twice in MEM containing 100 µg carrier/ml followed by two washes in MEM. The temperature of the washing solutions was 2 - 4°. The cells were then resuspended in 2.2 ml of cold 0.9% KCl and filtered through nylon net (No 5 mesh). The total uptake of the label by the cells

was determined from duplicate 0.5 ml or single 1 ml samples of the filtrates. The samples were measured into pre-weighed test tubes, dried, reweighed and hydrolysed for 1 hr at 110° in 1.0 ml 3N KOH. The hydrolysates were acidified with 1.2 ml 3N HCl and 0.2 ml samples were counted in a Packard Tricarb liquid spectrometer using a toluene: triton scintillator (2:1 V/V).

The remaining volumes of the cell filtrates (approximately 1.2 ml) were measured so that the exact weight of cells could be calculated from the samples taken for uptake measurements. The filtrates were then treated with $HClO_4$ and the ^{14}C-content of the acid soluble fraction was determined as CPM/mg dry cells. The RNA was isolated from the $HClO_4$ precipitate by a modified Schmidt-Tannhauser procedure (17) and estimated spectrophotometrically by the method of Fleck and Begg (18). The radioactivity in 0.5 ml samples of the RNA fraction was measured as described above.

Adenine-8-^{14}C or adenine-U-^{14}C, and uridine-2-^{14}C were obtained from the Radiochemical Centre, Amersham. The TSH used was either NIH-B_4, which was kindly supplied by the Endocrine Study Section, National Institutes of Health, or Armour 'Thytropar'. DBC was purchased from Boehringer.

Results and Discussion

Thyroid cells were incubated with ^{14}C-adenine for various periods ranging from 15 min to 4 hr in the presence of 0.2 U TSH/ml or 2 mM DBC (Fig. 1). TSH added at the start of the incubation caused a rapid incorporation of the labelled purine base into total RNA, with a maximum stimulation of 30% after 1 hr (Fig 1a). The duration of the response was relatively short-lived and was hardly significant at 4 hr. A TSH-like action of DBC on the incorporation of ^{14}C-adenine into RNA of thyroid cells was also observed (Fig 1b). Incubation of the cells with the label for about 1 hr in the presence of 2 mM DBC consistently stimulated the specific radioactivity of RNA by about 50%. Furthermore, the time-course of the DBC stimulatory effect was very similar to that of TSH.

Concentrations of DBC ranging from 0.25 - 2 mM were found to be

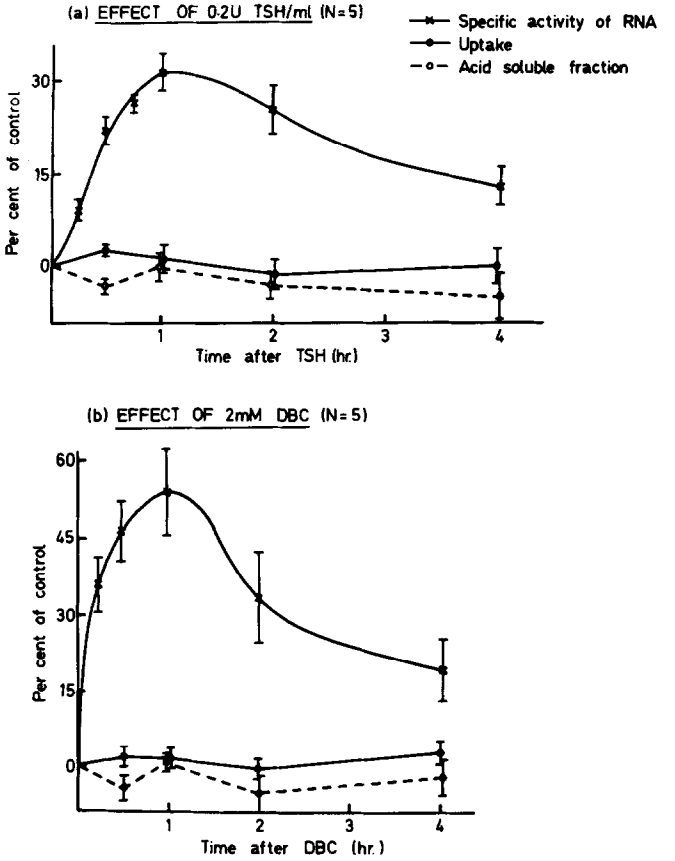

Fig. 1.

Effects of (a) TSH and (b) DBC on the total cellular uptake of ^{14}C-adenine and the distribution of ^{14}C-adenine in the RNA and acid-soluble fractions of isolated thyroid cells. Approximately 0.2 ml of bovine thyroid cells were suspended in 10 ml Eagle's Medium containing 5 or 10 μC adenine-U-^{14}C (see text) and 0.4 μmoles stable adenine. The concentrations of TSH and DBC indicated were added immediately before the cells. The values ± the standard errors represent the means of 5 experiments.

effective in enhancing ^{14}C-adenine incorporation into RNA (Table I). A maximum stimulatory response in RNA synthesis of 48% was attained with 1 mM DBC. A significant stimulatory effect of 26% was still observed at 0.25 mM DBC. In order to compare directly the effects of TSH and DBC on RNA synthesis, the concentrations of TSH and DBC chosen in this study were such that a maximum response was obtained with each reagent. Other nucleotides, such as ATP, AMP and the non-acylated cyclic AMP did not have TSH-like actions on the ^{14}C-adenine incorporation into RNA.

The enhancement of pyrimidine nucleoside incorporation into RNA by TSH and DBC is similar to that found with the purine base, adenine (Fig 2). Continuous incubation of the cells for 0.5 - 1 hr with 0.2 U TSH/ml increased ^{14}C-uridine incorporation into RNA by 31% (Fig 2a). Similarly, after about 1 hr incubation with 2 mM DBC it was noted that the specific radioactivity of the RNA was maximumly stimulated to about 50% (Fig 2b). The duration of the responses to both TSH and DBC were almost identical and were also relatively short-lived.

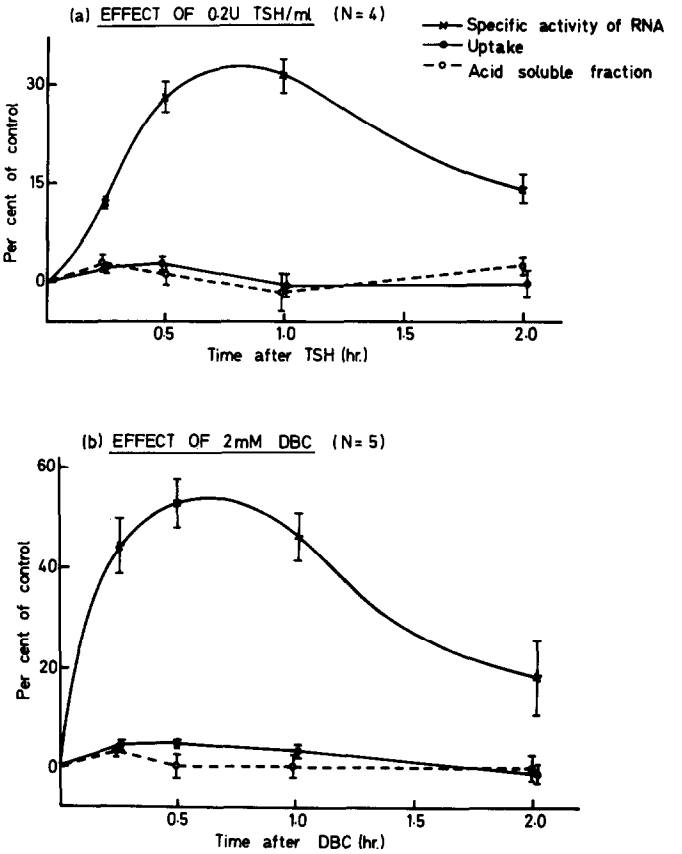

Fig. 2.

Effects of (a) TSH and (b) DBC on the total cellular uptake of ^{14}C-uridine and the distribution of ^{14}C-uridine in the RNA and acid-soluble fractions of isolated thyroid cells. The incubation conditions were the same as those described in Fig. 1 except 5 or 10 µC uridine-2-^{14}C (see text) and 0.4 µmoles uridine carrier were used. The values ± the standard error represent the means of 4 experiments.

In an attempt to determine whether the stimulatory actions of TSH and DBC on the incorporation of ^{14}C-labels into RNA were possibily due to increased uptake of the label into precursor pools, the ^{14}C-adenine or ^{14}C-uridine content of the acid soluble fraction and the total uptake of the labels by the cells were measured. Both TSH and DBC were found to have little effect on the rate of entry of ^{14}C-adenine and ^{14}C-uridine into the cell (Figs 1 and 2). On the other hand these substances decreased slightly the content of ^{14}C-label in the acid soluble fractions at a time when maximum incorporation of the label into RNA was observed. Furthermore, in several experiments the specific radioactivities of the ^{14}C-label in the acid soluble fractions were measured and a similar but slightly more marked depression in the specific radioactivity was noted with TSH and DBC. These decreases may represent a rapid transfer of label from precursor nucleotide pools to the RNA fraction resulting from the enhanced RNA synthesis induced by the TSH or DBC.

The TSH stimulated incorporation of ^{14}C-adenine and ^{14}C-uridine into RNA with isolated thyroid cells, is in accordance with the results reported previously (9, 10, 11, 12). Although these investigators demonstrated a TSH-enhanced incorporation of ^{14}C-adenine and ^{3}H-uridine into RNA, they did not eliminate the possibility that the observed increases could be due to changes in permeability caused by the added TSH. On the other hand, Kerkof and Tata (8) found that although TSH accelerated the incorporation of ^{32}P into RNA in the absence of any changes in ^{32}P uptake, DBC enhanced the synthesis of ^{32}P-RNA, while simultaneously increasing the total ^{32}P content of the tissue. This observation suggested that the stimulation of RNA synthesis by DBC was only apparent and resulted from the enhanced uptake of ^{32}P by the tissue. Our findings differ from those of Kerkof and Tata (8) since we were not able to show any increases in total cellular uptake of either ^{14}C-adenine or ^{14}C-uridine when thyroid cells were incubated for up to 4 hr in the presence of 2 mM DBC. One possible explanation is that an

EFFECT OF VARYING CONCENTRATIONS OF DBC ON ^{14}C-ADENINE INCORPORATION INTO RNA OF ISOLATED THYROID CELLS

Additions	Specific radioactivity (CPM/μg RNA-P)	% Change
None	800	-
0.25 mM DBC	1007	+26
0.5 mM DBC	1086	+36
1.0 mM DBC	1182	+48
2.0 mM DBC	1159	+45

Table I.

The incubation conditions were the same as those described in Fig. 1, except that the incubation time was 1 hr and the medium contained 1.5 μmoles stable adenine.

independent mechanism exists for the uptake of orthophosphate and that this mechanism is sensitive either directly or indirectly to DBC. The existence of such a mechanism is probable on the grounds that orthophosphate is required for many important and diverse metabolic processes.

The TSH-like action of DBC on the incorporation of labelled precursors into RNA indicates that, at least one of the effects of the elevated levels of cyclic AMP induced by TSH, is to modify protein formation by enhancing the synthesis of DNA-dependent RNA. However, the site at which cyclic AMP acts to stimulate RNA synthesis still remains to be established. Recent evidence is consistent with the concept that the TSH-enhanced synthesis of RNA is due to increased nucleotide synthesis resulting from increased ribose production via the pentose phosphate pathway (10, 13). Since DBC has been shown to stimulate the oxidation of glucose-1-^{14}C in canine (6) and porcine (19) thyroid slices it is important to establish whether the observed DBC

stimulatory actions on RNA synthesis may be due to increased ribose production. It is interesting to note that Shimada and Yasumasu (12) demonstrated a TSH stimulation of ^{3}H-uridine incorporation into RNA in isolated thyroid nuclei. However, TSH stimulation of glucose oxidation has so far not been reported in cell-free systems (20). Accordingly, it may be difficult to attribute the entire TSH stimulatory effect on RNA synthesis to increased ribose production. Our results on the stimulation of ^{14}C-uridine incorporation into RNA possibly indicate that TSH, perhaps through cyclic AMP, also acts at a level subsequent to the formation of ribose. This possibility is now being investigated.

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