NOTE

Effects of Concanavalin A and Neuraminidase on Cyclic AMP Levels and ^{14}C -1-Glucose Oxidation in Dog Thyroid Slices

KAMEJIRO YAMASHITA¹, YUJI AIYOSHI¹, HIROSHI OKA²
AND ETSURO OGATA¹

¹Department of Medicine, University of Tsukuba, Ibaraki-ken, 300-31, Japan and ²First Department of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Synopsis

Treatment with concanavalin A at 100 $\mu\text{g/ml}$ or higher concentrations significantly increased ^{14}C -1-glucose oxidation in dog thyroid slices as reported in other tissues. This treatment exerted no effect on tissue cyclic AMP levels.

Neuraminidase at the same concentrations also had similar effects on these parameters.

Neither concanavalin A nor neuraminidase at the concentrations up to 100 $\mu\text{g/ml}$ had the TSH effect on both tissue cyclic AMP and ^{14}C -1-glucose oxidation.

These results indicate that modification of carbohydrate moieties of glycoproteins on the cell surface may cause an increase in glucose metabolism without any critical effect on cyclic AMP system and in the process of TSH response.

The initial action of thyroid-stimulating hormone (TSH) involves binding to receptors on the plasma membrane and subsequent generation of cyclic 3', 5'-adenosine monophosphate (cAMP) in the thyroid cell (Field, 1975). However, the mechanism of this process is yet to be clarified in detail.

In several tissues such as adipose tissues, liver (Cuatrecasas and Illiano 1971; Cuatrecasas and Tell, 1973) and adrenal cells (Haksar *et al.*, 1974) certain regulatory roles in cell metabolism have been ascribed to the membrane glycoproteins and especially to sialic acid residues. In contrast, Macchia and Pastan (1967) reported that neither the Sephadex G-100-purified clostri-

dial neuraminidase nor the viral neuraminidase exerted any activity on the TSH stimulation of ^{14}C -1-glucose oxidation in dog thyroid slices.

Therefore, we investigated the influence of the manipulation of the surface glycoproteins on the cyclic AMP level and ^{14}C -1-glucose oxidation in dog thyroid slices and on the stimulation of them by TSH. For this purpose, concanavalin A and neuraminidase were employed; the former binds to specific carbohydrate determinants on the cell surface (Sharon and Lis, 1972) and the latter selectively cleaves the terminal sialic acid residues of the membrane glycoproteins.

Materials and Methods

Dog thyroid glands were excised, sliced and incubated for determination of cyclic AMP content (Oka *et al.*, 1973) and of the ^{14}C -1-glucose oxidation activity essentially as described previously (Yamashita *et al.*, 1970).

Received for publication February 2, 1976.

Abbreviations: TSH = thyroid-stimulating hormone; cyclic AMP = adenosine 3', 5'-cyclic monophosphate.

This study was supported in part by the grant from the Ministry of Welfare of Japan and by the grant from the University of Tsukuba for special projects.

For the test of the *in vitro* effect of concanavalin A or neuraminidase on the thyroid slices with regard to the cyclic AMP concentration and to the ^{14}C -1-glucose oxidation and on their responses to TSH, slices (approximately 30–40 mg wet weight) were incubated for 30 min at 37°C in 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) that was added with glucose to 100 mg/100 ml and with the reagent. Then the slices were removed and rinsed intensively at 4°C with 20–30 ml of the fresh glucose Krebs-Ringer buffer. The rinse was repeated once with the fresh buffer. The slices were transferred for the second incubation in the same buffer at 37°C either for cyclic AMP assay (20-min incubation) in the presence of 10 mM theophylline or for ^{14}C -1-glucose oxidation (45-min incubation). In all incubations, the gas phase was 95% O_2 –5% CO_2 .

Each result presented in the text is a representative of three independent and highly reproducible experiments.

Concanavalin A (from Jack bean meal, 1 mg combines and precipitates with 1 mg glycogen) and neuraminidase (EC3.2.1.18, Type V, purified from *Cl. perfringens*, 0.035 unit using mucin/mg protein,) were purchased from Daiichi Pure Chemicals Co. and Sigma Co., respectively. Bovine TSH was obtained from Armour Pharmaceutical Co., and ^{14}C -1-glucose (2.4 mCi/mM) from Amersham-Searle Co.

Results

As shown in Fig. 1, pretreatment with concanavalin A (100 $\mu\text{g}/\text{ml}$) exhibited no effect on basal cyclic AMP levels in dog thyroid slices. The same concentration of concanavalin A produced a slightly depressive effect on the TSH stimulation of cyclic AMP levels, but the effect was statistically insignificant.

Concanavalin A at the same concentration caused a significant increase (about 50%) in the ^{14}C -1-glucose oxidation in the second incubation (Fig. 2). The stimulatory effect of this plant lectin was not observed at concentrations lower than 10 $\mu\text{g}/\text{ml}$. The extent of stimulation by 100 $\mu\text{g}/\text{ml}$ of concanavalin A was the same as that by the reagent of the concentration up to 500 $\mu\text{g}/\text{ml}$. It was much lower than that by 50 mU/ml of TSH. Concanavalin A (100 $\mu\text{g}/\text{ml}$) did not affect the stimulation by TSH of glucose oxidation as was the

Fig. 1. Effect of Pretreatment with Concanavalin A on basal and TSH-stimulated Cyclic AMP levels in Dog Thyroid Slices. Cyclic AMP was assayed following 20 minutes incubation after pretreatment in the presence or absence of concanavalin A. The other experimental procedures were described under 'Materials and Methods.' Results are averages $\pm$ S.E. of triplicate determinations. Essentially identical results were obtained when neuraminidase (100 $\mu\text{g}/\text{ml}$) was employed instead of concanavalin A.

Fig. 2. Effect of Pretreatment with Concanavalin A on ^{14}C -1-glucose Oxidation in Dog Thyroid Slices. Glucose oxidation was determined during 45 min of incubation after pretreatment with concanavalin A. The other experimental procedures were described under 'Materials and Methods.' Results are averages $\pm$ S.E. of triplicate determinations.

case of cyclic AMP formation ($^{14}\text{CO}_2$ production from ^{14}C -1-glucose: TSH [50 mU/ml]; 104 ± 4.0 cpm/mg tissue $N=3$, concanavalin A [100 $\mu\text{g}/\text{ml}$] + TSH [50 mU/ml]; 98 ± 8.5 cpm/mg tissue $N=3$).

Neuraminidase at the concentration of 100 $\mu\text{g}/\text{ml}$ was not effective on both basal and the TSH-stimulated levels of tissue cyclic AMP (See the legend for Fig. 1). This enzyme at a higher concentration (500 $\mu\text{g}/\text{ml}$) inhibited the TSH stimulation of cyclic AMP content (cyclic AMP levels: control; 0.77 ± 0.01 nM/g tissue, TSH [100 mU/ml]; 14.3 ± 1.3 , neuraminidase [100 $\mu\text{g}/\text{ml}$] + TSH [100 mU/ml]; 12.5 ± 1.4 , neuraminidase [500 $\mu\text{g}/\text{ml}$] + TSH [100 mU/ml]; 5.4 ± 1.6 , $N=3$ in each group).

As shown in Fig. 3, neuraminidase at 100 $\mu\text{g}/\text{ml}$ increased glucose oxidation by about 44%. This glycosidase at lower concentrations (less than 10 $\mu\text{g}/\text{ml}$) showed no effect and at higher concentrations (up to 500 $\mu\text{g}/\text{ml}$) showed no further stimulation (Data not shown). The enzyme at 100 $\mu\text{g}/\text{ml}$ had no significant effect on the stimulation by TSH of ^{14}C -1-glucose oxidation.

Fig. 3. Effect of Pretreatment with Neuraminidase on ^{14}C -1-glucose Oxidation in Dog Thyroid Slices. Glucose oxidation was determined during 45 minutes of incubation after pretreatment with neuraminidase. The other experimental procedures were described under 'Materials and Methods.' Results are averages $\pm$ S.E. of triplicate determinations.

Discussion

Since the molecular sizes of concanavalin A and neuraminidase are estimated larger than 50,000 daltons, it is highly unlikely that these reagents induced the above-described effects after penetrating into the cell interior. In view of the ^{14}C -1-glucose oxidation in dog thyroid slices enhanced by concanavalin A and neuraminidase at the concentration of 100 $\mu\text{g}/\text{ml}$ (Figs. 2 and 3), it appeared that these reagents exerted certain metabolic effects on the thyroid cells via the action on the cell surface. The stimulation of ^{14}C -1-glucose oxidation by concanavalin A of similar concentrations has also been observed in leucocytes and macrophages (Romeo *et al.*, 1973). Cuatrecasas and Tell reported that concanavalin A was as effective as insulin in enhancing the rate of glucose transport and in inhibiting epinephrine-stimulated lipolysis in isolated adipocytes (Cuatrecasas and Tell, 1973). The stimulation by neuraminidase of the glucose transport in the isolated adipose tissue was also reported (Cuatrecasas and Illiano, 1971). These observations implicate that the increase in ^{14}C -1-glucose oxidation in the present experiments is explicable at least partly by the enhanced transport of glucose into the thyroid cells. Therefore, in some sets of experiments, we estimated the glucose consumption by measuring the glucose concentrations remaining in the medium. However, only equivocal results were obtained, because too minute extents of diminution were detected in the medium glucose. On the contrary, Field *et al.* (1960) reported that insulin augmented glucose uptake by thyroid slices without the stimulation of glucose oxidation.

In view of the inherent problems in employing biochemical inhibitors as a tool for the special metabolic study and of the questions as to the extent to which the inhibitors develop the expected effect in

each particular experimental setting, we used two types of reagents which affect the membrane glycoproteins by an entirely independent mode of action. The present data demonstrate that similar biological changes can be obtained either by the binding of concanavalin A to the membrane glycoproteins or by the cleavage of the terminal sialic acid residues with neuraminidase. However, several parameters other than ^{14}C -l-glucose oxidation should be examined to elucidate the exact mode of metabolic effects of concanavalin A and neuraminidase on thyroid slices.

As mentioned under the Method, slices were pretreated with either concanavalin A or neuraminidase, washed intensively and then incubated with TSH for cyclic AMP assay and glucose oxidation. These precautions are considered important in order to protect the TSH molecule (a glycoprotein) from being directly attacked by these reagents. In these experimental conditions, we observed that these reagents did not exert any significant effect on the TSH stimulation of cyclic AMP content and ^{14}C -l-glucose oxidation. It should be mentioned here that the concentrations of the reagents used in these experiments were high enough to produce maximum metabolic effects (stimulation of basal ^{14}C -l-glucose oxidation) in thyroid slices and no further stimulation was elicited by increasing the dosage beyond these ranges. Comparable concentrations of these reagents have been used in the experiments of similar purposes with other tissues (Cuatrecasas and Illiano, 1971; Cuatrecasas and Tell, 1973). Consequently, the above data indicate that most of the concanavalin A-specific carbohydrate determinants and the sialic acid residues of the cell surface may play only a minor direct role, if any, in TSH binding to the receptors

and in the subsequent processes to the cyclic AMP formation and ^{14}C -l-glucose oxidation, although they are important in the maintenance of basal levels of some intermediate metabolism. As shown in the Results, higher concentrations of neuraminidase inhibited the effect of TSH on cyclic AMP formation. Needless to say, very high concentrations of these reagents may impair of the cell reactions by rather non-specific effects on the cell membrane structures.

Cuatrecasas and Tell (1973) reported that concanavalin A ($5\text{--}50\text{ }\mu\text{g/ml}$) inhibited basal as well as epinephrine-stimulated adenylate cyclase activity of membranes obtained from homogenates of fat cells and that concentrations of concanavalin A greater than $50\text{ }\mu\text{g/ml}$ caused stimulation of this enzyme. One of the reasons for the difference between our results and those by Cuatrecasas and Tell may be the difference in the conditions employed; we used thyroid slices and they used isolated fat cell membranes.

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