THE EFFECT OF SIALIC ACID ON ADENYLATE CYCLASE ACTIVITY AND THYROTROPIN-RECEPTOR BINDING IN HUMAN THYROID MEMBRANES

Medical Center of Postgraduate Education, Department of Biochemistry, ul. Marymoncka 99; 01-813 Warszawa, Poland

Exogenous sialic acid at 3 mM and higher concentrations inhibits the basal adenylate cyclase activity and the activity stimulated by thyrotropin (TSH) and fluoride in the human thyroid membrane fraction; 30 mM-sialic acid acts as an inhibitor of TSH binding. The decrease of these activities at high sialic acid concentrations might be ascribed to changes in membrane conformation caused by acidic character of this sugar.

In the last few years attention has been called to the role of gangliosides in the action of hormones. Similarly as in the case of bacterial toxin (Cuatrecasas, 1973; Heyningen, 1974; Mullin et al., 1976a; Fishman & Brady, 1976) it was found that gangliosides participate in the binding of TSH to bovine (Mullin et al., 1976b) and rat (Meldolesi et al., 1976) thyroid membranes and also in the binding of human chorionic gonadotropin (Lee et al., 1976) and the luteinizing hormone (Lee et al., 1977a) to the rat testis membranes. Meldolesi et al. (1977) assume that gangliosides are not directly involved in the binding process but are important in transmission of messages from the hormone-receptor complex to adenylate cyclase. Since it is postulated that this might be a primary function of gangliosides, it seemed of interest to investigate the molecular function of free sialic acid in those processes. In the present work we examined the effect of exogenous sialic acid on the basal adenylate cyclase activity and the activity stimulated by thyrotropin (TSH) and fluoride in human thyroid membranes. The effect of sialic acid on TSH binding to thyroid membranes was also investigated.

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1 Abbreviation: TSH, thyrotropin.
MATERIALS AND METHODS

Human TSH, a gift from Byk-Mallinckrodt (Diepenbach, G.F.R.), was iodinated with $^{125}$INaI (Amersham) by the modified chloramine T method (Greenwood et al., 1963; Golda et al., 1976). Bovine TSH (biological activity 25 U/mg) was kindly provided by Dr. L. D. Kohn (National Institute of Health, Bethesda, Maryland, U.S.A.).

Human thyroids were obtained from surgically treated patients with Graves' disease.

Crude membrane fraction was obtained as follows: 5 g slices were homogenized in 50 ml of 10 mM-Tris/HCl, pH 7.4, containing 1 mM-MgCl$_2$ and 250 mM-sucrose, and centrifuged at 1000 g for 10 min. The collected supernatant was spun down at 10,000 g for 10 min, the pellet was washed with buffer without sucrose and was resuspended in 2 ml of 25 mM-Tris/HCl, pH 7.8 (Nauman & Wasilewska, 1976).

The adenylate cyclase activity was determined during 5 to 30 min incubation at 37°C. The assay medium contained 2.5 mM-ATP, 5 mM-MgCl$_2$, 10 mM-theophylline, 0.1% bovine serum albumin, 2.5 mM-phosphoenolpyruvate, 130 µg/ml of pyruvate kinase and 10 to 130 µg of membrane protein in 100 µl of 25 mM-Tris/HCl, pH 7.8. To some samples, 0.3 to 20 mU of bovine TSH or 10 mM-NaF were added.

The effect of sialic acid (N-acetylmuraminic acid, Koch Light, Colnbrook, Bucks., England) was studied at a concentration from 0.3 to 30 mM. Before the addition to the incubation mixture, sialic acid solutions were adjusted to pH 7.8 with 0.5 M-NaOH. The reaction was stopped by adding 500 µl of ethanol. The mixture was centrifuged, the protein pellet was discarded, the supernatant was evaporated under N$_2$ and the amount of cAMP before and after the enzymatic reaction was estimated by the protein binding method (Brown et al., 1971).

The TSH-receptor activity was investigated during 15 min incubation at 4°C in a mixture containing 1 µM-$^{125}$I-TSH, 50 µg of membrane proteins and 0 to 30 mM-sialic acid in 100 µl of 25 mM-Tris/acetate, pH 6. The specificity of binding was estimated in the above system using 50 microunits of non-labelled TSH instead of sialic acid.

Protein was estimated according to Lowry et al. (1951).

RESULTS

The activity of adenylate cyclase in thyroid membranes was linear from 5 to 20 min and at a protein concentration from 15 to 100 µg per sample (Figs. 1, 2). The basal adenylate cyclase activity in the membrane fraction obtained from individual thyroids showed large variations. The average basal activity calculated from the results obtained for 16 human thyroids was 32.3 (SE±6.5) picomoles of cAMP formed per 20 min per 1 mg of protein. In the presence of 10 mM-NaF the activity was increased 33 times (SE ± 7).
TSH (0.3 to 20 mU) added to the incubation mixture stimulated adenylate cyclase activity. In the presence of 0.8 mU of bovine TSH, the average stimulation of basal enzyme activity was 205% (SE ± 35). The effect of increasing TSH concentrations is shown in Fig. 3.

Fig. 1. Time-dependence of adenylate cyclase activity in human thyroid membranes (50 μg of protein per sample). O, Basal activity; □, activity stimulated by bovine TSH (0.8 mU).

Fig. 2. The dependence of adenylate cyclase activity on the concentration of human thyroid membrane proteins (Incubation time 20 min). O, Basal activity; □, activity stimulated by bovine TSH (0.8 mU).

Fig. 3. Effect of TSH on the adenylate cyclase activity. Protein, 55 μg per sample, incubation time 20 min.

Fig. 4. Effect of sialic acid on the adenylate cyclase activity (50 μg of human thyroid membrane protein per sample, 20 min incubation). O, Basal activity; □, activity stimulated by bovine TSH (0.8 mU). The data represent mean values from two experiments ± S. E.

Fig. 5. Effect of sialic acid on the binding of [125I]TSH with human thyroid membranes (50 μg of protein per sample). The data represent mean values from three experiments ± S.E.

The influence of sialic acid on the adenylate cyclase activity was investigated during 20 min of the reaction with 50 μg of membrane proteins. Sialic acid at 3 mM concentration lowered almost parallelly both the basal activity and that stimulated by TSH (Fig. 4). The adenylate cyclase activity stimulated by F⁻ was decreased by 29% in the presence of 30 mM-sialic acid (not shown).
The crude membrane fraction bound specifically 41 picomoles of $[^{125}\text{I}]$TSH per 50 µg of membrane proteins. Incubation of the labelled hormone and membranes in the system containing 0.3 to 10 mM-sialic acid did not cause any change in the amount of bound TSH. However, a further increase in the sialic acid concentration to 30 mM resulted in inhibition of TSH binding (Fig. 5).

DISCUSSION

In this paper we have described the influence of exogenous sialic acid on the adenylate cyclase activity in the membranes from pathologic thyroids from patients with Graves’ disease. The mechanism of stimulation of adenylate cyclase by TSH and $F^-$ in normal and pathologic tissues is similar, despite some quantitative differences (Lee et al., 1977b; Kuzuya et al., 1977; Orgiazzi et al., 1975; Carayon et al., 1977), so it is probable that the effect of sialic acid on the enzyme system from normal and pathologic tissues is the same.

Exogenous sialic acid at 3 mM concentration acted as an adenylate cyclase inhibitor. It lowered the basal activity, as well as the activities stimulated by TSH and $F^-$. Sialic acid at low concentration did not influence the binding of TSH by membranes. The inhibitory effect on the binding at pH 6 appeared at sialic acid concentration as high as 30 mM. Data of Moore & Feldman (1976) indicate that, at this concentration, the optimum pH for TSH binding shifts to 7.5, with a concomitant increase in the amount of the bound TSH. It could be supposed that the stimulatory effect of TSH on adenylate cyclase activity would be enhanced by the high concentration of sialic acid. However, such an effect was not observed; moreover, sialic acid lowered the enzyme activity irrespective of the absence or presence of TSH. Probably, sialic acid affects all the activities studied by inducing small changes in membrane conformation. These changes could be caused by unspecific adsorption of sialic acid on membrane surface (Moore & Feldman, 1976) or by binding of cations by sialic acid (Jacques et al., 1977). However, the binding of exogenous Mg$^{2+}$ is rather improbable because in such a case the increase in sialic acid concentration above 10 mM would result in almost complete inhibition of the reaction.

In thyroid, sialic acid is a component of membrane glycolipids and soluble glycoproteins, mainly thyroglobulin. As an intermediate, it may exist also as a free sugar, and its local higher concentrations may affect the processes connected with the release of thyroid hormones. The effect of exogenous sialic acid on the binding of TSH with the membranes and on the adenylate cyclase activity seems to be related to the acidic character of this sugar.

REFERENCES

Wpływ Kwasu Sialowego Na Aktywność Cykłazy Adenyloowej I Na Wiązanie Tyreotropiny Z Receptorem We Frakcji Blon Komórkowych Tarczycy Ludzkiej

Streszczenie

We frakcji blon komórkowych tarczycy ludzkiej egzogenny kwas sialowy w stężeniu 3 mm i wysokim hamuje aktywność podstawową cykłazy adenyloowej oraz aktywności stymulowane przez TSH i F-_. Kwas sialowy w stężeniu 30 mm jest również inhibitem wiązania TSH. Obniżenie wszystkich badanych aktywności przez stężony roztwór kwasu sialowego może być spowodowane zmianami konformacji błon wynikającymi z kwasowego charakteru tego cukrowca.

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