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Adenylate cyclase of a human medullary thyroid carcinoma[1]

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Summary. The adenylate cyclase of a human medullary thyroid carcinoma was activated by TRH, glucagon, epinephrine, norepinephrine, phentolamine, serotonin and NaF, suggesting the presence of multiple hormone receptors including a β-adrenergic one in the tumor.

The adenylate cyclase-cyclic adenosine 3′, 5′-monophosphate (cyclic AMP) system is known to be involved in the secretory regulation of many hormones[3]. With respect to calcitonin (CT)[4,5] there is increasing evidence that the release of the hormone is modulated via the adenylate cyclase system in humans and animals. On the other hand, human medullary thyroid carcinoma[6,7] (MCT) secreting CT excessively has been found not to be completely autonomous in function but in part under the control of physiological stimuli. The present study was conducted to elucidate the adenylate cyclase response of MCT to various stimuli.

Materials and methods. A sporadic MCT which was later proven histologically was obtained from a 40-year-old woman who had hypercalcitonemia. The tumor was immediately placed in saline at 4 °C and homogenized in iced buffer solution composed of 62.2 mM Tris-HCl and 15.5 mM theophylline at pH 7.4. The whole homogenate was used in triplicate for the adenylate cyclase assay, which was carried out according to a modification[8] of the method described by Schorr et al.[9]. In brief, the adenylate cyclase activity was assayed by incubating the tissue homogenates in a buffered solution containing an ATP regenerating system and 8-^{14}C-ATP, with or without the addition of the agents, for 20 min at 37 °C, and expressed as cyclic AMP (pmoles/mg protein) produced during a 20-min incubation, based on the conversion of ^{14}C-ATP to ^{14}C cyclic AMP. TRH was supplied by Tanabe, Osaka, ACTH (Cortrosyn) by Daiichi, Tokyo, and tetra-gastrin by Eizai, Tokyo. Bovine TSH and prolactin were provided by NIAMDD, NIH. Glucagon was obtained from Novo, Denmark. Phentolamine was supplied by Ciba-Geigy, propranolol by I.C.I., and prostaglandin E_1 (PGE_1) and PGE_2 by Ono, Osaka. Dopamine, carbachol, and serotonin were purchased from Nakarai, Kyoto, norepinephrine from Fluka and epinephrine from Merck. 8-^{14}C-ATP was purchased from New England Nuclear. Statistical analysis was performed using Student's t-test.

Results. Data on the adenylate cyclase of an MCT are shown in the table. The cyclase was stimulated by TRH, glucagon, epinephrine, norepinephrine, phentolamine, serotonin, and NaF. Propranolol, a beta-adrenergic antagonist, blocked the stimulation by epinephrine.

Discussion. Little is known about the detailed mechanism of the adenylate cyclase in the parafollicular cells of the thyroid. Hunt et al.[10] recently reported that the adenylate cyclase of a MCT was stimulated only by glucagon and NaF while CT, isoproterenol, PGE_1 and gastrin had no effect. The present study demonstrates cyclase activation by epinephrine, norepinephrine and phentolamine, in addition to glucagon and NaF, and the blockade of epinephrine-induced activation by propranolol indicates the β-adrenergic control of CT release in the human MCT. Also in normal subjects, isoproterenol, phentolamine and, surprisingly, methoxamine (α-adrenergic agonist) in addition to Ca infusion have been shown to increase plasma CT whereas propranolol and EDTA infusion were found to decrease them[11,12]. Of further interest in the present study is the blockade of phentolamine-induced tumor cyclase activation by dopamine since l-dopa, a dopamine precursor, was found to depress CT release from human MCT[13]. Glucagon, on the other hand, is reported to stimulate CT release from the tumor in vivo[6,7] and in vitro[14], and also in normal subjects[5,15]. The activation of tumor cyclase by TRH and serotonin demonstrated in the present study is also noteworthy.

Adenylate cyclase of a medullary thyroid carcinoma. Whole tissue homogenates were incubated with or without the addition of hormones during 20 min at 37°C. Adenylate cyclase activity is expressed as pmoles of cyclic AMP produced per mg protein per 20 min

Addition	Concentration	Adenylate cyclase activity (cyclic AMP pmoles/mg protein/20 min)
Control		167± 8[a]
ACTH	10^{-5} M	168± 14
TSH	10^{-5} M	198± 11
Prolactin	10^{-5} M	167± 8
TRH	1.4×10^{-5} M	227± 4***
Tetra-gastrin	10^{-5} M	151± 5
Glucagon	10^{-4} M	296± 12***
PGE_1	2.8×10^{-5} M	244± 37
PGE_2	2.8×10^{-5} M	159± 28
Epinephrine	10^{-5} M	243± 12**
+ Propranolol	10^{-5} M + 10^{-5} M	124± 4
Norepinephrine	10^{-5} M	207± 6*
Propranolol	10^{-5} M	150± 8
Phentolamine	10^{-4} M	260± 30*
Dopamine	2.6×10^{-5} M	158± 23
+ Phentolamine	2.6×10^{-5} M + 10^{-4} M	190± 11
Serotonin	1.2×10^{-5} M	290± 59*
Carbachol	10^{-5} M	167± 11
NaF	10^{-2} M	314± 28***

[a] Mean ± SE of triplicate determinations; [b] p-values (stimulated vs control values): * $p<0.05$, ** $p<0.02$, *** $p<0.01$.

The present investigation, showing the presence in a human MCT of multiple hormone receptors, thus provides some basic information about the pathophysiology of CT release from MCT, which may assist in diagnostic and therapeutic approaches to the tumor.

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Cholesterol mediates thyrotropin binding to liposomes containing gangliosides

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Summary. We present evidence that cholesterol mediates thyrotropin binding to liposomes containing GT_1 ganglioside. Thyrotropin fixation is maximal at 22% of cholesterol. This result suggests that the gangliosides' organization in the lipid matrix modulates their interaction with the glycoprotein hormone.

Addition of cholesterol to phosphatidylcholine bilayers in the fluid state leads to a decrease in the fluidity of the bilayer membrane. Inclusion of cholesterol in the phosphatidylcholine bilayers below the chain-melting transition temperature leads to fluidization. There is much evidence that this fluidity-modulating effect plays a significant role in the biological function of cell membranes. Model membranes [1-4] offer a unique opportunity to study this effect in well-defined physical and chemical conditions.

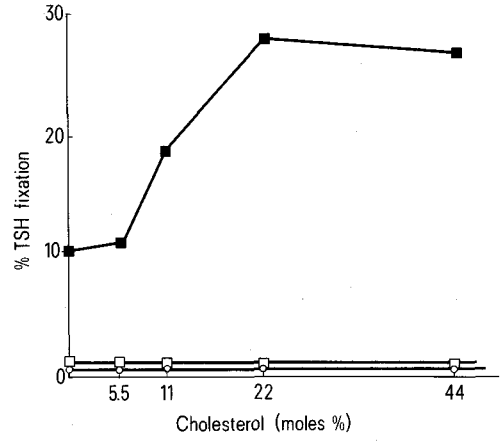
In the present paper, we give evidence that cholesterol mediates thyrotropin (TSH)) binding to liposomes containing GT_1 ganglioside. The result is briefly discussed in terms of ganglioside lateral mobility.

Materials and methods. D-L-α-dipalmitoylphosphatidylcholine (DPPC), cholesterol and thyrotropin were purchased from Sigma. GT_1 ganglioside (N-acetyl-neuraminylgalactosyl-N-acetylgalactosaminyl- (N-acetylneuraminyl-N-acetylneuraminyl)-galactosylglucosylceramide) GD_{1a} ganglioside (N-acetylneuraminylgalactosyl-N-acetylgalactosaminyl-(N-acetyl-neuraminyl)-galactosylglucosylceramide) and GM_1 ganglioside (galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosyl-ceramide) were Supelco products.

Lipid-gangliosides mixtures were dissolved in chloroform-methanol 2/1 (v/v). The solvent was evaporated under nitrogen flow and the lipid-ganglioside film was dried overnight. Liposomes were obtained by mechanical stirring (vortex mixer) of the film in Tris-HCl pH 7.2 buffer.

Binding studies were done at 21 °C by adding thyrotropin to batches of liposomes containing a constant amount of gangliosides (2 mole%) in Tris-HCl 0.025 M buffer pH 7.2. After 30 min., the tubes were centrifugated at 20,000 × g for 10 min., TSH was estimated by the Lowry method in pellets and in supernatants. Pellets were dissolved in a 10% SDS-NaOH 0.1 N solution.

Results and discussion. Studies on model membranes have shown that gangliosides interact specifically with TSH glycoprotein hormone[2,5]. The figure shows the TSH fixation on liposomes containing gangliosides as a function of the cholesterol content. DPPC liposomes do not bind TSH whereas those containing GT_1 show considerable binding.



Binding of TSH to liposomes containing gangliosides as a function of cholesterol content. Lipid-ganglioside mixtures were dissolved in chloroform-methanol 2/1 (v/v). The solvent was evaporated under nitrogen flow and the lipid ganglioside film was dried overnight. Liposomes were obtained by mechanical stirring (vortex mixer) of the film in Tris/HCl pH 7.2 buffer. The ganglioside-phospholipids molar ratio was equal to 0.02. Temperature was maintained at 21 °C. Each experimental point is the mean value of 6 experiments. ■, DPPC liposomes containing GT_1; ○, DPPC liposomes containing GD_{1a}; □, DPPC liposomes containing GM_1. TSH and lipid concentrations were respectively 50 µg/ml and 500 µg/ml.