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.

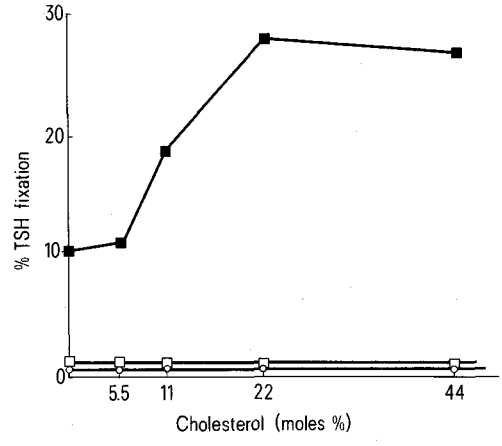
In the present paper, we give evidence that cholesterol mediates thyrotropin (TSH)) binding to liposomes containing GT_1 ganglioside. The result is briefly discussed in terms of ganglioside lateral mobility.

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.

No significant binding was observed with GM_1 and GD_{1a}. A considerable enhancement of TSH binding is observed around 11 moles% cholesterol with DPPC liposomes containing GT_1 at physiological concentrations. TSH fixation is maximal at 22 moles% cholesterol. Interestingly, at the temperature of our experiments (21 °C) it has been shown that in the case of DPPC/cholesterol mixtures, the lateral diffusion coefficient of DPPC molecules increases by factor of the order of 10 as the cholesterol content is increased by a few percent around 20 moles% cholesterol[6].

This result may suggest that at this well defined cholesterol concentration, lateral diffusion of the gangliosides is greatly increased. Moreover, it would mean that the large quantitative changes in recognition patterns could be based on small modifications in ganglioside organization.

Ganglioside structures contrast strongly with the conformation of classical lipids. Their large head groups as compared to the hydrocarbon tail could determine the organization in the lipid bilayer. We demonstrated recently[7] from electron spin resonance (ESR) measurements that above the lipid phase transition of dipalmitoyl phosphatidylcholine, the model membrane can be viewed as a fluid system in which gangliosides are randomly distributed. Below the phase transition, the model membrane consists of a mosaic structure composed of ganglioside clusters embedded in the lipid matrix. Gangliosides were labelled with a 3-carboxy-2.2.5.5-tetramethyl pyrrolidine-1-oxyl-residue fixed on the primary hydroxyl group of the carbohydrate residue. The method gives quantitative information about the proximity of the spin labelled gangliosides[8]. Using the same procedure, we showed that if, in the absence of cholesterol, at 21 °C, gangliosides are organized in clusters in the DPPC matrix, 20 mole% cholesterol induces a random distribution of the gangliosides in the lipid layer[9]. The possibility for gangliosides to diffuse freely in the lipid matrix appears thus as an essential condition for a maximal TSH recognition. Cholesterol modulating action could be a general process for other glycoproteic hormones (follitropin, luteinizing hormone). The use of model membranes allows us to examine the role of gangliosides in the recognition process.

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Inhibition of hypothalamic GnRH synthesis by inhibin

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Summary. Both testicular and ovarian inhibin preparations blocked GnRH synthesis by the hypothalamus and consequently reduced the circulating level of FSH. The serum level of LH was unaffected.

Although the mechanism of action of inhibin is not known with certainty, its actions may be multiple, and affect the hypothalamus, pituitary and gonads. Inhibin appears to act at the hypothalamic level by regulating the synthesis and/or release of GnRH[2-4], at the pituitary level it may act by modulating the responsiveness of the pituitary to GnRH[5-7], and at the gonadal level by interfering with the action of FSH[8,9].

We have reported the presence of a trypsin sensitive, heat stable, low-molecular-weight (1500 daltons) peptide with inhibin activity in ovine testis and ovary[9-11]. This peptide specifically suppressed circulating levels of serum FSH in rats presumably by interfering with the production of GnRH. The present study was designed to obtain more direct evidence in support of this presumption by actual measurement of the hypothalamic releasing hormone.

Materials and methods. The testicular and ovarian inhibin preparations used in this study were equivalent to TFR-II and OFR-II respectively[9] and were isolated by fractionating the high-speed supernatant derived from 40% homogenates of ovine testicular and ovarian tissues sequentially, on Sephadex G-100 and Sephadex G-25 as described earlier[9-11].

Adult male rats of the Holtzman strain, bilaterally castrated 2 weeks before the assay, were injected i.m. once daily with 1.0 ml of saline or the inhibin preparations, for 3 days. 4 h after the last injection, the animals were bled under light ether anaesthesia and their sera collected. The animals were then decapitated and the skull opened. The hypothalamus was carefully dissected out and immediately homogenized in 1.0 ml of chilled 0.1 N HCl. The homogenates were centrifuged in the cold and the sediments discarded.

Effect of testicular and ovarian inhibin on serum levels of FSH and LH and hypothalamic GnRH content in treated rats

Treatment	GnRH (ng/hypothalamus)	FSH (μg/ml)	LH (μg/ml)
Saline	2.58 ± 0.06 (4)	2.83 ± 0.05 (5)	1.28 ± 0.06 (4)
Testicular inhibin (300 μg/rat)	1.61 ± 0.08* (5)	2.25 ± 0.07** (5)	1.17 ± 0.04 (5)
Ovarian inhibin (300 μg/rat)	1.72 ± 0.08* (5)	2.22 ± 0.05** (5)	1.24 ± 0.05 (5)

* $p < 0.001$; ** $p < 0.05$. Values are means ± SE. Numbers in parentheses indicate number of observations. Serum FSH and LH values are expressed in terms of NIAMDD-Rat-FSH-RP-1 and NIAMDD-Rat-LH-RP-1 respectively.