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.

The supernatants were neutralized with 0.1 N NaOH and used for the estimation of GnRH. The levels of FSH and LH in the sera were estimated by RIA using NIAMDD systems and expressed in terms of NIAMDD-Rat-FSH-RP-1 and NIAMDD-Rat-LH-RP-1 respectively. The GnRH was measured by RIA using the polyethylene glycol precipitation method, employing a kit obtained from Biodata, Hypolab, SA.

Results and discussion. Both testicular and ovarian inhibin suppressed serum FSH levels without affecting LH (table). The hypothalamic GnRH content of both these groups was significantly ($p < 0.001$) less than that of rats treated with saline. Since the content of hypothalamic releasing hormone and the serum FSH levels were lowered at the same time we conclude that inhibin acts by blocking GnRH synthesis. The unaltered levels of LH probably indicate that the threshold amount of GnRH required for LH release could be less than that for FSH.

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Prednisolone-binding proteins in the rat liver and gastrocnemius muscle

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Summary. Quantitatively, liver cytosol preparations were found to contain 2.3 times the number of prednisolone-binding proteins that were present per mg total protein in the rat gastrocnemius muscle. The liver proteins were larger molecules and, in a low ionic environment, were more chemically stable than the muscle proteins.

When prednisolone acetate was injected s.c. every day over a period of 8–10 days into male Wistar rats, there were losses of RNA and protein in the gastrocnemius muscle of the animals[2]. However the concentrations of these 2 cell constituents were increased in the liver[2].

These changes were shown to involve alterations in RNA turnover in the 2 tissues. In the liver, the rate of synthesis of ribosomal RNA was shown to be increased and its rate of breakdown decreased while, in the muscle, prednisolone caused net decreases in the rates of synthesis and breakdown of ribosomal RNA[2]. An increase in the activity of the RNA polymerase believed to be responsible for the synthesis of ribosomal RNA in the liver was also observed while, in the muscle, prednisolone treatment was shown to cause a decrease in the activity of this enzyme[2]. Thus, the alterations in the enzymic activities could explain the observed changes in RNA turnover in prednisolone-treated animals. In view of the opposite effects which prednisolone had in the 2 tissues on RNA turnover and RNA polymerase activities, it is difficult to visualize an identical mode of action of this steroid in the tissues under study. It is conceivable, however, that the responses differ because prednisolone-protein complexes in the respective tissues differ in physicochemical properties and, therefore, affect different regions of the DNA template. An extension of this concept would suggest that such differences might also account for the phenomenon of side effects which prolonged administration of corticosteroids produces in patients[3,4]. Since it is widely believed[5–7] that the phenotypic response of target tissues to a steroid begins with an initial binding of the steroid to cytoplasmic receptor proteins located in the target tissue, it seemed appropriate, in explaining the action of prednisolone, to investigate differences in the proteins which bind the steroid in the cytoplasm and which presumably translocate it to the nucleus.

Materials and methods. Wherever possible, Analar grade reagents were used without further purification. [6,7(n)-^{3}H] prednisolone was obtained from Radiochemical Centre, Amersham, England, in toluene/ethanol mixtures. For use, 0.1 ml unlabelled prednisolone in toluene/ethanol (9:1), containing 0.036 mg prednisolone was mixed with 0.4 ml ^{3}H-labelled prednisolone to give a final steroid concentration of 9.6 µM (sp. act. 173 µCi per mg prednisolone). The mixture was evaporated to dryness under nitrogen at 20 °C and redissolved in 0.5 ml absolute ethanol.

Preparation of cytosols and prednisolone-protein complexes. Gastrocnemius muscle, perfused in situ for 2 min with ice-cold 0.9% (w/v) saline was excised quickly from the animal and minced finely. The tissue was suspended in 4 times its volume of the homogenisation buffer (1,0 mM sodium phosphate buffer, pH 7.4, containing 0.5% (v/v) thiodiglycol) and homogenized using a Silverson homogenizer (Silverson Machines, Bucks, England) driven at top speed. This homogenisation procedure was carried out in 2 bursts each lasting 30 sec. The homogenate was immersed in ice throughout this procedure and a 2-min interval was interposed between each burst to maintain the temperature of the homogenate between 0 and 4 °C. Thereafter, the homogenate was centrifuged at 60,000 × g for 2 h at 2 °C. The supernatant constituted the cytosol fraction and was used within 24 h of preparation without further purification.