of embryos to determine apparent embryonic age showed that exposure to R24571 for the first 24 h inhibited development as severely as exposure for the whole of the 48 h culture. Development was also inhibited in embryos exposed to R24571 for the last 24 h of culture, but to a lesser extent than in embryos exposed for the entire 48 h culture (table 1). Measurement of crown-rump lengths gave similar results; growth of embryos exposed to R24571 for the first 24 h of culture was more inhibited than that of embryos exposed for the last 24 h. Comparison of the frequency and types of abnormalities suggested that embryos exposed to R24571 for the first 24 h of culture showed similar types of abnormalities to those exposed for the entire culture period. Embryos exposed to R24571 for the last 24 h of culture showed similar abnormalities but at a lower frequency (table 2).

Thus the present results show that R24571, which inhibits the activity of the calcium-binding protein calmodulin, inhibits the growth of rat embryos in vitro and causes a number of morphological defects, including defects of the nervous system. Recently it has been shown that interference with calcium ion fluxes in mammalian embryos can perturb neural tube formation[14], suggesting that, as in amphibian embryos, neurulation in mammalian embryos is brought about by cell shape changes affected by calcium-activated microfilaments. the demonstration that an inhibitor of calmodulin activity causes abnormal neurulation and perturbs other morphogenetic movements suggests that calmodulin may play a part in the control of calcium levels which are important to microfilament-mediated changes in cell shape during morphogenesis.

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FSH receptor binding inhibiting activity associated with low molecular weight inhibin from sheep, human, rat and chicken

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Summary. Low molecular weight inhibin (1500 daltons) was obtained from sheep, human, rat and chicken testes by sequential chromatography on Sephadex G-100 and G-25. In addition to its ability to suppress circulating FSH levels in adult castrated male rats, it also exhibits binding inhibition of I^{125}hFSH to rat testicular receptors.
Key words. Testes; inhibin; FSH receptor; FSH binding inhibiting activity.

Inhibin appears to regulate circulating levels of FSH either by its direct modulatory action on pituitary responsiveness to hypothalamic GnRH[1] or via the hypothalamus where it may have a regulatory role in GnRH output[2]. Available evidence suggests that in addition to its regulatory action on circulating FSH, inhibin has inhibitory effect on the action of FSH at the gonadal level, in that it inhibits binding of FSH to gonadal receptors in vitro[1,3-5]. In our earlier studies we demonstrated that low molecular weight peptides with inhibin activity obtained from sheep ovaries and testes exhibit FSH-binding inhibiting (FSH-BI) activity. The present study was designed to examine whether the FSH-BI like property is common to inhibin preparations obtained from other species.

Materials and methods. Inhibin preparations were isolated by fractionating the high-speed supernatant derived from 40% homogenates of human, ovine, rat and chicken testicular tissues, sequentially on Sephadex G-100 and G-25 columns as described earlier[6]. The human, ovine, rat and chicken inhibin preparations used in the present study were equivalent to Hm-3-II, Rm-3-II, Rt-3-II and Ch-3-II respectively[6] and are peptides of about 1500 daltons molecular weight. Inhibin activity of these preparations was assessed by their ability to suppress circulating levels of FSH in castrated adult male rats. 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 anesthesia and their sera collected. 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. Serum FSH and LH levels in the groups of animals treated with the test material were compared with those in the saline-treated group. Significant suppression of FSH levels were taken as an index of inhibin activity.

The FSH-BI-like activity of these preparations was tested using a system containing ^{125}I-hFSH and rat testicular receptors as described by Reichert and Abou-Issa[7]. The receptor preparation was prepared from testes of mature (90-day-old) rats of the Holtzman strain and was equivalent to fraction R-1 of Reichert and Abou-Issa[7]. Human FSH (LER-1575C) was iodinated to a specific activity of 10–12 μCi/μg using chloramine T by the method described by Reichert and Bhalla[8]. The assay was carried out in 10 × 100 mm glass centrifuge tubes to which was added 500 μl of receptor preparation followed by an appropriate amount of inhibin dissolved in 400 μl of 0.05 M Tris-HCl buffer, pH 7.5 (containing 0.1% BSA, 0.1 M sucrose and 5 mM MgC12) and 5 ng ^{125}I-hFSH in 100 μl. The tubes were incubated in a metabolic shaker at 37°C for 3 h. The incubated tubes were centrifuged at 1500 × g for 15 min and the supernatant discarded. The tissue pellets were resuspended in 1 ml of chilled Tris-HCl buffer and subjected to a 2nd centrifugation. The supernatants were decanted, the tubes were drained and the radioactivity bound to the pellets counted in a gamma ray spectrometer. All statistical evaluations were performed using Student's t-test. The relative potencies of different inhibin preparations were calculated by the method of Borth[9].

Table 1. Inhibition of binding of I^{125} hFSH to rat testicular receptor by inhibin

Species	Dose	No. of observations	Percentage binding (mean ± SE)	Percentage inhibition	Mean relative potency in terms of sheep inhibin preparation (fiducial limits at 95% confidence level)
Control		4	5.01 ± 0.04*	–	–
Sheep	25 µg	4	3.43 ± 0.02*	31.5	–
	50 µg	4	2.36 ± 0.02*	52.9	–
	100 µg	4	1.59 ± 0.02*	68.3	–
	200 µg	4	1.00 ± 0.02*	80.0	–
Human	25 µg	4	3.17 ± 0.06*	36.7	0.92 (0.88–0.95)
	50 µg	4	2.55 ± 0.04*	49.1	
	100 µg	4	1.82 ± 0.03*	63.7	
	200 µg	4	1.19 ± 0.02*	67.2	
Rat	25 µg	4	2.51 ± 0.03*	49.9	1.58 (1.79–1.44)
	50 µg	4	2.02 ± 0.02*	59.7	
	100 µg	4	1.39 ± 0.04*	72.3	
	200 µg	4	0.60 ± 0.02*	88.0	
Chicken	25 µg	4	3.54 ± 0.02*	29.3	0.71 (0.65–0.75)
	50 µg	4	2.76 ± 0.02*	44.9	
	100 µg	4	2.15 ± 0.02*	57.1	
	200 µg	4	1.36 ± 0.06*	72.9	

*$p < 0.001$

Table 2. Suppression of post-castrational rise in serum FSH by inhibin in castrated adult male rats

Species	Dose	No. of animals	Serum rFSH µg/ml (mean ± SE)	Percentage inhibition	Mean relative potency in terrms of sheep inhibin preparation (fiducial limits at 95% confidence level)
Control		11	2.39 ± 0.07	–	–
Sheep	250 µg	5	1.89 ± 0.05*	20.9	–
	500 µg	5	1.56 ± 0.09*	34.7	–
	1000 µg	5	1.34 ± 0.06*	43.9	–
Human	250 µg	6	1.45 ± 0.06*	39.3	1.75 (1.29–2.59)
	500 µg	6	1.34 ± 0.04*	43.9	
Rat	250 µg	6	1.59 ± 0.07*	33.5	1.44 (1.06–2.06)
	500 µg	6	1.32 ± 0.05*	44.8	
Chicken	250 µg	6	1.95 ± 0.07**	18.4	0.65 (0.22–1.00)
	500 µg	6	1.57 ± 0.07*	34.3	

*$p < 0.001$; **$p < 0.01$.

Results and discussion. Our previous studies have demonstrated that low molecular weight peptides with inhibin activity, isolated from ovine testes and ovaries, exerted an inhibitory action on the binding of ^{125}I-hFSH to rat testicular receptors in vitro[1,3]. The present study is an extension on these observations to similar peptides obtained from human, rat and chicken testes. It was observed that all the preparations exhibited FSH-BI-like activity in a dose related manner (table 1). In the absence of a reference preparation, human, rat and chicken inhibin preparations were compared with the ovine inhibin preparation which has been characterized to some extent in our laboratory. The rat preparation was observed to be 1.58 times as active as the ovine preparation while the human and chicken preparations were 0.92 and 0.70 times as active, respectively. In a similar exercise, when the inhibin activity of these preparation as assessed by their ability to suppress circulating levels of serum FSH in castrated adult male rats was compared, the human preparation was 1.75 times as active as the ovine preparation while those from rat and chicken were 1.44 and 0.65 times as active, respectively (table 2). The observed differences in these 2 activities exhibited by the inhibin preparations could be due to the inherent differences in the two assay systems. Daume et al.[4] and Sato et al.[5] have also reported that their inhibin preparations derived from porcine, human and bovine follicular fluid exhibited FSH-BI activity. Whether these observations could be interpreted to mean that FSH-BI is an intrinsic property of inhibin or are simply indicative of co-purification of 2 activities will be known only when inhibin is purified completely.

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