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Isolation of inhibin like peptides from human placenta

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Abstract. Two moieties of inhibin could be obtained by chromatography of partially purified preparations of inhibin from human placenta on Sephadex G-100, G-25 and ion exchange chromatography on diethylaminoethyl Sephadex A-50. The higher molecular weight moiety (14,000) designated as HPI-H appears to be similar to inhibin from human seminal plasma. While the lower molecular weight moiety (1500) designated as HPI-L appears to be similar to that of sheep testicular inhibin.

The preparations from both human term placenta and human seminal plasma inhibited the binding of [^{125}I] human follicle stimulating hormone to rat testicular receptors. This effect of inhibins could be neutralized by antisera raised against corresponding polypeptide. Further these antibodies could neutralize endogenous inhibin resulting in 2 to 3 fold increase in serum follicle stimulating harmone levels, which could then be reversed by exogenous administration of the isolated inhibin preparations.

Keywords. Inhibin; placenta; human.

Introduction

The placenta, which is known to secrete a number of peptides has also been shown to be a source of inhibin-like peptide (Bandivdekar *et al.,* 1981; Hochberg *et al.,* 1981; Apte and Sheth, 1982). The present report deals with the purification and characterization of inhibin-like peptides from full term human placenta.

Materials and methods

Human placentae from full term normal deliveries were obtained from maternity hospitals. Fresh placentae were collected and trasported to the laboratory in ice and processed immediately.

The foetal part of the placenta was separated as far as possible from the maternal part and was homogenized in 0·01 M sodium phosphate buffer pH 7·4 in a Braun blender to obtain a 40 % (w/v) homogenate. The homogenate was then centrifuged for 60 min at 25,000 *g,* using a Type-19 rotor in a Spinco L2-65 B centrifuge.

Abbreviations used: HPI-H, Human placental inhibin–high molecular weight; HPI-L, human placental inhibin–low molecular weight; FSH, follicle stimulating hormone; LH, lutinizing hormone; DEAE, diethylaminoethyl; PAGE, Polyacrylamide gel electrophoresis; Con-A, concanavalin A; NRS, normal rabbit serum; RIA, radioimmunoassay; HSPI, human seminal plasma inhibin; hFSH, human FSH.

Bioassay for detection of inhibin activity

The fractions obtained at each stage of purification were assayed for inhibin activity in adult intact male rats. Adult male rats of the Holtzman strain, weighing between 200–250 g and 90 days of age were injected intramuscularly once daily with 1 ml saline or the test material, for 3 days. Four h after the last injection, the animals were bled and sera collected. The levels of follicle stimulating hormone (FSH) and lutinizing hormone (LH) in these sera were determined by radioimmunoassay using NIAMDD systems and were expressed in terms of NIAMDD-Rat-FSH-RP-1 and NIAMDD-Rat-LH-RP-1, respectively. The serum FSH levels obtained from groups of animals treated with test material were compared with those from the saline treated group. Any significant suppression of FSH levels were taken as an index of inhibin activity. All Statistical evaluations were performed using Student's '*t*' test.

Chromatography on Sephadex G-100

The 25,000 *g* supernatant (containing a maximum amount of about 1 g protein) was charged on to Sephadex G-100 column (5 × 100 cm) pre-equilibrated with 0·01 M sodium phosphate buffer, pH 7·4. The column was then eluted with the same buffer at the rate of 40 ml/h and 10 ml fractions collected. The different fractions were pooled, and designated on the basis of the order of their elution *viz.* HPI-G-100-1; HPI-G-100-2 and HPI-G-100-3. The fractions with inhibin activity were concentrated by ultrafiltration or lyophilization and subjected to further chromatography.

The fraction HPI-G-100-2 was rechromatographed on Sephadex G-100 column (3 × 150 cm). The fraction exhibiting inhibin activity designated as HPI-G-G-2 was further subjected to ion exchange chromatography.

Chromatography on Sephadex G-25

The fraction HPI-G-100-3 was further subjected to gel filtration on Sephadex G-25 column (2·5 × 38 cm). Five fractions were obtained. The fraction eluting immediately after the void volume was found to be active and was designated as HPI-L (human placental inhibin – low molecular weight moiety).

Ion exchange chromatography on DEAE Sephadex A-50

The fraction HPI-G-G-2 was then charged on diethylaminoethyl (DEAE)-Sephadex A-50 column (2·5 × 20 cm). Both the column and the fraction were equilibrated with 0·05 M Tris-HCl buffer, pH 8·0. The column charged with the fraction was then eluted initially with the same buffer and then stepwise with 0·01, 0·05, 0·1, 0·5 and 1·0 M sodium chloride in 0·05 M Tris-HCl buffer, pH 8·0. The fractions were collected at the rate of 20 ml/h. The fraction with inhibin like activity was further rechromatographed on DEAE Sephadex A-50, using 0·05 M Tris HCl buffer, pH 8·0 for equilibration and initial elution and 0·1, 0·2 and 0·5 M NaCl in 0·05 M Tris-HCl buffer, pH 8·0 for stepwise elution of the column. The fractions were pooled and dialysed extensively against distilled water and lyophilized. The active fraction was designated as HPI-H (human placental inhibin–high molecular weight).

Inhibin from human seminal plasma was purified as described earlier (Sheth *et al.*

1984). Acetone dry powder of human seminal plasma was chromatographed on Sephadex G-100 using 0·05 M sodium acetate buffer pH 4·0 and then on DEAE-Sephadex A-50. Purity of the inhibin preparation was checked on reverse phase high performance liquid chromatography (Sheth *et al.,* 1984).

The protein content of the fractions obtained at different stages of purification were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as Standard.

Polyacrylamide gel electrophoresis

Homogeneity of the high molecular weight active fractions were tested on polyacrylamide disc gel electrophoresis (PAGE) at pH 8·3 using 7·5 % running gel (Davis 1964).

Chromatography on Concanavalin A Sepharose 4B

About 20 mg of purified HPI-H was applied on Concanavalin A (Con-A) Sepharose-4B column (3 × 30 cms), pre-equilibrated with 0·1 M sodium acetate buffer, pH 6·0 containing 1 M NaCl, 1 mM each of $MnCl_2$ and $CaCl_2$. The column was then washed with the same buffer until the effluent showed no measurable absorbance at 280 nm. Glycoproteins if present bind strongly to Con-A Sepharose-4B and can be recovered by elution of the column with 2 % α-methyl-D mannoside in the same buffer.

Preparation of antibodies to human placental inhibin
(*high molecular weight*)–(*As-HPI-H*)

Antibodies to the high molecular wieght inhibin preparation obtained from human placenta (HPI-H) were raised in rabbits by active immunization. 100 μg of purified HPI-H was dissolved in 1 ml saline and emulsified with an equal volume of Freund's complete adjuvant and administered intra-dermally at multiple sites. A second injection was given 6 weeks later. Four weeks after the second injection, the animals were bled and the sera harvested and stored frozen until tested and was designated as As-HPI-H.

Antibodies to human seminal plasma inhibin (As-HSPI) were raised in our laboratory as described by Vaze *et al.,* (1979).

In-vivo neutralization of endogenous inhibin in immature rats with As-HPI-H

Ten day old male rats of Holtzman strain were injected with 50 and 100 μl of As-HPI-H or with normal rabbit serum (NRS) (as a control) on days 10, 11 and 12 of age. The animals were bled 4 h after the last injection. The serum FSH levels were measured by radioimmunoassay (RIA).

The *in vivo* effects observed in the above experiments were again confirmed in intact adult male rats for specific neutralization. Three month old intact male rats were injected for 3 days with a total dose of either 10 and 20 μg of HPI-H, or 250 and 500 μg of HPI-L, or 50 and 100 µg of human seminal plasma inhibin (HSPI). Another group of animals were administered 50 and 100 μ1 of either As-HPI-H or As-HSPI. Several combinations of HPI-L, HPI-H and HSPI with 100 μl of As-HPI-H and As-HSPI were also administered. In the two control groups, saline and NRS were injected as controls.

Four h after the last injection, animals were bled by cardiac puncture and their sera were harvested and assessed for FSH levels by RIA.

In vitro effect of inhibin and its antiserum

In vitro FSH receptor binding inhibition by inhibin: Receptors were prepared from testes of 90 day old rats of Holtzman strain and was equivalent to fraction R-l of Reichert and Abou-Issa (1977). Five hundred μl of this receptor preparation was incubated with 5 ng of [^{125}I]-human FSH (hFSH), in the presence of appropriate amounts of HPI-H, HPI-L and HSPI, dissolved in 400 μl of 0·05 M Tris-HCl buffer pH 7·5 containing 0·1 % bovine serum albumin, 0·1 M sucrose and 5 mM $MgCl_2$ (assay buffer). The tubes containing the above Tris-HCl buffer instead of test material served as control while, non specific binding was determined by the addition of a 1000 fold excess cold hFSH (*i.e.* 5 μg). The tubes were then incubated in a metabolic shaker at 37°C for 3 h and then resuspended in 2 ml chilled buffer and centrifuged at 1500 *g* for 20 min. The supernatants were decanted and the tubes were counted in a gamma ray spectrometer.

In vitro neutralization of FSH-receptor binding inhibitor activity by antibodies to inhibin: The antibodies to HPI-H and HSPI in various combinations were pre-incubated at 4°C for 24 h with HPI-H, HPI-L and HSPI. HPI-H, HPI-L and HSPI were also preincubated with 50 and 100 μl of NRS and assay buffer. After a 24 h incubation in cold and then centrifugation to remove the precipitated antigen-antibody complex, the supernatant was checked for FSH-RBI activity as described above.

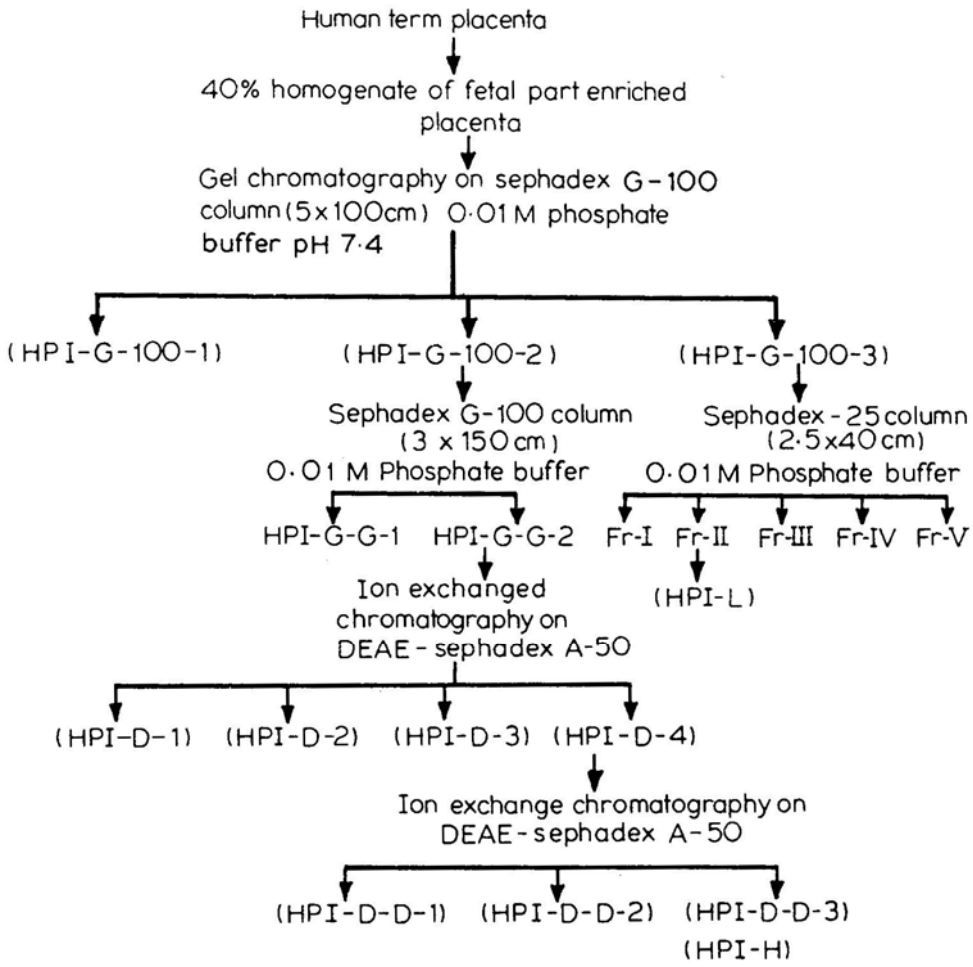
Results

Flow-chart 1 shows the schematic representation of purification of inhibin from human placenta. The fractions obtained following gel filtration are shown in figure 1. Fractions HPI-G-100-2 and HPI-G-100-3 significantly suppressed serum FSH levels in intact adult male rats, while fraction HPI-G-100-1 did not do so (table 1).

The most retarded fraction *i.e.* HPI-G-100-3 resolved into 5 fractions (figure 2) following further chromatography on Sephadex G-25 column. The fraction eluting immediately after the void volume, exhibited inhibin-like activity as assessed by bioassay in intact adult male rats, while the other fractions were devoid of any inhibin-like activity (table 2). This active fraction with inhibin like activity was designated as HPI-L.

The HPI-G-100-2 fraction following rechromatography on Sephadex G-100 column (3 × 150 cm) resolved into two fractions (figure 3). Inhibin-like activity was observed in the fraction which eluted later, when tested in intact adult male rats (table 3). This active fraction was designated as HPI-G-G-2.

Four fractions were obtained when the fraction HPI-G-G-2 was subjected to ion exchange chromatography on DEAE-Sephadex A-50 column and eluted stepwise with 0·01 to 1 M NaCl in 0·05 M Tris-HCl pH 8·0 (all the fractions were obtained when the column was eluted with 0·1 M NaCl in 0·05 M Tris-HCl buffer, pH 8·0, figure 4). The fractions were designated as HPI-D-1, HPI-D-2, HPI-D-3 and HPI-D-4. Bioassay of



Flow-chart 1. Schematic representation of purification of inhibin from human term placenta.

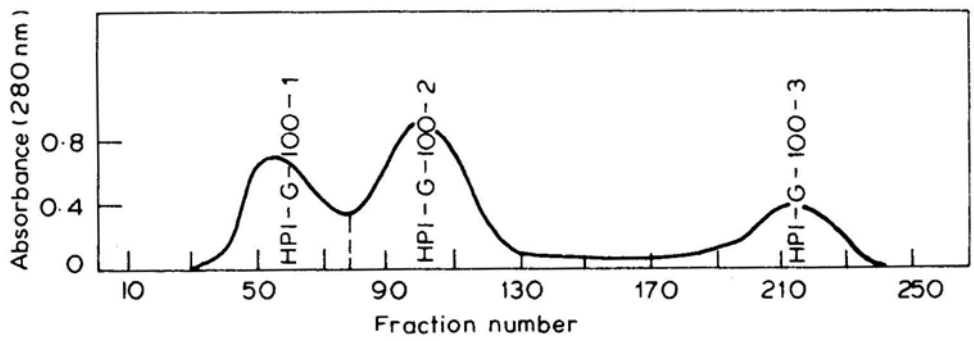


Figure 1. Elution profile of human placental homogenate on Sephadex G-100 column (5 × 100 cm). The elution buffer was 0·01 M sodium phosphate buffer, pH 7·4, the flow rate was 40 ml/h and the fraction volume was 10 ml.

Table 1. Inhibin activity of the fractions obtained by chromatography of placental homogenate on Sephadex G-100 column (5 × 100 cm)

Treatment	Dose in mg	No. of observations	rLH ng/ml ±S.E.	rFSH ng/ml ±S.E.	Inhibition in serum FSH levels (%)
Saline	—	7	220·3 ± 11·2	339·7 ± 21·1	—
HPI-G-100-I	1·5	6	190·2 ± 15·9	394·0 ± 29·6	—
	3·0	6	186·2 ± 13·1	410·5 ± 24·1	—
HPI-G-100-II	1·5	6	195·0 ± 19·1	268·2 ± 20·5*	32·9
	3·0	6	210·7 ± 15·7	191·7 ± 21·8**	52·1
HPI-G-100-III	1·5	6	196·7 ± 10·8	300·2 ± 27·3*	24·9
	3·0	5	185·2 ± 19·5	234·8 ± 15·6**	41·3

* $P < 0{\cdot}01$. ** $P < 0{\cdot}001$.

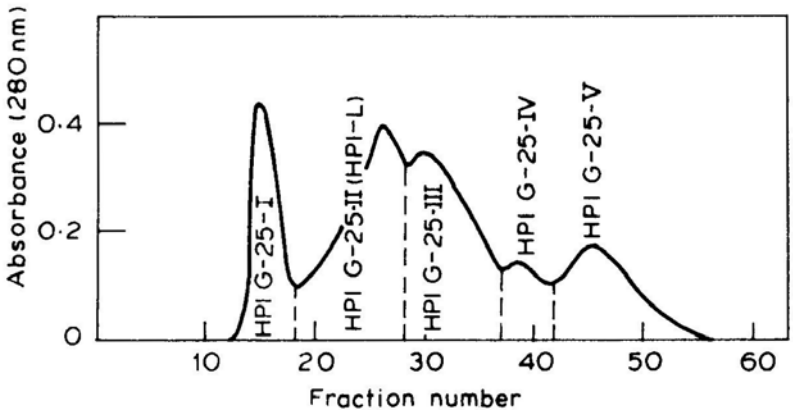


Figure 2. Elution profile of rechromatography of fraction HPI-G-100-3 on Sephadex G-25 column (2·5 × 40 cm). The elution buffer was 0·01 M sodium phosphate buffer, pH 7·4, the flow rate was 18 ml/h and the fraction volume was 3 ml.

these fractions in intact adult male rats showed the existence of inhibin activity in fraction HPI-D-4 (table 4).

Rechromatography of HPI-D-4 on DEAE-Sephadex A-50 when eluted with 0·1 M NaCl in 0·05 M Tris-HCl buffer, pH 8·0 further resolved into 3 fractions. These fractions were designated on the basis of their emergence as HPI-D-D-1, HPI-D-D-2 and HPI-D-D-3 (figure 5). HPI-D-D-3 could significantly suppress FSH levels in intact adult male rats while the other fractions were devoid of FSH suppressing activity (table 5).

The fraction HPI-D-D-3 was found to be homogeneous on Polyacrylamide gel electrophoresis at pH 8·3 (figure 6) and this fraction was designated as HPI-H.

Table 2. Inhibin activity of the fractions obtained by rechromatography of HPI-G-100-3 on Sephadex G-25 column (2·5 × 40 cm).

Treatment	Dose in μg	No. of observations	rLH ng/ml ± S.E.	rFSH ng/ml ± S.E.	Inhibition in serum FSH levels (%)
Saline	—	5	221·4 ± 16·4	415·4 ± 20·0	—
HPI-G-25-I	250	6	211·2 ± 14·3	417·7 ± 22·2	—
	500	5	199·6 ± 19·6	410·0 ± 26·9	—
HPI-G-25-II (HPI-L)	250	5	202·2 ± 18·0	302·0 ± 7·5*	27·30
	500	5	202·8 ± 17·4	209·4 ± 13.0*	49·6
HPI-G-25-III	250	6	213·5 ± 14·6	383·2 ± 25·7	—
	500	6	190·4 ± 9·28	403·0 ± 16·3	—
HPI-G-25-IV	250	5	208·2 ± 16·7	412·0 ± 27·1	—
	500	5	226·8 ± 28·1	400·4 ± 22·5	—
HPI-G-25-V	250	6	192·6 ± 12·1	428·8 ± 22·7	—
	500	6	194·7 ± 22·7	395·3 ± 25·2	—

* $P < 0{\cdot}01$.

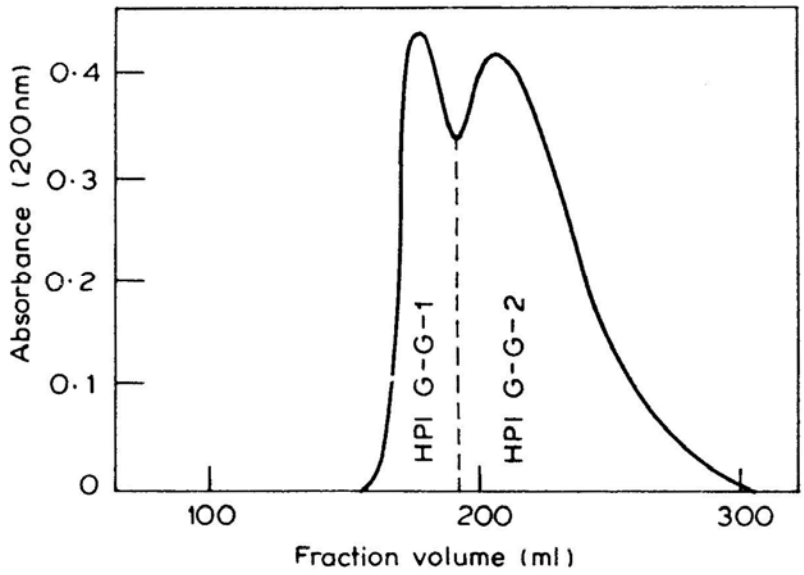


Figure 3. Elution profile of rechromatography of fraction HPI-G-100-2 on Sephadex G-100 column (3 × 150 cm). The elution buffer was 0·01 M sodium phosphate buffer, pH 7·4, the flow rate was 20 ml/h and the fraction volume was 5 ml.

Table 3. Inhibin activity of the fractions obtained by rechromatography of HPI-G-100-2 on Sephadex G-100 column (3·0 × 150 cm).

Treatment	Dose in mg	No. of observations	rLH ng/ml ± S.E.	rFSH ng/ml ± S.E.	Inhibition in serum FSH levels (%)
Saline	—	6	206·3 ± 14·2	410·5 ± 20·4	—
HPI-G-G-1	0·5	6	204·7 ± 20·2	425·7 ± 25·2	—
	1·0	5	212·6 ± 15·3	420·8 ± 35·5	—
HPI-G-G-2	0·5	6	192·8 ± 13·3	327·2 ± 12·6*	20·3
	1·0	6	190·2 ± 14·1	267·0 ± 12·1**	35·0

* $P < 0{\cdot}01$ ** $P < 0{\cdot}001$.

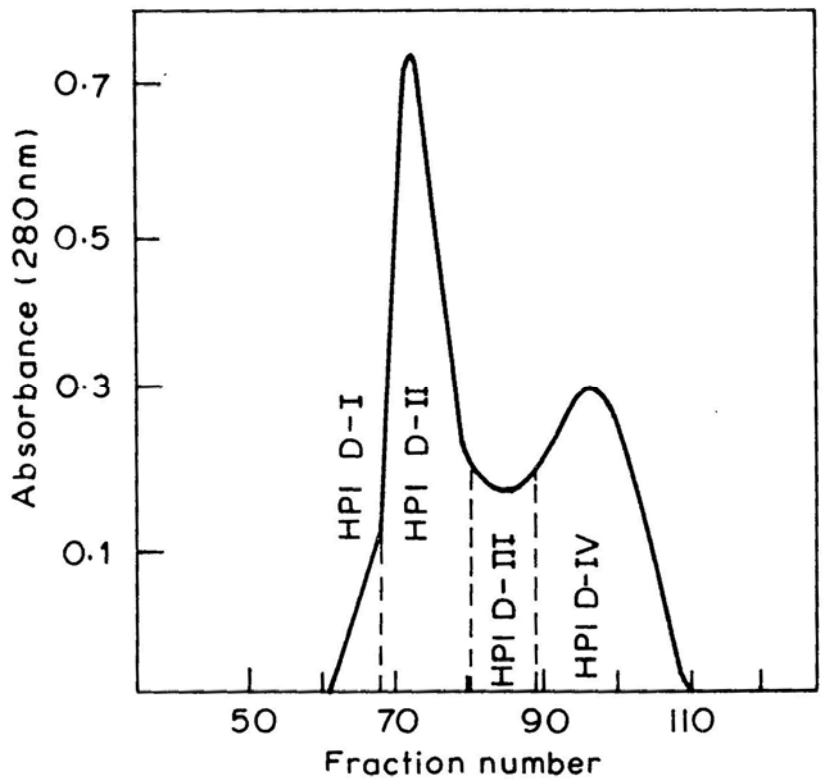


Figure 4. Elution profile of ion-exchange chromatography of fraction HPI-G-G-2 on DEAE Sephadex A-50 column. Fractions were eluted with 0·1 M NaCl in 0·05 M Tris-HCl pH 8·0.

Table 4. Inhibin activity of the fractions obtained by ion exchange chromatography of HPI-G-G-2 on DEAE Sephadex A-50 column.

Treatment	Dose in μg	No. of observations	rLH ng/ml ± S.E.	rFSH ng/ml ± S.E.	Inhibition in serum FSH levels (%)
Saline	—	5	193·4 ± 3·9	392·6 ± 21·7	—
HPI-D-1	50	5	189·4 ± 6·1	413·4 ± 27·7	—
	100	5	188·2 ± 8·3	392·0 ± 28·6	—
HPI-D-2	50	5	186·8 ± 5·9	400·4 ± 20·4	—
	100	5	176·4 ± 10·9	413·4 ± 27·8	—
HPI-D-3	50	5	190·4 ± 9·3	365·8 ± 22·6	—
	100	5	202·0 ± 22·0	392·8 ± 25·9	—
HPI-D-4	50	6	196·7 ± 11·7	298·0 ± 8·4*	24·1
	100	6	185·3 ± 9·2	264·5 ± 8·9**	32·6

* $P < 0{\cdot}01$. ** $P < 0{\cdot}001$.

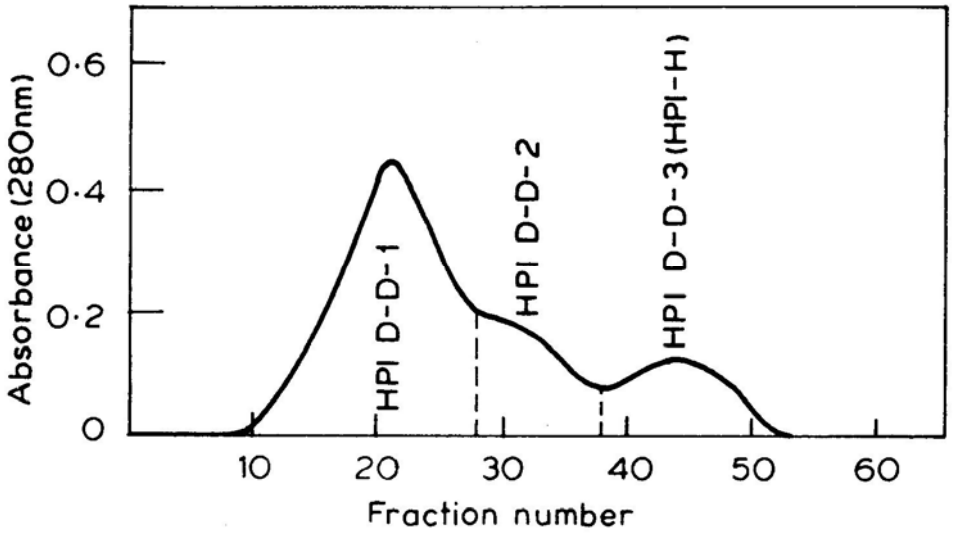


Figure 5. Elution profile of ion exchange chromatography of fraction HPI-D-4 on DEAE Sephadex A-50 column. Fractions were eluted with 0·1 M NaCl in 0·05 M Tris-HCl pH 8·0.

The purified HPI-H when charged on Con-A Sepharose 4B, was eluted in the unadsorbed fraction only, suggesting that it is not glycoprotein.

In vivo effect of inhibin and its antiserum

Immature male rats: As-HPI-H when injected in 10 day old male rats on day 10, 11 and 12 of age caused 2 to 3 fold increase in serum FSH levels at a total dose of 50 and 100 μl respectively (table 6).

Table 5. Inhibin activity of the fractions obtained by ion exchange chromatography of HPI-D-4 on DEAE-Sephadex A-50.

Treatment	Dose in μg	No. of observations	rLH ng/ml ± S.E.	rFSH ng/ml ± S.E.	Inhibition in serum FSH levels (%)
Saline	—	6	207·0 ± 16·7	400·2 ± 21·0	—
HPI-D-D-1	10	5	200·0 ± 25·5	417·6 ± 10·9	—
	20	5	188·4 ± 15·1	384·8 ± 29·0	—
HPI-D-D-2	10	5	196·2 ± 16·4	415·0 ± 20·1	—
	20	5	197·6 ± 18·3	400·8 ± 15·2	—
HPI-D-D-3	10	5	197·6 ± 11·3	347·6 ± 11·3*	13·1
(HPI-H)	20	5	179·4 ± 9·9	272·2 ± 11·9**	32·0

* $P < 0{\cdot}01$. ** $P < 0{\cdot}001$.

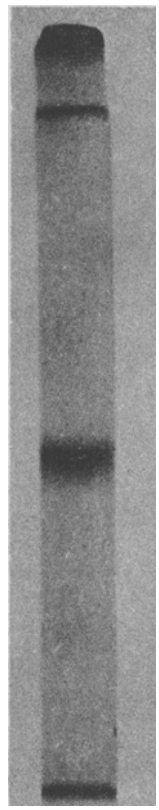


Figure 6. Electrophoretic pattern of HPI-H on Polyacrylamide gel electrophoresis at pH 8·3 using 7·5 % running gel.

Table 6. *In vivo* effect of As-HPI-H in 10 day-old intact male rats on circulating levels of FSH.

Treatment	rFSH μg/ml ±S.E.	Number of observations
Control	3·32±0·22	6
50 μl AS HPI-H	7·08±0·68*	5
100 μl As HPI-H	9·50±0·45*	4

* $P < 0{\cdot}001$.

Adult male rats: While confirming the specific *in vivo* neutralization of endogenous inhibin, As-HPI-H and As-HSPI were injected and in another group HPI-H, HPI-L and HSPI were injected. In yet another group, several combinations of As-HPI-H, As-HPSI and HPI-H, HPI-L, HSPI were injected. NRS and saline were injected in two control groups. Antiserum to HPI-H and HSPI caused about 2 to 3 fold increase in serum FSH at the dose of 50 and 100 μl respectively, while HPI-H, HPI-L and HSPI caused about 20 to 70 % inhibition in serum FSH levels.

When simultaneous administration of 100 μl of As-HPI-H/As-HSPI alongwith HPI-H, HPI-L or HSPI resulted in a decrease in FSH levels (figure 7) than those observed with antiserum alone, suggesting thereby the specific *in vivo* neutralization of inhibin.

In vitro effect of inhibin and its antiserum

FSH binding inhibition to its receptor: Five to six per cent specific binding of [^{125}I]-hFSH to rat testicular receptor was observed. This specific binding was inhibited in a dose related manner by HPI-H, HPI-L and HSPI when 25 to 200 μg were added (table 7).

An increased binding of [^{125}I]-hFSH to rat testicular receptor was observed in the presence of As-HPI-H and HSPI as compared to when HPI-H and HSPI were incubated alone (figure 8).

While As-HPI-H and As-HSPI preincubated with HPI-L did not significantly effect [^{125}I]-hFSH binding to rat testicular receptors. These results suggested that As-HPI-H and As-HSPI neutralized the *in vitro* activities of HPI-H and HSPI while HPI-L did not (figure 8).

Discussion

Inhibin, a FSH inhibiting peptide, shown to be of a gonadal as well as extragonadal origin was isolated from various sources. The existence and partial purification of inhibin using human term placenta as a source was reported for the first time from our laboratory (Bandivdekar *et al.,* 1981). The present study describes the purification and immunobiological characterization of inhibin from human placenta.

Studies on the *in vitro* synthesis of inhibin by the human placenta established that

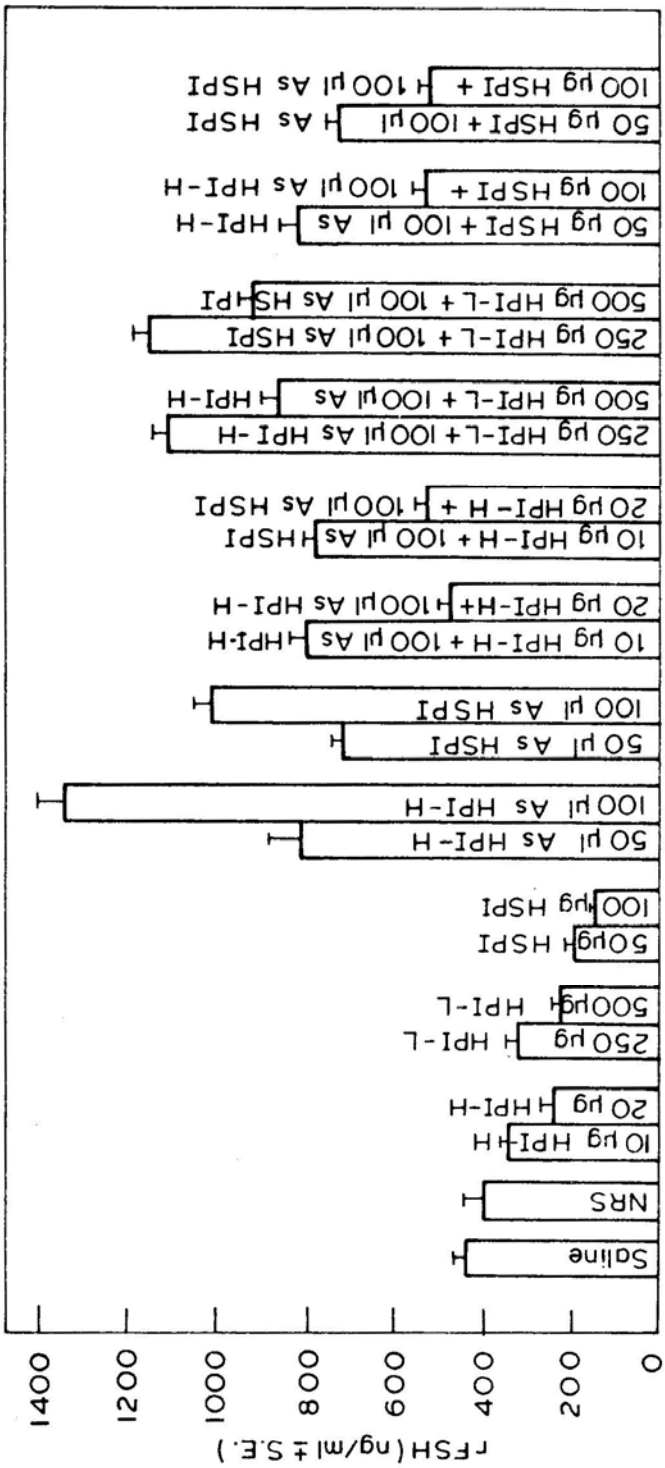


Figure 7. *In vivo* effect of HPI-H, HPI-L and HSPI and As-HPI-H and As-HSPI on FSH levels in adult male rats (*in vivo* neutralization of inhibin with As-HPI-H and As-HSPI).

Table 7. *In vitro* binding inhibition of [^{125}I]-hFSH to rat testicular receptor by HPI-H, HPI-L and HSPI.

Treatment		Dose in μg	Binding of [^{125}I]-hFSH to its receptor M ± S.E. (%)	Inhibition of binding of [^{125}I]-hFSH to its receptor (%)
Buffer control	(4)	—	5·29 ± 0·05	—
HPI-H	(3)	25	4·99 ± 0·12	5·67
	(3)	50	4·76 ± 0·13**	10·02
	(3)	100	3·29 ± 0·08*	37·81
	(3)	200	2·59 ± 0·12*	51·04
HPI-L	(3)	25	2·60 ± 0·08*	50·85
	(3)	50	2·13 ± 0·06*	59·74
	(3)	100	1·69 ± 0·04*	68·05
	(3)	200	1·40 ± 0·08*	73·53
HSPI	(3)	25	4·99 ± 0·06**	5·67
	(3)	50	4·57 ± 0·20**	13·61
	(3)	100	3·55 ± 0·25*	32·89
	(3)	200	2·67 ± 0·16*	49·53

* $P < 0·01$. ** $P < 0·001$.
Numbers in parentheses represent the number of observations.

only the fetal part synthesizes inhibin (Bandivdekar *et al.,* 1984). Hence, for the purification only fetal part enriched portion of the placenta was used.

The present study reports the isolation of a high molecular weight and a low molecular weight moiety exhibiting inhibin like activity from the human placenta. The HPI-H appears to be homogeneous as judged by PAGE. The chromatographic elution profile of HPI-H and that of HSPI suggests the possible similarity of these two preparations, while the HPI-L preparation from human placenta exhibited similar elution profiles as described earlier for inhibin preparations obtained from testes of sheep, human, rat and chicken (Vijayalakshmi *et al.,* 1980; Bandivdekar *et al.,* 1982). However, a detailed chemical structure is needed to confirm their identity.

Several different molecular weight proteins ranging from 1500 to 160,000 exhibiting inhibin-like activity have been reported (Moodbidri *et al.,* 1976; Vijayalakshmi *et al.,* 1980; Cahoreau *et al.,* 1979; Lugaro *et al.,* 1974; deJong and Sharpe, 1976; Sairam 1981; Schwartz and Channing, 1977). These differences in molecular weight could be either due to polymer formation or due to combination of native inhibin and a carrier substance or a large active protein giving rise to smaller still active fragments. Yet another possibility is the presence of several active peptides with inhibin-like activity. But the cause, if any, for this association or dissociation is not yet clear. Whether it is due to experimental artefacts or due to presence of some intrinsic factor(s) in the starting material itself which is responsible for the aggregation or breaking up of the molecule has not been established.

Several workers have suggested that the initial extraction procedures like ethanol precipitation (Franchimont *et al.,* 1975), amicon ultrafiltration (Davies *et al.,* 1979) and

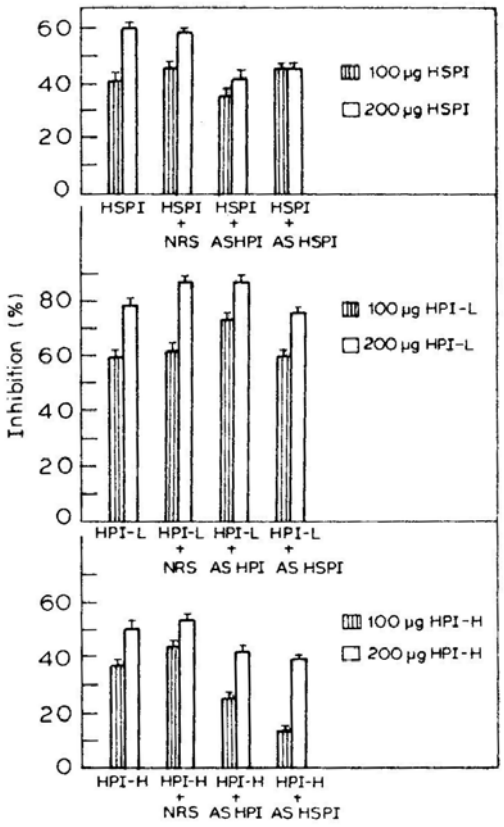


Figure 8. *In vitro* neutralization of HPI-H and HSPI with As HPI-H and As HSPI on the basis of their binding of [^{125}I]-hFSH to rat testicular receptors.

ammonium sulphate (Sato and Ishibashi, 1978) results in the aggregation of the molecules and hence, the reported differences in molecular weight.

Franchimont *et al.* (1977) reported that the low molecular weight species is lost when proteins are precipitated with ethanol as an initial step. This was later confirmed by Krishnan *et al.* (1982), who used ethanol in the initial step of purification of inhibin from human seminal plasma to obtain a high molecular weight inhibin moiety. They further observed that the alcohol supernatant after concentration using rotary vacuum evaporation and chromatography on Sephadex G-25 exhibited inhibin activity in the low molecular weight region.

These results suggest that the differences in reported molecular weights of inhibin are not due to aggregation of the molecule during alcohol precipitation or ultrafiltration.

In addition to the descripancies in molecular weight, it is still not clear whether or not inhibin is a glycoprotein. The low molecular weight form of inhibin obtained from various tissues or fluids appears to be non-glycoprotein in nature. However, based on the retention of activity on Con-A Sepharose columns and subsequent elution by competiting glycosides, it has been inferred that the inhibin molecule found in bovine follicular fluid is a glycoprotein (deJong *et al.,* 1980). While the studies from other

laboratories have shown the absence of carbohydrate moeity in inhibin obtained from bull seminal plasma (Chari *et al.,* 1978; Sairam 1981) and human seminal plasma (Thakur *et al.,* 1978). In the present study, purified high molecular weight placental inhibin was not bound to Con-A Sepharose-4B, suggesting the lack of carbohydrate in the placental inhibin. These results suggest but do not prove the absence of carbohydrate in the inhibin molecule, as substances may still have some sugar residue but simply lack the hexoses in the proper configuration that are usually necessary to be bound by Con-A Sepharose. Much work has been done on its biological actions in addition to the physicochemical characterization of inhibin. The results of several studies indicate that inhibin could act directly at the hypothalamus (Moodbidri *et al.,* 1981; Lugaro *et al.,* 1974; LeLannou and Chambon, 1977), pituitary (Baker *et al.,* 1976; Rush and Lipner, 1979; Franchimont *et al.,* 1978) and at the gonadal levels (Moodbidri *et al.,* 1980; Daume *et al.,* 1979). The present study demonstrates the binding inhibition of [^{125}I]-hFSH to rat testicular receptor by HPI-H, HPI-L and HSPI. HPI-L causes more inhibition than that of HPI-H or HSPI.

The binding inhibition effect of HPI-H and HSPI could be neutralized by both antibodies to HPI-H and HSPI; however, the binding inhibition observed due to HPI-L could not be neutralized by either of the antisera.

Similarly, in *in vivo* experiments the antibodies to HPI-H as well as HSPI caused dose related increase in serum FSH levels which may be due to neutralization of endogenous inhibin. This elevated increase in FSH could be suppressed by administration of HPI-H and HSPI.

The results of the present study establishes that the placenta is a source of both the high and low molecular weight inhibin like peptide. However, its chemical similarity with inhibin from the gonads remains to be established.

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