Recombinant forms of rat and human luteinizing hormone and follicle-stimulating hormone; comparison of functions *in vitro* and *in vivo*

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Abstract

We have previously described the preparation, purification and partial characterization of recombinant (rec) forms of rat luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In the present study, the special functional features of these hormones were studied further, *in vitro* and *in vivo*, and compared with human recLH and recFSH, as well as with human urinary choriongonadotropin (hCG) and rat pituitary LH (NIDDK-RP3). In radioreceptor assay, the affinity of hCG binding to rat testis membranes was 5-fold higher than that of human recLH and 100-fold higher than that of rat recLH. In *in vitro* bioassay, using dispersed adult mouse interstitial cells or a mouse Leydig tumor cell line (BLT-1), hCG and human recLH were 10- to 20-fold more potent than rat recLH. Correspondingly, rat pituitary LH was about 10-fold less potent than rat recLH, and evoked a maximum testosterone response that was about half of that elicited by the other LH/CG preparations. Rat recFSH was about 10-fold less potent than human recFSH in stimulating cAMP production of a mouse Sertoli cell line (MSC-1) expressing the recombinant rat FSH receptor.

The circulating half-times ($T_{\frac{1}{2}}$) of rat and human rec hormones were assessed after i.v. injections into adult male rats rendered gonadotropin-deficient by treatment with a gonadotropin-releasing hormone antagonist. A novel immunometric assay was used for the rat FSH measurements. In the one-component model the $T_{\frac{1}{2}}$ values of rat and human recLH were 18·2 ± 1·9 min (n=7) and 44·6 ± 3·1 min (n=7) respectively and those of rat and human recFSH were 88·4 ± 10·7 min (n=6) and 55·0 ± 4·2 min (n=6) respectively; the two-component models revealed similar differences between the rec hormone preparations. Collectively, rat recLH was eliminated significantly faster from the circulation than human recLH (P<0·0001). In contrast, the elimination of rat recFSH was significantly slower than that of human recFSH (P=0·02).

In conclusion, rat recFSH and rat recLH display lower biopotencies per unit mass than the respective human hormones *in vitro*, and also *in vivo* for LH. This is paralleled by shorter $T_{\frac{1}{2}}$ of rat recLH than the respective human hormone in the circulation, whereas human recFSH has a shorter $T_{\frac{1}{2}}$ than human FSH. The special functional features of the rat rec gonadotropins emphasize the use of these preparations on studies of gonadotropin function in the rat, an important animal model for reproductive physiology.

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Introduction

The two gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), as well as their receptors, show high structural homology between different mammalian species. The cDNAs and genomic genes of the rat and human FSH and LH (see Gharib *et al.* 1990 for review), and their respective receptors (McFarland *et al.* 1990, Sprengel *et al.* 1990, Minegishi *et al.* 1990, 1991, Koo *et al.* 1991, Heckert *et al.* 1992) have recently been cloned. The homology at the DNA level of the rat and human gonadotropins and their receptors is 70–90% (Gharib *et al.* 1990, McFarland *et al.* 1990, Minegishi *et al.* 1990). However, the receptor binding of rat and human FSH and LH to the homologous and heterologous receptors vary more than could be predicted from the structural differences of these molecules. The human FSH receptor preferably recognizes human FSH to the respective rat hormone (Tilly *et al.* 1992, Mulder *et al.* 1994), whereas the human LH receptor does not bind the rat LH at all (Jia *et al.* 1991). In contrast, the rat LH receptor preferably binds LH or choriongonadotropin (CG) of human origin (Huhtaniemi & Catt 1981, Jia *et al.* 1991). These differences suggest that it may be important to use homologous

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hormones in studies of the physiology of gonadotropin actions in various species.

We have produced, purified and partially characterized rat recombinant (rec) FSH (Hakola *et al.* 1997*a*) and rat recLH (Hakola *et al.* 1997*b*), in order to study further the physiology of gonadotropin functions in the rat, the most common animal model in reproduction research. The advantages of the rec gonadotropins are that they can be produced and purified in large quantity, as is needed, for instance, for *in vivo* tests. In addition, the recombinant gonadotropins are expected to improve the batch-to-batch consistency (Shoham & Insler 1996), which is important with respect to the well-known microheterogeneity in the glycosylation of gonadotropin molecules (Sairam 1989). Recombinant gonadotropins have been produced for human LH (Simon *et al.* 1988) and FSH (Keene *et al.* 1989, Mannaerts *et al.* 1991, Matikainen *et al.* 1994), the latter being already available for clinical use.

In this study, we have further compared, *in vitro* and *in vivo*, the rec forms of rat and human LH and FSH, all produced in Chinese hamster ovary (CHO) cells, as well as urinary human choriongonadotropin (hCG) and rat pituitary LH (NIDDK-RP3).

Materials and Methods

Hormone preparations

The highly purified recombinant (rec) forms of rat LH (95% purity, 1100 IU/mg by *in vitro* bioassay, in relation to human recLH; Hakola *et al.* 1997*b*) and FSH (98% purity, 8820 IU/mg by *in vivo* bioassay, Hakola *et al.* 1997*a*) and of human FSH (Org 32489, >95% purity, 11 500 IU/mg by bioassay; batch 65), were donated by Organon (Organon International BV, Oss, The Netherlands). Highly purified human recLH (99·9% purity, 15 900 IU/mg by *in vivo* bioassay, batch C31) was a generous gift of Ares-Serono (Geneva, Switzerland), and highly purified urinary hCG (NIH CR 121, 11 500 IU/mg) was a generous gift of NICDH (NIH, Bethesda, MD, USA). Rat luteinizing hormone (NIDDK-rLH-RP3) and follicle-stimulating hormone (NIDDK-rFSH-RP2), both of pituitary origin, were used as reference preparations, and were obtained from Dr A F Parlow (Harbour-UCLA Medical Center, Torrance, CA, USA). The gonadotropin-releasing hormone (GnRH) antagonist used, Cetrorelix acetate (SB-75), was donated by Asta Medica AG (Frankfurt, Germany).

Animals

Adult male rats (2–4 months of age) of the Sprague–Dawley strain and adult mice of the NMRI strain were used. The temperature of the vivarium was controlled (21–23 °C) and the light period was adjusted to 14 h light and 10 h darkness. The animals had free access to standard pelleted laboratory animal food and tap-water. The study was approved by the local Ethical Committee of Animal Experimentation.

LH receptor binding-inhibition assay

This assay was based on displacement of radioiodinated hCG from rat testicular membrane preparations by noniodinated LH/CG preparations, as described earlier (Huhtaniemi & Catt 1981). The assays were repeated three times with three replicate samples.

In vitro *bioassay of LH*

The *in vitro* bioassay was based on stimulation of cAMP and testosterone production in dispersed mouse testis interstitial cells as described earlier by van Damme *et al.* (1974) and modified by Ding and Huhtaniemi (1989). The cell stimulations were repeated three times with three replicate samples.

Stimulation tests with the murine Leydig tumor cell line (BLT-1)

The BLT-1 cells (Kananen *et al.* 1996) are derived from a mouse Leydig cell tumor originating from a transgenic mouse expressing a 6 kb mouse inhibin α-subunit promoter/Simian virus 40 T-antigen (SV40 Tag) fusion gene. The cell line expresses the LH receptor and produces cAMP and progesterone in response to LH stimulation. The culture medium was Dulbecco's modified Eagle's medium/F12 (1:1, with 0·365 g/l L-glutamine) (Life Technologies, GIBCO BRL, Glasgow, UK) supplemented with 10% fetal calf serum (FCS, Autogen Bioclear, Calne, Wilts, UK), 4·5 g/l glucose, 20 mmol/l HEPES, 0·1 g/l gentamycin (Biological Industries, Bet-HaEmek, Israel) and 1·25 mg/l fungizone (GIBCO BRL). BLT-1 cells were inoculated to 24-well plates (Greiner Labortechnik, Frickenhausen, Germany) at 10^5 cells/well in 0·5 ml complete culture medium, on the day before the experiment, and cultured at 37 °C in a humidified atmosphere of 5% CO_2/95% air. The next day the cells were washed with PBS (GIBCO BRL), and 0·5 ml culture medium without FCS was added, containing 0·2 mmol/l 3-isobutyl-1-methylxanthine (MIX; Aldrich-Chemie, Steinheim, Germany), hCG, human recLH, rat recLH or no hormones. In the dose–response experiments, 0·1 ml culture medium was removed after 1-h culture, diluted 1:1 with 2 mmol/l theophylline, boiled for 5 min, and stored at − 20 °C for cAMP measurements (see below). After an 8-h culture, the rest of the media were collected, boiled for 5 min, and stored at − 20 °C for progesterone measurements (see below). In the time–response experiments, the concentrations of the above hormones were 3 μg/l and the culture time varied from 1 h to 48 h. The

stimulations were repeated three times with four replicate samples in the dose–response experiments, and six replicate samples were used in the time–response experiments.

Stimulation tests with the murine Sertoli tumor cell line (MSC-1)

The MSC-1 cells are derived from a mouse Sertoli cell tumor originating from transgenic mice carrying a fusion gene of the human anti-Müllerian hormone promoter sequences linked to the SV40 Tag gene (Peschon *et al.* 1992). The cell line used was stably transfected with the rat FSH receptor cDNA, and displayed FSH binding and FSH-responsive cAMP production (Eskola *et al.* 1998). The culture medium used was as above without fungizone. MSC-1 cells were inoculated to 24-well plates (Greiner) at 50 000 cells/well in 0·5 ml culture medium on the day before the experiment, and cultured at 37 °C in a humidified atmosphere of 5% CO_2/95% air. The next day, 0·5 ml fresh culture medium was added containing 0·2 mmol/l MIX and human recFSH, rat recFSH or no hormones. After 4 h, the culture media were collected and treated as above for the cAMP measurements. The MSC-1 cell stimulations were repeated three times with four replicate samples.

In vivo *experiments*

Adult male rats (weighing 233–265 g) of the Sprague–Dawley strain were used (6–7 rats per group). The day before the experiment a cannula was inserted into the right jugular vein of the rats. The rats were pretreated with Cetrorelix acetate in 5% mannitol (100 μg/rat) the day before, and 1 h before the experiments. When measuring FSH half-times, an additional dose of Cetrorelix (100 μg/rat) was given 8 h after the FSH injections. Thereafter, the rats received a single intravenous injection of rat recLH (10 μg/rat), human recLH (1 μg/rat), rat recFSH (10 μg/rat) or human recFSH (1 μg/rat) in Ringer solution+0·1% BSA (0·1 ml/rat), to allow measurements of circulatory half-times of these hormones. The blood samples (0·2 ml) were collected through the indwelling venous jugular catheter, followed by flushing of the catheter with 0·4 ml Ringer/heparin. Serial blood samples were collected in this fashion prior to the gonadotropin injections, and at times 7·5, 15, 30, 60, 90, 120, 240 and 300 min for LH and 0·5, 1, 3, 5, 7, 9, 12, 24 h for FSH half-time ($T_{\frac{1}{2}}$) measurements. The blood samples were allowed to clot, centrifuged, and the serum was stored at − 20 °C until the hormone measurements.

Hormone and cAMP measurements

Rat LH was measured using the time-resolved immunofluorimetric assay (IFMA, Delfia, Wallac OY, Turku, Finland) as described before (Haavisto *et al.* 1993) with the exception that rat recLH was used as standard. Human LH (hLH Spec, Wallac OY) and rat and human FSH (hFSH, Wallac OY) from the rat sera were measured using the time-resolved immunofluorimetric assay principle (Delfia) described previously by Lövgren *et al.* (1984). A rat FSH immunofluorimetric assay (IFMA) was established and carried out as follows. After screening numerous monoclonal antibodies (MAB) against rat FSH, two antibodies against human FSH were found to display sufficient binding. The MAB against hFSH β-subunit (no. 6602, Medix, Kauniainen, Finland) and the MAB against hFSH α-subunit (8D10, Wallac OY) were used as capture and tracer antibodies respectively. The capture antibody was biotinylated (e.g. Haavisto *et al.* 1993) and it was attached (0·5 μg/well; 2·5 mg/l) to streptavidin-coated microtitration wells (Delfia) for 30 min at 20 °C under continuous shaking, using a Delfia Plateshaker (Wallac OY). After washing twice with Delfia platewasher (Wallac OY), 25 μl rat FSH standard (NIDDK-rFSH-RP2) or sample were incubated with 0·3 μg/well (1·3 mg/l) europium-labeled antibody (e.g. Haavisto *et al.* 1993) for 30 min at 20 °C, using shaking, followed by an overnight incubation at 4 °C without shaking. Thereafter, the plates were washed six times, 200 μl enhancement solution were added and the plates were incubated for 5 min, after which fluorescence was measured in an Arcus Fluorometer (Wallac OY) for 1 s/well. The intra-assay coefficient of variation (CV) of the assay was 10% at rat FSH concentration of 12·5 μg/l and <4% at levels above 25 μg/l. The interassay CV was 13·1% at FSH concentration of 24·4 μg/l and 5·3% at 56·0 μg/l (n=8). When different concentrations of rat FSH standard, ranging from 25–100 μg/l, were added to individual samples of rat serum, the recovery of the added hormone, after subtraction of the endogenous level, was 88·4 ± 1·0% (n=16). The analytical sensitivity of the assay (signal at 0 standard+2 S.D.) was 203 pg/tube, which corresponds to a concentration of 8·1 μg/l at a sample volume of 25 μl. The cross-reactivities of the assay were <0·7% with rat LH, 2·0% with rat thyroid stimulating hormone, 1·1% with the rat α-subunit, <0·2% with rat growth hormone, and <0·8% with rat prolactin.

cAMP, testosterone and progesterone were analyzed directly from the media by radioimmunoassays, and testosterone from the sera, after diethyl ether extractions, as described before (Harper & Brooker 1975, Huhtaniemi *et al.* 1985, Vuorento *et al.* 1989).

Statistical analyses

The half-time ($T_{\frac{1}{2}}$) values of LH and FSH elimination from rat circulation after i.v. injection were estimated by using the least-squares method. Both one- and two-component models were used. The elimination function [(E(t))] of the one-component model is: $E(t)=e^{-t \times \ln(2)/HL}$ and of the two-component model is: $E(t)=F \times e^{-t \times \ln(2)/HL_1}$

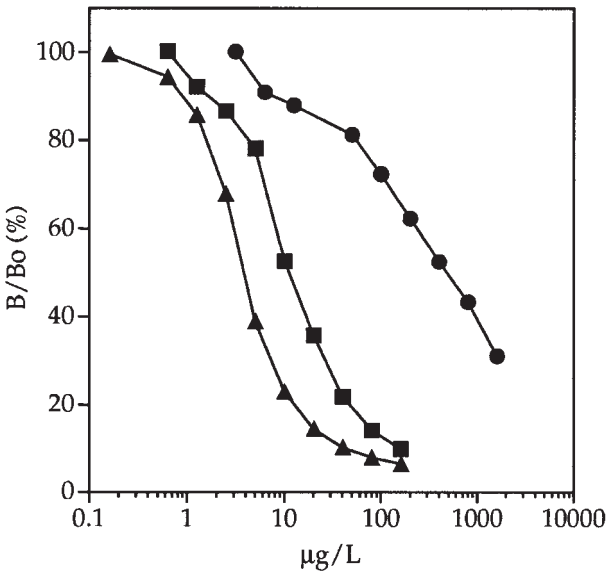

Figure 1 Displacement of [^{125}I]iodo-hCG from rat testicular membranes by increasing concentrations of rat recLH (●), human recLH (■) and hCG (▲). B/Bo indicates the c.p.m. bound as a percentage of total binding in the absence of competitor (100%). One of three experiments with similar results is presented. Each point is the mean of three parallel replicates.

$+(1-F) \times e^{-t \times \ln(2)/HL_2}$, where t is the time difference from the start of the measurements (t_0), HL_1 and HL_2 are the two half-lives of elimination, and F and $1-F$ their fractional distributions respectively.

The data are expressed as means ± S.E.M. The statistical analyses were carried out by a Macintosh version of the StatView and SuperANOVA programs (Abacus Concepts, Inc., Berkeley, CA, USA) using 1 Factor ANOVA, followed by Duncan's new multiple range and Fisher's protected LSD post-hoc tests, or unpaired Student's *t*-test in the case of two groups. A *P* value less than 0·05 was considered statistically significant.

Results

LH receptor binding-inhibition assay

In the radioreceptor assay using rat testis membranes, hCG binding displayed affinity that was about 3-fold higher than that of human recLH (P=0·0002) and about 100-fold higher than that of rat recLH, in physiological salt concentrations (Fig. 1).

In vitro *bioassay of LH using mouse interstitial cells and BLT-1 cells*

In the *in vitro* bioassay using mouse interstitial cells, human recLH and hCG evoked cAMP and testosterone responses

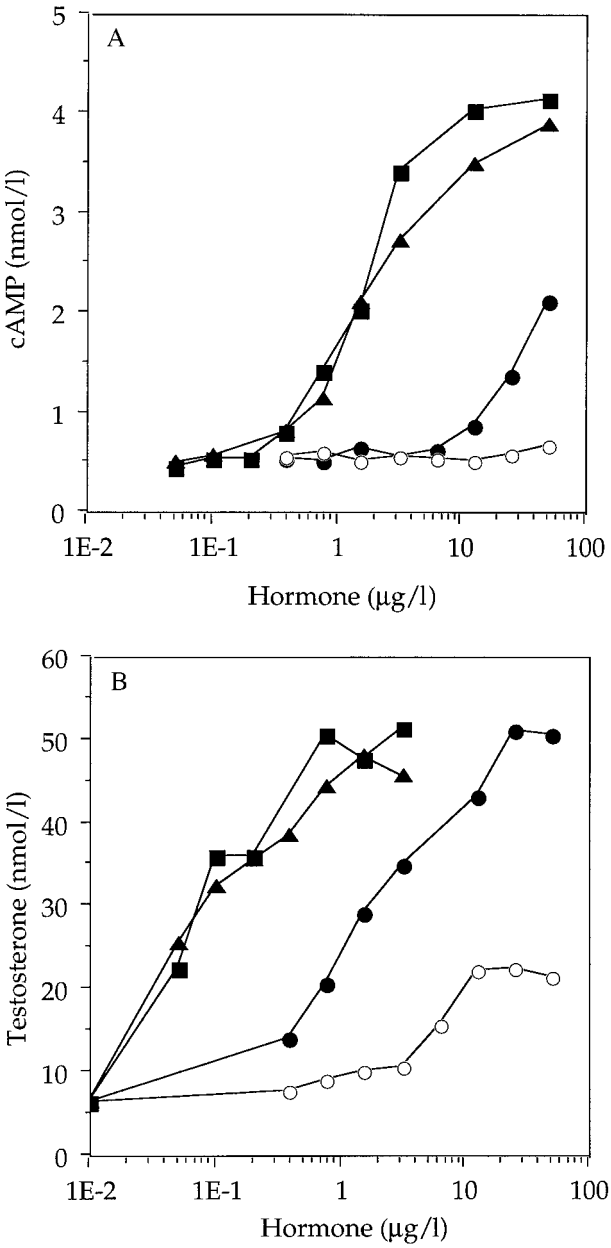

Figure 2 Stimulation of (A) cAMP and (B) testosterone production in mouse primary Leydig cells by rat recLH (●), human recLH (■), hCG (▲) and rat pituitary LH (RP3) (○). One of three experiments with similar results is presented in each panel. Each point is the mean of three parallel replicates.

in a dose-dependent manner (Fig. 2A,B). The human hormone evoked similar maximum responses of cAMP and testosterone, about 4 nmol/l and 50 nmol/l respectively. The maximum testosterone response to rat recLH was also similar, but the highest dose of this hormone was not high enough to saturate the cAMP response. Rat pituitary LH (RP3) was clearly less effective than the other

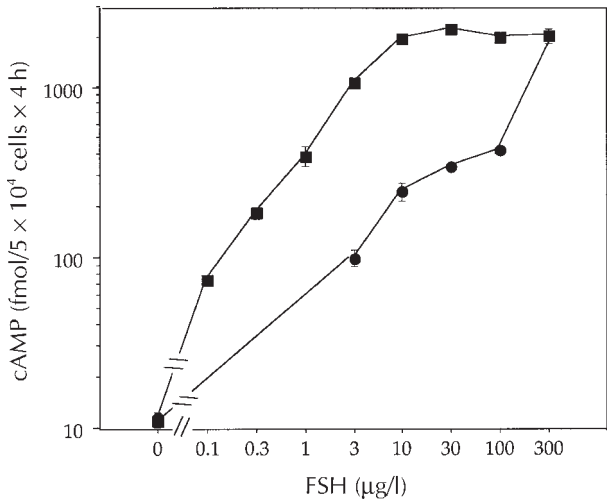

Figure 3 cAMP production of MSC-1 cells, stably transfected with the rat FSH receptor cDNA, in response to increasing concentrations of rat recFSH (●) and human recFSH (■) in a 4-h incubation. One of three similar experiments is presented. Each point is the mean ± S.E.M. of four parallel replicates.

preparations, even the highest dose only marginally stimulated cAMP production, and the maximal testosterone response remained at about 40% of that of the other hormone preparations tested. Human recLH and hCG were equipotent in the *in vitro* bioassay, and rat recLH was about 30-fold less potent. The potency of RP3 could not be accurately compared with the other hormones, due to the low level of cAMP and testosterone response even at the highest concentrations tested. The mean ED_{50} values were similar for hCG and human recLH, 0·27 and 0·33 μg/l respectively. The ED_{50} value was 7·9 ± 5·5 μg/l (n=3) for rat recLH.

The capacities of rat recLH, human recLH and hCG to stimulate cAMP and progesterone production in murine Leydig tumor cells (BLT-1) were also determined. The cell line expresses the LH receptor, and produces cAMP and progesterone in response to LH stimulation (Kananen *et al.* 1996). All the three LH/CG forms tested stimulated cAMP and progesterone production in a dose-dependent manner and their maximum responses were similar (data not shown). As in primary interstitial cells, hCG and human recLH were over ten times more potent than rat recLH.

Stimulation tests with the murine Sertoli tumor cell line (MSC-1)

The MSC-1 cell line permanently transfected with the rat FSH receptor cDNA was used to measure the cAMP response to stimulation with rat recFSH and human recFSH. Both hormones stimulated cAMP production in a dose-dependent manner (Fig. 3). Human recFSH was about 10-fold more potent than the respective rat hormone. The maximum stimulation was about 200-fold from the control level (no hormone added) with both hormones.

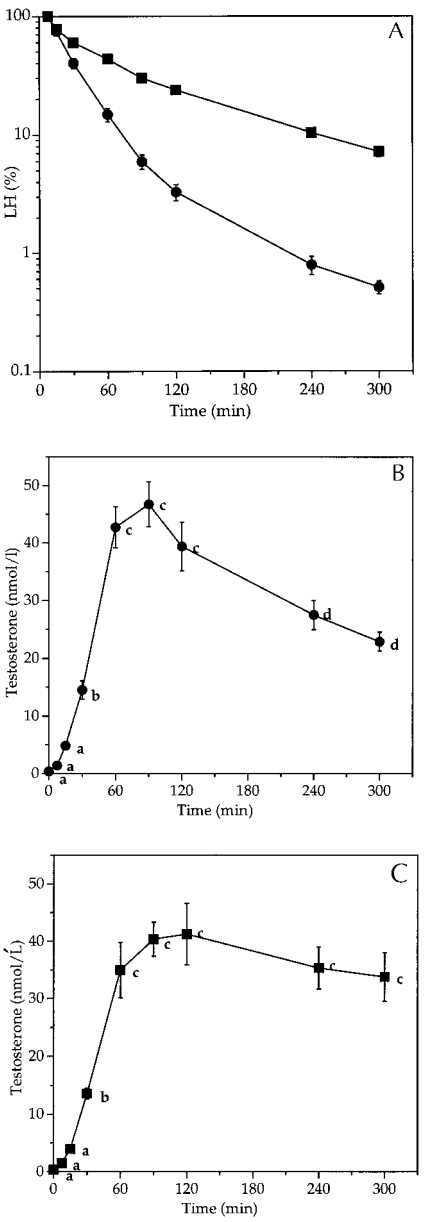

Figure 4 Half-time measurements of rat recLH (●) and human recLH (■) in the rat circulation in gonadotropin antagonist-treated male rats. The percentage levels of the 7·5 min values (=100%) are presented in panel A. Panel B displays rat serum testosterone after injection of 10 μg rat recLH and panel C after injection of 1 μg human recLH in gonadotropin antagonist-treated male rats. In panels B and C, the time points provided with the different letters (a-d) are significantly different (P at least 0·05).

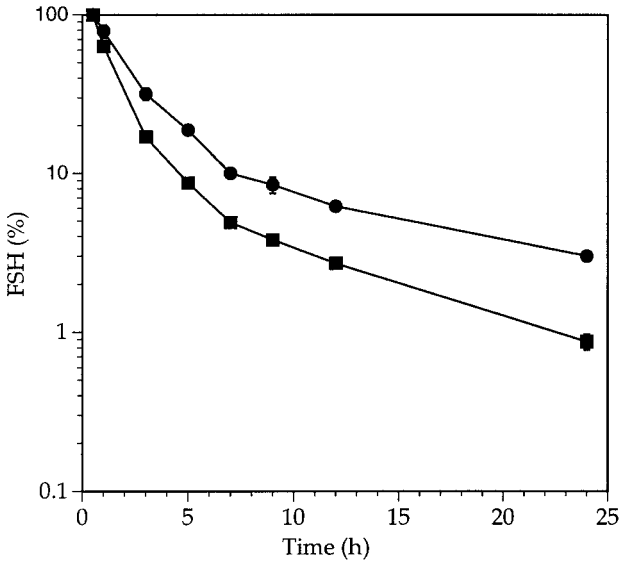

Figure 5 Half-time measurements of rat recFSH (●) and human recFSH (■) in the rat circulation in gonadotropin antagonist-treated male rats. The percentage levels of 30 min values (=100%) are presented.

In vivo *experiments*

The $T_{\frac{1}{2}}$ values in rat circulation were determined after 10 μg and 1 μg i.v. injections of rat and human recLH respectively, in gonadotropin antagonist-treated rats. The maximum concentrations, at 7·5 min after the injections, were 3650 ± 420 μg/l for rat recLH, and 866 ± 36 μg/l for human recLH. The $T_{\frac{1}{2}}$ values were 18·2 ± 1·9 min (n=7) and 44·6 ± 3·1 min (n=7) for rat recLH and human recLH respectively (Fig. 4A), and the difference between the two hormones was significant (P<0·0001). In two-component models, the first half-times were 12·7 ± 3·5 min and 10·6 ± 2·2 min and the second half-times were 43·7 ± 11·8 min and 88·7 ± 5·7 for rat recLH and human recLH respectively. Both recLH preparations showed similar maximum testosterone responses, 40–50 nmol/l, 90 min after the LH injections (Fig. 4B,C). It was found that the testosterone levels were unaltered between 90 and 300 min after the human LH injection, whereas a significant decrease was observed at the same time in the rat LH-injected animals.

The $T_{\frac{1}{2}}$ values of rat and human recFSH in rat circulation were determined after 10 μg and 1 μg i.v. injections respectively, in gonadotropin antagonist-treated rats (Fig. 5). The maximum concentrations, at 30 min after the injections, were 1450 ± 118 μg/l (n=6) for rat recFSH, and 422 ± 19 IU/l (n=6) for human recFSH. The $T_{\frac{1}{2}}$ values were 88·4 ± 10·7 min (n=6) and 55·0 ± 4·2 min (n=6) for rat recFSH and human recFSH respectively, and the difference between the two hormones was significant (P<0·02). In two-component models, the fast phases were 64·0 ± 9·7 min and 38·0 ± 5·3 min and the slow phases were 10·0 ± 2·9 h and 4·7 ± 1·3 h, for rat and human recFSH respectively.

Discussion

In this study, we have compared *in vitro* and *in vivo* the functions of rat recLH and recFSH with the respective human hormones, as well as with urinary hCG and rat pituitary LH. In addition, a novel immunofluorimetric assay for rat FSH is described.

The receptor binding affinity of human recLH was threefold lower than that of hCG, but the *in vitro* bioactivities of the two hormones were similar. There is no apparent explanation for this finding, but it may be due to the fact that the bioactivity dose–response occurs at low level of receptor saturation, whereas the radioreceptor assay uses higher receptor occupancy. Any interaction between free and occupied receptors may then be critically dependent on the characteristics of the ligand molecule. This is even clearer with rat recLH, which consistently shows shallower binding-inhibition curves than the human hormones. Moreover, we have observed previously that the receptor binding of rat LH is greatly influenced by the salt concentrations in the assay buffer, as compared with hCG (Hakola *et al.* 1997*b*). It seems that the binding affinities of human LH and CG are less influenced by salt concentrations of the assay buffer compared with rat and ovine LH (Huhtaniemi & Catt 1981).

The 10-fold difference in biopotency of rat recLH and the human hormones (hCG and recLH), and their similar maximum responses, were confirmed in the mouse testis interstitial cell and mouse Leydig tumor cell assays. Similarly, human recFSH was about 10-fold more potent than the respective rat hormone. In the *in vitro* bioassay of LH, the maximum steroidogenic response to rat pituitary LH (RP3) was less than half that to the other LH/CG preparations. This might be due to possible impurities, or deglycosylated forms of the hormone (Sairam 1989), some of which might act as competitive LH antagonists. No accurate ED_{50} value could therefore be calculated for RP3. Rat pituitary LH (RP3) is a widely used standard for measuring rat LH. The present data show that it is apparently a suboptimal choice for this purpose, and may not reflect accurately the bioactive component of the LH immunoreactivity to be measured.

De Greef *et al.* (1983) have reported previously a half-time of 18·6 min for rat LH after injection of adenohypophysial extracts in female rats. These data on LH are surprisingly similar to the $T_{\frac{1}{2}}$ values measured for rat recLH in our study (18·2 min). The carbohydrate side chain termini of the pituitary LH molecules are both sulfated and sialylated (Sairam 1989), whereas they are exclusively sialylated in the rec glycoprotein hormones synthesized by CHO cells (Smith *et al.* 1990). The terminal sulfation

shortens the $T_{\frac{1}{2}}$ of the hormone, but this apparently is of minor importance in rat LH because of the similarities in the half-times of the two LH preparations. The half-time of human recLH observed with the one-component model was 44·6 ± 3·1 min, which is consistent with earlier reports. Previously it was shown that the half-time of human LH in human circulation was 47 min (Veldhuis *et al.* 1987) and in rat circulation 53 and 48 min with LH samples from females and males respectively (Haavisto *et al.* 1995). Human recLH has been reported to have a serum immunoreactive half-time of 52 min in monkeys (Porchet *et al.* 1995). Rat recLH was eliminated significantly faster than human recLH ($P<0·0001$). The difference is apparently due to differences of the amino acid structure, since both LH forms were produced by the same CHO cells and were evidently similarly glycosylated.

A larger difference was found in the circulatory $T_{\frac{1}{2}}$ of rat FSH between the earlier findings on pituitary (40·5 min; de Greef *et al.* 1983) and recFSH (88·4 min). The reason for the difference is not apparent, since pituitary FSH and recFSH should be structurally closer, due to the fact that the carbohydrates of FSH are not sulfated in the same way as those of LH. It is possible that the two hormone preparations have different isoform compositions with different mean rates of elimination. With regard to LH, the lower bioactivity of rat LH was coupled with faster $T_{\frac{1}{2}}$ value in the circulation of this hormone, as compared with the respective human hormone. In contrast, whereas rat recFSH had lower bioactivity (Hakola *et al.* 1997*a*) and receptor affinity than the cognate human hormone, the $T_{\frac{1}{2}}$ of the rat hormone was longer than that of the human hormone. In accordance, the elimination half-times of human recFSH were previously reported to be about 5·7 h in rats, but about 30 h in dogs and humans (de Leeuw *et al.* 1996), which makes human FSH a suboptimal choice to study the physiology of FSH action in rats. Why human FSH seems to be more rapidly eliminated from the rat circulation than the homologous gonadotropin remains obscure.

Finally, we report here a novel IFMA for rat FSH. It was developed with the same principle as the rat LH IFMA a few years ago (Haavisto *et al.* 1993). A pair of heterologous LH antibodies was found with high affinity and specificity to recognize rat LH, and in this way the sensitivity of the conventional RIA was improved about 50-fold. With the rat FSH IFMA, the methodological advancement was not as great, since the sensitivity of the IFMA assay is only marginally better than that of the NIH RIA. The new assay, however, offers several advantages being faster (overnight vs 2–3 days), using no radioisotopes, requiring smaller sample volumes (25–50 vs 50–100 μl serum), and having practically unlimited shelf-life of all reagents needed. Once set up, it is accessible immediately, and does not require hazardous radioiodination of the [^{125}I]iodoFSH tracers which are notoriously fast in decaying.

In conclusion, we expanded our previous findings (Hakola *et al.* 1997*a*,*b*) that rat recLH and rat recFSH are functional *in vitro* and *in vivo*. The recombinant rat hormones are pure, free of any crossreacting contaminants, and their structure can be defined in detail. They have similar half-lives *in vivo* as their pituitary counterparts. They can also be produced in large quantities for *in vivo* studies on pituitary–gonadal functions in rats. The importance of the use of homologous hormones in physiological studies of the rat pituitary–gonadal functions is highlighted by the profound functional differences that were found between the rat and human gonadotropins in the present study.

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