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Rotational dynamics of luteinizing hormone receptors and MHC Class I antigens on murine Leydig cells

Cynthia J. Philpott [a], Noorul A. Rahman [b], Nicholas Kenny [c], Thomas R. Londo [b], Russell M. Young [b], B. George Barisas [b], Deborah A. Roess [a,*]

[a] *Department of Physiology, Colorado State University, Fort Collins, CO 80523, USA*
[b] *Department of Chemistry, Colorado State University Fort Collins, CO 80523, USA*
[c] *Department of Obstetrics and Gynecology, University of Vermont School of Medicine, Burlington, VT 05405, USA*

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Abstract

We have examined the molecular motions of luteinizing hormone (LH) receptor and the Major Histocompatibility Complex Class I antigen on murine Leydig cells. Using time-resolved phosphorescence anisotropy methods, erythrosin (ErITC)-derivatized ovine luteinizing hormone (oLH) bound to the LH receptor appears rotationally mobile with rotational correlation times of 19.6 ± 1.3 μs, 13.3 ± 2.4 μs, 9.5 ± 0.7 μs and 4.7 ± 0.5 μs at 4° C, 15° C, 25° C and 37° C, respectively. Rotational correlation times for human chorionic gonadotropin (hCG)-occupied LH receptors were similar to those of the ErITC-oLH occupied receptor at each temperature. In addition, both oLH- and hCG-occupied LH receptors were laterally mobile in fluorescence photobleaching recovery experiments with diffusion coefficients at 29° C of $(5.8 \pm 0.9) \cdot 10^{-10}$ cm^2 s^{-1} and $(2.9 \pm 0.4) \cdot 10^{-10}$ cm^2 s^{-1}, respectively. We also measured the rotational correlation time of Class I antigen on murine Leydig cells using ErITC-derivatized 34-12-2S, an anti-Class I monoclonal antibody. Because there was no decay of the anisotropy function at 4° C, 15° C, 25° C or 37° C in the absence of oLH or following preincubation of Leydig cells with 1 nM oLH, it appears that Class I is rotationally immobile on the 1 ms timescale of our experiments. This result is consistent with the presence of Class I antigen in large molecular weight structures and may be the result of Class I self-aggregation. Further, treatment of cells with anti-Class I antibody had no effect on either basal or oLH-stimulated testosterone secretion. Thus, it appears that this anti-Class I antibody is not LH-mimetic on murine Leydig cells.

Keywords: MHC class I antigen; Luteinizing hormone receptor; Fluorescence photobleaching recovery; Time-resolved phosphorescence anisotropy; Rotational dynamics

1. Introduction

Luteinizing hormone (LH) receptors play a central role in the regulation of ovarian and testicular function. The binding of the glycoprotein hormones LH or hCG to the LH receptor is the first step in initiating their biological effects. However, the interactions between the hormone-receptor complex and other plasma membrane proteins and the hormone-induced biological response are not well understood. From studies of epidermal growth factor [1], platelet-derived growth factor [2], insulin [3,4] and gonadotropin releasing hormone [5], it has been proposed that hormone binding alone may not induce a biological response unless accompanied by other membrane events that may include receptor aggregation [6] or redistribution [7]. In the case of LH receptors, electron microscopy studies using rat luteal cells have shown that receptor aggregation into small clusters is a hormone-dependent phenomenon [8]. Polarized fluorescence depletion studies of the LH receptor rotational diffusion on rat [9] and sheep [10] luteal cells indicate that receptor motions are slower than would be expected for the receptor alone, suggesting that the receptor might be part of a larger molecular weight complex.

Abbreviations: oLH, ovine luteinizing hormone; hCG, human chorionic gonadotropin; MHC, major histocompatibility complex; TPA, time-resolved phosphorescence anisotropy; FPR, fluorescence photobleaching recovery; TRITC, tetramethylrhodamine isothiocyanate; ErITC, erythrosin isothiocyanate; BSS, balanced salt solution; PBS, phosphate-buffered saline.

* Corresponding author. Fax: +1 (303) 4911801.

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Other membrane proteins may be part of such a LH receptor-containing complex. Solano and co-workers [11] have suggested that Major Histocompatibility Complex (MHC) Class I antigen may be associated with the murine LH receptor as has been reported for insulin [12,13], glucagon [14] and epidermal growth factor [15] receptors. Although the biological function of these interactions has not been determined [16], it is known that Class I antigen consists of a 45 kDa α-chain noncovalently linked to 12 kDa β_2-microglobulin and is pivotal in immunological self/nonself discrimination. To probe the organization of LH receptors and Class I in the plasma membranes of viable murine Leydig cells, we measured their rotational correlation times using time-resolved phosphorescence anisotropy (TPA) techniques. Because rotational diffusion of plasma membrane proteins is affected by their in-membrane molecular weight, as well as by protein conformation and microenvironment [17], similar rotational correlation times for proteins such as Class I and LH receptor would suggest that these proteins could be closely associated in the membrane.

2. Materials and methods

2.1. Materials

Medium 199 (M199), BSA, $NaHCO_3$, Hepes, goat anti-mouse IgG, Protein A Sepharose and Sephadex G-25 were purchased from Sigma, St. Louis, MO. Penicillin-streptomycin was purchased from KC Biologicals, Lenexa, KS. The 34-1-2S cell line was purchased from American Type Culture Collection, Rockville, MD. Ovine LH (NIH S-26) and hCG (CR-127) were obtained from the National Hormone and Pituitary Program, NIADDK, Baltimore, MD. Tetramethylrhodamine isothiocyanate (TRITC) and erythrosin isothiocyanate (ErITC) were purchased from Molecular Probes, Eugene, OR.

2.2. Preparation of murine Leydig cells

Murine Leydig cells were isolated by modification of a non-enzymatic procedure previously published [18]. Testes from adult BALB/c mice (National Cancer Institute, Frederick, MD) were collected into M199 containing 0.1% (w/v) BSA, 42 mM $NaHCO_3$, 20 mM Hepes, 100 U penicillin/ml, and 100 μg streptomycin/ml, pH 7.4. Testes were decapsulated and the seminiferous tubules were gently washed with M199. The media was centrifuged at $300 \times g$ for 3 min with two washes in M199. The final wash was in Balanced Salt Solution (BSS). Cells were pooled, counted with a hemocytometer and viability was assessed by Trypan blue dye exclusion. Routinely $2.5 \cdot 10^6$ cells with $> 95\%$ viability were obtained per testis. Between 10–20% of the cells were positive for steroid synthesis as shown by 3β-hydroxysteroid dehydrogenase staining [19] and were fluorescently labeled by TRITC-oLH or TRITC-hCG.

2.3. Purification of monoclonal antibodies and preparation of Fab fragments

An anti-K^dD^d monoclonal antibody 34-1-2S specific for BALB/c MHC Class I [20] was purified from collected supernatant by Protein A-Sepharose affinity chromatography [21]. The eluate was then dialyzed against 0.5 mM phosphate buffer (0.04 mM NaH_2PO_4 and 0.46 mM Na_2HPO_4) and lyophilized. Fab fragments were obtained by cleaving intact antibodies as described by Parham [22] and purified by Protein A-Sepharose affinity chromatography [23].

2.4. Preparation of TRITC and ErITC-derivatized proteins

Proteins were derivatized with tetramethylrhodamine isothiocyanate (TRITC) or erythrosin isothiocyanate (ErITC) using a modification of methods described by Johnson and Holborow [24]. Intact antibody, Fab fragments, hCG or oLH were dissolved in phosphate buffered saline (1.86 mM NaH_2PO_4, 8.39 mM Na_2HPO_4, 0.15 M NaCl, PBS) containing 50 mM sodium borate, pH 9.3. Protein concentrations were determined spectrophotometrically at 280 nm. TRITC or ErITC was dissolved in 50 μL DMSO and diluted with 50 mM sodium borate buffer. The TRITC concentration was determined at 541 nm assuming $\epsilon^M_{541} = 82\,000$. The concentration of ErITC was determined spectrophotometrically at 535 nm assuming $\epsilon^M_{535} = 101\,000$. A 5-fold molar excess of TRITC or ErITC was added to the protein solutions and the mixtures were kept at 4° C for 18 h in the dark. The reaction was quenched with 1 M Tris and dye-derivatized proteins were separated from the unreacted free dye on a Sephadex G-25 column. The column eluate was extracted once with an equal volume of n-butanol to remove any remaining non-covalently bound dye. The labeled proteins were then dialyzed against PBS for a total of 48 h at 4° C. The molar ratios for dye-derivatized hormones and the concentration of protein in solution were determined spectrophotometrically. The hormone preparations used in these experiments had 1.6 and 2.8 mol of TRITC per mol of oLH and hCG, respectively, and 1.8 and 2.4 mol of ErITC per mol of oLH and hCG, respectively. The antibody preparations used in these experiments had 4.9 and 1.4 mol of ErITC per mole of intact antibody and Fab fragments, respectively. We have previously shown that there is no effect of dye conjugation on hormone biological activity [25]. Prior to use, all dye-derivatized proteins were centrifuged at $130\,000 \times g$ for 5 min in a Beckman Airfuge (Beckman Instruments, Palo Alto, CA) to remove any protein aggregates which may have formed during storage at 4° C.

2.5. Labeling murine Leydig cells with TRITC- and ErITC-derivatized proteins

Typically, 10^7 cells in 1 ml were labeled with ErITC-derivatized probe for each TPA experiment. In experiments measuring the rotational diffusion of oLH- or hCG-occupied receptors, cells were preincubated in BSS containing 0.1% NaN_3 at 37° C for 30 min to prevent hormone internalization [25] prior to addition of 1 nM ErITC-derivatized oLH or hCG for 1 h. To examine Class I rotational diffusion, cells were labeled with 10 μg/ml of anti-Class I antibody for 1 h at 4° C. In some experiments, cells were preincubated with 1 nM unlabeled oLH for 1 h prior to antibody labeling. After labeling, cells were washed two times by centrifugation at $300 \times g$ for 3 min in BSS to remove unbound ligand and were deoxygenated for 15 min by purging with argon gas to eliminate phosphorescence quenching caused by O_2 [26]. To determine the lateral mobility of the LH receptor, $5 \cdot 10^6$ cells were labeled with 1 nM TRITC-oLH or TRITC-hCG for 1 h at room temperature. After labeling the cells were washed two times by centrifugation at $300 \times g$ for 3 min in BSS to remove any unbound hormone.

2.6. Time-resolved phosphorescence anisotropy measurements

After cells were washed and deoxygenated, the sample, in a 5-mm Suprasil quartz cuvette (Helma Cells, Jamaica, NY), was placed in a thermostatted cuvette holder. Experiments were performed at 4° C, 15° C, 25° C and 37° C. The frequency-doubled 532 nm output of a Spectra-Physics DCR-11 Nd:YAG laser provided the excitation pulse for each experiment. The laser was operated at 10 Hz with a vertically polarized TEM 00 output of 0.19 mJ and a beam $1/e^2$ radius of 5 mm restricted to a 2.5 mm region at the sample. Phosphorescence emission from the sample was collected at 90° to the excitation axis and isolated with a 1 cm pathlength of 1 M $Na_2Cr_2O_7$ solution, a KV 550 color filter (Schott Glass Technologies, Duryea, PA) and a 3 mm thick RG 665 filter. A rotating polarizer was placed in front of the photomultiplier tube in order to observe the intensity of phosphorescence with polarization parallel and perpendicular to the exciting pulse. The phosphorescence signal was collected by a thermionically cooled EMI 9816A photomultiplier tube. A fast gating circuit was used to turn the photomultiplier tube off during the high-power Nd:YAG pulses [27]. The output signal from the photomultiplier tube was amplified by a Tektronix 476 oscilloscope. The oscilloscope was further amplified by a 35 MHz bandwidth buffer amplifier and fed to a Nicolet 12/70 signal averager equipped with a 20 MHz analog-to-digital converter. Phosphorescence decay traces from 4096 laser pulses were averaged for each polarizer orientation, the channel width being 0.5 μs. After data acquisition was complete, the data were downloaded into an 80386 microcomputer for data analysis and storage. Phosphorescence intensities $I_{\parallel}(t)$ and $I_{\perp}(t)$ were analyzed [28] to yield a phosphorescence intensity function $s(t)$ and a phosphorescence anisotropy function $r(t)$. Standard errors in these quantities were assigned by the usual methods [29]. Results from the lifetime analysis were used to weight points in a non-linear least squares fit of the anisotropy data. Anisotropy decay data were analyzed according to a single exponential decay model.

$$r(t) = r_\infty + (r_0 - r_\infty)\exp(-t/\phi) \quad (1)$$

Fitting $r(t)$ to equation 1 yields the initial anisotropy value r_0, the limiting anisotropy value r_∞, and the rotational correlation time ϕ as well as the statistical uncertainties in these quantities [29].

2.7. Fluorescence photobleaching recovery measurements

The methods for fluorescence photobleaching recovery measurements have been described elsewhere in detail [30]. Briefly, samples were examined under coverslip on 0.08 mm deep × 5 mm diameter well slides using a 40 × objective. Fluorescence from cells labeled with tetramethylrhodamine-conjugated proteins were excited with 514.5 nm light from an Innova 100-10 Coherent Radiation argon ion laser. The $1/e^2$ spot radius was 0.41 μm in the image plane and the probe beam laser power was 44 μW. The bleaching pulses were 39 mW power for 250 ms. Fluorescence recovery after photobleaching was monitored for 40 s at 80 ms per point. A special light pulse generator produced the bleaching pulses without introducing measurable beam angular deviations. A photometer-equipped Zeiss Axiomat microscope afforded visual observation and photometric measurement, via photon counting, of fluorescence recovery kinetics after bleaching. A temperature-controlled microscopy stage maintained sample temperature. An iterative, non-linear algorithm implemented in PASCAL on a 20 MHz 80386 microcomputer provided direct, on-line data reduction. We obtained, among other parameters, the diffusion coefficient and the fraction of mobile fluorophore. An image-intensification system greatly facilitated accurate focusing of the laser beam on the faintly fluorescent cell surfaces.

2.8. Testosterone determinations

Cells were washed twice and suspended in sterile M199 culture media containing 100 U penicillin/ml, 100 μg streptomycin/ml and 0.1% (w/v) BSA. Aliquots of $2 \cdot 10^5$ cells were added to tubes containing hormone or antibody treatments to a final volume of 1 ml. Cells were incubated at room temperature for 3 h at which time cells were removed by centrifugation at $300 \times g$. Some samples were preincubated with 100 μg of anti-Class I antibody for 1.5 h prior to hormone and/or second antibody treatment. The supernatant was stored at −20° C until assayed for testos-

Table 1
Time-resolved phosphorescence anisotropy studies of murine Leydig cell LH receptors following binding with ErITC-oLH or ErITC-hCG [a]

Ligand	Temperature (° C)	r_0 [b]	r_∞ [c]	ϕ [d] (μs)
oLH	4	0.041±0.001	0.046±0.001	19.6±1.3
	15	0.036±0.001	0.048±0.001	13.3±2.4
	25	0.029±0.001	0.040±0.001	9.5±0.7
	37	0.021±0.001	0.041±0.001	4.7±0.5
hCG	4	0.043±0.001	0.053±0.001	13.7±0.5
	15	0.049±0.001	0.047±0.001	13.6±0.7
	25	0.035±0.001	0.041±0.001	10.1±1.8
	37	0.048±0.001	0.052±0.001	3.4±0.8

[a] Murine Leydig cells were labeled with 1 nM ErITC-oLH or ErITC-hCG as described in Materials and methods. For each sample, 4096 measurements of phosphorescence decay were averaged to obtain the $I_\parallel$ and $I_\perp$ traces from which anisotropy was calculated.
[b] r_0 is the initial anisotropy.
[c] r_∞ is the final anisotropy.
[d] The RCTs (ϕ) are the mean and S.D. of measurements on three samples examined at each temperature.

Table 2
A comparison of the rotational and lateral dynamics of LH receptors on different LH receptor-bearing cells following labeling with 1 nM oLH or hCG [a]

Tissue type	RCT (μs)		D (10^{-10} cm^2 s^{-1})	
	oLH	hCG	oLH	hCG
Sheep luteal cells	63±19	87±20	1.9±0.6	< 0.01 [b]
Rat luteal cells	43± 1.6	64±11	1.7±0.6	< 0.01 [b]
Mouse Leydig cells	19± 1.3	13± 0.5	5.8±0.9	2.9±0.4

[a] The rotational dynamics of sheep (10) and rat (43) LH receptors at 4° C were measured by polarized fluorescence depletion techniques. Rotational correlation times for murine Leydig cells were measured at 4° C using time resolved phosphorescence anisotropy methods. The lateral diffusion coefficients D of sheep (25) and rat (9), measured at 37° C using fluorescence photobleaching recovery techniques, are compared with those for murine LH receptors. For both polarized fluorescence depletion and fluorescence photobleaching recovery measurements, cells were labeled with 1 nM fluorophore-derivatized oLH or hCG. For murine Leydig cells, diffusion coefficients for TRITC-oLH and TRITC-hCG at 29° C are the mean and S.E. of 18 and 10 measurements, respectively.
[b] Fluorescence recovery curves for these samples showed no recovery on the 1-5 min timescale of measurement. This implies that the lateral diffusion coefficient for this membrane proteins is less than 10^{-12} cm^2 s^{-1}.

terone at which time they were rapidly thawed and mixed. Supernatants were diluted in assay buffer and testosterone was measured in unextracted aliquots by radioimmunoassay [31].

3. Results

The rotational correlation times for oLH- and hCG-occupied LH receptors were measured on murine Leydig cells using time-resolved phosphorescence anisotropy techniques. As shown in Fig. 1, the LH receptor on murine Leydig cells following binding of oLH or hCG exhibited fast rotational diffusion at 4° C with rotational correlation times of 18.0 ± 0.5 and 13.8 ± 0.8 μs, respectively. The rotational correlation times (ϕ) for oLH- and hCG-occupied LH receptors were similar at each temperature measured (Table 1) and both ErITC-oLH and ErITC-hCG were rotationally mobile. However, the small amplitudes of anisotropy decay ($r_\infty - r_0$) at 15, 25, and 37° C for hCG-occupied receptors indicate substantial restriction of rotational freedom for these receptors.

Because rotational correlation times for oLH- and hCG-occupied receptors were similar, we also examined their lateral diffusion with fluorescence photobleaching recovery techniques (Table 2). Previous results in sheep [25] and rat [9] indicate that hCG-occupied receptors, but not LH-occupied receptors, were laterally immobile between 27° C and 37° C. Fluorescence recovery after photobleaching was less than 20% indicating that most, if not all, LH receptors are laterally immobile under these conditions. This was not the case for murine Leydig cell receptors where hCG-occupied receptors, as well as oLH-occupied receptors, were laterally mobile at 29° C. Fluores-

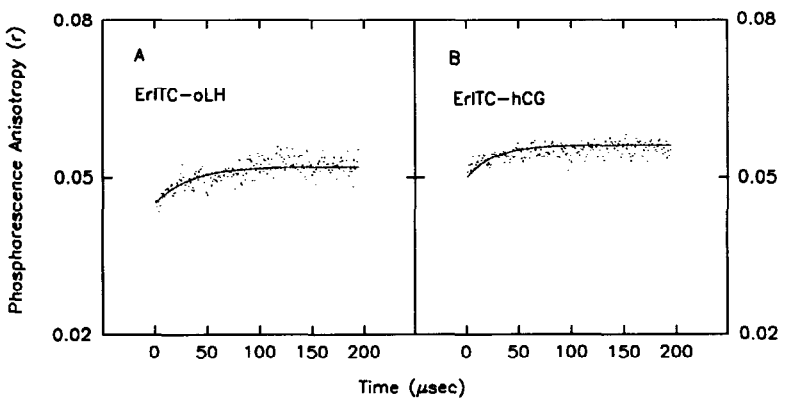

Fig. 1. Time-resolved phosphorescence anisotropy results at 4° C following binding of ErITC-oLH (A) or ErITC-hCG (B) to LH receptors on murine Leydig cells. Data displayed are from 4096 depletion-recovery cycles. The smooth curves are the calculated anisotropy fitted to Eq. (1) using weighting from the corresponding intensity function. The rotational correlation times for oLH and hCG calculated from these data were 18.0 μs and 13.8 μs, respectively.

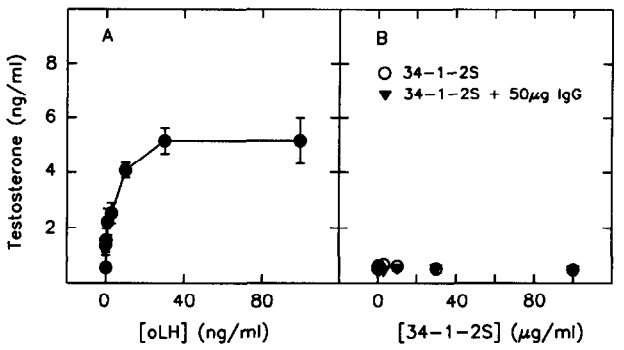

Fig. 2. Testosterone secretion from murine Leydig cells following incubation with oLH or with anti-MHC Class I antibody. In A, testosterone was assayed in supernatants from murine Leydig cell preparations incubated with various concentrations of oLH for 3 h. In B, testosterone secretion was measured for cells incubated with anti-MHC Class I antibody alone for 3 h (○) or with goat anti-mouse immunoglobulin for 1.5 h following a 1.5 h incubation with anti-MHC Class I antibody (▼). The amount of testosterone in the supernatant was determined by radioimmunoassay. Results shown are the mean and S.E. of three experiments performed in triplicate.

cence recovery was 35% ± 2% and 48% ± 3% for hCG- and LH-occupied receptors, respectively. Interestingly, the diffusion coefficient for oLH-occupied receptors ($(5.8 \pm 0.9) \cdot 10^{-10}$ cm^2 s^{-1}) was faster than that for hCG-occupied receptors ($(2.9 \pm 0.4) \cdot 10^{-10}$ cm^2 s^{-1}) and this difference was statistically significant ($P < 0.05$). Although these values for fluorophore-derivatized oLH and hCG lateral diffusion are faster than those observed for the LH receptor on rat or sheep luteal cells, they are consistent with diffusion coefficients reported for plasma membrane glycoproteins on a number of other cell types [32].

To determine whether Major Histocompatibility Complex Class I molecules were associated with the LH receptor, we measured the rotational motions of Class I antigen in the presence and absence of oLH. The rotational correlation time of Class I antigen was greater than 1000 μs at each temperature and preincubation of Leydig cells for 1 h with 1 nM oLH had no significant effect on the rotational correlation times of Class I antigen (Table 3). Class I antigen probed by Fab fragments of anti-Class I antibody also appear rotationally immobile on the experimental timescale (data not shown).

Treatment of Leydig cells with oLH produced dose-dependent testosterone secretion with a maximum response observed following treatment with 30 ng/ml oLH as shown in Fig. 2A. However, treatment of cells with anti-Class I antibody at concentrations of antibody from 0.1–100 μg/ml did not result in the stimulation of testosterone production (panel B). Additional crosslinking of Class I molecules with goat anti-mouse immunoglobulin (50 μg/ml) also had no effect on testosterone production. Only when oLH (100 ng/ml) was present in the incubation medium for 1.5 h was any increase in testosterone secretion observed (data not shown).

4. Discussion

In our hands, both oLH- and hCG-occupied LH receptors on murine Leydig cells were laterally mobile and had comparatively fast rotational diffusion times appropriate for receptor monomers or for small receptor-containing membrane complexes at physiological temperature. The murine LH receptor is a 85–93 kDa glycoprotein with seven transmembrane domains [33]. Assuming that membrane viscosity is approx. 1 poise at 37° C [34] and that membrane viscosity increases by a factor of 3.8 as the temperature decreases from 37° C to 4° C [35], one would expect rotational correlation times of 10–20 μs for the LH receptor at 37° C and rotational correlation times at lower temperatures that reflect the increased microviscosity of the plasma membrane [36]. Similar temperature-dependent rotational correlation times have been observed for the Fc_ϵ receptor on 2H3 rat basophilic leukemia cells [37]. Our results differ from rotational correlation times reported for the EGF receptor where binding of EGF causes both time-dependent microaggregation of the receptor at a given

Table 3
Rotational diffusion for MHC Class I molecules [a]

Preincubation	Temperature (° C)	r_0 [b]	r_∞ [c]	ϕ [d] (μs)
None	4	0.064 ± 0.001	0.068 ± 0.001	> 1000
	15	0.053 ± 0.001	0.058 ± 0.001	> 1000
	25	0.047 ± 0.001	0.048 ± 0.001	> 1000
	37	0.045 ± 0.001	0.049 ± 0.001	> 1000
oLH	4	0.045 ± 0.001	0.044 ± 0.001	> 1000
	15	0.046 ± 0.001	0.050 ± 0.001	> 1000
	25	0.038 ± 0.001	0.044 ± 0.001	> 1000
	37	0.043 ± 0.001	0.036 ± 0.001	> 1000

[a] The rotational dynamics of MHC Class I on murine Leydig cells were probed with ErITC-derivatized anti-MHC Class I antibody in the absence and presence of 1 nM oLH. The rotational correlation times for MHC Class I were greater than 1000 μs at 4° C, 15° C, 25° C and 37° C. Because the effective detection range for TPA measurements is 1–1000 μs, rotational diffusion times greater than 1000 μs cannot be resolved.
[b] r_0 is the initial anisotropy.
[c] r_∞ is the final anisotropy.
[d] The RCTs (ϕ) were obtained from measurements on three samples examined at each temperature.

temperature and an overall increase in receptor rotational times at higher temperatures [1]. Thus, in contrast to ligand-induced changes in EGF receptor aggregation, our results on murine Leydig cells suggest that decreases in LH receptor rotational correlation times at increasing temperatures occurred in the absence of any change in the receptor's local environment, conformation or interactions with other membrane proteins.

Lateral diffusion coefficients and the rotational correlation times for the LH receptor appear to be tissue- and species-dependent. We have examined the rotational correlation times of LH receptors on sheep and rat luteal cells (Table 2) using polarized fluorescence depletion, a technique similar to time-resolved phosphorescence anisotropy. On these cells rotational correlation times for oLH and hCG [9,10] were slower than those measured at 4° C for LH receptor on murine Leydig cells. We do not know whether it is reasonable to compare the molecular motions on Leydig cells with similar measurements on luteal cells. However, we have observed, in preliminary studies on MA-10 cells, a mouse Leydig cell tumor line [38], that oLH-occupied receptors have rotational correlation times of about 30 μs at 37° C while hCG-occupied receptors are cytoskeletally anchored and rotationally immobile [39].

It is also not known how the LH receptor is organized in each of these tissues. For sheep luteal cells, immobilization of the hCG-occupied LH receptor lateral diffusion can be attributed, in part, to interactions of hCG with membrane glycoproteins [25] and interactions of the hormone-receptor complex with cytoskeletal components [40]. Cytoskeletal interactions are also responsible, at least in part, for immobilization of the hCG-occupied receptor on MA-10 cells but, interestingly, not for the slower-than-predicted rotational correlation times of the oLH-occupied receptor [39]. Differences in LH receptor rotational correlation times following oLH or hCG binding might result from differential interactions with non-receptor proteins near the LH receptor. If this were the case, similar rotational correlation times for oLH and hCG on murine Leydig cells may result from the absence or inaccessibility of a membrane protein or proteins capable of interacting with hCG and oLH. Also, given the wide range of values seen for lateral diffusion coefficients and rotational correlation times on tissues from several different species, we believe that organization of the LH receptor is unique to both a given species and tissue.

Between 4° C–37° C, Class I antigen had no rotational correlation times measurable on the timescale of our experiments suggesting that Class I is present in aggregates of large molecular weight. Similar behavior has been reported for Class I antigen on sheep luteal cells [10] as well as for Fc_ϵ receptors that have been extensively crosslinked by antibody [17]. In addition, Chakrabarti et al. have shown that Class I introduced into liposomes will spontaneously self-associate even at low surface concentration, which may explain the slow rotation time in viable cells [41].

One goal of this study was to determine whether LH receptors were interacting with Class I antigen on murine Leydig cells. Solano and coworkers have reported that Class I antigen becomes associated with the murine Leydig LH receptor upon binding of hormone [11]. If this were the case, we would expect that these proteins would have similar rotational correlation times regardless of whether the receptor-containing complex was probed with hormone or anti-Class I antibody. In our studies there was a significant difference between the rotational correlation times of the hormone-occupied LH receptor and MHC class I antigen whether oLH was present or absent. There are two interpretations for this result; LH receptors may simply not interact with Class I antigen following LH binding. Alternatively, while most Class I molecules may be present in large molecular weight complexes, some Class I may be associated with LH receptor. In that case, rotational diffusion of Class I on LH-receptor positive cells would comprise only a small component of the phosphorescence signal in cell preparations containing only 10–20% LH receptor-positive cells. However, if Class I is associated with LH receptor on murine Leydig cells, antibody binding to Class I or extensive crosslinking of Class I does not produce an LH-like response. Although Solano et al. [11] used 34-1-2S and were able to activate the LH receptor on BALB/c mice, in our hands, anti-MHC Class I antibody did not stimulate testosterone secretion even with extensive antibody crosslinking. Gel electrophoresis of the antibody following affinity chromatography purification from tissue culture supernatant indicated that our antibody preparations were not contaminated with non-immunoglobulin proteins (data not shown). The methods used by Solano et al. to isolate anti-Class I antibodies were not discussed in their paper and, for this reason, we cannot explain why there is a difference in our results.

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