Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 334–338, January 1992
Biochemistry

Circulatory half-life but not interaction with the lutropin/chorionic gonadotropin receptor is modulated by sulfation of bovine lutropin oligosaccharides

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Communicated by Elizabeth F. Neufeld, September 30, 1991 (received for review August 21, 1991)

ABSTRACT **Certain of the glycoprotein hormones, including bovine lutropin (bLH), bear asparagine-linked oligosaccharides terminating with the sequence SO_4-4GalNAcβ1-4GlcNAcβ1-2Manα. To establish the biologic significance of these sulfate-bearing oligosaccharides we have compared properties of native bLH, desulfated bLH, recombinant bLH produced in Chinese hamster ovary cells that bears asparagine-linked oligosaccharides terminating with sialic acid α2-3Galβ1-4GlcNAcβ1-2Manα rather than sulfated oligosaccharides (bLH/CHO), and desialyzed bLH/CHO. Using cultured MA-10 cells, a Leydig cell tumor line expressing the lutropin/chorionic gonadotropin receptor, we have found no differences in binding, cAMP production, or progesterone production between native and desulfated bLH. Sulfation of bLH oligosaccharides does not, therefore, modulate bLH bioactivity at the level of the lutropin/chorionic gonadotropin receptor. Removal of sulfate from bLH oligosaccharides and sialic acid from bLH/CHO oligosaccharides results in rapid clearance from the circulation by the hepatocyte asialoglycoprotein receptor. Thus sulfate, like sialic acid, prevents clearance from the circulation by the asialoglycoprotein receptor. The rapid removal of desulfated bLH from the circulation causes a 4- to 16-fold increase in the amount of bLH required to stimulate ovulation compared with native bLH. Particularly striking were differences in the metabolic clearance rates for native bLH and bLH/CHO, 7.3% per min and 1.7% per min, respectively. These differences were unexpected because bLH and bLH/CHO do not differ significantly in charge or size. The different metabolic clearance rates obtained for bLH and bLH/CHO indicate that the presence of sulfated rather than sialylated oligosaccharides on bLH results in a shorter circulatory half-life, which has a significant impact on *in vivo* bioactivity.**

The glycoprotein hormones lutropin (LH), follitropin, thyrotropin (TSH), and chorionic gonadotropin (CG) are a family of closely related glycoproteins. Each hormone is a dimer consisting of a common α subunit and a hormone-specific β subunit. The primary structures of the hormone-specific β subunits are closely related, and two of the hormones, LH and CG, interact with the same receptor to stimulate cAMP production (1, 2). We previously determined that asparagine-linked oligosaccharides terminating with the sequence SO_4-4GalNAcβ1-4GlcNAcβ1-2Manα are present on certain of the glycoprotein hormones synthesized in the anterior pituitary (3). For example, virtually all of the asparagine-linked oligosaccharides on bovine (b) LH and TSH, ovine (o) LH, and human (h) TSH terminate with sulfate. In contrast, human and bovine follitropins and hCG bear asparagine-linked oligosaccharides terminating with Siaα-Galβ1-4GlcNAcβ1-2Manα, where Sia is sialic aciel (3). We recently demonstrated that sulfated asparagine-linked oligosaccharides reflect the activity of a hormone-specific GalNAc-transferase and a GalNAcβ1-4GlcNAcβ1-2Manα-specific sulfotransferase that are expressed in the pituitary but not in the placenta (4, 5). The biologic significance of these sulfated oligosaccharide structures on hormones such as LH has not yet been established.

There is considerable evidence that glycosylation may modulate glycoprotein hormone bioactivity. Characterization of chemically and enzymatically deglycosylated forms of the glycoprotein hormones indicates one or more of the asparagine-linked oligosaccharides is required for the full expression of bioactivity. The asparagine-linked oligosaccharides are not required for binding to the hormone receptor but are essential for activation of adenylate cyclase (6). Recent studies with recombinant forms of the glycoprotein hormones indicate that only certain of the asparagine-linked oligosaccharides are required for adenylate cyclase activation (7). Furthermore, numerous reports indicate that differences in bioactivity correlate with different hormone isoforms, presumably on the basis of differences in glycosylation (3, 8). If structural features of the asparagine-linked oligosaccharides of glycoprotein hormones, such as LH, modulate the expression of bioactivity *in vivo*, this most likely results from changes in terminal glycosylation—i.e., the addition of sulfate. We have examined this hypothesis by comparing the properties of bLH bearing different oligosaccharide structures. Our studies demonstrate that sulfation of bLH oligosaccharides does not alter bLH bioactivity at the level of interaction with the LH/hCG receptor but does have a major impact on *in vivo* bioactivity by controlling circulatory half-life. Our results indicate that terminal glycosylation plays a central role in hormone bioactivity by regulating the rate of hormone clearance from the circulation.

MATERIALS AND METHODS

Preparation of Hormones. Sulfate was released from bLH oligosaccharides enzymatically using arylsulfatase B prepared from human liver (J.U.B., R.M.B., and S.K., unpublished work). Digestions were performed in 10 mM $MnCl_2$/50 mM sodium acetate, pH 5.8/bovine serum albumin at 0.1 mg/ml/leupeptin, antipain, pepstatin, and chymostatin each at 0.4 mg/ml/aprotinin at 1 unit per ml. After digestion, released sulfate and buffer salts were removed by passage over Sephadex G-25 in 1% NH_4HCO_3 and repeated lyophilization. bLH/CHO was prepared from Chinese hamster

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Abbreviations: CG, chorionic gonadotropin; LH, lutropin; bLH, bovine LH; bLH/CHO, recombinant bLH prepared from Chinese hamster ovary cells; MCR, metabolic clearance rate; TSH, thyrotropin; o and h prefix on hormone abbreviation, ovine and human, respectively.

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ovary (CHO) cells as described (9). Iodinations of hCG (25 μg), bLH (25 μg), and bLH/CHO (25 μg) were done at 4°C in 100 μl of 20 mM Tris·HCl, pH 7.4/150 mM NaCl by using 0.5 mCi of $Na^{125}I$ (1 Ci = 37 GBq) and a single Iodo-Bead (Pierce). The radiolabeled hormones were separated from free $^{125}I^-$ and reagents by gel filtration on a 1-ml column of Sephadex G-25 developed with 20 mM Tris, pH 7.4/150 mM NaCl/0.1% bovine serum albumin. For clearance studies the ^{125}I-labeled hormones were digested with sulfatase as described above or with clostridial neuraminidase (9).

***In Vitro* Hormone Bioassays.** Binding of native and desulfated bLH to the LH/CG receptor on MA-10 cells was examined by displacement of ^{125}I-labeled hCG (preparation CR119, National Institute of Diabetes and Digestive and Kidney Diseases) as described by Ascoli (10). The data were analyzed, and the IC_{50} was determined by using the LIGAND program (11) supplied by Biosoft (Milltown, NJ). Production of cAMP and progesterone was also examined by using MA-10 cells. Levels of cAMP produced were determined by RIA with a kit supplied by ICN. Levels of progesterone produced were determined by using an RIA kit provided by DuPont/NEN. Statistical analyses of the dose–response curves were done by using the LIGAND program (11).

***In Vivo* Hormone Bioassays.** Immature female mice (ICR strain, Harlan, Indianapolis) were injected s.c. with 5 international units of pregnant mare serum gonadotropin (Calbiochem) on day 23. Forty-eight hours later the hormone to be tested was introduced i.v. through the tail vein in 100 μl of phosphate-buffered saline/bovine serum albumin (10 mM phosphate, pH 7.4/0.1% bovine serum albumin). The following morning (16–18 hr later) ovulation was scored by removing both oviducts and counting the number of eggs present. Because the number of eggs in the fallopian tubes (10 to 32) did not correlate with the hormone dose and all animals ovulated bilaterally, the results in Table 3 are presented as the number of animals that ovulated.

Clearance Studies. The carotid artery of anesthetized Sprague–Dawley rats (150–200 g) was cannulated. After heparinization, ^{125}I-labeled hormones were introduced into the circulation. Blood samples (100 μl) were taken at multiple times after injection, and the cpm/ml was determined. The data were fit to the equation $C = Ae^{-\alpha t} + Be^{-\beta t}$, where C is the blood concentration, t is time, A and α are the fast-component parameters, and B and β are the slow-component parameters. The metabolic clearance rate (MCR) was determined from the formula MCR = dose/$(A/\alpha + B/\beta)$ and is expressed as % per min.

PAGE. bLH and desulfated bLH were analyzed by PAGE under nondenaturing conditions as described (12) in the presence of SDS.

RESULTS

Desulfation of bLH. bLH was incubated with a sulfatase prepared from human liver for 48 hr at 37°C in the presence of protease inhibitors. The rate and extent of sulfate removal were monitored by an ELISA by using a monoclonal antibody specific for the sulfated trisaccharide SO_4-4GalNAcβ1-4GlcNAcβ1-4Manα and the lectin *Wistaria floribunda* agglutinin (WFA), which recognizes the terminal β-linked GalNAc on these structures after sulfate removal (J.U.B., R.M.B., and S.K., unpublished observation). After digestion with the sulfatase <5% of the GalNAc was resistant to destruction by periodate oxidation, confirming that >95% of the sulfate had been removed in the preparations used for the studies described below. When examined by nondenaturing SDS/PAGE (Fig. 1), desulfated bLH migrated exclusively as a dimeric species. The greater mobility of native bLH reflects the charge contributed by the sulfate. Thus, under the conditions used for digestion with the human liver sulfatase, terminal sulfate could be quantitatively removed from bLH without evidence of proteolytic degradation or dissociation of the α and β subunits.

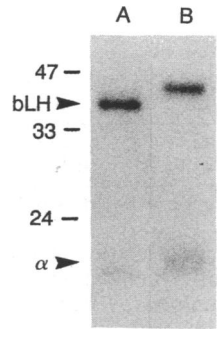

FIG. 1. bLH remains dimeric after digestion with sulfatase. ^{125}I-labeled bLH was incubated for 48 hr at 37°C in the absence (lane A) or presence (lane B) of sulfatase. The product was examined by SDS/PAGE under nondenaturing conditions—i.e., at 4°C without added 2-mercaptoethanol. Locations of authentic dimeric bLH and free α subunit are indicated. Molecular mass standards were 24, 33, and 47 kDa.

Desulfation of bLH Does Not Alter Binding to the LH/CG Receptor. MA-10 cells express biologically active LH/CG receptor at their surface (2, 10). The ability of native and desulfated bLH to competitively block binding of ^{125}I-labeled hCG to the LH/CG receptor was examined as shown in Fig. 2. The IC_{50} values (Table 1) did not differ significantly for native and desulfated bLH, indicating that binding to the LH/CG receptor was not altered by the terminal sulfate.

Desulfation of bLH Does Not Alter cAMP or Progesterone Production. The ability of native and desulfated bLH to stimulate production of cAMP and progesterone after binding to the LH/CG receptor was also examined (Fig. 3). Removal of sulfate from bLH did not significantly alter its ability to stimulate either cAMP or progesterone production by the LH/CG receptor of MA-10 cells (Table 1). The ED_{50} values obtained for cAMP and progesterone production by native bLH agreed well with values we had obtained (9) with a different preparation of bLH. Thus, the presence of terminal sulfate on bLH oligosaccharides does not modulate its ability to stimulate cAMP after binding to the LH/CG receptor.

Clearance of Native and Desulfated bLH from the Circulation. The circulatory half-life of glycoproteins frequently

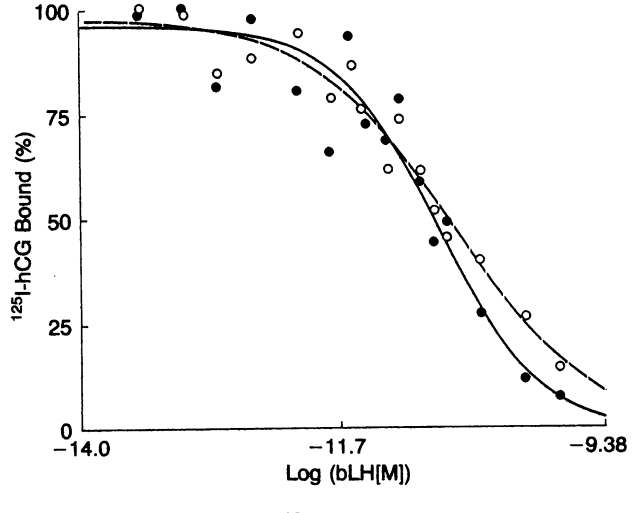

FIG. 2. Displacement of ^{125}I-labeled hCG binding to cultured MA-10 cells. MA-10 cells were incubated with ^{125}I-labeled hCG at 4°C and increased amounts of native (○) and desulfated (●) bLH. Results were analyzed by using the LIGAND program.

Table 1. Comparison of native and desulfated bLH

bLH	Inhibition of hCG binding, IC_{50}*	cAMP, ED_{50}*	Progesterone, ED_{50}*
Native	14.3 (3.1)	0.28 (0.05)	0.16 (0.06)
Desulfated	17.6 (2.9)	0.36 (0.07)	0.22 (0.08)

*IC_{50} and ED_{50} values are expressed as pM (SE).

reflects structural characteristics of their oligosaccharides. The potential effect of glycosylation on the circulatory half-life of bLH was examined by comparing the clearance of ^{125}I-labeled hCG, bLH/CHO, and bLH from the circulation (Fig. 4). The circulatory half-lives of bLH and bLH/CHO differed significantly. Although all three hormones displayed fast and slow clearance phases—the fast phase had a shorter $t_{1/2}$ and accounted for a greater proportion of native bLH clearance than the fast phases for bLH/CHO or hCG clearance. As a result the MCR was 4.3-fold greater for bLH than for bLH/CHO, whereas the MCR was only 2-fold greater for bLH/CHO than for hCG (Table 2).

Removal of terminal sialic acid from hCG oligosaccharides resulted in the exposure of terminal galactose moieties and rapid clearance from the circulation (Fig. 4). Similarly, removal of either sialic acid from bLH/CHO oligosaccharides or sulfate from bLH oligosaccharides resulted in the rapid clearance from the circulation (Fig. 4). After removal of sialic acid or sulfate from bLH/CHO and bLH, respectively, there was no longer any evidence of a slow phase of clearance. Desulfated bLH and desialyzed bLH/CHO were rapidly cleared from the circulation, each having a $t_{1/2}$ of 2 min. The radiolabel from desulfated bLH and desialyzed bLH/CHO was found almost exclusively (>70% of the injected dose) in the liver at autopsy (data not shown).

***In Vivo* Bioactivity.** The capacities of bLH, desulfated bLH, and bLH/CHO to stimulate ovulation *in vivo* were compared (Table 3). Desulfation of bLH increased the amount of hormone required to stimulate ovulation by 4- to 16-fold compared with native bLH. bLH/CHO and native bLH did not differ in their potency *in vivo*. In contrast, 4- to 16-fold more bLH or bLH/CHO was required to stimulate ovulation than hCG. Thus, one can rank the various forms of bLH and hCG with respect to *in vivo* potency as follows: hCG > bLH = bLH/CHO > desulfated bLH. The *in vivo* activities of bLH species bearing different oligosaccharide structures reflect the effects of both circulatory half-life and interaction with the LH/CG receptor.

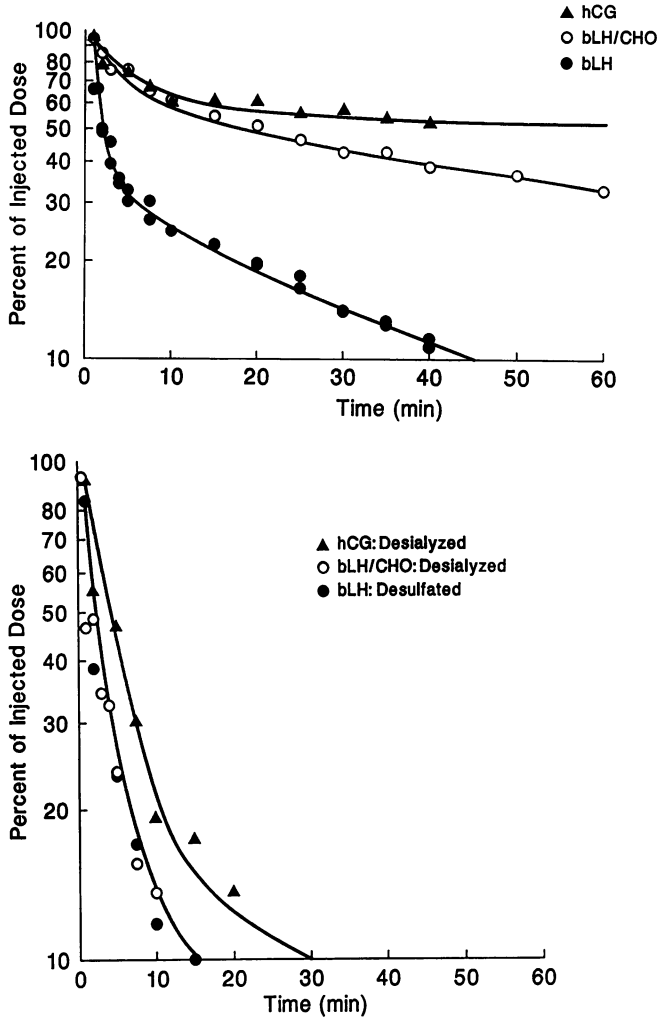

FIG. 4. Plasma clearance rates of bLH, bLH/CHO, and hCG. Hormones labeled with ^{125}I (0.5–2.0 × 10^6 cpm, 50–200 ng) were injected into heparinized anesthetized rats. Samples (200 μl) were drawn from the carotid artery through a cannula at the times indicated. Radioactivity is expressed as percent of injected dose per ml of plasma. Sulfate and sialic acid were removed enzymatically after iodination. (*Upper*) ●, bLH; ○, bLH/CHO; and ▲, hCG. (*Lower*) ●, Desulfated bLH; ○, desialyzed bLH/CHO; and ▲, desialyzed hCG.

DISCUSSION

The glycoprotein hormones are heavily glycosylated with two asparagine-linked oligosaccharides on their α subunit and either one or two asparagine-linked oligosaccharides on their β subunits. It is well established that these oligosaccharides play an important role in the expression of biologic activity. Chemical removal of the asparagine-linked oligo-

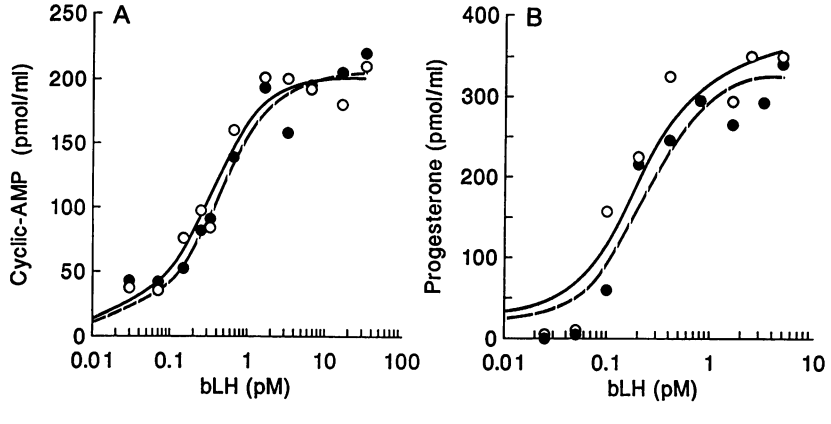

FIG. 3. bLH-mediated stimulation of cAMP and progesterone production by MA-10 cells is not modified by the terminal sulfate. cAMP (*A*) and progesterone (*B*) production by MA-10 cells was measured at the indicated concentrations of native (●) and desulfated (○) bLH.

Table 2. Comparison of hormone clearance rates from the circulation

Hormone	Clearance rate ($t_{1/2}$) Fast phase, min	Slow phase, min	MCR, %/min
bLH	1.2	27	7.3
bLH/CHO	3.4	64	1.7
hCG	2.0	119	0.9
bLH, desulfated	2.0		35
bLH/CHO, desialyzed	2.0		35
hCG, desialyzed	4.2		17

saccharides produces products that can bind to the LH/CG receptor but cannot activate adenylate cyclase efficiently and, as a result, do not stimulate progesterone production (6). Selective deletion of specific glycosylation sites by using site-directed mutagenesis has confirmed these observations and indicated that specific asparagine-linked oligosaccharides play a dominant role in the activation of adenylate cyclase (7). Modulation of hormone biologic activity *in vivo* on the basis of structural differences in glycoprotein hormone oligosaccharides would, however, most likely result from differences in the pattern of terminal glycosylation rather than the absence of an oligosaccharide. Numerous studies have suggested that glycoprotein hormone isoforms differ in bioactivity and that these differences reflect the extent and/or type of terminal glycosylation (3).

We previously demonstrated that bLH bears asparagine-linked oligosaccharides terminating with the sequence SO_4-4GalNAcβ1-4GlcNAcβ1-2Manα (3), rather than Siaα-Galβ1-4GlcNAcβ1-2Manα, which is found on most other pituitary glycoproteins including follitropin. bLH/CHO bears asparagine-linked oligosaccharides terminating in Siaα2-3Galβ1-4GlcNAcβ1-2Manα due to the absence of both the GalNAc- and sulfotransferases in these cells (9). Were the glycoprotein hormone-specific GalNAc-transferase not expressed in gonadotrophs, oligosaccharides on bLH would terminate with sialic acid-galactose, whereas decreased expression of the sulfotransferase would result in oligosaccharides terminating in β-linked GalNAc. The presence of sulfated, as opposed to sialylated, oligosaccharides on bLH must, therefore, be important to the biologic function of this hormone. The ability to prepare forms of bLH differing only in the character of their terminal sugars presented an opportunity to define the biologic impact of terminal glycosylation *in vitro* at the level of the LH/CG receptor and *in vivo*.

The present studies show that the presence of terminal sulfate on bLH oligosaccharides does not have a significant impact on binding to the LH/CG receptor of MA-10 cells or on the production of cAMP and progesterone by MA-10 cells. Identical results were obtained with rat Leydig cells (data not shown). Thus, terminal sulfate does not alter the bioactivity of bLH at the level of interaction with the LH/CG receptor. In contrast, desialyzed bLH/CHO with oligosaccharides terminating with β-linked galactose has the same ED_{50} as native bLH for stimulating cAMP or progesterone production by MA-10 cells, whereas bLH/CHO bearing oligosaccharides terminating in α-linked sialic acid has an ED_{50} that is 3-fold greater than that of native bLH or desialyzed bLH/CHO (9). Because the removal of sialic acid from bLH/CHO and sulfate from bLH would have a similar effect on the charge of the hormone, this result suggests that the reduced *in vitro* bioactivity of bLH/CHO (9) reflects a steric effect from the greater size of the sialic acid.

Table 3. *In vivo* ovulation

	Ovulation, no. mice ovulating/no. mice injected								
	0 ng	1 ng	4 ng	15 ng	62 ng	250 ng	1000 ng	4000 ng	1600 ng
bLH	0/4		0/4	0/4	2/4	4/4	4/4	4/4	4/4
bLH, desulfated	0/4			0/4	1/4	0/4	4/4	4/4	4/4
bLH/CHO	0/4	0/4	1/4	0/8	3/8	8/8	4/4		
hCG		0/4	0/6	3/5	3/4	4/4			

Hormone was injected i.v. (ng) in pregnant mare serum gonadotropin-primed mice. All mice ovulated bilaterally.

Even though the addition of sulfate to terminal GalNAc on bLH asparagine-linked oligosaccharides does not alter interaction with the LH/CG receptor, the sulfate addition has a major impact on the circulatory half-life of bLH. The rapid removal of desulfated bLH from the circulation and its retention in the liver indicate clearance by the asialoglycoprotein receptor found in hepatic parenchymal cells. We have confirmed this hypothesis by demonstrating that desulfated bLH but not native bLH is bound and internalized by isolated rat hepatocytes (J.U.B. and D. Fiete, unpublished work). Thus, the presence of sulfate at the 4 position of terminal β1,4-linked GalNAc, as with sialic acid linked to terminal β1,4-linked galactose, prevents binding by the asialoglycoprotein receptor. Therefore, sulfate plays an analogous role to sialic acid in preventing clearance of bLH by the asialoglycoprotein receptor. Removal of sialic acid from bLH/CHO also results in rapid removal from the circulation by the asialoglycoprotein receptor.

Particularly striking were the differences in the clearance of native bLH and bLH/CHO seen in Fig. 4. Most native bLH is cleared from the circulation during the initial fast phase; $t_{1/2}$ is 1.2 min. Ascoli *et al.* (13) reported that native oLH, which bears exclusively sulfated oligosaccharides, has a circulatory half-life of 5 min. Ascoli *et al.* (13) also indicated that hLH, which bears both sulfated and sialylated oligosaccharides (3), has a circulatory half-life similar to that of oLH rather than that of hCG. In contrast to bLH, most bLH/CHO, like hCG, is cleared primarily during the slow phase. The differences in clearance of bLH and bLH/CHO cannot readily be accounted for by differences in their size. bLH and bLH/CHO differ only by 2–4 kDa when examined by native SDS/PAGE and do not differ when examined by gel filtration on Sephadex G-50 (data not shown). The presence of terminal GalNAc on a small fraction of bLH is unlikely to account for the rapid clearance because only 5–15% of native bLH bears any terminal GalNAc, and this is predominantly in the form of a single residue. Furthermore, native bLH is not internalized by isolated rat hepatocytes (data not shown). The similar apparent molecular masses of bLH and bLH/CHO suggest that their glomerular filtration rates in the kidney should also be similar. In preliminary experiments, we have found that a large proportion of bLH is present in the liver at early times after injection into the circulation; this situation is not so for bLH/CHO, raising the possibility that a receptor that recognizes the sulfated form of bLH oligosaccharides may be present in liver and account for the relatively rapid clearance of bLH as compared with bLH/CHO. Such a receptor would also account for the rapid clearance of hLH, which bears both sulfated and sialylated oligosaccharides.

Even though native bLH and desulfated bLH display similar bioactivities with respect to cAMP and progesterone production by MA-10 cells, they differ by 4- to 16-fold in their ability to stimulate ovulation *in vivo*. This difference must reflect the 4.8-fold more rapid MCR for desulfated bLH (35% per min vs. 7.3% per min). Native bLH and bLH/CHO stimulate ovulation at the same dose when injected i.v., even though their MCRs differ by 4.3-fold (Table 2). We previously determined that the ED_{50} for progesterone production by MA-10 cells is 3- to 4-fold greater for bLH/CHO than for either native bLH or desialyzed bLH/CHO (9). Therefore, the decrease in potency resulting from the terminal sialic acid is compensated for by the reduced MCR of bLH/CHO

compared with that of native bLH. hCG has an MCR that is half that of bLH/CHO (0.9% per min vs. 1.7% per min); yet hCG is 4- to 16-fold more potent than bLH/CHO as a stimulator of ovulation *in vivo*. The difference in potency in this case appears to reflect predominantly differences in the ability of hCG and bLH/CHO to stimulate progesterone production at the receptor level (ED_{50} values of 1.7 and 7.2 ng/ml, respectively).

Plasma levels of LH vary in a pulsatile manner throughout the menstrual cycle (14, 15), reflecting variations in the frequency, amplitude, and duration of the secretory burst (16, 17). The circulatory half-life of native bLH we have observed is shorter than the secretory burst time reported for LH in humans from deconvolution studies (16). Such a short circulatory half-life could modulate the amplitude of the secretory burst and determine the amount of hormone release required to maintain a steady-state level of circulating LH. Furthermore, variation in the frequency of secretory bursts on plasma LH concentration would be markedly amplified by an increase in the circulatory half-life of the hormone. The 4.8-fold difference in the MCR for native bLH and bLH/CHO suggests that terminal glycosylation with SO_4-4GalNAcβ1-4GlcNAcβ1-2Manα rather than Siaα2-3Galβ1-4GlcNAcβ1-2Manα is essential for precise regulation of the circulatory half-life for LH. Pulsatile variations in serum LH concentration are thought necessary for the *in vivo* expression of bioactivity. It may be that the ability to produce such a pulsatile variation in hormone level would be disrupted were the half-life of LH increased by addition of sialic acid–galactose in place of SO_4-GalNAc.

Because the *in vivo* biologic activity of bLH is strongly influenced by the terminal glycosylation of its asparagine-linked oligosaccharides, there may be circumstances under which the levels of GalNAc- and sulfotransferase expression are altered. The absence of terminal sulfate due to reduced sulfotransferase activity would result in reduced *in vivo* potency due to the rapid clearance. Changes in the extent of sulfation could occur rapidly and transiently. The 4.8-fold difference in MCR for native bLH and bLH/CHO was unexpected. Replacement of SO_4-GalNAc with sialic acid–galactose produces a form of bLH with nearly the same MCR as the hCG. This result occurs even though the LH β subunit does not have the carboxyl-terminal extension peptide found on hCG β subunit. Horses also produce a placental form of LH. Notably, placental equine LH bears sialylated oligosaccharides (18), whereas equine LH of pituitary origin appears to bear sulfated structures (19). This fact suggests that forms of LH/CG with different circulatory half-lives are required during pregnancy and the ovulatory cycle. Conceivably mammals that do not produce a placental form of LH may alter the glycosylation of LH during pregnancy by regulating expression of the glycoprotein hormone-specific GalNAc-transferase. Synthesis of sialylated rather than sulfated oligosaccharides on LH by gonadotrophs during pregnancy would result in the production of a form of LH with properties similar to those of the placental hormone.

Gyves *et al.* (20) recently reported that TSH from hypothyroid rats has increased levels of sialic acid-bearing oligosaccharides. Furthermore, they have found that hTSH produced in CHO cells has a longer half-life than native hTSH isolated from cadavers (21). The circulatory half-life and, as a result, the *in vivo* potency of TSH may also be modulated by the terminal glycosylation of its asparagine-linked oligosaccharides in a fashion analogous to LH.

The sulfated oligosaccharides on the glycoprotein hormones provide a mechanism to regulate their circulatory half-life and thereby their *in vivo* bioactivity. This fact may be particularly critical for hormones, such as LH, that show pulsatile variations in their circulatory levels. Although this regulation may not be the only biologic function for these sulfated structures, this activity is one that is critical for hormone function.

We thank the National Hormone and Pituitary Program for providing hCG. This work was supported by National Institutes of Health Grant R01-DK41738 to J.U.B. P.L.S. was supported by U.S. Public Health Service Grant T32-ES07066.

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