The Presence of Glycosylated, Biologically Active Chorionic Gonadotropin in Human Liver

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Whole extracts of normal human liver contain hCG-like material as determined by radioimmunoassay using antibodies to the β subunit of the hormone. However, the extracts are biologically inactive, when analyzed by the *in vitro* rat Leydig cell assay for steroidogenesis. When subjected to Concanavalin-A-Sepharose chromatography, the radioimmunoassayable material was entirely lectin-bound. After elution with methyl-α-D-glucopyranoside, the lectin-bound fractions now displayed biological activity.

KEY WORDS: human chorionic gonadotropin, lectins, glycosylation; biological assay, Leydig cell

Until fairly recently, it was generally believed that under both normal and pathologic conditions the synthesis and secretion of human chorionic gonadotropin (hCG) was confined strictly to trophoblastic tissue and any circulating hormone originated from that source. However, with the advent of sensitive and relatively specific radioimmunoassays for hCG, using antibodies to the β-subunit of the hormone, several studies (1–4) reported small but significant concentrations of this hormone in normal tissues. Using the RIA system, Braunstein *et al.* (3) found a moiety in extracts of human tissues from non-pregnant subjects that reacted like hCG. This material was found to have the immunological, physical and biological properties of hCG, rather than those of luteinizing hormone (LH). Also, hCG-like material was demonstrated in extracts of normal human liver and colon by means of both an hCG-RIA and a radioreceptor gonadotropin assay (4). Human fetal liver and muscle contained hCG-RIA-reactive material as noted by Huhtaniemi *et al.* (5); they ruled out the hCG from the maternal circulation as source.

The placental and non-placental forms of the hormone appear to exhibit different biological activities (6). The *in vivo* bioactivity of hCG is dependent not only on the integrity of its quaternary structure (neither the free α- nor β-subunit is bioactive) but also on its degree of glycosylation. Progressive desialylation of the hormone results in a decrease of its plasma half-life and a consequent loss of biological activity (7). On the other hand, desialylation has no effect on the immune reactivity of hCG (8). Little or no hCG can be found in the circulating blood of normal non-pregnant individuals.

Slight but definite increases in circulating hCG have been noted not infrequently in patients bearing tumors of non-trophoblastic origin (9, 10). If it is true that normal non-trophoblastic organs contain primarily (8) the non-glycosylated form of the hormone, then it follows that during malignant transformation of these tissues the genome must undergo derepression, the organs acquire the capacity for post-translational glycosylation of the hormone and the synthesis of hCG should then be regarded as being ectopic (i.e. non-trophoblastic in origin). We felt that it was important to try to resolve this question in view of its implications with respect to tumor biology in general and ectopic hormone production in particular. Consequently, we undertook first to confirm that hCG-like material can indeed be found in adult human liver using human placenta as reference tissue; to compare the hCG content of the organs by RIA with that measurable by bioassay; and to ascertain the state of glycosylation of these polypeptides by affinity chromatography on Con-A Sepharose columns.

Con-A Sepharose has a selective affinity for some glycopeptides; both Krusius (11), and Debray and Montreuil (12), found that the lectin Concanavalin A has the highest affinity for the oligomannosidic type of glycopeptides, and that binding depends on the accessibility of the *N*-acetyl-glucosamine-mannose (GNAc-Man) sequences. hCG contains several of these GNAc-Man sequences and even though they are not terminal residues, they appear to be accessible to binding.

Materials and methods

Normal human livers were obtained at autopsy from male patients who had died from diseases other than of the liver or cancer; placentae were obtained from prostaglandin abortions at the 12th to 16th week of gestation (courtesy of the Department of Pathology, Sunnybrook Medical Centre). The tissue specimens were washed in physiological saline, cleaned of supporting elements, weighed and frozen until time of extraction.

The Koenig and King extraction technique (13) was used to obtain crude hCG preparations from the tissues listed above. Following treatment with 40% ethanol, the pellet was further extracted with saline.

β-hCG RADIOIMMUNOASSAY

The polyethylene glycol method (Serono Laboratories, Braintree, MA) was used. The rabbit antibody is

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Manuscript received June 11, 1984; revised manuscript received September 6, 1984; accepted for publication September 18, 1984.

TABLE 1
hCG in Human Placenta and Liver, as Determined by Radioimmunoassay and Bioassay

Tissue	n	hCG-β (RIA)	hCG (Bioassay)	B/I*
		X̄ mIU/mg protein (range)	X̄ mIU/mg protein (range)	
Placenta	4	541 (314–772)	456 (139–637)	0.84
Liver	4	17 (14–21)	0** 3***	0.17

*B/I: ratio of biological to immunological activity.
**before chromatography on Con-A Sepharose.
***after chromatography on Con-A Sepharose.

directed towards the β-subunit of hCG, which allows selective measurement of this hormone in the presence of human luteinizing hormone. One mIU is approximately equal to 0.2 ng of hCG. In our hands, the sensitivity of the assay was found to be ≤2.0 mIU/mL. The within-assay coefficient of variation was 9.1% and the between-assay CV was 14.5%.

hCG/LH RAT LEYDIG CELL BIOASSAY *IN VITRO*

We followed the method described by Sundaram *et al.* (14), using minced rat testis *in vitro*. The standard curve was established by addition of serial dilutions of the same hCG 2nd International Standard as used in the β-hCG RIA. The resulting testosterone synthesis was determined by a testosterone RIA (15). Two pg of testosterone can be reliably distinguished from 0; 3 ng hCG per sample induces 7.5 pg testosterone.

PROTEIN DETERMINATION

All extraction data are reported as mIU/mg protein. The protein content of the extraction samples was determined by the Lowry method (16).

AFFINITY CHROMATOGRAPHY

The liver tissue extracts were concentrated in an Amicon 202 stirred cell concentrator using a PM 10 membrane (Amicon Canada Ltd., Oakville, Ont. L6H 2B9) and then chromatographed. Placental extracts were applied without concentration. Con-A Sepharose was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden as the swollen gel suspension.

Miniature columns (75 × 3.5 mm) of Con-A Sepharose were prepared and washed of their storage buffer with 0.01 mol/L phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). The extracts containing hCG were chromatographed at room temperature.

Two buffer systems, 0.01 mol/L PBS + 10 g/L BSA, and 0.01 mol/L PBS + 0.2 mol/L methyl-α-D-glucopyranoside, were used. All eluted fractions were tested with the hCG-β RIA and bioassay.

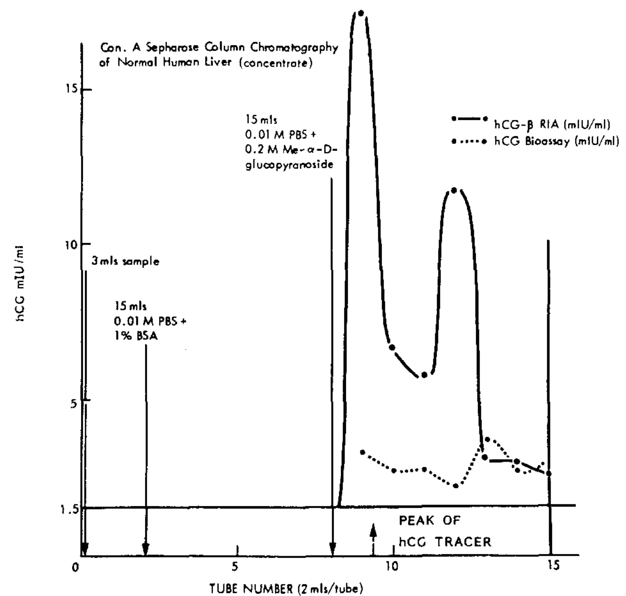

Figure 1 — Levels of hCG determined in eluates from human liver extracts on a Con A-Sepharose column. Comparison of the radioimmunoassay and bioassay. The tracer was an hCG (WHO Standard) obtained from NCI (US).

Results

The results obtained with the radioimmunoassay and the bioassay of hCG in human liver and placenta extracts are presented in Table 1.

All liver tissue extracts investigated showed the presence of hCG-like material with the RIA, using a specific antibody directed against the β-subunit. All placental extracts studied as control contained hCG, as determined both by RIA and bioassay. The relatively crude extracts from liver did not induce any steroidogenesis in the *in vitro* rat Leydig cell system. However, after partial purification of the extract by passage on Con-A Sepharose and subsequent elution with a pulse of methyl-α-D-glucopyranoside, the hCG obtained showed slight but definite biological activity in the bioassay, which consistently peaked one elution fraction after the peak determined by RIA (Figure 1). The same phenomenon was observed after Con A chromatography of the placental extract; however, with placental hCG, the total bioactivity determined after passage on the Con A column was greater than measured by RIA. This activity was not due to the presence of LH, which was not detectable by RIA in the specimens studied.

Discussion

In previous studies of Con-A Sepharose affinity chromatography of placental hCG (3, 17) it was noted that about 90% of the hormone was lectin-bound and therefore glycosylated. Our results are entirely in agreement; about 90% of our placental extract was lectin-bound and displaced by 0.2 mol/L methyl-α-D-glucopyranoside. However, Yoshimoto *et al.* (4), using a different extraction method than ours on normal male

liver, reported that their extracts contained 15% lectin-bound hCG. In our hands, the small amount of hCG-like substance, extracted from human male liver and chromatographed on Con-A Sepharose, was indistinguishable from human placental hCG in both assay systems used. The material was glycosylated and, in addition, reacted like the intact hormone since it displayed weak but definite biological activity once it had passed through the column. If the hepatic hormone is indeed non-glycosylated, it is difficult to see how we could have added lectin-binding material during the extraction process. Consequently, we feel that it is more likely that the carbohydrate residues were removed during the extraction process by other investigators.

In a recent paper, Braunstein *et al.* (18) reported a similar average ratio between bioactive and immunoactive hCG-like substances in normal human tissues. The yield of hCG appears to be greater in our study; we feel that this may be the result of the extraction procedure (which differs from the above authors) and our expression of results per mg protein rather than per wet tissue weight. Differences in age and strain of rats used as source of Leydig cells may account for the lower B/I ratio observed by us.

Using liver as a potential non-trophoblastic source of hCG, we have observed that the organ contains this hormone, albeit in small quantities as reported also by others (3, 4, 8, 18). That the presence of hCG in adult male liver can be attributed to simple trapping seems unlikely since there is no abundant source for the hormone in males and circulating levels are either very low or virtually absent (19).

If the hCG in other normal organs is found, as in liver, to be predominantly glycosylated, then the question regarding the eutopic or ectopic origin of hCG in non-trophoblastic tumours remains unresolved by this approach. In either case the glycosylated hormone could have arisen as a simple consequence of excessive production rather than by derepression of genetic information. Accordingly, we must still try to determine whether the non-trophoblastic hCG is identical in all respects to the placental hormone, and if not, whether the intact hormone in non-trophoblastic tumours resembles that found in either the normal organ or placenta or indeed is something entirely different.

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