The Bioassay for Human Prolactin and Human Chorionic Somatomammotropin in Serum from Pregnant Women and in Amniotic Fluid

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Abstract

The bioactivity of human prolactin (hPRL) and human chorionic somatomammotropin (hCS) in the sera from the pregnant women (30-40 weeks of gestation) and in the amniotic fluid (37-41 weeks of gestation) were determined individually by the bioassay for lactogenic hormones using rat lymphoma cell bioassay in combination with the antibody blocking method.

The bioactivity of hPRL in the pregnant sera ranged from 67 to 289 ng/ml and in the amniotic fluid from 141 to 1603 ng/ml. The ratio of the bioassay and RIA estimates of hPRL in the pregnant sera was 0.93 ± 0.23 (mean ± SD) and that of estimates of hPRL in the amniotic fluid was 1.04 ± 0.26. These ratios are not significantly different from unity, which demonstrates that the bioactivity of hPRL is not significantly different from the immunoactivity.

The bioactivity of hCS in the pregnant sera ranged from 5.6 to 21.8 μg/ml and in the amniotic fluid from 87 to 1274 ng/ml. The ratio of the bioactivity and RIA estimates of hCS in the pregnant sera was 1.48 ± 0.20 and that of estimates of hCS in the amniotic fluid was 1.32 ± 0.21. These ratios are significantly higher than unity, which demonstrates that the bioactivity of hCS was significantly higher than the immunoactivity. This may be due to the impurity or relatively decreased bioactivity of the hCS standard.

This bioassay seems useful and convenient to measure the individual concentration of hPRL and hCS in the pregnant serum and in the amniotic fluid.

Human prolactin (hPRL) and human chorionic somatomammotropin (hCS) are known as lactogenic hormones as well as human growth hormone (hGH) and are supposed to play an important role during pregnancy (Nicoll, 1974; Morishige and Rothchild, 1974). Although radioimmunoassay (RIA) is routinely used for both hormones (Tohjoh et al., 1971; Sinha et al., 1973), bioassays are not easily available. A number of bioassays for PRL (Nicoll, 1967; Kleinberg and Frantz, 1971; Forsyth and Myres, 1971; Turkington, 1971) have been reported, but most of them are technically difficult and rather tedious to perform. Bioassays for CS using radioreceptor assay (RRA) have been also reported (Shiu et al., 1973; Kelley et al., 1976). However, the RRA for CS was based on the binding of lactogenic hormones to the membrane, so that not individual CS activity but total lactogenic activity was determined.

We have previously reported the bioassay for lactogenic hormones using rat lymphoma cell line (Tanaka et al., 1980). Although the bioassay is based on the stimulation of the growth and multiplication of the number of
rat lymphoma cells by the lactogenic hormones, we succeeded in measuring the bioactivity of hPRL and hCS individually in combination with the antibody blocking method. This is the first paper to report the measurement of the bioactivity of hCS.

Materials and Methods

Purified hPRL (hPRL-Friesen 80-3-20) was a gift from Dr. Friesen's Laboratory (University of Manitoba, Winnipeg) and hCS was obtained from the NIAMDD, NIH. They were used as the standards in both RIA and bioassay and also used for radioiodination as a tracer for RIA. The standard double antibody RIA method was employed. Anti-hGH and anti-hPRL antisera were raised in rabbits at Dr. Friesen's Laboratory and anti-hCS was obtained from NIAMDD. Antisera were used at a dilution of 1:10,000 for RIA and 1:100 for biological inactivation.

The bioassay procedure employed was that described previously (Tanaka et al., 1980) with a slight modification. On the day the bioassay started 1.5 ml aliquots of lymphoma cells in Fischer's medium supplemented with 10% horse serum, 10^{-4} M 2-mercaptoethanol and antibiotics were distributed in 24 hole multiwell dishes (Nunc) at a concentration of 1-2 × 10^6 cells/ml. Diluted samples or standard were added in 50 μl as well as 50 μl of 1:100 diluted antiserum (final dilution 1:30,000) and cultures were incubated in a CO₂ incubator for 72 hours at 37°C. After 72 hours incubation, the cell numbers were determined with a Coulter Counter (Sysmex).

The lactogenic hormones have a similar amino acid sequence (Kawauchi and Li, 1974; Shome and Parlaw, 1977). Especially hGH and hCS are very close and the genes of both hormones are located on chromosome No. 17 (Owerbach et al., 1980). Frequently anti-hGH antiserum cross-reacts with hCS. The anti-hGH antiserum, we used, showed the cross-reactivity with hCS not only immunologically but also biologically.

The immunological cross-reactivity of hCS was 0.25% when 125I-hGH was used as a tracer and the final anti-hGH antiserum concentration in Fischer's medium supplemented with 10% horse serum, 10^{-4} M 2-mercaptoethanol and antibiotics was 1:70,000. However, the cross-reactivity of hGH was 2.5% when 125I-hCS was used as a tracer with the same concentration of antiserum. The growth stimulating effect of hPRL standard (up to 50 ng/ml) could be blocked with 50 μl of 1:100 diluted anti-hPRL and that of hCS standard (up to 50 ng/ml) with 50 μl of 1:100 diluted anti-hGH antiserum (Fig. 1). The addition of anti-hGH antiserum to hPRL standard and anti-hPRL antiserum to hCS standard had little effect on the growth stimulating effect of hPRL and hCS standard.

Human sera were obtained from pregnant women at 38–41 weeks of gestation and amniotic fluid was obtained at delivery at 37–41 weeks of gestation. Serum samples were used at 1:100–1:500 dilution with 0.01 M phosphate buffered saline, pH 7.4 containing 0.1% BSA, and amniotic fluid samples were used...
at 1:50–100 dilution. The addition of both anti-hGH and -hPRL antisera to the cultures with pregnant serum samples was found to block the growth stimulating effect of the serum completely. This shows that the growth stimulating effect of the serum depends only on the lactogenic hormones. Usually sera from late pregnant women contain three lactogenic hormones, 5–15 µg/ml of hCS, 100–400 ng/ml of hPRL and 0–5 ng/ml of hGH by RIA (Hirai, 1974; Kawauchi et al., 1975; Soria et al., 1977). The hGH concentration in pregnant sera is almost 1000 times less than the hCS concentration and the bioactivity of hGH is negligible in comparison to hCS in pregnant sera even if the potency of the biological activity of both hormones takes into account.

Thus, the growth stimulatory activity which is unblocked by anti-hPRL anti-serum is regarded as hCS bioactivity and the growth stimulatory activity which is unblocked by anti-hGH antiserum is regarded as hPRL bioactivity both in pregnant serum samples and amniotic fluid samples.

As shown in Fig. 2, the serum samples in the presence of anti-hPRL antiserum or anti-hGH antiserum showed a dose-dependent increase in cell numbers which is parallel to that produced by hPRL or hCS standards. This was true of the amniotic fluid samples, also. This made it possible to express the growth-promoting activity of serum or amniotic fluid samples in terms of the concentrations of hPRL or hCS producing the same increase in population.

Statistical test of significance was carried out with Student's t-test.

Results

The bioactivity of hPRL in the sera from twenty-two pregnant women ranged from 67 to 289 ng/ml and that of hCS from 5.6 to 21.8 µg/ml. The bioactivity of hPRL in nineteen amniotic fluids ranged from 141 to 1603 ng/ml, and that of hCS ranged from 87 to 1274 ng/ml. Fig. 3 shows the correlation between the hPRL or hCS values in pregnant sera obtained by the bioassay and RIA. The ratio of the bioassay and RIA estimates of hPRL was 0.93 ± 0.23 (mean ± SD) and that of estimates of

![Fig. 3 (a)](image1.png)

![Fig. 3 (b)](image2.png)
Fig. 4 (a). Correlation between hPRL concentrations in amniotic fluid samples as estimated by the lymphoma cell bioassay and by RIA. The ratio of the bioassay and RIA estimates of hPRL was 1.04±0.26 (mean±SD) and that of estimates of hCS was 1.32±0.21 (mean±SD).

The ratio of the bioassay and RIA estimates of hPRL in both pregnant serum and amniotic fluid was not significantly different from 1.00, but that of estimates of hCS was significantly higher than 1.00 (p<0.001.)

Discussion

The pigeon crop sac assay (Nicoll, 1967) and the in vitro assays employing rabbit (Forsyth and Myres, 1971), and mouse (Kleinberg and Frantz, 1971; Turkington, 1971) mammary gland tissue have been used as PRL bioassays. RRA using rabbit mammary gland membrane preparations has also been used for assaying lactogenic hormones (Shiu et al., 1973; Kelley et al., 1976). However, these bioassays based on detecting the lactogenic activity are only used to determine the potency of PRL preparations or PRL activity in the serum from non pregnant women or post-partum women. Since both hPRL and hCS have lactogenic hormones, to our knowledge there is no report on measurement of the bioactivity of hPRL and hCS individually in the samples from pregnant women and amniotic fluid. Although the rat lymphoma cell bioassay also detects the lactogenic activity, the cross-reaction of anti-hGH antiserum with hCS and the negligible concentration of hGH made it possible to determine the biological activity of hPRL and hCS in the pregnant serum and amniotic fluid individually.

The biological activity of hPRL was not different from the immunological activity in the pregnant serum or the amniotic fluid. However, the biological activity of hCS was significantly higher than the immunological activity. The hCS preparation from NIAMDD...
was not actually characterised and its biological activity is only about one sixth that of pure hPRL and hGH preparations on a weight basis. Gel filtration of hCS standard on a Sephadex G-100 column revealed a small peak corresponding to a molecular weight of 40,000 daltons and a big peak corresponding to a molecular weight of 20,000 daltons. Both peaks showed decreased bioactivity on lymphoma cell bioassay (unpublished data). Thus, increased bioactivity of hCS in the pregnant serum and amniotic fluid seems not due to the molecule itself in the samples but due to the impurity or relatively decreased bioactivity of the hCS standard.

This bioassay seems useful and convenient for measuring the individual concentration of hPRL and hCS. However, in the case of a very high hCS concentration and a low hPRL concentration, dilution to block the hCS activity completely may make the hPRL concentration too low to be detected in the bioassay.

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References


