Binding inhibition by monoclonal antibodies of ovine placental lactogen to growth hormone receptors in human liver

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In the radioreceptor assay for growth hormone (RRA-GH) using [^{125}I]iodo-hGH, hGH and human liver membrane particulate fractions as tracer, hormone standard and receptors, respectively, ovine placental lactogen (oPL) is capable of inhibiting the binding of [^{125}I]iodo-hGH in a parallel manner with hGH and in equipotency. Similarly, in the RRA-GH by employing [^{125}I]iodo-oPL, oPL and human liver membrane particulate fractions as tracer, hormone standard and receptors, respectively, hGH is also equipotent as oPL in inhibiting the binding of [^{125}I]iodo-oPL in a parallel fashion. The addition of monoclonal antibodies against oPL in the assay was effective in inhibiting the binding of [^{125}I]iodo-oPL to human liver, but could not, however, inhibit the binding of [^{125}I]iodo-hGH to human liver. Furthermore, the addition of the monoclonal antibodies in the RRA-GH did not affect the parallelism of the oPL standard but lowered the total binding of oPL. Our studies indicate that the structure of the binding sequence in oPL which binds to the GH receptor of human liver is not identical to the equivalent sequence of hGH and that the monoclonal antibodies compete with GH receptors in human liver for the binding of oPL.

Monoclonal antibody *Ovine placental lactogen*

1. INTRODUCTION

Numerous studies on the mechanism(s) of action of polypeptide hormones have led to the concept that the primary event involves the binding of a hormone to a specific recognition site. This recognition site is also termed a 'receptor'. The receptor is not only able to bind the hormone with high specificity and high affinity, but also is able via signal transduction to activate cellular processes after binding with the hormone. Within the last 15 years, receptors specific for pituitary polypeptide hormones such as growth hormone (GH), prolactin (PRL), gonadotropins (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)), thyroid stimulating hormone (TSH), adrenocorticotropin and β-endorphin, have been identified and characterized [1–8]. By employing a direct binding assay for growth hormone, it has been shown that the GH receptor is not only present in a wide range of animal tissues [9,10] but also in human liver and cultured lymphocytes [11,12]. We have previously shown that hGH specifically binds to human liver, whereas non-primate GH preparations display no cross-reactivity with hGH [13]. The only known hormone preparations that can compete with hGH for binding to human liver are human placental lactogen (hPL) and ovine placental lactogen (oPL). The potencies of hPL and oPL were approx. 1–10 and 100% as potent as hGH, respectively. These observations raised the speculation that oPL and hGH might share a common (or identical) binding sequence (antigenic determinant) for binding to growth hormone receptor in human liver.

Here we report the use of specific monoclonal

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antibodies against oPL to inhibit the binding of [^{125}I]iodo-hGH and [^{125}I]iodo-oPL to human liver membrane particulate fractions. Our studies indicate that the structures of the GH-binding sequence in hGH and oPL are not identical but could be similar.

2. MATERIALS AND METHODS

2.1. *Hormone preparations*

Human growth hormone (NIAMDD-hGH-I-1, 2.2 U/mg), ovine prolactin (NIAMDD-oPRL-I-1, 32 U/mg), ovine growth hormone (NIADDK-oGH-I-3) and human placental lactogen (hPL) were kindly supplied by NIAMDD, NIH, USA.

Ovine placental lactogen (oPL-I and oPL-II) were purified according to the methods that we have described elsewhere [14]. It is equipotent to oGH and oPRL in the radioreceptor assay for growth hormone (RRA-GH) [15] and for prolactin (RRA-PRL) [16], respectively.

2.2. *Radioreceptor assay for GH using human liver*

Human liver was obtained from post-mortem cadavers less than 6 h after death, at the Department of Pathology, Hôtel-Dieu Hospital of Montreal.

A RRA-GH was performed according to the method that we have described for RRA-oPL [17] using human liver membrane particulate fractions, [^{125}I]iodo-hGH and hGH as receptors, tracer and hormone standard, respectively.

2.3. *Iodinations*

All iodinations were performed using the enzymatic method of lactoperoxidase [18]. The specific activity for [^{125}I]iodo-hGH and ^{125}I-oPL was 75 and 78 μCi/μg, respectively.

2.4. *Monoclonal antibodies against oPL*

The development of monoclonal antibodies to oPL has been described [19]. Monoclonal antibodies (ascites fluid) derived from three immunoglobulin (Ig)G secreting hybridomas (2C5, IB12 and 2C6) were used here.

2.5. *Effect of monoclonal antibodies on the binding of hGH and oPL*

To assess whether the monoclonal antibodies against oPL could inhibit the binding of [^{125}I]iodo-hGH and [^{125}I]iodo-oPL to human liver membrane particulate fractions, the following procedures were performed: assays were carried out in plastic tube (12 × 75 mm) containing 200 μl diluted ascites fluid and 100 μl diluted membrane particulate fractions (approx. 1 mg protein). All dilutions were made with 0.025 M Tris-HCl buffer, pH 7.6, containing 0.1% BSA and 10 mM $MgCl_2$. The incubation proceeded overnight at cold-room temperature. The reaction was terminated by the addition of 3 ml ice-cold 0.025 M Tris-HCl buffer, pH 7.6, containing 0.1% BSA, then centrifuged at 780 × g for 30 min at 4°C. The supernatant was aspirated and the membrane-bound iodinated hormone in the precipitate was counted in an LKB autogamma counter. In the absence of diluted ascites fluid, approx. 25 and 30% of [^{125}I]iodo-hGH and [^{125}I]iodo-oPL of the added radioactivity were bound, respectively. Non-specific binding (tubes without ascites fluid and receptor added) accounted for < 10% of the maximum total binding. Results are expressed as % of control (in the absence of diluted ascites fluid) after subtracting the counts non-specifically bound.

3. RESULTS

In the RRA-hGH using [^{125}I]iodo-hGH, hGH and human liver membrane particulate fractions as tracer, hormone standard and receptors, respectively (fig.1A), oPL was able to inhibit the binding of [^{125}I]iodo-hGH in a parallel manner to hGH standard and in equal potency to hGH, whereas hPL was only 5% as effective as hGH. oGH and oPRL were approx. 1–2% as active as hGH.

Similarly, when using [^{125}I]iodo-oPL as tracer in the RRA-GH as shown in fig.1B, oPL was also equipotent to hGH in inhibiting the binding of [^{125}I]iodo-oPL to human liver membrane particulate fractions, whereas hPL, oGH and oPRL were only 2% as active as the oPL standard.

Fig.2A shows that the monoclonal antibodies derived from both 2C5 and 1B12 hybridomas are able to inhibit the binding of [^{125}I]iodo-oPL to human liver membrane particulate fraction (greater than 65% inhibition at 1:10 dilutions of ascites fluid), whereas monoclonal antibodies derived from 2C6 were ineffective in inhibiting the binding of [^{125}I]iodo-oPL. None of the

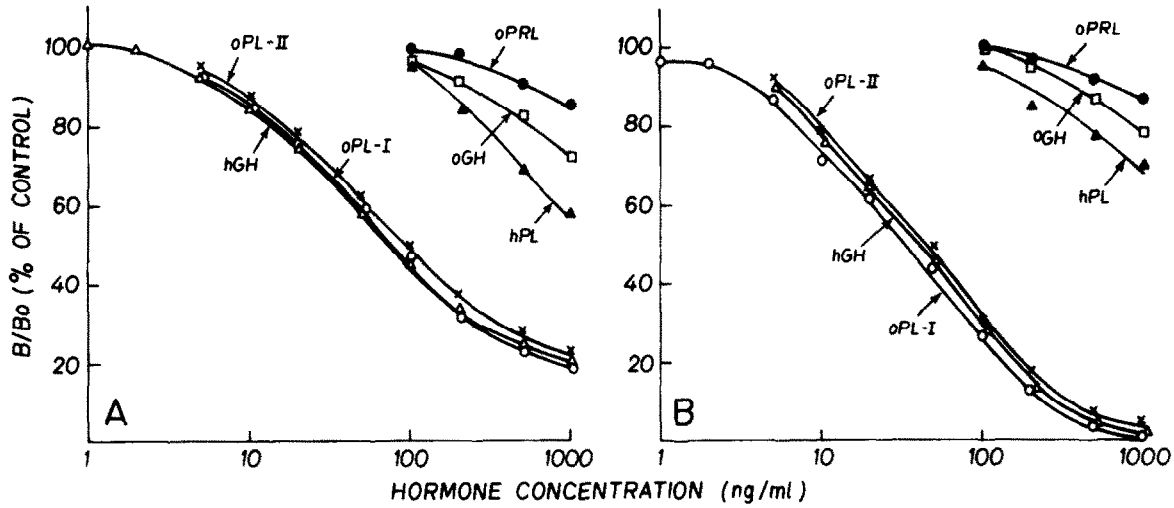
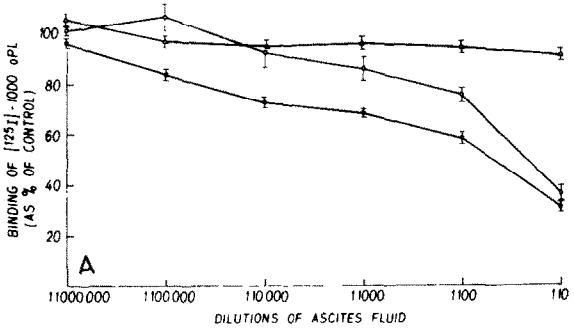
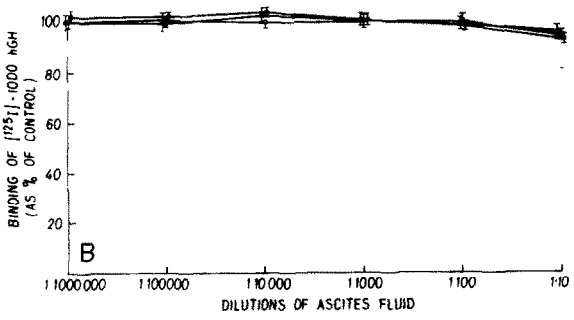


Fig.1. (A) Displacement curves for purified preparations of oPL-I, oPL-II, hGH, oPRL and oGH in the radioreceptor assay for growth hormone (RRA-GH). Human liver membrane particulate fractions were incubated with [^{125}I]iodo-hGH in the presence of increasing concentrations of 'cold' hormone. The ordinate represents the [^{125}I]iodo-hGH bound to growth hormone binding site in the receptor preparation (total binding). In the absence of any added hormone the amount of total binding is approx. 25% using 1 mg membrane protein and is taken as control (100%). (B) Displacement curves of purified preparation of oPL-I, oPL-II, hGH, oPRL and oGH in the radioreceptor assay for placental lactogen (RRA-PL). Human liver membrane particulate fractions were incubated with [^{125}I]iodo-oPL-I in the presence of oPL-I, oPL-II, hGH, oPRL and oGH. The total binding for [^{125}I]iodo-oPL-I is 30% using 1 mg membrane protein. The amount of [^{125}I]iodo-oPL-I bound in the absence of cold hormone is taken as 100%.





monoclonal antibodies were effective in inhibiting the binding of [^{125}I]iodo-hGH to human liver (fig.2B).

Fig.3 depicts the effect of monoclonal antibodies in the RRA-GH using [^{125}I]iodo-oPL as tracer. The addition of monoclonal antibodies did not affect the dose-response curve (parallelism) of the oPL standard in the assay. It did, however, lower the total binding of the iodinated oPL to the receptors.

Fig.2. (A) Inhibition of binding of [^{125}I]iodo-oPL to human liver membrane particulate fractions by monoclonal antibodies against oPL. The total binding for [^{125}I]iodo-oPL without monoclonal antibodies added is approx. 27% and is expressed as control (100%). Hybridoma 2C5 (○—○), 1B12 (●—●) and 2C6 (△—△). (B) Inhibition of binding of [^{125}I]iodo-hGH to human liver by monoclonal antibodies against oPL. The total binding for [^{125}I]iodo-hGH without monoclonal antibodies added is approx. 30% and is expressed as control (100%). Symbols same as in A.

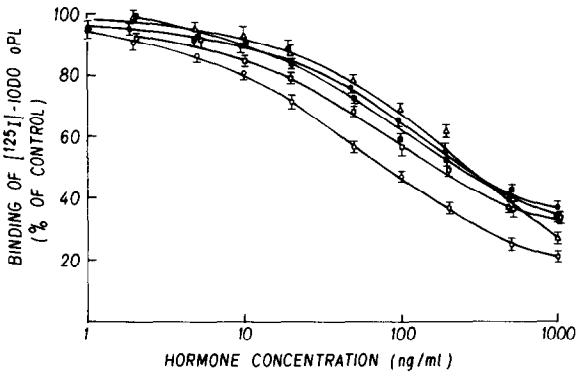


Fig.3. Displacement curves of oPL standard in the RRA-GH using human liver membrane particulate fractions and iodinated oPL as receptors and tracer, respectively. The total binding for oPL standard without monoclonal antibodies is 37.95% (○—○). The total bindings for oPL with monoclonal antibodies added for 2C5 at dilutions of 1 : 10000 (△—△) and 1 : 100 (■—■) and for 1B12 at dilutions of 1 : 10000 (●—●) and 1 : 100 (□—□) are 37.78, 28.89, 24.44 and 12.0%, respectively.

4. DISCUSSION

Lesniak et al. [20] have reported that human lymphocyte receptor for GH is species specific, and that non-primate GHs fail to compete for the GH receptors. The same features were observed by Carr and Friesen [11] using human liver radioreceptor assay.

It is interesting to note that only oPL and hGH compete equally either against [^{125}I]iodo-hGH or [^{125}I]iodo-oPL in the RRA-GH using human liver membrane particulate fractions as receptors (fig.1). These observations raised the prospect that the binding sequence in both oPL and hGH might be identical or very similar. In our current studies, we have successfully inhibited, by monoclonal antibodies against oPL, the binding of [^{125}I]iodo-oPL but not the binding of [^{125}I]iodo-hGH to human liver. These observations indicate that the structure of GH-binding sequence in oPL is different from that in hGH. Nevertheless, we could not rule out the possibility that the GH-binding sequences in both oPL and hGH might be similar (partial homology). Indeed, we are currently investigating the structure of GH-binding sequence in oPL. A definite answer should become available in the near future.

Furthermore, our studies have shown that the addition of monoclonal antibodies in the RRA-GH does not affect the parallelism of oPL standard curves (fig.3). These studies suggest that our monoclonal antibodies are probably competing with the receptors for hormone-binding sequence in the oPL molecule.

Monoclonal antibodies directed to hGH or hPL have been reported by various authors [21–23]. Some of these monoclonal antibodies were shown to be able to inhibit the biological effect of hGH and hPL in vitro, i.e. growth of rat NB2 lymphoma cells [23]. In this regard, whether our monoclonal antibodies could also inhibit the biological effect of oPL in vitro and in vivo remains to be determined.

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REFERENCES

[1] Waters, M.J. and Friesen, H.G. (1979) J. Biol. Chem. 254, 6815–6825.
[2] Shiu, R.P.C. and Friesen, H.G. (1975) J. Biol. Chem. 249, 7902.
[3] Bhalla, V.K. and Reichert, L.E. (1974) J. Biol. Chem. 249, 43.
[4] Catt, K.J., Dufau, M.L., Neaves, W.B., Walsh, P.C. and Wilson, J.D. (1975) Endocrinology 97, 1157.
[5] Wolff, J., Winand, R.J. and Kohn, L.D. (1974) Proc. Natl. Acad. Sci. USA 71, 3460–3464.
[6] McIlhinney, R.A.J. and Skhulsta, D. (1974) Endocrinology 94, 1259.
[7] Klee, W.A. and Nirenberg, M. (1976) Nature 263, 609–611.
[8] Blume, A.J. (1978) Proc. Natl. Acad. Sci. USA 75, 1713–1717.
[9] Posner, B.I., Kelly, P.A., Shiu, R.P.C. and Friesen, H.G. (1974) Endocrinology 95, 521.

[10] Arrenbrecht, S. (1974) Nature 252, 255.
[11] Carr, D. and Friesen, H.G. (1976) J. Clin. Endocrinol. Metab. 42, 484–493.
[12] Gavin, J.R. iii, Trivedi, B. and Daughaday, W.H. (1982) J. Clin. Endocrinol. Metab. 55, 133–139.
[13] Chan, J.S.D., Robertson, H.A. and Friesen, H.G. (1976) Endocrinology 98, 65.
[14] Chan, J.S.D., Nie, Z.-R., Seidah, N.G. and Chrétien, M. (1986) FEBS Lett. 199, 259–264.
[15] Tsushima, T. and Friesen, H.G. (1973) J. Clin. Endocrinol. Metab. 37, 334.
[16] Shiu, R.P.C., Kelly, P.A. and Friesen, H.G. (1973) Science 180, 968.
[17] Chan, J.S.D., Robertson, H.A. and Friesen, H.G. (1978) Endocrinology 102, 632.
[18] Thorell, J.I. and Johnson, B.G. (1971) Biochim. Biophys. Acta 251, 363.
[19] Chan, J.S.D., Nie, Z.R., Seidah, N.G. and Chrétien, M. (1986) Endocrinology, submitted.
[20] Lesniak, M.A., Roth, J., Gordon, P. and Gavin, J.R. iii (1973) Nat. New Biol. 241, 20–22.
[21] Ivanyi, J. and Davies, P. (1980) Mol. Immun. 17, 287–290.
[22] Bundesen, G., Drake, R.G., Kelly, K., Worsely, I.G., Friesen, H.G. and Sehon, A.H. (1980) J. Clin. Endocrinol. Metab. 51, 1472–1474.
[23] Ivanyi, J. (1982) Mol. Immun. 19, 1611–1618.