Vol. 136, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1986 Pages 38-44

DELAYED APPEARANCE OF LIVER GROWTH HORMONE BINDING SITES AND OF GROWTH HORMONE-INDUCED SOMATOMEDIN PRODUCTION DURING RAT DEVELOPMENT

B. Flandez, E. Alvarez and E. Blázquez

Departamento de Fisiología, Facultad de Medicina, Salamanca, Spain

Received February 10, 1986

SUMMARY: The present study was designed to determine whether the apparent paradox of high circulating growth hormone levels in the fetus and the minimal effect of this hormone on growth might reflect a diminished responsiveness of fetal target organs to GH. Specific uptake by rat liver of [^{125}I] bGH was very low in fetuses as compared to suckling and adult rats. Also, liver uptake of the iodinated hormone decreased proportionally with the simultaneous injection of increasing amounts of growth hormone, but was not modified by the simultaneous injection of unlabelled chemically-related hormones. Since the water content is significantly greater in fetal than adult tissues, results were expressed by liver dry weight and again, [^{125}I] bGH liver uptake continued to increase with age. After bovine growth hormone administration to adult rats, plasma somatomedin C concentrations increased significantly, while they had no effect in fetuses. These results suggest that reduced liver somatogenic binding sites in the fetus prevents growth hormone from inducing growth-promoting effects during intrauterine life.

Though during the last third of pregnancy the fetal pituitary gland releases greater amounts of growth hormone than in other periods of intra and extrauterine life (1), the contribution of this hormone to fetal growth seems to be minimal. Thus, the birth length and body weight of anencephalic human fetuses without the pituitary gland and most children with hypopituitarism are within the normal range (2-4), while fetuses of rat, rabbit and rhesus monkey hypophysectomized *in utero* continue to grow at normal rates until birth (5, 6). The present study was thus designed to determine whether the apparent paradox of high circulating growth hormone levels in the fetus and the minimal effect of this hormone on growth might reflect a diminished responsiveness of fetal target organs to growth hormone.

MATERIALS AND METHODS

Rats of the Wistar strain, with free access to commercial rat chow and water, were used in our experiments. Female animals weighing 200-250 g were caged with

0006-291X/86 $1.50

males until copulation occurred. Vaginal smears were examined daily for spermatozoa early each morning. Pregnancy was dated from the first day on which spermatozoa were identified. The accuracy of this method of dating, estimated to have 6-12 hours error, was validated by the fact that all the rats delivered 22 days after the finding of spermatozoa. Suckling and adult rats, slightly anesthetized with ether, received a jugular vein injection of 20 ng/100 g body weight of either [^{125}I] bGH or [^{125}I] insulin, with or without unlabelled hormones dissolved in NaCl (0.9%) with 0.1% bovine serum albumin. Hormone administration in experiments with fetuses was done with a Hamilton microsyringe through the umbilical vein. Bovine growth hormone kindly provided by Drs. Dellacha (Buenos Aires, Argentina) and Sonemberg (New York, USA) was labelled with specific activities of 110-130 μCi/μg according to a procedure previously described (7). Mono [^{125}I] insulin (280-350 μCi/μg) was obtained by the procedure of Freychet and Roth (8). The animals were maintained at room temperature. At the times indicated, blood samples were obtained from the inferior vena cava, and liver slices, one from each lobe, were removed. In the case of fetuses blood samples were obtained by decapitation. Radioactivity present in blood plasma and liver slices was measured with a gamma-scintillation counter. Liver uptake was expressed as the liver to plasma ratio (L/P: counts per min/g liver cpm/g plasma), and as cpm/g dry liver weight, calculated as described by Retegui-Sardou et al. (9). Blood plasma determinations of somatomedin C were carried out at different times after the subcutaneous administrations of bGH (25 μg/100 g body weight) dissolved in a solution of NaCl (0.9%) with 0.1% bovine serum albumin or of a placebo to 21 day-old fetuses and adult rats. In the case of the fetuses, the hormone or the placebo (containing NaCl and bovine serum albumin) were injected through the uterine wall. Somatomedin C determinations were carried out by radioimmunoassay (Nichol Institute Diagnosis, San Juan de Capistrano, USA). The antibody used in this assay was of high specificity and affinity. Thus, IGF-II shows less than 2% cross reactivity with the antibody, and none of the known classical hormone cross react to any significant extent. The sensitivity of the assay was up to and including 0.1 U/ml and the percentages of intra and inter-assay variation were 5.2 and 9.4% respectively.

RESULTS

After the intravenous injection of [^{125}I] insulin and [^{125}I] bGH, liver uptake was studied at 5 and 20 minutes, respectively; the times of maximal hormonal uptake in fetal, suckling and adult rats. Both radioactive hormones were rapidly cleared by the liver and kidney, but liver uptake of iodinated hormones decreased significantly with the simultaneous injection of unlabelled hormones (Figures 1 and 2 and Table 1), suggesting that bGH and insulin are bound at specific binding sites rather than simply absorbed by the liver. As shown in Figure 1, hepatic uptake of [^{125}I] bGH was minimal in fetal rats as compared with weanling and adult rats ($p < 0.001$). Interestingly, during intrauterine life, liver insulin uptake was dramatically superior to that of growth hormone, while in postnatal life GH uptake was greater or the same as that of insulin. Doses of 0.5 to 50 μg/100 g body weight of either bGH or insulin injected together with the corresponding radioactive hormones produced a dose-response curve (Fig. 2) in all the experimental groups. The dose at which displacement was half-maximal was within the lower concentration range used for GH and insulin bioassays.

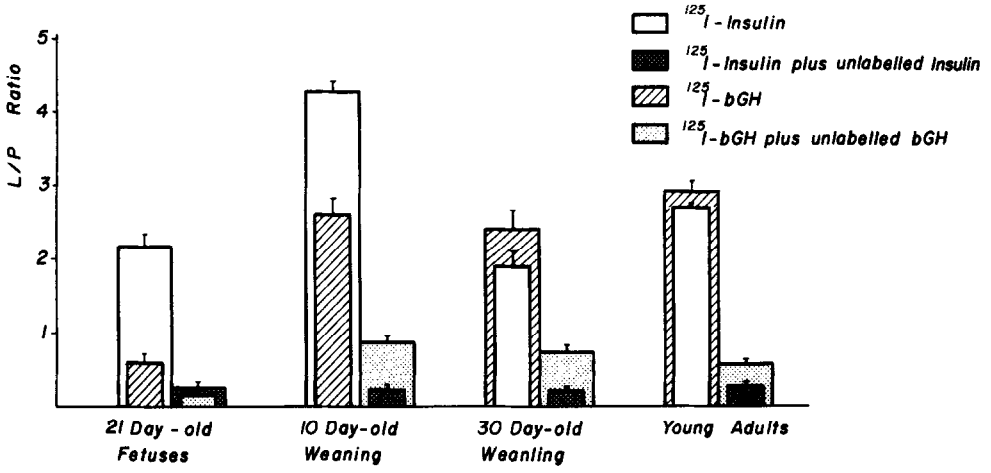

Fig. 1. Uptake of [^{125}I] bGH or [^{125}I] insulin by the liver of fetal, weanling and adult rats in the absence or presence of the corresponding unlabelled hormones (100 µg/100g body weight).

It might be suggested that the high circulating GH levels in the fetus could affect by dilution the lower liver uptake of [^{125}I] bGH (Fig. 2). However, plasma GH levels (1) in 21 day-old fetuses and adult rats were 0.10 and 0.025 µg/ml, respectively; much lower than those given for the liver uptake studies (1-50 µg/100 g b.w.). This suggests that the higher GH levels in 21 day-old fetuses as compared with adult rats would not affect liver uptake. This idea is supported by the fact

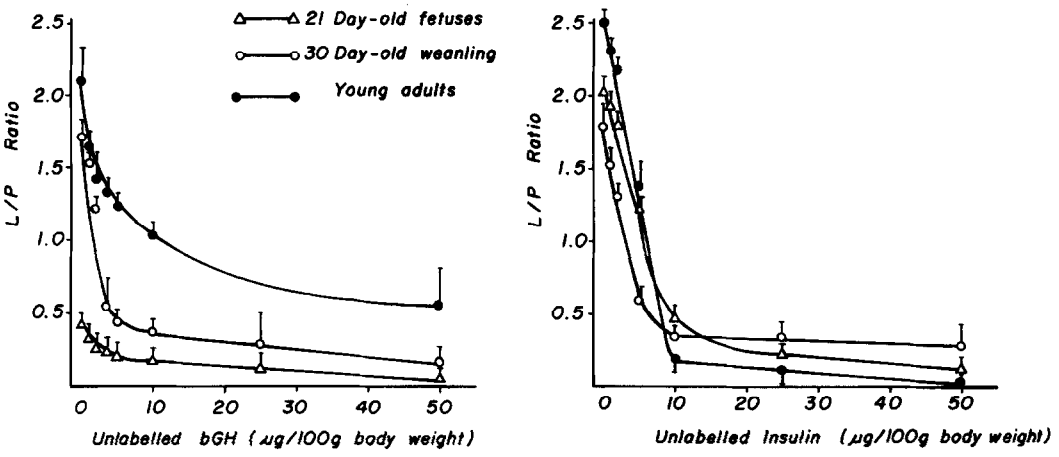

Fig. 2. Inhibition by unlabelled bGH or insulin of liver uptake of [^{125}I] bGH and [^{125}I] insulin, respectively, in fetal, weanling and adult rats. Means ± SEM. n=3-5.

TABLE 1. Specificity of liver uptake of radioactivity after administration of [^{125}I] bGH in the presence of various unlabelled native hormones

Rat age	Unlabelled hormones administered	L/P ratio	Liver (cpm/g dry wtx10^{-4})
21 day-old fetuses	None	0.39±0.03	8.42±2.31
	bGH	0.21±0.04	0.72±0.03
	oPRL	0.31±0.05	9.24±1.12
	hCG	0.33±0.20	6.01±1.04
30 day-old weanlings	None	1.34±0.28	33.62±16.36
	bGH	0.54±0.15	5.39± 3.24
	hPL	1.37±0.10	32.21±11.04
	Insulin	1.52±0.03	29.63±10.84
Adults	None	1.55±0.08	19.52±2.97
	bGH	0.55±0.01	9.24±5.20

Unlabelled hormones were used at a concentration of 10 µg/100 g body weight, except for the amount of bGH used in adult rats at a dose of 100 µg/100 g body weight. Means ± SEM. n=6-10.

that in 19 day-old fetuses both plasma hormone levels (0.014 µg/ml) and [^{125}I] bGH uptake were lower than in adult animals; additionally, on studying the binding of hGH to isolated liver membranes, hormone binding was seen to be 4-fold lower in 21 day-old fetuses than in adult rats.

Since changes in water content of the liver could have modified our results, the dry weight of this organ was determined at different stages of rat development. The percentage of liver water content in adult (71±2) and 30 day-old weanling (72±4) rats was found to be significantly lower ($p < 0.001$) than in 21 day-old fetuses (88±4). Accordingly, in Table 1 liver uptake of radioactivity has been expressed as L/P ratio and as counts per min/g dry liver weight. Using these calculations, liver uptake of [^{125}I] bGH continued (Table 1) to increase significantly with the age of animals. Also, when [^{125}I] bGH was injected with unlabelled chemical and functionally related hormones (Table 1), displacement of radioactive bGH could not be obtained.

At any time tested (Fig. 3), circulating somatomedin C levels were significantly ($p < 0.001$) smaller in fetuses than in adult animals and no differences were found between the groups injected with bGH or the placebo. However, in adult rats bGH induced an increase of plasma somatomedin concentrations at 8 and 13 ($p < 0.05$) hours after hormone administration.

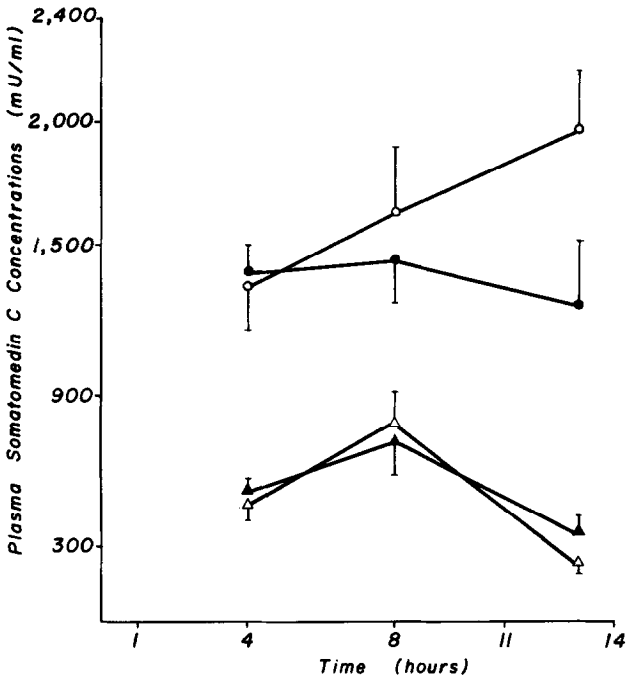

Fig. 3. Effect of bovine growth hormone on the circulating levels of somatomedin C of 21 day-old fetuses and adult rats. bGH was administered subcutaneously to the fetuses (Δ) and adult rats (o). Control fetuses (▲) and adult rats (●) were injected with a placebo. Means ± SEM. n=8-13.

DISCUSSION

The evidence that growth hormone possesses hepatocyte receptors, and induces different biological events such as amino acid transport (10) and the production of somatomedins (11) suggests that the liver could be a main target organ for this hormone. The characterization of growth hormone receptors in rat liver has been difficult because of the presence of somatogenic and lactogenic binding sites, and because of the varying ability of the hormone from different species to recognize both types of receptors in liver membranes or isolated hepatocytes. Thus in rodents, primate GHs display both growth promoting and lactogenic activity, while non primate GHs only induce body growth (12, 13). Accordingly, in most studies hGH has been used to recognize lactogenic binding sites, and bGH to test somatogenic receptors.

In vivo, binding techniques for different proteins or hormones have been applied successfully (14, 15); Turyn and Dellacha (16) have described a rapid and simple experimental procedure that detects *in vivo* the interactions of GHs with specific somatotropic and lactogenic

binding sites in rat liver. With this procedure, the liver uptake of either [^{125}I] bGH or [^{125}I] insulin was proportionally decreased to the dose of the unlabelled bGH or insulin, respectively, but not by the administration of other hormones, suggesting that radioactive bGH or insulin were bound to specific sites. Also, the rapid development of liver insulin binding sites reported here is in agreement with the results obtained with liver membranes, *in vitro* (17), which represents another validation of the procedure used by us.

Our results show a reduced liver uptake of [^{125}I] bGH and a lack of bGH-induced somatomedin C production in fetal rats as compared with adult animals, suggesting that the decreased GH liver binding sites could prevent growth hormone-promoting activity, despite the high circulating levels of this hormone during the last days of fetal life (1). In support of this hypothesis, it has been reported that GH stimulates the amino acid transport and the ornithine decarboxylase activity in tissues of neonatal rats but not in those of fetuses (18, 19). In addition, Kelly et al. (20) have found that in liver membranes of fetal rats binding sites for hGH are minimal, indicating that lactogenic binding sites are also significantly reduced during intrauterine life. These results correlate very well with the progressively increased biological action of growth hormone, from its absence in the fetus and its reduced activity in 6 day-old suckling rats to its full activity in 28 day-old weanling rats (21-22). Clinical and experimental evidence also supports these findings. Thus, anencephalic human fetuses and most children with hypopituitarism present a normal growth pattern and experimental animals decapitated *in utero* continue to grow at a normal rate until birth (2-4, 23, 24). In the postnatal mammal, skeletal growth is under pituitary control via somatomedin (25). However, in a period of maximal linear growth, as in fetal life, somatomedin activity does not seem to depend on the presence of growth hormone. This is true despite the fact that there is strong evidence to suggest that during intrauterine life skeletal growth is stimulated by somatomedin (26, 27), which circulates in significant amounts and has increased numbers of receptors in its target organs (28).

Since somatic growth is a multifactorial process, several factors or hormones would be involved. In fact placental lactogen, epidermal growth factor and insulin increase somatomedin production by fetal cells (29, 30, 31). Besides, insulin may play a role in fetal growth through its high affinity binding sites (32) which increase (33) in

parallel with the circulating levels of insulin (34) during the last days of pregnancy and indirectly by cross-reacting with the growth factor receptors (35).

ACKNOWLEDGEMENTS

This work has been supported by grants from the Comisión Asesora de Investigación Científica y Técnica and from the Instituto Nacional de la Salud, Spain.

REFERENCES

1. Blázquez, E., Simon, F.A., Blázquez, M. and Foà, P.P. (1974) Proc. Soc. Exp. Biol. Med. 147, 780-783
2. Nanagas, J.C. (1925) Am. J. Anat. 35, 445-460.
3. Mosier, H.D. (1956) J. Pediatrics 48, 633-639.
4. Reid, J.D. (1960) J. Pediatrics 56, 658-664.
5. Jost, A. and Picon, L. (1970) Adv. Metab. Dis. 4, 123-184.
6. Eguchi, Y. (1961) Endocrinology, 68, 716-718.
7. Roth, J. (1975) Methods of Enzymology 37, 223-228.
8. Freychet, P., Roth, J. and Neville, D.M. (1971) Biochem. Biophys. Res. Commun. 43, 400-408.
9. Retegui-Sardou, L.A., Scaramal, L.M., Dellacha, J.M. and Paladini, A. (1977) Mol. Cell. Biochem. 16, 87-96.
10. Riggs, R.T. and Walker, L.M. (1960) J. Biol. Chem. 235, 3603-3607.
11. Mc Connaghey, P. and Sledge, C.B. (1970) Nature 225, 1249-1250.
12. Kleinberg, D.L. and Frantz, A.G. (1971) J. Clin. Invest. 50, 1557-1568.
13. Li, CH. (1972) in Recent knowledge of the chemistry of lactogenic hormones, Wolstenholme, G.E.W. and Knight, J. (eds.), Churchill, Livingstone, London, pp. 7-22.
14. Kammerman, S. and Canfield, R.E. (1972) Endocrinology 90, 384-389.
15. Boylan, E.S. and Wittliff, J.L. (1973) Cancer Res. 33, 2903-2908.
16. Turyn, D. and Dellacha, J.M. (1978) Endocrinology 103, 1190-1195.
17. Blázquez, E., Rubalcava, B., Montesano, R., Orci, L. and Unger, R.H. (1976) Endocrinology 98, 1014-1024.
18. Freemark, M. and Handwerger, S. (1983) Endocrinology 112, 402-404.
19. Hurley, T.W., Kuhn, C.M., Shanberg, S.M. and Handwerger, S. (1980) Life. Sci. 27, 2269-2275.
20. Kelly, P.A., Posner, B.I., Tsushima, T. and Friesen, H.G. (1974) Endocrinology 95, 532-539.
21. Heggestad, C.B. (1955) Anat. Record 121, 399-400.
22. Heggestad, C.B. and Well, L.M. (1965) Acta Anatomica 60, 348-361.
23. Chez, R.A., Hutchinson, D.L., Salazar, H. and Mintz, D.H. (1970) Am. J. Obst. Gynecol. 108, 643-650.
24. Liggins, G.C. and Kennedy, P.C. (1968) J. Endocrinol. 40, 371-381.
25. Van Wyk, J.J. and Underwood, L.E. (1975) Ann. Rev. Med. 26, 427-441.
26. Gluckman, P.D. and Brinsmead, M.W. (1976) J. Clin. Endocrinol. Metab. 43, 1378-1381.
27. Ashton, J.K. and Francis, M.J.O. (1978) J. Endocrinol. 76, 473-477.
28. Rosenfeldt, R., Thorsson, A.V. and Hintz, R.L. (1979) J. Clin. Endocrinol. Metab. 48, 456-461.
29. Adams, S.O., Nissley, S.P., Handwerger, S. and Rechler, M.M. (1983) Nature 302, 150-153.
30. Richman, R.A., Benedict, M.R., Florini, J.L. and Toly, B.A. (1985) Endocrinology 116, 180188.
31. Hill, D.J. and Milner, R.D.G. (1980) Diabetologia 19, 143-147.
32. Massagué, J., Blinderman, L.A. and Czech (1982) J. Biol. Chem 257, 13958-13963.
33. Caliendo, A.M. and Patel, M.S. (1983) Arch. Biochem. Biophys. 227, 552-561.
34. Blázquez, E. Montoya, E. and López Quijada, C. (1970) J. Endocrinol. 48, 453-461.
35. Roth, J. (1970) in Endocrinology, De Groot, L. (ed.) Grunne and Stratton, New York pp. 2037-2054.