Journal of Animal and Veterinary Advances 2 (2): 82-84, 2003

In vitro Liver Synthesis and Plasma Levels of Corticosteroid-binding Globulin (CBG) in the Piglet

Jeonghoon Heo, Henry. G. Kattesh, Jules J.E. Doré, Jr, Mary P. Roberts, and James D. Godkin
Department of Animal Science, The University of Tennessee, Knoxville,
Tennessee 37901-1071, USA

Abstract: In vitro production of corticosteroid-binding globulin (CBG) was evaluated in liver collected from piglets at 3 to 40 days of age and compared to the developmental pattern of plasma levels of CBG. The amount of CBG released per unit weight of liver slices and in circulation was measured using an enzyme-linked immunosorbent assay (ELISA) procedure. Plasma CBG levels were low on days 3 and 10, but significantly ($P < 0.05$) increased on day 30. The concentration of CBG released from liver slices cultured for 12 hours was higher ($P < 0.01$) on day 10 than that measured on the other days. These results suggest that plasma CBG levels and the production of CBG by liver may be related to age of the piglet, but there are age-related differences in the profiles of CBG produced by liver tissue and that found in the plasma.

Key words: Piglet, liver, CBG, Elisa

Introduction

During the period from birth to weaning the young pig encounters various environmental and management-related stressors (Curtis, 1974; England, 1974). The adrenal glucocorticoid, cortisol, aids the animal in adjusting to these stressors as well as in the early postnatal maturation process (Baxter and Rousseau, 1979). Corticosteroid-binding globulin (CBG) binds cortisol with remarkably high affinity and specificity (Slaunwhite and Sandberg, 1959). As a result, CBG not only transports cortisol but also modulates the bioavailability of cortisol to target cells (Siiteri *et al.*, 1982). Its hepatic synthesis, metabolism, and transfer to extravascular spaces determine the plasma levels of CBG. In the neonate, plasma levels of CBG increase dramatically two to four weeks after birth (Henning, 1978; Kattesh and Roberts, 1993). Liver has been identified as the major site of CBG biosynthesis in mammalian species (Smith and Hammond, 1989; Seralini *et al.*, 1990). The objective of this study was to compare plasma levels of CBG with its production by liver tissue in culture collected from piglets from 3 to 40 days of age.

Materials and Methods

Twenty crossbred female piglets (Landrace, Duroc and Hampshire breeding), born naturally and reared conventionally, were used. Four piglets each were randomly allotted for study on days 3, 10, 20, 30, and 40 following birth. On the designated day, a piglet was selected and immobilized by administering a combination of Ketamine hydrochloride and telezol and then placed under general anesthesia using closed circuit administration of halothane. A single blood sample was immediately collected from the pig by anterior vena cava puncture, the blood centrifuged at 3,000 rpm for 10 minutes at 4°C, the plasma removed and stored at -20°C. The abdominal body cavity was opened following a mid-ventral incision. Two lobes of liver were quickly clamped, removed and rinsed in cold HBSS solution (pH 7.4, Sigma Chemical Co., MO). From a 0.2 g section of tissue, slices of uniform thickness (~ 0.5 mm) were pre-incubated in dishes containing 4 ml of William's medium E (pH 7.4, Gibco BRL, NY) at 37°C in a 5% CO2, 45% O2, 50% N2 atmosphere for 3 hours. After pre-incubation, the samples were washed with warm HBSS solution and then incubated in 5 ml William's medium E at 37°C for 12 hours under the same atmosphere conditions as before. The culture media were collected and stored at -20°C.

The amount of CBG in plasma and synthesized by cultured liver tissue was measured by an enzyme-linked immunosorbent assay (ELISA) for porcine CBG developed by Kattesh and Roberts (1993). The intra- and inter-assay coefficient of variation of CBG measured in a pooled sample of pig plasma was 6.9% and 14%, respectively.

The results were analyzed using General Linear Mixed Models (GLMM) procedures to determine differences in CBG concentrations with regard to age effect only (Blouin and Saxton, 1990). Least square means and standard errors were

calculated for all variables and significant differences among means were partitioned using Duncans procedures.

Results

The concentration of CBG in plasma from piglets sampled on days 3 and 10 was lower ($P < 0.05$) than that measured on day 30 (Fig. 1). The CBG concentrations increased ($P < 0.05$) from 1.38 ± 0.06 µg/ml on day 10 to 2.18 ± 0.29 µg/ml on day 30. The CBG concentration in media from liver slices following 12 hours of culture was higher ($P < 0.01$) on day 10 than that measured on the other days examined (Fig. 2). The CBG concentration decreased from 4.38 ± 0.42 ng/mg on day 10 to 2.77 ± 0.42 ng/mg on day 20.

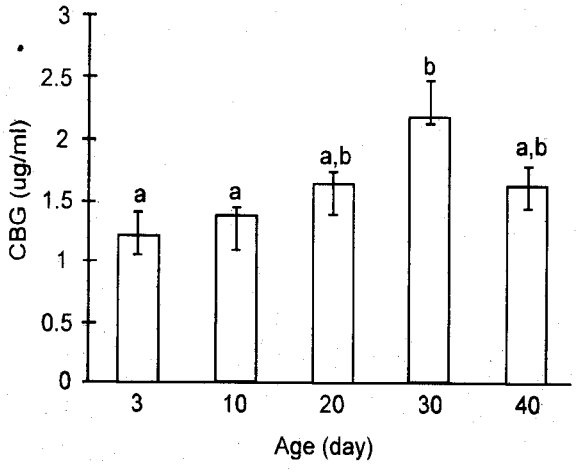

Fig. 1: Plasma CBG concentration at each age studied. CBG concentration is expressed as microgram per milliliter of plasma (µg/ml). Each bar represents the mean ± SE (n = 4). Columns with different letters are significantly ($P < 0.05$) different.

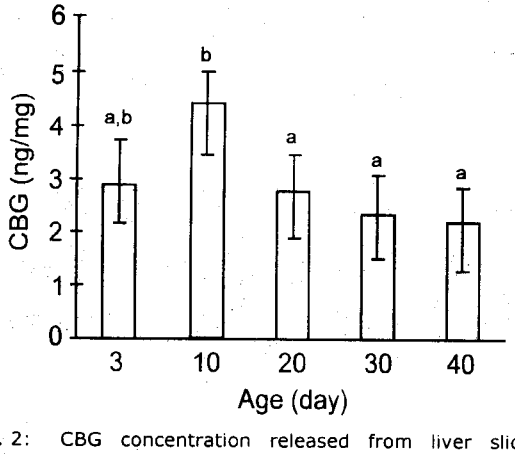

Fig. 2: CBG concentration released from liver slices following 12 hours incubation. CBG concentration is expressed as nanogram of CBG per unit weight of liver slice (ng/mg). Each bar represents the mean ± SE (n = 4). Columns with different letters are significantly ($P < 0.01$) different.

Discussion

In several mammalian species, circulating levels of CBG are low following birth and proceed to increase during development. In the rat, plasma CBG levels are very low during the first 9 days after birth and significantly increase between days 9 and 12 until they plateau on day 24 (Henning, 1978). Kattesh and Roberts (1993) demonstrated that plasma levels of CBG in the piglet decrease from day 3 to day 7, but significantly increase between days 21and 28 of age. The results of the present study confirm that plasma CBG levels experience a dramatic increase between days 20 and 30.

Smith and Hammond (1991) demonstrated that although adult rat CBG mRNA levels were attained by 3 weeks of age, serum CBG concentrations did not reach adult values for an additional 3 weeks, and that the half-life of CBG in 3-week-old infants (6.9 hours) was consistently less than that in adults (14.5 hours). They suggested that the age-related differences in the metabolic clearance of CBG might be responsible for the age-related difference in the profiles of hepatic CBG mRNA and serum CBG concentrations. Elfahime *et al.* (1992) showed that hepatic CBG mRNA levels and plasma CBG concentrations increased in parallel from postnatal day 10 to day 15 in the rat, but hepatic CBG mRNA levels decreased on day 20 while plasma CBG levels continued to increase. Their results were similar to those of the present study in that plasma CBG levels increased on day 30 while CBG concentrations released from liver slices decreased on day 20. Consequently, there was an age-related difference in the profiles of the release of CBG per unit weight of liver tissue and plasma CBG levels in the young pig. It is possible that this age-related difference may be due to a change in the half-life of plasma CBG and/or hepatic CBG mRNA levels. Future studies will examine the changes in hepatic CBG mRNA in the young pig.

Acknowledgement

This research is published with the approval of the Dean of the Tennessee Agricultural

Experiment Station and supported by State and Hatch Funds allocated to the Station.

References

Baxter, J. D. and G. G. Rousseau, 1979. Glucocorticoid hormone action: An overview; in Baxter JD, Rousseau GG (ed): Glucocorticoid Hormone Action. Springer-Verlag Berlin, Heidelberg, 12, pp: 1-24.

Blouin, D. C. and A. M. Saxton, 1990. General linear mixed models GLMM (version 1.1). Baton Rouge, LA. Lousiana Agricultural Experiment Station, LSU.

Curtis, S. E., 1974. Responses of the perinatal stressors. J Ani. Sci., 38:1031-1036.

Elfahime, E. L., M. Plante, F. Rene, J. M. Felix, B. Koch, 1992. Biosynthesis of hepatic corticosteroid-binding globulin: ontogeny and effect of thyroid hormone. J. Steroid Biochem. Molec. Biol., 41: 135-140.

England, D. C., 1974. Husbandry components in prenatal and perinatal development in swine. J. Ani. Sci., 38: 1045-1049.

Henning, S. J., 1978. Plasma concentrations of total and free corticosterone during development in the rat. Am. J. Physiol., 235: 451-456.

Kattesh, H. G., M. P. Roberts, 1993. Age related changes in plasma concentrations of porcine corticosteroid binding globulin (pCBG) as determined by an enzyme-linked immunosorbent assay (ELISA). J. Ani. Sci., 71(Suppl. 1): 234.

Seralini, G. E., C. L. Smith and G. L. Hammond, 1990. Rabbit corticosteroid-binding globulin: primary structure and biosynthesis during pregnancy. Mol. Endocrinol., 4: 1166-1172.

Siiteri, P. K., J. T. Murai, G. L. Hammond, J.A. Nisker, W. J. Raymoure and R. W. Kuhn, 1982. The serum transport of steroid hormones. Rec. Prog. Horm. Res., 38: 457-510.

Slaunwhite, W. R., Jr. and A. A. Sandberg, 1959. Transcortin: a corticosteroid-binding protein of plasma. J. Clin. Invest., 38: 384-391.

Smith, C. L. and G. L. Hammond, 1989. Rat corticosteroid binding globulin: primary structure and messenger ribonucleic acid levels in the liver under different physiological conditions. Mol. Endocrinol., 3: 420-426.

Smith, C. L. and G. L. Hammond, 1991. Ontogeny of corticosteroid-binding globulin biosynthesis in the male rat. Endocrinology, 130: 2245-2251.