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CHANGES IN THE NUMBER OF INSULIN RECEPTORS OF ISOLATED RAT HEPATOCYTES DURING PREGNANCY AND LACTATION

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Summary

The weight of the rat liver increased during pregnancy and lactation due solely to an increase in hepatocyte size.

Insulin receptors were identified using ^{125}I-labelled insulin and isolated hepatocytes in vitro. Scatchard analysis was interpreted in terms of high affinity (K_D 4 nM) and low affinity (K_D 80 nM) receptors for insulin. No change in the affinity of either receptor site was detected during pregnancy or lactation. There was, however, a significant increase in the number of both types of receptor on the hepatocyte by day 12 of pregnancy which was maintained until at least day 20 of pregnancy. During lactation, the number of receptors declined to values similar to those of virgin rats.

Serum insulin concentrations, determined by radioimmunoassay, were elevated during pregnancy, returned to values similar to virgin rats during early lactation and were significantly reduced compared with virgin rats by day 15 of lactation.

These results illustrate that physiological conditions exist whereby the number of insulin receptors may increase, despite elevated serum insulin concentrations, in apparent conflict with the 'down-receptor' hypothesis.

Introduction

Pregnancy is often accompanied by insulin resistance with both endogenous hyperinsulinaemia and pancreatic cell hypertrophy evident [1—3]. Both placental steroids and placental lactogen have been implicated in the development of this condition [4].

Insulin resistance has, in a number of instances, been associated with reduced numbers of insulin receptors in a variety of tissues [5] although we have found

increased numbers of insulin receptors during pregnancy in rat adipocytes [6]. This study was undertaken to examine whether alterations in the insulin receptor of the rat hepatocyte might, in part, explain the insulin resistance which develops during pregnancy.

Materials and Methods

Wistar rats (A. Tuck and Son Ltd., Rayleigh, Essex, U.K.) weighing 210—220 g were used throughout this study. Animals were allowed food (diet 41B, Oxoid Ltd.) and water ad libitum.

Virgin rats and rats at various stages of pregnancy and lactation were killed by cervical dislocation between 10.00 and 11.00 h. Blood was collected from the neck and centrifuged at $1000 \times g$ for 5 min to obtain serum, which was stored at −20°C until used for hormone analysis. The liver was perfused in situ with Ca^{2+}-free Krebs-Ringer bicarbonate via the portal vein, following which isolated hepatocytes were prepared by digestion with collagenase (C 2139, Sigma, London) by the method of Berry and Friend [7]. Trypan blue exclusion tests routinely revealed at least 90% cell viability. Hepatocyte numbers were determined by means of a haemocytometer of the Neubauer type and mean hepatocyte volume was calculated assuming a specific gravity of 1.07 [8].

Binding of ^{125}I-labelled insulin to isolated hepatocytes. Isolated hepatocytes (about 10^6 cells) were incubated at 22°C in Krebs-Ringer phosphate, pH 7.8, containing 1% BSA with 0.2 ng ^{125}I-labelled insulin (Radiochemical Centre, Amersham, Bucks, U.K.) and 1—500 ng of unlabelled insulin (bovine insulin, kindly donated by the Boots Co. Ltd., Nottingham, U.K.) in a final volume of 500 μl. Incubations were terminated after 60 min by the addition of 1.5 ml ice-cold saline and centrifugation at $1000 \times g$ for 20 min. The supernatant was removed by decanting and the radioactivity in the pellet determined by gamma counting. All data were corrected for non-specific binding by subtracting the amount of radioactivity still bound in the presence of 10 μg of bovine insulin, from all other values. Non-specific binding accounted for approximately 20% of the total binding to the hepatocytes. Insulin degradation was determined by the procedure involving precipitation with trichloroacetic acid described by Freychet et al. [9]. Degradation did not vary significantly throughout pregnancy or lactation and amounted to 17 ± 2% per 10^6 cells.

Results were subjected to Scatchard analysis [10]. The results were interpreted in terms of a component with a higher affinity for insulin (K_D 4 nM) and a second component with a lower affinity for insulin (K_D 80 nM). The total numbers of both types of insulin receptor were determined from the Scatchard plots, without any correction for insulin degradation.

Radioimmunoassay. Serum insulin was determined using ^{125}I-Insulin and Insulin Binding Reagent (Wellcome Reagents Ltd., London). Results were expressed in terms of ng/ml of a bovine insulin standard (23.6 U/mg). sensitivity of the assay is 30 pg/tube and inter- and intra-assay coefficients of variation are 8 and 2%, respectively.

TABLE I

CHANGES IN LIVER MORPHOLOGY AND ^{125}I-LABELLED INSULIN BINDING TO ISOLATED HEPATOCYTES DURING PREGNANCY AND LACTATION

All values are mean ± S.E.M. *N*, number of animals.

Group	Virgin (*N* = 5)	Pregnancy		Lactation		
		12 (*N* = 5)	20 (*N* = 5)	2 (*N* = 5)	5 (*N* = 6)	15 (*N* = 6)
Liver morphology						
Liver weight (g)	8.2 ± 0.4	11.4 ± 1.7	13.8 ± 0.5 ***	10.6 ± 0.7 *	9.8 ± 0.3 **	9.7 ± 0.5 *
Hepatocyte volume (pl)	3.9 ± 0.1	5.0 ± 0.5	6.4 ± 0.6 **	4.7 ± 0.4	4.6 ± 0.2	4.8 ± 0.4
Hepatocyte number ($\times 10^{-7}$)	1.9 ± 0.1	2.1 ± 0.1	2.0 ± 0.2	2.2 ± 0.3	2.1 ± 0.1	1.9 ± 0.1
Insulin receptors						
Number						
10^{-4} × high affinity sites/cell	5.4 ± 0.3	8.4 ± 0.6 **	9.3 ± 1.0 **	6.0 ± 0.9	5.4 ± 0.6	6.7 ± 0.7
Low affinity sites/cell	50 ± 5	96 ± 20	115 ± 13	69 ± 9	62 ± 7	77 ± 7
High affinity sites/μm^2	44 ± 2	55 ± 9	59 ± 49 *	45 ± 6	32 ± 6	49 ± 4
Low affinity sites/μm^2	478 ± 38	675 ± 126 *	688 ± 49 *	516 ± 76	464 ± 52	565 ± 37
Affinity						
High affinity K_D (nM)	5.1 ± 0.3	4.2 ± 0.3	4.5 ± 0.7	4.6 ± 0.7	4.6 ± 0.5	4.9 ± 0.5
Low affinity K_D (nM)	76 ± 7	80 ± 16	84 ± 12	79 ± 11	81 ± 10	75 ± 7
Serum insulin ng/ml	1.3 ± 0.1	1.7 ± 0.3	2.0 ± 0.3	1.3 ± 0.2	1.2 ± 0.1	0.9 ± 0.1 *

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with virgin rats.

Results

Changes in liver morphology

Liver weight increased over 50% by day 20 of pregnancy and remained elevated, although not to as great an extent, during lactation (Table I). The increase in liver weight appeared to be due solely to an increase in hepatocyte size; there was no evidence of cell hyperplasia.

Serum insulin and insulin receptors

Changes in the serum insulin concentration and the number of insulin receptors on the hepatocyte are shown in Table I. Serum insulin concentration increased during pregnancy and although neither of the mean values at days 12 or 20 of pregnancy was significantly greater than the value for virgin rats, the overall mean value for pregnant rats was significantly higher ($P < 0.05$). There was a significant decrease ($P < 0.05$) in serum insulin concentration between day 20 of pregnancy and day 5 of lactation and by day 15 of lactation, serum insulin concentration was significantly lower ($P < 0.05$) than that of virgin rats.

There was a significant increase in the numbers of both high and low affinity insulin receptors per cell by day 12 of pregnancy. These elevated numbers of receptors were maintained until at least 2 days before parturition after which they declined, around parturition, to values similar to that of virgin rats. When expressed per unit of surface area, the number of insulin receptors with a low affinity for insulin were also significantly elevated during pregnancy, although the surface density of the high affinity insulin receptor was not significantly different from that of the virgin rat.

Scatchard analysis of the binding data revealed no change in the affinity for insulin of either the high or low affinity receptors during pregnancy or lactation (Table I and Fig. 1), mean K_D values for all rats being 4.6 ± 0.2 nM and 79 ± 4 nM for the high and low affinity receptors, respectively (n = 32 observations).

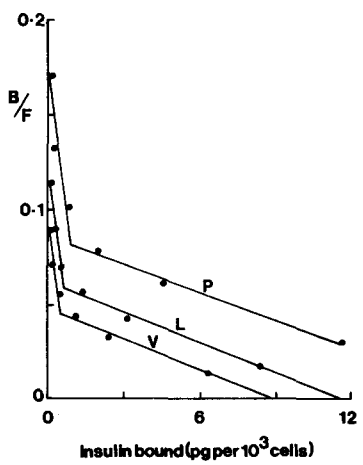

Fig. 1. Scatchard analysis of insulin binding to isolated rat hepatocytes. V, virgin; P, 20 days pregnant; L, 5 day lactating.

Discussion

Increased liver weight during pregnancy and lactation in this study was accounted for solely by hypertrophy of hepatocytes, whereas Herrera et al. [11] described both hypertrophy and hyperplasia of rat hepatocytes during pregnancy.

Serum insulin concentrations increased during pregnancy and decreased around the time of parturition to values similar to that of virgin rats, results broadly in agreement with previous findings [12—14]. During late lactation, serum insulin concentrations fell to values significantly lower than in virgin rats, supporting the findings of Robinson et al. [15], although elevated serum insulin concentrations have also been reported during lactation [16].

The increase in the number of receptors for insulin on the pregnant rat hepatocyte is in apparent conflict with the results of Kelly et al. [17] who found no difference in insulin binding to pregnant and virgin rat liver membranes although they did find an increase in insulin receptors during pregnancy in guinea pig liver membranes. These results could, however, be reconciled since their data failed to take into account any increase in hepatocyte size and, when expressed per unit of surface area, our results suggest only a small, non-significant, increase in the number of high affinity receptors for insulin although there was a significant increase in the number of low affinity receptors for insulin.

Insulin resistance develops during pregnancy [4,18,19] probably due to the high levels of placental lactogen in the serum [4] and the increased serum insulin concentrations during pregnancy may be a response to this insulin resistance [13]. The increased number of insulin receptors on the hepatocyte during pregnancy may, as previously suggested for adipose tissue [6], be an adaptation to counteract the insulin antagonistic effects of lactogenic hormones. Certainly glucose production is low [20] and hepatic lipogenesis is elevated during pregnancy [21], findings consistent with an increase in the response to insulin.

During lactation, both serum insulin concentration and the number of insulin receptors on the hepatocyte were reduced whilst serum glucagon is elevated [13], a situation which should favour glucose production, inhibit glucose utilisation and inhibit lipogenesis. This does not appear to be the case, however, since lipogenesis is elevated during lactation [23,24] and glucose production may be restrained [20].

These results also illustrate that during pregnancy, physiological conditions prevail which are capable of preventing the normal regulatory mechanism whereby increased serum insulin concentrations reduce the number of insulin receptors, the 'down' hypothesis of Gavin et al. [22].

The factor(s) responsible for the increased number of insulin receptors during pregnancy is uncertain, but the increase in the number of insulin receptors of the rat adipocyte during pregnancy [6] appears to be under the control of progesterone [23] whilst during lactation we have found that the insulin receptors on the rat adipocyte appear to be under the control of prolactin (Vernon, R.G., Clegg, R.A. and Flint, D.J., unpublished observations).

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