Shimane J. Med. Sci., Vol. 10, pp. 49–57, 1986

PREGNANCY AND DIABETES(I) : SECRETORY ACTIVITY IN THE PANCREAS DURING PREGNANCY

(pregnant rats/nutrients/insulin release/perfusion)

Satoshi OHGUNI, Keiichiro TANIGAWA, Yohji MASAKI, Shinichi TSUCHIYAMA and Shinya NOTE

Department of Internal Medicine, Shimane Medical University, Izumo 693, Japan

(Received Oct. 2, 1986/Accepted Dec. 22, 1986)

The effect of pregnancy on insulin secretion from isolated perfused rat pancreas was studied.

Hypoglycemia and hyperinsulinemia were observed in pregnant rats in contrast to virgin rats. The infusion of 16.7 mM glucose significantly augmented insulin secretion in both the first and second phases in pregnant rats compared with virgin rats, whereas insulin secretion was reduced very nearly to the virgin levels at both phases in one-month postpartum rats. The other nutrient tested, arginine (19 mM), was a more potent stimulator than glucose. Not only the first phase, but also the second phase was stimulated significantly by arginine in pregnant rats. When one month had passed after delivery, insulin release was diminished to the virgin levels. These data confirm the suggestion that secretory activity is enhanced in the pancreas of pregnant rats.

Pregnancy is well characterized by hyperinsulinemia and by augmented plasma insulin responses to a variety of insulinogenic stimulators (1-3). Among the factors controlling insulin concentrations during pregnancy, an increased elabolation of contrainsulin hormones may play a central role (4-8). Through numerous studies the causes of elevated plasma insulin other than contrainsulin hormones have been elucidated, i.e., progressive insulin resistance (9), pancreatic islet hypertrophy (10), and an increase in adenylate cyclase activity (11). Augmented insulin

release evoked by secretagogues in vitro has also been demonstrated (12, 13). However, all experiments on the dynamic pattern of insulin secretion from the pancreas during pregnancy have been made using isolated islets of rat pancreas. Curry et al. (14) has drawn attention to the possibility that the collagenase which isolates islets of the rat pancreas might damage the membrane of B cells resulting in impaired insulin secretion evoked by secretagogues. We have, therefore examined the insulin release from the pancreas of pregnant rats using isolated perfused pancreas.

MATERIALS AND METHDS

Wistar strain, fed female rats, matched in age from 16 to 19 weeks, were used in all experiments. In pregnant rats experiments were performed at 21.5 days of gestation. When the sperm test was positive following the day of mating, that day was considered day 0.5 of gestation. Natural birth occurred in the afternoon of day 21.5 after mating. In one-month postpartum rats lactation was terminated at 3 weeks after delivery.

The rats were housed in air-conditioned quarters at 24°C and provided with light from 8 a.m. to 8 p.m. Liberal quantities of water and chow pellets with a caloric distribution of 60% carbohydrate, 13% fat and 27% protein were provided. All experimental manipulations, i.e., blood sampling, perfusion of the isolated rat pancreas, etc., were performed between 9:30 a.m. and 12:30 p.m.

Blood samples were collected from the jugular vein immediately after the induction of pentobarbital anesthesia (i.p. 4-5 mg/100 g body weight). The pancreas was isolated and perfused by the procedure described by Grodsky et al. (15) with minor modifications (16). All perfusions were accomplished with 4.4 mM glucose with Krebs-Ringer bicarbonate buffer (KRB) containing 0.25% bovine serum albumin and 4.6% dextran (mean mol.

wt., 70,000). The medium was gassed with 95% O_2-5% CO_2 and maintained at pH 7.4 and 37°C. The flow rate was kept constant at 1.9 ml/min. After an equilibration period of 20 min, various agents were introduced into the medium over 20 min through a side pump. Effluent from the portal vein was collected every 1 min in tubes, frozen immediately, and stored at -20°C until assayed.

Plasma glucose was measured by the glucose oxidase method. Immunoreactive insulin was measured by radioimmunoassay by the polyethylene method (17). Dextran was a gift from Midori Juji Co. (Osaka, Japan).

Statistical analysis was performed using the unpaired Student's t test, and results were expressed as mean ± SEM.

RESULTS

Plasma glucose in pregnant rats was significantly lower than in virgin rats, as shown in Table 1. Plasma insulin during pregnancy was significantly increased compared with virgin states. After delivery plasma glucose and insulin recovered to almost the virgin levels.

Table 1. Effects of pregnancy on body weight, plasma glucose and plasma insulin

	n	Body weight (g)	Plasma glucose (mg/dl)	Plasma insulin (μU/ml)
Virgin	20	243.8 ± 2.4	134.0 ± 6.1	58.6 ± 3.7
Pregnant (21.5 days)	20	328.5 ± 4.4*	82.9 ± 3.6**#	77.3 ± 6.9***
Postpartum (1 wk)	5	273.0 ± 6.7	104.8 ± 5.2	57.5 ± 7.1
Postpartum (1 mo)	8	246.3 ± 5.9	125.7 ± 6.7	60.2 ± 7.5

Values are expressed as mean ± SEM.
* P < 0.005 versus fed virgin, one-week postpartum and one-month postpartum rats
** P < 0.005 versus fed virgin and one-month postpartum rats
*** P < 0.05 versus virgin
\# P < 0.025 versus fed one-week postpartum rats

Insulin response to 16.7 mM glucose from isolated perfused pancreas of virgin, of pregnant and of one-month postpartum rats is shown in Fig. 1. Basal secretion of insulin under 4.4 mM glucose was similar in all three states. Insulin release in response to 16.7 mM glucose in pregnant rats was significantly elevated in both the first and second phases. Glucose-stimulated insulin release in one-month postpartum rats was reduced very nearly to the virgin levels. Total insulin secretions induced by

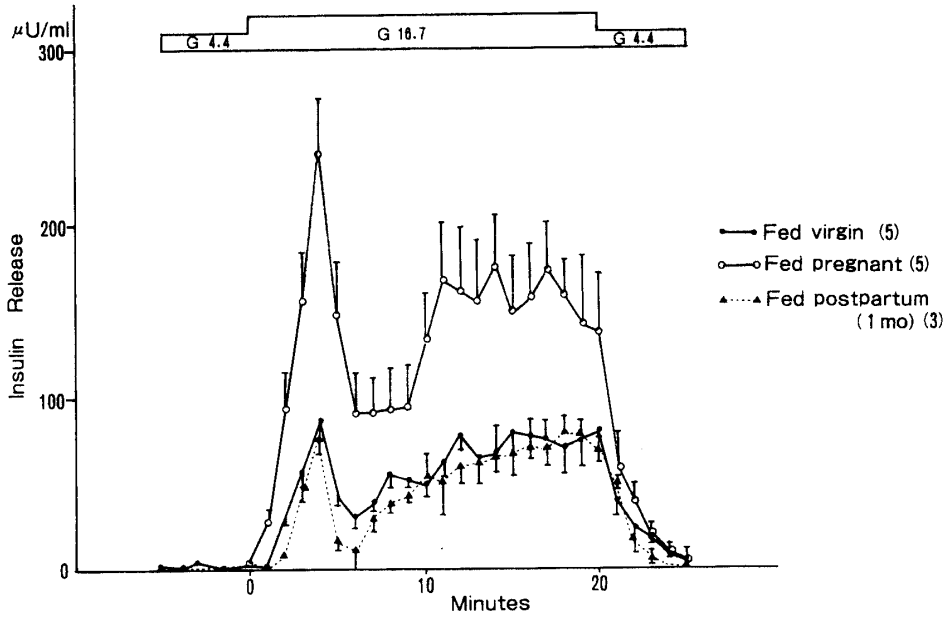

Fig.1. Insulin response to 16.7 mM glucose from the isolated perfused pancreas of virgin, pregnant and one-month postpartum rats. Each point represents mean ± SEM.

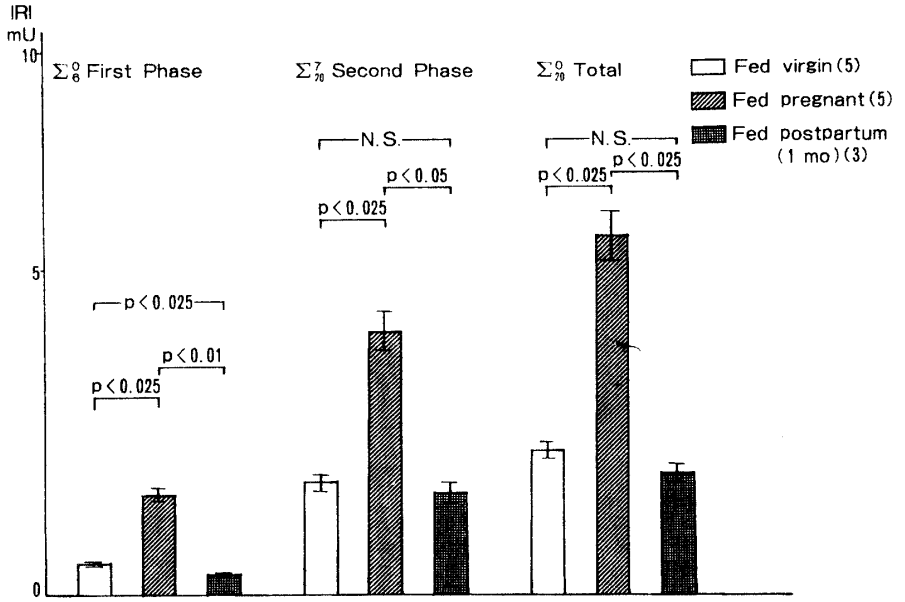

Fig.2. Insulin response to 16.7 mM glucose from isolated perfused pancreas expressed as increments above basal levels during first, second, and total phases. Each bar represents mean ± SEM.

16.7 mM glucose in virgin, pregnant and postpartum rats are shown in Fig.2. In comparing the summation of insulin secretion in the first phase (0-6 min), second phase (7-20 min) and total (0-20 min), the effect of pregnancy on glucose-induced insulin secretion was absolutely clear. Both the first and second phases of insulin secretion were augmented by pregnancy. Pregnancy

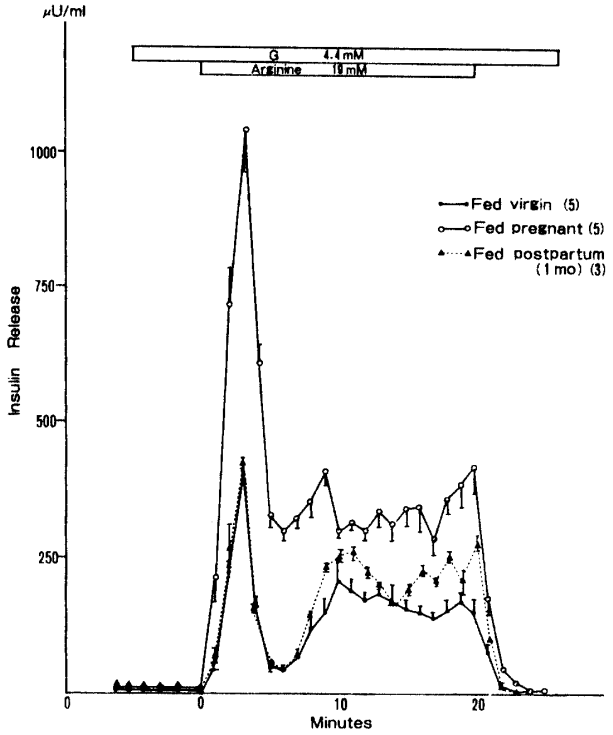

Fig.3. Insulin response to 19 mM arginine from the isolated perfused pancreas of virgin, pregnant and one-month postpartum rats. Each point represents mean ± SEM.

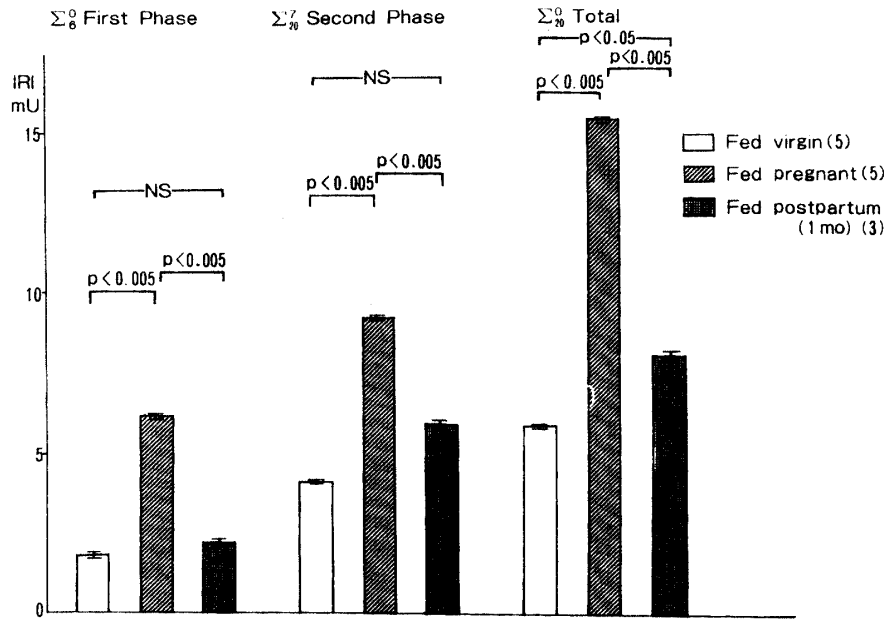

Fig.4. Insulin response to 19 mM arginine from the isolated perfused pancreas expressed as increments above basal levels during first, second, and total phases. Each bar represents mean ± SEM.

appears to affect the first phase greater than the second phase. The augmentation by pregnancy was 3-fold in the first phase and 2.5-fold in the second phase. Insulin secretion decreased significantly to the virgin levels at one-month postpartum.

The other nutrient tested, arginine, was a more potent stimulator than glucose, as shown in Fig.3. The peaks of the first phase in virgin, pregnant and one month postpartum rats were 405.3 ± 19.1 μU/ml, 1088.0 ± 43.2 μU/ml and 430.0 ± 21.4 μU/ml, respectively. The second phase was also markedly stimulated by pregnancy. The summation of insulin released during arginine stimulation is shown in Fig.4. The results again clearly show that the amount of insulin released during arginine stimulation was markedly greater than that during glucose stimulation. Both the first and second phases were significantly stimulated by arginine in pregnant rats. Thus secretory activity during pregnancy is greatly enhanced by nutrients.

DISCUSSION

Hypoglycemia and hyperinsulinemia are common, physiologically important features of pregnancy. We confirmed that plasma glucose is lower in pregnant rats than in virgin rats while plasma insulin is higher. After delivery plasma glucose and insulin recovered very nearly to the virgin levels. During pregnancy, the mother provides the fetuses with nutrients, so that plasma glucose is lower in pregnancy than in nonpregnancy. Despite the low plasma glucose insulin is needed in pregnancy more than in nonpregnancy because (1) the mother must store up glycogen and triglyceride efficiently as an energy source and provide glucose or gluconeogenic precursors for her brain and for the fetuses rapidly whenever she is in the fasted state; (2) the increases of contrainsulin hormones and peripheral insulin resistance during pregnancy require a large amount of insulin to prevent catabolism.

The fact that insulin release in response to glucose is elevated during pregnancy has been demonstrated in pregnant women by both oral and intravenous glucose tolerance tests (1-2, 18-20), and in rats by the static incubation or the perifusion of isolated islets of Langerhans (3, 7, 8, 11-13). To our knowledge the present study is the first to demonstrate exaggerated insulin secretion from the isolated perfused pancreas of pregnant rats.

Insulin release in response to nutrients such as glucose and arginine in pregnant rats was significantly elevated in both the first and second phases. The cause of elevated insulin responses during pregnancy has been investigated, and pancreatic islet hypertrophy, an increase in adenylate cyclase activity and insulin resistance during pregnancy have been demonstrated (3, 10, 11, 13). However, the details are not well understood.

We have recently observed that forskolin, a potent stimulator of adenylate cyclase, failed to enhance insulin release from the pancreas of pregnant rats (unpublished data). Our result is compatible with previous findings that cAMP is already increased in the pregnant pancreas (11, 13). An alternative piece of evidence was reported recently by Hubinount et al. (21). They have shown that activities of both protein kinase-A and -C are augmented in the pregnant pancreas. Although further work will be necessary to elucidate the mechanism of secretory activity in the pancreas during pregnancy, the present paper clearly indicates that exaggerated insulin secretion is a prominent feature of pregnancy.

REFERENCES

1) Spellacy, W.N. and Goetz, F.C. (1963) Plasma insulin in normal late pregnancy. N. Engl. J. Med., 268, 988-991
2) Bleicher, S.J., O'Sullivan, J.B. and Freinkel, N. (1964) Carbohydrate metabolism in pregnancy: V. The interrelation of glucose, insulin, and free fatty acids in late pregnancy and postpartum. N. Engl. J. Med., 271, 866-872
3) Green, I.C. and Taylor K.W. (1972) Effects of pregnancy in the rat on the size and insulin secretory response of the islets of Langerhans. J. Endocrinol., 54, 317-325
4) Kalkhoff, R.K., Kissebah, A.H. and Kim H.J. (1978) Carbohydrate and lipid metabolism during normal pregnancy: relationship to gestational hormone action. Semin. Perinatol., 2, 291-307
5) Felig, P. (1977) Body fuel metabolism and diabetes mellitus in pregnancy. Med. Clin. North. Am., 61, 43-66
6) Kalkhoff, R.K. and Kim H.J. (1979) The influence of hormonal changes of pregnancy on maternal metabolism. Pregnancy Metabolism, Diabetes and the Fetus. Ciba Foundation Symposium No. 63, Amsterdam, Excerpta Medica, p.29-46

7) Malaisse, W.J., Malaisse-Lagae, F., Picard, C. and Flament-Durand, J. (1969) Effects of pregnancy and chorionic growth hormone upon insulin secretion. Endocrinology, 84, 41-44
8) Costrini, N.V. and Kalkhoff, R.K. (1971) Relative effects of pregnancy, estradiol, and progesterone on plasma insulin and pancreatic islet insulin secretion. J. Clin. Invest., 50, 992-999
9) Leturque, A., Ferre, P., Satabin, P., Kervran, A. and Girard, J. (1980) In vivo insulin resistance during pregnancy in the rat. Diabetologia, 19, 521-528
10) Van Assche, F.A. (1974) Quantitative morphologic and histoenzymatic study of the endocrine pancreas in nonpregnant and pregnant rats. Am. J. Obstet. Gynec., 118, 39-41
11) Green, I.C., Howell, S.L., Montague, W. and Taylor, K.W. (1973) Regulation of insulin release from isolated islets of Langerhans of the rat in pregnancy: the role of adenosine 3':5'-cyclic monophosphate. Biochem. J., 134, 481-487
12) Kalkhoff, R.K. and Kim, H.J. (1978) Effects of pregnancy on insulin and glucagon secretion by perifused rat pancreatic islets. Endocrinology, 102, 623-631
13) Lipson, L.G. and Sharp, G.W.G. (1978) Insulin release in pregnancy: studies on adenylate cyclase, phosphodiesterase, protein kinase, and phosphoprotein phosphatase in isolated rat islets of Langerhans. Endocrinology, 103, 1272-1280
14) Curry, D.L. (1986) Insulin content and insulinogenesis by the perfused rat pancreas: effects of long term glucose stimulation. Endocrinology, 118, 170-175
15) Grodsky, G.M., Batts, A.A., Bennett, L.L, Vcella, C., Mc Williams, N.B. and Smith, D.F. (1963) Effects of carbohydrates on secretion of insulin from isolated rat pancreas. Am. J. Physiol., 205, 638-644
16) Goto, Y., Seino, Y., Taminato, T., Inoue, Y., Kadowaki, S., Mori, K. and Imura H. (1978) Modulation by alloxan of glucagon and insulin secretion in the isolated perfused rat pancreas. Endocrinology, 102, 1496-1500
17) Desbuquois, B. and Aurbach, G.D. (1971) Use of polyethylene glycol to separate free and antibody-bound hormones in radioimmunoassay. J. Clin. Endocrinol. Metab., 33, 732-748
18) Kalkhoff, R., Schalch, D.S., Walker, J.L., Beck, P., Kipnis, D.M. and Daughaday, W.H. (1964) Diabetogenic factors

associated with pregnancy. Trans. Assoc. Am. Physicians, 77, 270-279

19) Trayner, I.M., Welborn, T.A., Rubenstein, A.H., Fraser, T.R. (1967) Serum and urine insulin in late pregnancy and in a few pregnant latent diabetics. J. Endocrinol., 37, 443-453

20) Kalkhoff, R.K., Richardson, B.L. and Beck, P. (1969) Relative effects of pregnancy, human placental lactogen and prednisolone on carbohydrate tolerance in normal and subclinical diabetic subjects. Diabetes, 18, 153-163

21) Hubinont, C.J., Duframe, S.P. and Malaisse, W.J. (1985) Effect of pregnancy upon the activity of protein kinase A and C in rat pancreatic islets. Horm. Metabol. Res., 17, 104