Beta cell adaptation in pregnancy: a tribute to Claes Hellerström

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ABSTRACT
Pregnancy is associated with a compensatory increase in beta cell mass. It is well established that somatolactogenic hormones contribute to the expansion both indirectly by their insulin antagonistic effects and directly by their mitogenic effects on the beta cells via receptors for prolactin and growth hormone expressed in rodent beta cells. However, the beta cell expansion in human pregnancy seems to occur by neogenesis of beta cells from putative progenitor cells rather than by proliferation of existing beta cells. Claes Hellerström has pioneered the research on beta cell growth for decades, but the mechanisms involved are still not clarified. In this review the information obtained in previous studies is recapitulated together with some of the current attempts to resolve the controversy in the field: identification of the putative progenitor cells, identification of the factors involved in the expansion of the beta cell mass in human pregnancy, and the relative roles of endocrine factors and nutrients.

Introduction

Pregnancy represents a unique physiological condition with profound changes in the hormonal regulation of metabolism in order to provide sufficient nutrients to the fetus. The increased food intake increases the demand for insulin, which is further reinforced by the elevated levels of the somatolactogenic hormones (prolactin (PRL), growth hormone (GH), placental lactogen (PL), and placental growth hormone (GH-V)). The increased demand for insulin is in normal pregnancy compensated for by an expansion of the beta cell mass. If this does not occur, gestational diabetes will develop. Maternal hyperglycemia is transmitted to the fetus and may induce a premature insulin secretion resulting in macrosomia as well as an up to 8-fold increased risk of developing type 2 diabetes (1). It is therefore pertinent to unravel the mechanisms involved in beta cell adaptation to pregnancy. Through history several investigators have described an increased islet cell mass in various species, and recently several transcriptional and proteomic characterizations of islets from pregnant mice and rats have been performed (2–5) (for a review see Nielsen et al., in press). The scope of this review is to focus on some of the challenges that still remain in the understanding of the mechanisms involved in beta cell adaptation to pregnancy.

Role of hormones and nutrients

The first systematic quantitative study of the influence of pregnancy and lactation on the endocrine pancreas in mice was published by Claes Hellerström in 1963 (6). The islet volume at gestational day 20 was increased with about 25% compared with virgin mice. The numerical distribution of the islets was asymmetric in both groups, with the majority of islets in the small size class. In pregnant mice the number of islets in the large size class was increased. As the ratio between beta and alpha cells was increased in the pregnant mice it was concluded that the enlargement of the endocrine pancreas was mainly due to proliferation of the beta cells. On day 14 of gestation a significant increase in nuclear size of the beta cells was found, suggesting an increased insulin synthesis activity. This was not seen at day 20, suggesting that the glucose load was reduced at the end of the pregnancy or that the increased number of beta cells was sufficient to maintain euglycemia. The islet volume was still increased by 25% at day 20. In the rat an increased nuclear size was maintained until term, suggesting a sustained high insulin synthesis (7). Increased insulin content and secretory response were found in islets isolated from late pregnant rats (8,9). Data compiled from previous studies have shown a marked increase in the beta cell mass during pregnancy until term (Figure 1A) (10) and a corresponding increase in pancreatic insulin content (Figure 1A) (11). Sorenson and collaborators have demonstrated an increased proliferation of the islet cells during pregnancy in rats (Figure 1B) (12). Interestingly, there was a decline in proliferation late in pregnancy. There was also a decrease in the glucose-stimulated insulin secretion (GSIS) of the perfused pancreas (Figure 1A) (12). In contrast, the levels of lactogenic activity reflecting the secretion of the two placental lactogens PL-1 and PL-2 and pituitary PRL were high until term (Figure 1B) (12), as was the level of prolactin.
receptor (PRLR) mRNA (Figure 1B) and growth hormone receptor (GHR) mRNAs (data not shown) in the pancreas of pregnant rats (13). It has been suggested that the decreased proliferation may be due to the increased levels of progesterone (14) and/or glucocorticoids in late pregnancy (15), findings that have been supported by in vitro studies in isolated islets. It may be that the very high PL levels prevent the dimerization of the PRLR that is required for signal transduction. Alternatively, the insulin demand may decrease in late pregnancy as it has been reported that the blood glucose is lower close to term (10) and therefore attenuates the metabolic pressure on the beta cells, as already pointed out by Hellerström in 1963 (6). Restricted carbohydrate intake during pregnancy has been found to prevent the increased glucose sensitivity of the beta cells (16), and it has been shown that insulin treatment or food restriction during pregnancy reduces the mitogenic effect of PL on beta cells (17,18). Thus, hyperglycemia seems to play a central role in the proliferative response of the beta cells, and in fact glucose metabolism in the beta cells has been demonstrated to promote beta cell replication (19). The first step in glucose metabolism in beta cells is glucokinase followed by ATP production and closure of K/ATP channels and membrane depolarization. Interestingly, pregnancy is associated with an increase in glucokinase synthesis induced by lactogenic hormones (20), resulting in a lowering in the threshold for glucose-induced insulin secretion. Support for the role of glucokinase in beta cell proliferation comes from studies of activating glucokinase mutations in patients with congenital neonatal hyperinsulinemic hypoglycemia that have hyperplastic islets (21). Thus, the increased demand for insulin reflected in hyperglycemia may be compensated for by an increased glucose metabolism in the beta cells, resulting in increased proliferation and insulin production. This was already predicted by Hellerström in his extensive review from 1977 where he stated: 'It therefore appears that endocrine factors may be of minor significance for the islet growth in pregnancy' (22). The role of the lactogenic hormones may be to amplify the stimulating effect of glucose on beta cell growth and function and to provide energy for the growth of the fetus.

The challenge of human pregnancy

In human pregnancy there is also an increase in the beta cell mass, although only few studies have been performed. In 1978 van Assche and co-workers reported five cases with a 2-fold increase islet mass with an increased number of beta cells (23). In 2010 Peter Butler and co-workers published 18 cases and found a 1.4-fold increase in the beta cell area mainly due to an increased number of small islets and no increase in Ki67-positive beta cells, suggesting that in human pregnancy the beta cell adaptation occurs by neogenesis of beta cells from progenitor cells rather than proliferation of existing beta cells (24). The lack of proliferation of adult human beta cells has been suggested to be due to low expression of PRL receptors, and in fact gene expression profiles for mouse and human beta cells have confirmed that the expression level of PRLR is 40-fold higher in mouse beta cells than in human beta cells (25). However, this may not be the only reason, since a recent study has shown that over-expression of PRLR in human islets did not result in a mitogenic response to PRL (26). Whereas activation of STAT5 has been shown to stimulate proliferation in rat beta cells (27), activation of human STAT5 was not sufficient to stimulate human beta cell proliferation, but surprisingly over-expression of mouse STAT5 did stimulate human beta cell proliferation (26). Apparently there are species differences in the post-receptor PRL signaling pathway as well.

Role of beta cell neogenesis

Studies of pregnancy in other species like cow, pig, sheep, and dog have described the appearance of single beta cells or small clusters associated with ducts or in the acinar compartment, suggesting that neogenesis may contribute to the increased beta cell mass (see Nielsen et al., in press). Using a lineage-tracing approach, evidence for the recruitment of non-beta cell progenitors of beta cells in pregnant mice has been presented (28). In order to investigate if neogenesis of beta cells may contribute to the beta cell adaptation in rodent pregnancy we have recently studied the expression of neurogenin-3 (Ngn-3) that is transiently expressed during the embryonic development of the endocrine pancreas (29) in pancreata of pregnant mice. By immunohistochemical staining we found a 3.5-fold increase in expression mainly in the acinar compartment at pregnancy day 14 where it was 8-fold higher.
than in non-pregnant mice (30). Strikingly, this time point coincides with the maximal expression of Ngn-3 in the embryonic pancreas (29). The validity of Ngn-3 as a marker of post-natal beta cell neogenesis is controversial, but even if all the Ngn-3-positive cells differentiated to beta cells their quantitative contribution to the increased beta cell would be small compared to the contribution from the proliferation of the existing beta cells. In order to test if circulating factors in pregnancy contribute to neogenesis we employed the method for large-scale islet isolation from fetal rat pancreata in tissue culture developed by Claes Hellerström and co-workers in 1979 (31). At this late fetal period from gestational day 20 to 22 the number of beta cells is more than doubled mainly by formation of new beta cells from differentiation and proliferation of precursor cells (32). When we added serum from pregnant women in third trimester to cultures of dispersed fetal rat pancreas isolated at gestational day 21.5 we found a marked increase in Ngn-3 mRNA expression, in particular in the fibroblast-like monolayer (30). We are currently searching for factors in serum from pregnant women that influence beta cell growth and function (33).

**Concluding remarks**

Although much is known about beta cell adaptation to pregnancy, there are still unanswered questions that already were pointed out and investigated by Claes Hellerström decades ago. What is the relative contribution of the somatolactogenic hormones and the food intake to the health of the pregnant woman and her offspring? What is the relative contribution of proliferation and neogenesis of beta cells to the beta cell growth in human pregnancy? What is the identity of the progenitor cells? Can identification of factors that promote beta cell growth and function in pregnancy be useful in the treatment or prevention of gestational diabetes and other forms of diabetes?

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