Increased Dye Coupling in Pancreatic Islets From Rats in Late-Term Pregnancy

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Our previous studies have suggested that elevated lactogen, increased glucose-stimulated insulin secretion, and increased β-cell coupling are associated. To determine whether this association occurs under conditions of physiologically increased lactogen, we have studied the extent of dye coupling in rat islets during the later stage of pregnancy. These animals have high plasma lactogen levels in the form of placental lactogen, increased plasma insulin, and decreased plasma glucose. The fluorescent tracer, Lucifer yellow CH, was microinjected into central cells of islets from both pregnant and virgin rats, and the extent of transfer was quantitated by determining the projected area of dye spread. Two area measurements were made for each injection, one around the entire discernible fluorescent region ("outer") and another around the distinct brighter region of cells surrounding the injected cell ("inner"). Pregnancy increased dye transfer, as determined by both measurements. The outer area of dye transfer was 9047 ± 775 μm² for the islets from pregnant rats and 4699 ± 391 μm² for the islets from virgin rats ($P < .001$). Similarly, pregnancy increased the inner area of dye transfer, 1447 ± 161 μm² for the islets from pregnant rats and 795 ± 80 μm² for the islets from virgin rats ($P < .001$). These results support the hypothesis that elevated lactogen, increased glucose-stimulated insulin secretion, and increased β-cell dye coupling are associated under physiological conditions. The study indicates that enhanced β-cell coupling is part of the structural and functional adaptation that the islets undergo during a subject's pregnancy and demonstrates that the extent of β-cell coupling is regulated by a physiological condition. *Diabetes* 37:908–11, 1988

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Several studies have demonstrated that gap junctions (1) and cell-to-cell transfer of both ionic-current (2) and fluorescent dyes (3–6) are increased under conditions leading to increased insulin secretion. These studies suggest that enhanced cell-to-cell communication via gap junctions may be important in the regulation of glucose-induced insulin secretion (7,8). However, most of these studies have used pharmacologic agents or prolonged hyperglycemia to demonstrate junctional effects, making the importance of their physiological relevance unclear.

In a series of in vivo and in vitro studies, we examined the effect of elevated lactogen on insulin secretion. To characterize the effect of lactogen on insulin secretion, we studied the relationship between glucose and insulin dose responses in rats with elevated lactogen. In pregnant rats, the glucose stimulation threshold is lower, and the extent of stimulated insulin secretion is enhanced. Also, MtTW15 mammosomatotropic tumor-bearing rats had insulin secretion characteristics that matched those observed in the pregnant rats (9). Because in both cases hormones in addition to lactogen are elevated, the characteristics of insulin secretion from pancreases obtained from rats infused with prolactin (PRL) for 4 days were studied (10). Again, a decrease in the glucose stimulation threshold and enhanced insulin secretion were observed. Recently, we examined the effect of lactogen on isolated islets in culture and observed insulin secretion similar to that during pregnancy (11). Based on these observations, we have proposed that lactogen is the primary hormone responsible for the adaptive changes observed for insulin secretion during pregnancy.

In addition to the effects lactogens have on glucose sensitivity of islets, we have determined that lactogen can modulate junctional coupling in the islet. An increase in dye transfer among β-cells was observed in islets isolated from MtTW15 tumor-bearing rats (12,13), from rats infused with

PRL for 4 days (10), and in isolated islets treated with PRL in vitro (11,12).

These results linking elevated lactogen with increased dye transfer among β-cells and increased glucose sensitivity of insulin secretion formed the basis for the following working hypothesis (9,10,14): *1*) lactogen leads to increased β-cell coupling; *2*) low-threshold cells are thereby coupled to higher-threshold cells; and *3*) the overall glucose threshold is reduced, and suprathreshold glucose-induced insulin secretion is increased. Although our previous studies are consistent with this hypothesis, they all involve experimental elevations of PRL. Consequently, we undertook this study to determine whether islets from pregnant rats, having physiologically increased lactogen levels, have the increase in dye transfer predicted by our hypothesis.

MATERIALS AND METHODS

From six rats at 15–21 (17.4 ± 1) days of pregnancy and six virgin control rats of comparable age and weight, islets were isolated, attached to agarose-covered 35-mm dishes, and incubated in Krebs-Ringer solution as previously described (3,5,10). A total of 41 islets were examined from the pregnant animals (5–9 islets/animal) and 36 islets from the control animals (4–8 islets/animal).

Lucifer yellow CH was microinjected into a cell in the core of each attached islet, with micropipettes filled with 4% dye, and an injection regimen of ~10-nA pulses of 250-ms duration repeated 2/s for 5 min. The injections were carried out in the absence of illumination to avoid photobleaching. At the end of each injection, a photomicrograph was taken via a 1-min exposure on 400 ASA Ektachrome film (daylight), which was then processed by standard procedures.

The extent of dye transfer for each injection (1/islet) was measured by projecting each slide on paper, drawing an outline of the dye spread, and digitizing the area of the dye-containing region with a Hewlett-Packard computer (10). Because each injection typically resulted in a bright central region and a distinctly less bright peripheral region, we

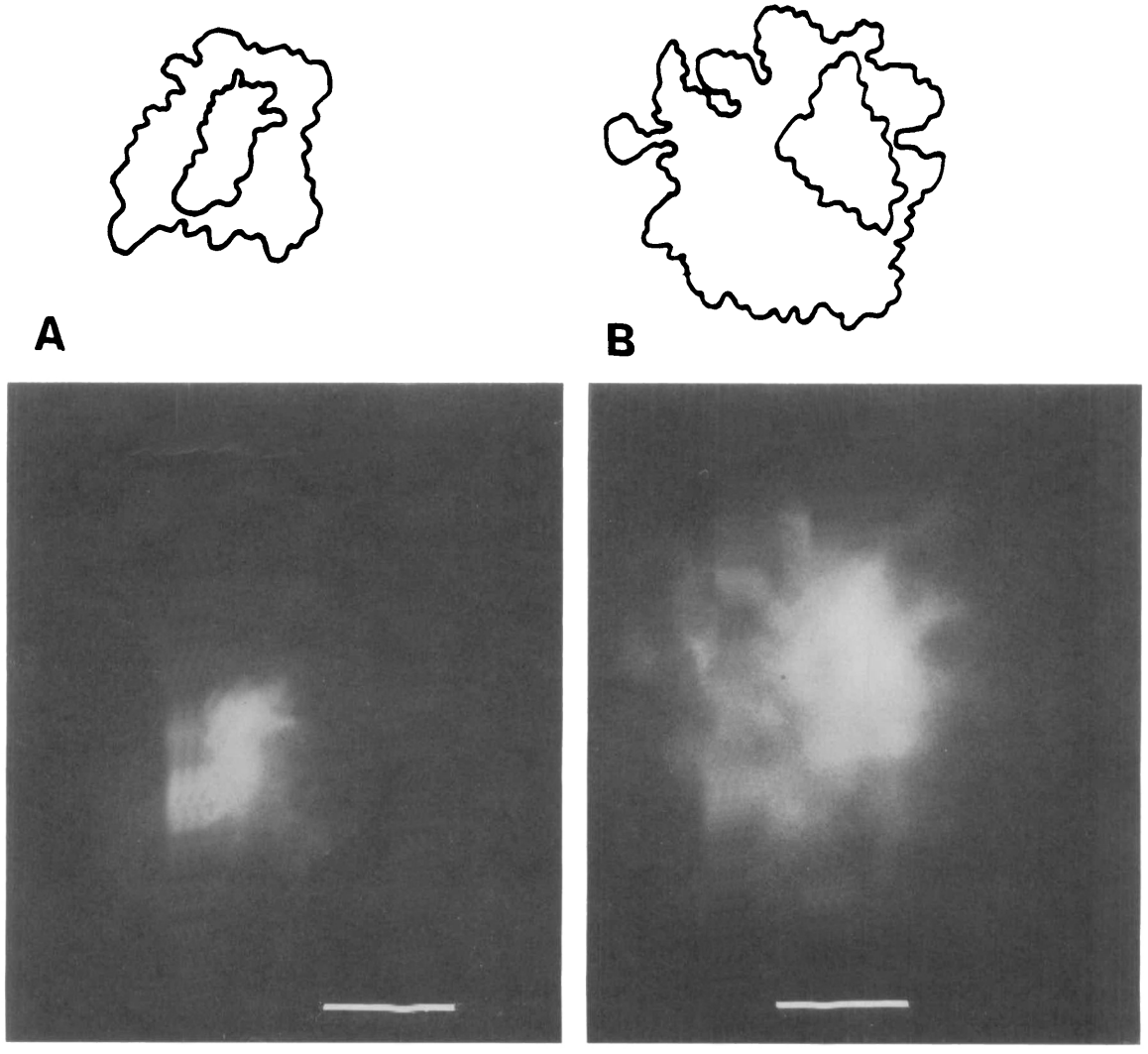

FIG. 1. Photomicrographs of dye transfer after microinjection of Lucifer yellow CH into islets from virgin control (*A*) and pregnant (*B*) rats and corresponding tracings of "inner" and "outer" extents of dye transfer (*above*). Dye was injected for 5 min in each case, and photographic slides were taken on Ektachrome 400 film (additional detail is present in original color photomicrographs). Slides were projected and tracings made of brighter discrete regions around inner and less distinct outer perimeters of dye spread. Areas contained within inner and outer tracings in each case were measured (*A*: inner = 1185 μm², outer = 5346 μm²; *B*: inner = 1868 μm², outer = 10,464 μm²). *Calibration bar* = 50 μm.

drew separate outlines around the "inner" and "outer" areas (Fig. 1).

Plasma glucose, insulin, and placental lactogen were determined as previously described (9). In brief, glucose was measured with a Beckman Glucose Analyzer II (Fullerton, CA), insulin was determined by radioimmunoassay, and placental lactogen by bioassay.

Statistical comparisons were made with Mann-Whitney and Student's *t* tests, and the data are expressed as means ± SE. Both parametric and nonparametric analyses gave equivalent results.

RESULTS

Hormone and glucose levels. As found in our previous study (9), the pregnant rats had elevated plasma insulin (35 ± 7 vs. 20 ± 5 μU/ml, $P < .05$) and placental lactogen (1774 ± 221 μg/ml vs. nondetectable) and decreased glucose (110 ± 6 vs. 146 ± 8 mg/dl, $P < .01$) compared with the control rats.

Dye coupling. Typical patterns of dye spread observed in islets from pregnant and control animals are shown in Fig. 1. In pregnant and control rat islets there was a central group of cells with noticeably higher fluorescence than the more peripheral cells, and the projected areas from these inner cells were measured separately.

The distribution frequency of dye spread was positively skewed in the control and pregnancy groups. It is clear from the distributions of dye spread (Fig. 2) that the experimental values for both inner and outer areas were higher than those of the control groups. The mean areas per injection for the experimental samples (inner, 1447 ± 161 μm^2; outer, 9047 ± 775 μm^2; $n = 41$) were about twofold greater than the values for the respective controls (inner, 795 ± 80 μm^2; outer, 4699 ± 391 μm^2; $n = 36$), differences that are statistically significant ($P < .001$).

DISCUSSION

This study clearly demonstrates that β-cell coupling is regulated under physiological conditions. We have shown that dye coupling among central islet β-cells is markedly increased in islets from rats in late-term pregnancy in comparison with control islets. Because previous research determined that islets from animals in the later stage of pregnancy have a decreased glucose threshold and increased suprathreshold insulin secretion (9,15), the results of this study add support to our hypothesis that elevated lactogen, increased glucose-induced insulin secretion, and increased β-cell dye coupling are associated (9,14). However, direct evidence supporting a causal relationship between the changes in β-cell coupling and altered insulin secretion remains to be determined.

The quantitative changes in dye coupling observed in this study are similar to those observed in islets from adult rats infused with ovine PRL (10,16) or in neonatal islets treated with ovine PRL in vitro (11). In those previous studies, the mean areas of dye spread among central β-cells increased about twofold in the PRL-treated islets. However, the absolute magnitude of the dye spread was greater, especially for the outer areas. The reason for this increase, particularly in the case of the control injections, is not clear. The smaller values for the in vivo infusion experiments (10,16) probably resulted from the injection of less dye into the β-cell due to the use of shorter iontophoretic-current pulses (50 ms vs. 250 ms). However, a similar explanation is not likely for the in vitro experiments because 250-ms pulses were used (11). For these experiments, the difference may reflect an underlying difference in the baseline coupling in cultured neonatal versus adult islets.

As noted in our previous studies (10,11,16), the size distribution of the coupling territories was positively skewed. To what extent this skewness was due to differences in coupling territories from islet to islet or within an islet or whether cou-

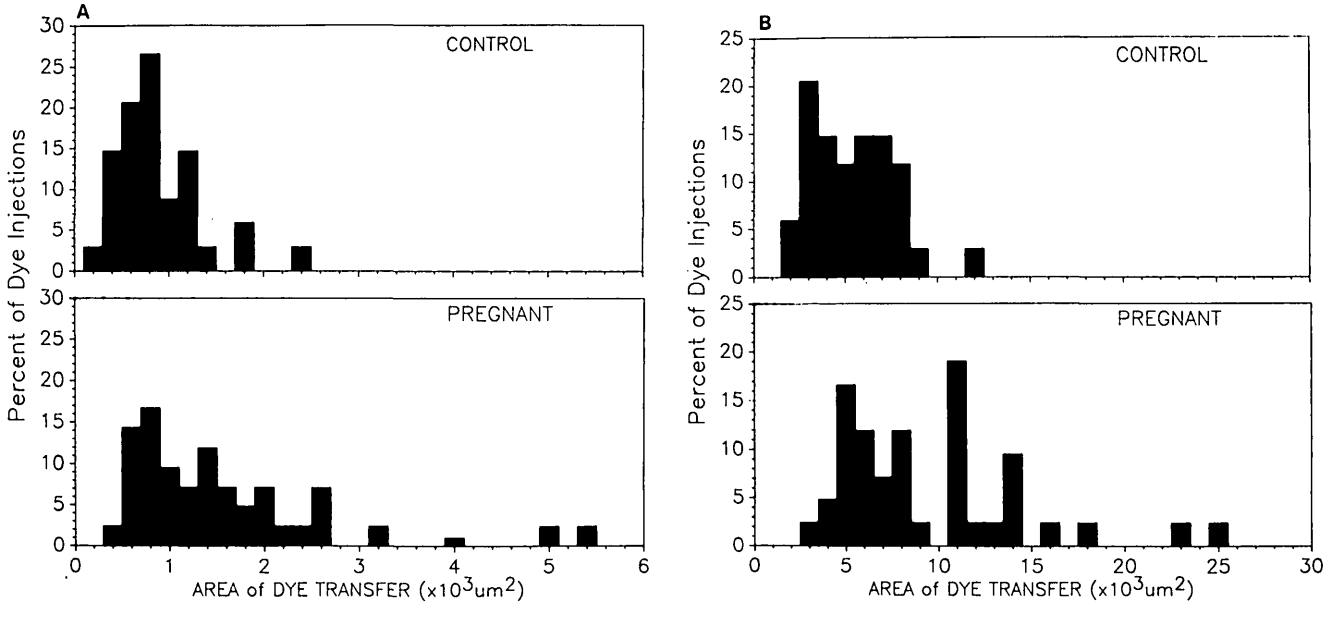

FIG. 2. Distributions of projected areas (*A*, inner; *B*, outer) for injections of islets from 6 virgin control (36 islets) and 6 pregnant (41 islets) rats. Note difference in scales for abscissas.

pling territories are in a state of flux with coupling territories more frequently in a smaller state is unknown. We have noted that in experiments in which multiple injections are made into an islet there appears to be a wide range in the extent of dye transfer (unpublished observations), indicating that at least part of the variation, and possibly skewness, represents different-sized coupling territories within individual islets.

The number of cells contributing to the β-cell coupling domain can be estimated if assumptions are made about the geometric arrangements of the cells constituting the area of dye spread (i.e., the injected cell will transfer dye to adjacent cells in all 3 dimensions with equal probability). These values can then be compared with those reported earlier (12,13) for numbers of dye-coupled cells determined by thin sectioning of the dye-injected islets. For this comparison, it is appropriate to use the inner areas, which should contain sufficient dye to remain detectably fluorescent after processing for thin sections. Michaels et al. (13) reported an average of 5 coupled cells/injection in the control islets and 55 coupled cells/injection in the islets from MtTW15 mammosomatotropic tumor-bearing rats. We estimate that an average of ~6 cells were coupled per injection in the control islets and ~30 cells were coupled per injection in the islets obtained from pregnant rats, representing a fivefold increase in the size of the coupling domain among the β-cells in pregnant rats.

In summary, this study demonstrates that the extent of β-cell coupling is regulated under physiological conditions and that β-cell coupling is part of the structural and functional adaptation that the islet β-cells undergo to accommodate the increased islet demand during pregnancy.

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