The In Vivo β-to-β-Cell Chat Room: Connexin Connections Matter

Paolo Meda

Proper glycemic control requires that numerous β-cells are coordinated to rapidly, but temporarily, secrete insulin after meals. This coordination integrates hundreds of β-cells within each islet into a functionally homogeneous unit. This homogeneity occurs despite the intrinsic heterogeneity of individual β-cells (1). Fine-tuning of this coordination is further required because of the cyclic oscillations that ionic, metabolic, and effector events undergo within each islet, as well as among the many islets of a pancreas. Together, these events ensure normal pulsatility of insulin secretion (2,3). Many mechanisms have evolved over time to achieve this coordination (1–4). One such mechanism is cell-to-cell coupling, the process by which contiguous cells exchange cytosolic molecules (Fig. 1) via channels made of connexin (Cx) proteins. These proteins concentrate at gap junction domains of the cell membrane (4).

β-Cells are coupled by Cx36 (5–8). Disruption or blockade of this coupling alters basal and glucose-stimulated insulin secretion (GSIS), the expression of insulin genes, and the handling of cytosolic calcium (1,4,8). In Cx36-null mice, which feature islets devoid of gap junctions and coupling (9,10), uncoupled β-cells cannot synchronize across each islet the calcium transients that are induced by glucose stimulation (9,11). Loss of this synchronization eliminates the pulsatile release of insulin and raises the basal secretion of the hormone, such that postprandial glucose no longer stimulates it (9,10) (Fig. 1). Uncoupled β-cells also feature a slower decay of GSIS (12) and are sensitized to apoptosis (13). While these data provide evidence that Cx36 has a role in the control of insulin secretion, they also highlight a paradox: Cx36-null mice are normoglycemic (9,10) and have a normal sensitivity to insulin (1) despite their sizable defects in insulin secretion.

In the current issue of *Diabetes*, Head et al. (14) revisited mice featuring native and null levels of Cx36. They report that loss of Cx36 induces intolerance to postprandial glucose levels, despite normal circulating levels of insulin, glucagon, and norepinephrine. They further document that oscillations in circulating insulin are decreased in Cx36-null mice. To determine the underlying mechanism, the authors studied islets isolated from these animals and confirmed that loss of Cx36 alters the intercellular coordination of the calcium transients induced by glucose (8,9,11) and prolongs the decay of insulin secretion once the glucose stimulation ends (12). Further, they report that these alterations are associated with decreased amplitude and longer decay of the first phase of GSIS, as well as with reduced insulin oscillations during the second phase of GSIS (14).

These data are noteworthy because they provide evidence that, in vivo, the altered insulin secretion of Cx36-null mice actually results in abnormal control of blood glucose levels, thereby supporting the physiological role of Cx36 signaling. By showing that the dynamics of GSIS are altered after loss of Cx36, these data also provide a possible explanation why Cx36-null mice are normoglycemic under basal conditions, but not after a glucose load. The data also indicate that Cx36 occupies a prominent position among the several mechanisms that contribute to regulate the dynamics of GSIS (2–4). Thus, while Cx36 signaling is dispensable for the occurrence of both Ca^{2+} and insulin oscillations, since these oscillations are preserved in Cx36-null mice, the period and amplitude of the oscillations observed in these animals were highly heterogeneous. This indicates that Cx36 is essential to harmonize their frequency and duration and also to sustain them within individual islets. Further, this study extends to the living mouse a number of observations (Cx36-dependent control of synchronized calcium oscillations, insulin pulsatility, and GSIS decay), previously made on isolated islets or pancreas (9–11), indicating that the in vivo phenotype is linked to islet autonomous alterations. Finally, this study confirms observations made in other colonies of Cx36-null mice (normal insulin sensitivity, lack of phenotype of mice retaining 50% of the native Cx36 levels) (1,9,10,13), illustrating that Cx36 signaling establishes a consistent β-to-β-cell "chat" within each islet, which is necessary for proper control of insulin secretion (Fig. 1). A scenario is proposed in which a decrease in the amplitude and increase in duration of the first phase of GSIS, together with decreased oscillations of its second phase, may account for the glucose intolerance observed in Cx36-null mice (14).

While this scenario is plausible, several issues remain to be addressed. The mice used in this study featured a global invalidation of the *Gjd2* gene (9), which also presumably abolished Cx36 expression in several neuronal populations and in catecholamine-producing cells (5). Given that these cell types contribute to control blood glucose by a variety of mechanisms, presumably including coordination of the 800–1,000 islets of the mouse pancreas (3), further experiments using mice that feature a β-cell–specific deletion of Cx36 (10) are now needed to validate the autonomous influence of the islet events. Another issue concerns the heterozygous mice, which presumably all expressed about half the native levels of Cx36 (9). Head et al. (14) report that only female mice developed marginal glucose intolerance. However, these animals were not tested for Cx36 levels or calcium and insulin oscillations, thereby raising questions about a hypothetical sex effect. An important

From the Department of Cell Physiology and Metabolism, University of Geneva, Geneva, Switzerland.
Corresponding author: Paolo Meda, paolo.meda@unige.ch.
DOI: 10.2337/db12-0336
© 2012 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.

See accompanying original article, p. 1700.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/61/7/1656/561081/1656.pdf by guest on 12 June 2024

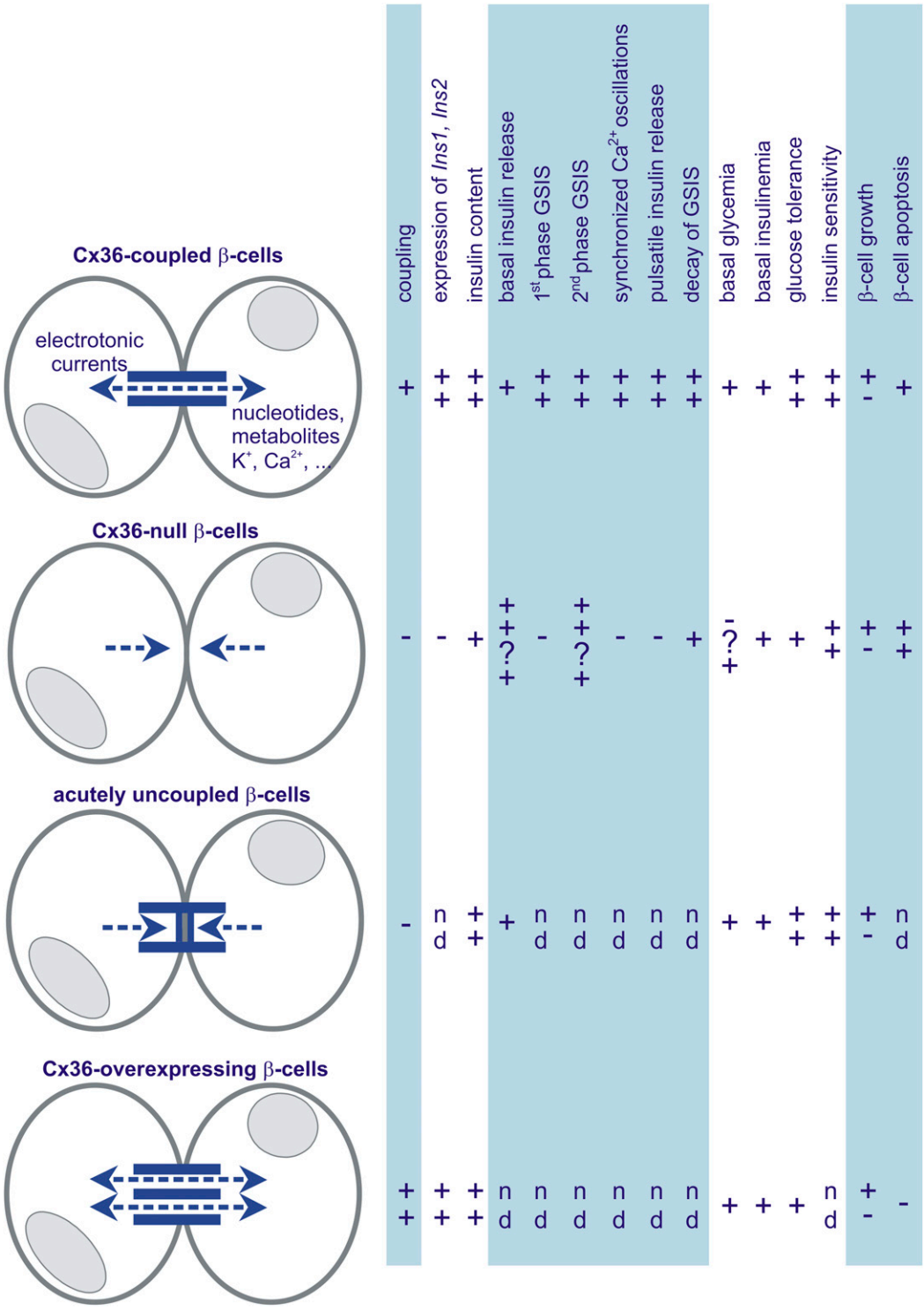

FIG. 1. In control islets, Cx36 channels allow for the exchange of electrotonic currents and cytosolic molecules between coupled β-cells. Islets null for Cx36 no longer feature this coupling, which causes the loss of the intercellular synchronization of glucose-induced Ca^{2+} transients and of pulsatile insulin release, as well as an extended off-response of β-cells. These alterations are associated with a decrease in the expression of the *Ins* genes and in both first and second phases of GSIS, which result in impaired glucose tolerance of the Cx36-null mice. It is still unclear (?) whether loss of Cx36 also sufficiently raises the basal secretion of insulin to alter the circulating levels of the hormone. Whether similar changes also take place after the temporary uncoupling of β-cells, resulting from the closure of Cx36 channels, is still largely undetermined (nd). Many aspects of β-cell function also remain to be investigated in β-cells forced to overexpress Cx36. Strikingly, these cells are more resistant to in vivo conditions eliciting apoptosis, in marked contrast to Cx36-null β-cells, which are sensitized to such conditions.

consideration is to determine how much Cx36 is required to preserve normal insulin secretion. Previous data have shown that 50% of the protein is sufficient to sustain most β-cell functions (9,11–13), whereas further loss may be as deleterious as the absence of the protein (10). Head et al. (14) now independently refine this estimation by computing that loss of ~75% of the Cx36 conductance would alter the synchronization of islet cells as observed in

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/61/7/1656/561081/1656.pdf by guest on 12 June 2024

Cx36-null mice. This may imply that the safety Cx36 factor may be lower in females than in males—an intriguing implication that deserves further investigations. Given that Cx may signal β-cells by mechanisms independent of cell uncoupling (4,15–17), further studies should also investigate whether the effects of Cx36 loss are solely accounted for by the uncoupling of β-cells or if they could also be attributable to altered signaling by other regulatory mechanisms (4).

Strikingly, the phenotype of Cx36-null mice is reminiscent of alterations typical of prediabetes (glucose intolerance, loss of circulating insulin oscillations) as well as type 2 diabetes (decreased first and second phases of GSIS, increased β-cell apoptosis) (18). Since human β-cells are also coupled by Cx36 (7), whose encoding gene is located at a chromosomal locus (19) that confers susceptibility to type 2 diabetes (20), an intriguing possibility is that alterations in Cx36 signaling, possibly as a result of a partial loss of Cx36 (14), may be implicated in the loss of β-cell function and mass that characterize this disease (1,4,5). If so, Cx36 may be a unique target of innovative therapies aimed at improving β-cell function and control of blood glucose.

ACKNOWLEDGMENTS

P.M. is supported by grants from the Swiss National Science Foundation (310030-141162, CR32I3-129987, IZ73Z0-127935), the Juvenile Diabetes Research Foundation (40-2011-11, 5-2012-281), and the European Union (BETAIMAGE 222980, IMIDIA C2008-T7, BETATRAIN 289932).

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Bavamian S, Klee P, Britan A, et al. Islet-cell-to-cell communication as basis for normal insulin secretion. Diabetes Obes Metab 2007;9(Suppl. 2): 118–132
2. Henquin JC. Regulation of insulin secretion: a matter of phase control and amplitude modulation. Diabetologia 2009;52:739–751
3. Tengholm A, Gylfe E. Oscillatory control of insulin secretion. Mol Cell Endocrinol 2009;297:58–72
4. Bosco D, Haefliger JA, Meda P. Connexins: key mediators of endocrine function. Physiol Rev 2011;91:1393–1445
5. Potolicchio I, Cigliola V, Velazquez-Garcia S, et al. Connexin-dependent signaling in neuro-hormonal systems. Biochim Biophys Acta. 1 October 2011 [Epub ahead of print]
6. Serre-Beinier V, Le Gurun S, Belluardo N, et al. Cx36 preferentially connects beta-cells within pancreatic islets. Diabetes 2000;49:727–734
7. Serre-Beinier V, Bosco D, Zulianello L, et al. Cx36 makes channels coupling human pancreatic beta-cells, and correlates with insulin expression. Hum Mol Genet 2009;18:428–439
8. Calabrese A, Zhang M, Serre-Beinier V, et al. Connexin 36 controls synchronization of Ca2+ oscillations and insulin secretion in MIN6 cells. Diabetes 2003;52:417–424
9. Ravier MA, Güldenagel M, Charollais A, et al. Loss of connexin36 channels alters beta-cell coupling, islet synchronization of glucose-induced Ca2+ and insulin oscillations, and basal insulin release. Diabetes 2005;54:1798–1807
10. Wellershaus K, Degen J, Deuchars J, et al. A new conditional mouse mutant reveals specific expression and functions of connexin36 in neurons and pancreatic beta-cells. Exp Cell Res 2008;314:997–1012
11. Benninger RK, Zhang M, Head WS, Satin LS, Piston DW. Gap junction coupling and calcium waves in the pancreatic islet. Biophys J 2008;95: 5048–5061
12. Speier S, Gjinovci A, Charollais A, Meda P, Rupnik M. Cx36-mediated coupling reduces beta-cell heterogeneity, confines the stimulating glucose concentration range, and affects insulin release kinetics. Diabetes 2007;56: 1078–1086
13. Klee P, Allagnat F, Pontes H, et al. Connexins protect mouse pancreatic β cells against apoptosis. J Clin Invest 2011;121:4870–4879
14. Head WS, Orseth ML, Nunemaker CS, Satin LS, Piston DW, Benninger RKP. Connexin-36 gap junctions regulate in vivo first- and second-phase insulin secretion dynamics and glucose tolerance in the conscious mouse. Diabetes 2012;61:1700–1707
15. Charollais A, Gjinovci A, Huarte J, et al. Junctional communication of pancreatic beta cells contributes to the control of insulin secretion and glucose tolerance. J Clin Invest 2000;106:235–243
16. Konstantinova I, Nikolova G, Ohara-Imaizumi M, et al. EphA-Ephrin-A-mediated beta cell communication regulates insulin secretion from pancreatic islets. Cell 2007;129:359–370
17. Calabrese A, Caton D, Meda P. Differentiating the effects of Cx36 and E-cadherin for proper insulin secretion of MIN6 cells. Exp Cell Res 2004; 294:379–391
18. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. J Clin Invest 2006;116:1802–1812
19. Belluardo N, Trovato-Salinaro A, Mudò G, Hurd YL, Condorelli DF. Structure, chromosomal localization, and brain expression of human Cx36 gene. J Neurosci Res 1999;57:740–752
20. Mori Y, Otabe S, Dina C, et al. Genome-wide search for type 2 diabetes in Japanese affected sib-pairs confirms susceptibility genes on 3q, 15q, and 20q and identifies two new candidate Loci on 7p and 11p. Diabetes 2002; 51:1247–1255

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/61/7/1656/561081/1656.pdf by guest on 12 June 2024