Sorting Out the Receptor Isoforms Underlying Dopamine Inhibition of Insulin Secretion

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Glucose is the primary signal for insulin secretion in pancreatic β-cells, and the process from glucose sensing to insulin secretory granule exocytosis relies on the activation of tightly regulated pathways within the β-cell. The major trigger for exocytosis is the elevation of intracellular Ca^{2+} concentration. cAMP, which activates protein kinase A, is a well-known potentiator of glucose-stimulated insulin secretion (GSIS) (1,2) that increases flux through the Ca^{2+} channels (3,4). cAMP also activates insulin secretion independently of glucose stimuli through a direct activation of insulin exocytosis distal to changes in intracellular Ca^{2+} concentration (5,6). It is critical that researchers uncover molecular mechanisms of cAMP regulation in β-cells for therapeutic exploitation that can improve insulin secretion in patients with type 2 diabetes. One G-protein–coupled receptor known to modulate insulin secretion is the dopamine receptor (DR), which inhibits exocytosis from β-cells (reviewed in Ustione et al. [7]). This inhibition may be driven through downstream signaling by either G_{α} or $G_{\beta\gamma}$ subunit or both subunits. Thus, improved understanding of DR action in the islet could open the path toward enticing therapeutic candidates.

Dopamine transduces its various physiological effects through activation of five G-protein–coupled DRs, D1R–D5R. These receptors are divided into two major subclasses: D1-like receptors (D1R and D5R) and D2-like receptors (D2R–D4R) (8,9). D1-like receptors are linked to $G\alpha_{s/olf}$ proteins, and their activation leads to increased adenylate cyclase activity and formation of cAMP (10). In contrast, D2-like receptors are linked to $G\alpha_{i/o}$ proteins, and their activation leads to inhibition of adenylate cyclase and decreased cAMP levels (10). Dopamine is a potent inhibitor of insulin secretion, and in an early description of this effect (11), accompanying immunohistochemistry results suggested the expression of D2R in the islets. These results remained controversial, since there was no evidence of dopaminergic neurons in the pancreatic islets.

It was later shown that islet β-cells express all the necessary components for dopamine synthesis, secretion, and signaling (11–15). By use of selective inhibitors, it was established that D2R and D3R are expressed in β-cells (11,13–15), and stimulation of these receptors with either dopamine or specific agonists inhibits GSIS. Conversely, inhibition of D2R/D3R signaling augments insulin secretion. Although D3R, and not D2R, was previously implicated in regulation of cAMP levels and insulin secretion in β-cells (11,15), a recent study using different selective inhibitors reported that D2R and D3R work together to modulate dopaminergic inhibition of GSIS (13).

Since the dominant effect of dopamine on β-cells is inhibitory, it has seemed reasonable to assume that D2-like DR play a major role in this action. Interestingly, β-cells also express D1R. In fact, D1R show the highest expression levels in islet RNA sequencing experiments (16). Membrane proteins typically exhibit low transcript numbers in RNA sequencing experiments, and not every islet study has identified DR expression. Even in those studies that did report these messages, none showed very high expression, and none of the isoforms have been singled out as being up- or downregulated in subjects with diabetes. The function of DR further depends on their expression level at the plasma membrane, which is ultimately regulated by trafficking and degradation. Still, it has been hard to reconcile the higher D1R transcript level with the inhibitory effects seen for dopamine, especially as both D2R and D3R have been implicated in β-cell function. This opens questions as to whether there are temporal or spatial regulation mechanisms of these receptors under specific conditions or whether these receptors interact with each other. The answers to these questions could have profound implications for β-cell function.

In this issue of *Diabetes*, Uefune et al. (17) demonstrate that dopamine released from the insulin secretory granules during GSIS negatively feeds back on β-cells to

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COMMENTARY

reduce the second phase of insulin secretion. This was concomitant with reductions to both intracellular cAMP accumulation and the cytosolic Ca^{2+} response. While the cAMP phenotype was accounted for by $G_{\alpha i/o}$ signal transduction in which D2 receptors participate, this only partially accounted for the Ca^{2+} phenotype. Fittingly, antagonism of D1R but not D2R counteracted the inhibitory effect of dopamine on the glucose-induced Ca^{2+} response. Thus, while activated D2 negatively regulates both Ca^{2+} and cAMP to restrict second-phase insulin secretion, D1R activation negatively regulates the Ca^{2+} signal specifically. Further, the authors demonstrate that D1 antagonists elevated the second phase of GSIS even without exogeneous dopamine added, emphasizing that this effect is mediated by autocrine signaling from endogenous dopamine.

The authors turned to overexpression systems to dissect the relative contributions of D1 and D2 to dopamine signaling. D2 overexpression abolished both glucose-induced Ca^{2+} flux and insulin secretory granule exocytosis in the presence and absence of dopamine, but interestingly, dopamine potentiated both parameters in D1-overexpressing cells. Such irregular and severe responses from D1 and D2, respectively, indicate that the inhibitory effect of dopamine is not mediated by the individual receptors.

Conveniently, it is known that D1R and D2R can form a heteromer (18), and the presence of such heteromers was confirmed in islets via a Duolink proximity ligation assay. D1-D2 agonism resulted in a sharp, transient inhibition of GSIS that was mediated by inhibiting Ca^{2+} flux but not by inhibiting cAMP accumulation. This is the putative mode for D1R and D2R function in the β-cell, and as such, previous studies inquiring into the mechanism of action of D1 and D2 signaling alone may not capture the full picture. The authors conclude their study by illustrating that overexpression of D1 and D2 together exerted beneficial protective effects on islet cell population, in contrast to prior studies that found D2 overexpression has detrimental effects. In summary, the authors suggested that D1 exists in stoichiometric excess to D2 as a safeguard to prevent cell damage and that D2 is a limiting factor for D1-D2 heteromer formation, thus extending our understanding of autocrine dopamine signaling in the islet (Fig. 1).

While the article provides significant data supporting the new model of dopamine signaling, the experiments

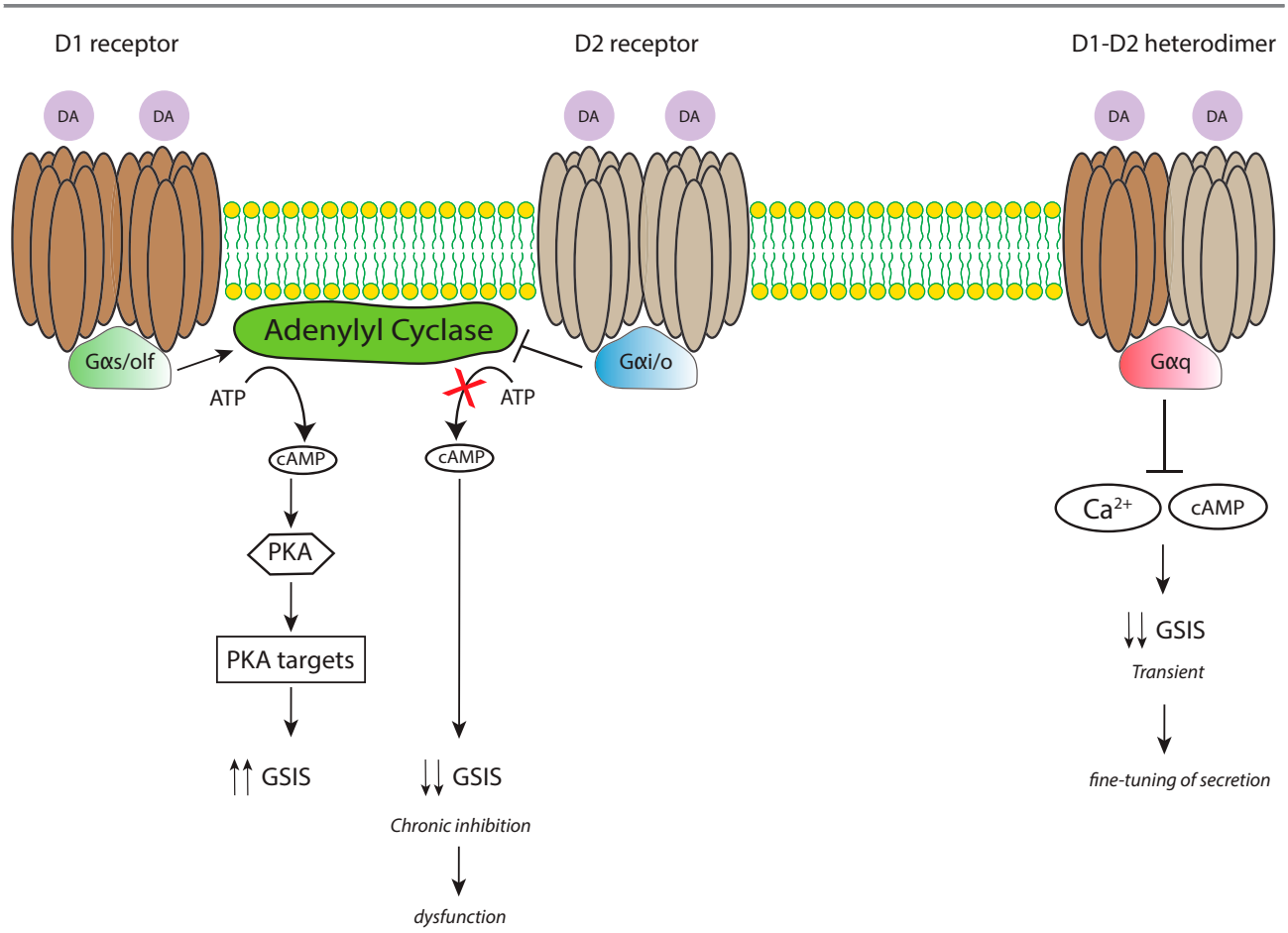

Figure 1—Schematic representation of signaling pathways activated by D1R homodimers, D2R homodimers, and D1R-D2R heterodimers in pancreatic β-cells. DA, dopamine; PKA, protein kinase A.

rely mainly on selective pharmacological inhibitors and overexpression experiments. Even the best pharmacological inhibitors of one DR isoform have some activity on the other isoforms, which could lead to confounding results. Further, the DR isoforms involved, D1R, D2R, and D3R, all have conditional gene deletions in mice available that could be mixed and matched to further test the hypothesis. These animals could be used by following the experimental approaches described by Uefune et al. (17). Further, islets and β-cells with specific isoform deletions can be used with rescue experiments to rigorously establish which specific isoforms are required to generate the observed signaling responses to dopamine. It will be interesting to see how this exciting hypothesis stands up to further tests in the coming years.

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

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