

GABAergic System in β -Cells: From Autoimmunity Target to Regeneration Tool

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The γ -aminobutyric acid (GABA) is a product of decarboxylation of the amino acid glutamate mediated by the synthesizing enzyme glutamic acid decarboxylase (GAD) (1–3). Although GABA is a major inhibitory neurotransmitter of the brain, it is produced at high levels in pancreatic islets (4). β -Cells store GABA in synaptic-like microvesicles, and upon its secretion, GABA exerts many paracrine functions in pancreatic islets (4). While the total function of GABA in β -cells is incompletely understood (4), its synthesizing enzyme GAD is possibly one of the most significant pancreatic islet β -cell autoantigens (5). GAD is a primary target of autoantibodies, and anti-GAD antibodies are associated with the development of type 1 diabetes (T1D) (5).

GABA AND β -CELLS

GABA activates three types of membrane receptors: GABA_A and GABA_C, which are ligand-gated Cl⁻ channels, and GABA_B, a ligand-gated Ca²⁺ or K⁺ channel (6). It has been demonstrated that β -cells mainly express the GABA_B receptor (GABA_BR) and the GABA_A receptor (GABA_AR) and produce GABA through GAD (Fig. 1) (4); GABA colocalizes with insulin as shown by confocal microscopy (4,7). Activation of GABA receptors in islet β -cells increases insulin release (8), exerts protective and regenerative effects on islet β -cells (9), and reduces apoptosis in cultured islets (9). GABA has also been shown to increase DNA synthesis in the pancreatic cell line INS-1, and when injected in vivo it increased the number of Ki67⁺ islet β -cells (Fig. 1). Thus, GABA increases β -cell proliferation in vivo and in vitro, protects INS-1 cells from streptozotocin (STZ)-induced apoptosis, and prevents hyperglycemia in murine models of diabetes (9).

GABA AND THE IMMUNE SYSTEM

Interestingly, different effects of GABA on the immune system have been reported (10). In 1999, Tian et al. (11) described the presence of GABA_AR in murine CD4⁺ cells (Fig. 1). The presence of GAD65 was also demonstrated in murine dendritic cells and macrophages (12). In vitro, investigators showed that GABA was able to inhibit the T-cell proliferative response to anti-CD3 in a dose-dependent

manner (11) as well as to islet autoantigens (13). Inhibition of T-cell proliferation resulted from a substantial GABA-induced reduction of interleukin (IL)-2 (Fig. 1) (11). GABA suppressed nuclear factor- κ B activation in lymphocytes (14), and this effect was blocked by picrotoxin, a GABA_AR antagonist. This observation is consistent with a GABA_AR-mediated response (14). In vivo experiments using a delayed type hypersensitivity assay showed downregulation in T-cell activity in NOD mice during GABA treatment (11). Administration of GABA to NOD mice not only inhibited the progression of the disease but also reduced the activity of diabetogenic effector T cells (13). A decrease in peripheral inflammatory cytokines (IL-1 β , tumor necrosis factor- α , interferon- γ , and IL-12) with increased numbers of regulatory T cells (CD4⁺CD25⁺FoxP3⁺ cells) was observed in GABA-treated mice (10).

In this issue, Tian et al. (15) investigated the effect of GABA and of the GABA_BR agonist baclofen and of the GABA_AR agonist muscimol on β -cell apoptosis in cultured rodent cell lines and in murine and human islets. These studies are important because while a number of mitogens and growth factors promote rodent β -cell replication, very few stimulate human β -cell replication. Oxidative stress of β -cells was induced with STZ, and GABA, the GABA_BR-specific agonist baclofen, or the GABA_AR-specific agonist muscimol were subsequently administered for 48 h (15). GABA, baclofen, and muscimol reduced the percentage of apoptotic islet cells in dose-dependent mice (Fig. 1). To strengthen their hypothesis, Tian et al. next examined whether GABA administration limited β -cell apoptosis in a model of human islet transplantation. Two days following islet transplant, a significant reduction in the percentage of apoptotic cells concomitant with an increased frequency of insulin⁺ β -cells in human islets was evident in mice treated with GABA, baclofen, or muscimol (15).

The proliferation of β -cells (as assessed by BrdU⁺insulin⁺ staining) was very low in control mice (approximately 1% of islet cells) (15). But in mice treated with GABA or GABA_{A/B}R-specific agonists, the percentage of newly replicated β -cells reached approximately 3%, suggesting that oral GABA treatment promotes β -cell replication (Fig. 1). The authors next examined whether oral GABA promoted human β -cell replication in NOD severe combined immunodeficiency mice transplanted with human islets. GABA, baclofen, and muscimol treatment promoted significant human β -cell replication, as suggested by the increased percentage of total insulin⁺ cells within islet grafts (Fig. 1). The functional recovery of STZ-induced hyperglycemic mice may thus be caused by two effects: protection from β -cell apoptosis and stimulation of β -cell proliferation.

CLINICAL RELEVANCE

GABA and GABA agonists have potentially important clinical applications. GABA or GABA_{A/B}R-specific agonists could become components of treatment in islet-transplanted

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See accompanying brief report, p. 3760.

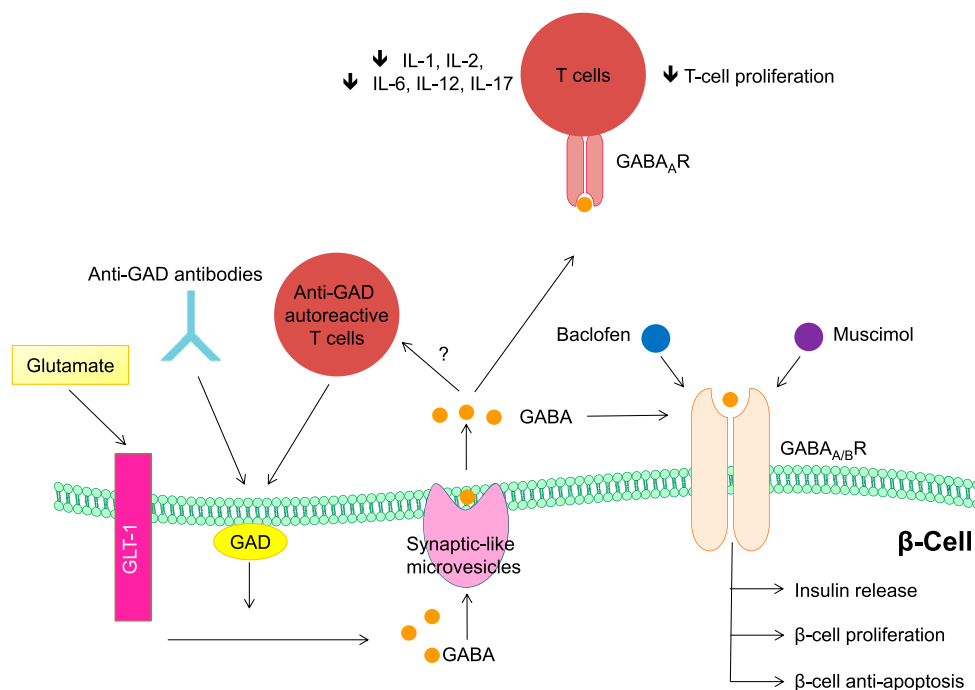


FIG. 1. Regenerative and immunological abilities of the inhibitory neurotransmitter GABA. Extracellular glutamate, the precursor of GABA, enters β -cells through the glutamate transporter-1 (GLT-1), where it is converted to GABA by the enzyme GAD and is then stored in synaptic-like microvesicles. GABA signals through the GABA_BR expressed by β -cells, thus increasing insulin release, protecting β -cells from STZ-induced apoptosis, and stimulating β -cell proliferation. Baclofen and muscimol, both agonists of the GABA_BR, have the same effects. T cells are sensitive to GABA due to the presence of GABA_AR on their cell surface. GABA exerts an immunomodulatory effect on T cells by decreasing the production of inflammatory cytokines (IL-1, IL-2, IL-6, IL-12, and IL-17) and inhibiting or reducing T-cell proliferation. Of note, GAD is targeted by autoreactive T cells and specific anti-GAD antibodies.

patients, with the goal of reducing the number of islets required to achieve insulin independence. The islet transplant field is still struggling with high numbers of transplanted islets succumbing to peritransplant apoptosis and the subsequent loss of islet mass (16). However, a successful or even partially functioning islet transplant has been shown to halt the progression of diabetes complications (17–19). The second most obvious approach would be to apply this treatment in newly diagnosed diabetic individuals or in individuals with autoantibodies who are at high risk for diabetes (20). Type 2 diabetes is a potential target of investigation for this therapy as well, although the optimal pool of individuals to be tested and studied may not be so straightforward. Patients with late-stage pancreatic disease may be appropriate candidates for GABA treatment, but ultimately GABA regenerative abilities may not prove all that powerful. Possibly, individuals with systemic inflammation (high C-reactive protein, IL-6, severe insulin resistance, and overwork of islets) may also benefit from GABA treatment. It will be important to develop biomarkers to identify patients who will benefit from GABA therapy as an enrichment strategy.

The important message and strength of Tian et al. (15) is its description of how GABA protects murine and, more important, human β -cells from inflammation and apoptosis and how it induces β -cell proliferation. However, some weaknesses of this research should be highlighted, which, it should be noted, may represent strengths in the clinical setting. The effect of GABA and GABA receptor agonists on the immune system may partially explain the results observed in this study. However, these off-target effects of GABA and its agonists may become beneficial in islet-transplanted individuals or in individuals with T1D, in

which some immunoregulatory effects (as shown by target of the GABA system) may be desirable. This newly acquired knowledge may change the view of how β -cells modulate their own fate and how they potentially modulate inflammation. The release of stored GABA by β -cells may protect β -cells themselves and potentially reduce inflammation. The investigators showed that activation of GABA_A or GABA_B receptors inhibited STZ-induced murine and human β -cell apoptosis (15). Furthermore, treatment with either a GABA_AR- or GABA_BR-specific agonist promoted mouse and human β -cell proliferation in mice. Last, GABA's anti-inflammatory activity may partially explain the observed results. The GABAergic system, a well-known target of autoimmunity, appears to be a promising tool for β -cell regeneration.

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