The Possible Mechanisms by which Borapetol B Stimulates Insulin Release from Rat Islets

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ABSTRACT

We have recently demonstrated the anti-diabetic effect of borapetol B (C1) isolated from the plant Tinospora crispa to be associated with stimulation of insulin release in the pancreatic islets. This present study aims to reveal the mechanisms by which C1 stimulates insulin release in the isolated pancreatic islets of normoglycemic control Wistar (W) and spontaneously type 2 diabetic Goto-Kakizaki (GK) rats. Isolated pancreatic islets from Wistar (W) and Goto Kakizaki (GK) rat were cultured overnight and then exposed to incubation conditions at 3.3 mM and 16.7 mM glucose. Several modulators and inhibitors were used; 0.25 mM diazoxide only (to open the K-ATP channel, 50 mM of KCl (for depolarization of beta cells), nifedipine (to block the L-type Ca2+ channels), 10 uM H89 and 1.5 uM of calphostin-C (to block PKA-and PKC respectively) and 100 ng/ml pertussis toxin (to inhibit the Ge protein).

The insulin released during incubation was measured using radioimmunoassay (RIA) assay. C1 significantly stimulated insulin release at both low and high glucose in W and GK rat islets. The opening of K-ATP channels by adding diazoxide inhibited insulin release at 16.7 mM glucose in W ($P<0.01$) and GK ($P<0.05$) rat islets compared to control. Diazoxide decreased insulin response to C1 in W and in GK (both $P<0.01$) only at 16.7 mM glucose. The insulin release of both W and GK rat islets

incubated with C1+diazoxide+KCl was significantly higher (when compared with islets incubated either with C1 or only or diazoxide+KCl) at both 3.3 mM and 16.7 mM glucose. Nifedipine decreased insulin release in W ($P<0.05$) and in GK ($P<0.01$) rat islets at 16.7 glucose only. In the presence of nifedipine, C1-induced insulin secretion of islets was decreased in W ($P<0.01$) and GK rat islets at 16.7 mM glucose. H89 and calphostin C inhibitors did not affect the insulin response to C1 respectively in W and GK islets at both 3.3 mM and 16.7 mM glucose. At 16.7 mM glucose, pertussis toxin decreased the insulin response to C1 in the W ($P<0.01$) and GK rat islets ($P<0.05$). When exploring the mechanisms of insulin release in the W and GK pancreatic islets, we showed that the C1 effect was exerted partly via K-ATP channels since diazoxide partly, but not totally suppressed C1 stimulation at 16.7 mM glucose. C1 effect was also dependent on L-type Ca2+ channels since nifedipine suppressed the insulin response to C1 at 16.7 mM. There was no modulation by PKA and PKC inhibitors. Furthermore, C1 effect was partly dependent on pertussis toxin sensitive Ge-protein. Therefore, the major stimulatory effect of C1 might be on the exocytosis.

KEYWORDS: Type 2 diabetes, Tinospora crispa, insulin secretion, Goto kakizaki rat, pancreatic islets