Review
DO MICROTUBULES PLAY A ROLE IN INSULIN SECRETION FROM THE PANCREATIC BETA CELLS?

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"When an erroneous hypothesis becomes entrenched and generally accepted, it is transformed into a kind of tenet that no one is allowed to question and investigate; and it then becomes an evil which endures for centuries."

— *Goethe*

Electron microscopy has revealed that in a variety of animal cells, microtubules and microfilaments play a major role in movement of intracellular particles. Microtubules have been implicated in the secretion of hormones from various endocrine glands (1). Vincristine, vinblastine and colchicine, which are known to disrupt microtubules, have been shown to inhibit iodine release from thyroid glands (2), catecholamines release from adrenal medulla (3), and prolactin secretion from pituitary tumor cells in culture (4). The involvement of microtubules in the stimulus-induced insulin secretion from beta cell of the pancreas has also been hypothesized (5, 6). It has been shown that vincristine inhibits glucose-induced insulin secretion from pieces of pancreas, from perfused pancreas, and from isolated perfused islets of Langerhans (7-10).

Diphasic Effect of Vincristine on Insulin Secretion

The effect of vincristine on insulin secretion in the intact animal may be dose related. We have shown that acutely, vincristine treatment in lower doses (0.06 mg per kg) potentiates glucose-induced insulin secretion and significantly enhances glucose tolerance in the intact rat (11). However, this acute potentiating effect of vincristine on glucose-induced insulin secretion was less after the treatment with the medium dose (0.15 mg per kg) and was completely abolished with a tendency towards inhibition after the treatment with highest dose of vincristine (0.5 mg per kg) (Figure 1). The effect of vincristine was also time-related on insulin secretion and glucose tolerance. The medium dose of vincristine (0.15 mg per kg) caused progressively more inhibition as the time interval for eliciting the glucose-induced insulin secretion and glucose tolerance was increased after vincristine treatment. In other words, insulin secretion and glucose disappearance rates deteriorated progressively from 10 minutes to 120 minutes after the treatment with vincristine (Figure 2). Although vincristine in the dose of 0.15 mg per kg clearly caused inhibition of glucose-induced insulin secretion and glucose tolerance, it had no effect on arginine-induced insulin secretion (12). Furthermore, vincristine, at the dose of 0.15 mg per kg, had no effect on the disruption of beta-cell microtubular structures.

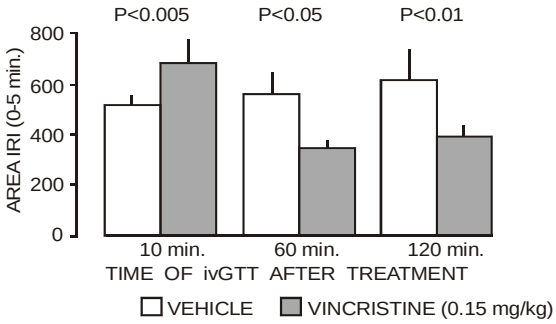

Fig 1: The time of ivGTT after vincristine treatment (0.15 mg/kg) and insulin secretion. At 10 minutes after vincristine treatment, glucose-induced insulin secretion (0-5 minutes area IRI) was significantly (p<0.005) enhanced. At 60 and 120 minutes after vincristine treatment, glucose-induced insulin secretion was significantly inhibited (P<0.05 and <0.01, respectively).

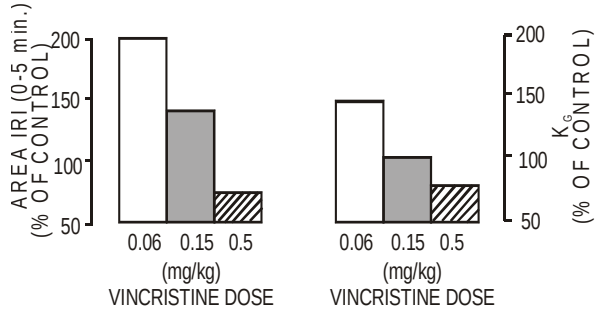

Fig. 2: Dose-effect relationship of vincristine on glucose-induced insulin secretion and glucose disappearance rate (KG). Increasing dose of vincristine significantly (P<0.05) inhibited glucose-induced insulin secretion and impaired glucose tolerance (KG).

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Mechanisms of Vincristine's Effect on Glucose-Induced Insulin Secretion

The process of stimulus-induced insulin secretion from the pancreatic beta-cell is highly complex and involves multiple mechanisms (Figure 3). For example, after recognition of glucose as a stimulus by the beta-cell, glucose oxidation and glucose metabolites, activation of adenylatecyclase cyclic AMP system, extracellular calcium ion uptake, and mobilization of intracellular calcium ion have all been implicated as signals that trigger insulin secretion (13-15). It is hypothesized that these signals trigger insulin secretion by activating a contractile microtubular microfilamental system involved in the migration and extrusion of insulin secretory granules (13-14, 16-18). It has been suggested that the insulin secretion induced by amino acids is mediated by mechanisms involving calcium ion and microtubular structures, but it is independent of the mechanisms involving glucose receptor and cyclic AMP system (20-22).

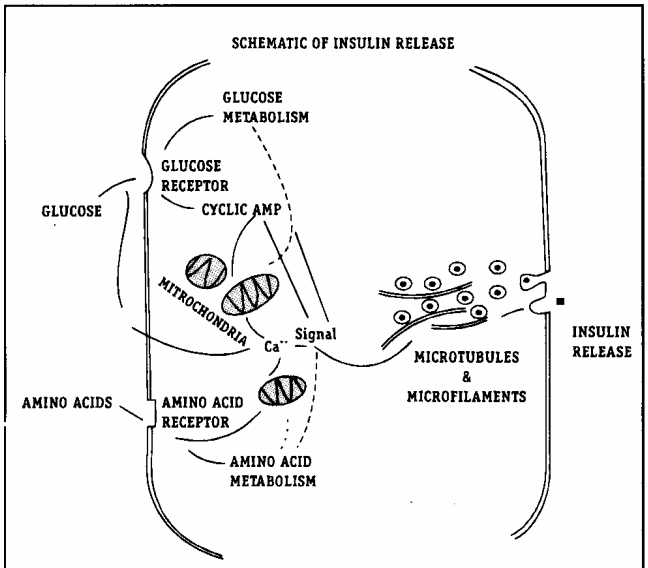

Fig 3: A simplified scheme of a complex intracellular mechanism of insulin secretion.

Since vincristine a) has a diphasic effect, potentiating and inhibiting on glucose-induced insulin secretion, b) causes inhibition of glucose-induced insulin secretion in the absence of any morphologic alteration of microtubules, and c) has no effect on the arginine-induced insulin secretion in the intact rat, it is likely that vincristine may have an effect on the insulin secretion via mechanisms other than alteration of microtubules (11, 12). It is therefore possible that, in the intact rat, vincristine may alter glucose receptors on the beta-cell and thereby cause time-related diphasic effect on the glucose-induced insulin secretion. Arginine-induced insulin secretion may not be mediated by glucose receptor mechanisms and, therefore, is unaffected by vincristine (21, 23-24). It is also possible that vincristine may alter glucose uptake and glucose metabolism on the beta-cell, thereby inhibiting glucose-induced insulin secretion.

Microtubules are Not Involved in Arginine-Induced Insulin Secretion

In the next series of experiments, we demonstrated that high dose of vincristine (0.5 mg per kg) causes a marked decrease in the microtubular content of the pancreatic beta-cell, and significantly impairs the glucose-induced insulin secretion and glucose tolerance in the intact rat (25). This inhibitory effect of higher doses of vincristine on glucose-induced insulin secretion is comparable to that observed with the lower doses (0.06 and 0.15 mg per kg) of vincristine, which caused no alteration of microtubules. Therefore, in the intact rat, vincristine causes inhibition of glucose-induced insulin secretion with or without morphological alteration of the beta-cell microtubules. In contrast, despite marked alteration of the beta-cell microtubular structures, arginine-induced insulin secretion was not altered by the higher doses of vincristine treatment (25). Furthermore, it has been shown that, in-vitro; a significant destruction of microtubular structures and paracrystalline deposits observed at 25 minutes after the vincristine treatment was associated with potentiation of glucose-induced insulin secretion (26). This observation strongly suggests that microtubules may not play a crucial role in the stimulus-induced insulin secretion.

Several studies have demonstrated that the effect of microtubule disrupting agents, colchicine and vinblastine, may actually be mediated by mechanisms other than microtubular disruption (27-30). The dissociation of the effect of vincristine on the beta-cell microtubules and on the stimulated insulin secretion observed in our studies also suggests that inhibitory effect of vincristine on glucose-induced insulin secretion may be mediated by mechanisms other than disruption of microtubules (11-12, 25). It has been postulated that arginine induces insulin secretion by mechanisms independent of the glucose receptor (21) and cyclic AMP system of the beta-cell (20). Therefore, it is possible that vincristine may alter these parameters and thereby cause inhibition of glucose-induced insulin secretion, but not of the arginine-induced insulin secretion.

Glucose, but not Arginine, Prevents Inhibitory Effect of Vincristine on Glucose-Induced Insulin Secretion

In the next series of studies, we demonstrated that the exposure to the high levels of glucose at the time of vincristine treatment prevented the inhibitory effect of vincristine on the subsequent glucose-induced insulin secretion (31). On the other hand, arginine exposure under similar circumstances did not protect the beta-cell from the inhibitory effect of vincristine. These findings, along with the findings from our other studies, supports the concept that arginine-induced insulin secretion is mediated by mechanisms other than those that mediate glucose-induced insulin secretion, and also suggests that in vivo effect of vincristine on glucose-induced insulin secretion is mediated by alteration of the beta-cell glucose receptors rather than microtubular structures. The protective effect of glucose on the inhibitory effect of vincristine on insulin secretion observed in our study (31) is similar to the protective effect of glucose against alloxan poisoning of the beta-cell (32-33).

Several studies have shown that microtubular disrupting agents may mediate their action by affecting cellular systems and the structures other than microtubules (34-37). For example, colchicine has been shown to affect insulin receptors on the hepatocytes and concanavalin-A receptors on white cells (36). Therefore it is possible that vincristine exerts its inhibitory effect on glucose-induced insulin secretion by altering or interfering with the glucose receptors on the pancreatic beta-cell. Since arginine appears to stimulate insulin secretion via mechanisms that do not involve the glucose receptors, vincristine did not have any effect on the arginine-induced insulin secretion. These results do not exclude the possibility that the effects of vincristine may be mediated by mechanisms that interfere with the generation of cyclic AMP or glucose metabolism, both of which are essential in the process of glucose-induced insulin secretion.

Vincristine's Effect on Glucose-Induced Insulin Secretion is Mediated by Glucose Receptors Rather than Microtubular Mechanisms

In the next series of studies, we evaluated whether 3-0-methyl glucose can prevent inhibitory effect of vincristine on glucose-induced insulin secretion despite destruction of microtubular structures in isolated islets of pancreas. As shown in Figure 4, groups of islets each were incubated at various concentrations of glucose in the presence or absence of 3-0-methyl glucose. The glucose-induced insulin secretion was not affected by 3-0-methyl glucose at various glucose concentrations. The groups of isolated islets were then incubated with or without vincristine at various concentrations of glucose (Figure 5). The glucose-induced insulin secretion was markedly inhibited by vincristine at the glucose concentration of 16.7 and 25.0 mmol/l.

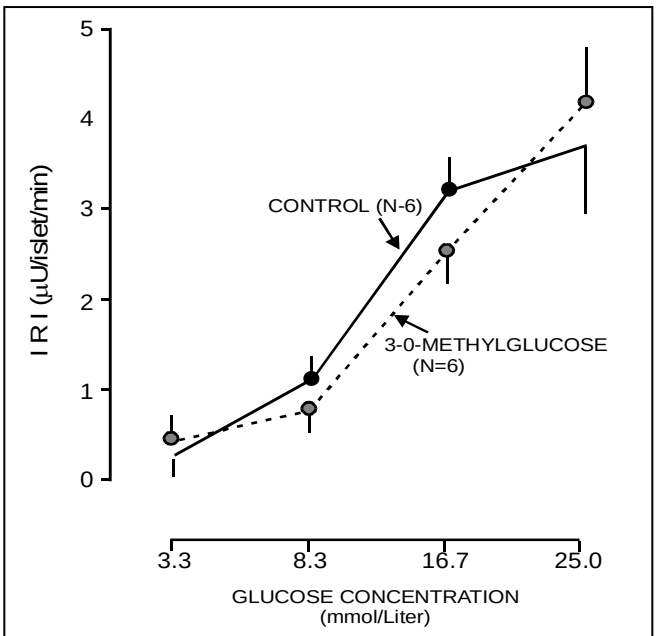

Fig 4: 3-0-methyl glucose has no effect on glucose-induced insulin secretion in isolated islets of pancreas.

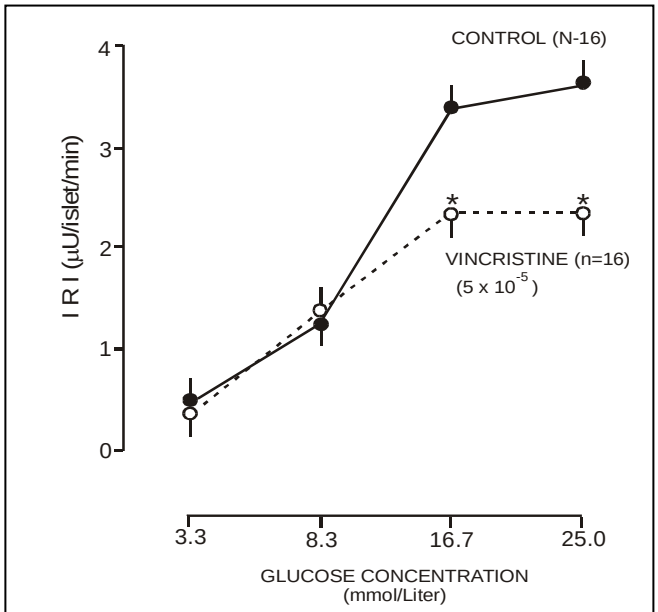

Fig 5: The glucose-induced insulin secretion is significantly inhibited by vincristine in isolated islets of pancreas.

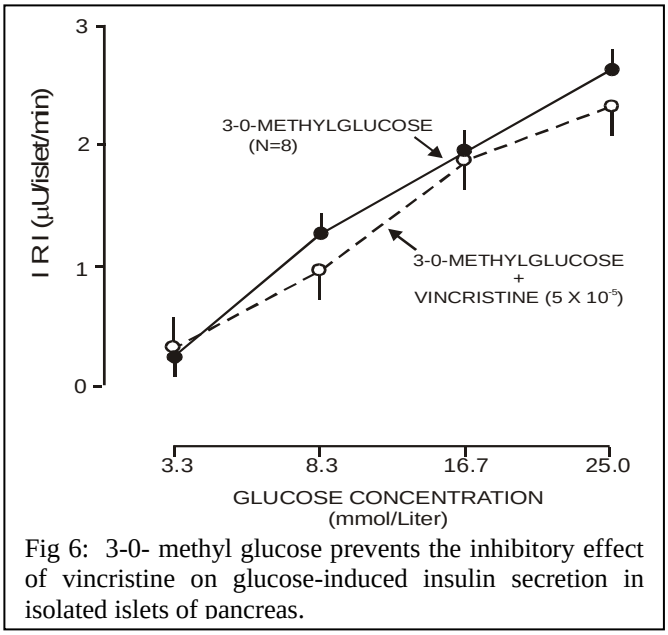

Fig 6: 3-0- methyl glucose prevents the inhibitory effect of vincristine on glucose-induced insulin secretion in isolated islets of pancreas.

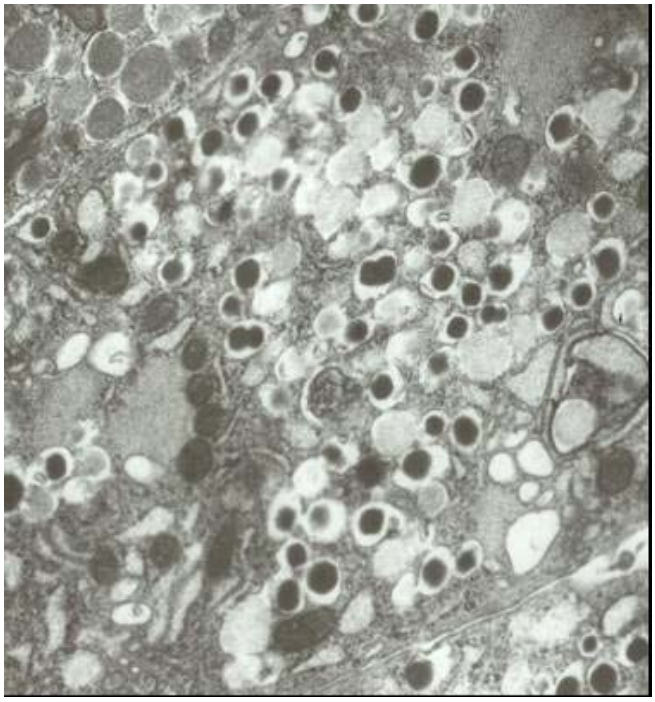

Fig 7: Electron micrograph of a beta cell of pancreas showing absence of microtubules and several paracrystalline deposits of vincristine.

In the next experiment, groups of isolated islets were incubated at various concentrations of glucose and with or without vincristine but in the presence of 3-0-methyl glucose. As shown in Figure 6, 3-0-methyl glucose completely prevented the inhibitory effect of vincristine on glucose-induced insulin secretion. At this concentration of vincristine, a marked disruption of microtubules and paracrystalline deposits were observed (Figure 7).

In summary, in the intact rat, vincristine appears to have a diphasic effect (potentiating and inhibitory) on glucose-induced insulin secretion. Arginine-induced insulin secretion is not inhibited by vincristine, despite its effect on microtubular disruption. Glucose, but not arginine, prevents vincristine's inhibitory effect on glucose-induced insulin secretion in the presence of microtubular disruption. Finally, 3-0-methyl glucose, in isolated pancreatic islets, prevents inhibitory effect of vincristine on glucose-induced insulin secretion. At this concentration, vincristine caused a marked destruction of microtubular structures with paracrystalline deposits in the beta cells of isolated pancreas. Therefore, it appears that microtubular structures of the beta cells of pancreas do not play an essential role in stimulated insulin secretion. Vincristine, a known microtubular disrupting agent may exert its effect on glucose-induced insulin secretion in vivo and in vitro via glucose receptor mechanism. Vincristine may be used as a tool to study the characteristics of glucose receptors and mechanisms that induce insulin secretion from pancreatic beta cells.

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