THE STIMULUS-SECRETION COUPLING OF GLUCOSE-INDUCED INSULIN RELEASE

IV. The Effect of Vincristine and Deuterium Oxide on the Microtubular System of the Pancreatic Beta Cell

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INTRODUCTION

Microtubules have been recognized in the cytoplasm of rat and rabbit pancreatic beta cells (1–3). According to studies performed in other animal systems, the cytoplasmic microtubules are formed of an actin-like material (4), and are thought to play an important role in controlled cellular movements (5–7). In 1968, Lacy and his colleagues (1) suggested that contraction of the microtubules might lead to insulin secretion by controlling the migration of secretory granules during the process of emiocytosis. Indeed, insulin secretion presents a number of analogies with muscular contraction. Both processes are thought to be energy dependent (8), associated with depolarization of the cell membrane (9), and triggered by an influx of calcium (10).

In the present series of experiments, we have approached the problem of the possible participation of microtubules in the process of insulin secretion by examining the effects of a mitotic spindle-inhibitor (vincristine) and a microtubule-stabilizer (deuterium oxide) upon both the function and the ultrastructure of the beta cell.

MATERIALS AND METHODS

Pieces of pancreas (about 10 mg each) removed from fed male albino rats were transferred to a bicarbonate-buffered medium (8) containing albumin (0.5%, w/v, Bovine Plasma Albumin, Armour Pharmaceutical Co., Kankakee, Ill.), glucose (3 mg/ml dextrose, National Bureau of Standards, Washington, D. C.), guinea pig antiinsulin serum (prepared and kindly donated by Dr. P. H. Wright, Indiana University, Indianapolis, Ind.), and, as required, deuterium oxide (50%, v/v; Mallinckrodt Chemical Works, St. Louis, Mo.), or vincristine sulfate (10^{-5} M; Oncovin, Eli Lilly and Co. Indianapolis, Ind.). The media were equilibrated against a mixture of O_2 (95%) and CO_2 (5%), and incubated at 36°C for 90 or 180 min. The rate of insulin secretion was deduced from the partial neutralization of the antiinsulin serum, according to a method previously described. After incubation, the pieces of pancreas were fixed in 2.5% glutaraldehyde in 0.15 M phosphate buffer (pH 7.4) for 1 hr, rinsed with the buffer solution, and postfixed for 1 hr in 1% OsO_4 in the same buffer. After dehydration in ethyl alcohol, the pieces of pancreatic tissue were embedded in an epoxy resin. All procedures were done at room temperature, except for the polymerization of the plastic which was carried out at 60°C. Ultrathin sections were stained with uranyl acetate followed by lead citrate.

RESULTS

Table I indicates that deuterium oxide caused a marked and immediate inhibition of glucose-induced insulin release. Vincristine also reduced insulin release evoked by glucose in the pancreatic tissue. The inhibitory effect of vincristine was slight (about 15% reduction) during the first period of incubation of 90 min, and much more marked (about 48% reduction) during the second incubation (90th–180th min).

Figs. 1 and 2 demonstrate the presence of microtubules in an islet of Langerhans, incubated for 90 min in the presence of glucose. The microtubules are not restricted to a particular area of the cell, but are scattered throughout the cytoplasm. Similar structures are also present in beta cells of pancreatic tissue incubated in the presence of glucose for 180 min.

After incubation of pieces of pancreatic tissue for 90 min in the presence of deuterium oxide, the general appearance of the beta cells and their organelles was unaltered (Fig. 3). In particular, the mitochondria and their cristae were intact. There was no swelling of the ergastoplasmic reticulum. After treatment with D_2O, however, the beta cells contained more microtubules than the control cells which were never exposed to D_2O.

When the pieces of pancreas were exposed to vincristine for 90 min, the beta cells appeared normal in all respects; microtubules could still be

TABLE I

Effect of Vincristine and Deuterium Oxide upon Insulin Release Induced by Glucose

Period of incubation	Insulin output		
	Control	Vincristine (10^{-5} M)	D_2O (50%, v/v)
min		*μU/mg per 90 min*	
0–90	91.4 ± 4.2 (33)	78.0 ± 5.3 (33) $P < 0.05$	49.7 ± 7.3 (20) $P < 0.001$
90–180	64.3 ± 6.4 (33)	33.3 ± 2.5 (33) $P < 0.001$	

Effect of vincristine and deuterium oxide upon insulin release induced by glucose (3.0 mg/ml) in pieces of rat pancreatic tissue incubated for two successive periods of 90 min each. Mean values (± SE) are shown together with the number of observations (in parentheses), and the statistical significance of changes from corresponding control values.

visualized in their cytoplasms (Fig. 5). By contrast, after 180 min incubation in the presence of vincristine, no microtubules could be seen and the beta cells contained numerous and large deposits of crystalline-like material scattered throughout the cytoplasm (Fig. 6). Figs. 7 and 8 illustrate the microcrystalline appearance of this material in both longitudinal and cross-sections examined at higher magnification. The periodicity of the crystalline arrangement was about 100 A. After the same prolonged exposure to vincristine, microcrystals were recorded in both the acinar cells (Figs. 9 and 10) and ductular epithelial cells (Fig. 11), but they were much more scarce than in the beta cells. In no case was there evidence suggesting alterations of other organelles.

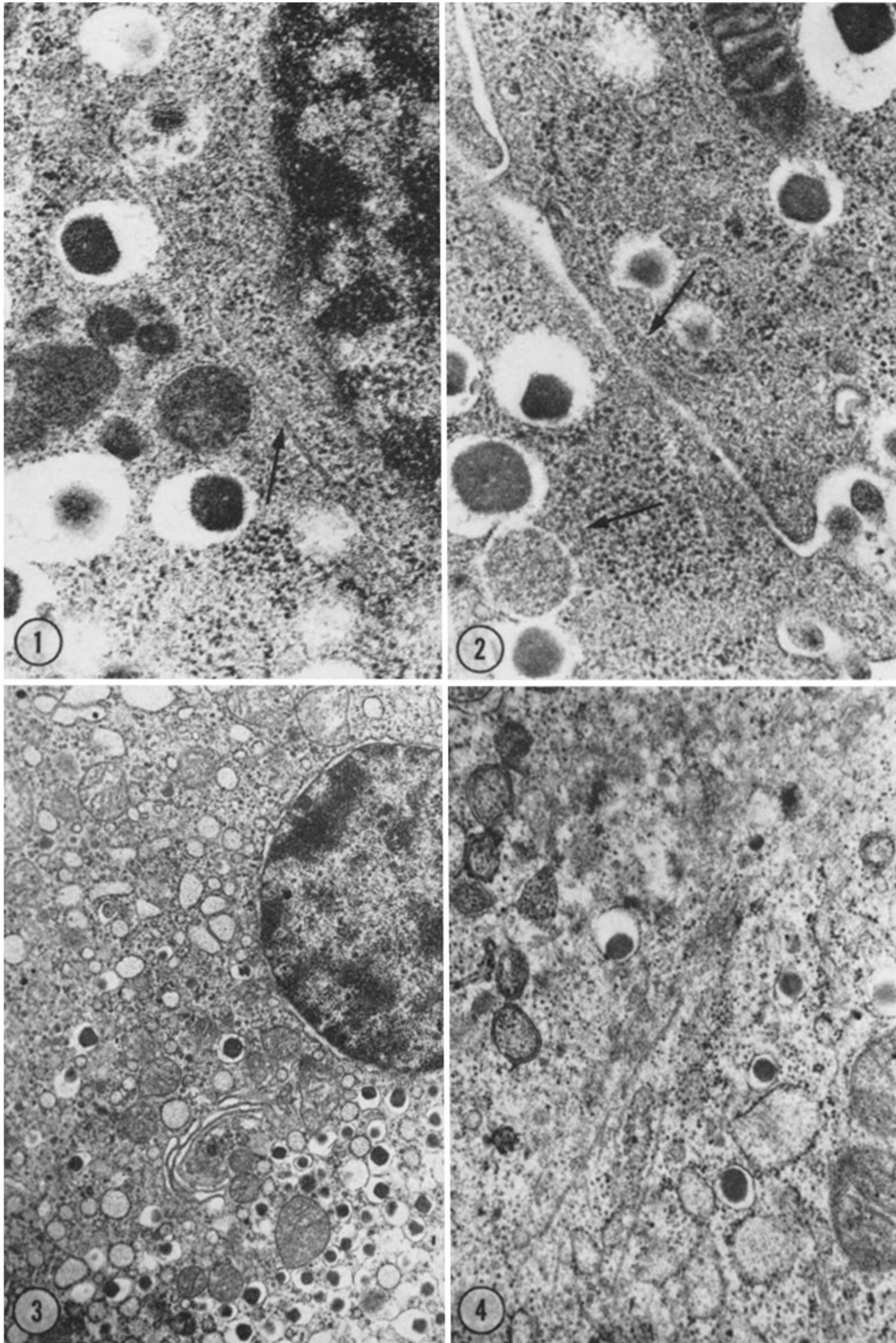
COMMENTS

The present study confirms the presence of a microtubular system in normal pancreatic beta cells (1–3). A possible role for this system in insulin secretion is suggested by the fact that both microtubule-stabilizers and mitotic spindle-inhibitors inhibit glucose-induced insulin secretion.

Deuterium oxide (50%, v/v) provoked a partial inhibition of insulin release, total inhibition being observed at a higher concentration of D_2O (100%, v/v, unpublished observations). The inhibitory effect of D_2O was already evident after 90 min of incubation (Table I). Figs. 3 and 4 indicate that, after incubation for 90 min in the presence of D_2O, no obvious damage to the beta cell organelles could be detected. The presence of a large number of microtubules in these D_2O-treated beta cells is compatible with the concept that heavy water stabilizes the microtubular structure and, by doing so, inhibits cellular movements (11–14).

Under the present experimental conditions, vincristine caused a time-related alteration of the microtubules. After 90 min of exposure to this agent, the microtubules could still be disclosed. At the same time, insulin secretion was barely decreased. However, from the 90th to the 180th min of incubation, a marked inhibition of insulin secretion occurred. Massive microcrystalline deposits were found at the end of the third hour. In other systems, a comparable time dependency has been reported for the interaction of vincristine and the microtubular protein (15). Therefore, total inhibition of glucose-induced insulin release might require more prolonged exposure to vincristine. The ultrastructural appearance of the deposits was comparable to that previously described in other tissues (15, 16), and shown here in acinar and ductular cells. If one assumes that the crystalline deposits formed under the influence of vincristine are wholly derived from microtubular protein, the present data would indicate that the beta cells contain more microtubular protein than suggested by the moderate number of microtubules observed in untreated beta cells.

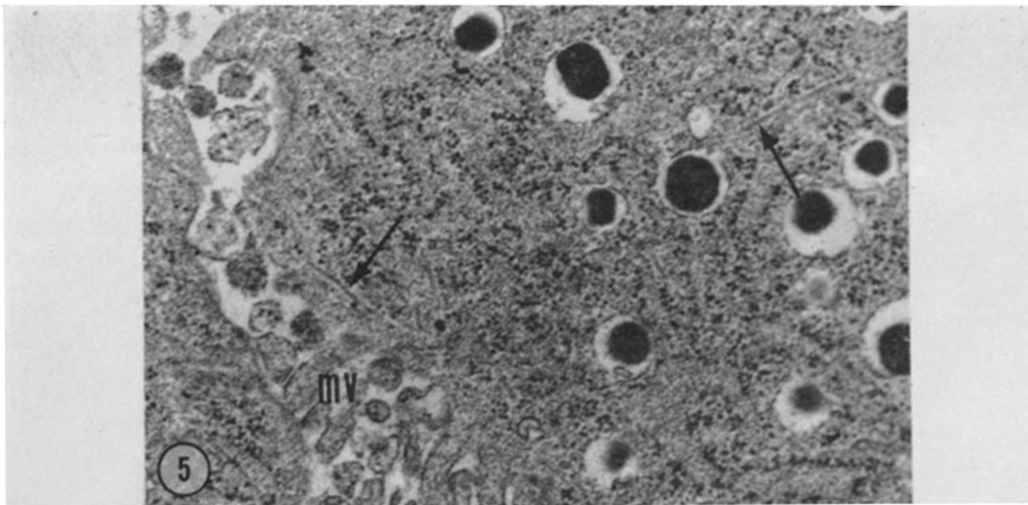
Both the deposition of microtubular protein and the inhibition of insulin secretion by vincristine required prolonged exposure to this agent. Such a synchronous pattern is compatible with the suggestion that vincristine inhibits insulin secretion by virtue of its specific action upon the microtubular system. Therefore, the present data support the concept that the microtubular system of the beta



FIGURES 1 and 2 Portions of beta cells of an isolated islet incubated for 90 min in the presence of glucose. Microtubules (arrows) are shown next to the cell membrane as well as in the vicinity of the nucleus. × 50,000.

FIGURE 3 Portion of a beta cell. Pancreatic tissue incubated for 90 min with deuterium oxide. Note the integrity of the Golgi apparatus, of the mitochondria, and of the beta granules. × 8000.

FIGURE 4 Portion of a beta cell. Pancreatic tissue incubated for 90 min with deuterium oxide. Arrays of microtubules and microfilaments are dispersed between the beta granules and the mitochondria. × 26,000.



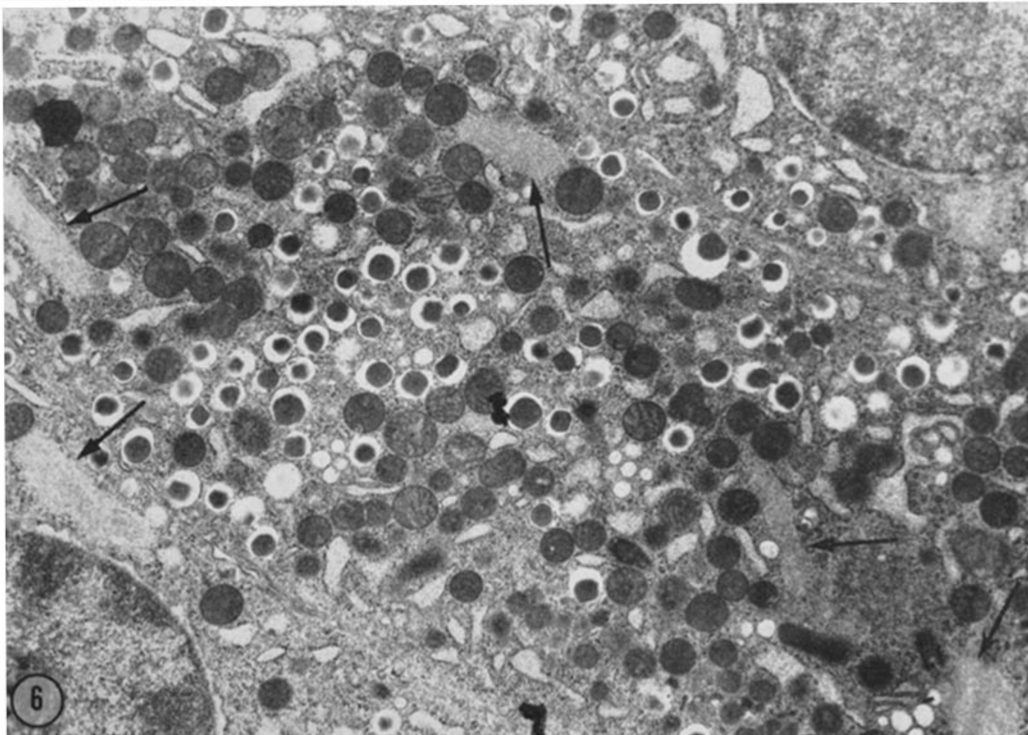


FIGURE 5 Portion of a beta cell. Pancreatic tissue incubated for 90 min in the presence of vincristine. Note the integrity of the microtubular structures (arrows). The plasma membrane of the beta cell shows several microvilli (*mv*). × 26,000.

FIGURE 6 Portions of three adjacent beta cells. Pancreatic tissue incubated for 180 min in the presence of vincristine. Clusters of crystalline material (arrows) are scattered throughout the cytoplasm. The other organelles (granules, mitochondria, and nuclei) have a normal appearance. × 10,000.

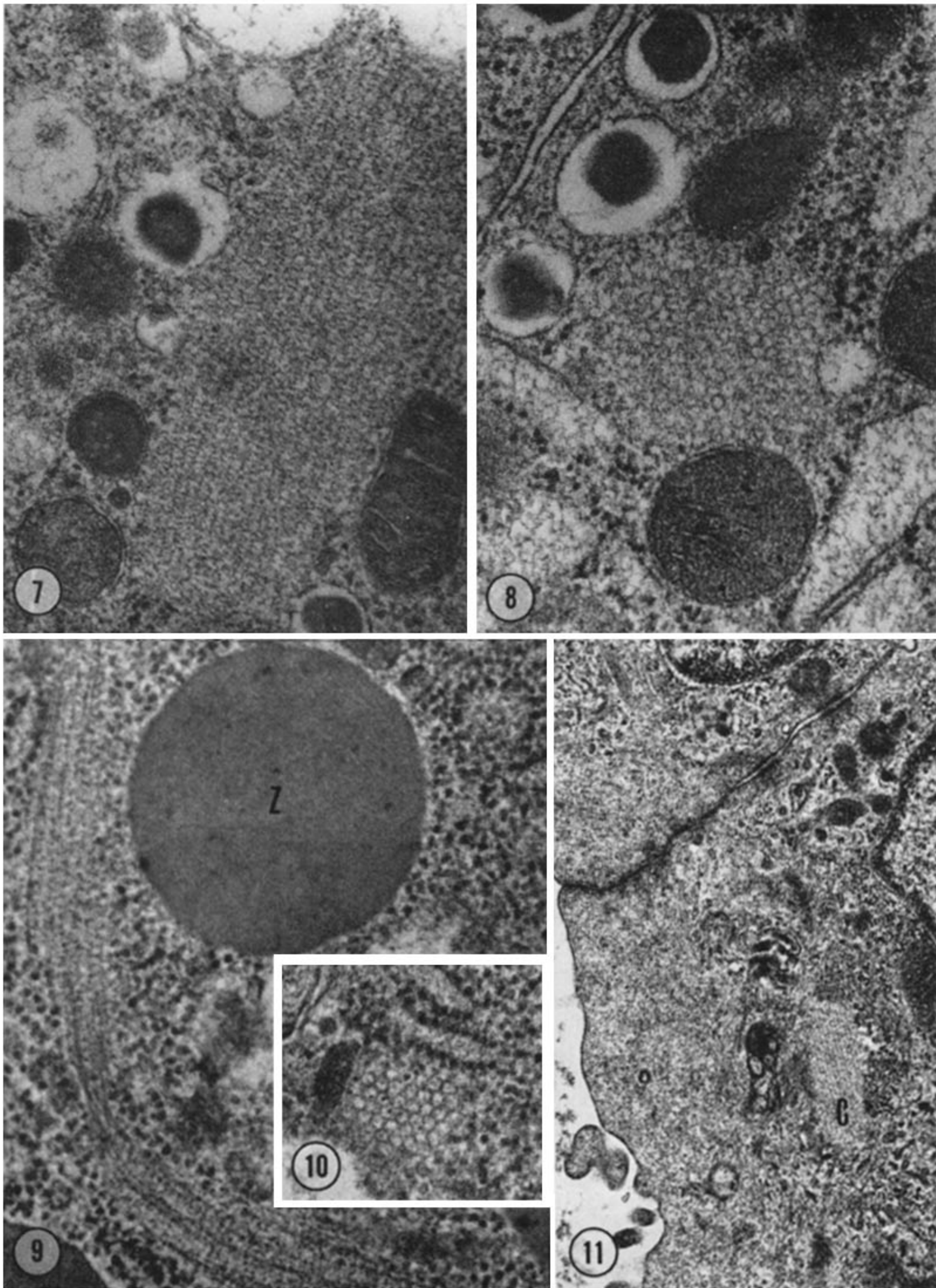


FIGURE 7 Pancreatic tissue incubated for 180 min with vincristine. Longitudinal section of a crystalline deposit in a beta cell. × 50,000.

FIGURE 8 Cross-section of a crystalline deposit of the type demonstrated in Figs. 6 and 7. × 50,000.

FIGURES 9 and 10 Pancreatic tissue incubated for 180 min with vincristine. Longitudinal and cross-sections through crystalline deposits in an acinar cell. *Z*, zymogen granule. × 50,000.

FIGURE 11 Pancreatic tissue incubated for 180 min in the presence of vincristine. Portions of two adjacent ductular epithelial cells. *C*, crystalline deposit. × 15,000.

cell plays an integral role in the sequence of events leading to insulin release in response to glucose.

SUMMARY

Microtubules have been previously recognized in pancreatic beta cells (1–3). A clue to the role played by the microtubules in the process of insulin secretion was sought in the present study. Rat pancreatic tissue was exposed, in vitro, to heavy water (a microtubule-stabilizer) and to vincristine (a mitotic spindle-inhibitor). Both agents failed to affect the general ultrastructure of the beta cells. Numerous microtubules were found in the beta cells after exposure to heavy water. Prolonged incubation in the presence of vincristine provoked the disappearance of microtubules and the massive precipitation of microcrystalline material, presumably derived from the microtubular protein. Less abundant crystalline deposits were also disclosed in acinar and ductular epithelial cells.

Both deuterium oxide and vincristine inhibited glucose-induced insulin secretion, and their effect on the beta cell ultrastructure seemed to be limited to the microtubular system. These data afford additional support to the concept that the microtubular system of the beta cells plays an integral role in the process of insulin secretion.

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