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Evidence That the Beta Cell Microtubules Are Not Involved in Arginine-Induced Insulin Release (41403)

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Abstract. The role of the beta cell microtubules in stimulus-induced insulin release was evaluated in the intact rat by utilizing vincristine, a known microtubular disrupting agent. In the first study, pancreases were removed at 60 min after vincristine (0.5 mg/kg) or vehicle (control) treatment for electron microscopy. Morphometric analysis revealed that the number of microtubules in the vincristine-treated rat pancreases was significantly less than that observed in the control rat pancreases. In the next series of studies, insulin release in response to a glucose (150 mg, iv) or an arginine (100 mg/kg, iv) pulse was examined at 60 min after vincristine (0.5 mg/kg) or vehicle treatment. In response to glucose, insulin release at 2, 3, and 5 min was significantly less, and glucose tolerance was significantly impaired in the vincristine-treated rats as compared with those observed in the control rats. In contrast, insulin release in response to arginine was similar in the vincristine-treated and the control rats. Therefore, a marked morphological alteration of the beta cell microtubules has failed to inhibit arginine-induced insulin release in the intact rat. These findings suggest that the integrity of microtubules may not be critical in the process of arginine-induced insulin release.

The essential role of microtubules in the process of stimulated insulin release has been hypothesized from the observations that microtubular disrupting agents such as colchicine, vincristine, and vinblastine can cause inhibition of glucose-induced insulin release *in vitro* (1–5). We have also previously shown that in the intact rat, therapeutic doses of vincristine and colchicine cause inhibition of glucose-induced insulin release and glucose tolerance (6, 7). However, our recent study (8) demonstrated that in the intact rat: (i) this inhibition of glucose-induced insulin release caused by low-dose (0.15 mg/kg) vincristine treatment, occurred in the absence of any morphological alteration of the pancreatic beta cell microtubular structures, and (ii) although vincristine in low doses inhibited glucose-induced insulin release, it had no effect on arginine-induced insulin release (8). These findings suggested that in the intact rat, the inhibition of glucose-induced insulin release may be mediated by mechanisms other than microtubular disruption. In order to further examine the critical role played by microtubules in the stimulus-mediated insulin release, the present study was designed to evaluate the *in vivo* effect of a higher dose of vincristine (0.5 mg/kg) on (a) the pancreatic beta cell microtubular structures, and (b) glucose- and arginine-induced insulin release.

Materials and Methods. *Preparation of rats.* Under pentobarbital anesthesia, a polyethylene catheter was implanted in the jugular vein and exteriorized on the dorsum of the neck of male Sprague–Dawley rats. The animals recuperated and regained weight, and were in a normal anabolic state by the fifth postoperative day. The infusion studies were performed after this recuperative period, when specially prepared extension catheters were connected to the indwelling catheters through which vincristine and glucose or arginine were infused and serial blood samples were collected. During the infusion procedure the animals remained unanesthetized, undisturbed, and unrestrained. The details of this technique have been previously reported (9). All the studies were performed in these 300- to 350-g rats after an overnight fast.

The effect of vincristine on the beta cell

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microtubular structures. Vincristine (0.5 mg/kg) or vehicle (0.9% saline) was administered via jugular vein catheter in the overnight fasting rat. Sixty minutes later, the rat was anesthetized with pentobarbital and the bile duct was cannulated with a polyethylene catheter. The pancreas was identified and isolated after injection of 5–7 ml of Hank's solution through the catheter. The pancreas was cut into small pieces and immediately fixed with 3% glutaraldehyde in phosphate buffer at room temperature for at least 2 hr. The pancreatic tissue was then washed and stored at 4° with 0.2 *M* sucrose in phosphate buffer for electron microscopy. Prior to electron microscopy the pancreatic tissue was further postfixed in 2% osmium tetroxide, dehydrated with acetone, and embedded in Epon. Thin sections of the pancreatic tissue were cut with a Sorvall Porter-Blum MT 2 ultramicrotome. These sections were then stained with aqueous solutions of uranyl acetate and lead citrate, and were examined with a RCA EMU 4B electron microscope. At the magnification of 11,900× and without identifying microtubules, 12–16 micrographs of the pancreatic islet were taken randomly by photographing any upper left corner of a grid square containing the beta cell cytoplasm. These photomicrographs were then enlarged to a final magnification of 36,000× and coded for morphometric analysis of microtubules.

All photomicrographs were blindly subjected to morphometric analysis, as the identity of each photomicrograph, whether it belonged to vincristine-treated or to the control rat pancreas, was not revealed to the person performing the analysis. Using the Weibel's coherent multipurpose test system, the number of microtubules was counted in a beta cell profile area, which was estimated by point counting (10). The length of each microtubule in the photomicrograph was also determined. From these data the number of microtubules per 100-μm^2 area of the beta cell profile and mean microtubular length in micrometers were calculated for each rat pancreas.

The effect of vincristine on glucose-induced insulin release and glucose tolerance. Baseline blood samples were collected before a rapid iv infusion of vincristine (0.5 mg/kg). Sixty minutes after vincristine treatment, 150 mg of glucose was rapidly infused iv, in 30 sec. Blood samples, in small quantities, were collected just before (0 time) and 2, 3, 5, 10, 15, 20, 25, and 30 min after the glucose pulse for serum glucose and immunoreactive insulin (IRI) determinations. Simultaneous control experiments were similarly performed in separate groups of rats after iv administration of vehicle (0.9% saline), instead of vincristine.

The effect of vincristine on arginine-induced insulin release. A parallel study was performed to compare the effect of vincristine (0.5 mg/kg) on arginine-induced insulin release with that of glucose-induced insulin release. In this study the experiments were performed in a manner similar to that described above at 60 min after vincristine (0.5 mg/kg) or vehicle treatment, except for substitution of arginine pulse (100 mg/kg, iv) for glucose.

Analytical methods. Serum glucose was measured immediately on glucose analyzer (glucose oxidase method) and the remaining serum was frozen at −20° for future determination of IRI by a micromodification of a radioimmunoassay technique (11) using rat insulin standards. The glucose disappearance rate (K_G) was calculated by the method of least squares (12) using natural logarithms of the actual glucose values. The resulting values multiplied by 100 expresses the rate of fall of serum glucose in percentage per minute. The details of this method have been described previously (6, 7). The results are expressed as mean ± standard error of observed values. The statistical analysis was done by applying Student's *t* test (12) to group differences between the test and the control animals.

Results. *The effect of vincristine on the beta cell microtubular structures*. As shown in Table I, the mean microtubular number of 13.5 ± 1.3/100-μm^2 area of the beta cell profile in the vincristine-treated rat pancreases was significantly less ($P < 0.02$) than that of 28.9 ± 4.4/100 μm^2 observed in the control rat pancreases. This decrease in the number of microtubules in the vin-

TABLE I. THE MORPHOMETRIC ANALYSIS OF THE PANCREATIC BETA CELL MICROTUBULES FROM THE VINCRISTINE- AND VEHICLE (CONTROL)-TREATED RATS

	Vehicle (60 min)[a]	Vincristine 0.5 mg/kg (60 min)[a]
No. of microtubules per 100 μm^2	28.9 ± 4.5[b]	13.5 ± 1.3*
Length of microtubules (μm)	0.318 ± 0.004	0.326 ± 0.05
Number of photomicrographs analyzed	70	60
Number of rats	5	4

[a] Time of removal of pancreata after vincristine or vehicle treatment.
[b] Results are expressed as mean ± standard error.
* $P < 0.02$.

crinstine-treated rat pancreas was not associated with paracrystalline deposits in the beta cell cytoplasm. The mean length of microtubules in the vincristine-treated rat pancreases was not significantly different from that observed in the control rat pancreases (Table I).

The effect of vincristine on glucose-induced insulin release and glucose tolerance. Mean fasting serum glucose and IRI concentrations were similar and remained unchanged at 60 min after either vincristine or vehicle administration. Therefore, vincristine per se had no effect on basal serum glucose or IRI concentrations. However, as shown in Fig. 1, mean serum glucose levels at 15, 20, 25, and 30 min after glucose pulse were significantly higher ($P < 0.02$) in the vincristine-treated rats than those observed at the same time intervals in the control rats. Similarly, mean K_G of 2.72 ± 0.1%/min in the vincristine-treated rats was significantly less ($P < 0.03$) than that of 3.64 ± 0.3%/min observed in the control rats. Mean IRI levels at 2, 3, and 5 min after glucose pulse were also significantly less ($P < 0.05$) in the vincristine-treated rats than those observed at the similar time intervals in the control rats (Fig. 1).

The effect of vincristine on arginine-induced insulin release. As shown in Fig. 2, mean fasting serum glucose and IRI levels were similar in both groups of rats before and at 60 min after vincristine or vehicle treatment. In response to arginine pulse, mean serum glucose and IRI concentrations at all time intervals were also similar in the vincristine-treated and the control rats (Fig. 2).

Discussion. The present study demonstrates that the high dose (0.5 mg/kg) vincristine treatment causes (i) a marked decrease in the microtubular content of the pancreatic beta cell, and (ii) a significant impairment of glucose-induced insulin release and glucose tolerance in the intact rat. This inhibitory effect of the higher dose of vincristine on glucose-induced insulin release is comparable to that observed with the lower doses (0.06 and 0.15 mg/kg) of vincristine, which caused no alteration of microtubules in our previous studies (7, 8). Therefore, in the intact rat, vincristine causes inhibition of glucose-induced insulin release with or without morphological alteration of the beta microtubules (8). In contrast, the findings from the present study show that despite marked alteration of the beta cell microtubular structures, arginine-induced insulin release was not altered by the higher dose of vincristine treatment. Furthermore, it has been shown that, *in vitro,* a significant disruption of microtubular structures and paracrystalline deposits observed at 25 min after vincristine treatment was associated with a poten-

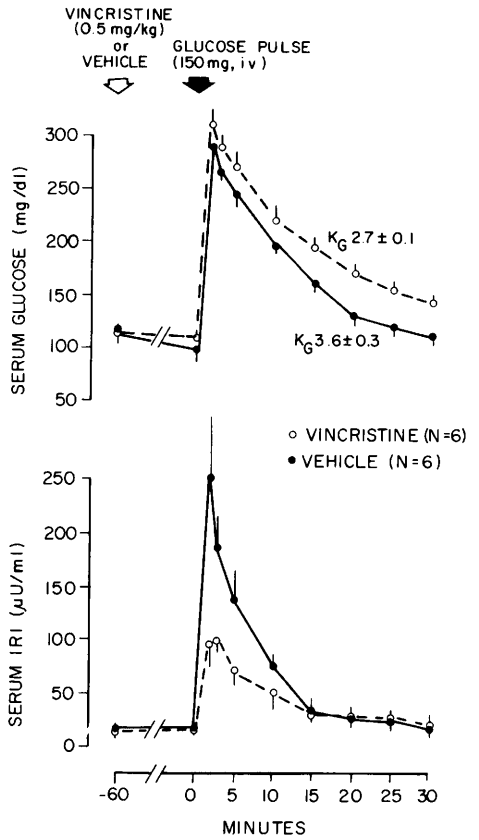

FIG. 1. Glucose-induced IRI release and K_G at 60 min after vincristine (0.5 mg/kg) or vehicle (control) treatment. The mean serum glucose levels between 15 and 30 min were significantly greater ($P < 0.02$) and mean K_G was significantly lower ($P < 0.03$) in the vincristine-treated rats than those observed in the control rats. In response to a glucose pulse, mean serum IRI levels at 2, 3, and 5 min were significantly less ($P < 0.05$) in the vincristine-treated rats than those observed in the control rats.

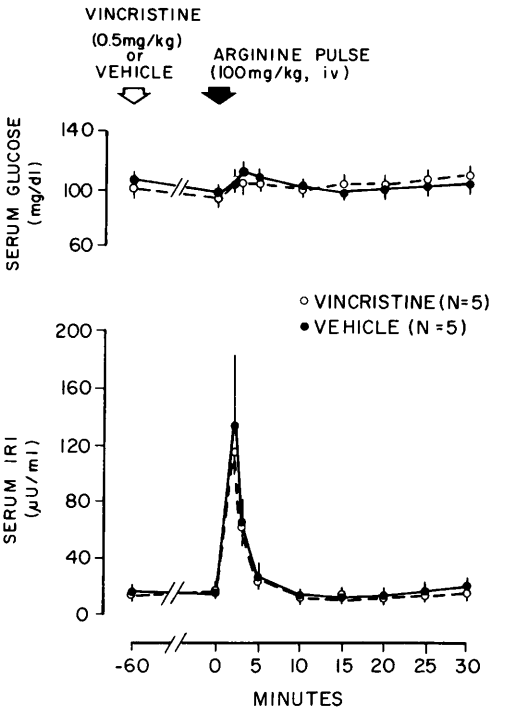

FIG. 2. Arginine-induced IRI and glucose responses at 60 min after vincristine (0.5 mg/kg) or vehicle (control) treatment. Mean serum IRI and glucose levels were similar between the vincristine-treated and the control rats throughout the observation period.

tiation of glucose-induced insulin release (4). These observations strongly suggest that microtubules may not play a crucial role in stimulus-induced insulin release.

Recently, several studies have demonstrated that the effects of microtubular disrupting agents (colchicine and vinblastine) may actually be mediated by mechanisms other than microtubular disruption (13–16). The dissociation of the effects of vincristine on the beta cell microtubules and on the stimulated insulin release observed in our present and previous studies (8) also suggests that the inhibitory effect of vincristine on glucose-induced insulin release may be mediated by mechanisms other than disruption of microtubules. It has been postulated that arginine induces insulin release by a mechanism independent of the glucose receptor (17) and the cyclic AMP system (18) of the beta cell. Therefore it is possible that vincristine may alter these parameters and thereby cause inhibition of glucose-induced insulin release, but not of arginine-induced insulin release. Further studies are needed to define the mechanism involved in the inhibitory effect of vincristine on glucose-induced insulin release.

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