Glucose but not Arginine Prevents the Inhibitory Effect of Vincristine on Glucose-induced Insulin Release

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SUMMARY

We have previously shown that in the intact rat: (1) the inhibition of glucose-induced insulin release caused by vincristine occurred in the presence or absence of morphologic disruption of the beta-cell microtubules; and (2) vincristine, however, failed to inhibit arginine-induced insulin release, even in the presence of a marked disruption of the beta-cell microtubules. The present study further evaluated the mechanism of inhibition of vincristine on glucose-induced insulin release in the intact rat. In the first series of studies, glucose (500 mg/kg) was infused over 1 min into fasting rats with indwelling vascular catheters. Five minutes later, vincristine (0.15 mg/kg i.v.) or vehicle (control) was injected. Sixty minutes after vincristine or vehicle treatment, insulin release in response to a 150-mg i.v. glucose pulse was examined. Serum insulin and glucose levels were similar at all time intervals in the vincristine-treated and the control rats. In the next series of studies, the experiments were repeated as above, except arginine (100 mg/kg), instead of glucose, was infused over 1 min before vincristine or vehicle treatment. In these studies, serum insulin in response to a glucose pulse was significantly inhibited in the vincristine-treated rats as compared with control rats. Therefore, in the intact rat, prior exposure to glucose but not arginine protected the beta-cell from the inhibitory effect of vincristine on glucose-induced insulin release. These findings, along with our previous observations, support the concept that arginine-induced insulin release is mediated via mechanisms other than those involved in glucose-induced insulin release and suggest that the in vivo effect of vincristine on glucose-induced insulin release is mediated via alteration of the beta-cell glucose receptors rather than microtubular structures. DIABETES *31*:834–837, September 1982.

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Intracellular microtubules have been considered to be essential components in the process of stimulus-induced insulin release.[1,2] This hypothesis has been strengthened by the observations that the microtubular disrupting agents vincristine, vinblastine, and colchicine cause inhibition of insulin release in vitro.[3–5] We have also shown that vincristine and colchicine inhibit glucose-induced insulin release in vivo.[6,7] However, our recent studies[8,9] have shown that in the intact rat: (1) vincristine caused inhibition of glucose-induced insulin release in the presence or absence of morphologic disruption of the beta-cell microtubules; but, in contrast, (2) vincristine failed to inhibit arginine-induced insulin release either in the presence or absence of the beta-cell microtubular disruption. These observations suggested that in vivo vincristine may cause inhibition of glucose-induced insulin release by a mechanism other than microtubular disruption.

It has been suggested that arginine stimulates insulin release by a mechanism that is independent of glucose receptors or the cyclic AMP system in the beta-cells.[10,11] Therefore, if the glucose receptor mechanism is involved in the in vivo effects of vincristine, this may explain why vincristine inhibited glucose-induced but not arginine-induced insulin release in the intact rat in our studies. The present study was designed to evaluate whether exposure to high levels of glucose or arginine at the time of vincristine treatment would prevent the inhibitory effect of vincristine on glucose-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, weighing 300–350 g. By the fifth postoperative day, the animals recuperated and regained weight and were in a normal anabolic state. The infusion studies were performed after this recuperative period, when specially

prepared extensive 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. All studies were performed on these rats after an overnight fast. Specific details of this technique have been reported previously.[12]

The effect of glucose exposure during vincristine treatment on subsequent glucose-induced insulin release. Baseline blood samples for serum glucose and immunoreactive insulin (IRI) were collected before an infusion of 500 mg/kg of glucose, given over 1 min. After 5 min, vincristine (0.15 mg/kg) was rapidly infused. Sixty minutes after vincristine treatment, a bolus of 150 mg of glucose was infused over 30 s. Blood samples in small quantities were collected just before (0 min) and 2, 3, 5, 10, 15, 20, 25, and 30 min after the 150-mg glucose pulse for measurement of serum glucose and IRI. Simultaneous control experiments were similarly performed in separate groups of rats, substituting vehicle (0.9% saline) for vincristine treatment.

The effect of arginine exposure during vincristine treatment on subsequent glucose-induced insulin release. Similar studies, as above, were performed to compare the effect of arginine exposure versus glucose during vincristine treatment on subsequent glucose-induced insulin release. In this series of studies, the experiments were repeated as detailed above, except arginine (100 mg/kg), instead of glucose, was infused over 1 min before the vincristine or vehicle treatment.

Analytic methods. Serum glucose was measured immediately on a glucose analyzer (glucose-oxidase method) and the remaining serum was frozen at −20°C for future determination of IRI by a micromodification of radioimmunoassay technique,[13] using rat insulin standards.

The areas above baseline under IRI time curves were calculated by modification of the trapezoidal rule and were expressed in arbitary units (μU/ml × min). Area IRI observed between 0 and 5 min was designated as cumulative acute insulin release in response to glucose pulse. The results are expressed as the mean ± SE of observed values. Statistical analyses were done by applying the Student's *t* test[14] to group differences between the test and control animals.

RESULTS

The effect of glucose exposure during vincristine treatment on subsequent glucose-induced insulin release. As shown in Figure 1, mean fasting serum glucose and IRI levels were similar in both groups of animals before and at 60 min after vincristine or vehicle treatment, indicating that vincristine per se had no effect on basal serum glucose and IRI concentrations. In response to a glucose pulse, mean serum glucose levels were also similar in both vincristine- and vehicle-treated groups. Similarly, in response to a glucose pulse, mean IRI levels were not significantly different in the two groups. Mean cumulative acute IRI responses (area IRI between 0 and 5 min after glucose pulse) were similar in both groups (333 ± 66 μU/ml × min in the vincristine-treated rats versus 365 ± 59 μU/ml × min in the control group).

The effect of arginine exposure during vincristine treatment on subsequent glucose-induced insulin release.

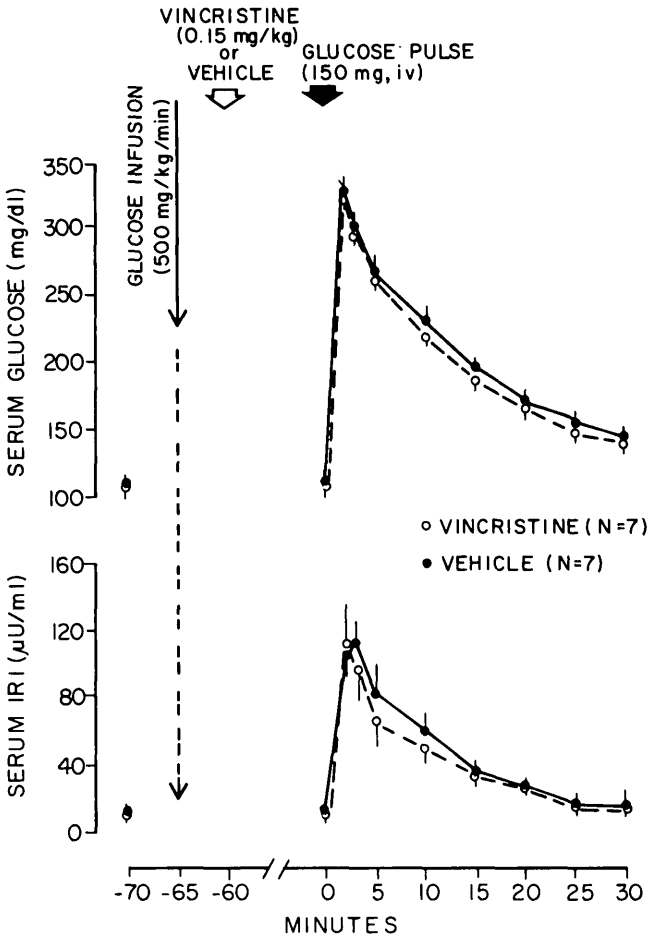

FIGURE 1. The effect of glucose exposure during vincristine treatment on subsequent glucose-induced insulin release. In response to a glucose pulse, mean serum glucose and IRI were similar in the vincristine-treated rats and the control rats.

As shown in Figure 2, serum glucose concentrations at all time intervals were similar in the vincristine-treated and the control rats. However, in response to a glucose pulse, serum IRI levels at 2 and 3 min in vincristine-treated rats were significantly less ($P < 0.02$) than those observed in the control rats (Figure 2). Similarly, the mean cumulative acute IRI response (754 ± 140 μU/ml × min) in the vincristine-treated rats was significantly lower ($P < 0.01$) than that observed in the control rats (1276 ± 85 μU/ml × min).

DISCUSSION

The findings of the present study demonstrate that in the intact rat: (1) exposure to high levels of glucose at the time of vincristine treatment prevented the inhibitory effect of vincristine on subsequent glucose-induced insulin release, while (2) arginine exposure under similar conditions did not protect the beta-cell from the inhibitory effect of vincristine.

These observations, along with the findings from our previous studies, support the concept that arginine-induced insulin release is mediated by mechanisms other than those that mediate glucose-induced insulin release and also suggest that the in vivo effect of vincristine on glucose-induced insulin release is mediated via alterations of the beta-cell glucose receptors rather than microtubular structures.

The protective effect of glucose on the inhibitory effect of

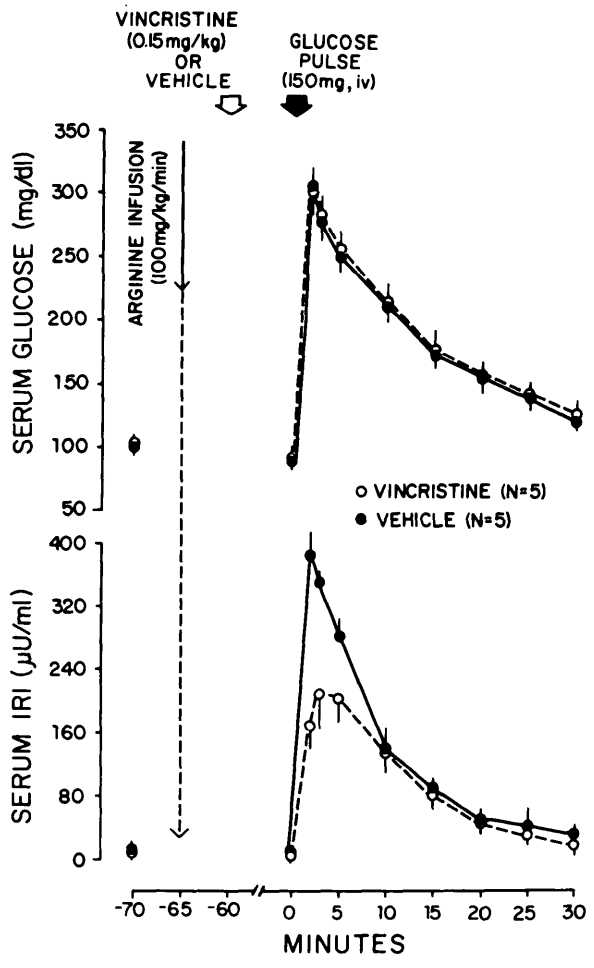

FIGURE 2. The effect of arginine exposure during vincristine treatment on subsequent glucose-induced insulin release. Serum glucose concentrations at all time intervals were similar in the vincristine-treated and the control rats. However, in response to a glucose pulse, serum IRI levels at 2 and 3 min in vincristine-treated rats were significantly less ($P < 0.02$) than those observed in the control rats.

vincristine on insulin release observed in the present study is similar to the protective effect of glucose against alloxan poisoning of the beta-cells, which has been previously reported.[15,16] In these studies, simultaneous treatment of isolated pancreatic islets with alloxan and glucose prevented the inhibition of insulin release seen with alloxan alone.

Recently, several studies have demonstrated that microtubular-disrupting agents may mediate their actions by affecting cellular systems and structures other than microtubules.[17–20] For example, colchicine has been shown to affect insulin receptors on hepatocytes[19] and concanavalin-A receptors on white blood cells.[20] Therefore, it is possible that vincristine exerts its inhibitory effect on glucose-induced insulin release by altering or interfering with the glucose receptors on the pancreatic beta-cells. Since arginine appears to stimulate insulin release via mechanisms that do not involve the glucose receptors, vincristine did not affect arginine-induced insulin release.

The above 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 release.[21,22]

Vincristine and similar agents may prove to be useful tools to study the characteristics of the pancreatic beta-cell glucose receptor system and the mechanisms that result in insulin release. Investigations using isolated rat islets are currently underway in our laboratory to further characterize the relationship between vincristine- and stimulus-induced insulin release.

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