Endotoxin-Induced Insulin Sensitivity in BCG Infected Mice (35411)

J. W. SHANDS, JR., V. SENTERFITT, AND V. MILLER
(Introduced by G. E. Gifford)

Department of Immunology and Medical Microbiology, University of Florida College of Medicine, Gainesville, Florida 32601

In previous studies of the effect of endotoxin on hyperreactive, BCG-infected mice we found that small doses of endotoxin caused profound hypoglycemia (1). It also became apparent that the cause of the hypoglycemia was largely an imposed defect in the synthesis of glucose from noncarbohydrate sources (2). The severity of the hypoglycemia encouraged the belief that it contributed to, or was responsible for, the lethal effect. However, attempts to protect endotoxin-poisoned BCG mice by glucose administration yielded variable results (1). Sometimes, life was prolonged but death was hastened at other times. In these latter instances, the mice often had convulsive seizures 30–40 min after a large dose of glucose; whereas those not given glucose did not. The possibility that we were observing an insulin effect was suggested by a report that insulin enhanced endotoxin lethality (3). Although we previously had shown that BCG-infected mice *per se* were not abnormally sensitive to insulin (2), we did not test endotoxin-poisoned BCG-infected mice. The defect in gluconeogenesis in this latter group should render them exquisitely sensitive to insulin. In this paper, we report the enhanced hypoglycemia in BCG-infected mice given endotoxin and insulin, and point out a situation in which endogenous insulin may participate in the production of endotoxin-induced hypoglycemia.

Materials and Methods. Animals. CD-1 female, pathogen-free mice, weighing approximately 20 g, were obtained from Charles River Breeding Laboratories, North Wilmington, Mass. The animals were housed 10 to a cage and fed water and chow *ad libitum*.

Endotoxin. The endotoxin was prepared in this laboratory from a smooth strain of *Salmonella typhimurium* by the phenol–water procedure (4). Endotoxin was administered by the intravenous route in 0.2 ml of saline.

TABLE I. The Potentiating Effect of Insulin on Endotoxin (LPS)-Induced Hypoglycemia in Fasted BCG Mice.

Treatment	No. of mice	Mean blood glucose (mg % ± SEM)[a]
LPS, 0.1 μg iv	7	37 ± 11
Insulin, 0.2 units iv	7	78 ± 16
LPS, 0.1 μg iv + 0.2 units of insulin iv 20 min later	7	7 ± 3[b]
None	7	114 ± 4

[a] Bleedings done 3 hr after endotoxin, blood glucose expressed as mean ± standard error of the mean.

[b] Began convulsing 40 min after insulin.

Insulin. Crystalline zinc insulin (Lilly) was used.

BCG infection. Mice were made hyperreactive to endotoxin by intravenous infection with *Mycobacterium bovis* BCG as described by Suter and Kirsnow (5). The mice were used in experiments 14 days later.

Blood determinations. Blood was obtained by repeated bleedings from the retrorbital plexus or by cardiac puncture. Blood glucose levels were assayed by the glucose oxidase method (Glucostat, Worthington).

Results. Table I shows the effect of 0.1 μg of endotoxin, 0.2 units of insulin, and their combined effects on the blood glucose of groups of fasted BCG mice. Without doubt the combination of endotoxin and insulin produced a much more profound hypoglycemia

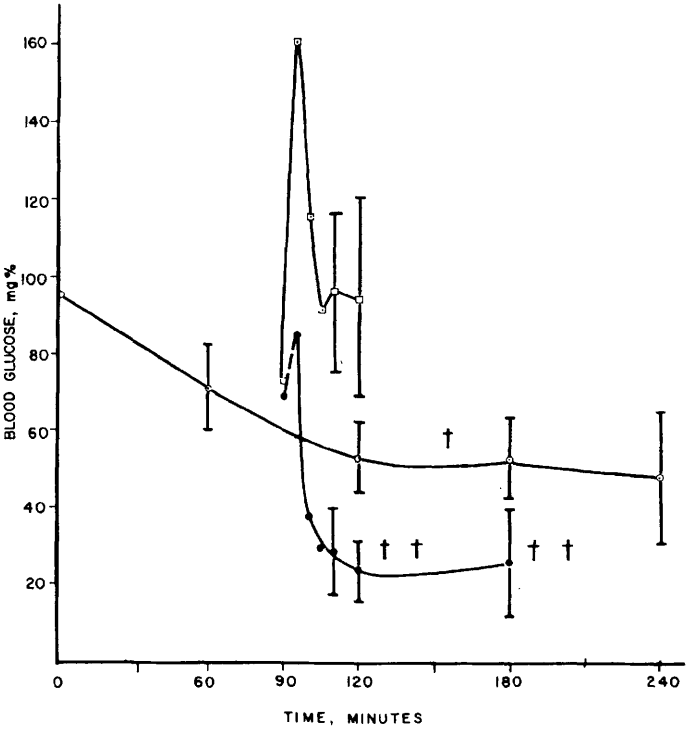

FIG. 1. (○), BCG mice given 0.1 μg of endotoxin; (●), same as (○), but given 10 mg of glucose iv at 1.5 hr; (□), BCG mice given 10 mg of glucose iv but no endotoxin. Each point represents the mean value from 6 mice. Arrow indicates time of glucose injection. Bars represent SD.

than either one alone. Differences in the groups were also evident at a clinical level. The group which received insulin alone appeared normal for the duration of the experiment. Those which recieved endotoxin alone appeared ruffled at 3 hr, but none had convulsions. Four of the 7 mice receiving both endotoxin and insulin began convulsions about 40 min after the dose of insulin.

This sensitivity to insulin resulting from endotoxin administration led us to investigate the possibility that endogenous insulin release might be responsible for the seizures often observed after glucose administration. In Fig. 1 the fluctuations of blood glucose in three groups of BCG mice are illustrated. Those mice given 0.1 μg of endotoxin showed a progressive decline of blood glucose. Those given endotoxin followed by 10 mg of glucose iv 90 min later showed a rise in glucose followed by an abrupt fall to severe hypoglycemic levels from which they did not recover. BCG mice given glucose but not endotoxin did not experience this reactive hypoglycemia.

If the reactive hypoglycemia is insulin mediated, then it should be abolished by D-mannoheptulose, an inhibitor of insulin release (6). The effect of D-mannoheptulose on the reactive hypoglycemia is shown in Fig. 2. In this experiment, 0.1 μg of endotoxin was given to all mice 1 hr before 0 time. The mice were then divided into 3 groups. Forty mg of D-mannoheptulose were given iv to one group of mice 15 min prior to 0 time, and at 0 time the group receiving mannoheptulose and a second group were given 10 mg of glucose iv. Although the reactive fall in blood glucose was not as precipitous as that shown in Fig. 1, nevertheless, the rate at which excess glucose disappeared from the blood was slowed by the preadministration of D-mannoheptulose.

Discussion. These data on the insulin sensitivity of endotoxin-poisoned BCG mice are consistent with the previous observation that endotoxin impairs gluconeogenesis (2). Blood glucose is regulated by very complex adap-

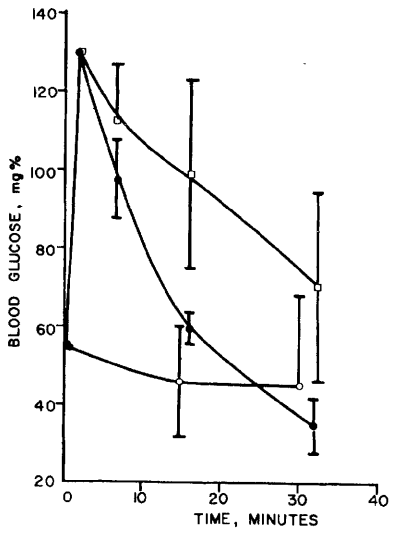

FIG. 2. (○), BCG mice given 0.1 μg of endotoxin 1 hr prior to 0 time; (●), same as (○) but given 10 mg of glucose iv at 0 time; (□), same as (●), but given 40 mg of mannoheptulose iv 15 min prior to 0 time. Each point represents the mean value from 6 mice. Bars represent SD.

tive control mechanisms, and in an animal such as the mouse, which has a relatively high metabolic rate, these mechanisms must operate optimally for the maintenance of homeostasis. Gluconeogenesis is particularly important in the mouse, since the rate of consumption of glucose is such that with fasting and without the process of gluconeogenesis all carbohydrate stores could be consumed in a short time. That under normal circumstances mice have a very active gluconeogenic pathway is suggested by the observation that they are relatively resistant to insulin (2). When gluconeogenesis is impaired, then insulin sensitivity appears. This is illustrated by the synergistic or additive effects of endotoxin and insulin on hypoglycemia.

Our data on the administration of glucose to endotoxin-poisoned BCG mice also illustrate the loss of homeostatic control in these mice. We suggest that the course of events is as follows: Since the mice used in these experiments were fasted overnight, one would expect insulin secretion was low and the rate of gluconeogenesis was high. After endotoxin the rate of gluconeogenesis dropped, causing a progessive fall in blood glucose. The administration of glucose after endotoxin caused a rapid rise in blood glucose and probably stimulated the release of insulin. This resulted in a rapid drop in blood glucose and the continued action of insulin led to a reactive hypoglycemia which could not be corrected because of failure of acute adaptive mechanisms for gluconeogenesis. Our only evidence that the reactive hypoglycemia is insulin mediated is derived from the effect of mannoheptulose. Mannoheptulose is known to inhibit insulin release (6) and, specifically, is thought to act on the glucose-induced release and not on the release mechanism *per se* (7). In view of this, and since mannoheptulose slowed considerably the rate at which excess glucose disappeared, it is reasonable to conclude that the reactive hypoglycemia was insulin mediated. This, however, must be regarded as a tentative conclusion. Although Chernick *et al.* (8) have reported that mannoheptulose did not alter the *in vitro* metabolism of glucose by liver, kidney, muscle, or fat, indicating that its effect on glucose metabolism was through its effect on insulin release, other effects of mannoheptulose on glucose metabolism have been reported, *i.e.*, inhibition of hexokinase activity (9, 10). It is, therefore, possible that prevention of the reactive hypoglycemia by mannoheptulose was related to other factors in addition to the prevention of insulin release.

The phenomenon we have described adds a complication to those efforts which have been made to save endotoxin-poisoned mice with glucose. In most instances glucose is administered to small animals by intermittent injection. It appears that intermittent injections of large quantities of glucose may sometimes be harmful. Either continuous infusion and/or blockage of insulin release might be more rational approaches.

Summary. The administration of endotoxin to BCG-infected mice induces a marked sensitivity to insulin. When insulin is administered after endotoxin, a profound hypoglycemia ensues. The stimulation of endogenous insulin secretion in endotoxin-poisoned BCG-infected mice by glucose administration may also lead to a reactive hypoglycemia from which the mice do not recover. These observations illustrate the loss of homeostatic control caused by endotoxin and are consistent

with previous observations that endotoxin impairs gluconeogenesis.

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