

## Hyperglycemic and Insulogenic Effects of Intravenous Glucagon at Different Blood Glucose Levels<sup>1</sup> (37396)

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(Introduced by L. S. Lilienfield)

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**Introduction.** The interaction of glucagon and blood glucose in the control of insulin secretion has not been elucidated. It has long been known that an elevation in blood glucose results in an increase in insulin secretion. Glucagon is also known to stimulate insulin release (1-3). The mechanism for the latter effect has not been clarified (3-5). It has been shown that prior ingestion of glucose enhances the glucagon effect on the beta cell production of insulin (6). In the work to be reported, we studied the effect of iv glucagon in beagle dogs after a period of fasting and during a period of sustained hyperglycemia produced by a constant glucose infusion. Our data suggest that glucagon can augment the beta cell response to hyperglycemia and that a high blood sugar level, in turn, increases the stimulating effect of a given amount of glucagon on the release of insulin from the pancreas.

**Materials and Methods.** Beagle dogs 18-30 months old, weighing approximately 10-12 kg were used in these experiments. Each animal was used 7-10 days apart. In the interval, they were fed a daily diet containing 100 g of carbohydrate. Prior to the experiment the animals were fasted overnight. Anesthesia consisted of an iv injection of Nembutal. An endotracheal tube and a respirator were used to maintain adequate oxygenation throughout the experiment.

Blood samples were obtained from a hind limb vein, through an indwelling 18-gauge needle kept patent with an infusion of normal saline. In order to avoid dilution, blood

samples were obtained from this site after discarding the first milliliter of blood. A vein was prepared for subsequent glucose infusion into the contralateral front leg.

Ten minutes were allowed to elapse in order to insure a steady state before any blood samples were obtained. A second fasting blood sample was obtained 5 min later. An infusion of a 50% glucose solution was then started at a rate of 1.95 ml/min (equivalent to 0.5 g/kg), using a Harvard pump. The infusion was maintained continuously for 3 hr. Blood samples for blood glucose (BG) and immunoreactive plasma insulin (IRI) measurements were obtained at 15-min intervals. BG was determined by the glucose oxidase method (7) and IRI by the method of Lazarow (8). Four types of experiments were performed on each animal as follows:

**Experiment A.** After the third hour, the infusion was stopped and blood samples were drawn every 10 min for one hour for BG and IRI determinations.

**Experiment B.** After a 3-hr infusion period, 40  $\mu$ g/kg of glucagon (Lilly) were injected intravenously. Blood samples were obtained after 5 min and then every 10 min for one hour.

**Experiment C.** The glucose infusion was maintained for 4 hr. At the end of the first 3 hr, glucagon was injected as in Experiment B and blood samples were collected at the same time intervals during the final hour of glucose infusion.

**Experiment D.** These animals received no glucose infusion. Glucagon (40  $\mu$ g/kg) was injected after an overnight fast and blood samples were drawn 1, 5, 10, 20, and 30 min

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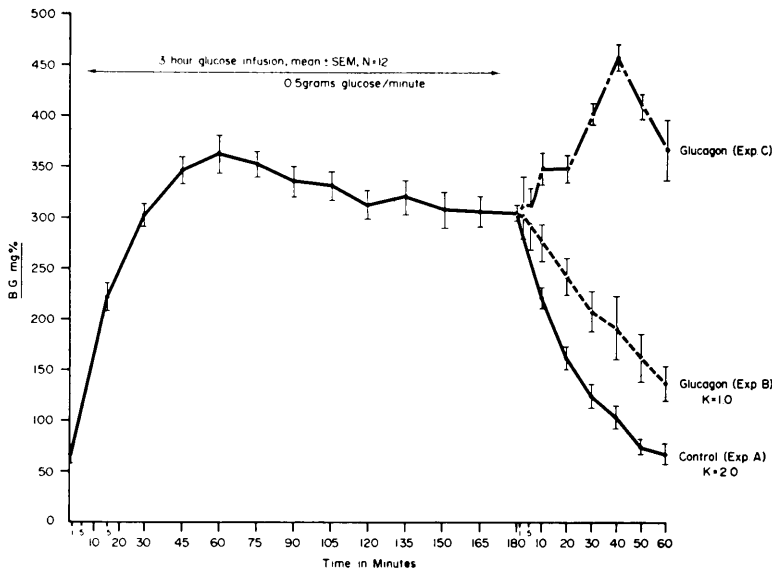


FIG. 1. Blood glucose (BG) values during a continuous three hour glucose infusion, 1.95 ml/min, 50% glucose in 12 Beagle dogs. Expt A: disappearance rate of glucose ( $K$ ) after stopping the glucose infusion ( $N = 4$ ). Expt B: glucose  $K$  rate after stopping the glucose infusion and injecting iv glucagon 40  $\mu\text{g}/\text{kg}$  ( $N = 4$ ). Expt C: BG levels after injecting iv glucagon 40  $\mu\text{g}/\text{kg}$  without stopping the glucose infusion ( $N = 4$ ).

after the injection.

**Results.** When glucose was infused for three hours, the BG curve showed a maximum value at the end of the first hour, after which time it declined slightly, reaching a plateau during the last hour of the infusion (Fig. 1, Table I). After the 3-hr glucose infusion was stopped (experiment A), the calculated disappearance rate for glucose ( $K =$

$t_{1/2}$ ) was 2.0. With the iv injection of glucagon at the end of the three hour glucose infusion (Expt B) the glucose  $K$  rate was 1.0. There was a significant difference ( $p < 0.001$ ) at all points after the glucose infusion was stopped.

When the glucose infusion was maintained after the glucagon injection (Expt C), the BG value rose slowly for the first 20 min (309 to 352 mg/100 ml), reaching a peak after 40 min (466 mg/100 ml) and decreasing rapidly thereafter. When glucagon was injected into fasted animals (Expt D, Table I), the BG rose from 76 to 195 mg/100 ml within 30 min. No further samples were collected after the first half hour in this experiment.

When glucose was infused for 3 hr, a nearly linear increase in the IRI curve was obtained by the end of the second hour, after which it plateaued (Fig. 2, Table II). After the glucose infusion was stopped (Expt A), the IRI values decreased gradually from a peak of 186  $\mu\text{U}/\text{ml}$  to 21  $\mu\text{U}/\text{ml}$  50 min later. Following the 3-hr glucose infusion (Expt B) an injection of glucagon elicited a rapid rise in IRI levels (188–290  $\mu\text{U}/\text{ml}$ ), reach-

TABLE I. Blood Glucose mg/100 ml. Av  $\pm$  SEM.<sup>a</sup>

Time in minutes	A	B	C	D
"0"	309 $\pm$ 7	309 $\pm$ 7	309 $\pm$ 7	76 $\pm$ 2
1	—	308 $\pm$ 26	317 $\pm$ 27	110 $\pm$ 8
5	—	295 $\pm$ 28	315 $\pm$ 16	124 $\pm$ 4
10	221 $\pm$ 9	277 $\pm$ 18	352 $\pm$ 15	162 $\pm$ 6
20	165 $\pm$ 13	246 $\pm$ 18	351 $\pm$ 15	182 $\pm$ 10
30	126 $\pm$ 14	210 $\pm$ 22	430 $\pm$ 10	195 $\pm$ 18
40	106 $\pm$ 15	196 $\pm$ 29	466 $\pm$ 13	—
50	77 $\pm$ 7	164 $\pm$ 24	413 $\pm$ 12	—
60	72 $\pm$ 11	139 $\pm$ 17	372 $\pm$ 32	—

<sup>a</sup>"0" Time in expts A, B and C represents samples obtained at the end of the 3-hr glucose infusion. Mean of 12 animals. All other values are the mean of four animals for each experiment.

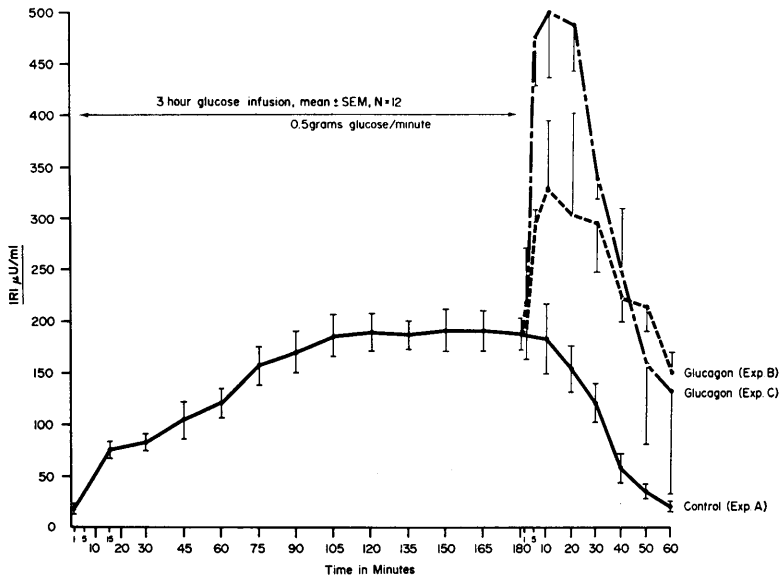


FIG. 2. Immunoreactive insulin (IRI) values during a continuous three hour glucose infusion, 1.95 ml/min, 50% glucose in 12 Beagle dogs. Expt A: after stopping the glucose infusion ( $N = 4$ ). Expt B: iv glucagon after stopping the glucose infusion ( $N = 4$ ). Expt C: iv glucagon without stopping the glucose infusion ( $N = 4$ ).

ing a peak value after 10 min ( $330 \mu\text{U/ml}$ ) and returning to preglucagon injection levels one hour later. When glucagon was injected during the glucose infusion (Expt C), the timing of the insulin response was similar to than seen in Expt B, but the IRI values were significantly higher after 5, 10 ( $p < 0.0002$ ), and 20 min ( $p < 0.005$ ). When glucagon was injected into fasted animals (Expt D, Table

TABLE II. Immunoreactive Insulin  $\mu\text{U/ml}$ .  
 $A_v \pm \text{SEM}^a$

Time in minutes	A	B	C	D
"0"	$188 \pm 17$	$188 \pm 17$	$188 \pm 17$	$15 \pm 3$
1	—	$192 \pm 32$	$216 \pm 46$	$67 \pm 39$
5	—	$290 \pm 12$	$474 \pm 68$	$116 \pm 15$
10	$186 \pm 34$	$330 \pm 64$	$500 \pm 44$	$85 \pm 46$
20	$156 \pm 23$	$308 \pm 98$	$487 \pm 26$	$34 \pm 18$
30	$121 \pm 19$	$297 \pm 56$	$338 \pm 19$	$20 \pm 12$
40	$64 \pm 13$	$223 \pm 23$	$248 \pm 63$	—
50	$34 \pm 6$	$215 \pm 85$	$159 \pm 26$	—
60	$21 \pm 2$	$145 \pm 100$	$133 \pm 22$	—

<sup>a</sup>"0" Time in expts A, B and C represents samples obtained at the end of the 3-hr glucose infusion. Mean of 12 animals. All other values are the mean of four animals for each experiment.

II), the insulin response was not as marked as in the hyperglycemic animals. The maximum IRI increment in experiment B was  $142 \mu\text{U/ml}$  in experiment C,  $312 \mu\text{U/ml}$  and  $101 \mu\text{U/ml}$  in experiment D. If the glucose infusion alone was continued for 4 hr, no significant rise or decline in BG or IRI was noted after the second hour of the infusion.

*Discussion.* Our results demonstrate that exogenous glucagon has an effect on plasma IRI levels and BG regardless of the BG concentration at the time of the glucagon injection. It has been reported that the hyperglycemic effects of exogenous glucagon are blunted in the fasting animal (1, 2). In addition, Penhos *et al.* (11) showed that perfused livers taken from fasted animals responded to glucagon administration with a markedly diminished glucose output as compared to livers from fed animals. This may be explained partially, by differences in the amount of available liver glycogen in fed or fasted animals. Other factors, however, regulate glucose release by the liver, such as the BG levels themselves and the hormonal milieu.

In our experiments, BG levels as well as plasma IRI were elevated during the glucose

infusion. Nevertheless, the liver appeared to increase its glucose output when glucagon was administered. The augmented hyperglycemia we observed following the glucagon injection may have resulted not only from increased liver glycogenolysis, but also from a diminished glucose uptake by peripheral tissues. Sarcione *et al.* (9) suggest that glucagon-induced epinephrine release may inhibit glucose uptake under these circumstances.

The stimulatory effect of a given level of BG on the beta cells seems to vary with the previous nutritional and hormonal state of the animal. Ketterer (12) found that even in the fasting animal, a small dose of glucagon produced a significant increase in insulin secretion. The insulin response was greater than would have been predicted by the magnitude of the glucagon-induced hyperglycemia. Our data confirm this observation and extend the study to show that glucagon increases the responsiveness of the beta cells even during prolonged periods of hyperglycemia.

Plasma IRI is markedly elevated during a glucose infusion but may be further elevated when exogenous glucagon is administered. This suggests that maximal insulin secretion or insulin release is not observed in the intact animal even when the BG levels are very high. It has been postulated that glucagon acts on an insulin-releasing mechanism different from the glucose-sensitive system (10). Curry has suggested (3) that glucagon releases the prestored or preformed insulin (first phase) and has little effect on the second phase of insulin release. Ryan (10), using human subjects, showed that glucagon alone could produce modest increases in insulin release and that oral glucose administration greatly enhanced this effect. Intravenous glucose was less effective in this regard. The precise site of action of both glucose and glucagon in the beta cell remains to be elucidated.

*Summary.* The effect of iv glucagon on insulin release, as measured by immunoreactive insulin (IRI), and blood glucose (BG) con-

centration, was studied in beagle dogs. The dogs were used in the fasting state and during a constant iv infusion of a 50% glucose solution for three hours. BG and IRI were monitored during the infusion and one hour after the infusion was stopped. Intravenous glucagon (40  $\mu\text{g}/\text{kg}$ ) was administered during the sustained hyperglycemia and it elicited a further rise in IRI. At the same time, glucagon appeared to delay the disappearance rate of glucose from the blood. After the glucose infusion had been stopped, the injection of glucagon had a smaller effect on the plasma IRI. Glucagon injected into fasting animals failed to produce an IRI increase of the magnitude of that seen during hyperglycemia. It did, however, produce some increase in insulin output despite the initial relative hypoglycemic state of the fasting animal. Our data extends the work of others in suggesting that both BG levels and glucagon act together to produce a maximal beta cell response to conditions of caloric plethora.

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