Inhibition of In Vivo Insulin Secretion by Prostaglandin E_1

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Abstract To determine the effect of prostaglandin E_1 (PGE_1) infusion upon in vivo insulin secretion, serum insulin responses after an intravenous glucose pulse (2 g) were measured before and during an intravenous infusion of PGE_1 (10 μg/min) in 11 anesthetized dogs. Circulating insulin decreased significantly during PGE_1 infusion, and insulin responses after glucose during PGE_1 infusion were significantly less than control responses. Three dogs received PGE_1 infusions into the thoracic aorta to preclude pulmonic and hepatic degradation of PGE_1 before its arrival at the pancreatic artery; inhibition of insulin secretion was again seen. Inhibition of insulin secretion could not be related to the degree of arterial hypotension induced by intravenous PGE_1, and despite alpha adrenergic blockade with intravenous phentolamine, PGE_1-induced inhibition of glucose-stimulated insulin responses persisted. Significant increments in systemically circulating PGE levels during intravenous PGE_1 infusions were documented by radioimmunoassay. These studies demonstrate that systemic PGE_1 infusion inhibits insulin secretion and that this effect could not be shown to be dependent upon alpha adrenergic activity.

INTRODUCTION

In general, prostaglandins (PG's) [1] appear to be ubiquitous and have been demonstrated to modulate a variety of tissue and hormone system effects (1). However, information regarding the effects of PG upon insulin secretion is sparse. That these fatty acids might be expected to affect insulin secretion is suggested by the facts that PGE_1 and PGE_2 modulate adrenergic nervous system action, i.e., they can cause both reversal of events which result from adrenergic stimulation and, in certain instances, potentiate norepinephrine effects (2). Since the adrenergic nervous system has profound regulatory effects upon insulin secretion (3, 4), the interaction of the PG's and the adrenergic nervous system in this regard are of obvious interest and potential importance to our understanding of physiologic and pathophysiologic responses of the pancreatic islets. Thus, the present studies were designed to focus upon two questions: (*a*) what is the effect of PGE_1 infusion upon in vivo insulin secretion and (*b*) can these effects be related to PGE_1 interactions with the adrenergic nervous system?

METHODS

Experimental model. Mongrel dogs were fasted for 36 h and systemically anesthetized with pentobarbital at 8:00 a.m. An endotracheal tube was used for assisted room air ventilation. A femoral arterial catheter with a heparinized saline lock was used to monitor arterial blood gases and blood pressure. A rectal thermometer and a heating pad were used to control body temperature. 30 min was allowed to elapse to establish basal conditions before beginning the experiments. Venous blood was drawn through a three-way stopcock from a femoral vein catheter kept patent when not in use with a slow infusion of 0.85% sodium chloride solution. Samples were collected at 15, 20, 25, and 30 min after the beginning of the basal period and at 2, 3, 4, 5, 7, 10, 15, 30, 45, and 60 min after each glucose injection. In all of the studies to be described, intravenous glucose was injected as a 2-g pulse (4 ml of a 50% glucose solution) in less than 3 s. Unless otherwise indicated, all infusions other than the glucose injections were given through a catheter placed in the femoral vein opposite to the one used for sample collection. Levels of serum insulin and plasma glucose were determined by previously published methods (5), and plasma PGE's were extracted and

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[1] *Abbreviation used in this paper:* PG's, prostaglandins.

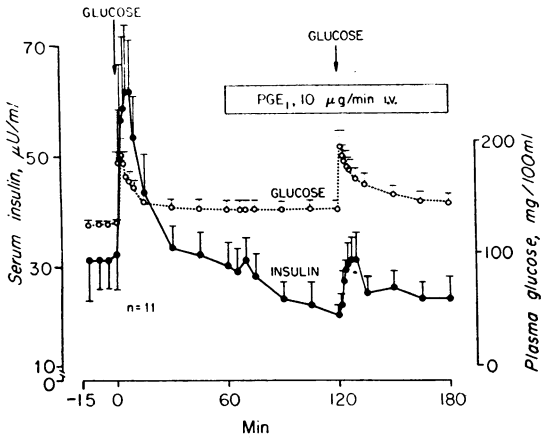

FIGURE 1 Effect of intravenous glucose (2 g) given in less than 3 s upon serum insulin and plasma glucose levels before and during an intravenous PGE_1 infusion (10 μg/min) in anesthetized dogs; mean±SEM.

TABLE I

Comparison of Insulin Levels during Saline or PGE_1 Infusion and Insulin Responses after the Second Glucose Pulse in the Control and Experimental Groups

	Saline*	PGE_1*
		μU/ml
Change at 60 minutes after onset of infusion	−2±6, NS‡	−10±9, $P < 0.01$‡
Response after second glucose pulse		
2 min	24±18	3±7, $P < 0.01$§
3 min	27±16	7±8, $P < 0.01$§
4 min	25±15	8±8, $P < 0.01$§
5 min	26±15	9±11, $P < 0.02$§

* Mean±SD.
‡ Comparison of changes in levels within the designated group.
§ Comparison of the PGE_1 group responses with the saline group responses.

then separated from other PG's by silicic acid column chromatography according to the method of Zusman, Caldwell, and Speroff (6). The amount of immunoreactive PGE in the eluate was determined by a double antibody technique for separation of antibody-bound and free PGE. A guinea pig PGE antisera made in this laboratory, which has 15% and 1% crossreactivity with PGA_1 and PGB_1, respectively, was used. Statistical comparisons were performed by Wilcoxan rank sum test, Student's *t* test, or paired *t* test.

Experimental design. To determine the effect of PGE_1 upon insulin secretion, glucose was injected before and again during the last hour of a 2-hour intravenous PGE_1 (10 μg/min) infusion. In a separate group of studies, PGE_1 was infused into the thoracic aorta by means of an arterial catheter passed retrograde from the femoral artery rather than into the femoral vein to preclude pulmonic and hepatic degradation of PGE_1 before its arrival at the pancreatic artery. To determine the effect of alpha adrenergic blockade upon PGE_1-induced effects on insulin secretion, intravenous phentolamine (0.2 mg/min) was infused for 3 h and 45 min. Intravenous glucose was given 45 min after the beginning of the phentolamine infusion. 60 min later, a 2-h infusion of PGE_1 was superimposed upon the phentolamine infusion; the glucose pulse was repeated during the final hour of the PGE_1 infusion. Control studies using saline instead of PGE_1 were performed for all the experiments described above.

RESULTS

The effect of PGE_1 upon insulin secretion. Compared to basal levels of circulating insulin (31±15 μU/ml, mean±SD, $n = 11$), there were immediate and significant increases in insulin (mean increment, $P < 0.01$ at 2, 3, 4, 5, 7, and 10 min) after initial glucose injection; insulin returned to basal levels by 60 min (Fig. 1). After 60 min of PGE_1 infusion, insulin levels fell significantly (−10±9 μU/ml, mean decrease, $P < 0.01$ at 60 min, Table I) compared to the levels observed 60 min after the first glucose injection. After the second glucose injection, the increments in insulin levels were significantly less than those observed after the first glucose injection ($P < 0.02$ at 2 min and $P < 0.01$ at 3, 4, 5, and 7 min). In contrast to the PGE_1 experiments, the circulating insulin levels in the control experiments at the end of the 1st h of the saline infusion (31±15 μU/ml) were not significantly lower than preinfusion values (33±12 μU/ml; Fig. 2). The increments in insulin levels after the second glucose injection during saline infusion were significantly less than those after the first glucose injection (mean increments, $P < 0.05$ and < 0.01 at 5 and 10 min, respectively); however, the second glucose-induced insulin responses in the animals receiving PGE_1 were significantly less than those seen in the animals receiving

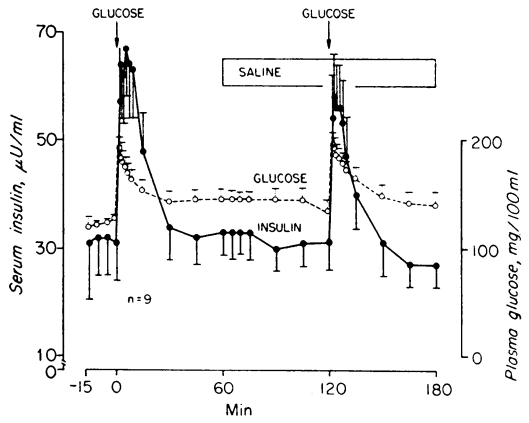

FIGURE 2 Effect of intravenous glucose (2 g) given in less than 3 s upon serum insulin and plasma glucose before and during an intravenous saline infusion in anesthetized dogs; mean±SEM.

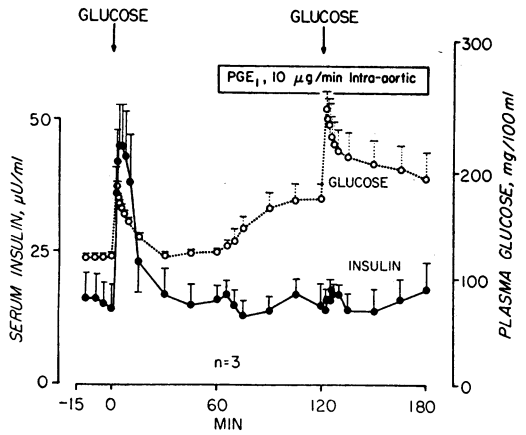

FIGURE 3 Effect of intravenous glucose (2 g) given in less than 3 s upon serum insulin and plasma glucose before and during an intrathoracic aorta PGE_1 infusion (10 μg/min) in anesthetized dogs; mean±SEM.

saline ($P < 0.01$ at 2, 3, and 4 min; $P < 0.02$ at 5 min, Table I). One animal treated with PGE_1 infusion was deleted from the PGE_1 group at the end of the study on the basis that the insulin response to the second glucose pulse was 13.77 SD's removed from the mean data of the other 11 animals. Since the probability that this response would fall within the distribution of the PGE_1 group's data was $P < 0.0001$, this animal's exclusion as an outlier was valid (7). There were no significant changes in circulating glucose levels during the 1st h of PGE_1 or saline infusion. The mean arterial blood pressure was recorded in five animals during PGE_1 infusion and fell significantly (−26±10 mmHg, mean±SD, $P < 0.01$); the correlations between the magnitude of blood pressure change and either the decrement in basal insulin levels ($r = 0.20$) or the decrement in glucose-stimulated responses at 5 min ($r = 0.16$) were not statistically significant. No significant change (−11±20 mm) in arterial blood pressure was seen during the five control experiments with saline in which pressures were recorded.

In the separate group of animals receiving PGE_1 infusion into the thoracic aorta, rather than into the femoral vein (Fig. 3), there was no increase in mean insulin levels after the second glucose pulse. Although circulating glucose levels were significantly increased over basal after the first 60 min of intra-aortic PGE_1 infusion (basal = 125±4; mean increase at 60 min = +51±22; mg/100 cc), there was no increase in mean insulin level at this time. Mean arterial blood pressure fell −51±15 mmHg during intra-aortic PGE_1 infusion. Five experiments designed to determine the concentration of postpulmonic plasma immunoreactive PGE levels after 30 min of femoral vein PGE_1 infusion revealed thoracic aortic plasma levels of 530±298, compared to basal levels of 88±29 (pg/cc, mean±SD, Table II). The femoral vein level of PGE was determined in one animal receiving PGE_1 infusion into the thoracic aorta and found to be >2500 pg/ml.

The effect of phentolamine-induced alpha adrenergic blockade upon PGE_1 effects on insulin secretion. After 45 min of phentolamine infusion in a group of five dogs (Fig. 4), there was an increase in both mean circulating glucose ($P < 0.01$) and mean insulin (P = NS) over

TABLE II

Thoracic Aortic Plasma Immunoreactive PGE Levels before and during Femoral Vein Infusion of PGE_1 (10 μg/min)

Dog	Before infusion	After 30 min infusion
	pg/ml	
1	83	350
2	50	500
3	70	1,100
4	135	450
5	100	250
Mean±SD	88±29	530±298

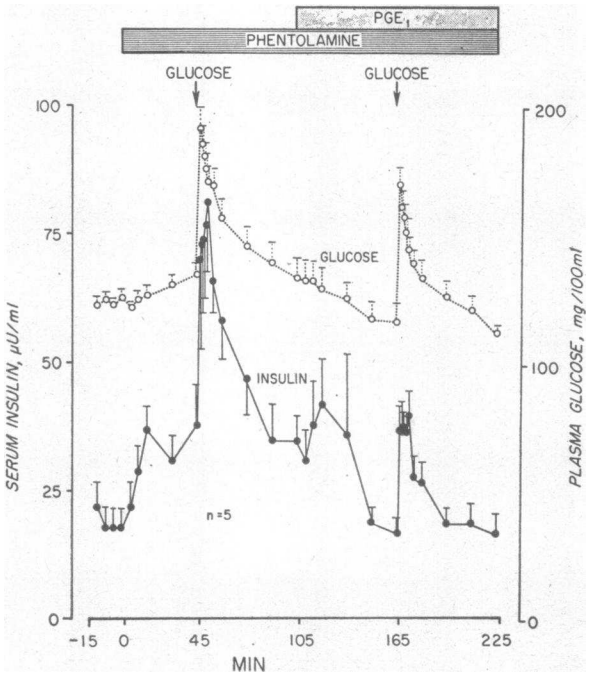

FIGURE 4 Effect of alpha adrenergic blockade (intravenous phentolamine, 0.2 mg/min) upon the effect of intravenous glucose (2 g) given in less than 3 s upon serum insulin and plasma glucose levels before and during intravenous PGE_1 infusion (10 μg/min) in anesthetized dogs; mean ±SEM.

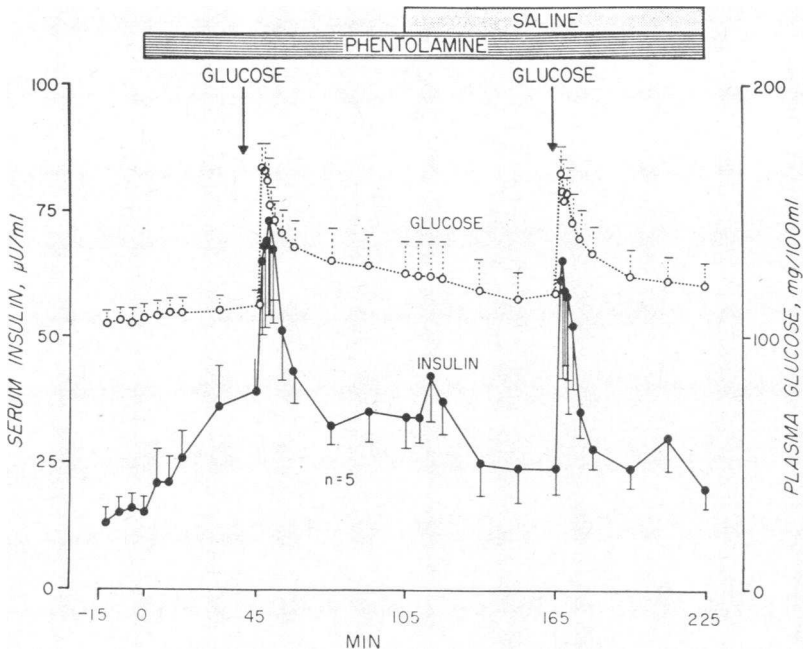

FIGURE 5 Effect of alpha adrenergic blockade (intravenous phentolamine, 0.2 mg/min) upon the effect of intravenous glucose (2 g) given in less than 3 s upon serum insulin and plasma glucose levels before and during intravenous saline infusion in anesthetized dogs; mean±SEM.

basal levels (basal: glucose = 122±8 mg/100 ml, insulin = 19±9 μU/ml; mean increases during phentolamine: glucose + 11±4 mg/100 ml, insulin = + 19±17 μU/ml). After the first intravenous glucose pulse, there was an immediate increase in mean circulating insulin levels which returned to control levels by 60 min. During the 1st h of the superimposed PGE_1 infusion, there was a decrease in circulating insulin levels (− 18 ±16 μU/ml, mean decrease at 60 min). After the second intravenous glucose pulse, the increments in insulin levels were significantly less ($P < 0.05$ for 3, 5, 7, and 10 min) than those observed after the first glucose pulse. During the control studies (Fig. 5) there was a decrease in mean circulating insulin levels − 9±14, (mean decrease ±SD; $n = 5$) during infusion of saline rather than PGE_1; although the mean decrease observed during PGE_1 infusion was greater, this difference was not statistically significant. However, in contrast to the studies with PGE_1 infusion, during the control studies there was no decrease in the insulin response after the second glucose pulse. The decreases in mean arterial blood pressure in the PGE_1 and the saline groups were not significantly different (PGE_1 = − 26±14, saline = − 13±15; mean±SD; mean decrease).

DISCUSSION

These studies demonstrate that intravenous PGE_1 infusion in dogs partially inhibits insulin secretion, since, during PGE_1 infusion, there was both a decrease in circulating insulin levels and a decrease in the height of insulin responses after intravenous glucose challenge. When saline was infused instead of PGE_1, there was no decrease in circulating insulin level, and although there was an attenuation of the insulin response after the second glucose challenge, these responses were significantly greater than the corresponding responses during PGE_1 infusion.

The importance of demonstrating the sixfold rise in aortic plasma immunoreactive PGE during femoral vein PGE_1 infusion lies in the fact that the lung immediately metabolizes an estimated 90% of PGE_1 reaching the pulmonary circulation (8). The aortic plasma PGE levels reported herein suggest that an adequate amount of PGE escaped pulmonic degradation to have systemic effects. Moreover, the systemic hypotension observed during the infusions is compatible with physiologically significant elevations of postpulmonic PGE levels. The fact that supraphysiologic postpulmonic levels of PGE supplied by intra-aortic PGE_1 infusion also inhibited insulin secretion further supports the contention that increases in systemically circulating PGE result in decreased insulin secretion.

Catecholamines are well-known profound inhibitors of insulin secretion in both the basal and stimulated state, an effect mediated by the stimulation of the alpha adrenergic receptor (3, 4). The fact that alpha adrenergic

blockade with phentolamine did not reverse PGE_1-induced inhibition of insulin secretion does not support the notion that this PGE_1 effect was secondary to reflex endogenous catecholamine secretion—a theoretical possibility in view of PGE_1-induced hypotension. However, since clinical hypotension has been associated with inhibition of insulin secretion (9, 10), it is possible that the inhibitory effect of PGE_1 upon insulin secretion may be secondary to the modest hypotensive effects encountered in these studies, even though there was no correlation between the magnitude of these two effects. It is of interest that there were diminished responses to the second glucose pulse in the group receiving saline alone but not in the group receiving saline and phentolamine. This suggests that nonspecific stress experienced by the animals during intubation, surgery, and blood loss may have activated alpha adrenergic receptors, perhaps through catecholamine secretion, which would have tended to diminish insulin responses. The fact that phentolamine infusion appeared to prevent a decrease in glucose-stimulated insulin responses after the second glucose pulse in the group receiving saline rather than PGE_1 further suggests that PGE_1 effects were not dependent upon alpha adrenergic activity.

PGE_1 has been reported to stimulate, rather than to inhibit, insulin secretion in isolated pancreatic islets (11). Increased insulin levels have also been reported in mice after interperitoneal PGE_1 (12), but it is not clear whether this increase in insulin secretion was caused by PGE_1 alone or by the associated hyperglycemia noted during these studies. During intravenous PGE_1 infusion in sheep (13) and during intravenous PGE_2 infusions in pregnant humans (14), no significant effect upon basal insulin or glucose level was noted: however, in the latter study, the rate of infusion was not constant, and in neither study was intravenous glucose stimulation attempted. Systemic hypotension and decreased pancreaticoduodenal vein blood flow, but no change in insulin output from the pancreaticoduodenal vein in dogs during PGE_1 infusion, has been reported; however, in these studies, a marked increase in insulin output occurred after discontinuation of PGE_1 (15). Since stress plays such a key role in the regulation of insulin secretion, it is difficult to compare directly these results from dogs underging major surgical stress to the dogs described in the present study who were stressed much less. However, it can be speculated that the post-PGE_1 infusion increase in insulin output observed may have reflected prior suppression of insulin secretion during PGE_1 infusion. Inhibition of glucose-stimulated insulin secretion during intravenous PGA_1 infusion in fasting anesthetized dogs has been demonstrated, but neither arterial blood pressure values nor attempts to reverse the PGA_1 effect with alpha adrenergic blockade were reported in this study (16).

In conclusion, these studies have demonstrated that intravenous PGE_1 infusion inhibits insulin secretion in anesthetized dogs. This effect, however, may or may not be due directly to the infused PGE_1. It is entirely possible that the PGE_1 infusion caused endogenous formation or secretion of some unidentified substance which was responsible for the observed inhibition of insulin secretion. However, if this effect was not due to PGE_1 directly, these data do not suggest it was related to alpha adrenergic activity.

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