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STIMULATION OF INSULIN SECRETION AFTER PROSTAGLANDIN PGE_1 IN THE ANESTHETIZED DOG*

PIERRE J. LEFEBVRE and ALFRED S. LUYCKX†
Secteur Diabétologie, Institut de Médecine, Université de Liège, Belgium

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Abstract—Overnight fasted anesthetized dogs were treated with prostaglandin PGE_1 infused at a rate of 0·5 or 1 μg/kg/min for 40 min and compared with saline-infused dogs. PGE_1 infusion caused a significant transient drop in arterial blood pressure (25–30 per cent) and in pancreaticoduodenal vein (PDV) blood flow (40–50 per cent). Total insulin output was unchanged during infusion. The cessation of PGE_1 infusion (1 μg/kg/min) was accompanied by a slight tendency toward recovery of PDV blood flow and a highly significant ($P < 0{\cdot}01$) increase in insulin output. No significant change in blood glucose was observed during PGE_1 infusion; however a moderate hypoglycemia was recorded at the time of maximal insulin output. These results (1), confirm that insulin output is relatively independant of gross changes in pancreaticoduodenal blood flow; and (2), demonstrate that insulin release is significantly increased in response to PGE_1 infusion. The relationship between the PGE_1-induced insulin increase and the adenylate cyclase system of the β-cell remains to be elucidated.

PROSTAGLANDINS are now recognized as natural compounds that may have much greater physiologic importance than hitherto suspected.[1] Their action on insulin secretion has received little attention although several of their effects have been related to changes in intracellular cyclic AMP, an event presumed to occur in the mechanisms associated with insulin release (Grodsky[2]). Bressler *et al.*[3] reported an increase in plasma insulin induced by PGE_1 in the mouse while others[4–6] have failed to observe any effect of PGE_1 on insulin release from rat pancreas slices of isolated islets incubated *in vitro*. Rossini *et al.*[6] postulated that the rise in insulin secretion reported *in vivo* was the result of regional increase in pancreatic blood flow owing to the well known vasodilative properties of prostaglandins rather than a direct biochemical effect of these compounds. We therefore decided to investigate the effect of PGE_1 on the pancreaticoduodenal blood flow and insulin output of dog pancreas *in situ*. We simultaneously studied certain hemodynamic (pulse, blood pressure) and metabolic (glucose, free fatty acids) parameters in the anesthetized dog.

MATERIALS AND METHODS

Mongrel dogs of both sexes, weighing 15–22 kg were fasted overnight. They were anesthetized with an intravenous injection of sodium pentobarbital (30 mg/kg body wt). Body temperature was kept constant with warming blankets. The trachea was

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† Chargé de Recherches du Fonds National de la Recherche scientifique, Belgium.

cannulated and the animals were given a mixture of O_2 95%–CO_2 5% to breathe freely. At laparotomy, an appropriate size polyethylene catheter was introduced into the proximal part of the superior pancreaticoduodenal vein (PDV) at about 2 cm from the portal vein. The PDV was then ligated, and the pancreatic venous effluent was drained off and reinfused through the splenic vein as previously described.[7] This permitted measurement of pancreatic blood flow. Throughout the experiments, the animals were infused intravenously with saline (1 ml/min). At the moment of PDV catheterization, heparin (250 IU/kg) was injected intravenously. Femoral blood pressure (measured with a mercury manometer), pulse rate and PDV blood flow were recorded every 5–10 min. Blood samples were drawn simultaneously from the femoral artery (9 ml) and, by free flow, from the PDV (4 ml). Collected blood was immediately replaced by an equivalent amount of blood obtained from a donor. Thirty to 45 min after the end of the surgical procedure, three blood samples were drawn at 7·5-min intervals to provide basal values. From zero time, an additional intravenous saline infusion (1 ml/min) was started in seven control animals, PGE_1* infusion was started in three dogs at a rate of 0·5 μg/kg body wt/min and in six dogs at a rate of 1 μg/kg body wt/min. The duration of prostaglandin infusion was 40 min. Blood samples were drawn at 5, 10, 20, 30 and 40 min during the infusion of saline or prostaglandin and then every 10 min (for 40 min) after termination of infusion. Glucose concentrations in arterial blood were measured by the method of Hoffman,[8] adapted to the Technicon Autoanalyzer, and free fatty acids (FFA) on arterial plasma were measured according to Dole and Meinertz.[9] Plasma insulin was assayed by the method of Quabbe,[10] using an anti-pig insulin antiserum. Human insulin was used as a standard and diluted in insulin-free[11] human plasma. All samples from a single experiment were assayed in the same series using the same standard curve. The dog and human insulins were confirmed to have the same reactivity by verifying the parallelism of dilution curves. At the end of the experiment, the animals were killed and the part of the pancreas studied was determined by retrograde infusion of 10–15 ml of a 1% aqueous solution of methylene blue through the PDV catheter, according to the procedure described by Seltzer.[12]

RESULTS

In the higher dose (1 μg/kg/min), PGE_1 caused a rapid and marked drop in arterial systolic blood pressure (Table 1, Fig. 1). At the end of PGE_1 infusion blood pressure tended to recover, although it had not reached its initial value by the end of the experiment. This drop in arterial blood pressure was accompanied by a very marked drop in PDV blood flow. When PGE_1 infusion was terminated, the blood flow showed a slight tendency to recover. As shown in Table 1 PGE_1 infusion produced a significant increase in the PDV hematocrit; the PDV plasma flow was therefore more markedly reduced than the PDV blood flow. Heart rate was slightly but not significantly reduced.

PGE_1 infusion (1 μg/kg/min) was not accompanied by any significant change in blood glucose, but significant hypoglycemia was recorded at the end of the post-infusion period (Table 2). No significant change in plasma FFA was observed either during or after PGE_1 infusion.

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TABLE 1. EFFECT OF AN INTRAVENOUS INFUSION OF PGE_1 (1 μg/kg/min) ON SYSTOLIC BLOOD PRESSURE, HEART RATE, PDV HEMATOCRIT, PDV BLOOD FLOW AND PDV PLASMA FLOW IN SIX ANESTHETIZED DOGS

	Control period			Infusion period PGE_1 1 γ/kg/min					Post-infusion period			
	−15 min	−7·5 min	0 min	+5 min	+10 min	+20 min	+30 min	+40 min	+50 min	+60 min	+70 min	+80 min
Systolic blood pressure (mmHg)	139 ± 7*	138 ± 8	139 ± 7	113 ± 8 P<0·05†	104 ± 9 P<0·02	100 ± 10 P<0·02	98 ± 11 P<0·02	98 ± 12 P<0·05	113 ± 12 N.S.	119 ± 11 N.S.	124 ± 11 N.S.	125 ± 10 N.S.
Heart rate (beats/min)	159 ± 2	153 ± 5	150 ± 7	146 ± 4 N.S.	139 ± 5 N.S.	137 ± 5 N.S.	139 ± 5 N.S.	140 ± 6 N.S.	143 ± 6 N.S.	147 ± 4 N.S.	149 ± 2 N.S.	145 ± 4 N.S.
PDV hematocrit (%)	44·3 ± 1·2	44·2 ± 1·2	44·6 ± 1·4	47·3 ± 1·4 N.S.	49·3 ± 1·4 P<0·05	50·7 ± 1·3 P<0·02	51·5 ± 1·8 P<0·02	51·8 ± 2·1 P<0·05	50·7 ± 1·9 P<0·05	50·5 ± 2·0 P<0·05	49·3 ± 2·0 N.S.	49·3 ± 1·8 N.S.
PDV blood flow (ml/min)	26·4 ± 2·8	24·1 ± 3·0	24·2 ± 2·9	18·2 ± 2·6 N.S.	16·4 ± 2·1 N.S.	13·7 ± 2·7 P<0·05	12·4 ± 2·7 P<0·05	12·2 ± 2·7 P<0·05	14·0 ± 2·4 P<0·05	14·3 ± 2·2 P<0·05	14·9 ± 2·2 P<0·05	15·0 ± 2·3 P<0·05
PDV plasma flow (ml/min)	14·5 ± 1·5	13·5 ± 1·7	13·4 ± 1·7	9·7 ± 1·6 N.S.	8·4 ± 1·2 N.S.	6·8 ± 1·3 P<0·02	6·1 ± 1·4 P<0·02	5·9 ± 1·4 P<0·02	7·0 ± 1·4 P<0·05	7·0 ± 1·3 P<0·05	7·4 ± 1·4 P<0·05	7·5 ± 1·4 P<0·05

* Mean ± S.E.M.
† Statistical comparison vs 0 mins values.

TABLE 2. EFFECT OF AN INTRAVENOUS INFUSION OF PGE_1 (1 μg/kg/min) ON ARTERIAL BLOOD GLUCOSE, PLASMA FREE FATTY ACIDS (FFA) AND PLASMA INSULIN IN SIX ANESTHETIZED DOGS

	Control period			Infusion period PGE_1 1 γ/kg/min					Post-infusion period			
	−15 min	−7·5 min	0 min	+5 min	+10 min	+20 min	+30 min	+40 min	+50 min	+60 min	+70 min	+80 min
Arterial blood glucose (mg %)	73·2 ± 3·1	74·0 ± 3·1	75·2 ± 4	75·7 ± 4·6 N.S.†	77·5 ± 5·2 N.S.	77·0 ± 5·9 N.S.	74·5 ± 4·7 N.S.	75·3 ± 5·4 N.S.	73·8 ± 4·6 N.S.	71·5 ± 4·0 N.S.	67·8 ± 4·2 N.S.	62·8 ± 3·1 P<0·05
Arterial plasma FFA (μeq/l)	890 ± 135	825 ± 120	795 ± 140	760 ± 160 N.S.	775 ± 160 N.S.	750 ± 135 N.S.	815 ± 175 N.S.	795 ± 175 N.S.	780 ± 160 N.S.	800 ± 150 N.S.	780 ± 135 N.S.	730 ± 120 N.S.
Arterial plasma insulin (μU/ml)	14·7 ± 3·5	11·3 ± 2·3	10·7 ± 3·3	13·1 ± 3·5 N.S.	14·4 ± 4·3 N.S.	15·9 ± 5·7 N.S.	18·8 ± 6·5 N.S.	19·8 ± 6·6 N.S.	24·1 ± 7·2 N.S.	26·9 ± 9·3 N.S.	25 ± 8·1 N.S.	23·2 ± 8·0 N.S.

* Mean ± S.E.M.
† Statistical comparison vs 0 min values.

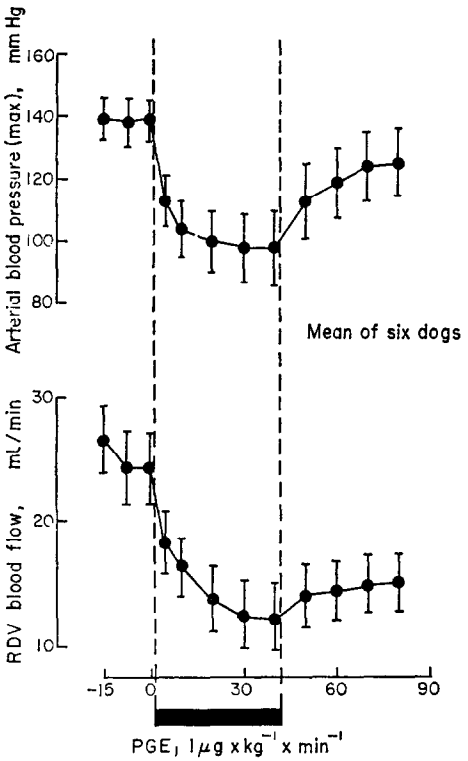

FIG. 1. Hemodynamic changes induced by the intravenous infusion of PGE_1. Results are expressed as mean±S.E.M.

TABLE 3. EFFECT OF SALINE OR PGE_1 INFUSION ON INSULIN OUTPUT FROM DOG PANCREAS *in situ*

	Insulin output (μU/g/min)		
Control period			
(all experiments)	120·5±10·6*	(n=41)	
Infusion period			
Saline	126·0±14·0	(n=21)	N.S. vs control period
PGE_1 0·5 γ/kg/min	120·5±20·6	(n=12)	N.S. vs control period; N.S. vs saline
PGE_1 1 γ/kg/min	162·5±18·0	(n=30)	P<0·05 vs control period; N.S. vs saline
Post-infusion period			
Saline	185·0±18·5	(n=19)	P<0·01 vs control period
PGE_1 0·5 γ/kg/min	205·7±33·0	(n=12)	P<0·01 vs control period; N.S. vs saline
PGE_1 1 γ/kg/min	326·3±40·8	(n=24)	P<0·01 vs control period; P<0·01 vs saline

* Mean±S.E.M. (the number of determinations are indicated in parentheses).

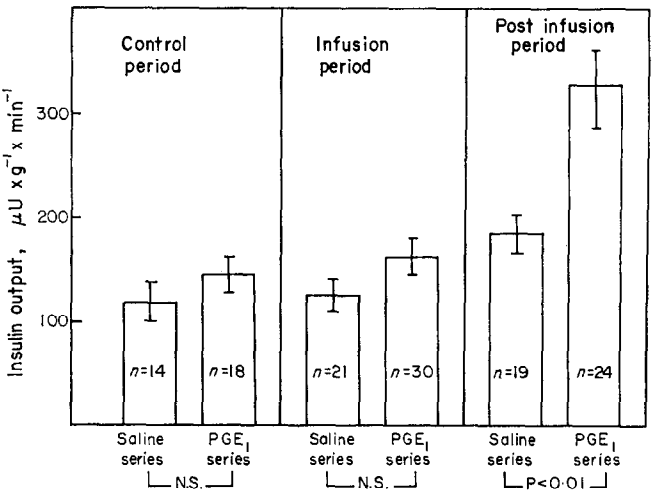

FIG. 2. Effect of PGE_1 (1 μg/kg/min) on insulin output from dog pancreas *in situ*. Seven dogs were studied in the control (saline) series and six dogs in the PGE_1 series. The total number of determinations in each series for each period (see text) are indicated by *n*. Results are expressed as mean ± S.E.M.

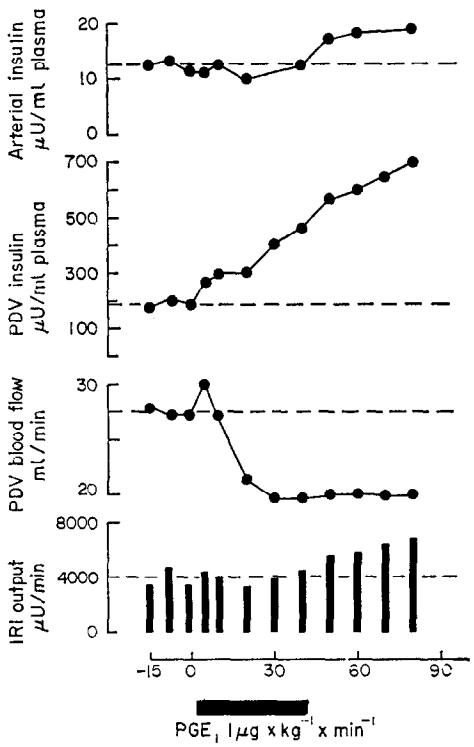

FIG. 3. Changes in arterial and pancreaticoduodenal vein insulin, pancreaticoduodenal vein blood flow and in insulin (IRI) output in a dog infused with PGE_1.

Basal plasma insulin output (the first three samples of all experiments) averaged 120·5±10·6 (mean±S.E.M.) μU/min/g of pancreas. In control dogs, no significant change in insulin output was observed during the infusion period, although a slight but significant increase was recorded during the post-infusion period (Table 3, Fig. 2). Dogs infused with PGE_1 at a rate of 0·5 μg/kg/min did not show any statistically significant difference from the saline-infused animals. On the contrary, in dogs infused with higher doses of PGE_1 (1 μg/kg/min) a highly significant increase in insulin output was recorded during the post-infusion period. Because of the marked reduction in PDV plasma flow, the lack of change (experiments with 0·5 μg/kg/min of PGE_1) or the insignificant increase (experiments with 1 μg/kg/min of PGE_1) in insulin "output" observed during PGE_1 infusion resulted from a marked increase in PDV insulin concentrations, as illustrated by Fig. 3. Mean arterial plasma insulin rose after PGE_1 infusion; owing to marked individual variations, however, the changes observed were not statistically significant (see Table 2).

DISCUSSION

The PGE_1-induced hemodynamic changes here reported are classic (Bergström *et al.*[13]). Since the fall in blood pressure is accompanied by a rise in cardiac output,[14,15] the primary mechanism of the blood pressure fall is decreased peripheral resistance. In contrast, our data indicate that pancreatic blood flow is simultaneously markedly reduced, an unexpected finding that runs counter the view of Rossini *et al.*,[6] who postulated a regional increase in pancreatic blood flow to explain the discrepancies between the results of *in vitro* and *in vivo* experiments assessing the action of prostaglandins on insulin secretion. This PGE_1-induced rise in pancreatic blood flow is in agreement with the finding of Saunders and Moser[16] that prostaglandin B_2 (PGB_2) increases vascular resistance in the isolated perfused rat pancreas in which blood flow is kept constant. In our experiments, the more striking reduction in PDV plasma flow resulted from a distinct rise in PDV hematocrit, a finding that has not been reported in the literature.

Our finding that PGE_1 infusion does not affect blood glucose in the early phase of the experiment but causes a significant hypoglycemia at the end of the post-infusion period is in contrast with the rise in blood glucose observed by others.[17,18] Since this hyperglycemic response is usually attributed to a marked reflex release of adrenal epinephrine in response to lowered blood pressure,[13] we consider its absence in our experiments to be due to the relatively small drop in blood pressure obtained with the dose used here. As discussed extensively in the review of Bergström *et al.*,[13] the effect of PGE_1 on FFA plasma levels differs markedly with the dose, the route of administration and the species. This complex situation results from the interaction of factors that may act in opposite direction: (1) at low doses PGE_1 stimulates FFA mobilization through the sympathetic nervous system, probably as a compensatory reaction to lowered blood pressure and; (2) at high doses PGE_1 reduces FFA mobilization via an inhibitory effect on lipolysis. We suggest that the lack of significant effect of PGE_1 on plasma FFA in the experiments here reported is due to the fact that an intermediate dose of PGE_1 was used so that the two effects simply cancelled each other out. Studies of the effect of PGE_1 on insulin secretion have given rise to conflicting results. Experiments *in vitro*, conducted both on slices of pancreas[4] and on isolated islets[5,6]

led to the conclusion that prostaglandins were devoid of any stimulatory effect on insulin release. Recent observations of Johnson *et al.*,[19] however, demonstrate the crucial role of the glucose concentration in the medium in this type of experiments. At a concentration of 30 mg glucose/100 ml, none of the prostaglandins tested by previous investigations (PGE_1, PGE_2 and PGF_{2a}) modified the slight basal release of insulin. In contrast, insulin release during stimulation in a medium containing 300 mg glucose/100 ml and 1 mM theophylline was increased over 200 per cent by all three prostaglandins tested. This effect was already apparent with PGE_1 concentrations as low as 10^{-8} M and it progressively increased with concentrations up to 10^{-5} M. In these experiments, the inhibition of insulin release by norepinephrine (10^{-5} M) was partially reversed by both PGE_1 and PGE_2. In experiments *in vivo*, Bressler *et al.*[3] reported an increase in plasma insulin after administration of PGE_1 to mice (2·5 and 5 μg/animal i.p.). It is impossible, however, to ascribe this effect with certainty to the administration of prostaglandin since a highly significant increase in blood glucose occurred simultaneously. At the doses used for the induction of labour in women, prostaglandins do not significantly affect peripheral plasma insulin levels.[20]

The present experiments demonstrate an unequivocal rise in the concentration of insulin in the PDV plasma during the intravenous infusion of PGE_1. Total insulin output, however, is not significantly increased during the infusion owing to the simultaneous drop in PDV blood flow and the rise in PDV blood hematocrit. The cessation of PGE_1 infusion is associated with an increase in insulin output which is statistically significant at the infusion rate of 1 μg/kg/min. The relationship between blood flow and insulin output of isolated dog pancreas *in situ* has been carefully studied in recent experiments by Rappaport *et al.*[21] In these experiments, mechanical constriction of the inferior pancreatic artery reduced blood flow to almost half its initial value but it reduced insulin output by less than one fifth of its initial value, although this decrease was statistically significant. It is interesting to note that the cessation of the mechanical constriction of the artery was associated with a return of both blood flow and insulin output to their initial values. This contrasts strikingly with our experiments in which, during the 40-min post-infusion period blood flow remained significantly reduced although insulin output was markedly increased. We therefore believe that the increase in insulin output after PGE_1 infusion is due to a direct or indirect action of the compound on insulin secretion and cannot be considered merely as a consequence of pancreatic circulatory changes. The discrepancies between the results of *in vivo* and *in vitro* experiments on insulin secretion are not peculiar to prostaglandins. Ouabain, for example, markedly stimulates insulin secretion in low doses *in vivo* in dogs[22,23] but a high ouabain concentration is required for even an inconsistent increase in insulin release *in vitro*;[24–26] similarly imidazole does not stimulate insulin secretion *in vitro*[27] although it has been demonstrated to be a powerful stimulator of insulin secretion *in vivo*.[28] The mechanism of the stimulation of insulin secretion by PGE_1 is still unclear. On the basis of the evidence discussed above, an indirect effect due solely to gross hemodynamic changes can reasonably be ruled out. Among the hormonal changes due to PGE_1 infusion[13] only the release of endogenous catecholamines could affect insulin release, and this would tend to counteract the effect here observed. Changes in metabolic substrates are unlikely since plasma FFA levels are unmodified and blood glucose remains constant except at the end of the experiment when it drops, most likely as a result of the increased insulin release. Several effects of prostaglandins have

been attributed to an increase or a decrease in intracellular cyclic AMP. The effects of PGE_1 on the adenylate cyclase or phosphodiesterase activities of the β-cells of the islets of Langerhans, as yet unknown, require further investigations.

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