REDDUCTION OF CIRCULATING INSULIN LEVELS DURING THE INFUSION OF DIFFERENT PROSTAGLANDINS IN THE RAT

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ABSTRACT

The intravenous infusion of prostaglandin (PG) E₁, E₂, and A₁ into normal rats at a dose of 2 μg/min significantly lowered plasma insulin levels with a tendency to recovery in the post-infusion period. Whereas PGA₁ infusion resulted in a moderate but significant hypoglycaemia, the administration of E-series PGs always produced a hyperglycaemic effect. The interference of PGE₁ on insulin response to classical insulinogogues (glucagon, aminophylline, and tolbutamide) was also investigated. The results of these experiments demonstrate that PGE₁ exerts an inhibitory action on insulin response to all insulin releasing agents investigated. As regards the haemodynamic effects of PGs, PGE₁ and PGE₂ lowered the arterial blood pressure by about 20%, while PGA₁ was almost completely ineffective. On the other hand, the lowering effect of PGE₁ on circulating insulin levels remained unchanged in rats treated with reserpine. These findings thus rule out a sympathetic over-activity secondary to the lowered arterial blood pressure as the mechanism of action of PGE₁. A possible direct interference with the adrenergic receptor system of the pancreatic islets was also ruled out since the inhibitory effect of PGE₁ was not overcome by phentolamine pre-treatment.

Although interest is increasing in the relationships between prostaglandins and insulin secretion, the reports presently available are largely conflicting and much uncertainty remains about this problem.

In studies performed on isolated rat islets or in the isolated rat pancreas, many investigators failed to observe any effect of PGs on basal and glucose-
stimulated insulin secretion (Rossini et al. 1971; Vance et al. 1971; Saunders & Moser 1972). On the other hand, Johnson et al. (1973) reported a stimulatory effect of PGs on insulin release from isolated islets of the rat in the presence of large amounts of glucose and theophylline. In experiments performed in vivo on mice, Bressler et al. (1968) observed an enhancement of insulin secretion after PGE₁ administration which was inhibited by beta-adrenergic blockade. More recently, a stimulatory effect of PGE₁ on insulin secretion was also found by Lefebvre & Luyckx (1973) in anaesthetized dogs as judged by insulin assay on pancreatico-duodenal vein blood. On the other hand, PGE₂ and PGF₂α did not produce any appreciable modification of plasma insulin levels when administered to pregnant women (Spellacy et al. 1971). In contrast to these experiments, it has been reported that PGA₁ engenders glucose intolerance after intravenous glucose loading and inhibition of insulin release in anaesthetized dogs (Saccà et al. 1973). An impairment of basal and glucose-stimulated insulin secretion was also observed by Robertson et al. (1973) in dogs during PGE₁ infusion. It is interesting that this inhibitory action of PGE₁ was not mediated by alpha-adrenergic receptors or by concomitant arterial lowering of the blood pressure (Robertson 1973).

The present work was undertaken in order to evaluate the influence of PGs on insulin secretion in the rat in the basal state and after pre-treatment with reserpine and alpha-adrenergic blockade. These latter experiments were performed in an attempt to establish if the inhibitory action of PGs was mediated by the sympathetic nervous system. Experiments were also carried out to examine the ability of PGE₁ to modify the insulin response to various insulin releasing agents (glucagon, aminophylline, and tolbutamide).

MATERIALS AND METHODS

Male albino rats weighing between 240 and 320 g were used after overnight fasting (18–20 h). They were anaesthetized by ip injection of sodium thiopental (50 mg/kg) and the trachea was cannulated. Serial blood samples (300–400 μl) for plasma glucose and for insulin assay were withdrawn through a polyethylene catheter placed in the right atrium via the jugular vein. After centrifugation at 4°C, the supernatant plasma was separated and stored at -20°C until analysis. Plasma glucose was determined by the glucose oxidase method (Boehringer, Mannheim GmbH) and plasma insulin by radioimmunoassay procedure using rat insulin as standard (Herbert et al. 1965). The following groups of experiments were performed.

1 – In this group, the effects of PGE₁, PGE₂, and PGA₁ were studied in untreated rats. PGs were administered by iv constant infusion for 45 min at the rate of 2 μg/min. Samplings were done in the basal state, at the end of the infusion, and 30 min after PGs infusion was discontinued.

2 – In these experiments PGE₁ was infused as above into rats pre-treated with reserpine or phentolamine. Reserpine was injected ip at a dose of 5 mg/kg 24 h before the
experiments. This dose of reserpine was able to reduce to about 75% the hyperglycaemic response to the iv injection of a hypotensive dose of sodium nitrite. The blockade of alpha-adrenergic receptors was induced by phentolamine which was given intravenously at a dose of 1 mg/kg 20 min before the beginning of PGE\textsubscript{1} infusion.

3 – In this group of experiments, the effect of PGE\textsubscript{1} infusion on insulin response to various insulinogogues was tested. PGE\textsubscript{1} was infused as above and 15 min later, insulin secretion was stimulated by the administration of glucagon (200 µg/kg iv), aminophylline (50 mg/kg ip) or tolbutamide (30 mg/kg ip). In control experiments the same dose of these substances was injected into rats receiving saline instead of PGE\textsubscript{1}.

4 – Experiments were also performed to evaluate the haemodynamic effects of PGs infusion at the dose reported above. For this purpose, a polyethylene catheter was placed in the abdominal aorta and blood pressure was continuously monitored with a Battaglia-Rangoni pressure transducer and polygraph.

The following substances were used: reserpine (Serpasil\textregistered, Ciba), phentolamine (Regitin\textregistered, Ciba), glucagon (Novo Industri A/S), aminophylline (Tefamin\textregistered, Recordati), and tolbutamide (Rastinon\textregistered, Hoechst).

All data are presented as mean ± se. Statistical analysis was performed by Student’s t-test for paired or unpaired samples (Snedecor & Cochran 1967).

Effect of intravenous infusion of different PGs (2 µg/min) on plasma glucose (mg/100 ml) and insulin (µU/ml) in normal rats and in rats pre-treated with reserpine or phentolamine. For treatment schedule see text. Student’s t-test for paired samples was used for the comparison of the means.

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Prostaglandins infused for 45 min</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>PGE\textsubscript{1} (6)</td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td>IRI</td>
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<tr>
<td>PGE\textsubscript{2} (6)</td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td>IRI</td>
</tr>
<tr>
<td>PGA\textsubscript{1} (9)</td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td>IRI</td>
</tr>
<tr>
<td>PGE\textsubscript{1} + reserpine (14)</td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td>IRI</td>
</tr>
<tr>
<td>PGE\textsubscript{1} + phentolamine (11)</td>
<td>Glucose</td>
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<td></td>
<td>IRI</td>
</tr>
</tbody>
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\textsuperscript{a} = P < 0.05
\textsuperscript{b} = P < 0.01

Number of rats in brackets

Table 1.

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RESULTS

The effects of PGs infusion on plasma insulin levels are presented in Table 1. All PGs studied produced a significant reduction of plasma insulin at the end of the infusion; a tendency to recovery was observed in the post-infusion period. It is of interest that while E-series PGs exerted a clear and sustained hyperglycaemic action, PGA₁ caused a moderate but significant hypoglycaemia with a partial recovery after the discontinuation of the infusion. Neither reserpine nor phentolamine were capable of preventing the diminution of circulating insulin levels induced by PGE₁. The plasma glucose levels markedly increased again and remained at significantly higher levels throughout the ex-

![Graph showing effects of glucagon on plasma glucose and insulin levels in normal (n=9) and PGE₁ treated rats (n=13). Glucagon was injected iv at 0 time in a dose of 200 μg/kg. PGE₁ infusion (2 μg/min) started 15 min before glucagon and continued throughout the experiment. One or two asterisks indicate P < 0.05 or P < 0.01 respectively.]

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Effects of tolbutamide on plasma glucose and insulin levels in normal (n = 5) and PGE₁ treated rats (n = 6). Tolbutamide was injected ip at 0 time at a dose of 30 mg/kg. PGE₁ infusion (2 µg/min) started 15 min before tolbutamide and continued throughout the experiment. One or two asterisks indicate P < 0.05 or P < 0.01 respectively.

**Fig. 2.**

In spite of the hyperglycaemia, the behaviour of plasma insulin was similar to that recorded in untreated rats given PGE₁, that is, a significant decrease during the infusion with a tendency to recovery thereafter.

Fig. 1 depicts the effects of glucagon on insulin secretion. In control rats plasma insulin levels promptly rose reaching a peak of 158 ± 16 µU/ml at 5 min and then slowly declined towards the base line levels. In PGE₁-treated rats the enhancement of plasma insulin levels was notably smaller in spite of the hyperglycaemia occurring in these experiments. The difference in insulin response to glucagon was also evident if evaluated in terms of integrated areas. In fact, these values were 1581 ± 235 µU/ml min⁻¹ in controls and 311 ± 96 µU/ml min⁻¹ in PGE₁-treated animals (P < 0.001).
Effects of aminophylline on plasma glucose and insulin levels in normal (n = 8) and PGE$_4$ treated rats (n = 9). Aminophylline was injected iv at 0 time at a dose of 50 mg/kg. PGE$_4$ infusion (2 µg/min) started 15 min before aminophylline and continued throughout the experiment. One or two asterisks indicate $P < 0.05$ or $P < 0.01$ respectively.

As shown in Fig. 2, insulin concentrations promptly rose after tolbutamide in control rats and remained at high levels throughout the experiment showing a biphasic profile. Correspondently, plasma glucose slowly declined from the basal level of 90 ± 4 mg/100 ml to a minimum of 56 ± 4 mg/100 ml after 60 min. In PGE$_4$-treated rats plasma glucose was significantly higher at any time as compared to the controls and did not show the expected diminution after tolbutamide. On the other hand, the insulin releasing effect of tolbutamide was significantly inhibited as demonstrated by insulin concentrations at various times as well as by the integrated insulin areas. These last values were
2098 ± 362 \( \mu \text{U/ml min}^{-1} \) in rats receiving tolbutamide only and 1238 ± 97 \( \mu \text{U/ml min}^{-1} \) in rats receiving tolbutamide during PGE\(_1\) infusion \( (P < 0.05) \).

An equally pronounced inhibition of insulin response occurred in experiments with aminophylline (Fig. 3). No appreciable modification of plasma glucose took place in rats treated with aminophylline only but in animals receiving the combined treatment a spectacular potentiation by aminophylline of PGE\(_1\)-induced hyperglycaemia was recorded. In spite of the marked increase in plasma glucose (230 mg/100 ml at 60 min) and the stimulation exerted by aminophylline, which was able to produce a five-fold increase in IRI levels in the controls, the insulin response was markedly depressed by PGE\(_1\). Integrated insulin areas were 2183 ± 370 \( \mu \text{U/ml min}^{-1} \) in PGE\(_1\)-treated and 4797 ± 1029 \( \mu \text{U/ml min}^{-1} \) in control rats \( (P < 0.05) \).

As far as the haemodynamic effects of PGs infusion are concerned, PGA\(_1\) was almost completely ineffective on arterial blood pressure whereas PGE\(_1\) and PGE\(_2\) produced a rapid fall by about 20% of the basal level without any tendency to recovery until the infusion was discontinued.

DISCUSSION

All PGs tested in the present study were capable of producing a significant reduction in circulating insulin levels when infused into normal rats under basal conditions. This effect appeared to be completely independent of the simultaneous changes in plasma glucose. In fact, whereas PGA\(_1\) infusion resulted in a moderate hypoglycaemia, the administration of E-series PGs always exerted a hyperglycaemic effect; nevertheless, the expected insulin response to the elevated glucose levels was completely absent. Basically, this would signify that PGs promote a decrease in basal insulin levels and block the insulin response to hyperglycaemia. In order to further confirm these findings, we have also studied the interference of PGE\(_1\) on insulin response to some classical insulinogogues, that is, glucagon, tolbutamide, and aminophylline. It is well known that glucagon releases insulin through its stimulatory effect on cyclic AMP formation in the beta-cells (Turtle & Kipnis 1967). Methylxanthines act as insulin releasing agents by virtue of their inhibiting action on phosphodiesterase (Butcher & Sutherland 1962; Malaisse et al. 1967a,b; Turtle et al. 1967). Tolbutamide, even though its mechanism of action is not completely known, is thought to influence insulin release by acting as a phosphodiesterase inhibitor or as a potentiator of the action of cyclic AMP on the release mechanism (Widström & Cerasi 1973). The results of our experiments clearly demonstrate that PGE\(_1\) exerts an inhibiting action on the insulin response to all the insulinogogues used. However, they do not show definitely whether
PGE$_4$ acts directly on the adenyl cyclase system of pancreatic beta-cells like in many other biological systems or through an indirect mechanism. Since PGE$_4$ is well known to be a vasoactive substance, the possibility existed that the observed metabolic effects may occur as a consequence of a sympathetic over-activity secondary to the lowered arterial blood pressure. In order to verify this possibility, experiments were conducted in rats whose catecholamines stores had been depleted by reserpine. Data relative to these experiments do not provide evidence for such an indirect mechanism based on a sympathetic mediation since PGE$_4$ infusion was accompanied by a significant decrease in circulating insulin levels in spite of the marked hyperglycaemic effect. In support of this conclusion is also the observation that the inhibitory effect of PGE$_4$ was not removed by phentolamine induced blockade of alpha-adrenergic receptors which mediate the inhibitory action of catecholamines on insulin secretion (Malaisse et al. 1967b; Porte 1967). Moreover, the experiments with phentolamine demonstrate that PGE$_4$ does lower plasma insulin levels irrespective of a possible direct interference with the adrenergic receptor system of the pancreatic islets. In this respect, our results are in complete agreement with the observations of Robertson (1973) namely that PGE$_4$ inhibition of insulin release in dogs persists after phentolamine pre-treatment. It is noteworthy that in the above experiments hydralazine induced hypotension of a comparable degree to that brought about by PGE$_4$ did not cause any inhibition of basal or glucose-stimulated insulin secretion. In our studies there is additional evidence that substantiates the concept of independence of the metabolic from the haemodynamic effect of PGs. In fact, PGA$_4$ was virtually ineffective on arterial blood pressure at the dosage used in the present study. Nevertheless, it was at least as effective as PGE$_4$ in reducing the plasma insulin concentration. In this context it is interesting to note that Rappaport et al. (1971) have observed only minor changes in insulin output by isolated dog pancreas when the blood flow through the inferior pancreatic artery was reduced to almost half of its initial value by mechanical constriction. All these findings argue against the possibility that the metabolic responses to PGs represent merely a consequence of alterations in pancreatic perfusion or of a reflex sympathetic over-activity and lead to speculation whether PGs may act directly on pancreatic beta-cells by inhibiting insulin response. On the other hand, the possibility that PGs indirectly affects the insulin metabolism through an extra-pancreatic mechanism cannot be excluded.

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