Theophylline prevents the inhibitory effect of prostaglandin E₂ on glucose-induced insulin secretion in man

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Abstract. This study was undertaken to assess the mechanism by which prostaglandins of the E series inhibit glucose-induced insulin secretion in man. Acute insulin response (mean change 3–10 min) to iv glucose (0.33 g/kg) was decreased by 40% during the infusion of prostaglandin E₂ (10 μg/min) and glucose disappearance rates were reduced (P < 0.05). Insulin response to arginine (5 g iv) and tolbutamide (1 g iv) were not affected by the same rate of prostaglandin E₂ infusion. The inhibitory effect of prostaglandin E₂ on glucose-induced insulin secretion was prevented by theophylline (100 mg as a loading dose followed by a 5 mg/min infusion), a drug that increases the intracellular cAMP concentrations by inhibiting phosphodiesterase activity. Our data suggest the involvement of the adenylate cyclase system in the inhibitory action of prostaglandin E₂ on glucose-induced insulin secretion in man. The initial confusion about the effects of prostaglandins (PG) on insulin secretion, arising for the main part from the conflicting results of the in vitro studies performed in different animal species (Robertson 1979), was followed by an unceasing increase in experimental in vivo data indicating that PG of the E series (PGE) inhibit the insulin response to glucose in several species, including rats (D Onofrio et al. 1977; Saccà et al. 1975), dogs (Robertson et al. 1974) and humans (Giugliano et al. 1978; Robertson & Chen 1977). The available data in humans indicate that both PGE₁ and PGE₂ can inhibit glucose-induced insulin secretion in a dose-dependent manner (Giugliano & Torella 1978; Giugliano et al. 1978; Robertson & Chen 1977) and that this effect is probably direct, because it is not reversed by alpha-adrenergic antagonists, which block the inhibiting effect of catecholamines on insulin secretion (Giugliano et al. 1979). More recently, Giugliano et al. (1983) showed that endogenous, pancreatically produced PGE may play a role in the appearance of the typical biphasic pattern of insulin release following a square wave glucose stimulation in normal man. Despite this evidence, many important questions still remain and await answers. The more pressing of these questions concerns the mechanism of action of PGE in islet cells. Recent in vitro studies (Robertson et al. 1987) suggest that PGE may interfere with the adenylate cyclase in the pancreatic beta-cell. Accordingly, the present study was undertaken to evaluate the effect of theophylline on PGE-mediated inhibition of insulin secretion in man, this drug inhibiting hydrolysis of cAMP by phosphodiesterase resulting in increased levels of intracellular cyclic nucleotides (Turtle et al. 1967).
Patients and Methods

Informed consent was obtained from 20 (12 males and 8 females) healthy, non-obese subjects, aged 22–37 years, after they had received a clear explanation of the nature, purpose and potential hazards of the study. The subjects were medical students or inpatients recovering from minor disease, with no personal or family history of diabetes. All were within 10% of their ideal body weight (Metropolitan Life Insurance Tables) and were taking no drugs. The subjects were on regular diets containing at least 200 g carbohydrates/day; some of them were studied twice and at least seven days were allowed to elapse between the two experiments. The study was carried out in accordance with the Helsinki II Declaration.

All studies were performed after a 12- to 14-h fast and with the subjects at bed rest and maintained supine thereafter. An antecubital vein of one arm was cannulated with a 19-gauge catheter 30 min before the start of the test and kept open by a slow 0.9% sodium chloride infusion. All blood samples were drawn from a contralateral vein through a three-way stopcock to avoid additional venipuncture during the infusions. In the first set of experiments, we evaluated the influence of exogenously administered PGE on insulin responses to various secretagogues. PGE2 was kindly provided by Upjohn (Kalamazoo, MI) and was diluted in 0.9% saline before infusion. The rate of PGE2 infusion (10 µg/min) has been previously shown to inhibit the acute insulin response to glucose in man (Robertson & Chen 1977; Giugliano et al. 1983). Glucose (0.33 g/kg), arginine (5 g) and tolbutamide (1 g) were given iv each stimulant administered as two consecutive pulses, separated by 120 (glucose and tolbutamide) or 90 (arginine) min. Blood samples were taken in the basal state (−30, −15, 0) and at 3, 5, 8, 10, 15, 30, 45 and 60 min after the iv pulse had been given.

In the other set of experiments, the insulin response to iv glucose (0.33 g/kg) was evaluated during the infusion of theophylline (100 mg as a loading dose followed by an infusion of 5 mg/min) or theophylline plus PGE2 (10 µg/min). These two experiments were done in the same subjects, performed in random order and separated by an interval of 7 days.

Blood samples were collected in prechilled tubes containing 1.2 mg EDTA/ml of blood, kept on ice until the end of the study and then immediately centrifuged after each experiment. The resultant plasma was stored deep-frozen until assayed. Plasma glucose concentrations were measured by the glucose-oxidase method adapted to a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). The method used for the determination of plasma immunoreactive insulin (IRI) has been previously described (Giugliano et al. 1983). The acute insulin response (mean 3–10 min change) was calculated as the mean of the 3-, 5-, 8- and 10-min post-injection values for a given subject from which was subtracted the insulin level immediately before the pulse for that subject. Glucose disappearance rates (Kd) were calculated with the method of the least squares, taking the natural logs of the glucose concentrations from 15 to 60 min.

Statistical analysis of the results was performed using the Student’s paired t-test and Wilcoxon’s rank sum test. Results are presented as mean ± SEM.

Results

Effect of PGE2 on insulin responses to glucose, arginine and tolbutamide

All subjects had fasting plasma glucose levels below 5.5 mmol/l. In all subjects, there was an almost immediate insulin response after the first glucose pulse, insulin returning to basal values by 90 min (Fig. 1). During the first 30 min of a subsequent PGE2 infusion there was no significant change in the mean insulin level. After the second glucose pulse, the acute insulin response was significantly less than that observed after the first glucose pulse (first response: 380 ± 64 pmol/l; second response: 229 ± 43 pmol/l, N = 6, Fig. 1). Circulating insulin levels in responses to glucose (0.33 g/kg) pulses given before and during infusion of PGE2 in normal humans (N = 6).
Table 1.

Plasma insulin levels before (basal) each pulse, acute insulin response to glucose (AIR) and glucose disappearance rates (KG) in the experiments.

<table>
<thead>
<tr>
<th></th>
<th>Pulse I</th>
<th></th>
<th></th>
<th>Pulse II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal pmol/l</td>
<td>AIR pmol/l</td>
<td>KG %/min</td>
<td>Basal pmol/l</td>
<td>AIR pmol/l</td>
</tr>
<tr>
<td>Glucose + PGE₂</td>
<td>93 ± 14</td>
<td>373 ± 64</td>
<td>2.29 ± 0.17</td>
<td>86 ± 14</td>
<td>240 ± 43*</td>
</tr>
<tr>
<td>Arginine + PGE₂</td>
<td>86 ± 14</td>
<td>337 ± 50</td>
<td>-</td>
<td>86 ± 14</td>
<td>330 ± 50</td>
</tr>
<tr>
<td>Tolbutamide + PGE₂</td>
<td>93 ± 14</td>
<td>509 ± 72</td>
<td>-</td>
<td>100 ± 21</td>
<td>481 ± 72</td>
</tr>
<tr>
<td>Glucose + saline</td>
<td>93 ± 14</td>
<td>351 ± 57</td>
<td>2.01 ± 0.18</td>
<td>87 ± 14</td>
<td>359 ± 57</td>
</tr>
<tr>
<td>Glucose + theophylline</td>
<td>86 ± 14</td>
<td>430 ± 72</td>
<td>2.09 ± 0.18</td>
<td>93 ± 14</td>
<td>581 ± 86*</td>
</tr>
<tr>
<td>Glucose + theophylline + PGE₂</td>
<td>80 ± 14</td>
<td>423 ± 72</td>
<td>2.02 ± 0.18</td>
<td>86 ± 14</td>
<td>502 ± 86</td>
</tr>
</tbody>
</table>

Results are given as mean ± SEM. The asterisk indicates significant differences (P < 0.05 or less) between the responses to the two consecutive pulses. N = number of experiments.

Fig. 2.
Circulating insulin levels in response to arginine (5 g) pulses given before and during infusion of PGE₂ in normal humans (N = 6).

Fig. 3.
Circulating insulin levels in response to tolbutamide (1 g) pulses given before and during infusion of PGE₂ in normal humans (N = 6).
The plasma glucose peaks obtained after the two consecutive iv glucose pulses were not significantly different (17.8 ± 3.2 mmol/l vs 18.1 ± 3.3 mmol/l, P = NS). Glucose disappearance rates were slightly but significantly reduced after the second glucose pulse as compared with those after the first one (first \( K_G \): 2.29 ± 0.17%/min; second pulse \( K_G \) 1.7 ± 0.15%/min, \( P < 0.05 \)). In control studies, in which saline instead of PGE\(_2\) was infused, the acute insulin response to the first and the second glucose pulses were similar, and there was no difference in the glucose disappearance rates (Table 1).

The iv arginine pulse elicited a prompt acute insulin response (336 ± 50 pmol/l) which became exhausted by 20 min. PGE\(_2\) infusion did not modify the insulin response to the second arginine pulse (329 ± 50 pmol/l, N = 6, \( P = NS \)). Fig. 2, Table 1.

In all subjects investigated, the insulin response to tolbutamide was of the monophasic type, with a peak at 5 min and a progressive return to basal values (Fig. 3). PGE\(_2\) infusion did not significantly change this response (Table 1). Fasting plasma glucose concentration (4.44 ± 0.22 mmol/l) reached its nadir at 30 min following tolbutamide stimulation (2.5 ± 0.16 mmol/l) and returned to basal values by 120 min. PGE\(_2\) infusion did not alter the plasma glucose nadir or affect the recovery from hypoglycemia following the second tolbutamide pulse.

**Effect of theophylline and PGE\(_2\) on insulin response to glucose**

Fig. 4 shows the influence of theophylline (top) and theophylline plus PGE\(_2\) (bottom) on glucose-induced insulin secretion in normal subjects. Theophylline significantly increased the acute insulin response to glucose, being 430 ± 71.7 pmol/l before and 581 ± 86 pmol/l during theophylline (\( P < 0.05 \)). The plasma glucose peaks following the two consecutive glucose pulses given in the absence or presence of theophylline were not significantly different (18.2 ± 2.4 mmol/l vs 18.5 ± 3.1 mmol/l, \( P = NS \)). \( K_G \) increased from 2.09 ± 0.18%/min to 2.31 ± 3.1%/min during theophylline infusion (\( P < 0.05 \), Table 1).

The infusion of theophylline prevented the inhibitory effect of PGE\(_2\) upon glucose-induced insulin release. In fact, the acute insulin response was 423 ± 71.7 pmol/l after the first pulse and 502 ± 86 pmol/l after the second one given during the combined infusion of theophylline and PGE\(_2\) (N = 7, \( P = NS \)). However, when comparing differences in the peak insulin responses between the glucose + PGE\(_2\) experiments and glucose + PGE\(_2\) + theophylline (i.e. testing the null hypothesis), a significant difference emerged (−149 ± 24 pmol/l vs + 81 ± 13 pmol/l, \( P < 0.01 \)). Glucose disappearance rates were 2.02 ± 0.18%/min in response to the first glucose pulse and did not change significantly during the combined infusion (2.10 ± 0.2%/min, N = 7, \( P = NS \), Table 1).

PGE\(_2\) and theophylline were well tolerated by all subjects.
Discussion

The results of the present studies show that PGE₂ infusion in normal man inhibits the acute insulin response to intravenous glucose but not to arginine or tolbutamide. Our data confirm and extend previous finding from this (Giugliano et al. 1978, 1983) and other (Robertson & Chen 1977; Robertson 1979) laboratories that the inhibition of the beta-cell response to glucose by PGE may represent specific inhibition of glucose-induced insulin secretion rather than a non-specific resetting of the beta-cell secretory activity to a lower level.

It is generally agreed that glucose stimulates insulin secretion by decreasing membrane permeability to K⁺ which in turn opens voltage-dependent Ca²⁺ channels thus increasing cytosolic free Ca²⁺ (Henquin 1978). Other mechanisms, among which are the increased beta-cell content of cAMP, may be important as well (Cerasi 1985). The amino acid arginine seems to depolarize the beta-cell membrane through the accumulation of its positively charged molecule with consequent activation of Ca²⁺ inflow into the cells (Charles et al. 1982). Finally, tolbutamide seems to facilitate Ca²⁺ inflow into beta-cells through voltage-sensitive Ca²⁺ channels (Coutirier & Malaisse 1982), although the possibility that hypoglycemic sulfonylureas may increase calcium entry by acting synergistically with native ionophores has also been suggested (Lebrun et al. 1982). Thus, the mechanism by which glucose stimulates insulin is different from that of arginine and tolbutamide.

The ability of PGE to inhibit only glucose-induced insulin secretion may be seen in this perspective. This means that PGE interferes with a step specific for the stimulus-secretion coupling of glucose. This step may be the adenylate cyclase system in the pancreatic beta-cells. This hypothesis seems supported by the findings of the present study that the enrichment of intracellular cAMP content consequent to phosphodiesterase inhibition by theophylline prevents PGE₂ from having an inhibitory action on glucose-induced insulin secretion in man. These results are also in keeping with recent data of Robertson et al. (1987) who, utilizing a glucose-sensitive monoclonal pancreatic beta-cell line, have shown that the inhibitory effect of PGE₂ on glucose-induced insulin release was prevented by pertussis toxin known to inhibit the inhibitory regulatory subunit of adenylate cyclase. According to Robertson et al. (1987) PGE₂ inhibits beta-cell function by activating a specific PGE receptor with a post-receptor action that decreases cAMP generation through the activation of the inhibitory subunit of the adenylate cyclase enzyme. The postulated important role played by the cAMP system in the mediation of PGE₂ effect on the pancreatic beta-cell may explain the apparently paradoxical selective inhibition of glucose-induced insulin secretion by PG of the E series, since cAMP is thought to play no role in the process of insulin release evoked by arginine or tolbutamide (Charles et al. 1982; Lebrun et al. 1982).

References


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