Effects of Calcium Channel Blockers and Hydralazine on Epinephrine-Induced Hyperglycemia In Vivo

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Abstract—Effects of calcium channel blockers from structurally different classes and hydralazine on epinephrine-induced hyperglycemia were studied in vivo. Nifedipine (0.05–0.20 mg/kg, i.p.) and nicardipine (0.40–0.80 mg/kg, i.p.) markedly potentiated the epinephrine-induced hyperglycemia in a dose-dependent manner. In contrast to these dihydropyridine calcium channel blockers, verapamil and diltiazem did not significantly affect the epinephrine-induced hyperglycemia at doses of 0.10–1.0 mg/kg, i.p. At higher doses (10 mg/kg, i.p.), significant potentiation of epinephrine-induced hyperglycemia was observed by these non-dihydropyridine calcium channel blockers. Hydralazine also markedly increased the epinephrine-induced hyperglycemia. These calcium channel blockers and hydralazine had no significant effect on the basal plasma glucose levels at any dose used here. As judged from the rates of glucose disappearance (K values), dihydropyridines significantly impaired the glucose tolerance in much lower doses than those of non-dihydropyridines and hydralazine. Furthermore, epinephrine-induced impairment of glucose tolerance was markedly potentiated by these calcium channel blockers and hydralazine at doses which potentiated the epinephrine-induced hyperglycemia. These results suggest that, at least in part, the potentiation of epinephrine-induced hyperglycemia by dihydropyridines, non-dihydropyridines and hydralazine is related to the inhibition of peripheral glucose utilization produced by insulin.

It is well-acknowledged that calcium channel blockers inhibit calcium influx through the voltage-dependent calcium channels to reduce vascular tone, hormone secretion, etc. (1). The cellular mechanisms of action of these agents are still unknown. In addition to the blockade of calcium entry into cells intracellular mechanisms of action have also been suggested such as interference with the calmodulin-mediated processes e.g., myosin light chain kinase and phosphodies-terase (2, 3).

Recently, calcium channel blockers such as verapamil, nifedipine and a number of related agents have been widely used in the management of hypertension and related cardiac disease (4). Mechanisms of action of these agents on the cardiovascular system have been extensively studied in vitro and in vivo. Several investigators have demonstrated that verapamil and nifedipine are capable of inhibiting glucose-stimulated insulin release by pancreatic islets, in vitro (5, 6). Although some reports have been published concerning the effect of calcium channel blockers on glucose tolerance (7, 8), the effects of these agents on glucose tolerance are not clearly understood. Furthermore, there has been a case report that nifedipine raises plasma glucose levels not only in diabetic but also in non-diabetic patients with hypertension (9).

However, few studies have been reported with respect to the effect of calcium channel blockers on the regulation of glucose homeostasis.

The present studies were designed to examine the comparative effect of dihydropyridines, non-dihydropyridine calcium channel blockers, and hydralazine on
epinephrine-induced hyperglycemia in vivo.

Materials and Methods

Animals and treatments: Before the experiment, male Wistar rats (180–200 g) were permitted free access to commercial laboratory chow (MF, Oriental Yeast Co., Osaka, Japan) and water at all times. The room temperature was kept at 24±2°C with a 12/12 hr-light/dark cycle. The rats were starved for about 20 hr before the experiments to deplete liver glycogen (10). They were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) before the administration of epinephrine (0.10 mg/kg, s.c.) and/or calcium channel blockers and hydralazine.

For the glucose tolerance test, rats were anesthetized with sodium pentobarbital. Then, a small incision was made to expose the femoral vein. A 20%-glucose solution was challenged rapidly through the vein at a dose of 0.3 g/100 g body weight. Plasma glucose concentrations were determined at -15, -5, 0, 10, 20, 30 and 40 min after the glucose load.

Analytical methods: Whole blood, 0.05 ml, was withdrawn serially from the cut end of the tail and mixed with 0.04 ml of 10 mM EDTA-10 mM sodium fluoride-saline solution. After centrifugation (5 min, 2500 rpm), the supernatant (0.02 ml) was assayed for the plasma glucose concentrations by the glucose oxidase method using commercially available diagnostic kits (Glucose Test Wako, Osaka, Japan).

For the analysis of glucose tolerance test, the rate of glucose disappearance (K value) was calculated from the regression coefficient of the semilogarithmic plot of plasma glucose level against time between 10 and 40 min after the intravenous glucose load. The absolute value of the slope represents the K value.

Statistical evaluation was performed using Student’s t-test for unpaired data or analysis of variance followed by the two-tailed Dunnett’s multiple comparison test (Table 1). Data were regarded as statistically significant at P values of less than 0.05. Results were presented as means±S.E.M. for the indicated number (n) of observations.

Materials: Stock solution of nifedipine was 1.0 mg/ml in 50% ethanol solution and diluted with saline immediately before use. Other calcium channel blockers were dissolved in saline. Dihydropyridine calcium channel blockers were given immediately before the glucose load. Statistical evaluation was performed using analysis of variance followed by the two-tailed Dunnett’s multiple comparison test. Results are presented as means±S.E.M. of five rats. N.S.: not significant (P>0.05); P<0.05, P<0.01: statistically significant difference from the control (glucose alone); a, b: 5 and 15 min before the intravenous glucose load, respectively.

Table 1. Effects of calcium channel blockers and hydralazine on the rate of glucose disappearance (K value) during intravenous glucose loading in 20 hr-starved rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses of drugs mg/kg, i.p.</th>
<th>K value</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td>2.07±0.23</td>
<td></td>
</tr>
<tr>
<td>Glucose+Diltiazem</td>
<td>1.0</td>
<td>1.60±0.20</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.74±0.15</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Glucose+Verapamil</td>
<td>1.0</td>
<td>1.48±0.12</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.49±0.098</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Glucose+Nifedipine</td>
<td>0.1</td>
<td>1.37±0.10</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>1.06±0.16</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Glucose+Nicardipine</td>
<td>0.4</td>
<td>1.16±0.05</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.97±0.19</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Glucose+Hydralazine</td>
<td>10a</td>
<td>1.20±0.14</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>10b</td>
<td>1.09±0.20</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

Based on the original results, the K values (percent removal/min) were calculated from the linear regression coefficient of the semilogarithmic plot of plasma glucose level against time between 10 and 40 min after intravenous glucose loading (0.3 g/100 g body weight). Calcium channel blockers were given immediately before the glucose load. Statistical evaluation was performed using analysis of variance followed by the two-tailed Dunnett’s multiple comparison test. Results are presented as means±S.E.M. of five rats. N.S.: not significant (P>0.05); P<0.05, P<0.01: statistically significant difference from the control (glucose alone); a, b: 5 and 15 min before the intravenous glucose load, respectively.
blockers were protected from light during use. Calcium channel blockers and hydralazine were purchased from Sigma (St. Louis, MO). All the other reagents were analytical grade.

Results

Subcutaneous injection of epinephrine (0.10 mg/kg) to 20 hr-starved rats produced about a 2-fold increase in plasma glucose level above basal value (Fig. 1). When given in combination with epinephrine, nifedipine (0.05-0.20 mg/kg, i.p.) and nicardipine (0.20-0.80 mg/kg, i.p.) markedly potentiated the epinephrine-induced hyperglycemia in a dose-dependent manner. These dihydropyridines alone (Fig. 1) and corresponding vehicle injection had no significant effect on the basal plasma glucose level (not shown). In contrast to the results observed with dihydropyridines, verapamil and diltiazem (0.10-1.0 mg/kg, i.p.) did not influence the epinephrine-induced hyperglycemia throughout the time course of investigation. However, at higher doses of verapamil and diltiazem (10 mg/kg, i.p.), they significantly potentiated the epinephrine-induced hyperglycemia. These non-dihydropyridines did not significantly affect the basal plasma glucose level by itself (Fig. 2). These results indicate that dihydropyridines are more effective than non-dihydropyridines in terms of potentiation of epinephrine-induced hyperglycemia in vivo. Interestingly, hydralazine, which is known to exert its blood pressure lowering effect by direct relaxation of arterial smooth muscle (11), also potentiated the epinephrine-induced hyperglycemia. Hydralazine alone (5, 10 mg/kg, i.p.) slightly increased the basal plasma glucose level (Fig. 3).

To elucidate the mechanisms for the potentiation of epinephrine-induced hyperglycemia by calcium channel blockers and hydralazine, I examined the effects of these agents on the glucose tolerance. The rates of glucose disappearance (K values) were calculated and summarized in Table 1. Impairment of...
glucose tolerance was produced by dihydropyridines, non-dihydropyridines (at higher doses) and hydralazine. No significant differences were observed between the control and animals treated with a lower dose (1.0 mg/kg) of verapamil and diltiazem. Corresponding vehicle injections did not affect the glucose tolerance (not shown). The order of the inhibitory action of these agents on the glucose tolerance seems to be consistent with the potentiating effects of epinephrine-induced hyperglycemia. Hydralazine treatment caused significant impairment of glucose tolerance when administered 5 or 15 min before the glucose load.

Moreover, the results of calcium channel blockers and hydralazine on the epinephrine-induced impairment of glucose tolerance was examined. As shown in Fig. 4, these agents significantly potentiated the epinephrine-induced impairment of glucose tolerance at 20 min, 30 min and 40 min after glucose loading (P<0.05). The effects of dihydropyridines on the epinephrine-induced impairment of glucose tolerance were much more potent than those of non-dihydropyridines and hydralazine.
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Fig. 4. Effects of calcium channel blockers and hydralazine on epinephrine-induced impairment of glucose tolerance. Epinephrine (0.1 mg/kg, i.p.) was injected immediately after the intravenous glucose load (0.3 g/100 g, b.w.). Hydralazine was administered 15 min before the intravenous glucose load. Other experimental details are described under Materials and Methods. The following doses were used: A: Glucose (0.3 g/100 g, b.w.)+epinephrine (0.1 mg/kg, i.p.: ○, n=5), glucose+Ep+diltiazem (10 mg/kg, i.p.: ●, n=4), glucose+Ep+hydralazine (10 mg/kg, i.p.: △, n=6), glucose+Ep+verapamil (10 mg/kg, i.p.: □, n=4). B: glucose+Ep+nifedipine (0.2 mg/kg, i.p.: △, n=7), glucose+Ep+nicardipine (0.4 mg/kg, i.p.: □, n=4). Thin lines indicate glucose+epinephrine for comparison.

Discussion

Plasma glucose levels are dependent on both hepatic glucose production and peripheral glucose utilization. The hyperglycemia induced by epinephrine may be explained in terms of the changes of metabolic activities caused by the agent (i.e., activation of hepatic glycogenolysis, enhanced gluconeogenesis, and inhibition of peripheral glucose utilization directly or indirectly through the suppression of insulin secretion) (12, 13). Since the glycogen content of the liver is very low in 20 hr-starved rats (10), prolonged hyperglycemia induced by epinephrine cannot be explained by the activation of hepatic glycogenolysis. Therefore, activation of hepatic gluconeogenesis and/or inhibition of peripheral glucose utilization play a predominant role in the epinephrine-induced hyperglycemia, although the relative contribution of these metabolic changes in epinephrine-induced hyperglycemia is yet unknown (4).

In the present in vivo study, dihydropyridines were shown to be more potent than non-dihydropyridines and hydralazine with respect to the dose-dependent increase in epinephrine-induced hyperglycemia. The effectiveness of these calcium channel blockers and hydralazine to potentiate epinephrine-induced hyperglycemia is similar to the vasodilatory potency of these agents in canine femoral arteries and veins in vitro (15). The differences in effectiveness of calcium channel blockers and hydralazine on the epinephrine-induced hyperglycemia cannot be explained only in terms of the inhibition of calcium entry into the cells.

The mechanisms through which calcium channel blockers and hydralazine potentiate the epinephrine-induced hyperglycemia
seem to be very complicated in vivo. However, it is unlikely that calcium channel blockers enhanced epinephrine-induced hyperglycemia due to the stimulation of hepatic gluconeogenesis, because separate experiments indicated that catecholamine stimulated gluconeogenesis is dependent on elevations of cytosolic calcium in isolated hepatocytes (16). Therefore, mechanisms for the potentiation of the epinephrine-induced hyperglycemia by calcium channel blockers and hydralazine may be largely related to the inhibition of peripheral glucose utilization produced by insulin (Table 1). In support of the hypothesis, verapamil and the other calcium channel blockers have been experimentally shown to inhibit the release of insulin in vivo (5, 6). In fact, it has been reported that mild hyperinsulinemia was produced during 2 hr of sustained epinephrine-induced hyperglycemia in vivo (14). It seems to be likely that, at least in part, the inhibitory effect of calcium channel blockers (and hydralazine) on glucose tolerance is mediated through the suppression of the hyperglycemia-induced insulin release and/or the inhibition of the insulin actions. Figure 4 shows direct evidence for the stimulatory action of calcium channel blockers and hydralazine on the epinephrine-induced impairment of glucose tolerance in vivo. These data support the above hypothesis.

Moreover, the potentiating effect of dihydropyridines and hydralazine on epinephrine-induced hyperglycemia may be due, in part, to other mechanisms. The first possible mechanism is the vasodilatory activity of these agents which produced an increase in the absorption of epinephrine into the circulation. The second possible mechanism is the reflex activation of the sympathetic nervous system, presumably as a result of the reduction in blood pressure by these agents. The third possible mechanism involves intracellular sites of action (e.g., inhibition of cAMP phosphodiesterase activity). In these regards, further studies are required to elucidate the relative contribution of these possible mechanisms in epinephrine-induced hyperglycemia.

References

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