ROLE OF ADRENERGIC MECHANISM IN KETOGENESIS

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ABSTRACT

Ketogenesis is a kind of physiological and biochemical imbalance between glycogenolysis, gluconeogenesis and lipolysis, which are delicately regulated by several hormones. The purpose of these experiments in rats is whether the administration of epinephrine, which has potent actions on glycogenolysis and lipolysis, can make the similar ketogenic state as glucagon or anti-insulin serum and how α- or β-adrenergic blockade acts against the rapid effect of epinephrine. As a result, the three hour infusion of epinephrine raised up plasma glucose rapidly and plasma ketones gradually and increased the ketogenic capacity of liver. On this time liver glycogen was exhausted almost completely and liver carnitine was elevated to the same level of fasted rats. The additional administration of α-adrenergic blockade blunted the effect of epinephrine on plasma glucose, plasma ketones and ketogenic capacity of the liver. On the other hand, the additional administration of β-adrenergic blockade aggravated plasma glucose furthermore and had less effect against epinephrine on plasma ketones and ketogenic capacity of the liver. The elevated liver carnitine with epinephrine was not affected by either α- or β-adrenergic blockade. From these findings, we concluded that the rapid ketogenic effect of epinephrine is mainly mediated by α-adrenergic effect of it.

The increase of ketone body production by the liver during fasting or in diabetic state requires an adequate mobilization of fatty acids and the accelerated oxidation of them. In rats the enhanced ability to produce ketone bodies does not appear until at 6 h after the onset of starvation, but then develops rapidly between 6 h and 9 h.1 It has already been reported that the administration of glucagon or anti-insulin serum is capable of producing the analogous ketogenic state in fed rats during a short time.2 The purpose of following

Abbreviations used in this article: Ep, Epinephrine; DHE, Dihydroergotamine; Prop, Propanolol. The term "ketones" means the sum of acetoacetic and β-hydroxybutyric acids.
experiments is whether the administration of epinephrine, which has potent actions on glycogenolysis and lipolysis, can bring about the similar ketogenic state and how α- or β- adrenergic blockade influences on the rapid ketogenic effect of epinephrine.

METHODS

(a) Animals: Male Sprague-Dawley rats weighing approximately 120 g were used in all experiments. Rats were fed at least one week prior to experiment with a diet containing 58.5% sucrose, 21% casein and less than 1% fat, together with all necessary vitamins and minerals. They were used for experiments between 7:00 and 8:00 a.m.

(b) In vivo studies: Rats were lightly anesthetized with ethyl ether and put polyethylene catheters in the femoral artery on one side and the inferior vena cava through the femoral vein in the other. They were then placed in individual restraining cages and allowed to awaken from anesthesia before experiments were started. After the collection of a zero time blood sample (about 0.2 ml) in iced heparinized microtubes, epinephrine and/or adrenergic blockade were infused with the rate of 10 μl per min through a venous catheter. Further arterial samples (about 0.2 ml each) were taken each one hour and the catheter was washed with minimal amount of heparin if the blood flow was not enough. After the collection of each sample, 0.9% sodium chloride, equivalent to the volume of blood sample, was infused into the rat. After 3 h infusion, rats were given an intraperitoneal injection of pentobarbital. Subsequently livers were either used for perfusion to determine their ketogenic capacity or were rapidly frozen in liquid nitrogen and analyzed for their glycogen and carnitine contents. In certain instances, blood samples (2 ml) were taken from the abdominal aorta for analysis of plasma glucagon and insulin concentrations.

(c) Liver perfusion studies: After 3 h infusion, livers were perfused with recirculating “artificial blood.” The “artificial blood” consisted of aged human erythrocytes suspended to a hematocrit of 20% in Krebs Ringer bicarbonate buffer, pH 7.4, containing 5% bovine serum albumin. The red blood cells were dialyzed for 24 h against 0.9% sodium chloride at 4°C before use in order to remove glucose, lactate and pyruvate. The Krebs buffer was bubbled with a gas consisting of 95% O2 and 5% CO2 about 1 h before use. PH was adjusted to 7.4–7.5 finally. The initial fatty acid concentration was approximately 0.7 mM in the cell-free fluid. After 30 min perfusion with the rate of 7 ml per min, samples of perfusion medium were collected for ketones measurement.
Adrenergic Mechanism in Ketogenesis

(d) Analytical procedure: All samples of blood and perfusate were centrifuged and analyzed for ketones, that is, acetoacetate and $\beta$-hydroxybutyrate and glucose by the enzymatic way. Liver glycogen was measured by the method of Good, Kramer and Somogyi. Total carnitine content of the liver was analyzed by the improved radioisotopic method of McGarry and Foster. Glucagon and insulin was measured by the radioimmunoassay. Statistical analysis was done using paired or unpaired $t$ test.

(e) Materials: Epinephrine (Vatarine Co., Inc.), dihydroergotamine (DHE-45, Sandoz Pharmaceuticals) as an $\alpha$-adrenergic blockade and propranolol (Ayerst Lab. Incorp.) as a $\beta$-adrenergic blockade were obtained commercially. The doses of them are as follows; epinephrine 0.75 $\mu$g/min/rat, dihydroergotamine 2.32 $\mu$g/min/rat and propranolol 1.01 $\mu$g/min/rat.

RESULTS

(1) Effect of epinephrine, $\alpha$-adrenergic blockade and $\beta$-adrenergic blockade on plasma glucose, plasma ketones and liver glycogen content.

There was no significant increase in plasma glucose and ketones and no significant decrease of liver glycogen with 3 h saline infusion. But the administration of epinephrine raised up plasma glucose rapidly ($p<0.01$ at 1, 2 & 3 h points) and plasma ketones gradually ($p<0.01$ at 1, 2 & 3 h points) and at the same time liver glycogen was exhausted almost completely ($p<0.001$). Compared to DHE alone, administration of propranolol alone produced higher plasma glucose ($p<0.05$ at 1 h point, $p<0.01$ at 2 & 3 h points) and lower liver glycogen ($p<0.05$) although there was no effect on plasma ketones. We could

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>glucose (mg/dl)</th>
<th>ketones (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>5</td>
<td>126±14 144±7 148±7 148±6</td>
<td>0.50±0.11 0.39±0.06 0.30±0.05 0.35±0.07</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>5</td>
<td>165±12 288±26 368±21 348±31</td>
<td>0.50±0.04 1.14±0.12 1.27±0.18 1.43±0.34</td>
</tr>
<tr>
<td>DHE alone</td>
<td>6</td>
<td>137±3 150±6 145±6 148±5</td>
<td>0.50±0.05 0.36±0.12 0.48±0.14 0.46±0.08</td>
</tr>
<tr>
<td>Prop alone</td>
<td>6</td>
<td>132±8 180±13 191±18 191±14</td>
<td>0.27±0.04 0.41±0.12 0.37±0.14 0.41±0.12</td>
</tr>
<tr>
<td>Ep+DHE</td>
<td>6</td>
<td>146±12 180±10 179±10 187±12</td>
<td>0.41±0.06 0.73±0.12 0.57±0.14 0.58±0.12</td>
</tr>
<tr>
<td>Ep+Prop</td>
<td>5</td>
<td>151±12 436±46 439±49 384±26</td>
<td>0.49±0.08 0.54±0.14 0.90±0.23 1.30±0.42</td>
</tr>
</tbody>
</table>

(mean ± SEM)
observe the similar tendency in the combinations of epinephrine and blockades, that is, the combination of epinephrine and propranolol caused much higher plasma glucose (p<0.01 at 1, 2 & 3 h points) and much lower liver glycogen (p<0.01) than that of epinephrine and DHE. But higher plasma ketones at 3 h point were observed in the group of epinephrine plus propranolol (p<0.05). Liver glycogen was almost thoroughly depleted by the epinephrine and propranolol combination as low as epinephrine alone. Namely propranolol could not inhibit the liver glycogen decrease induced by epinephrine, in other words, the depletion of liver glycogen is mainly mediated with α-effect of epinephrine, although even dihydroergotamine could not block completely the increase of plasma glucose and the decrease of liver glycogen caused by epinephrine. In short the increment of plasma ketones took place only in the groups which were treated by epinephrine alone or epinephrine plus propranolol and in only these two groups liver glycogen was almost thoroughly exhausted.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>T. carnitine (n mole/g liver)</th>
<th>Glycogen (mg/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5</td>
<td>134±2</td>
<td>6.6±0.3</td>
<td>119±11</td>
<td>55.7±2.3</td>
</tr>
<tr>
<td>Saline</td>
<td>4</td>
<td>120±4</td>
<td>6.2±0.4</td>
<td>176±13</td>
<td>53.5±4.2</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>4</td>
<td>114±7</td>
<td>5.4±0.5</td>
<td>233±8</td>
<td>4.5±1.3</td>
</tr>
<tr>
<td>DHE alone</td>
<td>6</td>
<td>124±2</td>
<td>6.7±0.1</td>
<td>—</td>
<td>51.8±3.4</td>
</tr>
<tr>
<td>Prop alone</td>
<td>6</td>
<td>114±4</td>
<td>6.2±0.4</td>
<td>—</td>
<td>39.0±2.3</td>
</tr>
<tr>
<td>Ep+DHE</td>
<td>4</td>
<td>122±1</td>
<td>5.8±0.1</td>
<td>247±8</td>
<td>14.0±1.8</td>
</tr>
<tr>
<td>Ep+Prop</td>
<td>4</td>
<td>131±2</td>
<td>5.5±0.2</td>
<td>237±9</td>
<td>3.8±1.8</td>
</tr>
</tbody>
</table>

(mean ± SEM)

(2) Total carnitine concentration in the liver.

Total carnitine in the liver significantly increased by the administration of 3 h saline infusion alone compared to untreated fed rats (p<0.001). With the treatment of epinephrine alone or epinephrine plus propranolol or epinephrine plus DHE, carnitine concentrations were elevated to the same level of fasted rats. But there was no significant difference of carnitine concentration between these groups. Furthermore the carnitine concentration had no significant correlation to the content of liver glycogen in these groups.
(3) Ketogenic capacity.

Ketone production per g liver in the epinephrine treated rats increased about 3 fold of saline control (p<0.001). But when DHE was infused with epinephrine, ketone production rate was blocked by 40% (p<0.001) and in the case of propranolol, it was blocked by 25% (N.S.).

Table 4
Plasma glucagon and insulin concentrations after 3 h infusion

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Glucagon (pg/ml)</th>
<th>Insulin (µU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>6</td>
<td>144±8</td>
<td>55±3</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>6</td>
<td>199±20</td>
<td>43±7</td>
</tr>
<tr>
<td>Ep+DHE</td>
<td>6</td>
<td>110±9</td>
<td>70±6</td>
</tr>
<tr>
<td>Ep+Prop</td>
<td>4</td>
<td>253±37</td>
<td>28±7</td>
</tr>
</tbody>
</table>

(mean ± SEM)

(4) Plasma glucagon and insulin concentration.

Epinephrine alone stimulated the glucagon secretion (p<0.001) and inhibited the insulin secretion (p<0.1). The combination of epinephrine and DHE, that is β-effect dominant state, gave rise to insulin secretion and blocked the glucagon secretion (p<0.001). On the contrary, the combination of epinephrine and propranolol, that is, α-effect dominant state, elevated glucagon and inhibited insulin markedly (P<0.001).
DISCUSSION

Recently it was shown that the elevation of liver carnitine is not enough for the induction of ketone body formation, that is, the stimulation of β-oxidation in the liver and that the decrease of liver glycogen is required at the same time for maximal ketogenic capacity of the liver. Therefore in these experiments, the purpose of epinephrine treatment was not only for the elevation of plasma glucose or the inhibition of insulin secretion, but also for the decrease of liver glycogen.

The dose of epinephrine was decided as a pharmacologically high dose which caused the decrease of liver glycogen as low as possible after 3 h infusion with the rate of 10 μl per min. 50 μg/ml solution of epinephrine was not enough (results were not shown). But when we put 100 μg/ml solution, most rats died with acute cardiac failure. Then 75 μg/ml solution was chosen for these experiments. The doses of dihydroergotamine and propranolol were designed to the equimolar solutions to epinephrine. Dihydroergotamine was chosen as an α-adrenergic blockade because phenoxybenzamine was unstable after dilution. With 3 h saline infusion, there was no significant change in plasma glucose and ketones although the liver carnitine content was significantly elevated (p<0.001). As a result of it, ketogenic capacity was also stimulated a little (p<0.05). It might be caused by glucagon, catecholamines and cortisol which were secreted through the surgical stress and 3 h restriction. The administration of epinephrine for 3 hours switched livers to complete ketogenic state and their ketogenic capacities were as high as those of fasted rats or glucagon treated rats. Epinephrine stimulated glucagon secretion and inhibited insulin secretion in spite of high glucose concentration. These effects were enhanced by β-adrenergic blockade and reversed by α-adrenergic blockade. Some investigators reported different results in dog and in man. But as shown in the textbook, Harvey et al. reported the same tendency in rats as our results. It may depend upon the difference of species. Basal glucagon plays an important role to maintain basal blood glucose through glycogenolysis in the liver. Moreover hyperglucagonemia is implicated in the pathogenesis of diabetes and in the development of diabetic ketoacidosis. Then some parts of ketogenic effect of epinephrine may be involved in this “hyperglucagonemia” due to epinephrine. Both epinephrine and glucagon has a potent action on glycogenolysis and gluconeogenesis. At the same time, epinephrine itself has much stronger effect on lipolysis. These three factors should participate in “epinephrine-induced ketogenesis.” On the administration of epinephrine plus propranolol as shown in Table 1, glucose at 1 h point was higher than that of
epinephrine alone. It might be caused by higher glucagon and lower insulin due to stronger α-effect than epinephrine alone. High ketogenicity induced by epinephrine was more or less diminished with both adrenergic blockades. Especially DHE, that is an α-adrenergic blockade, abolished it more markedly. Depletion of liver glycogen was also significantly inhibited by DHE (p<0.01), but not by propranolol. There was no significant difference in liver carnitine concentrations between those groups-epinephrine alone, epinephrine plus DHE and epinephrine plus propranolol. Therefore it was again confirmed that high carnitine concentration in the liver was not enough to get the high ketogenic state. Both elevated carnitine and subtotal glycogen depletion were required to switch livers from non-ketotic state to ketogenic state as seen in the groups of epinephrine alone and epinephrine plus propranolol. After all the additional administration of DHE to epinephrine had no effect against elevated carnitine but it could block the decrease of liver glycogen due to epinephrine. As a result of it, the ketogenic capacity of the liver diminished significantly (p<0.001). These results indicate that the rapid ketogenic action of epinephrine is mainly mediated by α-effect of it.

This work was partially presented at the 10th Congress of the International Diabetes Federation in Vienna, Austria, 1979.

REFERENCES

9. McGarry, J. D., Robles-Valdes, C. and Foster, D. W.: Role of carnitine in hepatic