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The effects of ginseng saponins on lactate dehydrogenase activity in the mouse

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The active principles present in ginseng saponins have been identified as triterpenoidal in nature (Court, 1975). It has been claimed that the intraperitoneal injection of ginseng saponins into both rats and mice increased the rates of RNA and protein synthesis in the liver (Oura et al., 1971, 1975). Ginsenoside administration has also been reported to accelerate the rate of hepatic lipogenesis in the rat, with concomitant decreases in liver glycogen, blood glucose and serum triacylglycerol (Yokozawa et al., 1975). By comparison, somewhat equivocal results have been observed with regard to the effects of ginseng on the activity of a number of hepatic metabolic enzymes. Thus, whereas pyruvate kinase (EC, 2.7.1.40) activity increased (Yokozawa et al., 1979), that of serine dehydratase (EC 4.2.1.13) exhibited a decline (Yokozawa & Oura, 1979). In cultured human diploid fibroblasts, lactate dehydrogenase activity was shown to increase significantly with ginseng supplementation of the growth medium (Shia et al., 1982), and the aim of the present study was to investigate whether a similar effect could be observed in vivo in the mouse.

Two groups of 25 male mice, strain LACa, each weighing 26–32 g, were maintained on a 12h-light/12h-dark regime, five to a group. Ginseng saponins in the form of a freeze-dried aqueous extract supplied by Pharmaton S.A. (Lugano, Switzerland) were dissolved in water and administered to the test group at a rate of 8 mg/kg body wt. per day for a period of 30 days. After 21 days of treatment the pharmacological activity of the ginseng saponins was verified in open-field stress tests (Bittles et al., 1979). The mice were killed by cervical dislocation, and the brain, heart, liver, skeletal muscle and testes immediately removed from each animal and stored at −20°C. For the enzyme assays, tissues were homogenized in 0.01 M-phosphate buffer, pH 7.4, to give an approximate concentration of 10% (w/v). The crude homogenate was centrifuged at 700 g for 10 min, and the lactate dehydrogenase (EC 1.1.1.27) activities of the tissues were determined on the supernatant, in triplicate, by the method of Wroblewski & LaDu (1955). The total protein of each preparation was measured by the method of Hartree (1972). All results were subjected to statistical analysis by the non-parametric two-tailed Mann–Whitney U test.

The liver homogenate showed a significant increase in lactate dehydrogenase activity (P<0.05) (Fig. 1). Small but non-significant increases in activity were observed in the heart and testis preparations, no effect was seen with the brain enzyme, and the skeletal-muscle enzyme showed a small, non-significant increase in activity.

It has been shown that ginsenosides administered to rats via their drinking water can be detected in the heart, liver, lung and spleen within 150 min (Takino et al., 1982). The observed tissue-specific differences in lactate dehydrogenase activity in the mouse can therefore be considered real, rather than representing a failure of the active principle(s) in the saponins to reach a particular potential site of action, for example in the brain or heart. Pre-treatment with ginseng has been shown to minimize the increase in blood lactate in rats after prolonged exercise (Avakian & Sugimoto, 1980). In the light of this finding, the significant increase in hepatic lactate dehydrogenase activity may therefore indicate an effective stimulation of the Cori cycle with a resultant enhanced production of pyruvate and glucose via gluconeogenesis. Preliminary studies on the lactate dehydrogenase isoenzymes of each tissue by polyacrylamide gel electrophoresis after ginseng administration have not revealed significant alterations in isoenzyme pattern. This would tend to suggest that the role of ginseng saponins in the stimulation of lactate dehydrogenase activity is not associated with synthesis de novo.


Fig. 1. Lactate dehydrogenase activity in mouse tissue
Activity in liver increased significantly (P≈0.05); there was no significant change in activity in testis, brain, heart or skeletal muscle. □, Control; ■, ginseng-treated.
Reversal of ketonaemia by vasopressin infusion in the starved rat

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Vasopressin inhibits ketogenesis in hepatocytes from fed, but not from starved, rats (Siess et al., 1978; Williamson et al., 1980). To see whether this lack of effect also applied in vivo, the effects of vasopressin on blood and hepatic metabolites were studied by using constant infusion of the hormone into starved rats.

Male Wistar rats (210-260 g) were starved for 24 h and anaesthetized with Nembutal (60 mg/kg body wt.). [Argininel-vasopressin (Sigma, in 0.9% NaCl) was infused (150 pmol/kg body wt. loading dose, then 50 pmol/kg body wt. per min) via the external jugular vein, to give an estimated plasma concentration of 1-5 nm. Blood samples were removed via the internal carotid artery and either deproteinized in HClO4, or heparinized for the separation of plasma. Control rats received saline alone. Livers were removed and freeze-clamped. Metabolites were measured by standard enzymic techniques, non-esterified fatty acids by the method of Shimizu et al. (1979) and insulin was determined by the method of Albano et al. (1972). Vasopressin caused a rapid decrease in blood ketone bodies, a transient increase in blood [glucose] and small increases in [lactate] and [pyruvate]. These changes were most marked during the first 10 min of infusion (Table I). Liver metabolite concentrations at this time were consistent with those seen in the blood. In addition, small increases occurred in a number of glycolytic intermediates, including phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate. Liver glucose was not significantly increased (Table I), nor was there any indication of anoxia, as judged by the more oxidized mitochondrial ([3-hydroxybutyrate]/[acetoacetate]) and cytosolic ([lactate]/[pyruvate]) redox couples. Perhaps the most important finding was the 60% decrease, at 10 min, in the plasma concentration of non-esterified fatty acids after vasopressin infusion. To investigate whether decreased substrate supply to the liver was the primary cause of the decreased ketonaemia, starved rats were injected with 3,5-dimethylpyrazole (50 mg/kg body wt. intraperitoneally) to inhibit endogenous lipolysis, and then infused with either octanoate or long-chain triglycerol. Vasopressin did not reverse the ketonaemia (1-2 mM) sustained by these exogenous fatty acids, indicating that hepatic ketogenesis was unimpaired. The apparent lack of insulin involvement (Table I) was confirmed by pre-infusing starved rats with somatostatin (1 nmol/kg body wt. per min) and then simultaneously infusing vasopressin and somatostatin. Under these conditions, where plasma insulin concentrations were always less than 7 munits/l, vasopressin still caused a marked decrease in blood ketone-body and non-esterified fatty acid concentrations.

The main metabolic effect of vasopressin in the starved rat therefore centres on a decreased supply of fatty acid to the liver. Possible mechanisms included a direct anti-lipolytic action of vasopressin, an indirect effect due to release of anti-lipolytic agents (i.e. prostaglandins), or decreased blood flow through adipose tissue.


Table 1. Effect of vasopressin infusion on blood and liver metabolite concentrations in 24 h-starved rats

Rats were infused with vasopressin or saline, and blood and liver samples were taken after 10 min. The results are shown as means ± S.E.M. for seven saline and eight vasopressin infusions. The mean zero-time blood metabolite concentrations were: glucose, 3.80 mM; ketone bodies, 2.38 mM; lactate, 0.40 mM; [3-hydroxybutyrate]/[acetoacetate] ratio, 1.84; [lactate]/[pyruvate] ratio, 9.21; non-esterified fatty acids, 0.99 mM; and insulin 10.5 munits/l. Significant differences between means were assessed with Student’s t test; *P < 0.05, **P < 0.01 and ***P < 0.001.

<table>
<thead>
<tr>
<th>Metabolite (mM)</th>
<th>Blood (change in 10min)</th>
<th>Liver (conc. at 10min)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Vasopressin</td>
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<tr>
<td>Glucose</td>
<td>-0.05 ± 0.11</td>
<td>+0.76 ± 0.21**</td>
</tr>
<tr>
<td>Ketone bodies</td>
<td>+0.15 ± 0.06</td>
<td>-0.96 ± 0.10***</td>
</tr>
<tr>
<td>Lactate</td>
<td>+0.10 ± 0.05</td>
<td>+0.35 ± 0.05**</td>
</tr>
<tr>
<td>[3-Hydroxybutyrate]/[acetoacetate]</td>
<td>+0.23 ± 0.09</td>
<td>-0.71 ± 0.19***</td>
</tr>
<tr>
<td>[Lactate]/[pyruvate]</td>
<td>+4.17 ± 1.43</td>
<td>+1.40 ± 1.50</td>
</tr>
<tr>
<td>Non-esterified fatty acids</td>
<td>-0.02 ± 0.04</td>
<td>-0.60 ± 0.06***</td>
</tr>
<tr>
<td>Insulin (munits/l)</td>
<td>−2.5 ± 1.6</td>
<td>-7.1 ± 2.6</td>
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Vol. 11