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Glycogen Phosphorylase Activities in Sulfonylureas Treated Rats*

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Sulfonylureas have been described as depressing the output of glucose from the splanchnic organs (*Ashmore, Cahill and Hastings* 1956; *Weber and Cantero* 1958), which can be the results of their direct or indirect effects on glucose and glycogen metabolism. Actually it has been shown that these drugs inhibit the activities of liver gluconeogenetic enzymes (*Ashmore, Cahill and Hastings* 1956; *Berthet, Sutherland and Makman* 1956; *Hawkins, Ashworth and Haist* 1956; *Tybergheim, Halsey and William* 1956) and prevent the glycogen phosphorylase reactivation in the fasting animal (*Berthet, Sutherland and Makman* 1956), producing a relative increase in liver glycogen concentration (*Baender and Scholz* 1956). These results have not been always reproduced (*Murphy and Anderson* 1974) and have been acquired with either "in vitro" preparations or with short term experiments. Recently, the long term effect of oral sulfonylureas on blood glucose, amino acid levels and gluconeogenic abilities have been studied (*Alemany, Palou, Codina and Herrera* 1977; *Codina, Lasunción and Herrera* 1977). In the present paper it has been determined the effect of chronic treatment with these drugs on the activities of both liver and muscle glycogen phosphorylase, in an intent to obtain a better understanding of the glucose-glycogen metabolism alterations observed in those studies.

Materials and Methods

Female virgin Wistar rats were housed in individual metabolic cages under temperature and light controlled environment (12 hours on -12 hours off; 23 ± 2°C). The sulfonylureas were administered twice daily during 29 consecutive days via gastric sonda, suspended in 0.5% CM-ellulose and 0.3% Tween 80 at the concentrations of 100 mg of tolbutamide/kg body weight and 5 mg of either glienclamide or glipentide/kg. Controls received the suspending medium. On day 29 rats were killed by decapitation, and fragments of liver and hind leg muscle were rapidly dissected and suspended in chilled Krebs-Ringer bicarbonate buffer containing 1 g/l dextran, 500 mg/l of defatted bovine serum albumin and 1 mg/l of Triton X-100. Samples were homogenized in the same medium using a glass-teflon motor driven Potter-Elvehjem homogenizer. After 30 minutes of centrifugation at 2,000 x g in the cold room, aliquots of the supernatants were used for total and independent (a) glycogen phosphorylase (E.C. 2.4.1.1.), using the method of Cori (*Fischer and Krebs* 1964) incubating the samples in 32.5 mM glucose-1-phosphate and 50 mg/ml of shellfish glycogen with (out) 100 mM AMP-sodium salt. The reaction was stopped by boiling the samples 1 minute at 100°C and after centrifugation phosphate concentrations in the supernatants was determined by the method of *Fiske and Subbarow* (1925). Each sample was incubated at three different times, and initial velocities were plotted and used as true activities.

Results and Discussion

In the Table it can be seen the glycogen phosphorylase activities in the liver of controls and rats chronically treated with sulfonylureas for 29 days. The animals treated with either glipentide or glibenclamide show significant decreased activity of both total and independent glycogen phosphorylase in liver. The levels of total activity found in the controls are in accordance with those found in the literature, both for liver and muscle (*Lederer and Stalmans* 1976). In the liver, the percentage of a versus total phosphorylase activity is the same for controls and for the chronically treated animals, indicating that the action of these drugs upon this system probably does not interfer with interconversion of both forms of the enzyme.

Table. Activity of glycogen phosphorylase in liver and muscle of rats treated chronically with oral sulfonylureas

Enzyme	Control	Tolbutamide	Glibenclamide	Glipentide
Liver total phosphorylase	0.342 ± 0.047	0.487 ± 0.114 N.S.	0.056 ± 0.011 **	0.038 ± 0.019 **
Liver a phosphorylase	0.078 ± 0.013	0.099 ± 0.009 N.S.	0.014 ± 0.002 **	0.010 ± 0.003 **
Liver a phosphorylase as percent of total activity	22.64 ± 3.08	23.69 ± 3.16 N.S.	26.18 ± 1.52 N.S.	24.67 ± 1.96 N.S.
Muscle b phosphorylase	1.826 ± 0.126	1.433 ± 0.199 N.S.	1.559 ± 0.232 N.S.	1.477 ± 0.285 N.S.

Values are expressed in microkatals/gram of tissue, and are the mean ± S.E.M. of 4 to 6 different animals.
Significance versus controls: ** = $p < 0.01$; N.S. = $p > 0.05$

The fact that the lower liver phosphorylase activity in glibenclamide and glipentide treated animals is not due to a predominance of active (a) versus inactive (b) form but to a decrease of the activity of both forms together, united to a constancy in the proportions of the two forms of the enzyme present seems to point towards an effect of these drugs, in long term treatment, upon the pathway of release of glucose from glycogen in the liver. This effect upon the liver glycogen phosphorylase activity seems unrelated with the insulinotropic action of these drugs, as it is known that insulin provokes important changes in the interconversion of the a and b forms of the enzyme (*Bishop, Goldberg and Larner* 1971). The proportion of the a activity versus total is kept constant, meaning that there is no interference of the treatment upon this interconversion as compared with controls. On the other hand, this effect upon glycogen phosphorylase is in accordance with the data on glycogen and glucose synthesis from alanine indicated by *Alemany, Palou, Codina and Herrera* (1977) for glibenclamide and glipentide in chronically treated rats.

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The activities in muscle are not affected by the drugs; this lack of effect agrees with the energy-supplying role of muscle phosphorylase as compared with the role of liver phosphorylase, that helps to regulate the hepatic glucose efflux, and hence is a critical site for control of glycemia.

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Transrenal Gradient of Serum Somatostatin-Like Immunoreactivity in the Rat

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Introduction

Somatostatin (growth hormone release inhibiting hormone; GHRIH) is a peptide hormone with diverse inhibitory effects and a widespread tissue distribution. In spite of extensive in vivo and in vitro experimentation, its metabolism in vivo is poorly understand. During the validation of the measurement of somatostatin by radioimmunoassay and its characterization in biological fluids, it was discovered that significant levels of somatostatin-like immunoreactivity were present in human urine (*Kronheim, Berelowitz and Pimstone* 1977), raising the possibility of a physiological role for the kidney in the metabolic clearance of somatostatin. To investigate this concept further, serum levels of somatostatin-like immunoreactivity were measured in blood samples obtained simultaneously from the renal vein and abdominal aorta of rats.

Materials and Methods

Under light ether anaesthesia the renal veins and abdominal aorta were exposed in 30 male Long-Evans rats. Blood samples were obtained simultaneously from the left renal vein and aorta. Blood was collected into Trasyloi (500 KIU/ml), placed on ice and centrifuged. Somatostatin-like immunoreactivity was determined in the serum so obtained by a previously validated and characterised immunoassay (*Berelowitz, Kronheim, Pimstone and Shapiro* 1978).

Results

The mean immunoassayable somatostatin level of abdominal aortic blood was 0.243 ± 0.02 ng/ml (mean ± SEM) as compared to a mean level of renal venous blood of 0.161 ± 0.009 ng/ml (mean ± SEM), thus demonstrating a significant ($p < 0.001$) transrenal gradient.

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Discussion

The renal, hepatic and tissue clearance of somatostatin and other hypothalamic hormones such as TRH and LHRH is as yet poorly understood. A transhepatic gradient of serum somatostatin-like immunoreactivity has been demonstrated (*Schusdziarra, Dobbs, Harris and Unger* 1977; *Berelowitz, Kronheim, Pimstone and Shapiro* 1978), raising the possibility of some physiological action on the liver, or a degree of metabolic clearance by the liver. The significant difference between levels of somatostatin-like immunoreactivity in aortic and renal venous blood in the rat again highlights the importance and value of regional blood sampling and, coupled with the presence of somatostatin in urine, suggests a possible physiological role for the kidney in the metabolic clearance of endogenous somatostatin. Studies on the metabolic clearance of exogenously administered somatostatin in normal human subjects and patients with chronic renal or chronic liver disease, are at present in progress.

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