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THE ACTIONS OF CARBENOXOLONE ON GLYCOGEN CATABOLISM AND OXYGEN CONSUMPTION IN LIVERS OF FED RATS

Renato Polimeni Constantin[1]; Rodrigo Polimeni Constantin[2]; Leandro Silva Pivato[3]

ABSTRACT: Carbenoxolone is a derivative of glycyrrhetinic acid, the active principle of licorice (*Glycyrrhiza glabra*), a medicinal root. The pharmacological properties attributed to carbenoxolone are related to its inhibitory actions on the 11β-hydroxysteroid dehydrogenase and gap junction channels. Recent studies have shown that carbenoxolone also induces swelling and membrane potential collapse in mitochondria. These effects were related to hydrogen peroxide generation and mitochondrial permeability transition (MPT) induction, indicating possible toxicological actions of carbenoxolone at the mitochondrial level, which could trigger the apoptotic pathway. The data of these previous reports are pointing, thus, in the direction of a possible action of carbenoxolone on the bioenergetic functions of mitochondria, which could in turn cause toxic metabolic changes in the liver. For this reason, the present work was planned to investigate if carbenoxolone affects glycogenolysis and glycolysis in the rat liver. Male Wistar rats, weighing 180 to 220 g, fed with a standard laboratory diet were utilized. The isolated liver was perfused in the non-recirculating system. The perfusion fluid was Krebs/Henseleit-bicarbonate buffer (pH 7.4), saturated with a mixture of O_2 and CO_2 (95:5) by means of a membrane oxygenator and simultaneously heated to 37°C. For measuring glycogen catabolism (glycogenolysis and glycolysis) livers from ad libitum fed rats were utilized. In livers from fed rats carbenoxolone increased oxygen uptake, glycolysis and glycogenolysis, which is an expected combination of phenomena for decreased mitochondrial ATP formation.

KEY WORDS: Carbenoxolone; Glycogenolysis; Glycolysis; Liver Perfusion.

1 INTRODUCTION

Carbenoxolone is a derivative of glycyrrhetinic acid, the active principle of licorice (*Glycyrrhiza glabra*), a medicinal root (MCHARDY, 1969). The pharmacological properties attributed to carbenoxolone are related to its inhibitory actions on the 11β-hydroxysteroid dehydrogenase (JELLINCK et al., 1993) and gap junction channels (DAVIDSON; BAUMGARTEN, 1988). It was observed that carbenoxolone, by blocking the gap junctional intercellular communications (GJIC) reduces the efficiency of tumor cell diapedesis (POLLMAN et al., 2005), a process involved in the migration of these malignant cells (metastasis). In addition to blocking the gap junctional intercellular communications, glycyrrhetinic acid and its derivatives exhibit anti-inflammatory (GOLDBERG et al., 1996), antiulcerous and antiviral activities. Recent studies have shown that carbenoxolone also

[1] Acadêmico do curso de farmácia. Departamento de Bioquímica, Laboratório de Metabolismo Hepático, Universidade Estadual de Maringá (UEM), Maringá – PR. Bolsistas do Programa de Bolsas de Iniciação Científica do PIBIC/CNPq-UEM (PIBIC-UEM). rereconstantin@yahoo.com
[2]Doutorando em Ciências Biológicas. Área de Concentração em Biologia Celular e Molecular. Departamento de Bioquímica, Laboratório de Metabolismo Hepático, Universidade Estadual de Maringá (UEM), Maringá – PR. rrconstantin@hotmail.com
[3]Docente da Faculdade Ingá (UNINGÁ). Departamento de Ciências Biológicas, Maringá – PR. lehandruls@hotmail.com

induces swelling and membrane potential collapse in mitochondria. These effects were related to hydrogen peroxide generation and mitochondrial permeability transition (MPT) induction, indicating possible toxicological actions of carbenoxolone at the mitochondrial level, which could trigger the apoptotic pathway. Since impairment of the bioenergetic capacity of mitochondria could cause metabolic changes in the liver, the present work was undertaken to investigate the action of carbenoxolone on glycogenolysis and glycolysis in the perfused rat liver.

2 MATERIALS AND METHODS

The liver perfusion apparatus was built in the workshops of the University of Maringá. Carbenoxolone and all enzymes and coenzymes used in the enzymatic assays were purchased from Sigma Chemical Co. (St. Louis, US). All other chemicals were from the best available grade (98-99.8% purity).

Male Wistar rats (weighing 180-220 g) fed with a standard laboratory diet (Nuvital - Nuvilab CR-1®) were used in all experiments. All experiments were conducted in strict adherence to the guidelines of the Ethics Committee for Animal Experiments of the University of Maringá. For the surgical procedure, the animals were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg kg^{-1}). Haemoglobin-free, non-recirculating perfusion was undertaken according to the technique described by Scholz and Bücher (1965). After cannulation of the portal and cava veins, the liver was positioned in a plexiglas chamber. Flow was maintained constant by a peristaltic pump (Miniplus 3, Gilson, France) and was adjusted to between 30 and 32 ml min^{-1}, depending on the liver weight.

The perfusion fluid was Krebs/Henseleit bicarbonate buffer (pH 7.4), saturated with a mixture of oxygen and carbon dioxide (95:5) by means of a membrane oxygenator with simultaneous temperature adjustment at 37°C. The composition of the Krebs/Henseleit bicarbonate buffer is as follows: 115 mM NaCl, 25 mM $NaHCO_3$, 5.8 mM KCl, 1.2 mM Na_2SO_4, 1.18 mM $MgCl_2$, 1.2 mM NaH_2PO_4 and 2.5 mM $CaCl_2$. Carbenoxolone was dissolved in the perfusion fluid.

Samples of the effluent perfusion fluid were collected according to the experimental protocol and analysed for their metabolite contents. The following compounds were assayed by means of standard enzymatic procedures: glucose (BERGMEYER, 1974), lactate (GUTMAN; WAHLEFELD, 1974) and pyruvate (CZOK; LAMPRECHT, 1974). The oxygen concentration in the outflowing perfusate was monitored continuously, employing a teflon-shielded platinum electrode adequately positioned in a plexiglass chamber at the exit of the perfusate (SCHOLZ; BÜCHER, 1965). Metabolic rates were calculated from input-output differences and the total flow rates and were referred to the wet weight of the liver.

The statistical significance of the differences between parameters obtained in the liver perfusion experiments was evaluated by means of Student's t-test or by Newman-Keuls test after submitting the data to variance analysis according to context. The results are mentioned in the text as the p values; $p < 0.05$ was the criterion of significance.

3 RESULTS AND DISCUSSION

The action of carbenoxolone on glycogen catabolism and oxygen uptake in the liver of fed rats was examined in the concentration range between 50 and 300 µM. The drug was infused during 20 minutes. The following parameters were measured: glucose release, lactate and pyruvate production and oxygen consumption. Figure 1 shows the time courses of the changes caused by 200 µM carbenoxolone. The infusion of 200 µM carbenoxolone increased both glucose and lactate release. The drug also promoted

increase in oxygen uptake and a small decrease in pyruvate production. These effects were reversible, i.e., they vanished upon cessation of the infusion but the reversion occurred slowly after a lag phase of approximately 5 minutes.

Experiments like those shown in Figure 1 were repeated with several carbenoxolone concentrations and the mean results are summarized in Figure 2. Control values (absence of carbenoxolone) correspond to the basal rates just before the onset of carbenoxolone infusion (10 minutes perfusion time). Rates of oxygen uptake, glycolysis ([lactate + pyruvate]/2) and glycogenolysis (glucose + glycolysis) in the presence of several carbenoxolone concentrations were evaluated at 30 minutes perfusion time. Figure 2 reveals that carbenoxolone was without action on these parameters at concentrations up to 100 µM. Glycogenolysis, however, was increased by 158% and 213%, respectively, with 200 and 300 µM carbenoxolone. For glycolysis the corresponding increments were equal to 178 and 269%, respectively; and, for oxygen uptake 24 and 17%, respectively.

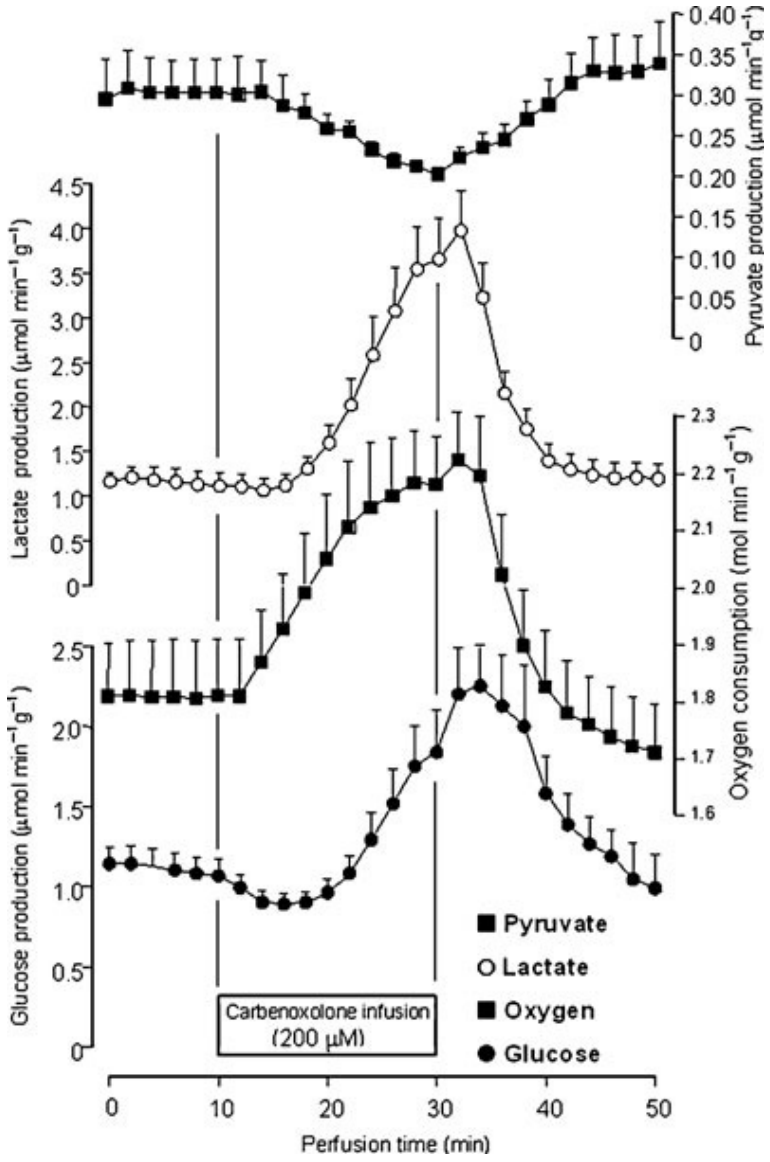

Figure 1. Time course of the effects of 200 µM carbenoxolone on glycogen catabolism and oxygen consumption in livers from fed rats. Samples of the effluent perfusate were withdrawn for metabolite assay. Oxygen in the venous perfusate was monitored polarographically. The carbenoxolone infusion time is indicated by the horizontal bar. Data represent the mean (±SEM) of four liver perfusion experiments.

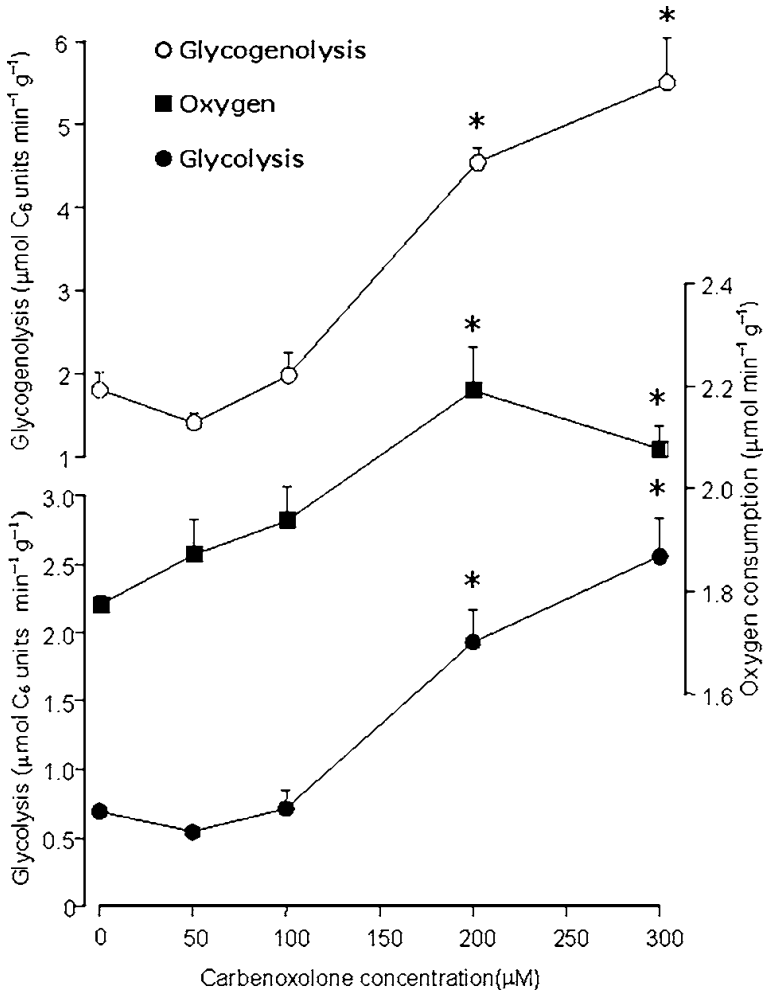

Figure 2. Concentration dependence of the action of carbenoxolone on glycolysis, glycogenolysis, and oxygen consumption in livers from fed rats. The data were obtained from experiments of the kind illustrated in Figure 1. The control values (zero carbenoxolone) correspond to the basal rates found before the onset of carbenoxolone infusion (10 min of perfusion). Rates in the presence of carbenoxolone were evaluated after 30 min of perfusion. Glycogenolysis and glycolysis were calculated from glucose, lactate, and pyruvate production and expressed as glucosyl units: glycogenolysis=glucose+1/2(lactate+pyruvate); glycolysis=1/2(lactate+pyruvate). Each datum point represents the mean (±SEM) of four liver perfusion experiments. Asterisks indicate statistical significance in comparison with the control condition as revealed by variance analysis with post hoc Newman–Keuls test ($p < 0.05$).

4 CONCLUSION

The results of the liver perfusion experiments revealed that carbenoxolone affects the hepatic metabolism probably acting as an inhibitor of the mitochondrial energy transduction. Carbenoxolone increased glycolysis and glycogenolysis, which are expected compensatory phenomena for the decreased mitochondrial ATP formation. It must be mentioned that the effects of carbenoxolone on carbohydrate metabolism are comparable to those caused by classical inhibitors of oxidative phosphorylation, such as 2,4-dinitrophenol (JACOB; DIEM, 1974).

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