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THE ACTIONS OF CARBENOXOLONE ON FRUCTOSE CATABOLISM IN LIVERS OF FASTED RATS

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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 gluconeogenesis in the rat liver. Livers from 24 h fasted Male Wistar rats weighing 200 to 280 g were used in the experiments. The isolated liver was perfused in the non-recirculating system. Carbenoxolone inhibited gluconeogenesis as well as decreased oxygen consumption and stimulated glycolysis from fructose, which is an expected combination of phenomena for decreased mitochondrial ATP formation.

KEY WORDS: Carbenoxolone; Fructose; Gluconeogenesis; 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 induces swelling and membrane potential collapse in mitochondria. These effects were related to hydrogen peroxide generation and mitochondrial permeability transition (MPT)

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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 oxygen consumption and gluconeogenesis from fructose in livers of fasted rats.

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. But in experiments for assess gluconeogenesis, the rats were starved for 24 hours before the surgical removal of the liver. 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 and pyruvate. 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

Experiments were done with fructose at a concentration of 5 mM. It is well known that this substrate can also be transformed into glucose in the liver, but ramification of the fructose pathway at the enolase step can also lead to the production of lactate and pyruvate (SUZUKI-KEMMELMEIER, 1992). The experimental protocol was that one illustrated by Figure 1. Both gluconeogenesis and oxygen uptake increased progressively upon fructose introduction, tending to stabilize at 30 minutes infusion time. The introduction of 100 µM carbenoxolone produced changes in all variables, but their kinetics was relatively complex. Oxygen uptake was rapidly inhibited with a minimum at 34 minutes

perfusion time. This inhibition was followed by a partial recovery. Lactate production was stimulated with a maximum around 46 minutes perfusion time and a partial decline thereafter.

Glucose production was inhibited with a minimum around 54 minutes perfusion time and a partial recovery during the rest of the carbenoxolone infusion time. Pyruvate production, finally, suffered an initial small inhibition, which was followed by a stable stimulation. The same pattern of response was obtained with 25, 50 and 200 µM carbenoxolone with the difference that, in most cases, the extent of the effects was a function of the concentration.

The mean alterations in oxygen uptake and glucose production at the end of the carbenoxolone infusion (66 minutes perfusion time) are summarized in Figure 2. A maximal inhibition of 94% in glucose production was obtained with 200 µM carbenoxolone. The same cabenoxolone concentration produced a decrease of about 75% in the fructose stimulated oxygen consumption. For glucose production 50% inhibition can be expected at a concentration of 86.5 µM as computed by numerical interpolation.

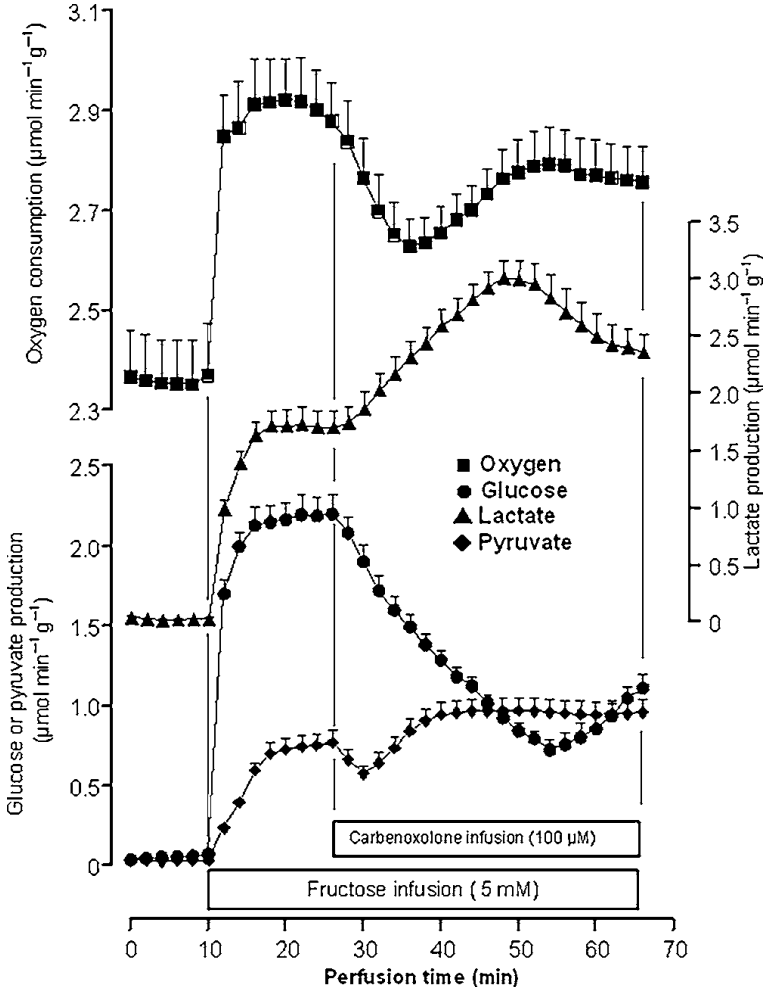

Figure 1. Time course of the effects of 100 µM carbenoxolone on gluconeogenesis from fructose and oxygen consumption in livers from fasted rats. Samples were collected in 2-min intervals and aliquots were used for the enzymatic measurement of glucose, L-lactate, and pyruvate. Oxygen in the venous perfusate was monitored polarographically. The fructose and carbenoxolone infusion times are indicated by horizontal bars.Data representmeans (±SEM) of four liver perfusion experiments.

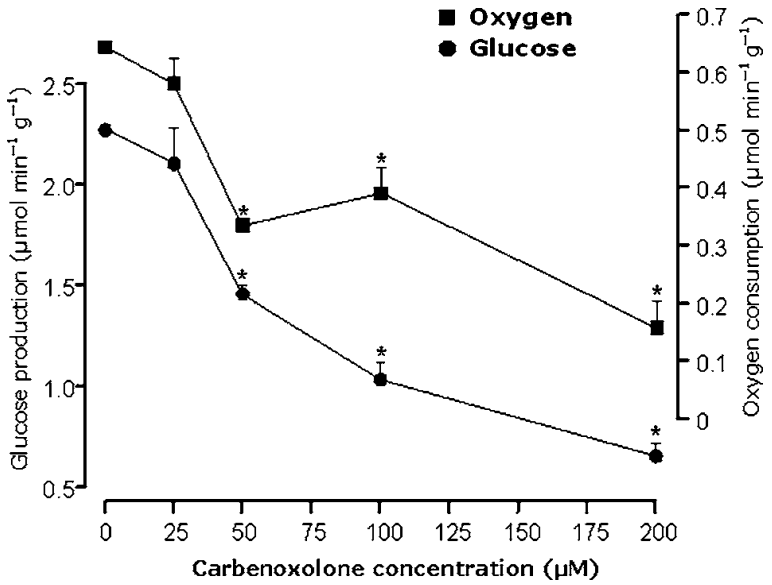

Figure 2. Concentration dependence of the action of carbenoxolone on the metabolic fluxes resulting from fructose metabolism in livers from fasted rats. The data were obtained from experiments of the kind illustrated in Figure 3 with 5 mM fructose as the gluconeogenic substrate. The control values (zero carbenoxolone) correspond to the rates found in the presence of fructose just before the onset of carbenoxolone infusion (30 min of perfusion) minus the basal rates (i.e., before the onset of fructose infusion). Rates in the presence of fructose+carbenoxolone were evaluated after 66 min of perfusion and also subtracted fromthe same basal rates. 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 inhibited gluconeogenesis, a biosynthetic route strictly dependent on energy in the form of ATP. 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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