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THE ACTIONS OF CARBENOXOLONE ON GLUCONEOGENESIS AND OXYGEN CONSUMPTION IN LIVERS OF FASTED RATS USING LACTATE AS EXOGENOUS SUBSTRATE

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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. Male Wistar rats, weighing 180 to 220 g, fed with a standard laboratory diet were utilized. But in this case, the rats were starved for 24 hours before the surgical removal of the liver. 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. In livers of 24-hours fasted rats, carbenoxolone inhibited gluconeogenesis from lactate, as well as inhibited oxygen consumption, which is an expected combination of phenomena for decreased mitochondrial ATP formation.

KEY WORDS: Carbenoxolone; Gluconeogenesis; Lactate; 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),

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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) 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 lactate 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 compound was assayed by means of standard enzymatic procedures: glucose (BERGMEYER, 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

In the first experiments the action of carbenoxolone on oxygen consumption and gluconeogenesis from lactate was investigated in perfused rat livers in order to verify the effects of carbenoxolone on an energy-dependent biosynthetic process. Figure 1 shows the time courses of glucose production and oxygen uptake obtained in a series of experiments in which 2 mM lactate and 100 µM carbenoxolone were infused in livers of 24-hours fasted rats. Both glucose production and oxygen uptake increased progressively

upon introduction of lactate, tending to stabilize at 30 minutes infusion time. The introduction of 100 µM carbenoxolone produced progressive decreases in both gluconeogenesis and oxygen uptake. It has been amply demonstrated that the perfused rat liver maintains a stable gluconeogenic activity for at least two hours (ACCO et al., 2004). Experiments like those shown in Figure 1 were repeated with several carbenoxolone concentrations and the results are summarized in Figure 2. The control values (absence of carbenoxolone) correspond to the rates found in the presence of lactate just before the onset of carbenoxolone infusion (30 minutes perfusion time) minus the basal rates (i.e., before the onset of lactate infusion). Rates in the presence of lactate + carbenoxolone were evaluated at 70 minutes perfusion time and also subtracted from the basal rates. Figure 2 reveals that both oxygen uptake and gluconeogenesis were a negative function of the carbenoxolone concentration. An inhibition of about 97% in glucose production was observed at the concentration of 200 µM; 50% inhibition can be expected at a concentration of 53.1 µM, as computed by numerical interpolation. Oxygen uptake was also diminished progressively in the presence of carbenoxolone. The inhibition degree of the lactate stimulated respiration was 80% at 200 µM carbenoxolone; 50% inhibition can be expected at a concentration of 84 µM, as computed by numerical interpolation.

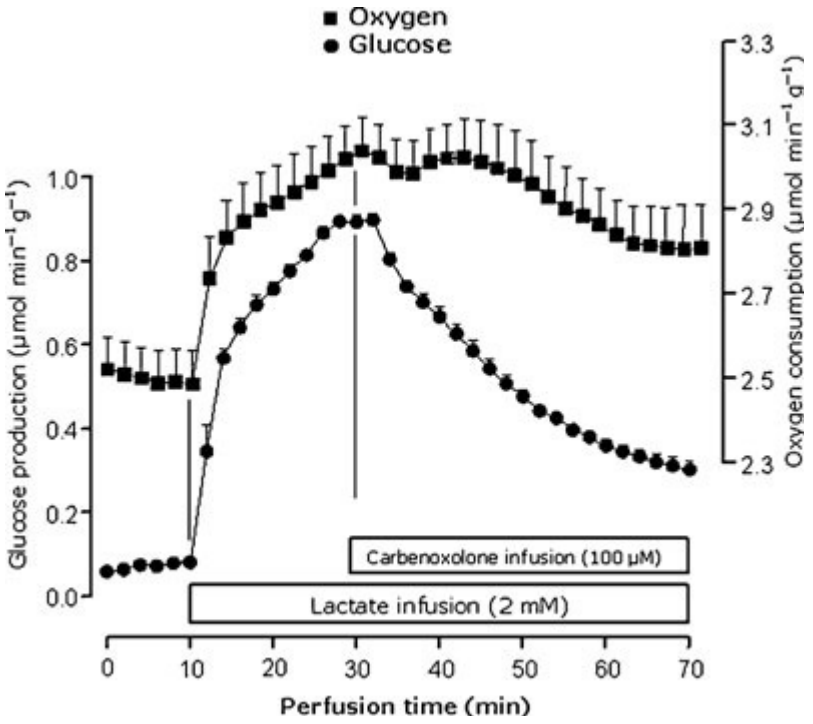

Figure 1. Time course of the effects of 100 µM carbenoxolone on gluconeogenesis from lactate and oxygen consumption in livers from fasted rats. Samples of the effluent perfusate were withdrawn for the measurement of glucose. Oxygen in the venous perfusate was monitored polarographically. The lactate and carbenoxolone infusion times are indicated by horizontal bars.Data representmeans (±SEM) of four liver perfusion experiments.

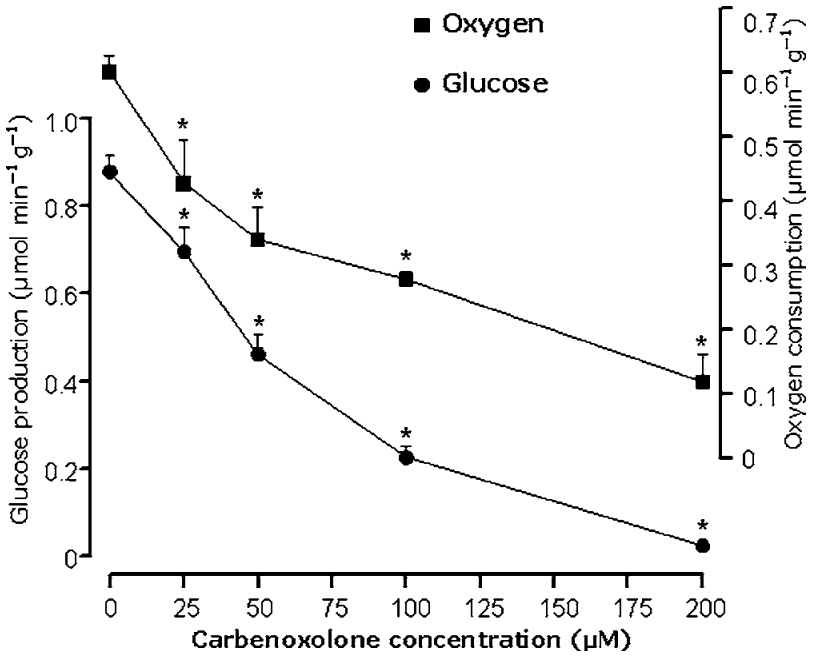

Figure 2. Concentration dependence of the action of carbenoxolone on glucose production from lactate and oxygen consumption in livers from fasted rats. The data were obtained from experiments of the kind illustrated by Figure 1. The control values (zero carbenoxolone) correspond to the rates found in the presence of lactate just before the onset of carbenoxolone infusion (30 min of perfusion) minus the basal rates (i.e., before the onset of lactate infusion). Rates in the presence of lactate+carbenoxolone were evaluated after 70 min of perfusion and also subtracted from the same basal rates. Each datum point represents themean (±SEM) of three to five 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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