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THE EFFECTS OF FISETIN ON GLYCOGENOLYSIS AND GLYCOLYSIS IN THE RAT LIVER

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ABSTRACT: Flavonoids have been proposed to exert beneficial effects in a multitude of disease states, including cancer, cardiovascular disease, and neurodegenerative disorders. The most known biological effects of the flavonoids, is no doubt their antioxidant action, which represents protection of tissues against the action of free-radicals and diminution of lipid peroxidation. Fisetin is a flavonoid found in the smoke tree (*Cotinus coggyria*) and is also widely distributed in fruits and vegetables such as strawberry, apple, persimmon, grape, onion, and cucumber. It has been reported to suppress the proliferation of tumor cells, such as prostate cancer, liver cancer, colon cancer, and leukemia. The present work was planned to investigate if fisetin affects glycogenolysis and glycolysis in the rat liver. Male Wistar rats, weighing 200 to 280 g, were utilized in all experiments. 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). For measuring glycogen catabolism (glycogenolysis and glycolysis) livers from ad libitum fed rats were utilized. Fisetin inhibited glycogenolysis, as revealed by the decrease of glucose, lactate and pyruvate release from endogenous glycogen. Lactate and pyruvate productions from endogenous glycogen (glycolysis) were diminished by fisetin, the diminution in pyruvate production being less pronounced. Overall, the inhibition of glycogenolysis indicates that fisetin could be potentially useful as an antidiabetic agent, although further studies are needed to validate its therapeutic use.

KEY WORDS: Fisetin; Flavonoids; Glycogenolysis; Glycolysis; Liver.

1 INTRODUCTION

Fisetin (3,7,3´,4´-tetrahydroxiflavone) is a flavonoid dietary ingredient found in the smoke tree (*Cotinus coggyria*) and is also found in fruits and vegetables such as strawberry, apple, persimmon, grape, onion, and cucumber at concentrations of 2 to 160 µg/g (ARAI et al., 2000). It exhibits a wide variety of pharmacological properties, including neurotrophic, antioxidant, anti-inflammatory and antioangiogenic effects. It has been reported to suppress the proliferation of tumor cells, such as prostate cancer, liver cancer, colon cancer, and leukemia. Certain bioflavonoids, including fisetin, inhibit glycolysis in Ehrlich ascites tumor cells (SUOLINNA et al., 1974; SUOLINNA et al., 1975).

Flavonoids have also been identified as the antidiabetic components in a number of traditional ethnic remedies. Although there has been considerable scientific progress over

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the past few years in the unraveling of the effect and mechanism of action of flavonoids, the mechanisms whereby these compounds exert their hypoglycemic action have rarely been investigated. There are indications that the liver is implicated in this hypoglycemic action.

The authors have suggested that flavonoids improve hyperglycemia by regulating the activity of hepatic enzymes involved in glycolysis and glycogenolysis (JUNG et al., 2004). Naringin reduced the activity of hepatic glucose 6-phosphatase and phosphoenolpyruvate carboxykinase (JUNG et al., 2004). Quercetin, which is a flavonol extensively studied in relation to its potential role in diabetes, inhibits both glucose degradation and production (VESSAL et al., 2003; GASPARIN et al., 2003a,b). Also, quercetin has been demonstrated to possess a potent inhibitory effect on both glycogen phosphorylase activities a and b in isolated muscle (KATO et al., 2008b).

There is no information about the actions of the flavonol fisetin on liver glucose metabolism. Thus, we here report the study of the potential acute effects of fisetin on glycogenolysis and glycolysis in the isolated perfused rat liver.

2 MATERIALS AND METHODS

The liver perfusion apparatus was built in the workshops of the University of Maringá. Fisetin was purchased from Sigma-Aldrich (ST Louis, MO, USA). Enzymes and coenzymes used in the enzymatic assays were purchased from Sigma-Aldrich (St Louis, MO, USA). Male Wistar rats (weighing 200-280 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. 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 35 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 $CaCl_2$. Fisetin was dissolved in the perfusion fluid. Solubilization was achieved by the simultaneous addition of an equivalent amount of NaOH. For analyse Glycogen catabolism livers of fed rats were used in the experiments.

Samples of the effluent perfusion fluid were collected at 2-min intervals and analyzed for their metabolite content. Lactate and pyruvate were assayed by means of standard enzymatic procedures using L-lactate dehydrogenase. Interference by fisetin (absorbance at 340 nm) was excluded by running blanks. The oxygen concentration in the outflowing perfusate was monitored polarographically, employing a Teflon-shielded platinum electrode adequately positioned in a plexiglas chamber at the exit of the perfusate (CLARK, 1956). Fisetin interferes with the glucose oxidase reaction and, for this reason, glucose was measured colorimetrically by means of an *o*-toluidine method (DUBOWSKI, 1962).

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

3 RESULTS AND DISCUSSION

The actions of fisetin on oxygen consumption, glycogenolysis and glycolysis were investigated in the perfused liver of fed rats. Several experiments were undertaken in which fisetin was infused in the range between 50 and 300 μM during 30 minutes. Figure 1 illustrates the results of a representative experiment, showing the time courses of the changes caused by 200 μM fisetin. These livers were perfused with substrate-free perfusion fluid, in an open system. Under these conditions, the livers release glucose, lactate and pyruvate from endogenous glycogen stores. The infusion of 200 μM fisetin at 10 min of perfusion produced decreases in glucose and lactate release. At the end of the infusion, glucose release was reduced by 46% ($p<0.001$) compared with the rates measured before the infusion of the drug. A small inhibition of oxygen consumption also occurred. Even when the infusion of fisetin was interrupted at 30 minutes, its inhibitory effects on lactate and pyruvate production persisted during the next 20 minutes.

Figure 2 shows the mean results of experiments in which the effects of several concentrations of fisetin on glycogenolysis, glycolysis and oxygen uptake were estimated at the end of the infusion period. The concentration dependence of the effects on oxygen uptake exhibits an irregular curve. The maximal inhibitions of glycogenolysis (49%, $p<0.001$) and glycolysis (59%, $p<0.001$) were obtained with the concentration of 200 μM.

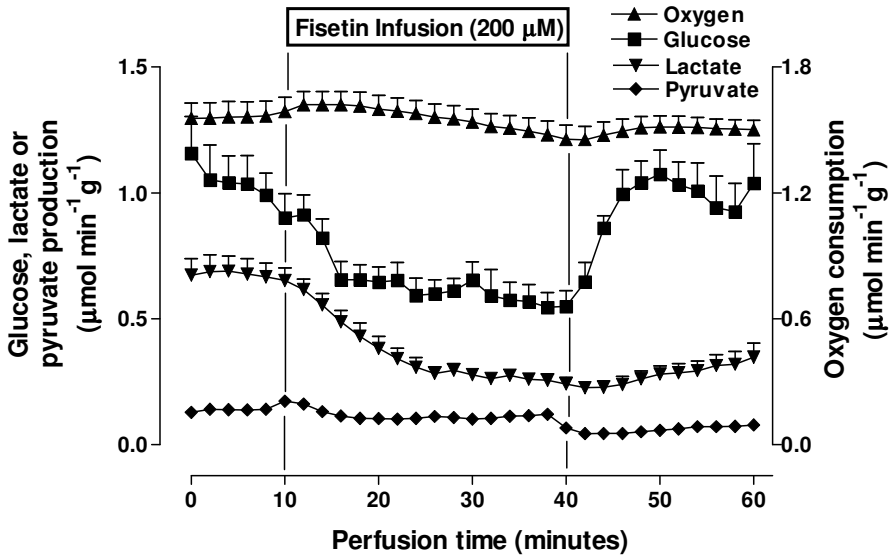

Figure 1. Time course of the changes caused by fisetin in glycogen catabolism and oxygen uptake. Livers from fed rats were perfused as described in Materials and methods. Fisetin 200 μM was infused at 10-40 min, as indicated by the horizontal bar. The effluent perfusate was sampled in 2-min intervals and analyzed for glucose, lactate and pyruvate. Oxygen consumption was followed polarographically. Each data point represents the means of six liver perfusion experiments. Bars are standard errors of the mean.

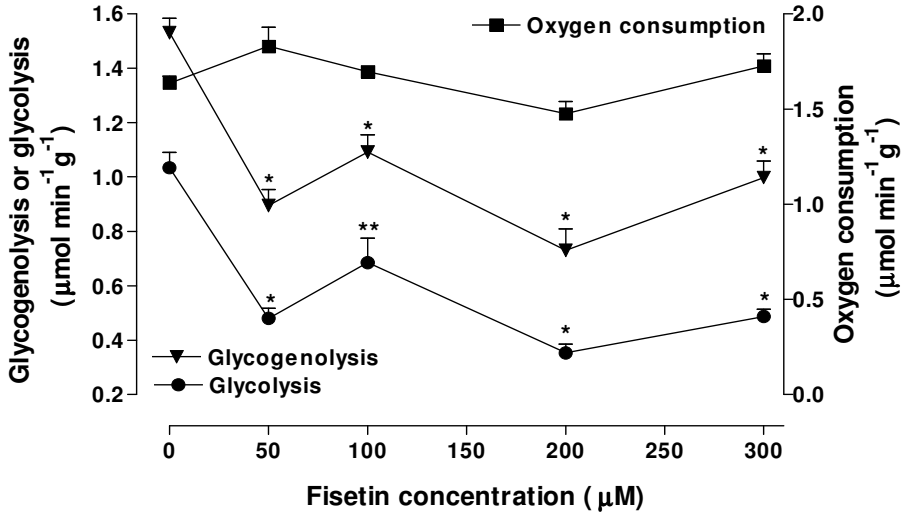

Figure 2. Concentration dependencies of the effects of fisetin on glycogen catabolism and related parameters. Livers from fed rats were perfused with Krebs/Henseleit-bicarbonate buffer (pH 7.4) with different fisetin concentrations in the range between 50 and 300 µM. The experimental protocol was the same illustrated in Figure 1. Rates of glycogenolysis and glycolysis were calculated from glucose, lactate and pyruvate production [glycogenolysis: glucose + 1/2 (lactate + pyruvate)]; glycolysis: (lactate plus pyruvate) and were expressed as glycosil units. Asterisks indicate statistical significance in comparison with the control condition as revealed by variance analysis with post hoc Newman-Keuls testing (**p<0.01, *p<0.001).

4 CONCLUSION

The results of this investigation revealed that fisetin inhibits glucose release from the liver of fed rats. The main cause for the inhibition of glycogen breakdown cannot be inferred from the available data, but it may be related to glycogen phosphorylase inhibition, since several flavonoids have been shown to exert inhibitory actions on this enzyme. The reduction of lactate and pyruvate production from endogenous glycogen was probably consequence of reduced glycogen breakdown. However, a direct action of fisetin on some step of the glycolytic pathway should be also considered.

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