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THE EFFECTS OF FISETIN ON THE RESPIRATORY ACTIVITY OF ISOLATED MITOCHONDRIA

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ABSTRACT: Fisetin (3,7,3´,4´-tetrahydroxyflavone) is a flavonoid dietary ingredient found in fruits and vegetables. It exhibits a wide variety of pharmacological properties, including neurotrophic, antioxidant, anti-inflammatory, antidiabetic, antioangiogenic and anticarcinogenic effects. The most known biological effect of flavonoids is their antioxidant action. At high doses, however, the potentially toxic effects of fisetin and other flavonoids have to be taken into account, because it has been shown that in elevated doses, flavonoids, can act as mutagens, prooxidants with the generation of free radicals and as inhibitors of enzymes involved in energy and hormonal metabolism. The precise mechanisms by which flavonoids exert their beneficial or toxic actions remain unclear. The present work was planned to investigate the action of fisetin on respiratory activity of isolated rat liver mitochondria. Male Wistar rats, weighing 200 to 280 g, fed with a standard laboratory diet were utilized. Oxidative phosphorylation and energy transduction were evaluated in isolated mitochondria. Mitochondrial respiration was measured polarographically. In isolated mitochondria, fisetin diminished state III respiration dependent on succinate or α-ketoglutarate, increased state IV respiration with the FAD-dependent substrate and diminished the respiratory control and ADP/O ratios. A reduction in the cellular ATP levels and an inhibitory action on energy-dependent processes are expected responses to a decrease in the efficiency of mitochondrial energy transduction. The data of the present work show that fisetin is able to affect mitochondrial energy metabolism.

KEY WORDS: Fisetin; Flavonoids; Liver; Mitochondria.

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 antiangiogenic 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).

The most known biological effects of the flavonoids, including quercetin and fisetin, is no doubt their antioxidant action, which represents protection of tissues against the

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action of free-radicals and diminution of lipid peroxidation. However, the antioxidant and anticancer effects of quercetin and other flavonoids are not an unanimity, since it has been shown that significant protective effects due to quercetin would only be achieved upon the ingestion of high doses. At high doses, however, the potentially toxic effects of quercetin and other flavonoids have to be taken into account. It has been reported that flavonoids can act as mutagens, prooxidants with the generation of free radicals (CONSTANTIN; BRACHT, 2008) and as inhibitors of enzymes involved in energy metabolism and hormone actions (GASPARIN et al., 2003a,b).

The present work was planned to investigate if fisetin shares with quercetin the ability to affect liver energy metabolism. For this reason, parameters of energy metabolism were evaluated in isolated rat liver mitochondria.

2 MATERIALS AND METHODS

All experiments were conducted in strict adherence to the guidelines of the Ethics Committee for Animal Experiments of the University of Maringá.

Fed rats, weighing between 200 and 280 g, were decapitated and their livers removed immediately and cut into small pieces. These fragments were suspended in a medium containing 0.2 M mannitol, 75 mM sucrose, 2.0 mM Tris-HCl (pH 7.4), 0.2 mM EGTA, 0.1 mM phenylmethylsulphonyl fluoride (PMSF), and 50 mg% fatty acid-free bovine serum albumin. Homogenization was carried out in the same medium by means of a *Dounce* homogenizer. After homogenization, the mitochondria were isolated by differential centrifugation according to Voss et al. (1961) using a sucrose-manitol isolation medium, and suspended in the same medium, which was kept at 0-4 ℃. Oxygen consumption by coupled isolated mitochondria was measured polarographically using a teflon-shielded platinum electrode (CLARK, 1956; VOSS et al., 1961).

Mitochondria were added for a final protein concentration around 2 mg ml^{-1}, and were incubated in the closed oxygraph chamber in a medium containing 250 mM mannitol, 10 mM KCl, 10 mM tris(hydroxymethyl) aminomethane-HCl (Tris-HCl, pH 7.4), 0.2 mM ethylene glycol tetraacetic acid (EGTA), 5 mM potassium phosphate and 50 mg% fatty-acid free bovine serum albumin. Fisetin, purchased from Sigma-Aldrich (ST Louis, MO, USA), was added to the incubation medium as a solution in dimethylformamide (100-600 µM).

The substrates were succinate (10 mM) and α-ketoglutarate (10 mM). Appropriate control experiments were performed in order to exclude solvent effects. ADP, at a final concentration of 125 µM, was added at appropriate times in order to evaluate the action of fisetin on oxidative phosphorylation. Rates of oxygen consumption were computed from the slopes of the recorder tracings. The ADP/O ratios and the respiratory control ratios (RC) were computed from the recorder tracings.

Protein contents of all experiments with mitochondria were measured using the method of Lowry et al. (1951).

The statistical significance of the differences between parameters was evaluated by means of 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 effects of fisetin on the respiratory activity of isolated mitochondria were investigated using NAD^+-dependent (α-ketoglutarate) and FAD-dependent (succinate) substrates in the absence (state II respiration), presence of exogenously added ADP (state III respiration) or after ADP exhaustion (state IV respiration). The data shown in table 1

reveals that the mitochondrial respiration driven by both substrates in the presence of ADP (state III respiration) was clearly decreased in a concentration dependent manner by fisetin. The inhibitory effect was more pronounced with the NAD^+-dependent substrate and it reached 42% ($p<0.001$) at the concentration of 300 µM, while with succinate the inhibition was about 23.5% ($p<0.05$). The oxygen consumption in the absence of ADP, was stimulated by fisetin in the concentration range between 100 to 600 µM only when succinate was the substrate. The state IV respiration tended to be stimulated with increasing drug concentrations, but statistical significance was lacking with α-ketoglutarate. These events produced a progressive decrease of the respiratory control ratio (RC) with both substrates.

Besides the respiratory control ratios (RC), table 1 also shows the effects of fisetin on the ADP/O ratios. With the NAD^+-dependent substrate no respiratory control was found at the concentration of 500 µM. At this concentration, evidently, no ADP/O ratio could be evaluated, but with succinate the ADP/O ratio was significantly decreased only at higher drug concentrations.

Table 1. The actions of fisetin on mitochondrial respiration driven by α-ketoglutarate (α-KG) and succinate in the presence and absence of exogenously added ADP.

Substrate	Fisetin (µM)	Substrate respiration	State III respiration	State IV respiration	Respiratory control ratio	ADP/O ratio
		($\mu mol\ O_2\ min^{-1}\ mg\ protein^{-1}$)				
α-KG (n = 6)	0	4.58 ± 0.29	17.27 ± 1.68	4.95 ± 0.31	3.53 ± 0.27	2.48 ± 0.06
	100	4.12 ± 0.32	13.84 ± 1.25$^+$	5.57 ± 0.54	2.50 ± 0.11*	2.16 ± 0.09$^+$
	200	3.51 ± 0.63	10.51 ± 0.80*	5.29 ± 0.31	1.98 ± 0.09*	1.82 ± 0.10*
	300	4.17 ± 0.54	10.02 ± 1.44*	6.28 ± 0.66	1.57 ± 0.07*	1.57 ± 0.08*
	400	4.50 ± 0.42	8.47 ± 0.93*	6.91 ± 0.59	1.22 ± 0.06*	1.00 ± 0.21*
	500	3.76 ± 0.70	7.31 ± 0.90*	7.31 ± 0.90	1.00 ± 0.00*	—
	600	2.04 ± 0.23$^+$	5.04 ± 0.68*	5.04 ± 0.68	1.00 ± 0.00*	—
Succinate (n = 6)	0	13.94 ± 1.46	62.95 ± 6.35	12.61 ± 1.09	5.10 ± 0.18	1.77 ± 0.08
	100	16.88 ± 1.87	64.24 ± 5.45	17.50 ± 1.68$^+$	3.69 ± 0.09*	1.73 ± 0.09
	200	19.30 ± 1.08$^+$	57.11 ± 4.19	19.88 ± 1.40**	2.87 ± 0.07*	1.59 ± 0.09
	300	18.44 ± 1.34	48.10 ± 2.99$^+$	20.88 ± 1.19**	2.31 ± 0.10*	1.50 ± 0.08
	400	21.57 ± 0.64**	43.49 ± 1.52**	22.91 ± 1.52*	1.93 ± 0.12*	1.41 ± 0.09
	500	24.62 ± 1.04*	40.88 ± 1.25**	27.82 ± 0.89*	1.47 ± 0.04*	1.07 ± 0.07*
	600	26.01 ± 1.43*	34.96 ± 2.55*	26.62 ± 1.93*	1.31 ± 0.03*	1.06 ± 0.11*

Data are the means ± standard errors of six experiments with identical protocol. Statistical significance relative to the controls is indicated by asterisks and crosses. $^+p<0.05$, $^{**}p<0.01$, $^*p<0.001$, ANOVA with Newman-Keuls test.

4 CONCLUSION

The data of the present work show that fisetin is able to affect mitochondrial energy metabolism. The effects observed in isolated mitochondria indicate that fisetin acts through different mechanisms. The observations that fisetin stimulates oxygen consumption with the FAD-dependent substrate, reduces state III respiration, control respiratory ratios and ADP/O ratios, suggest that fisetin could be acting as an uncoupler of oxidative phosphorylation. An inhibitory action on ATP-synthase is indicated by the inhibition of respiration in the presence of ADP with both, NAD^+ and FAD-dependent substrates. A reduction in the cellular ATP levels and an inhibitory action on energy-dependent

processes are expected responses to a decrease in the efficiency of mitochondrial energy transduction.

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