INFLUENCE OF NATURAL POLYPHENOLIC FRACTIONS ON CELLULAR RESPIRATION

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ABSTRACT – We have studied the influence of two polyphenolic fractions – methanolic (PMF) and ethanolic (PEF) – extracted from the leaves of the medicinal plant Asclepias syriaca upon cellular respiration. Experiments were performed in vitro on the cells from liver, sartorius striated muscle and stomach smooth muscle of frog (Rana ridibunda, Pall.), determining the cellular oxygen consumption by the Warburg micromanometric method. Polyphenol effects were different, depending on the nature of used fraction and cellular type. Therefore, after 90 minutes of experiencing on liver, the both polyphenolic fractions stimulated cellular respiration, compared to the untreated control. The striated muscle PMF has inhibited and PEF has stimulated respiratory processes. In stomach muscle, reverse effects were noticed: PMF has stimulated and PEF has slightly inhibited cellular respiration. The results pointed out the specific action of these polyphenolic agents on cellular respiration and energetic metabolism processes, also allowing the estimate of their useful pharmacological properties.

Key words: polyphenolic fraction, cellular respiration, energetic metabolism

Rezumat – Influenţa unor produşi polifenolici naturali asupra respiraţiei celulare. *S-a urmărit influenţa a două fracţii polifenolice asupra respiraţiei unor tipuri diferite de celule animale, studiindu-se o fracţie metanolică (PMF) şi alta etanolică (PEF), extrase din frunzele plantei medicinale Asclepias syriaca. Experimentele s-au realizat " in vitro", determinându-se, timp de 90 de minute, consumul respirator de oxigen al celulelor de ficat, muşchi striat sartorius şi muşchi neted stomacal de broască (Rana ridibunda, Pall.), prin metoda micromanometrică Warburg.*

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Efectele respiratorii au fost diferite, în funcție de natura fracției polifenolice şi tipul celulelor. Astfel, după 90 de minute, la ficat ambele fracții au stimulat respirația celulară, comparativ cu lotul martor, netratat, însă, la țesutul muscular, efectele au fost mai diverse: la mușchiul striat, PMF a indus o inhibare, iar PEF o stimulare a respirației, în timp ce la stomac, efectele au fost inversate – o stimulare sub acțiunea PMF şi o uşoară depresare a consumului de oxigen sub influența PEF. Rezultatele evidențiază efectele specifice ale acestor agenți polifenolici asupra proceselor respirației celulare şi asupra metabolismului intermediar şi energetic celular şi permit estimarea proprietăților lor farmacologice utile.

Cuvinte cheie: fracții polifenolice, respirație celulară, metabolism energetic

INTRODUCTION

Different investigations have signalled the presence of polyphenols in different organs of some plants and, especially, in flowers, fruits and leaves (Bodea, 1965), as well as in juice, must and wine (Cotea, 1985; Ursini, Sevanian, 2002). There were shown some aspects regarding the bioactive characteristics of polyphenols from different plant extracts, such as the ones present at cell level, expressed by membranotrope, bioelectric, and bioenergetic (Crăciun et al.,1995; Gonzales-Lebrero et al., 2003; Neacşu et al.,2004; Rotinberg et al., 2004; Rotinberg et al., 2005), metabolic (Rotinberg et al, 2005), antioxidant and liver protecting (Ursini, Sevanian, 2002), and anti-tumour actions (Rotinberg et al., 2004; Rotinberg et al., 2005; Rotinberg et al., 2004; Rotinberg et al., 2005) or by redox modulators (Karp, 1996; Ursini, Sevanian, 2002).

For pointing out aspects typical of the action of some natural polyphenolic agents at the level of animal cells, this paper has studied the specific effects on cell respiration, determined by two types of plant polyphenolic extracts, at three categories of animal cells – liver, striated and smooth muscular cells. We had in view the correlation between the intensity of oxygen consumption and cellular energy (Karp, 1996; Lehninger, 1987) and data enriching as concerns useful pharmacological properties of studied polyphenolic produces, as well as the explanation of aspects on the mechanism of their effects.

MATERIALS AND METHODS

We have studied the dynamics of the intensity of oxygen consumption at three categories of animal cells, as influenced by two types of polyphenolic preparations, extracted and purified from leaves of the medicinal plant *Asclepias syriaca* – methanolic (PMF) and ethanolic fraction (PEF), at rates of 1.5 mg DM/ mL normal Ringer solution (NR).

Experiments were conducted *in vitro* on three series of frog (*Rana ridibunda*, Pall.) tissue preparations, represented by liver fragments (L), sartorius striated muscle (SM) and

stomach smooth muscle (SSM), accurately weighed and incubated into the respiration vessel, in pure or with studied agent Ringer solution, at a constant temperature of 20^0C. Each experimental series was carried out on three groups of five preparations obtained from the same tissue: an untreated control group, kept in NR without agents, a group treated with PMF and another treated with PEF, at a concentration of 1.5 mg DM/mL NR. The oxygen consumption was determined by the Warburg micro-manometric method (Rotinberg et al., 2004), at a constant volume, for 90 minutes. Reading of the manometrical values and calculation of the oxygen consumption were carried out at intervals of 15-30 minutes. The results were expressed in mm^3 O/g tissue/h and were calculated statistically according to the Student Test, by reporting to the quantity of studied fresh tissue (g) and to the control group, which was not treated with polyphenols.

For the estimate of the composition of studied polyphenolic fractions, their adsorption spectrum was registered in UV. Spectra were traced by the help of spectrometer UV-Vis Analytical Jena UV-Vis 200 PC with a slit of 5 nm and a scanning speed of 1 nm/s. We reported to water found in 2 mm quartz tanks. The dry mass was dissolved in water and, then, filtered through a sterilized nylon filter of 0.45 μm.

RESULTS AND DISCUSSION

The obtained data have shown a respiratory reactivity of the studied tissues to the action of polyphenolic produces, which differed according to the type of tissue, nature of polyphenolic fractions and periods of time at which the measurement of cell oxygen consumption was done.

At the liver tissue from the untreated control, a graduate diminution in the intensity of oxygen consumption was registered during 90 minutes, from 1.773 mm^3 O/g/h (100%) until 0.919 mm^3 O/g/h, and after 90 minutes (51.8%). It represented the normal dynamics of liver cellular respiration under experimental conditions (*Table 1*), the diminution in the intensity of respiration being caused by the decrease of *in vitro* cellular energetic stocks.

When treating the liver preparations with polyphenolic fractions, a different dynamics of cellular respiration was registered, according to the nature of studied fraction (*Table 1*). Thus, the methanolic fraction (PMF) has induced a graduate increase in the intensity of oxygen consumption, while the ethanolic fraction (PEF) has resulted in a diminution in the oxygen consumption, which was maintained during the entire period of the experiment. By reporting the values of treated groups to those of the control, at each period of the measurements, considered as 100%, we found that both polyphenolic fractions have stimulated respiration, unlikely the untreated control (*Table 1*). The stimulation effect has been differentiated according to the nature of polyphenolic agents. PMF has induced an earlier (at minute 30) and stronger stimulation. At minute 90, respiration was more intense by 154% compared to the control, and in case of PEF, stimulation was later (minute 60) and weaker (by 60.2%), as we could also see in a previous work (Neacşu et al., 2004)

Table 1

Dynamics of the intensity of cell respiration (mm^3 O_2/g/h), as influenced by polyphenolic agents in liver, striated muscle and smooth muscle

Tissue	Agent	Index	Duration (minutes)			
			15 (±%C)	30 (±%C)	60 (±%C)	90 (±%C)
Lever (L)	Control (C)	$\bar{X}$	1.773 (100%)	1.628 (100%)	1.061 (100%)	0.919 (100%)
		±SE	0.613	0.458	0.283	0.144
		VC%	37.374	32.908	39.268	34.997
	PMF	$\bar{X}$	1.492 (–15.9)	1.996 (+22.6)	2.398* (+126.0)	2.335* (+154.1)
		±SE	0.084	0.497	0.391	0.452
		VC%	13.820	30.992	39.396	43.317
	PEF	$\bar{X}$	1.580 (–10.9)	1.435 (–11.9)	1.531* (+44.3)	1.472* (+60.2)
		±SE	0.328	0.380	0.408	0.320
		VC%	40.981	34.991	35.431	38.622
Muscles (SM)	C	$\bar{X}$	1.912 (100%)	2.340 (100%)	1.839 (100%)	1.172 (100%)
		±SE	0.046	0.231	0.144	0.271
		VC%	5.414	27.810	17.489	25.324
	PMF	$\bar{X}$	2.190 (+14.7)	1.646 (–29.7)	1.376 (–25.2)	0.943 (–19.6)
		±SE	0.369	0.488	0.382	0.293
		VC%	37.732	36.388	32.100	29.730
	PEF	$\bar{X}$	1.776 (–7.2)	1.980 (–15.4)	1.780 (–3.2)	1.842* (+57.1)
		±SE	0.445	0.338	0.109	0.131
		VC%	36.088	38.272	13.766	15.917
Stomach (SSM)	C	$\bar{X}$	0.738 (100%)	1.197 (100%)	1.101 (100%)	0.990 (100%)
		±SE	0.150	0.099	0.023	0.038
		VC%	45.601	18.534	4.702	8.596
	PMF	$\bar{X}$	1.570* (+112.7)	1.895* (+58.3)	1.690* (+53.5)	1.618* (+63.4)
		±SE	0.110	0.161	0.166	0.145
		VC%	15.699	19.017	22.064	22.068
	PEF	$\bar{X}$	0.967 (+31.0)	0.890 (–25.7)	0.835 (–24.2)	0.896 (–9.5)
		±SE	0.236	0.199	0.125	0.146
		VC%	34.781	43.347	33.634	36.521

$\bar{X}$ = mean value; SE = standard error; VC = variability coefficient; C = control; PMF= methanolic fraction of polyphenols; PEF = ethanolic fraction of polyphenols; * = significant to C

At the striated muscle, the dynamics of respiration has shown some different aspects compared to that of the liver (*Table 1*). At the untreated control, respiration has registered an increase by 22.4% in minute 30, compared to minute 15 (1.912 mm^3 O/g/h; 100%), then it diminished gradually, as in case of liver, until 1.172 mm^3 O/g/h (by 38.4% compared to minute 15) at minute 90. The treatment of the striated muscle with PMF resulted in a decreasing dynamics of oxygen consumption values, from 2.190 mm^3 O/g/h, at minute 15, to 0.943 mm^3 O/g/h (by 56.9% to minute 15), 90 minutes after the experiment. In comparison with the values of the control group (100%), we found that PMF has induced a moderate inhibition of the respiratory processes by 29.7% at minute 30, and by 19.6% at minute 90, although, initially (at minute 15), a slight stimulation of respiration (by 14.7%) took place. The ethanolic fraction (PEF) had a reverse effect compared to the methanolic one (PMF), determining a diminution in the intensity of cell respiration in the first 60 minutes (by 3.2-15.4% compared to the control), followed by a stimulation (by 57.1%) after 90 minutes. We found that the reactivity of striated cells to the action of polyphenolic fractions was different from the one of liver cells, because of their structural-functional characteristics, the muscular cells being excitable and contractile, and the liver cells being of metabolic type (Karp, 1996).

The cells of stomach smooth muscles have shown a more diverse respiratory dynamics to the action of polyphenolic agents (*Table 1*), resembling both to striated muscular cells and to hepatocites, but being also different from these. Thus, at the control group, unlike liver and striated muscle, *in vitro* experimental conditions have required an intensification of cellular respiration during the entire period of measurements; after 90 minutes, the oxygen consumption was greater by 34.1% than after 15 minutes.

The methanolic fraction has shown a respiratory effect quite similar to the one from liver, but different from the one of the striated muscle, requiring an increase in the intensity of cell respiration during the determinations. This increase was higher by 112.7%, at the first stage, after 15 minutes, compared to the control, and, then, moderate (by 63.4%) after 90 minutes.

The ethanolic fraction has caused a different effect, having a weak resemblance to the one from the striated muscle, and requiring a slight respiratory intensification after 15 minutes (by 31% compared to the control), followed by a total depression during the entire period of determinations. After 90 minutes, the diminution was by 9.5% compared to the control (*Table 1*). We remarked that stomach smooth muscular cells have shown a different reactivity to the action of polyphenolic fractions, in comparison with the striated ones, although they were excitable contractile cells, too. However, they presented some structural-functional differentiations to the striated ones, expressed by a bioelectric and contractile automatism. It determined a rhythmical contractile activity, also involving some metabolic specific features, different from other cellular types and

a different energetic metabolism. This required certain dynamism of cellular respiratory processes.

At the same way, we could explain the respiratory response of liver cells, which are the headquarters of multiple metabolic processes, involving oxygen consumption within the processes of the Krebs cycle of aerobe cellular respiration (Karp, 1996; Lehninger 1987).

By comparing the values of oxygen consumption for respiration at the control groups, we found that the striated muscular tissue presented a higher intensity of cellular respiration, which was correlated to the processes of oxidative phosphorilation, creating a superior energetic balance (Lehninger, 1987; Neacşu et al., 1996).

The action of polyphenolic fractions on the three types of investigated cells has interfered with the processes of their aerobe respiration, and implicitly, with the energetic processes, correlated to the reactions of oxidoreduction and oxidative phosphorilation within the Krebs cycle (Lehninger, 1987; Neacşu et al., 1996). The effects were according to the characteristics of the cellular type and the nature of studied polyphenols.

The characteristic effects of the two types of polyphenolic fractions have been signalled in glucide, lipide and protide intermediary metabolism of tumour cells, correlated with a stimulation of cellular respiration and their cytostatic characteristics (Crăciun, 1995; Karp, 1996; Neacşu et al., 2004; Nenițescu, 1968; Rotinberg et al., 2005, Rotinberg et al., 2004; Rotinberg et al., 2005; Ursini, Sevanian, 2002**).**

The differences between the effects of the two types of polyphenolic fractions came from the different chemical composition, also shown by their UV absorption spectra. From UV-Vis spectrum, the presence of a continuous emission area could be noticed, which was due to a clearly marked fluorescence in the entire UV field. The spectral nature of these alcoholic extracts, strongly fluorescent (until four units of absorbance), has shown that they were not compounds of anthocyanic type, but of polyphenol-quinol type.

A detailed analysis of similar fractions has pointed out the differences of chemical composition between them, by the spectrophotometrical determination of polyphenols, the total content being expressed in galic acid (0.76 g/L). Data are presented in *Table 2* (Karp, 1996).

Table 2

Composition of polyphenolic fractions

Compounds	Methanolic fraction	Ethanolic fraction
Total polyphenols	19%	20%
Flavones	10%	12–15%
Catechol	2%	2.5%
Anthocians	9%	10%

By thin layer chromatography, rutine, flavones, glucose, mannose, palmitic, stearic, limuli and arahidonic acids, as well as steroids (β-sistosterol, heterozide arahidonic) were found at different rates in the two fractions (Karp, 1996). This chemical composition has explained the multiple biological effects of polyphenolic agents and differences between studied fractions and justified the continuation of investigations, for establishing their pharmacological characteristics and specific action mechanism.

CONCLUSIONS

The polyphenolic fractions extracted from leaves of *Asclepias syriaca* have a great influence on the intensity of cellular respiration, and the type of studied cells (liver, striated muscular or smooth muscular cells).

The respiration of liver cells was stimulated by both types of polyphenolic fractions, but with different amplitude, according to the nature of fraction, the methanolic one having a stronger effect than the ethanolic one.

The oxygen consumption by striated muscular cells was slightly diminished by both polyphenolic fractions, but, at the end of the experimental period, the ethanolic fraction has resulted in a slow effect of stimulating the cellular respiration.

The reactivity of stomach smooth muscular cells to the action of polyphenols was different, both to the action of liver cells and to the one of striated muscular cells. The methanolic function has determined stimulation and the ethanolic fraction – slight inhibition of cellular respiration.

The results indicate the interference of investigated polyphenolic agents to the processes of aerobe respiration and cellular energetic and intermediary metabolism and allow the assessment of useful pharmacological characteristics of these compounds.

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