THE INTERACTION BETWEEN SOME VEGETAL POLYPHENOLIC EXTRACTS AND CHOLESTEROL AT THE LEVEL OF THE CELLULAR MEMBRANE

ION V. NEACSU[1*], PINCU F. ROTINBERG[2], DANIELA N. GHERGHEL[2], COSMIN G. MIHAI[1]

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Abstract – *"In vitro"* treatment of the fibers of sartoruis muscle of frog (*Rana ridibunda, Pall)* with 0,1 mM cholesterol causes on increase of the membranary resting potential (hyperpolarization), seen as irreversible on washing of the preparations with Ringer physiological solution without cholesterol. The polyphenolic extracts from bilberry (*Vaccinum sp.*) fruits, red peony (*Poeonia sp.)* flowers, black grapes and pink flowers of *Hibiscus sp.* (2 mg % active substance) remove the cholesterol deposited in the structure of the cellular membranes, thus re-establishing the membranary properties and the redox potential (rH) affected by cholesterol. Such effects are similar to those induced by similarine – a polyphenolic product, and by chlophenate – a drug recommended in therapeutics. The results obtained evidence the properties of the pholyphenolic extracts, as both normalizing agents of the cholesterol proportion from biomembranes and redox modulators, which recommends them for possible pharmacological utilization.

INTRODUCTION

The numerous researches devoted to cholesterol have evidenced its presence in the structure of the cell membrane, in various quantitative ratios, with other lipidic membranary compounds – phospholipids, especially – as well as with membranary proteins , in various types of cells [1, 3, 12]

Equally, data on the regulating role of the membranary cholesterol in the preservation of the structural-functional properties of the cellular membranes have been provided [1, 3, 6, 12].

Thus, it has been shown that cholesterol influences membrane's degree of molecular packing, fluidity, the liquid crystal state and the phase transitions of the membranary lipids, the permeability and the electrical properties of the membrane and, finally, the interaction between certain agents and the cellular membranes [1, 3, 6, 12]. The involvement of cellular cholesterol's modification in a series of severe maladies, such as cardio-vascular, hepatic and renal affections, alongwith some forms of cancer and ageing phenomena, has been also proved [3, 12].

In a series of our previous investigations, there had been analyzed the membranotropic properties of some polyphenolic agents of vegetal origin and their various effects at cellular level, such as : the influences exercised on the membrane potential, on permeability, on Na^+-K^+-ATPase, on the redox potential and cellular metabolism, as well as on their hepatoprotecting, cytostatic and antitumoral properties [4, 7 – 11].

For the eurichement of the existing data on the specific effects – manifested at cellular level – of the various categories of polyphenols, for the elucidation of their mechanism of action and for ascertaining their possible pharmacological utilization, the present study analyzes the effects of some polyphenolic extracts from different vegetal species on the cholesterol – enriched cellular membranes.

MATERIALS AND METHODS

The experiments, performed *"in vitro"* on striated muscular fibers of frog (*Rana ridibunda Pall)* sartorius muscle, determined the electrical membrane potential by the technique of glass intracellular microelectrodes. There have been followed the bioelectrical effects of a series of total polyphenolic extracts, of preponderently antocyanic nature, from various vegetal species [2], such as : bilberry (*Vaccinum sp.* – VAC) fruits, red peony *(Paeonia sp.* – PEN) flowers, black grapes *(Vitis* – VIT) and red flowers of *Hibiscus* (HIB) in a concentration of 2 mg dry matter /100mL normal Ringer phyological solution (NR). The effects of the polyphenolic extracts have been compared with the effects induced by two reference drugs namely : silimarine (SIL) 1.4mg % of vegetal polyphenolic origin, and chlophenate 20 mg% (sodium–p– chlorphenoxyisobutirate – CLF), evidencing, at usual concentration values, pharmalogical action similar to that of polyphenols.

Two series of determinations have been made; the former followed the bioelectrical effects of all agents on normal muscular fibers, while the latter one analyzed the agents' action upon fibers treated initially with 0.1 mM cholesterol dissolved in 1 % ethylic alcohol, for putting into evidence their effect upon the cholesterol – enriched membranes. In all cases, the muscular fibers have been finally treated with normal Ringer without agent, which permitted to follow the recovery of the normal resting potential (NRP). The duration of each phase was of 60 minutes.

On the basis of polyphenols' antioxidating properties [5] there have determined potentiometrically the values of the redox potential (E_h) and the solutions' pH values. Quantification of the redox effects involved calculation of the rH parameter, with the relation of Clark :

$$rH = \frac{Eh + 0.058 + 250}{0.029}(Volts)[14]$$

The initial NRP values have been calculated statistically, with Student test, some comparative values being obtained percentually, *versus* NRP, or *versus* the effect of the corresponding treatment agent.

RESULTS AND DISCUSSIONS

The experiments, made on normal muscle fibers, evidenced that all polyphenolic extracts under study show a bioelectrical effect of hyperpolarization on the cellular membranes, with amplitudes depending on the extract's nature (Table 1). Consequently, the strongest hyperpolarizing effect is induced by the *Poeonia* extract (i.e. 7.32 % of the initial NRP value), followed by that of *Vaccinum* (6.62.%), *Hibiscus* (5.19 %), black grapes (5.17 %) and silimarine (4.77 %). Chlophenate does not cause significant NRP modifications, only a weak hyperpolarization (0.93 % of the NRP value) - easily cancelled on muscular fibers' washing with NR without on agent – being recorded.

Such a difference of action, comparatively with the effect of the polyphenolic extracts, may be explained by the chemical nature of the drug, i.e., natrium – p – chlorphenoxyisobutirate [13] .

Table 1 – Bioelectrical effects of the polyphenolic agents upon the striated muscle fibers. (mV – milivolts, NR = normal Ringer, NRP = normal resting potential, h = hyperpolarization (+), d = depolarization (-), rec = recovery of NRP)

Agent	NRP;X ±SE (mV)	Agent' effect- 60 min. (±% from NRP)	NR effect after agent – 60 min	
			±% from agent effect	(±% from NRP)
VAC	93.25 ± 0.69	+ 6.62 (h)	– 83.79 (rec)	+ 2.07 (h)
VIT	89.45 ± 0.60	+ 5.17 (h)	– 63.07 (rec)	+ 1.91 (h)
PEN	91.00 ± 0.50	+ 7.32 (h)	– 105.10 (rec)	– 0.37 (d)
HIB	90.55 ± 0.66	+ 5.19 (h)	– 83.30 (rec)	+ 1.38 (h)
SIL	91.63 ± 0.48	+ 4.77 (h)	– 77.57 (rec)	+ 1.07 (h)
CLF	90.32 ± 0.70	+ 0.93 (h)	– 90.47 (rec)	+ 0.09 (h)

Actually, the effect of the grapes extract is manifested in a more complex way, inducing initially (i.e., in the first 20 min) membrane depolarization, followed by a stable hyperpolarization.

The persistency of the hyperpolarizing effect on the muscular fibers' washing with normal Ringer without extract depends, too, on the nature of the polyphenols from the extract (Table 1). Thus, the observation may be made that, after a 60 min washing of the muscular preparations with NR without agent, the recovery of the NRP initial value is extremely slow, in all cases.

In the muscular fibers treated with PEN the NRP recovery is nevertheless more rapid, up to the manifestation – within 60 minutes – of a depolarization tendency (a 105.10% recovery, comparatively with the agent–induced depolarization). In the other extracts, NRP recovery after 60 min of incubation in pure NR proceeds in the following order : VAC – 83.79% of the hyperpolarization value, HIB – 83.30%, SIL – 77.57% and VIT (grapes extract) – 63.07%.

One may therefore observe that the most persisting hyperpolariying effect is the one induced by the grapes extract – evidencing, probably, a more complex polyphenolic composition [5], its bioelectrical effect being biphasic, as a function of time, at the same concentration value (depolarization followed by hyperpolarization).

The effect of membranary hyperpolarization of such agents assumes, from their part, a stabilization action on the structure of the cell membrane accompanied by the modification of the ratio between the passive and the active ionic transport in favour of the latter ore, alongwith the intensification of the Na^+-K^+- ATPase activity – as put into evidence in some previous investigations of ours [4, 7 – 11]. Such effects are the more persistent on the preparations' reintroduction into pure NR, the strongest is the polyphenolic componnds' binding to the structure of the membranes [4, 7, 8]. From this perspective, the strongest effect is induced by the grapes extract, followed by silimarine (Table 1), which might by probably explained by their more pronounced lipophily known as favourizing their binding to the membranary structure [2, 7, 8].

In another series of experiments, the cell membranes had been initially enriched in cholesterol, prior to the application of treatments with the polyphenolic extracts taken into study.

Incubation of the muscular fibres in NR with 0.1 mM cholesterol induces – in all cases – a stable hyperpolarization of the cell membrane, the amplitute attained after 60 min ranging between 6.66% and 9.34% - comparatively with the initial NRP value (Table 2). Mention should be here made of the fact that the 1% ethylic alcohol used for solving the cholesterol does not modify the NRP value to a considerable extent [6].

The reference experiment, in which the membranes hyperpolarized with cholesterol have been treated with pure normal Ringer showed that hyperpolarization is extremely persistent, the reduction recorded after 60 min being of only 35.58% which means that a 6.02% hyperpolarization remains unrecovered comparatively with NRP.

Table 2 – Effects of polyphenolic agents upon the cholesterol hyperpolarizing effect (Explications – idem table 1)

Agent	NRP;X ±SE (mV)	COL effect, 60 min. (hyperpol =% from NRP)	Agent'effect after COL-60min (± %from COL hiperpol)	NR eff.after agent-30min (± %from NRP)
COL-NR	92.03 ± 0.39	+ 9.34 (h)	(NR= –35.58-rec)	+ 6.02(d)
COL-VAC	90.06 ± 0.45	+ 6.76 (h)	– 73.53 (rec)	– 0.15 (d)
COL-VIT	90.25 ± 0.57	+ 8.67 (h)	– 69.22 (rec)	– 1.76 (d)
COL-PEN	89.50 ± 0.40	+ 6.66 (h)	– 85.57 (rec)	+ 0.18 (h)
COL-HIB	90.86 ± 0.45	+ 6.70 (h)	– 74.54 (rec)	+ 0.32 (h)
COL-SIL	91.17 ± 0.36	+ 6.72 (h)	– 94.62 (rec)	– 1.10 (d)
COL-CLF	91.66 ± 0.42	+ 6.95 (h)	– 124.80 (rec)	– 0.99 (d)

Such a situation is indicative of a strong binding of the exogenous cholesterol to the membranary structures, which accompanied by perturbation of the degree of structural packing and membrane stabilization, alongwith an almost irreversible modification of the passive and active ionic flows, and of the membranes' electrical charge [3, 12].

Treatment of the cholesterol–loaded membranes with polyphenolic extract evidence the latter's capacity of eliminating the cholesterol deposited in membranes, alongwith the recovery of NRP and of the membranary properties (Table 2), the amplitude and rate of such effect depending on the components of the extract employed. Consequently, after a 60 min treatment with polyphenolic extracts, reduction of cholesterolic hyperpolarization may be noticed, in increasing order, as follows: a 69.22% reduction for the extract of grapes (VIT), 73.53% -VAC, 74.54% -HIB and 85.57% /PEN. Nevertheless, the reference agent show stronger effects on the cholesterol deposited in membranes. Thus, silimarine reduces the cholesterol – induced hyperpolarization with 94.62% while chlophenate, which is a specific anti – cholesterolic drug, removes the cholesterol on the whole, up to provoking membranary depolarization (effect of 124.80% from hyperpolarization).

Another important observation is that, although, in the first phase, the polyphenolic extracts do not reduce completely cholesterolic hyperpolarization, they nevertheless mobilize the cholesterol deposited in membranes, to be totally removed subsequently, on washing of the preparations with normal Ringer without agents (Table 2), in all cases, in the preparations treated with black grapes extract and silimarine, a depolarization tendency may be even observed.

All these results permit the observation that, by the elimination of the cholesterol deposited in the structure of the cellular membranes, the polyphenolic extracts evidence their capacity of re–establishing the electrical and permeability properties of the membranares, thus recovering the normal ratio between cholesterol and the other membranary components [3,6,12].

Another series of determinations evidenced the influence of the polyphenolic extracts on the redox potential of the solutions employed (Table 3). Considering the value of 28.3 V as a neutral rH, the 0 – 28.3 V values – as a reducing domain and respectively, the 28.3 – 42.4 V ones – as on oxidating domaine [14] one may reach the conclusion that extracts VAC and VIT, as well as SIL, induce – similarly with the pure NR - a neutral rH, while, on the other side, extracts PEN and HIB and the chlophenate induce a slightly reducing of rH. However, the cholesterol induces a much more reducing of rH value (22.3V), which is actually the result of lowering the rH value of the NR to which the cholesterol is added.

Calculation of the ratio between extracts' percent effect on the cholesterol – loaded membranes and the rH values of the solution of extract, cholesterol or NR, alongwith considering the ratio given by NR (i.e., 3.5) as a reference one, leads to the observation that, in the case of cholesterol, such a ratio takes a much lower value (1.5), altough all polyphenolic extracts induce higher values of this ratio (2.4 – 3.4) quite close to the value determined by pure NR.

One may therefore observe that such agents asses, too properties of redox modulators, by means of which they may reject the negative effects of a higher cholesterol ratio in the cell membrane, comparatively with their normal content.

Table 3 – The rH values of agent’ solutions and correlation (ratio) between the agent’ effect upon hyperpolarized cells by cholesterol and corresponding rH (MP = membrane potential, NRP = normal resting potential, rec = recobery of NRP)

Agent	Agent’ sol rH (Volts)	MP agent’ eff.after COL (% from NRP)	Ratio- % agent’ effect : rH
NR	28.2828	100 (control)	3.5357
COL	22.3649	35.58 (rec)	1.5649
VAC	28.1053	73.53 (rec)	2.6162
VIT	28.4856	69.22 (rec)	2.4299
PEN	24.6672	85.57 (rec)	3.4689
HIB	24.7235	74.54 (rec)	3.0149
SIL	28.3217	94.62 (rec)	3.3409
CLF	27.4048	124.80 (rec)	4.5539

CONCLUSIONS

The vegetal polyphenolic extracts obtained from bilberry fruit (*Vaccinum sp).*, black grapes, red peony *(Paeonia sp.)* and pink flowers of *Hibiscus sp.* cause hyperpolarization of the membranes of striated muscular fibers, the effect being similar to the one induced by the pharmaceutical polyphenolic product silimarine. Al the same time, these extracts mobilize the cholesterol deposided in the structure of the cell membranes, a process evidenced through cancelling of the cholesterol-induced irrevesible membrane hyperpolarization, their effect being similar to that of silimarine and of the anti-cholesterolic drug chlophenate. Such actions are correlated with the redox modulating properties of the polyphenolic extracts, which are similar to those of the reference agents – silimarine and chlophenate. The results obtained challenge new researches on such polyphenolic extracts, for further elucidation of the phenomena observed and for their possible biomedical utilizations.

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[1] Faculty of Biology, "Al.I.Cuza" University, Bd. Carol I, 20A, 700505, Iasi, Romania
[2]Biological Research Institute, Bd. Carol I, 20A, 700505, Iasi, Romania
*ineacsu@uaic.ro