ORIGINAL ARTICLE

Electrophysiological evidence of the effect of natural polyphenols upon the human higher brain functions

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Abstract

OBJECTIVE: Several natural polyphenols exert effects upon the cardiovascular as well as nervous system. In vitro and animal studies suggest that polyphenols may potentially affect the human cognitive function. The aim was to study the effect of Provinols™, the polyphenolic compounds isolated from red wine, upon the human higher brain functions.

MATERIAL AND METHODS: The accuracy of space memory was assessed by means of visually-guided and memory-guided saccadic eye movements. The EEG and blood pressure were registered also. The healthy undergraduates served as subjects. They were divided into the control, placebo and Provinols™ groups. The amplitudes of saccades, EEG spectral density, evoked potentials time-locked to saccadic onset and blood pressure were analyzed in control condition and 2 hours later, after administration of placebo, Provinols™ (4 mg/kg of body weight) or nothing.

RESULTS: After the Provinols™ administration the memory-guided saccades were significantly more accurate and the significant decrease in the slow EEG bands, alpha power mainly, was registered over the broad regions of temporo-parietal cortex. No changes in saccadic eye movement related potentials as well as in blood pressure were found after the single dose Provinols™ administration.

CONCLUSIONS: Even a single dose of the Provinols™ was able to affect positively the space memory for limited time duration. The improvement in space memory function and/or the positive role of attentional mechanisms may be taken into account mainly. More sensitive analysis of the particular participation of attentional and memory components demands the further study.

INTRODUCTION

Several natural polyphenols have been reported to exert beneficial effects in preventing cardiovascular diseases (Goldberg 2003; Stanner 2005). In general, more than two thirds of the polyphenols consumed in the diet are flavonoids. Red wine flavonoids have generated a great interest especially due to their in vivo and in vitro antioxidant capabilities. Their beneficial effect on cardiovascular system was described mainly in relation to the French Paradox phenomenon as well as to the Mediterranean diet. The French Paradox is defined as a low incidence of coronary heart disease while consuming a diet rich in saturated fat.

The Mediterranean diet, rich in fruits and red wine, was shown to protect against the development of cardiovascular diseases (Hertog *et al.* 1995; de Lorgeril *et al.* 1996). Consumption of fruits, vegetables and red wine may help to reverse hyperlipidemia, to decrease the atherogenicity of the LDL particles (Lampe 1999), and to protect LDL cholesterol from oxidation (Brouillard *et al.* 1997).

Provinols™ represents the polyphenolic compounds isolated from red wine and it involves (in mg/g of dry powder) 480 proanthocyanidins, 61 total anthocyanins, 19 free anthocyanins, 38 catechin, 18 hydroxycinnamic acids, 14 flavonols and 370 polymeric tannins. Oral administration of Provinols™ was able to produce a decrease in blood pressure in normotensive rats. The hemodynamic effects of red wine polyphenols are associated with the increased NO synthase (NOS) activity, the moderate increase in endothelial NOS expression, and the reduction of oxidative stress which may be factors responsible for the beneficial effect of the Provinols™ (Pecháňová *et al.* 2006).

After identification NO as a potencional neurotransmitter in the CNS a lot of studies have implicated a variety of its functional role in numerous physiological and pathophysiological processes within the brain. Among them the modulation of the long term potentiation and long-term depression of the neuronal activity within the hippocampus (Bohme *et al.* 1991) may be mentioned. Weichselbaum and Buttriss (2010) pointed to the fact that there is emerging evidence from in vitro and a few animal studies suggesting that polyphenols may potentially have a protective effect on the development of neurodegenerative diseases and may improve cognitive function in patients when such diseases are established. These authors stressed also that research in this area is still in its early stages and human studies are needed to substantiate potential effects. Kovacsova *et al.* (2010) summarized the most important neuroprotective actions of natural polyphenolic compounds in the brain. They accentuated the evidences suggesting that their mechanism of action involves: 1) antioxidant activity, mainly inhibition of the NADPH oxidase and subsequent reactive oxygen species generation; 2) an activator effect on endothelial and inhibitory action on both neuronal and inducible nitric oxide synthase activity and subsequent NO production; 3) reduction of neuroinflammation via attenuation of the release of cytokines, such as interleukin-1beta and tumor necrosis factor-alpha and down regulation of the pro-inflammatory transcription factors such as NF-κB; and 4) the potential to modulate signaling pathways such as mitogen-activated protein kinase cascade and cAMP response element-binding protein leading to the improvement of memory and cognitive performance. As we have suggested earlier, polyphenols stimulating the activity of NO synthase, and thus the NO production, may influence those human higher nervous functions which are usually affected mostly in diseases and/or disorders of the central nervous system (Jagla *et al.* 2006).

Cardiovascular and CNS effect of polyphenols were recently pointed out by Karvaj *et al.* (2007) also. Now it is evident that NO exerts not only its peripheral vasodilatory action but it is also involved in the central regulation of sympathetic tone (Lassegue and Griendling 2004; Parohova *et al.* 2009; Bernatova *et al.* 2010). Moreover, now it is better evident that there exist the complex site-specific interactions between multiple angiotensins and multiple receptors in the mediation of important central functions including blood pressure regulation. The balance of angiotensin II and NO oxide within central nervous system seems to have a key role in this important regulation (Pechanova 2010). Although various levels of neuro-cardiovascular interactions within the CNS hierarchy are known we have selected the sensorimotor integration for study the effect of polyphenols upon the human higher brain functions. The reason is the sensorimotor integration subserve the interindividual communication as well as the communications between men and his/her environment. They may be significantly modulated not only by brain and mental disorders but by various hemodynamic changes as well – high and pronounced low blood pressure included.

It is known that the visual-oculomotor integration represent the most subtle as well as most precise kind of the sensorimotor interactions. Moreover, the nuclei of the oculomotor nerves as well as the rostral ventrolateral cardiovascular centers are located in the lower part of medulla. It was the reason we selected the saccadic eye movements for analyzing the probable effect of Provinols™ administration upon the visual perception and space memory functions.

Saccadic eye movements are rapid movements of eyes by means of which subject scans the visual environment. Saccades may be elicited by visual stimuli as well as by intentional effort. For this study we selected the analysis of the accuracy of visually evoked saccades and of saccadic eye movements driven by memory information about the eye landing position in space. If the Provinols™ can affect not only cardiovascular functions but the higher brain functions as well than we may expect improvement in the accuracy of the both visually- and memory-guided saccades.

MATERIAL AND METHODS

The registration of saccadic eye movement was divided into two parts and repeated two hours after the Provinols™ administration at the end of the first part of examination with every healthy normotensive undergraduate volunteer. At the beginning of examinations the saccades were elicited by the 0.3° circular visual targets (the visual-guided saccade task – VGS). The subject has to fixate as rapid and accurate as possible the visual target which appeared suddenly in the visual field. At least 50 saccades towards the every space locations used (irregularly according to numerals at clock face position) were registered.

Immediately after the VGS task the memory-guided saccade task (MGS) was introduced (Pierrot-Deseilligny *et al.* 1991). Subject has to fixate a central visual target and to continue its fixation while another visual stimulus was briefly flashed into the periphery of the visual field. He/she has to remember the location of the peripheral visual stimulus. After the central fixation target was switched off subject had to make a saccade to the remembered peripheral target location. Immediately after the MGS task in one group of volunteers the Provinols™ was administered (4 mg/kg of body weight), in the second group the placebo and in the third group nothing was used.

The whole experimental procedure (VGS followed by MGS task) was repeated two hours after the first VGS+MGS session in all the control, placebo and Provinols™ groups. Each group consisted of 10 subjects with normal vision and without the history of nervous and mental disorders. They gave informed consent before the examinations, according to Helsinki Declaration and obtained detailed information on the purpose of the study. The whole experimental procedure was approved by the local ethical committee. The examinations were performed in a sound-attenuating and partially electrically shielded room.

Subjects were seated in an armchair with moderate headrest and the visual targets were projected onto a panoramic screen 112 cm apart.. The vertical black and white stripes served as visual background. The eye movements were recorded electrooculographically (EOG) by means of EEG electrodes affixed at the outer canthi of the eyes and above and under the right eye. Electroencephalographic recording was made by EEG caps with 24 electrodes. Blood pressure was controlled by the Omron manometer. Amplitudes of saccades were evaluated by the specialized computer program developed at our laboratory (ANALOKN) for EOG data collection and analysis and for each subject its VGS amplitudes served as control values. EEG recordings were evaluated by means of matlab programs as well as by means of the specialized computer program developed at our laboratory (ANALERP).

RESULTS

Accuracy of saccadic eye movements

1. Amplitudes of saccades elicited by visual information:

In general, the accuracy of visually evoked saccades did not change significantly in relation to the Provinols™ administration. As we have seen in many our previous examinations the 95% of visually evoked saccades were

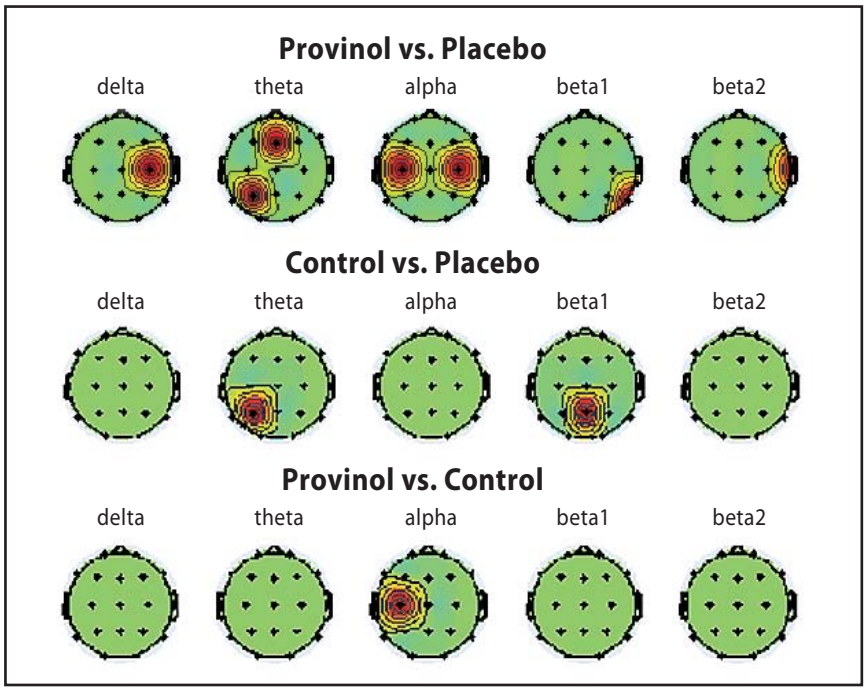

Fig.1. Changes in alpha and theta bands during preparation of memory-guided saccades that is during the fixation of the central visual target and brief presentation of lateralized one. Significant decrease of the particular EEG spectra are colored.

normometric (accurate) and only 5% of them dysmetric (inaccurate). The only exception was the situation two hours after Provinols™ administration. Their accuracy increased (98%: 95%, $p<0.02$). The value of inaccuracy did not exceed 0.5° with saccades of 10° of visual angle. The dysmetric saccades mostly overshoot the target and because of this a corrective saccade in opposite direction followed.

2. Amplitudes of saccades elicited by memory information:

In general, saccadic eye movements elicited by memory information are more inaccurate in comparison with visually evoked ones (30%: 5%, $p<0{,}001$). In the first, control session (before Provinols™ administration) the value of MGS inaccuracy was significantly higher than the value of VGS inaccuracy (1.2–1.5°: 0.5°, $p<0.01$). The number of dysmetric MGS saccades decreased significantly two hours after Provinols™ administration (18%: 30%, $p<0.01$). At the same time the value of their inaccuracy significantly decreased also (0.7°: 1.2–1.5°, $p<0.02$).

No significant changes in saccadic accuracy were found in control and placebo groups.

EEG evoked potentials time-locked to saccadic eye movement onset

No differences comparing to control group were registered either in correlates of saccadic eye movement preparation, or in correlate of maximal recruitment of eye muscle units at the beginning of a saccade and in correlate of the first encoding the new visual information at the primary visual cortex.

EEG power spectral densities

During the preparatory period for a saccade guided by memory information the significant decrease within the slow EEG bands, alpha power mainly, was registered. As for the alpha band, over the broad temporo-parietal cortex bilaterally and over the left temporo-parietal cortical regions when comparing the Provinols™ to Placebo and Provinols™ to Control group, respectively It means, the cortical activation after the Provinols™ administration appeared in areas playing role in attentional orienting and memorizing. Following figure illustrates the results.

Blood pressure changes

As expected, no blood pressure changes were registered after single dose administration of Provinols™**.**

The above effects had the limited duration. The control measurements made three hours after the Provinols™ administration showed no changes in relation to the control condition.

DISCUSSION

As stated above the visual-oculomotor integration represents the most subtle as well as most precise kind of the sensorimotor integration. In memory guided saccades the sensory part of the integration that is the primary encoding the basic shape and space characteristics of a visual stimulus is operationally "separated" from the motor part that is from the programming and generation of a saccade towards the remembered space position. It allows to study both parts more sensitively as well as to assess their particular contribution to the solving of experimental task as well.

The increase of NOS activity elicited by administration of Provinols™ may activate the NO/sGC/cGMP/cGK pathway within the brain. It was suggested (Kleppisch and Feil 2009) that this pathway modulates long-term changes of synaptic activity in the hippocampus, amygdala, cerebellum, and other brain regions and thus contributes to distinct forms of learning and memory, motor adaptation, and object recognition. Moreover, the above and other components of the cGMP signaling cascade were suggested as attractive new targets for the study of neurobiological mechanisms of various cognitive functions.

Previously we have proposed (Jagla *et al.* 2010) that the short lasting effect of single Provinols™ administration upon the human higher brain functions, as revealed by increased accuracy of memory-guided saccades, could be mediated via various cognitive functions. The role of perception, memorization, execution of saccades and attention was hypothesized mainly.

The analysis of the concomitant EEG activity helped us to clarify several issues. The component of saccadic eye movement related potentials reflecting the saccadic eye movement preparation was not affected by the Provinols™ administration. The correlate of maximal recruitments of the oculomotor muscles at the onset of the saccades as well as the time of the first encoding of new visual stimulus within the visual cortex were not affected as well. It suggests that the perception of the lateralized target and the preparation of the saccadic eye movement toward its location as well as the executions of the eye movement were not substantially engaged in the improved accuracy of the memory-guided saccades after Provinols™ administration.

The analysis of the EEG power spectra density difference during the memory-guided saccade task, prior and 2 hours after drug administration and comparison with the similar results for visually-guided saccades disclosed the significant decrease in the slow EEG bands, alpha bands mainly, as the significant change. Therefore, the role of the memorization and attention could be taken into account. As for memorization, the changes over the parietal and temporal cortical regions should be expected. Actually, the significant decrease in the alpha power density was registered over the parieto-temporal regions. It may indicate the higher level of activation in the cortical regions which are involved in the neuronal circuits subserving the space orientation and memory functions (Kolb and Whishaw 2003).

It is generally known that the thalamus plays not only an important role in the arousal mechanisms but

in gating the sensory information during their processing also. Both this mechanisms contribute substantially to the overall attentional level and to focusing the attention on the particular event. Williams *et al.* (1997) demonstrated that NO is produced in the thalamus in a state dependent manner, with the highest rate occurring during EEG desynchronized states, wakefulness and REM sleep and at the significantly slower rate during the slow wave sleep. Furthermore, Alexander *et al.* (2005 and 2006) demonstrated that the NO is important for the brain cortex to regulate its own input and to enhance cortically evoked responses. The NO is also involved in a cascade of processes inhibiting the astrocyte glutamate uptake (Hu *et al.* 2000). Rönnbäck and Hannson (2004) pointed to the fact that the increased concentration of the extracellular glutamate leads to affected signal-to-noise ration in glutamate transmission which results in decreased precision in information intake followed by affected information processing. All these mechanisms may play an important role in attentional as well as memory mechanisms. Up to now we could not separate the attentional and memory components in our results. More sensitive analysis of the particular participation of attentional and memory components will demand another study.

Nitric oxide belongs to probably the smallest molecules that have a regulatory effect in various brain mechanisms. It operates by very complex cascades of consecutive biochemical events and their exact importance is far from understood. However, the present findings may deepen our understanding of factors which in the future may play a role in pharmacological treatment of several higher brain function disorders.

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