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A DUAL ACTION OF α-LIPOIC ACID IN THE BRAIN

An electrophysiological evaluation

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ABSTRACT - Oxidative stress causes metabolic and structural abnormalities during reperfusion. In an animal model of electrophysiological evaluation of cerebral ischemia-reperfusion, α-lipoic acid effect on the oxidative stress was studied by mean absolute amplitude of EEG spectra evaluation. The left carotideal infusion of 3.03 mM α-lipoic acid in Wistar rats after cerebral ischemia and reperfusion caused initial reduction and partial final recuperation of the various EEG spectral frequency mean absolute amplitudes ($p<0.05$). The left intracarotideal infusion of 6.06 mM α-lipoic acid significantly reverted the induced depression of mean absolute amplitude of theta and delta spectra. Nevertheless there was an increasing pattern of ischemia demonstrated by mean absolute amplitude depression of almost all EEG spectra with 60.6 mM α-lipoic acid infusion. These observations suggest that, depending on the administered concentration, α-lipoic acid may act in a dual way, protecting from ischemia at lower concentrations and worsening this process at higher doses.

KEY WORDS: α-lipoic acid, anti-oxidant, pro-oxidant, cerebral ischemia, electroencephalogram (EEG), spectral mean absolute amplitude.

Dupla ação do ácido α-lipoico no encéfalo: uma avaliação eletrofisiológica

RESUMO - Estresse oxidativo causa anormalidades metabólicas e estruturais durante reperfusão após isquemia. Em um modelo animal de avaliação eletrofisiológica de isquemia-reperfusão, o efeito da infusão do ácido α-lipóico sobre o estresse oxidativo foi estudado por meio da avaliação da média das amplitudes absolutas dos espectros eletrencefalográficos. A infusão intracarotídea esquerda de ácido α-lipoic 3,03 mM em ratos Wistar após isquemia-reperfusão cerebral causou significante redução inicial e recuperação parcial final da média das amplitudes absolutas dos vários espectros eletro - encefalográficos ($p<0,05$). A infusão intracarotídea esquerda de ácido α-lipoic 6,06 mM significantemente reverteu a depressão induzida da média das amplitudes absolutas médias dos espectros teta e delta. Houve, contudo, maior depressão da média das amplitudes absolutas de quase todos os espctros com infusão de ácido α-lipóico 60,6 mM. Estas observações sugerem que, dependendo da concentração administrada, o ácido α-lipoic tem duplo efeito, protegendo da isquemia em baixas concentrações e piorando este processo em doses altas.

PALAVRAS-CHAVE: ácido α-lipóico, anti-oxidante, pró-oxidante, isquemia cerebral, eletrencefalograma (EEG), amplitude absoluta média espectral.

We have previously studied the electroencephalographic effects on mean absolute amplitudes of electroencephalogram (EEG) waves in Wistar rats submitted to global cerebral ischemia due to common carotid arteries ligation, arterial hypotension and reperfusion[1]. There are only few experimental reports in the literature concerning the value of the EEG as a marker of induced cerebral ischemia in animals[2-4]. According to Siesjö and Siesjö[5] two mechanisms are responsible for secondary cerebral involvement after brain ischemia. The first mechanism involves cellular signal transduction modifications from glutamate and growth factor receptors stimulation with intracellular signal modulation, deleterious calcium enzyme activation and genetic protein synthesis expression abnormalities[6]. The second one is related to oxygen supply during reperfusion and involves oxygen reactive species and free radicals

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production. Oxygen reactive species and free radicals could structurally compromise various macromolecules by oxidation and reduction reactions causing, among others, DNA strand braking[7], with products formation such as 8-hydroxy-2'-deoxyguanosine[8], lipid peroxydation products[9,10] and carbonyl group formation[11].

Otherwise, one of the most important aims in the treatment of cerebral ischemia, during the acute phase, is to minimize or even avoid oxygen reactive species structural lesions, and consequently broaden the therapeutic window in order to implement thrombolytic therapy. Although without beneficial clinical evidence, many experimental procedures suggest that pharmacological entities, blocking cytotoxic cascade events during ischemic process or acting as free radical or reactive oxygen species scavengers, could reduce infarct volume due to focal ischemia or late effects of global ischemia with reperfusion.

Thioctic acid, 1,2-dithiolane-3-pentanoic acid or α-lipoic acid and its reduced form, di-hydrolipoic acid, are natural components of biologic membranes, acting as mytochondrial lipoamid co-factor of α-ceto-acid de-hydrogenase[12,13]. Its participation in oxy-reduction state from external sources has been focused in various recent studies. It is easily absorbed and taken by cells, being finally reduced to di-hydrolipoic acid with participation of the systems NADH and NADPH[14]. The mitochondria are the main site of α-lipoic acid anti-oxidant action. Within the intracellular space the di-hydrolipoic is produced from α-lipoic acid and acts as anti-oxidant in the extracellular space[15].

The α-lipoic acid also increases glutathione intracellular levels[16,17], and as a result, it could be an important component of the therapeutic arsenal of cerebral vascular ischemic diseases. Other α-lipoic acid and di-hydrolipoic acid actions in ischemia and reperfusion have been described. Ion Fe^{+2} chelation by di-hydrolipoic acid[18], and nitric oxide[19] and other oxygen reactive species scavenging, as well as E vitamin and other anti-oxidant regeneration[20] are important α-lipoic acid roles. Using our previous idealized model[1], we decided on studying the electrophysiological effects of intracarotideal α-lipoic acid infusion.

METHOD

Male Wistar rats, weighing 300-500g at the time of surgery were used in the present study. The animals were fasted with free access to water during 24 hours before the experimental procedure. Three paired groups of animals (n=6, each paired group) were used in the first part of this study. Intracarotid α-lipoic acid infusion (different concentrations: 3.03 mM, 6.06 mM and 60.6 mM) started 30 minutes after both common carotid arteries occlusion. The animals of group 1 were infused with 3.03 mM a-lipoic acid, the animals of group 2 with 6.06 mM α-lipoic acid. The animals of group 3 received 60.6 mM α-lipoic acid infusion. In the second parf of this study, three other groups of six animals each were studied in order to determine brain tissue reduced glutathione (GSH) contents by spectrophotometry: one made of rats with both common carotid arteries occlusion, one with both common carotid arteries occlusion plus 6.06 mM α-lipoic acid intracarotid infusion and the last one with both common carotid arteries occlusion plus 60.6 mM α-lipoic acid intracarotid infusion. The mean weight of the animals was 339.3 ± 50.6 g. The weights of the animals of all studied groups were subject to Kolmogorov-Smirnov statitistical evaluation and considered as a normal distibution. Under intraperitoneal urethane anesthesia (1.5g/Kg), all the animals were submitted to surgical procedures that briefly consisted of cervical median incision with exposure of the trachea; identification and occlusion of the common carotid arteries; insertion and fixation of a polyethylene cannula (PE 50) into the left internal carotid artery in order to infuse α-lipoic acid solution; insertion and fixation of a polyethylene cannula into right internal carotid artery in order to record the intracarotideal blood pressure. The carotid blood pressure was monitored by using a Narco Bio-system physiograph. The right carotid cannula was connected to a P23 Statham transducer, being the carotideal blood pressure registered by a physiograph.

The animals were kept under continuous electrocardiographic and electroencephalographic monitoring. This system consisted of a preamplifier, an amplifier with adjustable time constants, an AD converter (12 bits), a 486 PC compatible microcomputer and Braintech® software to study the spectral frequencies and the absolute amplitude average of the chosen events of the EEG. This software analyses the frequency components with precision of 0.35 Hz and uses epochs of 2.84 seconds. Subcutaneous electrodes were inserted in right frontal (F4), left frontal (F3), right parietal (P4), left parietal (P3), vertex (Cz) regions with reference to bilateral auricular (A1 and A2) surface electrodes. The ground electrode was subcutaneously inserted in the nose region. Surface electrodes kept on anterior paws were used for electrocardiographic monitoring. The technical details of the system are similar to those presented in a recent review[21]. The electroencephalographic and electrocardiographic activities of each animal were monitored during about 30 minutes before and 30 minutes after the beginning of α-lipoic acid infusion into distal segment of left common carotid artery. The right intracarotid blood pressure was monitored during all the experiments. Two minutes periods from each ten minutes of EEG and EKG monitoring were saved on hard disk for future analysis. The mathematical study of the average of the EEG spectral amplitudes alfa (8.09-12.66 Hz), $beta_1$ (13.01-19.69 Hz), $beta_2$ (20.84-26.02 Hz), $beta_3$ (26.37-

32.70 Hz), theta (4.22-7.73 Hz) and delta (0.35-3.87 Hz) was done by choosing 2.84 s epochs (about 30 epochs from each animal group) of the saved EEG on hard disk. Epochs with artifacts were rejected. The rate of 3.03 mM α-lipoic acid infusion was 41.91 μl/100g/min.With 3.03 mM α-lipoic infusion, we considered initial, intermediate and final infusion periods and ANOVA was used to evaluate statistical significant differences ($p<0.05$) of the absolute amplitudes of the EEG main spectra. When the normality test failed, variance of repeated measures (Friedman) was considered.

Two other paired groups were included in this study: 6.06 mM α-lipoic acid infusion (45.87 μl/100g/min; n=6) and 60.6 mM α-lipoic acid infusion (44.84 μl/100g/min; n=6); paired t-test was used to evaluate statistical significance. We also performed spectrophotometric analysis of GSH in brain tissue fragments after both common carotid arteries occlusion, and after both common carotid arteries occlusion plus carotid infusion of α-lipoic (6.06 mM and 60.6 mM). The infusion of a-lipoic acid began 30 minutes after both common carotid arteries occlusion and it was done during 30 minutes.

Six cortical fragments from each rats were removed after craniectomy and kept on liquid nitrogen before their preparation for spectrophotometric analysis by modified Beutler et al.[22] method. Briefly, brain tissue fragments were weighted, homogenized and put into tubes with 6% trichloroacetic acid (TCA) solution (50 mg of brain tussue into 1 ml of 6 % TCA). After centrifugation (4000 rpm), 500 μl of each tube float solution were added to 500 μl of 6 % TCA; 1 ml of 6 % TCA acted as a blank tube. After that, 4 ml of 4.3% $NaHPO_4$ solution plus 500 μl of 5,5-dithio (2´-nitrobenzoic) acid (DTNB) (40 mg of DTNB/100 ml of 1 % sodium citrate) were added to each tube. Each tube was analized by spectrophotometry at 412 nm of wavelength and the absorbance was registred. From previous determination of GSH standard curve, it was possible to know GSH tissue content in the brain fragments. The groups of the second part of this study were used to this analysis. The choice of those groups was based on the electrophysiological analysis of the first part of this study.

All the animals were euthanazed with lethal dosis of urethane after the end of the experiment.

RESULTS

We observed that intracarotideal 3.03 mM α-lipoic acid infusion induced initial reduction (phases I and II) and final (phase III) partial reversal of mean absolute amplitude EEG waves (Table 1, Fig 1, Fig 2 - Panel A). Many mean absolute amplitude differences of EEG spectra between paired sub-groups reflecting initial reduction (control and initial or intermediate α-lipoic infusion phase) and final reversal (intermediate and final α-lipoic infusion phase) were statistically significant ($p<0,05$). More details about such mean EEG spectra absolute amplitude differences are presented in Tables 1 and 2, and Fig 2 - Panel A. The least significant differences were observed with left and right parietal alpha, right parietal delta and left and parietal theta spectra.

Table 1. Mean absolute amplitudes (μV) ± standard errors of mean of EEG spectra from scalp frontal and parietal regions. Control means EEG activity after ischemia and reperfusion; Initial means initial phase of 3.03 mM α-lipoic acid infusion; Intermediate means intermediate phase of 3.03 mM α-lipoic acid infusion; Final means final phase of 3.03 mM α-lipoic acid infusion. Initial reduction and final partial reversal of mean absolute amplitude EEG waves were observed during 3.03 mM α-lipoic acid infusion. See more details in the text.

Scalp	Experimental	EEG spectral bands					
regions	phase	Alpha	Theta	Delta	$Beta_1$	$Beta_2$	$Beta_3$
Left	Control	86.50±12.98	95.90±14.43	126.60±19.12	73.20±9.00	34.70±2.64	21.50±1.15
frontal	Initial	64.90±11.08	39.70±15.00	95.70±19.30	55.70±7.08	31.80±2.39	22.90±2.13
	Intermediate	32.00±3.40	15.20±5.74	52.10±7.27	35.40±4.25	26.30±3.08	18.60±2.11
	Final	57.90±5.18	16.70±6.33	92.50±11.94	51.10±3.50	30.10±2.49	20.50±2.78
Right	Control	84.40±15.96	93.00±17.40	126.00±23.98	70.20±10.92	33.11±3.47	20.20±1.89
frontal	Initial	64.00±12.89	74.90±15.50	97.50±22.66	55.70±9.00	31.31±2.74	21.50±1.62
	Intermediate	30.60±4.74	35.70±5.29	56.30±9.54	36.80±5.95	27.37±4.15	19.00±2.68
	Final	52.20±7.64	61.40±9.56	91.80±18.26	44.50±3.84	27.74±1.44	18.70±1.31
Left	Control	36.90±3.65	49.70 ± 6.87	57.14±11.00	35.85±2.79	26.55±3.67	20.20±1.89
parietal	Initial	33.20±4.16	48.20 ± 7.69	54.73±9.14	35.17±3.60	22.22±2.52	20.40±2.27
	Intermediate	21.50±3.43	29.00 ± 5.04	31.50±7.28	27.71±3.65	21.21±3.00	18.00±2.30
	Final	29.60±2.83	40.70 ± 3.66	47.14±7.55	32.72±3.22	22.00±2.10	18.10±2.28
Right	Control	38.00±8.84	55.40±12.20	65.40±18.11	39.20±5.45	28.90±3.10	20.80±1.24
parietal	Initial	32.40±6.11	51.90±9.62	41.50±15.67	36.40±3.07	28.00±2.51	22.80±2.01
	Intermediate	18.20±1.84	31.00±3.31	29.40±4.37	28.70±0.93	25.00±1.71	20.00±1.25
	Final	27.70±4.47	42.60±6.28	42.20±7.37	35.00±3.22	26.10±2.35	19.60±1.23

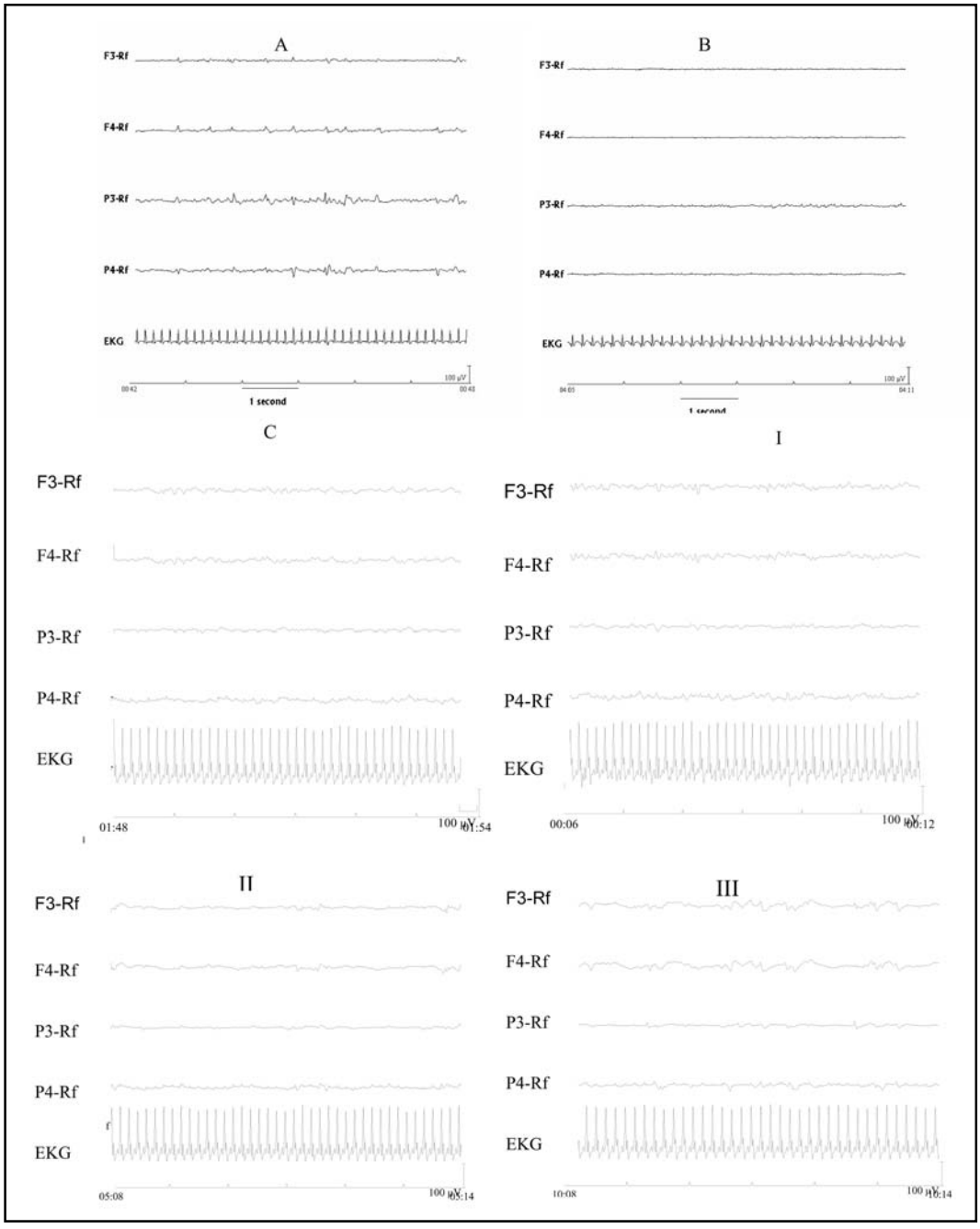

Fig 1. Rat EEG and EKG before (A), during (B) intracarotideal 60.6 mM α-lipoic acid infusion and before (C), during inicial (I), intermediate (II) and final (III) 3,03 mM α-lipoic acid infusion phases. F3 means left frontal; F4, right frontal; P3, left parietal; P4, right parietal; Rf, reference. More details are seen in the text.

This pattern of EEG spectra mean absolute amplitude variation with 3.03 mM α-lipoic acid intracarotideal infusion was not observed when we used other α-lipoic acid concentrations. With 6.06 mM α-lipoic acid infusion, there was a significant mean absolute amplitude increase ($p<0.05$) of right parietal delta, and left and right frontal theta spectra (Table 3, Fig 2B).

With 60.6 mM α-lipoic acid infusion, we observed a significant ($p<0.05$) mean absolute amplitude decrease of all EEG studied spectra with exception of right $beta_3$ and left frontal delta spectra (Table 4, Fig 3). The greatest statistical differences were found with right frontal ($p=0.0024$) and left parietal $beta_2$ ($p=0.0064$), right ($p=0.0052$) and left parietal $beta_1$ ($p=0.0054$), right frontal ($p=0.0083$) and left parietal alpha ($p=0.0066$), right frontal theta ($p=0.0098$), and right ($p=0.0030$) and left parietal delta spectra ($p=0.0066$).

There was a statistical significant increase of the brain tissue level of GSH evaluated by spectrophoto-

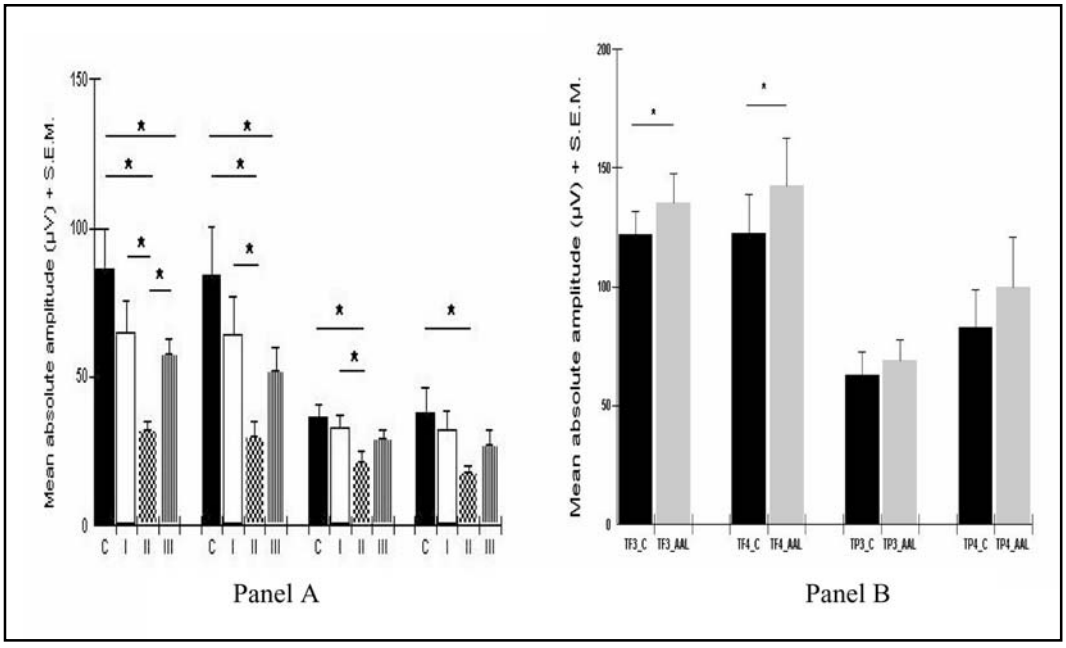

*Fig 2. Panel A - EEG alpha spectrum mean absolute amplitudes: groups control (C), initial (I), intermediate (II) and final (III) 3.03 mM α-lipoic acid infusion phases. S.E.M. means standard error of mean; * means p<0.05. Panel B - EEG theta spectrum mean absolute amplitudes; TF3 means left frontal theta; TF4, right frontal theta; TP3, left parietal theta, TP4, right parietal theta; C, control; AAL, 6.06 mM α-lipoic acid infusion; S.E.M., standard error of mean; *, p<0.05.*

metric method after infusion of 6.06 mM α-lipoic acid by intracarotid route. So, we found (0.372±0.021 μg of GSH/ml)/mg of brain tissue in rats with both common carotid arteries occlusion; in rats with both common carotid arteries occlusion plus 6.06 mM α-lipoic acid infusion, we found (0.444 μg of GSH/ml)/mg of brain tissue (p=0.024). With 60.6 mM α-lipoic acid infusion, the variations of GSH brain tissue contents were not significantly different (p>0.05).

DISCUSSION

In a previous observation, we demonstrated that computerized study of EEG spectral brain activity could be a valuable electrophysiological tool for evaluating drug cytoprotective action[1]. We also demonstrated that intracarotid GSH infusion could significantly revert to the previous wave amplitude the wave depression imposed by ischemia[2] and this effect was atributed to its physiological protection against oxidative stress. This fact has driven us to study α-lipoic acid intracarotideal infusion in Wistar rats with bilateral carotid arteries occlusion as previously described.

Previous studies have demonstrated α-lipoic acid cytoprotective action against reperfusion injury in animal models of global[23,24] and focal[25] cerebral ischemia.

*Table 2. Paired sub-groups with statistically significant EEG spectra mean absolute amplitudes differences (P<0.05) by ANOVA one way repeated measures (Student-Newman-Keuls method). Normality test failed when sub-groups C-II and I-II (parietal beta$_1$), I-III, I-II (right frontal theta) and C-II and I-II (right parietal theta) were analysed. C means control; I means inicial fase, II, intermediate and III, final fase of intracarotideal 3.03 mM α-lipoic infusion, *means P value by ANOVA Friedman method (failed normality test). Se more details in the text.*

Spectra	Alpha				Beta$_1$		Delta			Teta		
Regions	Frontal		Parietal		Frontal		Frontal		Parietal	Frontal	Parietal	
Side	left	right	left	right	left	right	left	right	right	left	left	right
	C - II	C - II	C-II	C-II	C - II	C - II	C - II	C - II	C - II	C - II	C - II	C - II
Groups	C - III	C - III	I-II		C - III	C - III	I - II		I -II	I -II	I - II	I - II
	I - II	I - II			C - I		III - II			III - II		
	III - II				I - II							
P value	0.000988	0.00158	0.0124	0.0176	0.0015	0.00275	0.00217	0.00601	0.0156	0.00208	0.0297	0.0239
												0.0293*

*Table 3. Spectral mean absolute amplitudes (μV) ± standard error of mean and P values of EEG spectra during 6.06 mM α-lipoic acid intracarotideal infusion; * means P value from non-parametric test (normality test failed), AAL means α-lipoic acid; Sp means spectra. There is a significant mean absolute amplitude wave increase (P<0.05) of right parietal delta and both right and left frontal theta spectra during 6.06 mM α-lipoic acid intracarotideal infusion. See more details in the text.*

EEG	Left frontal		Right frontal		Left parietal		Right parietal	
Sp	Control	α-lipoic	Control	α-lipoic	Control	α-lipoic	Control	α-lipoic
Alpha	104.07±12.17	108.13±12.55	102.42±16.99	109.97±15.52	44.14±7.07	47.89±7.27	55.40±14.06	66.80±15.45
	P=0.6202		P=0.1417		P=0.0996		P=0.1092	
Theta	121.70±9.73	135.50±12.00	122.60±16.27	142.70±19.58	62.82±9.60	68.92±8.84	83.00±16.05	99.60±21.45
	P=0.0414		P=0.0472		P=0.0884		P=0.0643	
Delta	128.60±12.90	136.60±14.00	117.10±13.20	137.30±12.80	51.95±4.89	59.80±8.28	60.50±8.92	83.71±21.68
	P=1.0000*		P=0.2431		P=0.5294*		P=0.0360*	
$Beta_1$	84.43±10.06	89.27±10.70	78.73±10.72	87.76±9.69	42.56±9.74	44.76±4.97	55.88±11.74	62.61±12.79
	P=0.3137		P=0.0876		P=0.4069		P=0.0592*	
$Beta_2$	41.58±8.30	43.17±10.67	39.01±3.45	41.33±3.61	26.06±6.30	25.46±6.43	36.99±12.02	38.15±12.87
	P=0.5009		P=0.2084*		P=0.8039		P=0.6345	
$Beta_3$	25.06±1.31	24.62±1.53	25.95±2.51	23.78±1.06	20.16±2.74	18.53±1.19	26.04±1.84	26.21±1.85
	P=0.8046		P=0.4600		P=0.8339*		P=0.9098	

Our demonstrations that 3.03 mM α-lipoic acid infusion promotes initial reduction and final partial EEG spectra mean absolute amplitude recuperation, allowed us to speculate that, in this condition, this substance has an initial pro-oxidant and a late anti-oxidant action. As we can see in Tables 1 and 2 and in Fig 2 – Panel A, many amplitude reductions and some amplitude final augmentations (left frontal alpha, theta and delta) are statistically significant. Some anti-oxidants, in some circumstances, produce eventually more oxidative stress , acting as pro-oxidant. Di-hydro-lipoic acid (reduced form from α-lipoic acid) reduces Fe^{+++} to Fe^{++}, activating Fenton reaction with $OH^{\cdot}$ and OH^{-} production as a pro-oxidant mechanism[26]. The di-hydro-lipoic acid also originates sulfur free radicals, and so, compromises some proteins such as 1- antiproteinase, with previous $^{\cdot}OH$ radicals generation[27]. The exogenous α-lipoic acid is rapidly reduced to di-hydrolipoic acid; in mitochondria the enzyme lipoate de-hydrogenase induces reduction of α-lipoic acid using NADH as co-factor[28]; in the cytosol the enzyme glutathione reductase actively participates of this reduction using NADPH as co-factor[29]. We speculate that such α-lipoic acid reductions could act as a possible cytotoxic mechanism. Roy and colleagues[30] suggested that lipoic acid could reduce NADH levels, using it as a co-factor during its reduction stress; during oxidative stress, both α-lipoic acid and di-hydrolipoic acid act as free radicals scavengers. It is possible that, at least, in α-lipoic acid infusion initial phases, the cerebral metabolic conditions of our experimental model behaved as it was in reduction stress. The final oxidative stress justifies the observed α-lipoic acid anti-oxidant activity.

This pattern of pro and anti-oxidant effect was not observed when we used 6.06 mM α-lipoic acid infusion. There was a significant mean absolute amplitude increase of right parietal and left and right frontal theta EEG spectra. So, we suppose that this anti-oxidant effect is more prominent in generating theta activity structure (the hippocampus). We ex-

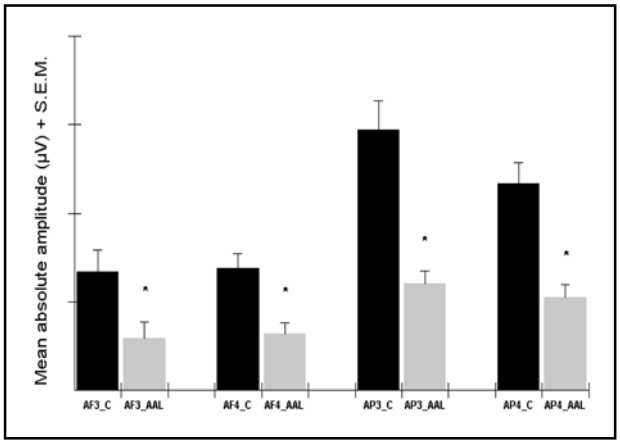

*Fig 3. EEG alpha spectrum mean absolute amplitudes; AF3 means left frontal alpha; AF4, right frontal alpha; AP3, left parietal alpha, TP4, right parietal alpha; C, control; AAL, 60.6 mM α-lipoic acid infusion, S.E.M., standard error of mean; *, p<0.05.*

*Table 4. Spectral mean absolute amplitudes (μV) ± standard error of mean and P values of EEG spectra during 60.6 mM α-lipoic acid intracarotideal infusion; * means P value from non-parametric test (normality test failed), AAL means α-lipoic acid; Sp means spectra. Significant mean absolute decrease of all EEG spectra (P<0.05) with exception of right parietal beta$_3$ is observed with 60.6 α-lipoic acid infusion. See more details in the text.*

EEG	Left frontal		Right frontal		Left parietal		Right parietal	
Sp	Control	α-lipoic	Control	α-lipoic	Control	α-lipoic	Control	α-lipoic
Alpha	67.20±12.37	29.50±9.26	69.20±8.63	32.00±6.99	147.20±16.12	60.50±6.96	116.8±11.88	52.70±7.27
	P=0.0193		P=0.0083		P=0.0066		P=0.0064	
Theta	77.90±17.90	40.20±14.60	84.30±11.10	43.30±12.30	195.70±22.00	77.70±13.00	146.10±15.80	71.50±13.10
	P=0.0444		P=0.0098		P=0.0138		P=0.0176	
Delta	77.50±27.30	48.10±17.30	84.00±17.38	53.40±16.43	196.50±17.30	92.80±15.40	146.50±17.20	81.90±14.40
	P=0.0714		P=0.0205		P=0.0066		P=0.0030	
Beta$_1$	59.90±10.69	28.00±7.90	63.80±8.86	30.70±5.19	124.70±11.84	55.50±7.35	101.60±8.24	52.50±7.26
	P=0.0208		P=0.0360*		P=0.0054		P=0.0052	
Beta$_2$	30.00±5.18	16.00±3.47	30.50±2.99	18.20±2.71	55.40±5.08	29.60±3.15	47.80±4.27	30.10±3.62
	P=0.0239		P=0.0024		P=0.0064		P=0.0102	
Beta$_3$	17.66±2.72	10.85±1.81	17.35±1.67	12.21±1.73	29.90±2.93	18.60±1.76	26.19±2.85	20.04±3.01
	P=0.0360*		P=0.0067		P=0.0220		P=0.0795	

perimentally observed by spectrophotometric method a significant increase of GSH in Wistar rat cerebral parenchyma with both common carotid arteries occlusion after 6.06 mM α-lipoic acid intracarotideal infusion (data not yet published). The antioxidant effect of 6.06 mM α-lipoic acid in our experiments could be due to GSH regeneration, after its reduction to di-hydrolipoic acid. Di-hydro-lipoic acid could actively participate of GSH regeneration mechanisms according to the chemical reaction: di-hydro-lipoic acid + oxidized glutatione (GSSG)® reduced glutathione (2GSH) + α-lipoic acid[20].

With our present experimental model, the intracarotideal infusion of more concentrated (60.6 mM) α-lipoic acid induced significant mean absolute amplitude reduction of all EEG spectra in almost all studied regions (Fig 3, Table 4). Therefore our data suggest that intracarotideal direct infusion of 60.6 mM α-lipoic acid in rats with both common carotid arteries occlusion has pro-oxidant effect. This could result of a counter regulatory effect on GSH production. Sen and colleagues[31], studying the intracellular thiol regulation by α-lipoic acid, verified that high concentrations of this acid produced cytotoxic lesions of stimulated mitogenic lymphocytes, in which thiol depletion and DNA fragmentation were detected. It was also verified that α-lipoic acid increases caspase-3 activation, having a role in cellular apoptosis[32]. Therefore, apoptotic events may be occurring with the higher concentrations used herein.

In the present experimental model high concentrations of α-lipoic acid was directly infused into the common carotid artery and rapidly gained access to cerebral parenchyma without systemic enzymatic reduction. So, the α-lipoic acid could be reduced directly in brain cells by glutathione reductase NADPH-dependent action with concomitant decreasing of GSH contents, and de-hydrogenase NADH-dependent action. Decreased contents of GSH could explain the pro-oxidant activity of higher concentrations of α-lipoic acid in our experimental model.

In a recent review Moini et al.[33] emphasized the antioxidant and prooxidant activities of α-lipoic and dihidrolipoic acid. According to these researchers, These are many evidences that both α-lipoic and dihidrolipoic acid exhibit direct free radical scavenging propreties and that there are only *in vitro* evidence of their prooxidant propreties. Our data suggest by first time the presence of this phenomena in an *in vivo* preparation induced by α-lipoic acid.

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