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Full Length Research Paper

Modulation of monoamines and amino-acids neurotransmitters in cerebral cortex and hippocampus of female senile rats by ginger and lipoic acid

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Brain aging is the major risk factor for common neurodegenerative diseases. Free radicals are involved in neurodegenerative disorders such as aging. Several neural systems are affected in aging. Neurotransmitters exhibited a marked alteration in different regions of the brain as part of the normal aging process. In the present study, age-related changes in the levels of monoamines and amino-acids neurotransmitters in normal female senile rats and the effect of oral administration of two antioxidants lipoic acid (LA) and ginger for 30 days accumulation on the neurotransmitters in the brain areas (hippocampus and cerebral cortex) is investigated. The levels of hippocampal monoamines [norepinephrine (NE) and serotonin 5-HT] and cortical dopamine (DA) and 5- HT were increased after administration of LA while, no significant differences were found in cortical monoamines compared with senile female rats. On the other hand, the levels of hippocampal DA, 5- HT and NE were increased after administration of ginger while, no statistical difference were found in cortical monoamines after administration of ginger compared with the adult ones. Furthermore, the effect of LA and ginger on the amino-acid profile in female senile rats was impressive. There were significant and remarkable increases in hippocampal glutamic, aspartic, GABA and cortical glutamic, aspartic, glycine and alanine compared with the levels of senile female rats. Ginger supplementation showed increased the amino-acids in the hippocampus (glutamic, aspartic, GABA and alanine) significantly as well as cortical aspartic, glycine, GABA and alanine compared with the normal senile female rats. In conclusion, LA and ginger may have shown a significant ameliorative value in counteracting age induced deficiency in some brain areas of female aged rats via modulating the investigating monoamines and amino acids in the brain cortex and hippocampus.

Key words: Ginger, lipoic acid, senile, monoamines neurotransmitters, amino acids, cortex, hippocampus.

INTRODUCTION

Aging is genetically programmed deterioration of all physiological function with age. Multiple studies demonstrated that aging increased excitability of principle hippocampal neurons (Bekenstein and Lothman, 1993; Barnes, 1994; Papa-theodoropoulos and Kestopoulos, 1996) and signified a diminution in the number and function of inhibitory interneurons with aging. Therefore, it is imperative to determine the extent of alternations in function of inhibitory gamma- amino butyric acid (GABA-ergic) interneurons in different regions of brain as a function of age (Stanly and Shetty, 2004).

Previous research showed that metabolism of monoamines neurotransmitters significantly changed with aging such as reduction of dopamine (DA) and norepinephrine (NE) in cerebrum and brain stem of aged rats (Barili et al., 1998; Lee et al., 2001). The levels of DA, NE and 5-hydroxytryptamine (5-HT) decreased throughout the brain of aged rats as compared with the brain of adult ones, display higher monoamines levels in striatum, entrohinal cortex, hippocampus and frontal cortex (Levine et al., 1990; Miguez et al., 1999).

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Moreover, Zambrzycka et al. (2002) indicated that aging processes play a major role in inhibition of choline acetyltransferase ChAT activity and this enzyme in striatum is selectively for β amyloid peptides, as well as, 5-HT or its metabolites are likely to enhance plasma lipid peroxidation via changes in the redox potential and lipid peroxidation chain reaction (Aviram et al., 1991).

Ginger extract and its biological active compounds caused some pharmacological activities, including anti-inflammatory (Lantz et al., 2007), anti-emesis (Sharma et al., 1997), anti-tumor (Surh, 2002) and analgesic effects (Aktan et al., 2006; Lantz et al., 2007) in numerous diseases. Recently, it had reported that zingerone and 6-shogaol (active compounds of ginger) prevented 6-hydroxy-dopamine induced dopamine depression (Kabuto et al., 2005) and apoptotic neural cell death (Kyung et al., 2006).

On the other hand, lipoic acid or its reduced form, dihydro-lipoic acid, is a physiological constituent of the mitochondrial membranes and is an essential cofactor for dehydrogenase. Biologically, alpha lipoic acid functions as cofactor of oxidative decarboxylation reactions in glucose metabolism to yield energy (Zulkhairi et al., 2008). Moreover, lipoic acid acts as an effective antioxidant in protecting the rat brain against reperfusion injury following cerebral ischemia (Panigrahi et al., 1996). Furthermore, Lykkesfeldt et al. (1998) discovered that the level of lipoate is lowered during the process of aging. Recent studies proved that lipoic acid acts as a potent antioxidant by inhibiting lipid peroxidation and thereby enhances the antioxidant status in aged rats (Bilska et al., 2008).

The present study amid to evaluate the age-related changes in the levels of monoamines and amino-acids neurotransmitters in normal female senile rats and the effect of orally administration of two antioxidants lipoic acid (LA) and ginger for accumulative 30 days on the neurotransmitters in the brain areas (hippocampus and cerebral cortex).

MATERIALS AND METHODS

Animals

Adult female albino rats weighing approximately 130-150 gm (3-4 months old) and senile (24 months old) weighing 280-300 gm were used. Rats were maintained in plastic cages and housed for 10 days prior to the initiation of the experiments, for adaptation to laboratory conditions. Animals were fed with commercial standard rat-pellet and tap water was provided *ad libitum.* Handling and usage of animals agreed strictly with the regulations and guidelines set by the research Ethics Committee of the Faculty of Science, Ain Shams University.

Drugs

Ginger was purchased from MEPACO (Arab Company for Pharmaceuticals and Medicinal Plants), Egypt and alpha lipoic acid (thiotacid) was purchased from EVA Company, Egypt. All other chemicals and solvents used were of the high performance liquid chromatography (HPLC) and analytical grade.

Experimental design

The animals were divided into four groups each of six rats as follows: the first was the control young rats and received orally 0.5% carboxy-methyl cellulose (CMC) sodium salt (0.1 ml/100 gm body weight), the second was the control senile rats and received the same amount of CMC. The third group, senile rats administrated ginger at a dose of 250 mg/kg body weight dissolved in CMC vehicle. The fourth group was senile rats administrated alpha-lipoic acid (ALA) (65 mg/kg body weight CMC). All groups received treatments for four consecutive weeks. Doses were calculated related to the human therapeutic dose according to Reagan-Shaw et al. (2007).

Following the completion of the experiments, the rats sacrificed after 12 hours from the last dose by rapid decapitation. Brain was excised for the determination of frontal cortex and hippocampus. The frontal cortex and hippocampus was homogenized in 75% methanol HPLC grade for determination of monoamines and free amino-acids using the precolumn phenylisothiocyanate (PTC) derivatization technique according to the method of Heinrikson and Meredith (1984).

Biochemical assay

The Agilent HPLC system used with Rheodyne injector 20µl loop and an ultraviolet (UV) variable wavelength detector was used for monoamine assays where the samples were injected directly into an AQUA column C18, purchased from Phenomenex, USA under the following conditions: mobile phase 97/3 20Mm potassium phosphate, pH 3.0/ methanol, flow rate 1.5ml/min, UV 270 nm. NE, DA, and 5-HT were separated after 10 minutes. The resulting chromatogram identified each monoamine position and concentration from the sample as compared to that of the standard, and finally, the calculation of the content of each monoamine as g per gram brain tissue was made according to Pagel et al. (2000).

As regard to amino acids assay the derivatization started by drying and re-drying the sample under test using re-drying solution consisted of 2:2:1 mixture (by volume) of methanol: 1M sodium acetate trihydrate: triethylamine (TEA). The drying solution was added to the dry sample, shook well and then put under vacuum till complete dryness. The derivatizing agent consisted of 7:1:1:1 mixture (by volume) of methanol: TEA: water: PITC (Phenylisothiocyanate). The derivatizing solution was added to the re- dried sample, shook well and left to stand at room temperature for 20 min, then applied to vacuum (70 millitore) till dryness. The dry sample was then reconstituted by sample diluent composed of 0.71 g disodium-hydrogen phosphate adjusted to a pH of 7.4 by 10% phosphoric acid. Acetonitrile was then mixed, as 5% by volume with the resulting solution. PICO- TAG column (Waters) was used for free-amino acid analysis 3.9 × 30 cm. The assay conditions were as follows: temperature: 46°C; wave-length: 254 nm; flow rate: 1ml/min. Standards and eluents are Waters chemistry package for free amino acids.

Statistical analysis

Reported values represent means ± SE. Statistical analysis was evaluated by one-way analysis of variance (ANOVA). Once a significant F test was obtained, LSD comparisons were performed to assess the significance of differences among various treatment groups. Statistical Processor System Support "SPSS" for Windows software, Release 12.0 (SPSS, Chicago, IL) was used.

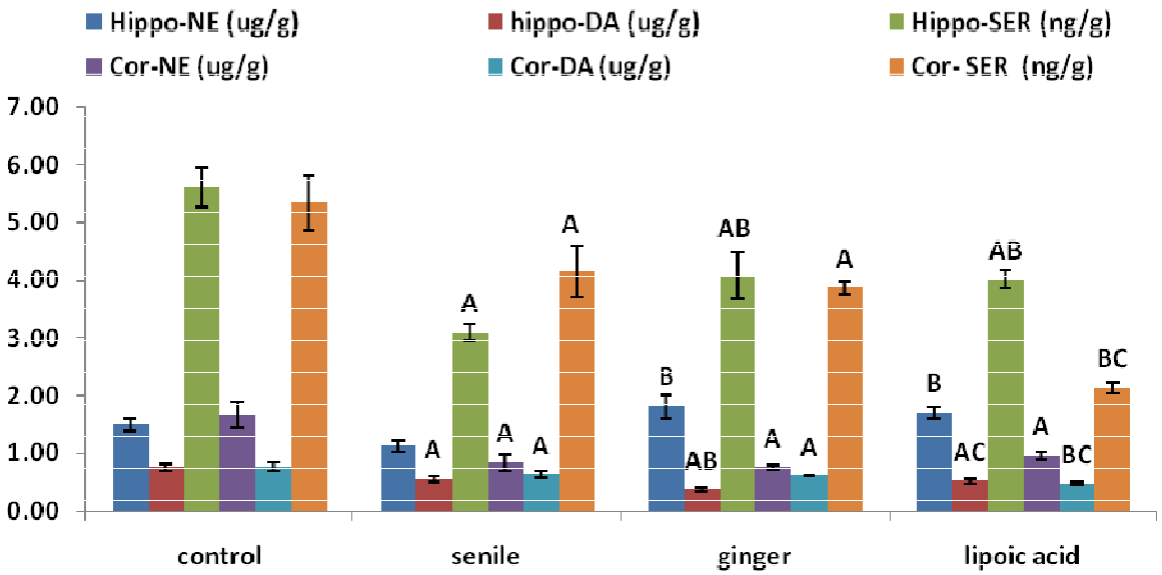

Figure 1. Effect of ginger and lipoic acid on norepinephrine (ug/g), dopamine (ug/g) and serotonin 5HT (ng/g) in hippocampus (Hippo) and cortex (Cor) of senile female rats. Values are means of 6 rats± SE. A = significant from control, B = significant from senile rats and C = significant from senile treated with ginger at p 0.05.

RESULTS

Figure 1 shows the effects of ginger and -lipoic acid administration on monoamines neurotransmitter levels in two brain regions, hippocampus and frontal cortex in rat groups. The data demonstrate that NE, DA and 5-HT levels in hippocampus and frontal cortex decreased significantly in senile female rats compared with that of the adult groups. The levels of NE, and 5-HT increased and DA decreased significantly in hippocampus of ginger treated senile group in comparison with senile non treated group. On the other hand orally administered lipoic acid group exhibited significant increase in NE and 5-HT levels in hippocampus when compared to the senile female rats. There were significant decreases in lipoic acid treated group in cortical DA and 5-HT levels at p 0.05 as compared with the senile female group.

The effect of the daily oral administration of ginger and lipoic acid on the concentration of amino acids (glutamic acid, aspartic acid, glycine, GABA and alanine) in the hippocampus and frontal cortex of senile female rats are presented in Figure 2. The senile female rats showed significant decreases in hippocampal glutamate and aspartate compared with adult female rats. In frontal cortex area of the senile group, significant decrease in levels of all amino acids investigated were found (p 0.05) as compared with the corresponding adult group. The daily oral administration of ginger induced significant increases in hippocampal glutamic, aspartic, GABA and alanine amino acids levels compared with the senile non treated female rats levels. As well as, in frontal cortex, administration of ginger showed significant increase in glutamic, glycine, GABA and alanine levels compared with senile female ones.

On the other hand, Figure 2 depicts the effect of - lipoic acid on amino acid neurotransmitter levels. The levels of all amino acids investigated were significantly elevated in hippocampus and frontal cortex except in the hippocampal glycine and alanine and cortical GABA comparing with the senile female group (p 0.05).

DISCUSSION

Aging is an inherently complex process that is regulated with multiple levels including genetic, molecular, and cellular and system levels (Kregel and Zhang, 2007). There are various structural, chemical and genetic changes that occur in brain of old age (Hedden and Gabrieli, 2004). Compared to other tissues in the body, the brain is deemed abnormally sensitive to oxidative damage (Keller et al., 2005). Recently, experimental evidence supports the existence of relationship between aging and various alternations of neurotransmitters in different areas of central nervous system (Ota et al., 2006; Lee et al., 2010).

Monoamine neurotransmitters are known to play an important role in the cognitive functions (Arnsten et al., 1994). The experimental results showed that the levels of DA, NE and 5-HT in the brain hippocampus and DA and 5-HT in cortical area of naturally senile female rats were remarkably lower than adult group. The present data indicated that monoamine neurotransmitters changed a lot during the aging process and orally administration of

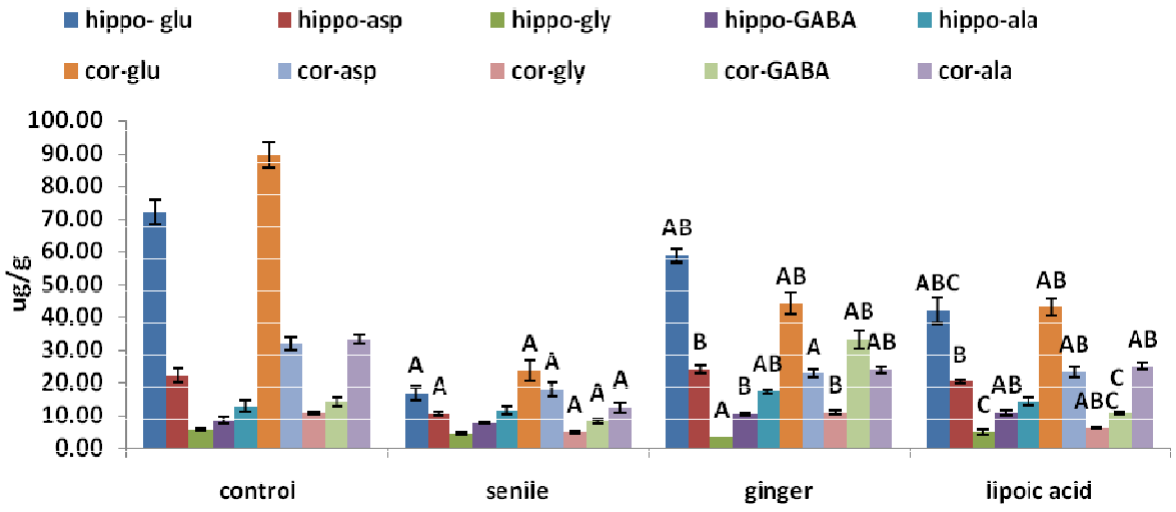

Figure 2. Effect of ginger and lipoic acid on glutamic (ug/g), aspartic (ug/g), glycine (ug/g), GABA (ug/g) and alanine (ug/g) in hippocampus (hippo) and cortex (cor) of senile female rats. Values are means of 6 rats± SE. A= significant from control, B= significant from senile rats and C= significant from senile treated with ginger at p 0.05.

ginger and – lipoic acid for 30 days can enhance the levels of hippocampal NE and 5-HT and cortical DA and 5-HT in case of – lipoic acid only. In line with previous results of Aviram et al. (1991) about alternation in 5-HT level, which played a major role in brain function, supported the formation of free radicals by aging. Furthermore, Jiang et al. (2009) reported that the levels of DA, NE and 5-HT in the brain tissues of naturally aged mice were remarkedly lower than adult mice. Hof and Mobbs (2009) reported the age relation to changes in dopamine synthesis, binding sites, and number of receptors. Recently, Santos *et al.* (2010) reported that significant age - and region – dependant impairments in monoamines modulatory neurotransmitter system that correspond well with the motor phenotype observed in the brain of methyl CpG binding protein 2 (a protein gene) in mice.

The result of the current study demonstrated that the levels of brain amino acids (hippocampal glutamic and aspartic acids) and cortical glutamic, aspartic, glycine GABA and alanine were significant declined in senile female rats compared with the adult female rats. Glutamate neurotransmitter decrease with age in living human brain in the motor cortex (Kaiser et al., 2005; Saliasuta et al., 2008; Chang et al., 2009). A significant age-related decline especially in the parietal gray matter, basal ganglia and to a lesser degree, the frontal white matter in human brain has also been noted (Kaiser et al., 2005; Saliasuta et al., 2008). On the other hand, Stanly and Shetty (2004) demonstrated that age- related decline in the functional GABA-ergic interneuron numbers may underlie some of the hippocampus related behavioral alterations observed in aged animals. In addition, Lee et al. (2010) discussed the decreased protein level and the age related functional decline and alterations of inhibitory function in the hippocampus.

Regarding ginger, the present result revealed that there were a remarkable increase in monoamine neurotransmitters (NE, DA and 5-HT) and amino acids neurotransmitters (glutamic, aspartic, GABA and alanine) in hippocampal area while no significant changes in the same monoamines in cortical regions, whereas there were significant increases in amino-acids (glutamic, glycine, GABA and alanine) neurotransmitters compared with the levels of the same areas in adult female rats. Kabuto et al. (2005) reported that zingerone and 6-shogaol (biological active compounds) prevent 6-hydroxy dopamine – induced dopamine depression. In addition, Geiger (2005) showed that Ginger exhibits 5HT3 receptor antagonism which effectively antagonizes serotonin at 5-HT3 receptors. This effect is mediated by galanolactone (one of the ginger constituents). On the other hand, Abdel-Aziz et al. (2006) found that the anti- emetic effect of ginger and some of its constituents is not mediated by competitive antagonism on the 5- HT3 receptor, since the constituents were not able to displace the specific radioligand from its binding site. Their action, therefore, may be mediated by binding to a modulatory site distinct from that of serotonin. Furthermore, Kyung et al. (2006) found that zingerone and 6-shogaol reduced apoptotic neuronal cell death and restores motor function in rat spinal cord injury. Qiang et al. (2009) showed that oil from ginger rhizome did not alter chronic unpredictable mild stress-induced reduction on 5-HT levels in prefrontal cortex, hippocampus and striatum in rats.

On the other hand, the effect of ginger on monoamines contents may be through androgenic activity (Chrubasik et al. 2005; Kamtchouing et al., 2002). Furthermore, Lin

et al. (2006) indicated that 1-(3, 4-dimethoxyphenyl)-3, 5-dodecenedione (I(6)), a derivative of gingerdione mediated neuroprotective effect. This effect may be due to increasing phosphorylation levels of extracellular signal-regulated kinases (Waggas, 2009). Another possible mechanism for Ginger, it shares with nonsteroidal anti-inflammatory drugs (NSAIDs) the property of inhibiting prostaglandin synthesis. Some ginger constituents are dual inhibitors of COX and LOX, and thereby reduce the biosynthesis of both prostaglandins and LTs. This remarkable property distinguishes ginger from conventional NSAIDs and may account for its lack of gastrointestinal and renal side effects (Grzanna et al., 2005).

The results of the present study demonstrated that the levels of monoamines (hippocampal NE, 5-HT and cortical NE) were significantly increased after administration of lipoic acid in aged normal female rats compared with the senile ones. Alpha lipoic acid or its reduced form proposed as an effective antioxidant in protecting the rat brain against reperfusion injury following cerebral ischemia (Panigrahi et al., 1996) and the level of lipoate was lowered during the process of aging (Lykkesfeldt et al., 1998). The present results can be explained by de Sales et al. (2010) who showed, for the first time, the effects of LA on monoamines levels, in the CNS. They results were consistent with the hypothesis that LA stimulates the release and/or syntheses or reduces the metabolization rate of endogenous monoamines. LA increased the dopamine and norepinephrine levels in normal rat hippocampus. Moreover, serotonin levels were decreased. Together, these results are of interest, considering that some neurodegenerative diseases are related to the imbalance of these monoamines levels in the central nervous system (CNS). The present results support the same hypothesis of modulation of investigated brain neurotransmitters. DA has a modulator effect on the dopaminergic system, in which increase of DA in the hippocampus is correlated with decreased glutamate levels de Sales Santos et al. (2010). Furthermore, the increased dopamine and norepinephrine levels can be produced by three mechanisms: 1) by increasing their synthesis and or release; 2) by reduction in their metabolization rate; and/or 3) by decreasing its reuptake brain. In another set of experiments previous study showed that ventral tegmental area stimulation releases endogenous dopamine from the axonal terminals of dopaminergic neurons in the accumbens, and de Sales Santos results suggested that LA induces the release of dopamine in hippocampus (Tao et al., 1996). Inhibition of the GABA receptor decreases hippocampal 5-HT level, while application of GABA agonists to hippocampus decreases 5-HT release (Stanzione et al., 1984). This reciprocal modulation of 5-HT and GABA in the hippocampus appears incompatible with the simplistic hypotheses of decreased 5-HT and GABA levels in neurodegenerative diseases. GABA prevented over firing of the nerve cells by blocking the transmission of an impulse from one cell to another in the central nervous system. Which produce anti-aging effects in brain mice (Bist and Bhatt, 2009).

On the other hand, Zhang et al. (2001) found that treatment with LA protected primary neurons of rat cerebral cortex against cytotoxicity induced by both - amyloid (A) and hydrogen peroxide. In a similar study, Lovell et al. (2003) reported that pretreatment of neurons (hipocampal cultures) with LA significantly protected against A and Fe/H_2O_2 toxicity, whereas concomitant treatment of cultures with LA potentiated the toxicity of Fe/H_2O_2. Furthermore, Zambrzycka et al. (2002) indicated significant inhibitory effect of aging on acetylecholinestrase (AChE) in brain cortex and striatum. Moreover, Han et al. (1997) demonstrated that LA can protect cells from both excitotoxicity and cystine-inhibtion oxidative stress aspects of glutamate. They concluded that LA has remarkable therapeutic potential in protecting against neurological injuries involving glutamate and oxidative stress.

Conclusion

From the present results ginger and LA have shown a significant ameliorative effect in counteracting age induced deficiency in some brain areas of female aged rats through increasing the investigating monoamines and amino acids in the brain cortex and hippocampus of senile female rats.

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