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The Effects of Coadministration Palmitic Acid and Oleic Acid (Omega 9) on Spatial Learning and Motor Activity in Adult Male Rat

[1]A.A. Moazedi, [1]Z. Hossienzadeh and [2]R. Chinpardaz
[1]Department of Biology, Faculty of Science,
[2]Department of Statistic, School of Mathematics, Shahid Chamran University, Ahwaz, Iran

Abstract: In this study, the effects of oleic acid (10%) and palmitic acid (10%) on spatial learning and motor activity have been investigated. So, adult male Wister rats divided into four groups (n = 10). Control group was fed with ordinary diet. other groups were fed with a diet containing oleic acid (10%) for 4 weeks or palmitic acid (10%) for 1 week. Also, coadministration group was fed with a diet containing oleic acid (10%) for 4 weeks plus palmitic acid (10%) for 1 week. In next step, rats were trained for spatial learning task by using T-maze at subsequently 9 days. Moreover, rats were tested for motor activity task by using Rota rod based on standard method. The results showed that the spatial learning and motor activity were significantly ($p<0.01$, $p<0.001$) increased in rats fed with oleic acid (10%) for 4 weeks. Also Spatial learning and motor activity were significantly ($p<0.001$, $p<0.001$) increased due to the diet containing palmitic acid 10% for 1 week. Also the Spatial learning and motor activity were significantly ($p<0.01$, $p<0.001$) increased in coadministration group diet containing oleic acid (10%) for 4 weeks and palmitic acid (10%) for 1 week but statistical test between three diet groups were not significant. A consensus has emerged from recent research that it is important the balance of the different type of fats. Because the type of fatty acids in the diet and ratio of these fatty acids determines the type of fatty acids that is available to the composition of cell membranes spatially in nerves system.

Key words: Palmitic acid, oleic acid, learning, balance diet, learning

INTRODUCTION

Lipids are important constituents of the neuronal membrane and changes in lipid composition may alter membrane activities. Essential fatty acids are necessary in growth, cell division and nerve function. They are found in high concentrations in the brain and are essential for normal neurotransmission and brain function. (Mostosky *et al*., 2004).

The type of dietary fat we consume affects the biology of each cell and determines how well it can perform its vital function and its ability to resist diseases. (Ruthrich *et al*., 1999) Indeed among the significant component of cell membranes are the phospholipids, which contain fatty acids. The type of fatty acids in the diet determines the type of fatty acids that is available to the composition of cell membranes. A phospholipids made from a saturated fat has a different structure and is less fluid, than incorporates an unsaturated and essential fatty acid. These consequences for cell function are not restricted to absolute levels of fatty acids but rather depend on the relative amounts of omega-3 and omega-6 in cell membrane (Yehuda, 2003; McCann and Ames, 2007).

The fatty acids are involved in retinal function, learning and memory mechanism, thermoregulation, pain, stress and sleep. It has demonstrated that a specific mixture of alpha-linolenic acid and linoleic acid in a ratio of 1:4 has significant effect on both human and lab animals (Rabinovitz *et al*., 2004). This compound improved the quality of life of Alzheimer patients and exerted beneficial effects in naive aged rats, as well as in rats that were experimentally rendered learning deficient. It improved the memory, thermoregulation and sleep in human and rats and rehabilitated symptoms of induced multiple sclerosis in rats (Rabinovitz *et al*., 2004; Yahuda *et al*., 2000). Fatty acids deficiency may impair normal neurological development (Yahuda *et al*., 2006).

Indeed the developing brain produces all required palmitic acid (Marbios *et al*., 1992).

So, based on different reports about the effects of saturated fatty acids and un saturated fatty acids spatially palmitic acid and oleic acid on nerve system and, learning and memory, in this study the effects of palmitic acid and oleic acid and coadminstration of them on spatial learning and motor activity of rats by using T-maze task have been investigated.

Corresponding Author: A.A. Moazedi, Department of Biology, Faculty of Science, Shahid Chamran University, Ahwaz, Iran
Tel: 00989163080198

MATERIALS AND METHODS

Subjects: This study was done in laboratory of learning and memory, Shahid Chamran University for 4 month in 2006. So, 40 Adult male wistar rats weighing 275±25 g were used. The rats were housed in individual cages with free access to water and Pellets food. Their ages were 1.5-2 month at the training test.

Group treatment: Palmitic acid (10 g) or oleic acid (10 g) and standard food (90 g) were mixed then rats were fed freely all the time. Briefly, the diets contained the following: 5% soybean protein isolate, o.3% DL-methioninm, 32.7% corn starch, 25% sucrose, 2% cellulose powder, 5% mineral mixture, 1% vitamin. Rats were categorized were as follow:

At first four groups fed oleic acid (10%) for 1, 2, 3 and 4 weeks and four groups were fed with palmitic acid (10%) for 1, 2, 3 and 4 weeks.

The compare between groups fed oleic acid (10%) for 1, 2, 3 and 4 weeks showed that the best group is 4 weeks in spatial learning and motor activity. Mean while the statistical analyses between groups were fed palmitic acid (10%) for 1, 2, 3 and 4 weeks indicated the best group is 1 week in spatial learning and motor activity. In next experiment a group was fed oleic acid (10%) for 4 weeks plus palmitic acid for 1 week. All groups compare with control group was fed with ordinary food.

Palmitic acid ($C_{16}H_{32}O_2$) and oleic acid ($C_{18}H_{36}O_2$) were obtained from Merck Chemical Company.

Behavioral testing

T-maze task: All rats were trained in T-maze during 9 days based on standard method (Annet *et al.*, 1989).

Three days before the behavioral test, rats were fasted for 23 h day^{-1} to maintain 80% of normal body weight. Water was available *ad libitum* throughout all experiments (Cocco *et al.*, 2002).

Apparatus: A wooden T-maze with 15 cm high walls and start and goal boxes 15-20 cm was used. The 48 cm long stem led to two L-shaped arms. The first part of each arm was 39 cm long and the second part, which led to the goal box, 28 cm long. Guillotine doors separated the start box from the stem and the stem from the L-shaped arms

Procedure: Preliminary training took place on days 1-3. On day 1 food pellets (4 mg) were left in the stem, arms and goal boxes with all the guillotine doors open. The rats were placed individually in the start box and left for 5 min to eat the pellets and explore the maze.

On days 2 and 3 each rat was confined to the start box for 10 sec and then allowed to find 4 pellets in either of the goal boxes, where it was confined for a further 40 sec.

On days 4 and 5, two pellets were available in both of the goal boxes on every trial. Each rat was confined to the start box for 10 sec before being allowed to choose one of the goal boxes, where it was confined for a further 20 sec. Eleven consecutive trials were given per day and the choice of right or left goal box was recorded, as was the latency between leaving the start box and entering the chosen arm.

On days 6, 7 and 8 were considered for spatial learning and reversal: Only one of the goal boxes was now rewarded and the rats had to learn which was correct. For half the rats the reward was to be found in the right goal box and for the other half the left goal box was rewarded. Trials continued until the criterion of 5 consecutive correct responses had been achieved. The choice of arm and latency between leaving the start box and entering the chosen arm were recorded on every trial. Immediately after reaching criterion the contingencies were reversed so that the previously unreinforced goal box was now correct. Training continued until the new response had been learnt, again to a criterion of 5 consecutive correct responses. This procedure commenced on day 6 and was repeated on days 7 and 8. Each day the rat was always required to start by re-learning the spatial discrimination which had been correct last during the previous day's training.

On day 9 as a extinction, after a spatial learning had been completed to 5 consecutive correct responses, an extinction stage was introduced. The food pellets were removed from the T-maze and goal box choices and start box to arm latencies were recorded over a further 10 trials.

2- Rotarod: The Rotarod test a rat is placed on a rotating rod. The speed of rotation is gradually increased and the rodent's ability to remain on the rotating rod or balances is recorded.

Purpose: The purpose of the Rotarod test is to assess the rat's sensorimotor coordination.

The animals are placed on textured drums to avoid slipping. When an animal drops onto the individual sensing platforms below, test results are recorded. The Rotation is at a perfect constant speed from 10 rpm (RUN Mode), in a choice of 3 selected times: maximum 60 sec. Then was measured average of three times (Hirvonen *et al.*, 2000).

Statistical analysis: For comparison between each dietary group and control were made using student t-test and for compare all groups, then data elevated by two ways ANOVA. For comparison two by two dietary groups were assessed by post hot the Tukey test.

RESULTS AND DISCUSSION

The T-maze result showed that a decrease in trail to criterion simple learning between palmitic acid (10%) was fed for one week group were significant ($p<0.05$) but not in another groups. The number of trail to criterion on reveres learning, in palmitic acid (10%) was fed group 1 week and oleic acid fed 4 weeks and coadminstration of them significantly decreased ($p<0.01$, $p<0.01$, $p<0.05$) (Fig. 1). The number of error on simple learning in coadminstration group was significantly ($p<0.05$) decreased. The number of error on reveres learning in palmitic acid (10%) fed for 1 week group was significantly ($p<0.001$) decreased but not about the other groups (Fig. 2). Mean latency on simple learning in oleic acid was fed 4 weeks were significantly ($p<0.01$) decreased but not about the other groups. Mean latency on reveres learning in palmitic acid (10%) was fed for 1 week and oleic acid was fed 4 weeks were significantly ($p<0.001$, $p<0.01$) decreased (Fig. 3). extinction trails in palmitic acid (10%) fed for 1 week, oleic acid was fed 4 weeks and coadminstration of them were significantly ($p<0.001$, $p<0.05$, $p<0.01$) decreased (Fig. 4).

The Rotaroad result showed that The mean of motor activity and balance in palmitic acid (10%) was fed for 1 week, oleic acid was fed 4 weeks and coadminstration of them were significantly ($p<0.001$, $p<0.001$, $p<0.001$) increased (Fig. 5).

DISCUSSION

In this study palmitic acid (10%) diet for 1 week was positive significant on spatial learning (Fig. 1-4).

In past studies our laboratory showed that the groups were dosed lovastatin for 9 days after 2 weeks administration of butter (10%) enhanced learning performance compared to rats that dosed only with lovastatin and also, historical results studies exhibited that haven't had obvious signs of cell injury in rats hippocampus. However increases learning performance that may be due to presence of saturated fatty acids in butter, mean while the most amount of saturated fatty acids in the butter is palmitic acid. Indeed the developing brain produces all required palmitic acid (Marbios *et al.*, 1992) according these studies confirmed present result.

Recently investigation shows that mechanism of effect of palmitic acid in brain. The palmitic modification of neuronal proteins is critical. Protein palmitoylation

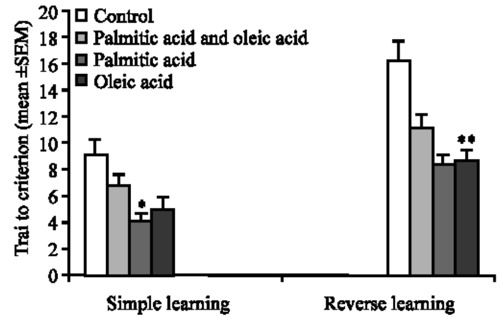

Fig. 1: Mean of trail to criterion on simple and reveres learning in rat which were fed with a diet containing palmitic acid (10%) for 1 week, oleic acid (10%) for 4 weeks and coadminstration palmitic acid for 1 week plus oleic acid for 4 weeks and control *: $p<0.05$; **: $p<0.01$

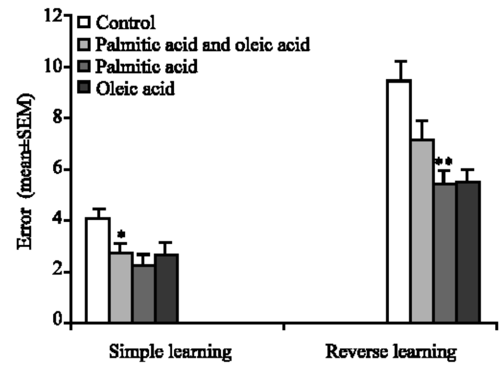

Fig. 2: number of error on simple and reveres learning in rat which were fed with a diet containing palmitic acid (10%) for 1 week, oleic acid (10%) for 4 weeks, coadminstration palmitic acid for 1 week plus oleic acid for 4 weeks and control *: $p<0.05$; **: $p<0.001$

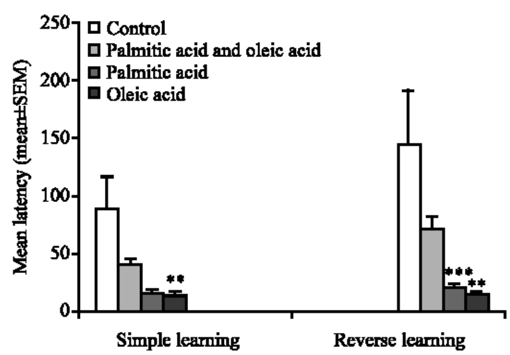

Fig. 3: Mean latencies on simple and reveres learning in rat which were fed with a diet containing palmitic acid (10%) for 1 week, oleic acid (10%) for 4 weeks and coadminstration palmitic acid for 1 week plus oleic acid for 4 weeks and control **: $p<0.01$; ***: $p<0.001$

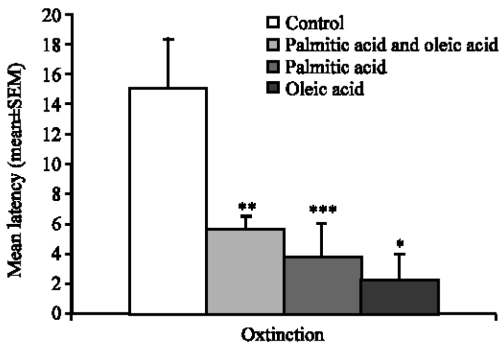

Fig. 4: Mean latencies on extinction in rat which were fed with a diet containing palmitic acid (10%) for 1 week, oleic acid (10%) for 4 weeks, coadminstration palmitic acid for 1 week plus oleic acid for 4 weeks and control, *: p<0.005; **: p<0.001; ***: p<0.001

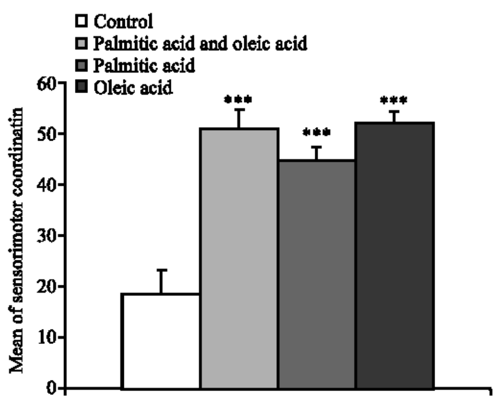

Fig. 5: Mean of sensorimotore coordination in rat which were fed with a diet containing palmitic acid (10%) for 1 week, oleic acid (10%) for 4 weeks, coadminstration palmitic acid for 1 week plus oleic acid for 4 weeks and control, ***: p<0.001

represents a common lipid modification of neuronal proteins (El-Husseini and Bredt, 2002; Linder and Deschenes, 2007). Carbon palmitic lipid in a thioester linkage to specific cycteine residues. Palmitoylation modifies nurmous important neuronal protein GAP-43, G-protein and etc. Importantly, palmitoylation occurs in reversible fashion which allows palmitoylation to dynamically regulation protein function and to participate in diverse as pects of neuronal signaling (Fukata *et al.*, 2004). This lipid modification is critical for PSD-95 clustering of AMPA receptors at excitatory synapses. Furthermore, the palmitoylation of PSD-95 is dynamically regulated by synaptic, such that cycling of palmitate on PSD-95 can contribute to aspects of synaptic plasticity and palmitate cycling on PSD-95 is augmented by calcium influx through NMDA receptors (El-Husseini *et al.*, 2002). So, dietary of palmitic acid is very important in control of cognition process because it transport across the blood-brain barrier (Alberghina *et al.*, 1993).

Although a group dietary palmitic acid (10%) for 1 week was significantly increased in motor activity (Fig. 5). As we know the dopamine transporter plays a crucial role in dopaminergic neurotransmission by taking up extracellular dopamine into nerve cells, thereby terminating action of the released transmitter. The mechanism that regulate dopamine uptake are of medicinal importance as potential sites of action for Parkinson and other neurological diseases and for psychiatric and other complex diseases involving dopamine (Hahn and Blakely, 2002). Also many G-protein receptors have been shown to be palmitoylated at cycteine residues in the cytoplasmic tail. For example metabotropic glutamate mGlu4 receptor, dopamine D1 and dopamine D2. Palmitoylation the function such as receptor internalization and down-regulation may be affected by elimination of palmitoylation (Jin *et al.*, 1999). So, palmitic acid probably affected on motor activity via dopamine receptors.

In next experiment spatial learning was increased in rats fed oleic acid (10%) for 4 weeks (Fig. 1-4). In past studies our laboratory showed that spatial learning in rats fed corn oil (10%) for 1, 2 and 3 weeks were significantly increased. Also spatial learning in groups fed sesame oil for 3 and 4 weeks were increased. However increases learning performance that may be due to presence of unsaturated fatty acids in both of plant oil and also the most amounts of unsaturated fatty acids in that oil is linoleic acid and oleic acid. Indeed 5 and 10 g kg^{-1} of soybean in diet for 30 days in mice were improved obviously and brain weight was increased the protein and polyunsaturated fatty acid and ratio them were significantly higher compared with control (Gong *et al.*, 2004) therewith soybean is contain oleic acid. Oleic acid reversibly opens the blood-brain barrier (Sztriha and Betz, 1991). Although, the mechanism of effect oleic acid on nerve system was revealed.

Oleic acid is used by neurons for the synthesis of phospholipids and is specifically incorporated into growth cones. In addition, oleic acid promotes axonal growth, neuronal clustering and the expression of the axonal growth-associated protein, GAP-43. All these observations point to oleic acid as a key factor in neuronal differentiation. GAP-43 is a marker of neuronal differentiation. This protein is located in growing axons, where it is bound to the membrane (Granda *et al.*, 2003).

Thus, it is probably that the effect of oleic acid on spatial learning by these mechanisms.

Furthermore motor activity was increased in rats fed oleic acid (10%) for 4 weeks (Fig. 5).

The presence of unsaturated fatty acids such as linoleic acid can be effective in the treatment of mood disorders, similar changes to our food supply and dietary habits may also be acting to increase the prevalence of behavioral and motor activity such as dyslexia, dyspraxia, ADHD and autism (Richardson, 2001).

Indeed MN9D dopamine increases linearly over a 48 h period suggesting the induction of an increased dopaminergic phenotype in these dividing cells. The ability to increase MN9D dopamine by oleic acid is shared by a number of other long-chain fatty acids including arachidonic, linoleic, linolenic, palmitoleic and cis-13-octadecenoic acid. The possibility that therapeutic approaches to the treatment of dopaminergic cell loss and the motor sequelae of Parkinson disease (Heller *et al*., 2005).

In next step of experiments spatial learning and motor activity were improved in rats fed oleic acid (10%) for 4 weeks plus palmitic acid (10%) for 1 week (Fig. 1-5). A consensus has emerged from recent research that it is important the balance of the different type of fats. Because the type of fatty acids in the diet and ratio of these fatty acids determines the type of fatty acids that is available to the composition of cell membranes spatially in nerves system.

Moreover the diet contained appropriated amounts and balance of linoleate and alpha-linoleinate, it is possible for rats to synthesize an appropriate amount of DHA and have normal behavioral activity without DHA supplementation (Cheon *et al*., 2000).

Also, in the brain a significant positive correlation between amyloid beta peptide and each of cholesterol, palmitic acid and stearic acid and between the number of reference memory errors and each cholesterol, palmitic, stearic and oleic acid. Moreover, a significant negative correlation was observed between the number of reference memory error and molar ratio of DHA to palmitic plus stearic acid in the brain (Hashimoto *et al*., 2005).

Although, the ratio of Saturated Fatty Acids (SFA) to Unsaturated Fatty Acid (USFA) determine mount of cholesterol in membrane. Decreased the ratio of SFA/USFA induced to decreased cholesterol and increased fluidity of membrane spatially in nerve system.

This formula was suggested to affect fatty acid on mount of cholesterol:

$$\text{Cholesterol} = 2.7\ (\text{saturated fatty acids}) - 3.7\ (\text{polyunsaturated fatty acids})$$

The monounsaturated fatty acids similar polyunsaturated fatty acids with lesser effect on cholesterol (Dietschy, 1998).

Also, cholesterol is an essential membrane constituent of most animal cells. This sterol has been shown to be involved in a wide variety of critical cell functions, including modulation of enzyme function, permeability, fusion and receptor function. The critical importance of cholesterol to normal functioning of cell membranes may lay in its ability to both alter fundamental properties of phospholipids bilayer and to interact directly with specific membrane proteins. If membrane cholesterol decreases and fattyacyl unsaturation increases, allows the protein to more easily activated (Albert and Boesze-Battaglia, 2005). This way of reducing cholesterol levels in the brains of living animals both decreased amyloid deposition and improved learning. Researchers have been investigating a potential relationship between cholesterol metabolism and Alzheimer's for several years.

When cholesterol level is low, it is bad for the liver and the brain (Xu, 1998) so, according our result that consume palmitic acid for short time increased a low cholesterol level which brain needed it and our data is established.

CONCLUSION

Palmitic acid a oleic acid is important in cognition and motor activity and both of them involve in brain but the ratio of them in dietary are importance because mount of saturated fatty acid and unsaturated fatty acid determine function of membrane in nerve system.

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