Carnitine: A Neuromodulator in Aged Rats

Packiasamy A. R. Juliet,1 Deepak Balasubramaniam,2 Naveen Balasubramaniam,2 and Chinnakannu Panneerselvam3

1Department of Geriatrics, Nagoya University School of Medicine, Japan.
2Madras Medical College, M.G.R. Medical University, Chennai, India.
3Department of Medical Biochemistry, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai, India.

A wide range of morphological and biochemical changes occur in the central nervous system with increasing age. L-carnitine, a naturally occurring compound, plays a vital role in fatty acid transport across the mitochondrial membrane. L-carnitine (300 mg/kg body wt/day) was administered intraperitoneally to young and old male Wistar rats for 7, 14, and 21 days. Carnitine, dopamine, epinephrine, and serotonin levels were assayed in discrete regions of the brain. Carnitine supplementation increased the levels of dopamine, epinephrine, and serotonin in the experimental animals in our study. Response to carnitine supplementation varied among the brain regions that have been studied. The regions rich in cholinergic neurons such as the cortex, hippocampus, and striatum showed more response after 21 days of carnitine treatment. The results of the present study suggest the role of L-carnitine as a neuromodulator and antiaging medication.

AGING has been defined as the sum total of all changes that occur in living organisms with passage of time that lead to functional impairment, increased pathology, and death (1). Loss of neurotransmitters, their receptors, and response to neurotransmitters are key manifestations of neurological aging (2). During aging, biochemical functions dealing with anabolism decrease, whereas those dealing with catabolism increase. Free radical theory of aging confirms that aging is associated with an impaired control of oxygen homeostasis (3). Gerlach and colleagues (4) proved that oxidative stress and iron are the key factors in neurodegeneration. Rapid auto-oxidation of catecholamines generate toxic free radicals, semiquinones, hydrogen peroxide, and neuromelanin, which further increases hydroxyl radical (•OH) production (5), thereby leading to neuronal loss (6). Toxic free radicals affect the sulfhydryl groups present in the carrier molecule and thus affect neurotransmitter transport (7). In nerve cells, neurotransmitter uptake by the presynaptic neurons and axonal transport in both directions requires adenosine triphosphate (ATP), which is provided by mitochondria (8). Oxidative stress by an increase in free radicals leads to an alteration of mitochondrial membrane ionic permeability, which causes a decline in ATP synthesis (9). Review of the literature suggests that oxidative stress and energy depletion may lead to imbalance and neurodegenerative processes.

The importance of nutrition in delaying the process of aging is well recognized by gerontologists. Attempts have been made to delay the onset of aging. Antioxidants are being administered to rodents to retard aging. L-carnitine is a naturally occurring compound. Carnitine therapy is used in many diseased conditions such as diabetes mellitus (13), atherosclerosis (14), and myocardial myopathy. Carnitine plays an important role in fatty acid transport across the mitochondrial membrane (10). Research data (11) have shown that a long treatment with carnitine spared pyramidal cells in the hippocampus. Carnitine concentration has been shown to decrease during aging in various regions of the aged rat brain (12). The present study was carried out to assess the neuromodulating action of L-carnitine in various brain regions by supplementing it to aged rats.

MATERIALS

L-carnitine (inner salt) was purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade.

Animals and Experimental Design

Male albino rats of the Wistar strain, which belonged to 2 different groups, aged 4 months and 24 months, respectively (Groups I and II), were used. Each age group was subdivided into 4 groups. One control group (Group IA and IIA) and 3 experimental groups were based on the duration of carnitine administration, for 7 days (Group IB and II B), 14 days (Group IC and II C), and 21 days (Group ID and II D). The animals were maintained on commercial rat feed that contained 5% fat, 21% protein, 55% nitrogen-free extract, and 4% fiber (w/w) with adequate mineral and vitamin content. Each group consisted of 6 animals that had access to food and water ad libitum. Experimental animals were administered L-carnitine (300 mg/kg body wt/day) intraperitoneally in 0.89% physiological saline. Control group animals received the vehicle alone.

Twenty-four hours after the last injection in the respective groups, all the animals were decapitated and the brains were quickly removed and dissected into hypothalamus, hippocampus, cortex, cerebellum, and striatum according to the method of Glowinski and Iversen (15) and immersed in saline. Carnitine and neurotransmitters such as dopamine, serotonin, and epinephrine were measured (16,17). The...
tissue levels of nerve growth factor (NGF) were assayed according to the methods previously described by Piovesan and colleagues (18). Statistically significant changes in the different groups were evaluated by student’s t test. The levels of significance were evaluated with p values.

RESULTS

Figure 1 shows the level of carnitine in various brain regions of control and carnitine-treated young and aged rats. The level of carnitine was found to be decreased in the cortex, hippocampus, striatum, cerebellum, and hypothalamus of aged rat brain. A highly significant reduction was observed in the cortex and hippocampus followed by the striatum. There was no significant increment in carnitine levels in young rat brain regions, even at the end of 21 days of carnitine administration, whereas aged rats showed a highly significant increase (cortex, striatum, and hippocampus: \( p < .001 \)) in carnitine status after 21 days of carnitine treatment. Table 1 shows the level of dopamine in various regions of control and carnitine-treated young and aged rat brain.

The dopamine turnover is an important factor in determining the degree of dopaminergic neuron loss because neurons with a physiologically higher dopamine metabolism could be subjected to a greater oxidative stress from dopamine-derived oxyradicals. It can be seen from the figure that the level of dopamine decreased during aging. The level of dopamine was significantly low in the cortex and striatum followed by the hippocampus (\( p < .001 \)), cerebellum, and hypothalamus (\( p < .05 \)) of the aged rat brain. Significant increase in the level of dopamine was observed in the cortex, hippocampus (\( p < .001 \)), and striatum (\( p < .01 \)), whereas only a mild increase was observed in the cerebellum and hypothalamus (\( p < .05 \)) of aged rat brain after carnitine administration. Table 2 depicts the effect of carnitine on the level of epinephrine in young and aged rat brain regions.

NGF is the best-characterized neurotrophin and is critical to the survival and maintenance of some populations of sympathetic and sensory neurons. In the CNS, NGF and its receptors have been found to be associated with cholinergic innervations. Unlike other enzymes, the level of NGF is moderately decreased during aging. NGF was significantly low in the cortex and in hippocampus (\( p < .001 \)) followed by the striatum and hypothalamus (\( p < .01 \)), whereas no age-related changes were observed in the cerebellum of the aged rat brain (Figure 2). The level of NGF was gradually

![Figure 1. Level of carnitine in carnitine-treated young and aged rat brain regions. Values are expressed as mean ± SD (standard deviation) for 6 animals in each group. On comparing groups IB, IC, and ID with group IA and groups IIB, IIC, and IID with group IIA: *\( p < .05 \); **\( p < .01 \); ***\( p < .001 \). Comparison of group IIA with group IA: #\( p < .01 \); ##\( p < .001 \).](https://academic.oup.com/biomedgerontology/article-abstract/58/11/B970/640330/971)
increased after carnitine supplementation. The cerebellum region did not show any response to carnitine supplementation. The regions that are highly affected during aging, such as the cortex and hippocampus, show high response to carnitine supplementation.

DISCUSSION
Aging induces several alterations in brain structure and functions. In old age, the level of neurotransmitter synthesis and their transport are decreased (19). Age-related changes in the brain are regionally variable since some brain regions are more age sensitive than others because of their biochemical make-up and also because functions differ from region to region (20). The present study was carried out to assess the effect of carnitine on age-associated alterations in different regions of the rat brain.

Carnitine is found in all areas of the CNS suggesting that a metabolic pool of carnitine exists in the brain (21). In the present study, the level of carnitine was found to be significantly low in aged rat brain regions (Figure 1). Our observations were consistent with those of Costell and colleagues (22) who demonstrated lower levels of carnitine in tissue of aged mice and humans. The changes in the level of tissue carnitine are pronounced in particular brain regions. It is well known that the brain is supplied with its carnitine requirements solely through hepatic synthesis and blood transport. The decrease in the level of carnitine may be due to factors such as reduced biosynthesis in the liver, defective carnitine transport, or failure of carnitine reabsorption by the kidney. Deficiency of any of the cofactors such as vitamin C, vitamin B, and pyridoxal phosphate and methionine during old age may be another reason (23–25).

Supplementation of L-carnitine significantly enhanced the status of carnitine in various regions of the brain of study animals while no significant increase was observed in young rats. The increase in the level of brain carnitine observed in the experimental animals treated with carnitine indicates that there is proper intestinal absorption (26) and adequate blood concentration due to exogenous administration.

Dopamine is easily auto-oxidized, and excessive oxidation of dopamine may lead to the accumulation of cytotoxic compounds with increasing age (27). Brain dopamine undergoes oxidation resulting in an increased level of oxygen free radicals, which might affect the cellular antioxidant defense mechanisms, which in turn could result in the degeneration of dopaminergic neurons (28). Dopamine oxidation products such as dopamine quinones and dihydroxy phenylacetic acid quinones bind to sulfhydryl groups of proteins and accumulate in the brain and are responsible for the physiological changes that take place during aging (29).

The levels of dopamine and epinephrine are found to be near normal after the supplementation of carnitine because carnitine is able to enhance the glutathione level due to its energy-promoting property (30). The enhanced glutathione binds to quinone and prevents its reaction with the sulfhydryl group of protein, which in turn inhibits the formation of 5-s-CDA (chenodeoxycholic acid). Apart from this, glutathione prevents an excess of cysteine from binding.

Table 1. Dopamine Level in Control and Carnitine-Treated Young and Aged Rat Brain Regions (Cortex, Hippocampus, Striatum, Cerebellum, and Hypothalamus)

<table>
<thead>
<tr>
<th></th>
<th>Young IA</th>
<th>Young IB</th>
<th>Young IC</th>
<th>Young ID</th>
<th>Aged IIA</th>
<th>Aged IIB</th>
<th>Aged IIC</th>
<th>Aged IID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>124.8 ± 41.7</td>
<td>130.5 ± 30.2</td>
<td>138.5 ± 24.5</td>
<td>143.5 ± 27.3</td>
<td>87.12 ± 19.1**</td>
<td>91.2 ± 14.8</td>
<td>105.6 ± 16.8**</td>
<td>117.7 ± 12.9***</td>
</tr>
<tr>
<td>Striatium</td>
<td>4018.1 ± 18.7</td>
<td>4047.8 ± 120.2</td>
<td>4088.0 ± 107.8</td>
<td>4112.4 ± 110.1</td>
<td>2818.7 ± 100.5**</td>
<td>3359.4 ± 110.7</td>
<td>3625.8 ± 101.7**</td>
<td>3994.2 ± 118.7**</td>
</tr>
<tr>
<td>Cortex</td>
<td>197.24 ± 10.7</td>
<td>199.9 ± 10.1</td>
<td>201.85 ± 9.5</td>
<td>204.54 ± 12.3</td>
<td>160.41 ± 11.1**</td>
<td>169.98 ± 13.1</td>
<td>178.55 ± 15.2**</td>
<td>188.10 ± 9.7***</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>61.8 ± 13.8</td>
<td>63.4 ± 14.2</td>
<td>66.3 ± 15.1</td>
<td>68.7 ± 13.3</td>
<td>41.3 ± 18.4*</td>
<td>47.8 ± 16.4</td>
<td>50.9 ± 11.9</td>
<td>56.7 ± 9.0*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>321.9 ± 28.8</td>
<td>325.7 ± 25.4</td>
<td>329.9 ± 18.1</td>
<td>333.4 ± 13.5</td>
<td>295.7 ± 20.5*</td>
<td>298.8 ± 19.8</td>
<td>308.1 ± 16.7</td>
<td>315.5 ± 17.7*</td>
</tr>
</tbody>
</table>

Note: Dopamine content is expressed as ng/g tissue.
Values are expressed as mean ± SD for 6 animals in each group.
On comparing groups IB, IC, and ID with group IA and groups IIB, IIC, and IID with group IIA: *p < .05; **p < .01; ***p < .001.
Comparison of group IIA with group IA: *p < .05; **p < .01.

Table 2. Epinephrine Concentration in Control and Carnitine-Treated Young and Aged Rat Brain Regions (Cortex, Hippocampus, Striatum, Cerebellum, and Hypothalamus)

<table>
<thead>
<tr>
<th></th>
<th>Young IA</th>
<th>Young IB</th>
<th>Young IC</th>
<th>Young ID</th>
<th>Aged IIA</th>
<th>Aged IIB</th>
<th>Aged IIC</th>
<th>Aged IID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>300 ± 18.7</td>
<td>303.8 ± 18.7</td>
<td>305.7 ± 19.0</td>
<td>309.1 ± 16.6</td>
<td>271.7 ± 16.4**</td>
<td>278.8 ± 16.9</td>
<td>285.5 ± 11.4</td>
<td>293.4 ± 10.7**</td>
</tr>
<tr>
<td>Striatium</td>
<td>360.8 ± 15.1</td>
<td>363.1 ± 15.1</td>
<td>367.5 ± 14.4</td>
<td>370.0 ± 14.5</td>
<td>355.4 ± 16.5**</td>
<td>341.8 ± 15.4</td>
<td>347.1 ± 12.8</td>
<td>356.1 ± 14.4**</td>
</tr>
<tr>
<td>Cortex</td>
<td>240.5 ± 21.1</td>
<td>245.5 ± 19.1</td>
<td>249.5 ± 16.1</td>
<td>254.5 ± 18.9</td>
<td>208.1 ± 18.1**</td>
<td>215.5 ± 14.3</td>
<td>228.4 ± 16.2</td>
<td>237.8 ± 15.0**</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>189.7 ± 23.5</td>
<td>192.5 ± 22.1</td>
<td>195.7 ± 21.8</td>
<td>199.4 ± 19.1</td>
<td>163.4 ± 19.1*</td>
<td>168.9 ± 17.6</td>
<td>174 ± 15.5</td>
<td>182.1 ± 12.7*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>158 ± 23.1</td>
<td>159.1 ± 19</td>
<td>161.8 ± 17.5</td>
<td>163.4 ± 15.6</td>
<td>140.3 ± 16.5</td>
<td>143.5 ± 14.5</td>
<td>147.6 ± 12.2</td>
<td>149.8 ± 11.2</td>
</tr>
</tbody>
</table>

Note: Epinephrine content is expressed as ng/g tissue.
Values are expressed as mean ± SD for 6 animals in each group.
On comparing groups IB, IC, and ID with group IA and groups IIB, IIC, and IID with group IIA: *p < .05; **p < .01.
Comparison of group IIA with group IA: *p < .05; **p < .01.
to dopamine to form quinines, which inhibit complex-I activity (31). The ATP-enhancing effect of carnitine is also responsible for the amelioration of the neurotransmitter storage, release, and reuptake process that is necessary for maintaining the normal level of neurotransmitter in the rat brain (32).

Elevated level of NGF after carnitine administration protects the brain from oxidative damage induced by dopamine auto-oxidation product. NGF enhances the activities of tyrosine hydroxylase and dopamine decarboxylase (33,34). This neurotrophin increases the availability of glutathione (35), which in turn protects the dopaminergic neurons from dopamine toxicity and also enhances the activities of catalase and glutathione peroxidase (36), which helps the brain to overcome catecholamine-induced toxicity during aging.

In contrast to catecholamines, serotonin was found to be decreased during aging (Table 3). The decreased activity of tryptophan hydroxylase in some brain areas may be responsible for this. The concentration of 5-HIAA (hydroxyindoleacetic acid) was shown to be increased in aged rats, and this increase may be correlated with the increased monoamine oxidase (MAO) activity during aging (37). In the cortex and striatum, the activities of MAO and carboxy-o-methyl transferase (COMT) were shown to be increased with age. There is evidence that cholinergic and serotonergic transmission could interact in the CNS (38). Recently, Lohninger and colleagues reported that the learning ability of old rats is improved by carnitine (39).

Conclusion

The results of the present study confirm that carnitine acts as a neuromodulator during aging. The antioxidant-replenishing, NGF-enhancing, and ATP-promoting actions of carnitine could be the factors responsible for its beneficial role in neurotransmitter metabolism. Aging is also associ-

Table 3. Status of Serotonin in Control and Carnitine-Treated Young and Aged Rat Brain Regions (Cortex, Hippocampus, Striatum, Cerebellum, and Hypothalamus)

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th></th>
<th></th>
<th></th>
<th>Aged</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IA</td>
<td>IB</td>
<td>IC</td>
<td>ID</td>
<td>IIA</td>
<td>IIB</td>
<td>IIC</td>
<td>IID</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>310.24 ± 31.8</td>
<td>312.38 ± 10.4</td>
<td>318.72 ± 11.2</td>
<td>323.71 ± 16.2</td>
<td>270.87 ± 16.28*</td>
<td>278.91 ± 12.3</td>
<td>290.10 ± 17.2**</td>
<td>304.77 ± 20.1***</td>
</tr>
<tr>
<td>Striatum</td>
<td>240.18 ± 38.2</td>
<td>244.08 ± 18.2</td>
<td>249.58 ± 15.2</td>
<td>254.71 ± 11.45</td>
<td>191.41 ± 11.2**</td>
<td>202.38 ± 9.8</td>
<td>215.72 ± 18.1**</td>
<td>231.01 ± 20.1***</td>
</tr>
<tr>
<td>Cortex</td>
<td>370.41 ± 30.2</td>
<td>375.01 ± 10.2</td>
<td>382.10 ± 12.4</td>
<td>388.32 ± 8.7</td>
<td>300.11 ± 40.2***</td>
<td>324.21 ± 30.4</td>
<td>350.50 ± 20.3**</td>
<td>372.08 ± 11.01***</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>180.41 ± 20.5</td>
<td>182.38 ± 12.1</td>
<td>185.61 ± 14.2</td>
<td>188.44 ± 8.9</td>
<td>161.85 ± 10.8*</td>
<td>165.48 ± 11.1</td>
<td>170.59 ± 12.8</td>
<td>176.91 ± 14.1*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>604.21 ± 24.3</td>
<td>607.31 ± 8.4</td>
<td>610.48 ± 7.1</td>
<td>614.81 ± 10.2</td>
<td>584.31 ± 8.4*</td>
<td>589.17 ± 8.5</td>
<td>593.01 ± 4.11</td>
<td>597.66 ± 7.3***</td>
</tr>
</tbody>
</table>

Note: Serotonin content is expressed as ng/g tissue.
Values are expressed as mean ± SD for 6 animals in each group.
On comparing groups IB, IC, and ID with group IA and groups IIB, IIC, and IID with group IIA: *p < .05; **p < .01; ***p < .001.
Comparison of group IIA with group IA: #p < .05; ##p < .01; ###p < .001.

Figure 2. Nerve growth factor (NGF) level in control and carnitine-treated young and aged rat brain regions (cortex, hippocampus, striatum, cerebellum, and hypothalamus). Values are expressed as mean ± SD (standard deviation) for 6 animals in each group. On comparing groups IB, IC, and ID with group IA and groups IIB, IIC, and IID with group IIA: *p < .05; **p < .01; ***p < .001. Comparison of group IIA with group IA: #p < .05; ##p < .01; ###p < .001.
ated with an increase in occurrence of heart, kidney, and immunological disorders. The beneficial role of L-carnitine has already been confirmed in the above conditions. The present findings suggest that L-carnitine could be used for the treatment of neuromotor disorders in view of its beneficial neuromodulatory activity and may also serve as an antiaging medication.

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Address correspondence to Chinnakannu Panneerselvam, Department of Medical Biochemistry, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 113, Tamil Nadu, India. E-mail: panneerselvam@eth.net

REFERENCES