Section A-Research paper

EVALUATION OF MCT2 AND MCT4 EXPRESSION OF DEPRESSION IN STRESS INDUCED RATS

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Abstract

Introduction: Glucose is the brain's primary source of energy. Decreased brain energy metabolism is linked to cognitive dysfunction in depressive disorders. Lactate has long been thought to be a glycolytic by-product in the brain but lactate has been transposted to the brain by monocarboxylate transporters (MCT).
Aim: To determine the effect of depression on monocarboxylate transporter (MCT) expression in the rat brain.
Methods: Depression was induced in wistar rats by a CUMS (Chronic Unpredictable Mild Stress) Protocols. Rats were scarified after 30days, and the brain levels of MCT2 and MCT4 were assessed with semi-quantitative RT-PCR and Western blotting analysis.
Results: Gene expressions of *mct2* and *mct4* were analyzed using semi-quantitative RT-PCR that showed significant decrease in *mct2* and *mct4* during chronic stress and their respective increase on administration of plant extracts and exercise treatment. MCT4 protein expression were significantly found to be decreased in Group II and increased in plant and swimming exercise treated groups when compared to normal.
Conclusions: The expression of brain MCT2 and MCT4 is significantly increased after treatment with *Sesbania bispinosa* and swimming exercised rats.

Key word: Depression, monocarboxylate transporters (MCTs), *Sesbania bispinosa.*

INTRODUCTION

Depression is a severe illness marked by an inability to carry out daily tasks that an individual typically enjoys persistent unhappiness, and a loss of interest in activities (1). Depression is the most common component and one of the top three illnesses in terms of disease burden worldwide. The cause and effect of depression has been connected to several non-communicable diseases (NCDs) (2). Depression occurs nearly twice as much in women as in men and affects approximately 6 % of the world's adult population every year (3). According to the World Health Organization, In 2015, the global prevalence of depression was projected to be 4.4 % (4). Furthermore, depression is associated with an elevated risk of developing conditions such as diabetes mellitus, heart disease and stroke (5), thus increasing its disease burden further. In addition, depression can lead to suicidal death. (6)

Genetic, epigenetic, physiological, and psychosocial factors are commonly implicated in the biological basis of mood disorders (7). Depression may be associated with genes that occupy a fixed location on chromosome 8, 15 and 17, according to some studies (8). A particular mechanism comprising multiple malfunctioning neural circuits (9) may actually linked to neurotransmission disorders in the brain, including substances such as serotonin , norepinephrine, dopamine, gamma-aminobutyric acid (GABA), cerebral nerve growth factor (BDNF)(10).

Glucose is the brain's primary source of energy (11). Decreased brain energy metabolism is linked to cognitive dysfunction in depressive disorders (12). Lactate has long been thought to be a glycolytic by-product in the brain (13) but lactate has been transported to the brain by monocarboxylate transporters (MCT). MCTs are a protein family with 14 different isoforms. MCT1, MCT 2 and MCT4 transports astrocytes from lactate, pyruvate, and ketone bodies to glial cells (14). Lactate, on the other hand, is an essential energy substrate that plays an important role in the metabolism and development of energy and memory, according to growing evidence (13).

Physical exercise has been recommended as a complementary treatment that can help to improve residual symptoms of depression and prevent relapse (15). The antidepressant effect of exercise therapy was published in the British Medical Journal in 2001(16). The monoamine hypothesis of depression suggested that the monoamine neurotransmitters serotonin and

norepinephrine were insufficiently accessible. Serotonin and norepinephrine levels rise as a result of exercise (17).

Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders including depression (18). *Sesbania* is a large genus of herbs, shrubs and in certain cases, soft wooded trees occurring in the tropical regions of the world. About six species occur in India and are normally cultivated for green manure and fodder as well as for the temporary shades, wind breaks (19).

Fortunately, our view about pathophysiology, treatment, and the biochemical basis of depression and the role of monocarboxylates for sustaining brain functions has changed in depression, bringing new attention on monocarboxylates and their transporters MCTs mediate the release of lactate from astroycytes and its incorporation into neurons, which could generate satiety during depression.

In the present study the oxidative stress generated by the immobilization stress. We used RT-PCR and western blotting technique to investigate the expressions of *mct 2* and *mct 4* in rat brain.

MATERIAL REQUIRED:

Plant collection and authentication

The whole plant of *Sesbania bispinosa* were from the local areas of Coimbatore district, Tamil Nadu, India. The plant was dried in shade at room temperature. The dried whole plant was submitted and authenticated (No.BSI/SRC/5/23/2014-15/Tech-1641) at Botanical Survey of India, Southern Regional Centre, Coimbatore, India.

Procurement of Animals

Young female Albino rats of Wistar strain (100 ± 20 g) procured from Chettikulam, Nagarcoil, India were used for the study. The Ethical clearance for handling of experimental animals were obtained from the Institutional Animal Ethics Committee (IAEC) constituted for the purpose and care of laboratory animals as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social justice and empowerment, Government of India (CPCSEA/No: 264/2015/IAEC).

Semi-quantitative RT-PCR(20)

MCT2 and MCT4 mRNA levels in rat brain were determined using semi-quantitative real-time PCR analyses. Brain samples were homogenized with Trizol reagent and total RNA was extracted according to the manufacturer's protocol. Concentration and purity of total RNA was determined using a imageJ. Total RNA was then reverse-transcribed into complementary DNA (cDNA) using a cDNA synthesis kit which was performed according to the manufacturer's protocol. Semi-quantitative RT-PCR was performed.

The Semi-quantitative RT-PCR conditions were 42°C/30 min, and 80°C/5 min for reverse transcription. PCR involved pre-denaturing at 95°C for 1 minute, then 30 cycles of 95°C for 15 seconds, 58°C for 20 seconds, and 72°C for 20 seconds. *gapdh* was used as the housekeeping gene. Levels of MCT1 and MCT4 mRNA were calculated based on the comparative quantification method.

The PCR primers used included: MCT2 forward primer (5′ TCA GCT CTG CAA TGA TGT TT - 3′), reverse primer (5′- AGG GAG GAT TGT GTG CGT TT -3′); MCT4 forward primer (5'- CCA GGC CCA CGG CAG GTT TC-3'), reverse primer (5'- GCC ACC GTA GTC ACT GGC CG-3); GAPDH forward primer (5'-CCA CTA GGC GCT CAC TGT TC-3'), reverse primer (5'-AGG CGC CCA ATA CGA CCA A-3'). All samples for each gene were run in duplicate.

Western blotting(21)

Brain tissue was homogenized and protein concentration of the supernatant was determined using a BCA method. The supernatant containing 50 μg of protein was separated using a 10%SDS-PAGE and transferred onto polyvinylidene difluoride membranes by electrophoresis. The membranes were then blocked in TBS containing 0.1% Tween-20 and 5% non-fat milk at room temperature for 1 hour. The membranes were incubated in primary antibodies (anti-MCT4) at 4 °C overnight, followed by a HRP-conjugated anti-rabbit IgG secondary antibody or anti-mouse IgG secondary antibody. Target proteins were detected using the ChemiDocXRS+ chemiluminescence imaging system.

RESULTS:

Extraction and Quantification of total RNA from rat brain

The total RNA was extracted from the rat brain using the TRI Reagent and was cleaned by using amplification grade DNase I. RNA integrity was assessed by electrophoresis and spectrophotometer. UV spectroscopy was used to calculate the amount of RNA present, and the results are summarized in table 1. As a result, cDNA was produced by reverse transcription of

purified total RNA. Following cDNA amplification, the sequence-specific primer pairs for the expression of the *mct2* and *mct4* genes were found and exploited in semi-quantitative real-time PCR.

According to a recent study, the brain is incredibly susceptible to oxidative damage, which is a major component of various neuropathological disorders (22). Antioxidant absorbed by neurons changes local energy metabolism by enhancing lactate absorption (23). As suggested by the ANLS, this may therefore provide a mechanism by which neuronal lactate use is preferred (over glucose) to meet the energy needs (24).

Table 1: Quantification of total RNA from rat brain using UV spectroscopy

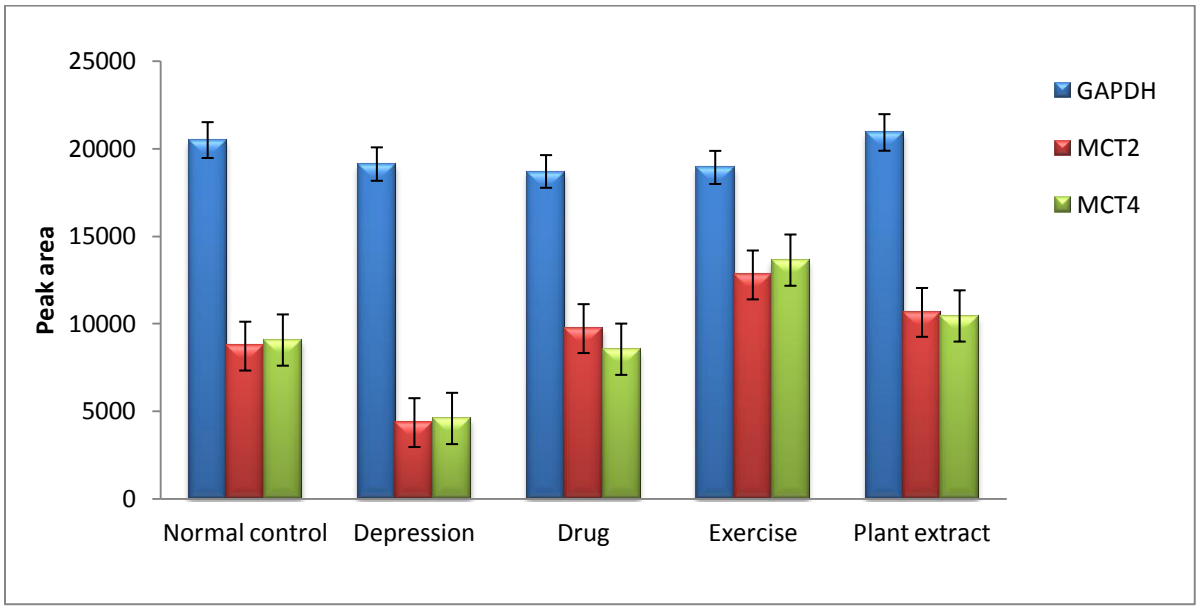
Groups	Concentration of RNA in brain (µg/ml)	
	Before cleaning	**After cleaning**
Normal control	3.40±0.46	1.87±0.25
Depressed	3.50±0.78	2.27±0.45
Drug Treated	2.78±0.83	2.06±0.25
Plant Treated	3.00±0.31	2.00±0.67
Exercise Treated	3.17±0.45	2.03±0.40

Another study showed that cerebral MCTs reliant on ANLS (Astrocyte-Neuron Lactate Shuttle) are essential for the brain's energy metabolism and crucial for neuronal plasticity, the process of learning, and memory (25,26). Additionally, it has been observed that MCTs play a crucial role in the transfer of energy metabolites between brain cells due to their association with the distribution of other crucial metabolic components, such as LDH, in the central nervous system (27). In addition, the expression of *mct2* on neurons that use lactate as an effective oxidative energy source and mct4 on astrocytes that create substantial amounts of lactate have helped to develop and establish the ANLS concept (28).

Gene Expression of *mct2* and *mct4* in the rat brain

MCT expression in the brain of animal models of depression was initially analyzed using Semi-quantitative RT-PCR with primers specific for *mct2*, *mct4*, and *gapdh* (housekeeping) genes. The resulting PCR products were separated using agarose gel electrophoresis and stained with ethidium bromide. The bands were then captured on film and evaluated using ImageJ software.

FIGURE 44: Peak area of Semi-quantitative RT-PCR

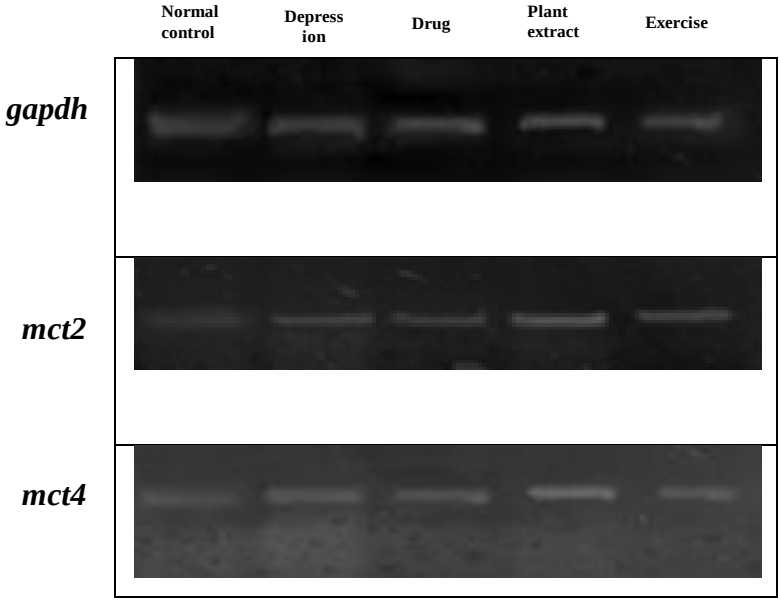


The present study shows the expression of *mct2* and *mct4* genes in rats. In group II (depressed) rats, the levels of *mct2* and *mct4* genes were significantly decreased when compared to that of group I (normal rats). After treatment, the gene expression was found to be reversed. The standard drug (Imipramine) treated rats showed an increased level of *mct2* and *mct4* gene expression than the group II (depression) rats. The exercised and plant extracts treated depressed rats revealed increased levels of *mct2* and *mct4* gene expression than the untreated rats. A recent study shows that the MCTs belong to the unique class of metabolic genes that are rapidly up-regulated by exercise (29).

It's interesting to note that Rao et al. also observed an increase in *mct2* transcript expression in the cerebral cortex, which indicates that an increased supply of different substrates

(lactate, pyruvate, and ketone bodies) in the cerebral cortex may potentially play a role in preventing neuronal damage (30).

FIGURE 2: Gene Expression of *mct2* and *mct4* in the rat brain

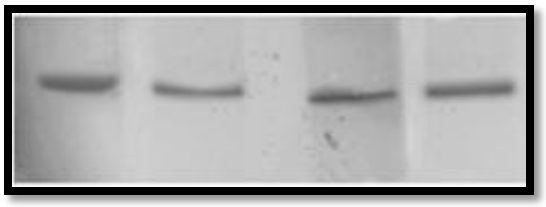


Protein Expression of MCT4 in the rat brain

The expression of the MCT4 protein in rat brains was identified using western blotting. The findings revealed a significant difference in MCT4 between the 4 groups. When compared to the normal control group, the chronic stress-induced group had considerably lower levels of the protein MCT4 in the rat brain. However, significant increases in MCT4 protein levels were seen in the plant extract treated group when compared with the untreated rats. The swimming exercise-treated depressed rats revealed increased levels of MCT4 protein expression than the depressed rats without exercise or plant treatment.

In standard conditions, glucose serves as the primary metabolic fuel for neurons and astroglia in the rat brain (31). However, other metabolic products like lactate and ketone bodies can operate as substitute energy sources for cerebral activity, particularly under conditions like hypoxia and states of cognitive impairment (32, 33).

FIGURE 3: Protein Expression of MCT4 in the rat brain



Monocarboxylates were mentioned as an alternate energetic substrate for the brain in the description of brain oxidative stress (33). Thus, studies have demonstrated that chronic stress is associated with increased brain MCT expression to favor the use of these alternative energetic substrates. Moreover, this overexpression is associated with increased monocarboxylate uptake (mainly lactate) during plant extract administration and swimming exercise.

The role of monocarboxylates as energy substrates for the brain has been well documented (34). The earlier data reported here also demonstrates that the MCT4 has been proposed as the primary lactate transporter at high lactate concentrations, which are seen, for example, during intensive exercise (35).

Conclution:

Thus, these studies suggested that variations in the expression of cerebral MCTs may be pertinent to neuroproductivity. As shown by our findings, *Sesbania bispinosa* leaf extract can be used therapeutically under traditional medicine to treat depression. The monocarboxylate transporter MCT4 also appears to be a significant contributor to neural health and is connected with the pathophysiology of depression.

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