International Journal of Medicine and Medical Sciences Vol 1(6) pp. 254-262, June, 2009
Available online http://www.academicjournals.org/ijmms

Full Length Research Paper

Cysteamine in 3- nitropropionic acid model of Huntington's disease in rats: Modulation of mitochondrial function and amino acid pattern

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Accepted 12 June, 2009

Huntington disease (HD), a neurodegenerative disorder, is characterized by selective atrophy and cell loss within the striatum. 3-nitropropionic acid (3-NP) is a mitochondrial toxin that induces experimental HD-like disorders. Currently, although there is no treatment that can prevent the striatal neuropathology, cysteamine is considered one of the most promising candidate drugs for HD. Previous studies showed that cysteamine modulates 3-NP-induced HD, via several mechanisms; however, its effect on amino-acids profile and mitochondrial function was not tested before, which is the main aim of the current study. Male Wistar albino rats (200-250 g) were injected subcutaneously by 3-NP (20 mg/ kg/ day for 7 days), to serve as positive control group. Another group received cysteamine intraperitonealy in a building dose from 25 up to 75 mg/kg/day for 7 days, one hour before 3-NP. Normal untreated rats were used as negative control. The striatal biochemical parameters and serum amino acid pattern were assessed one hour after the last 3-NP injection. The mitochondrial toxin resulted in significant decrease in striatal citrate synthase (CS), creatine kinase (CK) and glutathione (GSH) accompanied by a marked increase in nitric oxide (NO) activity/ content. Significant reduction in branched chain amino acids, glycine, alanine, serine, taurine, tyrosine and histidine was also demonstrated in 3-NP- treated rats. Cysteamine administration markedly improved the mitochondrial function as exhibited by restoration of CS activity; however, no effect was noticed on the rest of the striatal biochemical parameters. In addition, some of the amino acids altered by 3-NP were ameliorated by cysteamine. In conclusion, results of the present study explored the importance of amino acid pattern in the pathogenesis of HD and confirmed the neuroprotective efficacy of cysteamine against HD which may be related at least partly, to its influence on amino acid metabolism and enhancement of mitochondrial function.

Key words: Huntington disease, cysteamine, oxidative stress, mitochondria, amino acids.

INTRODUCTION

Huntington's disease (HD) is a fatal, genetic neurodegenerative disorder characterized by both motor and cognitive symptoms (Brouillet et al., 1999), caused by an unstable CAG expansion on the gene encoding the protein huntingtin. This expansion results in the formation of excessive polyglutamine repeats (> 40) at the N-terminus (The Huntington's Disease Collaborative Research Group, 1993), which causes a cascade of events leading ultimately to neuronal degeneration. Although several hypotheses regarding the mechanisms by which neurotoxicity is triggered in HD brains have been suggested on the basis of experimental evidence, so far it remains not clear

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Abbreviations: HD, Huntington disease; 3-NP, 3-nitropropionic acid; **CAG**, cytosine- adenine- guanine trinucleotide; CS, citrate synthase; **CK**, creatine kinase; GSH, glutathione; **NO**, nitric oxide; **SDH**, succinate dehydrogenase; **tTGase**, tissue transglutaminase; **BCAA**, branched chain amino acids and **BDNF**, brain-derived neurotrophic factor.

which of them are predominant or whether they are complementary. Further evidence supports the assumption that different toxic mechanisms (that is excitotoxicity, energy metabolism impairment, inflammatory events, oxidative stress, etc.) are confluent and depend on each other (Pérez-De La Cruz and Santamaría, 2007). One toxin that is used to induce changes in animals similar to that seen in HD is 3-nitropropionic acid (3- NP) (Beal et al. 1993). This mycotoxin is reported to interrupt mitochondrial electron transport, by selective inhibition of succinate dehydrogenase (SDH, EC 1.3.99.1) and homogeneously inhibits SDH in the rat brain (Alexi et al., 1998). This phenomenon induces a reduction in ATP production and gives rise to free radicals, both nitric oxide (NO) and reactive oxygen species (ROS) (Pe ´rez-Severino et al., 2002). Oxidative stress and excitotoxicity are two conditions leading to cell death, by both necrosis and apoptosis, which are thought to be important in several neurodegenerative diseases; they are relevant to the striatal cell loss seen in HD and are gaining prominence for 3- NP lesions (Vis et al., 2004). Additionally, the induction of tissue transglutaminase (tTGase) in brain has also been postulated to play a role in HD pathogenesis by cross-linking mutant huntingtin in aggregates (Kahlem et al., 1998). Interestingly, in addition to the striatal lesion, 3NP also produces other features of HD such as movement (chorea, dystonia) and cognitive (perseveration) disorders, specific loss of spiny projection neurons and sparing of NADPH-diaphorase-expressing cells (Beal et al., 1993).

Amino acids play a pivotal role in brain neurotransmitter function. Interestingly, as early as 1969 alterations in amino acid metabolism in HD were reported (Perry et al., 1969; Philipson and Bird, 1977; Reilmann et al., 1995), but these have never been established as important state markers until now. While some of these changes may reflect a procatabolic phenotype, it is unclear whether this accounts for the overall signature. However, it is encouraging when broad non-hypothesis driven experimental approaches yield a subset of candidate biomarkers which had previously been tentatively identified.

Because mitochondria dysfunction may play an impor-tant role in the pathogenesis of HD (Panov et al., 2002), thus, preventing mitochondria dysfunction may be beneficial to patients with HD. Currently there is no cure for HD and no efficient treatment available to reverse the disease's symptoms, although preclinical trials using HD mouse models have led to potential therapeutic avenues. Compounds with significant beneficial effects in HD mouse models include minocycline (Hersch et al., 2003), creatine (Matthews et al., 1998), coenzyme Q10 (Ferrante et al., 2002), suberoylanilide hydroxamic acid (Hockly et al., 2003) and riluzole (Rosas et al., 1999). Further, both oral and intraperitoneal cystamine treatment significantly extended the survival in the R6 ⁄ 2 HD mouse model, and significantly improved motor deficit, delayed loss of body weight, gross brain weight and neuronal atrophy (Dedeoglu et al., 2002; Bailey and Johnson, 2005), and atenuated the formation of huntingtin aggregates (Dedeoglu et al., 2002). These findings and others (Fox et al., 2004; Jeitner et al., 2005) stress the importance of examining further the beneficial effects of cysteamine, the FDA-approved reduced form of cystamine. Borrell-Pages et al. (2006) demonstrated that cysteamine is neuroprotective in HD mice by increasing brain-derived neurotrophic factor (BDNF), a trophic factor that is depleted in HD brains and is crucial for the survival of striatal neurons (Gauthier et al., 2004). Such aforementioned actions of cystamine and its reduced form, cysteamine, encouraged us to test the possible neuroprotective role of cysteamine in 3-NP model of HD. To our knowledge, no studies are available about the effects of cysteamine on amino acid pattern and striatal mitochondrial oxidative status in 3- NP model of HD.

MATERIAL AND METHODS

Drugs and chemicals

Cysteamine**,** 3-NP, acetyl CoA, oxaloacetic acid, sulfosalicylic acid, Ellman's reagent, vanadium chloride, sulfanilic acid, N- (1-naphthyl)-ethylene diamine and standard amino acids were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Reagent kit of Greiner Diagnostic GmbH(Germany) was used to assess the activity of creatine kinase (CK). Other chemicals unless specified were of analytical grade.

Animals

Male Wistar albino rats (200-250 g) were obtained from the National Research Center Laboratory, Cairo, Egypt. They were housed in the animal facility of Faculty of Pharmacy, Cairo University, Cairo, Egypt. Rats were kept under standard conditions of 25 ±2˚C, 40-60% relative humidity, and 12-h light/dark cycle each day. They were fed standard chow diet and provided with water *ad libitum* along the period of the study. All experiments were carried out in accordance with our institutional animal care guidelines.

Experimental protocol

Animals were stratified into three groups (each group ranges from 8-14 animals); 1) Normal control group receiving appropriate volume of saline injected intraperitonealy, 2) 3-NP- induced toxicity group injected subcutaneously with 3-NP at a dose of 20 mg/kg/day for 7 days (Beal et al., 1993), 3) Cysteamine+ 3NP- treated group; cysteamine (dissolved in saline) was injected intraperitonealy in a building dose form starting from 25 mg/kg/day up to 75 mg/kg/day, one hour before concurrent 3-NP injections. Fasted animals were sacrificed by decapitation one hour after the last 3-NP injection, blood samples were taken and used for estimation of serum amino acid pattern. For determination of biochemical parameters in striatal tissues, brains were quickly excised, washed with ice- cold saline, blotted dry with filter paper, then dissected and the two striata were isolated, weighed and homogenized in an ice-cold deionized water as 10% homogenate using an ice-cold Teflon homogenizer (Potter Elvehjem type) for 1 min. The homogenate was centrifuged at 600 g, to remove the nuclei and cell depris, at 4˚C for 15 min using a Dupont Sorvall Combiplus ultracentrifuge. The post nuclear fraction was further centrifuged at 13000 g at 4˚C for 20 min. GSH and NO contents and CK activity were determined in the post mitochondrial fraction. The mitochondrial pellets were re-suspended

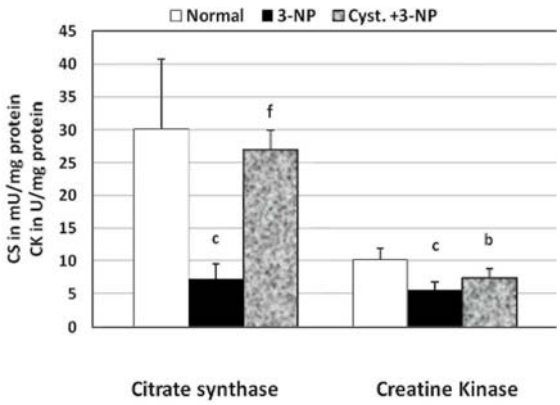

Figure 1. Effect of cysteamine (Cyst) on striatal citrate synthase (CS) and creatine kinase (CK) activities in 3-NP- treated rats. Data are presented as mean of 6-8 animals ± S.D. Comparison between groups was carried out using one way ANOVA followed by Tukey-Kramer multiple comparison test. Statistical difference from normal control group (b, c) at $P< 0.01$, 0.001 respectively and 3-NP-treated group (f) at $P< 0.001$.

in 20 mM phosphate buffer pH 7.2, sonicated and used for estimation of CS activity.

Assessment of striatal biochemical parameters

GSH

A portion of the post mitochondrial fraction was mixed with an ice-cold 4% sulfosalicylic acid (1:1), centrifuged at 600 g at 4˚C for 10 min. The GSH content was measured in the supernatant according to Beutler et al. (1963).

NO (NO2-/NO3-)

They were determined as described previously (Miranda et al., 2001) in the supernatant resulting from centrifugation of the post nuclear fraction at 13,000 g at 4 ˚C for 20 min. The assay is based on the reduction of NO_3^- into NO_2- using vanadium chloride then diazotization of sulfanilic acid with the total NO_2- in the medium with subsequent coupling with N- (1-naphthyl)-ethylene diamine. The azo dye produced was measured colorimetrically at 540 nm.

CK

A portion of the post mitochondrial fraction was diluted with saline (1: 9) before assessment. The assay employs a coupled enzyme assay, which ultimately monitors the formation of NADPH spectrophotometrically at 340 nm (Stein, 1998).

CS

An aliquot of the mitochondrial suspension was incubated for 5 min with 1 M phosphate buffer, pH 7.2, before addition of a solution containing 2.5 mM dithionitrobenzoic acid and 2.5 mm acetyl CoA. After incubation for 5 min, 10 mM oxaloacetate was added. The increase in absorbance for the subsequent 5 min at 415 nm was used to calculate CS enzyme activity using **J** = 13.6 mmol/l/cm (Kramer et al., 2005).

Protein content in the mitochondrial and post mitochondrial fractions were determined using the method of Lowry et al. (1951).

Assessment of serum amino acids

The following amino acids (19 compounds) were measured and used in the analysis: alanine, arginine , glutamic, aspartic, glycine, cysteine, taurine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan , tyrosine, and valine. Our initial aim was to assay serum concentrations of all the amino acids; however, because of technical drawbacks, we were not able to measure the concentrations of citrulline, glutamine, and ornithine.

Chromatographic separation of amino acids was done by deproteinizing serum sample using 75% HPLC grade methanol then centrifuged at 4000 rpm and the supernatant was dried under vacuum and derivatized with phenylisothiocyanate for 20 min at room temperature. The derivatized amino acids were reconstituted with phosphate buffered saline (pH=7.2). After vortex and sonicating for a few seconds, 20 µl was injected. The amino acid standard samples were treated the same as the serum sample. The mobile phase consisted of a gradient of two eluents: eluent (1) and eluent (2). The absorbance of the derivatized amino acids was measured at 254 nm. A constant flow rate of 1 ml/min and column oven 46°C were maintained throughout the experiment (Heinrikson and Meredith, 1984).

Apparatus specifications

The HPLC system of Perkin- Elmer (USA) was used which consisted of a quaternary pump, column oven, Rhyodine injector, *20µl* loop, and UV variable wavelength detector. PICO- TAG column 3.9 x 30 cm, Eluent (1) and Eluent (2) (Waters, USA) were used for free-amino acid analysis. The assay conditions were as follows: temperature, 46°C; wave-length, 254 nm; flow rate, 1 ml/min. The chromatogram was integrated by turbochrome software program [Perkin-Elmer, USA].

RESULTS

Administration of 3-NP caused a dramatic decrease in CS activity reaching about 24% of the basal level and reduced CK to about half its activity (Figure 1). As depicted in Figure 2, the 3-NP-induced insult resulted in a redox state perturbation, which is reflected by a 43% decrease in the endogenous defense element, GSH, and a significant 1.25 folds increase in NO content. Amino-acids profile was also affected by the mycotoxin as presented in Table 1, where the branched chain amino acids (BCAA), viz., valine, leucine and isoleucine were markedly decreased by 24, 30 and 32%, respectively. Such an effect was extended to include the excitatory (glutamic, 54% and aspartic, 62%) and co-excitatory (glycine, 34%; alanine, 46.5% and serine, 44.5%) amino-acids. Moreover, the level of other amino acids, viz., taurine, histidine, and tyrosine was leveled off to about half or less their values (41, 36.7 and 49.8%, respectively), as shown in Table 1. Cysteamine administration markedly improved the mitochondrial function as reflected by restoration of CS activity (Figure 1), however, no effect was observed on the rest of the striatal biochemical parameters (Figures 1, 2). On the other hand, treatment with cysteamine succeeded to restore the taurine and histidine contents as well as the co-excitatory amino acids, glycine by 1.31

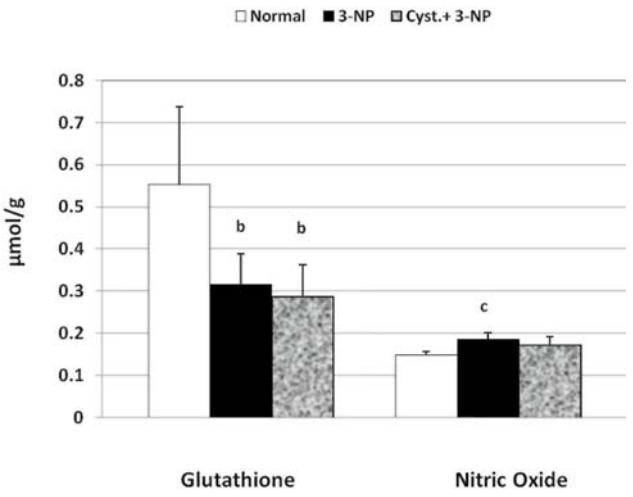

Figure 2. Effect of cysteamine (Cyst) on striatal glutathione and nitric oxide contents in 3-NP- treated rats. Data are presented as mean of 6-8 animals ± S.D. Comparison between groups was carried out using one way ANOVA followed by Tukey-Kramer multiple comparison test. Statistical difference from normal control group (b,c) at *P*< 0.01, 0.001, respectively

folds and alanine by about 1.8 folds as compared to 3NP-treated group. However, cysteamine did not alter the depressed values of BCAA, serine, aspartate, and tyrosine but caused further decrease in glutamate. Apart from the modula-tory effect of cysteamine on the alterations-induced by 3-NP, the drug showed its own action on some amino acids, namely, lysine and tryptophan, where both amino-acids were not affected by 3-NP, but their levels were decreased by 24 and 21.5%, respectively, as compared to normal untreated group and by 30 and 24%, respectively, as compared to 3-NP treated groups (Table 1).

DISCUSSION

Systemic administration of the mitochondrial toxin 3-NP, an irreversible inhibitor of SDH (complex 2 of the respiretory chain) and the tricarboxylic acid cycle, inhibited energy metabolism and induced oxidative stress as manifested in our study by significant reduction in the activities of CS, a mitochondrial matrix marker of oxidative capacity and CK, an enzyme involved in energy expenditure. Furthermore, significant reduction in the main intracellular antioxidant, GSH and marked increase in NO were found. Similarly, systemic administration of 3-NP to animals also decreases the levels of GSH while it enhances the formation of free radicals and oxidized proteins in the striatum (Schulz et al., 1996). The neurotoxin was reported to trigger an increase in NOx levels in striatal homogenates of the transgenic mouse model of HD (Pe ´rez-Severino et al., 2002) and these concur with our findings. On the contrary, both enzymatic activity and expression of neuronal NOS (nNOS) are decreased in HD models (Deckel et al., 2001).These changes result in neurodegeneration characterized by dysfunction of the blood- brain barrier and lesions of the striatum and basal ganglia (Duran-Vilaregut et al., 2009). Mechanistically, 3-NP as an inhibitor of the electron transport chain, leads to decrease in ATP synthesis, activation of NMDA receptors and influx of intracellular and intramitochondrial calcium. In turn, neuronal cell death induced by excitotoxicity has been related to the formation of ROS and reactive nitrogen species (RNS) (Alexi et al., 1998). Lesort et al. (2000) have also demonstrated that mitochondria dysfunction induced by 3-NP resulted in a significant increase in tissue transglutaminase (TGase) activity in human neuroblastoma cells. TGase is a calcium-dependent enzyme suspected of participating in HD pathogenesis and catalyzes the formation of e-(Y-glutamyl) lysine isopeptide bonds between a polypeptide-bound glutamine and a lysine of the protein substrate.

A decrease in CK activity is one of the biochemical markers of the CNS cell damage in neurodegenerative pathways that result in neuronal death (Aksenov et al., 2000) a fact agrees with our results. CK also appears to be coupled directly or indirectly to energetic processes required for calcium homeostasis (Wallimann et al. 1992) that was reported to be altered by 3-NP. Posttranslational oxidative modification of the enzyme may contribute to the loss of CK activity in neurodegenerative disorders (Aksenova et al., 1999) not a result of decreased CK gene expression. Therefore, the elevated NO and the altered oxidative status seen in our study in 3-NP treated group may contribute in the marked reduction in CK activity.

As an extension to the mitochondrial dysfunction produced by 3-NP, CS activity was severely reduced. This reduction could be mediated also through TGase enzyme. In support of this, tTGase, that was reported to be increased by 3-NP, has been shown to inactivate glyceraldehyde 3-phosphate dehydrogenase and the a-ketoglutarate dehydrogenase complex in the presence of expanded polyglutamine protein (Cooper et al., 1997).

In HD, a state of neurotransmitter dysregulation had been confirmed. Indeed, the biosynthesis of dopamine, nor-adrenaline and serotonin is related to the availability of their amino acid precursors, tyrosine and tryptophan (Fernstrom and Faller, 1978). The amount of these precursors in the brain, in turn, is influenced by serum levels of the large neutral amino acids valine, leucine, isoleu-cine and phenylalanine, which compete with tyrosine and tryptophan for the transport system across the blood-brain barrier (Fernstrom, 1981). Moreover, glutamate, aspartate and glycine have a direct neurotransmitter role in the central nervous system and are involved in brain development and neurotoxicity (Maycox et al., 1990). Therefore, peripheral amino acids affect central brain functions more or less directly; as a consequence, alterations in their availability and/or metabolism may have a role in the pathogenesis and maintenance of central disorders. Systemic administration of 3-NP resulted in significant reduction in valine, leucine, isoleucine (BCAA), taurine, glycine,

Table 1. Effect of cysteamine on serum amino acid pattern in 3- NP- treated rats.

Amino acid µmol/l	Normal control	3-NP	3-NP+ Cysteamine
Valine	288.3 ± 15.2	(b)218.8 ± 29.8	(b)221.8 ± 43.7
Leucine	209.6 ± 37	(b)146.1 ± 25.9	(a)153.3 ± 30.9
Isoleucine	47.3 ± 14	(a)32.3 ± 4.7	(b)28.3 ± 4.2
Taurine	268 ± 18.5	(c)157.9 ± 10.6	(f)251.5 ± 12
Cysteine	172.3 ± 26.6	214.3 ± 37.9	(e)138 ± 19.7
Glycine	453.6 ± 67.5	(c)298.3 ± 45.3	(d)390.4 ± 51.3
Alanine	378.5 ± 31.9	(c)202.5 ± 31.6	(f)371.8 ± 42.2
Serine	63.5 ± 14.4	(c)35.2 ± 5.7	(a)45.3 ± 8
Aspartic	33.1 ± 2.8	(c)12.5 ± 2.5	(c)13.4 ± 1.3
Glutamic	132.2 ± 13.9	(c)60.7 ± 6.3	(c, d)45.8 ± 3.2
Threonine	24.2 ± 5.7	23.6 ± 7.7	21.4 ± 2.7
Arginine	65.8 ± 12.3	55.4 ± 9.5	55.9 ± 8.3
Histidine	148.7 ± 16.3	(c)94.1 ± 18.7	(f)152.5 ± 21.5
Lysine	235.7 ± 19.2	257.2 ± 49.6	(a, e)179.1 ± 13.3
Methionine	37.6 ± 9.2	38.7 ± 5.9	29.6 ± 4.1
Proline	225 ± 47	231 ± 32.5	205.4 ± 18.6
Tyrosine	47 ± 3.1	(c)23.6 ± 6.2	(c)24.3 ± 6.9
Tryptophan	51 ± 7.0	52.7 ± 8.1	(a, d)40 ± 4.1
Phenylalanine	57.9 ± 11.5	49.9 ± 7.1	52.9 ± 6.2

Data are presented as mean of 6 animals ± S.D. Comparison between groups was carried out using one way ANOVA followed by Tukey-Kramer multiple comparison test. Statistical difference from normal control (a,b,c) and 3-NP-treated (d, e, f) groups at $P<0.05$, 0.01, 0.001, respectively.

alanine, serine, aspartate, glutamate, histidine, and tyrosine as compared to normal control group. This is compatible with the marked weight loss of 3-NP– treated rats (Duran-Vilaregut et al., 2009) and the hypermetabolic state seen with HD patients (Mochel et al., 2007), as weight loss was observed in the HD group even in pre-symptomatic carriers, although their caloric intake was higher than that of controls. Our findings confirmed previous studies of Phillipson and Bird (1977) who found that, patients with Huntington's chorea showed reduced fasting plasma concentrations of BCAA. In addition, Watt and Cunningham (1978) reported low values for alanine, leucine, isoleucine, histidine, threonine and lysine in plasma of Huntington's chorea patients which agree with our experimental results, with the exception of threonine and lysine which were not affected by 3-NP administration. However, the authors considered that their findings were probably due to non- specific factors. Later, Reilmann et al. (1995) reported significant reduction in plasma alanine and isoleucine of patients with HD. More recent, Mochel et al. (2007) reported decreased serum levels of BCAA in serum of HD patients. The observed decrease in BCAA may correspond to a critical need for Krebs cycle energy substrates in the brain that increased metabolism in the periphery is trying to provide. Importantly, mitochondrial oxidation of BCAA leads to the production of acetyl-CoA and succinyl-CoA, two key intermediates of the citric acid cycle (Mochel et al., 2007). In addition, the systemic decrease in serum BCAA may also reflect an adaptive response to a peripheral energy deficit since, an altered energy metabolism has been shown in muscle of HD patients (Lodi et al., 2000). Indeed, BCAA play an important role in brain metabolism, accounting for up to 20% of whole-body BCAA metabolism (Suryawan et al., 1998). Leucine, in particular, is a nitrogen donor for the glutamate-glutamine shuttle, with transamination of α-keto-isocaproate to leucine leading to the conversion of glutamate into α-ketoglutarate (Hutson et al., 2001). Therefore, the buffering of glutamate is likely to be impaired by low level of BCAA in brain. Previous studies showed altered glutamate-glutamine cycling in HD (Behrens et al., 2002). In our study, serum glutamate was markedly reduced, knowing that, glutamate is an excitatory neurotransmitter and was established to be increased and to mediate excitotoxicity in 3-NP pathogenesis and in HD patients. However, it could be argued that changes in serum glutamate do not necessarily imply similar changes in the central nervous system, since in the brain, this amino acid is not merely taken up from the blood, but is actively synthesized in nerve endings and glial cells (Maycox et al., 1990). As GABA, an inhibitory neurotransmitter, was reported to be decreased in HD brains (Perry et al., 1973), serum glutamate, may become the main agent that has been tried to enhance GABAergic neurotransmission.

Corresponding to the brain dysregulation of amino acid neurotransmitter in HD, our study showed decreased se-

rum levels of aspartate, glycine, alanine and serine. Peripheral and central consumption of these amino acids may be the clue in this reduction. Aspartate is one of the major excitatory amino acids in the brain, which is involved in NMDA neurotransmission. Although it is one of the most slowly exchanging amino acids, with a very low brain uptake (Bradbury, 1979) it may be consumed in the excitotoxicity exerted by 3-NP, especially with defective blood brain barrier (Duran-Vilaregut et al., 2009). As an extension, Nicoli et al. (1993) found a significant increase (30%) in the CSF level of glycine in chorea patients and serine can be essential for the brain, since serine may be converted into glycine in the brain (Woronczak et al., 1995). These observations are in agreement with the metabolic hypothesis of HD physiopathogenesis. Like glycine and D-alanine, D-serine stimulates the glycine site of the NMDA type receptor and enhances the action of glutamate on the receptor. Stimulation of the glycine modulatory site alone does not induce excitatory postsynaptic potentials, but is indispensable for adequate neurotransmission by glutamate. Consequently, glycine, D-serine, and D-alanine are called “co-agonists” for the NMDA receptor (Danysz and Parsons 1998). In accordance with our concept, Hashimoto et al. (2004) revealed that D-serine has been implicated in the pathophysiology of Alzheimer's disease (AD) and found different alterations in serum levels of D and L-serine in the patients with AD. The marked decrease in tyrosine may explain the reported increase in dopamine content as a result of glutamate excitation in HD brain (Roberts and Anderson, 1979).

In response to oxidative stress associated with systemic administration of 3-NP, serum taurine was significantly reduced in this study. The role of taurine as an antioxidant and a neuromodulator could explain its reduced level due to crucial participation to guard against the diseased condition. Taurine was reported to have a slight direct effect on GABA receptors, but is most likely to act as an indirect neuromodulator of GABAergic neurotransmission in the brain (Liljequist, 1993). Other researchers also documented taurine as a low affinity agonist for GABA(A) receptors in different brain areas, thus, enhancing the GABAergic transmission (McCool and Botting, 2000) being depressed in HD.

Apart from the role of amino acid as a neurotransmitter, the observed reduced serum alanine level in this study could be related also to its use as a precursor for pyruvate, an end metabolite of glycolysis with antioxidant activity. This was supported by the study of Nicoli et al. (1993) who detected a significant increase (60%) in pyruvate concentration in CSF of HD patients. Pyruvate has been shown to protect striatal neurons against varieties of stimulants, such as, quinolinic acid as an animal model of HD (Ryu et al., 2006). Furthermore, the activities of both mitochondrial and cytosolic forms of alanine aminotransferase were markedly increased in HD putamen autopsy samples suggesting a considerable alteration of glutamate and pyruvate metabolism (Carter, 1984).

The decrease in histidine in our study upon 3-NP administration implies that HD animals cannot easily control peroxidation events. Since histidine is a precursor of carnosine which is considered as an antioxidant in brain and skeletal muscle and a deficit in energy metabolism has been shown in muscle of HD patients (Lodi et al., 2000) so, it may be excessively consumed in response to HD pathology. Our concept was supported by the findings of Fonteh et al. (2007) who reported a decrease in plasma histidine and carnosine of patients with neurodegenerative disorders as Alzheimer disease.

Indeed, a potential neuroprotective strategy in HD may include the prevention of mitochondria dysfunction and oxidative stress damage, and in this regard it would be particularly interesting to examine in HD the beneficial effects of a drug possessing several actions to guard against a diseased condition from different mechanisms. This concept can be realized by the use of cysteamine.

In this study cysteamine was found to improve the mitochondrial function as revealed by normalization of CS activity. This improvement could be partly through antioxidant activity of cysteamine and TGase inhibition by acting as an alternate substrate for the enzyme. Previous study indicated that TGase2 affects mitochondrial function and regulates the energy balance of the cell (Rodolfo et al., 2004). This is explained by Jeitner et al. (2005) that TGase 2 catalyses the covalent attachment of cysteamine to a suitable protein/ peptide substrate, resulting in the formation of a γ-glutamylcysteamine moiety attached to a modified Q residue. This alteration would result in an increase in the thiol content of the modified protein and thereby offer greater antioxidant protection in the immediate vicinity of the peptide. Free γ-glutamylcysteamine is also a potent antioxidant. Thus, the formation of γ-glutamylcysteamine may significantly augment the cellular antioxidant defenses. However, no significant change was observed on striatal CK, GSH and NO activity/content. On the contrary to our results, cysteamine, was found to restore the depressed CK activity in cystinotic rat brain cortex by preservation of its sulfhydryl group (Rech et al., 2008). Matching with our findings, Fox et al. (2004) revealed that cystamine was markedly protective against 3-NP- induced striatal injury in the R6/2 mouse model of HD by increasing brain level of L- cysteine although no increase in the levels of GSH. Similarly, more recent findings suggest that cystamine is not directly involved in atenuating disease progression in HD models but that increased levels of brain cysteine or uncharacterized sulfur metabolites may be responsible (Pinto et al., 2005). On the contrary, Mao et al. (2006) provided an evidence that cystamine and the related compound cysteamine prevent, in a GSH-dependent manner, the increased vulnerability of HD striatal cells to 3-NP. Although brain cysteine was not measured in our study, and does not promote increased GSH synthesis, this molecule is also a good antioxidant (Peskin and Winterbourn, 2001). The

lack of increase in GSH may be due to the low brain content of glutamate-cysteine ligase (Liu and Choi, 2000) and very slow GSH turnover time in this tissue (60–80 h) (Chang et al., 1997). It is possible that glutamate-cysteine ligase in the brain is saturated at very low concentrations of cysteine. The aforementioned assumptions may explain the observed lowering action of cysteamine on serum cysteine level for saving purpose in brain. Furthermore, cysteamine restored the depressed serum taurine level which balances the peripheral oxidative stress. Taurine could be derived in this group from oxidation of cysteamine rather than cysteine. In this study, cysteamine was able to normalize the depressed levels of glycine, alanine and histidine. This may indicate an alleviation of the peripheral and/or central oxidative status that consumes alanine and histidine in carnosine formation. Also, the utilization of alanine and glycine as glutamate coagonists and/or transamination of alanine to pyruvate seem to be lessened. However, no change in the depressed levels of BCAA suggesting continuous need for their α-ketoacids in brain.

Cysteamine caused further decrease in serum glutamate, marked decrease in serum lysine and tryptophan as compared to normal control and 3- NP- treated groups. These actions for cysteamine need further investtigations, however, some speculations in this respect could be convincing. Cysteamine may enhance lysine incorporation into carnitine synthesis since, L-carnitine was found to attenuate the mitochondrial membrane permeability transition induced by 3-NP by decreasing long chain fatty acids generated by phospholipase A_2 (Nishimura et al., 2008). Measurement of peripheral and central levels of carnitine becomes essential to elucidate this mechanism. Also, the observed decrease in lysine essential amino acid upon cysteamine treatment seems to be neuroprotective since, Seminotti et al. (2008) reported that lysine induces oxidative stress in cerebral cortex of young rats. Generally, abnormalities of tryptophan metabolism along the kynurenine pathway could contribute to neuronal dysfunction and damage in HD (Stoy et al., 2005). Cysteamine interference with tryptophan metabolism in this study could be either protective through kynurenic acid formation (an NMDA receptor antagonist) (Perkins and Stone, 1982) or potentiating through quinolinic acid formation (an NMDA receptor agonist) (Stone 2001). Measurement of tryptophan metabolites will be discriminating.

It was concluded that, amino acid pattern in HD should be reconsidered and can be diagnostic; further, cysteamine was confirmed to be neuroprotective affecting amino acid and oxidative metabolism, suggesting new strategy in handling HD prophylaxis and therapy.

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