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# Influence of Proline on Rat Brain Activities of Alanine Aminotransferase, Aspartate Aminotransferase and Acid Phosphatase

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Hyperprolinemia type II (HPII) is an autosomal recessive disorder caused by the severe deficiency of enzyme $\Delta^1$-pyrroline-5-carboxylic acid dehydrogenase leading to tissue accumulation of proline. Chronic administration of Pro led to significant reduction of cytosolic ALT activity of olfactory lobes (50.57%), cerebrum (40%) and medulla oblongata (13.71%) only. Whereas mitochondrial ALT activity was reduced significantly in, all brain regions such as olfactory lobes (73.23%), cerebrum (70.26%), cerebellum (65.39%) and medulla oblongata (65.18%). The effect of chronic Pro administration on cytosolic AST activity was also determined. The cytosolic AST activity from olfactory lobes, cerebrum and medulla oblongata reduced by 75.71, 67.53 and 76.13%, respectively while cytosolic AST activity from cerebellum increased by 28.05%. The mitochondrial AST activity lowered in olfactory lobes (by 72.45%), cerebrum (by 78%), cerebellum (by 49.56%) and medulla oblongata (by 69.30%). *In vitro* studies also showed increase in brain tissue proline and decrease in glutamate levels. *In vitro* studies indicated that proline has direct inhibitory effect on these enzymes and glutamate levels in brain tissue showed positive correlation with AST and ALT activities. Acid phosphatase (ACP) activity reduced significantly in olfactory lobes (40.33%) and cerebrum (20.82%) whereas it elevated in cerebellum (97.32%) and medulla oblongata (76.33%). The histological studies showed degenerative changes in brain. Following proline treatment, the animals became sluggish and showed low responses to tail pricks and lifting by tails and showed impaired balancing. These observations indicate influence of proline on AST, ALT and ACP activities of different brain regions leading to lesser synthesis of glutamate thereby causing neurological dysfunctions.

**KEY WORDS:** Proline; hypreprolinemia; alanine aminotransferase; aspartate aminotransferase; brain regions.

## INTRODUCTION

Hyperprolinemia type II (HPII) is a rare inherited autosomal recessive disorder of amino acid metabolism characterized by elevated plasma proline (Pro) concentration (normal blood Pro levels are about 450 units whereas elevated blood Pro levels in subjects with HPII reach 1900–2000 units), aminoglycinuria and the urinary excretion of $\Delta^1$-pyrroline compounds (1,2) due to lack of/deficiency of enzyme $\Delta^1$-pyrroline-5-carboxylic acid dehydrogenase (EC 1.5.1.12) activity. HPII is a rare disorder that is present at birth and such individuals carry 4–5 abnormal genes. Consanguineous parents have a higher chance than unrelated parents to carry the same abnormal gene, which increases the risk of

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having children with a recessive genetic disorder. Patients detected so far show neurological disorders like seizures and mental retardation (3). In various pedigrees, asymptomatic hyperprolinemic siblings have been also identified (4). If untreated it may lead to complete mental retardation and neural blockade (5).

Moreira et al. (6) reported rats subjected to an experimental model of HPII present a significant deficit in habituation to open field, indicative of an impairment of learning/memory functions. High levels of Pro can lead to alteration in cell redox (4), inhibition of acetyl cholinesterase activity (7), reduction of creatine kinase activity and oxidative stress (5) in brain.

L-glutamate acts as an excitatory neurotransmitter in mammalian CNS. Some of the findings demonstrate that higher concentrations of Pro promote activation of neuronal receptors (8,9) thereby mediating depolarization of cells by an excitatory amino acid sensitive mechanisms (10,11) especially through putative glutamatergic pathways (12). Alterations in glutamatergic system bring about initiation, propagation and maintenance of epileptic activity (13,14). Aspartate aminotransferase (AST, EC 2.6.1.1) and Alanine aminotransferase (ALT, EC 2.6.1.2) are active brain enzymes that exist in cytosolic and mitochondrial forms. Both enzymes are involved in glutamate metabolism. AST catalyses the conversion of aspartate and 2-oxoglutarate to oxaloacetate and glutamate, or the reverse reaction with pyridoxal 5-phosphate (PLP) as a coenzyme (15,16). It also takes part in the synthesis of the neurotransmitter like glutamate in glutamatergic cells (17,18) and catalyzes the production of glutamate for GABA synthesis (16). Al-Ali and Robinson (19) have reported increase in ACP (ACP, EC 3.1.3.2) activities in ischemic brain of rats. ACP is a classical marker enzyme used to demonstrate the presence of lysosomes in animal tissues and ACP is known for digesting biological molecules during necrosis, pathological death of cells and tissues (20).

Therefore, aim of present work was to investigate *in vivo* activities of cytosolic and mitochondrial ALT and AST, the enzymes participating in glutamate metabolism, from immature brain regions of rat pups with induced HPII and also to determine whether a proline has a direct action on cytosolic as well as mitochondrial ALT and AST activities. Secondly, as Pro is likely to cause neuronal injury to the brain due to its excitotoxic effect and since ACP is commonly found at the site of brain lesions (19,20) it was felt necessary to incorporate assessment of the ACP activity and histopathology of different brain regions. Four functionally important regions of brain viz. olfactory lobes, cerebrum, cerebellum and medulla oblongata were chosen, in order to know which region is maximally affected or escapes proline effect.

## EXPERIMENTAL PROCEDURE

*Animals*. Two week old albino rats (Rattus norvegicus) weighing 30–35 g ($N = 25$) were procured from the animal house facility of Department of Zoology, Goa University, Goa, India and were housed (4 pups/cage) along with their mother from the day of their birth. Animals were maintained at 30 ± 1°C on 12:12 h light/dark cycle with a free access to commercial feed (Pranav Agro industries, Sangli, India) and water. The experiments were performed according to guidelines of Committee for the Purpose of Control and Supervision of Experiments of Animals (CPCSEA) of India and were approved by the Animal Ethics Committee of Goa University, India.

*Materials*. AST and ALT kits were obtained from Span diagnostics, India (Code No. 25913;AST, 25912;ALT). ACP kits were from Qualigens diagnostics (manufactured by Sigma diagnostics, India Product No.72001). All other chemicals were of analytical grade and were purchased from standard commercial Suppliers.

*Proline Treatment*. Proline dissolved in Dulbeco's phosphate buffer saline (DPBS; pH 7.4) was subcutaneously administered in rats (pups) twice a day at 7 h intervals from 15th day of age to 39th day of their age. The doses were decided on the basis of pharmacokinetic parameters of Pro and the Pro treatment was given as reported by Pontes et al. (3). During the first 8 days of treatment the rats received 12.8 μmol of Pro/g body weight; from day 23rd to 26th they received 14.6 μmol of Pro/g body weight; from day 27th to 30th they received 16.4 μmol of Pro/g body weight and from day 31st to 39th they received 18.2 μmol of Pro/g body weight. Rats thus treated gain plasma Pro levels of 1.0–2.0 mM similar to those found in HPII patients (3,6). Control rats were given saline instead of Pro in the same manner and volumes [(1 ml/100 g body weight) as recommended by Pontes et al. (3) and Moreira et al. (6)] as that of proline treated. Finally, both control and experimental rats were decapitated on the 40th day of their age.

*Behavioral studies*. On the 40th day, controls as well as Pro treated rats were subjected to behavioral tests. (*a*) Time taken for withdrawal of tail after a tail prick was noted in ms, (*b*) time taken to start swirling movements after lifting by tail was noted in s, (*c*) rotatory drum was rotated for 5 min at the speed of 3 rpm and the balancing time was noted in minutes.

*Preparation of Supernatants*. The animals were decapitated at the end of Pro treatment and after administration of DPBS for controls. The whole brain was rapidly removed, washed with ice-cold saline, blotted dry and immediately trans-

ferred to an ice chamber. The olfactory lobes, cerebrum, cerebellum and medulla oblongata were dissected and transferred to tissue culture tubes and frozen at −20°C until the preparation of homogenate. For estimation of tissue Pro and glutamate (Glu), a 10% (w/v) homogenate of each of the brain regions was prepared in 0.25 M cold sucrose. Protein-free filtrate of each brain region homogenates were prepared by mixing the homogenates with equal volumes of 10% trichlroacetic acid (TCA), followed by centrifugation in cold at 3000 rpm for 15 min. Blood plasma and CSF were collected using routine techniques. Briefly, CSF was collected after anesthetizing rats with ether followed by an incision at the base of the skull towards the caudad and the atlanto-occipital membrane was exposed and cleaned using saline and cotton swabs. Using 1 cc syringe with 25-gauge needle about 100 µl of CSF was aspirated from sub-arachnoid space of cisternae magna through the atlanto-occipital membrane (21). CSF samples contaminated with blood were rejected. Pro and Glu contents were measured after developing flurophores by ninhydrin with the protein free filtrate according to the method described by Chakraborti and Poddar (22).

For estimation of cytosolic enzymes, the frozen tissue was homogenized at 0°C in 0.32 M sucrose solution containing 5 mM Hepes buffer (0.1 g/10 ml; pH 7.4). The homogenate was freeze centrifuged (4°C) for 20 min at 12,000 × g. Undiluted supernatants were used for enzyme assay. Whereas for estimation of mitochondrial enzymes, each brain region was homogenized in nine volumes of 0.32 M sucrose. Homogenate was centrifuged at 1000 × g for 10 min and the supernatant removed was centrifuged again at 10,000 × g for further 30 min. The pellet thus obtained was washed twice and applied on a discontinuous sucrose density gradient consisting of 0.8, 1.0, 1.2 and 1.4 M sucrose. After centrifugation at 75,000 × g for 2 h, the mitochondrial pellet was obtained at the bottom of the tube, which was used as sample source for estimation of mitochondrial enzymes.

*Enzyme Assays*. Aminotransferases were measured according to 2,4-dinitrophenyl hydrazine (2,4-DNPH) method. The incubation mixture for ALT contained 500 µl of alanine-α-ketoglutarate, 100 µl of enzyme extract, 500 µl of 2,4- DNPH and 5 ml of 0.4 N NaOH. The incubation mixture for AST contained 500 µl of aspartate-α-ketoglutarate, 100 µl of enzyme extract, 500 µl of 2,4-DNPH and 5 ml of 0.4 N NaOH. Optical density of corresponding brown colored hydrazone formed in alkaline medium was read at 505 nm. ACP was assayed using span diagnostics kit (King's method, 23). The reaction mixture of ACP assay contained 500 µl of disodium phenyl phosphate substrate (pH 5.0), 1500 µl of deionized water, 100 µl of enzyme extract and 1000 µl of color reagent having 4-amino antipyrine with alkaline potassium ferry cyanide. Optical density of red-colored complex formed was read at 510 nm.

In Vitro *Studies*. Each of the brain regions, from 40-day-old untreated rats was used for *in vitro* estimations. Pro was dissolved in Tris–HCl buffer, pH 7.4 and added to the incubation medium to a final concentration ranging from 0.2 to 2.0 mM. Controls did not contain Pro in the incubation medium. Tissues along with medium were incubated for 1 h.

*Protein Assay*. Protein concentration was measured according to Lowry et al. (24) using bovine serum albumin as the standard.

*Histological Studies*. Brain regions after dissection were fixed with 10% buffered formaline for histological processing. Tissues were embedded in paraffin and sections were cut on microtome at 7-µm. Sections were stained with hematoxylene and eosin. The series of sections were minutely examined under microscope and early as well as late necrotic changes were recorded and the percentage count of cells showing necrosis was calculated on the basis of cell observations in different areas and slides without repeating any area.

*Statistical Analysis*. The results represented as means ± standard deviation for the number of animals indicated. Significance of differences was determined by an unpaired students *t*-test (Computer program Microsoft Excel for windows 97 SYSTAT 5.0).

## RESULTS

Rats after chronic treatment with Pro appeared to be sluggish and showed lowered responses to tail pricks i.e. tail withdrawal time after a prick was increased (Pro treated 12.6 ± 3.362 m; control 1.6 ± 0.894 m $P < 0.001$), roller drum balancing was lowered (Pro treated 1.4 ± 0.548 min; control 4.6 ± 0.548 min, $P < 0.001$) and lifting by tails showed increase in time taken to start swirling movements (Pro treated 69.6 ± 8.849 ms; control 21 ± 6.519 ms, $P < 0.001$) and convulsion after 5 s.

Rats chronically treated with Pro showed significant increase in plasma proline levels (Pro treated: 1.843 ± 0.051 mM; control: 0.196 ± 0.017 mM, $P < 0.001$), CSF Pro levels (Pro treated: 31.6 ± 3.5 µM; control: 3.73 ± 1.25 µM, $P < 0.001$) and tissue Pro levels from different brain regions were significantly elevated after chronic treatment with proline (olfactory lobes: Pro treated 980.0 ± 262.0 µM; control 132.0 ± 8.0 µM, $P < 0.001$; cerebrum: Pro treated 924.0 ± 172.0 µM; control 202.0 ± 8.0 µM, $P < 0.001$; cerebellum: Pro treated 728.0 ± 117.0 µM; control 182.0 ± 14.0 µM, $P < 0.001$; medulla oblongata: Pro treated 840.0 ± 77.0 µM; control 247.0 ± 103.0 µM, $P < 0.001$). Similarly, Glu levels of CSF also increased (Pro treated 23.31 ± 2.21 µM; control 1.525 ± 0.517 µM, $P < 0.001$) but Glu levels lowered significantly from all the brain regions under study except cerebellum, where it increased insignificantly (olfactory lobes: Pro treated 51.0 ± 13.0 µM; control 191.0 ± 13.0 µM, $P < 0.001$; cerebrum: Pro treated 103.0 ± 12.0 µM; control 233.0 ± 21.0 µM, $P < 0.001$; cerebellum: Pro treated 231.0 ±

13.0 μM; control 228.0 ± 11.0 μM; medulla oblongata: Pro treated 149.0 ± 14.0 μM; control 224.0 ± 25.0 μM, $P < 0.001$).

Chronic administration of Pro promoted significant reductions of cytosolic ALT activities of olfactory lobes (50.57%; $P < 0.001$), cerebrum (40%; $P < 0.001$), medulla oblongata (13.71%; $P < 0.005$) but not of cerebellum (Fig. 1). Similarly, mitochondrial ALT activity also reduced significantly in all brain regions (olfactory lobes 73.23%; $P < 0.001$, cerebrum 70.26%; $P < 0.001$, cerebellum 65.39%; $P < 0.001$, medulla oblongata 65.18%; $P < 0.001$) Fig. 2.

The effect of chronic Pro administration on cytosolic AST activity was also determined. The cytosolic AST activity from olfactory lobes, cerebrum and medulla oblongata reduced by 75.71% ($P < 0.001$), 67.53% ($P < 0.001$) and 76.13% ($P < 0.001$) respectively, while cytosolic AST activity from cerebellum increased by 28.05% ($P < 0.005$, Fig. 3). Mitochondrial AST activity was lowered in olfactory lobes (by 72.45%, $P < 0.001$), cerebrum (by 78%, $P < 0.001$), cerebellum (by 49.56%, $P < 0.001$) and medulla oblongata (by 69.30%, $P < 0.001$; Fig. 4).

Further we determined the *in vitro* effect of Pro on tissue Pro and Glu levels so also cytosolic and mitochondrial ALT/AST activities in all four brain regions to verify if proline has direct effect on these enzymes. *In vitro* Pro treatment promoted significant increase in tissue Pro levels and significant decrease in Glu levels (Figs. 5 and 6). *In vitro* studies of mitochondrial AST and ALT activities under the influence of Pro showed inhibition of enzyme activities in all the brain regions. Similar inhibition of cytosolic AST was observed in all the brain regions except cerebellum where it elevated significantly (Figs. 7–10).

Pearson correlation (1-tailed) analysis of *in vitro* studies showed significant positive correlation between tissue Glu levels and cytosolic/mitochondrial ALT activity (olfactory lobes, $P < 0.01$; cerebrum, $P < 0.01$; cerebellum, $P < 0.05$; medulla oblongata, $P < 0.05$). Significant positive correlation was seen between tissue Glu levels and cytosolic/mitochondrial AST activity (olfactory lobes, $P < 0.01$; cerebrum, $P < 0.05$; cerebellum, $P < 0.05$; medulla oblongata, $P < 0.05$) except for cerebellum which showed significant negative correlation between tissue Glu and cytosolic AST activity ($P < 0.05$). Chronic *in vivo* Pro administration showed positive correlation between tissue Glu levels and cytosolic as well as mitochondrial ALT/AST activities (significant at $P < 0.01$ for olfactory lobes, cerebrum and medulla oblongata). Cerebellar region did not show any significant correlation between tissue Glu levels and AST/ALT activities but these activities showed significant positive correlation with CSF Glu levels ($P < 0.01$) after chronic Pro administration.

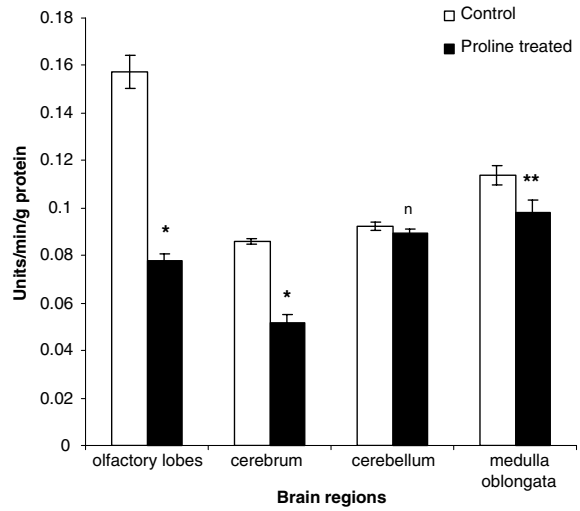


**Fig. 1.** Effect of chronic administration of proline on cytosolic ALT activity from rat brain regions. Data are mean ± SD for five independent experiments performed in pentuplate. *Different from control, $P < 0.001$, **$P < 0.005$; [n] insignificant (Student's *t*-test).

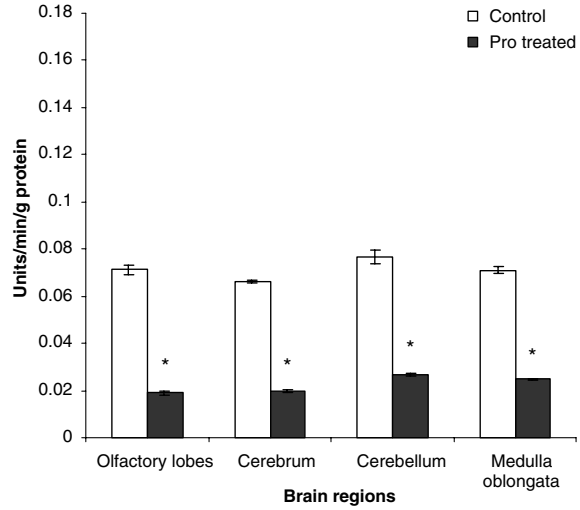


**Fig. 2.** Effect of chronic administration of proline on mitochondrial ALT activity from rat brain regions. Data are mean ± SD for five independent experiments performed five times. *Different from control, $P < 0.001$ (Student's *t*-test).

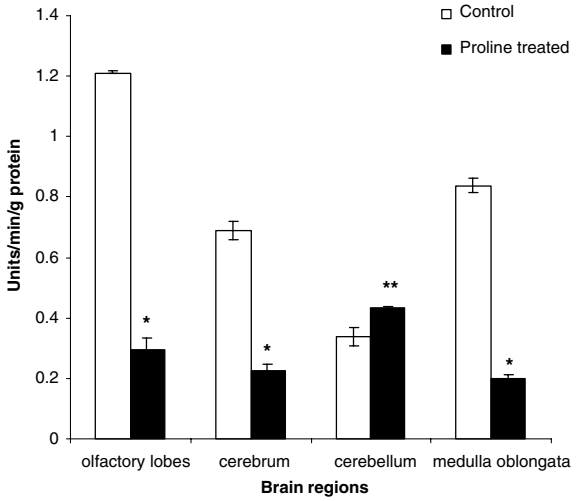


**Fig. 3.** Effect of chronic administration of proline on cytosolic AST activity from rat brain regions. Data are mean ± SD for five independent experiments performed in pentuplate. * Different from control, $P < 0.001$; ** $P < 0.005$ (Student's *t*-test).

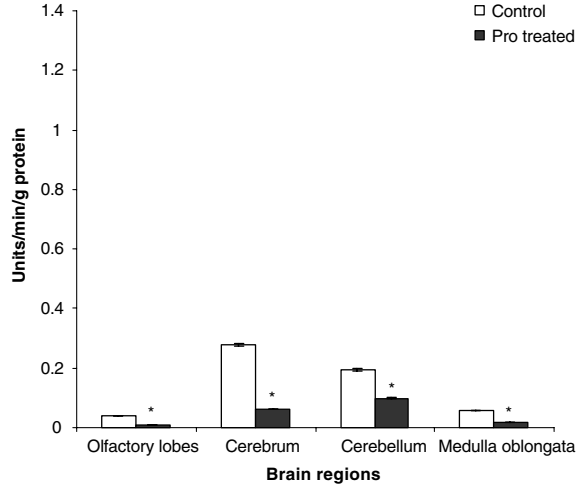


**Fig. 4.** Effect of chronic administration of proline on mitochondrial AST activity from rat brain regions. Data are mean ± SD for five independent experiments performed five times. *Different from control, $P < 0.001$ (Student's *t*-test).

We also assessed the effect of chronic Pro administration on ACP activities from rat brain regions and CSF. The ACP activities from olfactory lobes and cerebrum were reduced by 40.33% ($P < 0.001$) and 20.82%($P < 0.001$) respectively while that from cerebellum and medulla oblongata elevated by 97.32% ($P < 0.001$) and 76.33% ($P < 0.001$) respectively (Fig. 11). CSF ACP activity also showed increase (Pro treated: 56 ± 6.7 mKA Units/min/g protein; control: 5.4 ± 1.5 mKA Units/min/g protein, 1 mKA≡1 μg of phenol liberated/min).

Histopathological findings showed early necrotic changes (change in shape, shrinkage) in cell body of neuron as well as nerve fibers and late necrotic changes including loss of total integrity of cell, loss of cytoplasm, vacuolization and accumulation of debris changes, in all the brain regions after the chronic administration of Pro (Figs. 12a–12d and 13a–13d). The olfactory lobes, cerebrum, cerebellum and medulla oblongata showed 1.2, 1.8, 3.0 and 3.4% early progressive necrotic changes and 4.0, 3.1, 0.8 and 1.1% late necrotic changes respectively.

## DISCUSSION

In the present study animal responses to tail pricks, roller drum and lifting by tail clearly indicate that Pro affects the normal responses and promotes sluggishness. Further, it was noticed that Pro treated rats when lifted by tails for more than 5 s resulted in convulsion, which indicate disturbance in brain function. Flynn et al. (25). have documented a causal relationship between high concentration of Pro and recurrent seizures in HPII patients suggesting correlation between sustained tissue Pro levels and convulsion. It is a fact that many hyperprolinemic patients present a neurological syndrome constituting seizures and mental retardation, whereas some of the patients are found to be clinically normal (4) but in these cases, CSF and plasma Pro levels were found elevated. The present study clearly

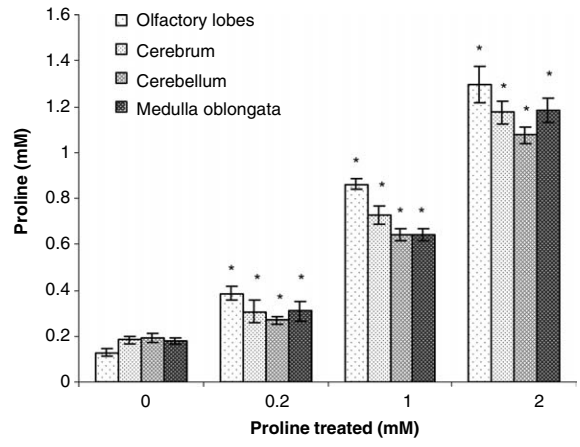


**Fig. 5.** *In vitro* effect of proline on tissue proline levels from rat brain regions. Data are ± SD for five independent experiments performed in pentuplate. *Different from control, $P < 0.001$ (Student's *t*-test).

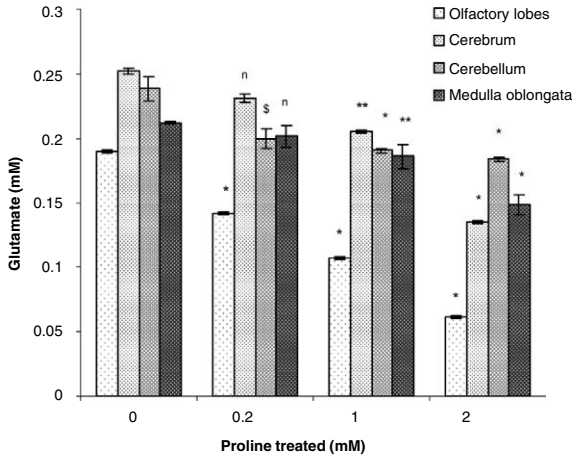


**Fig. 6.** *In vitro* effect of proline on tissue glutamate levels from rat brain regions. Data are ± SD for five independent experiments performed in pentuplate. *Different from control, $P < 0.001$; **$P < 0.005$; $P < 0.05$; nsignificant (Student's *t*-test).

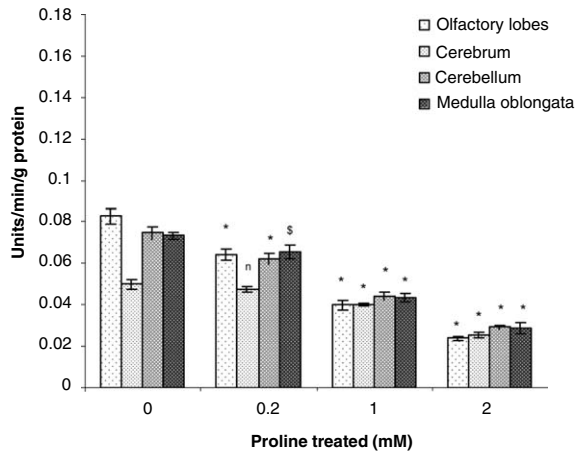


**Fig. 8.** *In vitro* effect of proline on mitochondrial ALT activity from rat brain regions. Data are ± SD for five independent experiments performed in pentuplate. *Different from control, $P < 0.001$; $P < 0.05$; nsignificant (Student's *t*-test).

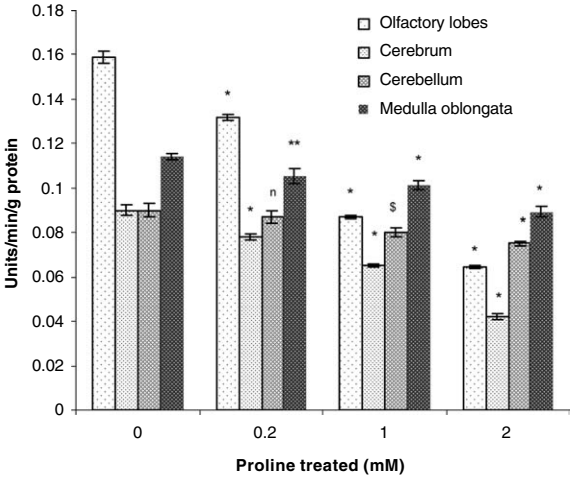


**Fig. 7.** *In vitro* effect of proline on cytosolic ALT activity from rat brain regions. Data are ± SD for five independent experiments performed in pentuplate. *Different from control, $P < 0.001$; **$P < 0.005$; $P < 0.05$; nsignificant (Student's *t*-test).

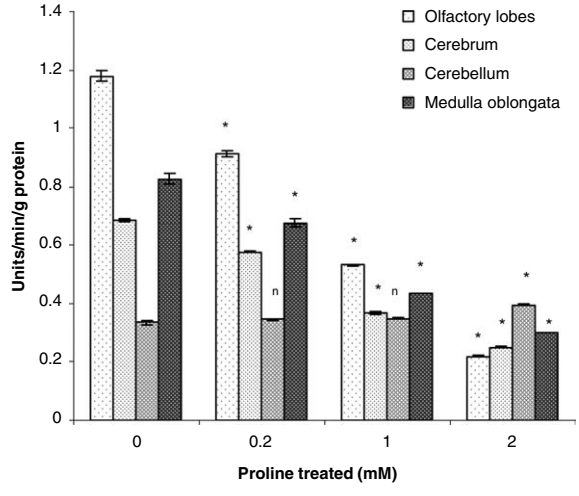


**Fig. 9.** *In vitro* effect of proline on cytosolic AST activity from rat brain regions. Data are ± SD for five independent experiments performed in pentuplate. *Different from control, $P < 0.001$; nsignificant (Student's *t*-test).

indicates association of high plasma and CSF proline levels with convulsions, sluggishness and reduced balancing ability indicating neural dysfunction. It appears that olfactory lobes are the most affected ones as they show high Pro levels, low Glu levels as well as lowest AST and ALT activities. Besides, histopathological studies indicate pronounced necrosis of olfactory lobes. Therefore, olfactory lobes vis-à-vis olfactory neurons appear to be most vulnerable to Pro action. Since olfactory neurons of the CNS are responsible for the sense of smell, early diagnosis of HPII could be possible by examining sense of smell. Earlier studies showed that acute subcutaneous/intracerebral administration of Pro to rats and chicks disrupts learning and memory functions (6,26) indicating that affected area could be cerebrum. Cerebrum is the second most affected region after olfactory lobes since it shows high proline and low Glu levels with reduced

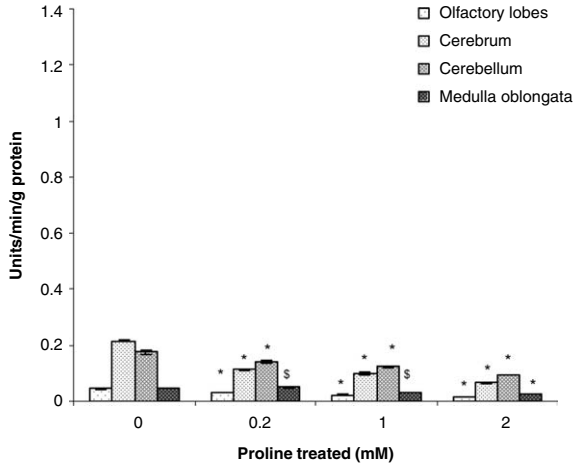


**Fig. 10.** *In vitro* effect of proline on mitochondrial AST activity from rat brain regions. Data are ± SD for five independent experiments performed in pentuplate. *Different from control, $P < 0.001$; $^{\$}P < 0.05$ (Student's *t*-test).

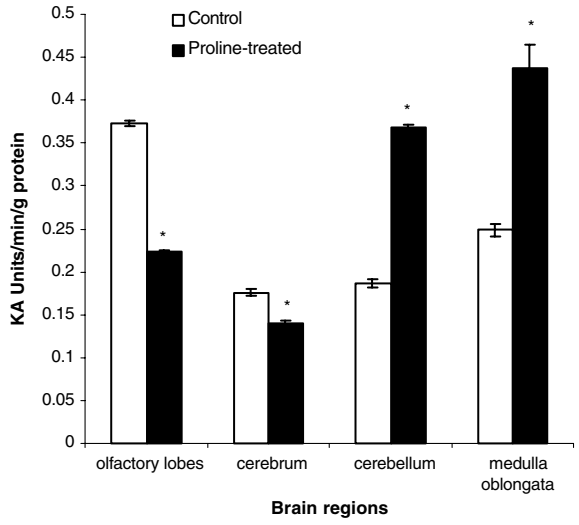


**Fig. 11.** Effect of chronic administration of proline on ACP activity in supernatants from rat brain regions. Data are mean ± SD for five independent experiments performed five times. *Different from control, $P < 0.001$ (Student's *t*-test).

AST and ALT activities along with some necrotic changes. This indicates that proline affects cerebrum. As this region of brain deals with the interpretation of impulses from sense organs, so also it is the site for storing information (memory) and retrieving information for reasoning, mental retardation observed in HPII patients could be partly due to affected cerebrum. From our study, third most affected region of brain was medulla oblongata followed by cerebellum, which is responsible for coordination of voluntary movements, balance and equilibrium so also some memory required for reflex motor acts. The observed behavioral changes such as reduced balancing ability and convulsions in the rats exposed to proline could be related to the histopathological alteration of cerebellum associated with increased AST activity and Glu levels. Though cerebellar Glu level elevated by just 1.2%, the CSF Glu level elevated by more than fifteenfolds. This indicates that actual elevation of Glu level in cerebellum could have led to release of glutamate in CSF, which may be considered as protective mechanism of the brain to avoid over excitation of that region.

AST and ALT are native brain enzymes existing in cytosolic and mitochondrial forms. The significant decrease in cytosolic and mitochondrial AST activity of olfactory lobes, cerebrum and medulla oblongata indicate reduction in Glu metabolism in these regions (as evidenced by reduced Glu levels) particularly the observed sluggishness could be due to the lesser production of Glu, an essential neurotransmitter for excitatory responses under the influence of Pro, particularly during the growth. But chronic Pro treatment appears to promote increase in cerebellar cytosolic AST activity that might have led to the increase in Glu synthesis and this increased cerebellar glutamate fraction alone could be contributing to the rise in Glu levels of CSF as observed in present study. Besides, it is reported that hyperprolinemia promotes rise in Pro and Glu levels in CSF indicating its possible excitotoxic role (3). Therefore, rise in Glu synthesis by cerebellum could be the cause of seizures observed in HPII while the mental retardation could be due to reduced production of Glu as observed in other regions, which may normally be required for normal neural function. Also alternatively decreased cytosolic AST activity could be connected to decreased transport of NADH from the cytosol to mitochondria leading to decreased metabolic rate as a corollary to the finding that, increased AST activity may be connected to increased transport of NADH from cytosol to mitochondria (27) which may increase metabolic rate. Therefore, the observed sluggishness of rats could be mostly due to decreased metabolic rate. The reduction in ALT activity by olfactory lobes, cerebrum and medulla oblongata indicate reduction in protein metabolism particularly the transformation of alanine to pyruvate

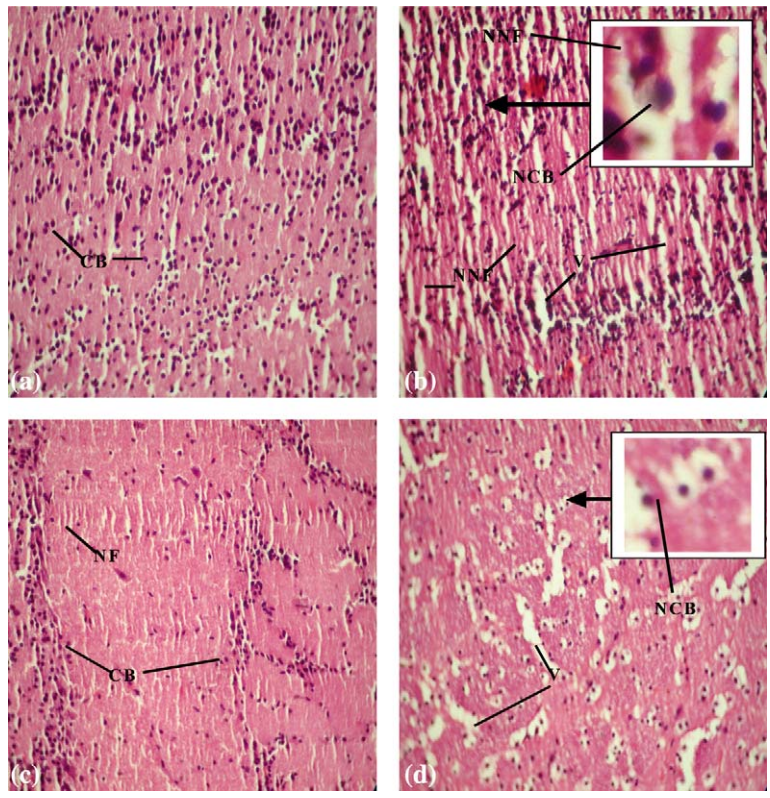


**Fig. 12.** (a) (10 × 10) Histological section of olfactory lobes of control rat showing normal cell body of neuron (CB). (b) (10 × 10) Histopathological section of olfactory lobes of rat brain chronically treated with proline showing necrotic cell body of neuron (NCB), necrotic nerve fibers (NNF) and vacuolization (V). Small box (10 × 40) showing necrotic cell body of neuron (NCB) and necrotic nerve fibers (NNF). (c) (10 × 10) Histological section of cerebrum of control rat showing normal cell body of neuron (CB) and normal nerve fibers (NF). (d) (10 × 10) Histopathological section of cerebrum of rat brain chronically treated with proline showing vacuolization (V). Small box (10 × 40) showing necrotic cell body of neuron (NCB).

and vice versa. ALT is involved in synthesis of Glu as the neurotransmitter in glutamatergic neurons (17,18). Therefore, reduction in aminotransferase activity may be leading to lesser synthesis of Glu thereby causing neurological dysfunction.

From the present study, i.e. from chronic as well as *in vitro* Pro exposure it is confirmed that cytosolic as well as mitochondrial AST/ALT activities are affected. Out of four regions olfactory lobes, cerebrum and medulla oblongata show significant positive correlation between cytosolic/mitochondrial ALT activity and tissue glutamate levels as well as between cytosolic/mitochondrial AST and glutamate levels. Cerebellar Glu level and AST/ALT activities do not show significant correlation as the recorded Glu hike is just 1.2% after chronic Pro administration. However, this need to be viewed with elevation of Glu in CSF, as elevation of cerebellar cytosolic AST activity shows positive correlation with glutamate level of CSF.

The increase of ACP activity of cerebellum and medulla oblongata indicate probable on-going necrosis of these areas thereby activating the golgi-lysosomal system of microglia (19). The histopathological observations support this. The reduction of ACP activity in olfactory lobes and cerebrum could be due to redistribution of ACP/lysosomes and the passage of ACP into the CSF after necrotic changes. The present study shows increase in CSF ACP activity. But it is difficult to know how much contribution each region of the brain has made for rise in CSF ACP activity. Our histopathological findings also show early and late necrotic changes in all brain regions under study. As cerebrum and olfactory lobes show pronounced late necrotic changes with a few early necrotic changes while other areas show pronounced early necrotic changes with a few late necrotic changes, the ACP activity shows reduction in olfactory lobes and cerebrum possibly due to sweeping of lysosomes with debris

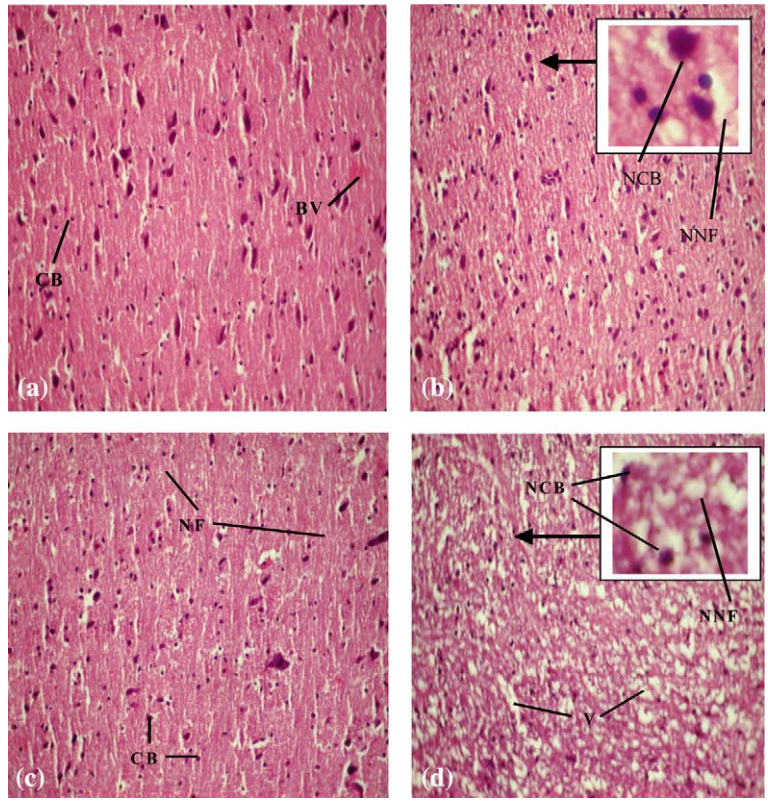


**Fig. 13.** (a) (10 × 10) Histological section of cerebellum of control rat showing normal cell body of neuron (CB) and blood vessel (BV). (b) (10 × 10) Histopathological section of cerebellum of rat brain chronically treated with proline. Small box (10 × 40) showing, necrotic cell body of neuron (NCB), necrotic nerve fibers (NNF). (c) (10 × 10) Histological section of medulla oblongata of control rat showing normal cell body of neuron (CB) and normal nerve fibers (NF). (d) (10 × 10) Histopathological section of medulla oblongata of rat brain chronically treated with proline showing vacuolization (V). Small box (10 × 40) showing necrotic cell body of neuron (NCB) and necrotic nerve fibers (NNF).

in CSF. The CSF shows elevation in ACP activity. The increase in ACP activity in cerebellum and medulla oblongata could be due to progressive necrotic changes as evidenced by presence of many early necrotic neurons and nerve fibers. The relationship between enhancement of ACP activity and progressive necrosis is well established (28).

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