IC/92/76
INTERNAL REPORT
(Limited Distribution)

International Atomic Energy Agency
and
United Nations Educational Scientific and Cultural Organization
INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS

EFFECT OF *LATHYRUS SATIVUS* AND VITAMIN C ON THE STATUS OF AROMATIC L–AMINO ACID DECARBOXYLASE AND DIPEPTIDYL–AMINOPEPTIDASE–IV IN THE CENTRAL AND PERIPHERAL TISSUES AND SERUM OF GUINEA PIGS

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Key words: *Lathyrus Sativus* seeds: β – N–Oxalyl–L–α,β–diaminopropionic acid: aromatic L–amino acid: dipeptidyl–aminopeptidase–IV: vitamin C: neurotransmitters.

MIRAMARE – TRIESTE
May 1992

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ABSTRACT

Studies on the effect of *Lathyrus Sativus seeds* (LSS) on aromatic L–amino acid decarboxylase (AADC) and on dipeptidyl–aminopeptidase–IV (DAP–IV) were carried out in the central and peripheral tissues and serum of LSS–treated and LSS plus vitamin C–treated guinea pigs. The feeding of LSS for 35 days decreased the AADC activity significantly in the brain and peripheral tissues, but the activity was recovered to normal level in the most tissues when vitamin C was added with the LSS. DAP–IV activity decreased in the peripheral tissues when treated with LSS, but the vitamin C administration with LSS did not recover the enzyme activity. The DAP–IV activity did not decrease significantly in any of the brain tissues of the LSS–treated group.

1. INTRODUCTION

Neurolathyrism is a crippling human disease characterized by the loss of control over the lower limbs (paraplegia). It afflicts when *Lathyrus Sativus* seeds (Khesari) (LSS) are taken as a main item of the diet contributing at least 30% of the calorie intake for a period of 3–4 months. It is seen mostly amongst poor people who during famine conditions are compelled to live on Khesari in lieu of rice or wheat. It is strictly a disease of the human species. No experimental model in animals was available explaining why we do not know enough on the biochemistry of the disease so that effective preventive and curative measures could be developed. Some scientists [1] in India, however, discovered that extracts of LSS when administered intraperitoneally to two day old chicks give rise to certain neurological symptoms.

Ahmad and Jahan [2] reported that neurolathyrism could be experimentally produced in adult guinea pigs by feeding LSS. They have to be, however, made deficient in vitamin C, even though this deficiency would not be severe enough to cause scurvy. The animals show all the classical symptoms of neurolathyrism seen in man.

Adiga *et al.* [3] isolated $\beta - N$–oxalyl–L–α, β–diaminopropionic acid (ODAP) from LSS. It is a neuroactive amino acid and is considered responsible for causing the neurological damage seen in neurolathyrism. Vitamin C appears to be a key protective nutrient against the neurotoxin of LSS [2].

Ahmad and Jahan [4] treated a lathyrism patient, named Khabiruddin, with 500mg of L–ascorbic acid (i.v.) for 2 weeks and then started giving him the same amount of vitamin orally every day for over 2 months and the patient recovered completely. The mechanism of the production of neurolathyrism is still unknown.

Aromatic L–amino acid decarboxylase (AADC) [5] is the enzyme that decarboxylates L–DOPA and L–5–hydroxytryptophan (L–5–HTP) to dopamine (DA) and serotonin (5–HT), respectively, in many mammalian tissues, including the humans. Then decarboxylated products by AADC (DA, norepinephrine, epinephrine and 5–HT) are important biochemically and pharmacologically, because the product monoamines are important inter–cellular messengers such as neurotransmitters and hormons and involved in the regulation of neuronal functions, behaviour and emotion of higher animals.

We have made systematic studies of AADC activities in tissues and brains of rats [6, 7], and human subjects [8, 9]. We also discovered this enzyme in the sera of various animals [10] and examined the developmental changes of AADC [11, 12]. We have also studied the effect of pyridoxal–5–phosphate deficiency on AADC in the central and peripheral tissues and serum of animals [7]. But up to now no study has been made on the status of AADC in lathyrism, a neurological related disease. On

the other hand, dipeptidyl–amino–peptidase IV (X–prolyldipeptidyl–aminopeptidase, DAP–IV) was a newly discovered enzyme in rat liver and kidney [13, 14].

Nagatsu *et al.* [15] first measured the enzyme in serum of various animals including human subjects. It had been reported that human serum DAP–IV activity was generally increased in patients with gastric cancer.

Hino *et al.* [16] and Kojima *et al.* [17] measured the serum activities of DAP–II and IV in tumor–bearing animals and in patients with blood and gastric cancer. DAP–IV is not a neurotransmitters–mediating enzyme. Up to now no study has been made on the status of DAP–IV in the lathyrism condition. These tempted us to make a systematic study on the comparison of the effects of lathyrism on these enzymes in the central and peripheral tissues and serum of adult guinea pigs maintained on various diets, i.e., normal, LSS, LSS plus vitamin C.

2. MATERIALS AND METHODS

2.1 *Materials*

L–5–HTP, D–5–HTP, pargyline HCl, pyridoxal–5–phosphate, NSD–1015, 5–hydroxytryptamine (5–HT), and ascorbic acid were obtained from the Sigma Chemical Co., St. Louis, M.O., Amberlite CG–50 from Rohm and Hass, Philadelphia, P.A., Glycylprolyl–p–nitroanilide tosylate was the kind gift from Dr. T. Kato, Tokyo Institute of Technology, Nagatsuta Campus, Nagatsuta, Yokohama, Japan and from Dr. E. Krepela, Res. Inst. of Tuberculosis and Respir. Diseases, Department of Biochemistry, Prague, Czechoslovakia. All other chemicals were of analytical grade. Amberlite CG–50 (type 1, 100–200 mesh) was activated by cyclic washing with 2M HCl, 2M NaOH, and finally with water, equilibrated with 1M potassium phosphate, pH 6.5, and stored as a suspension in the same buffer.

2.2 *Animal experiment*

Twenty one adult guinea pigs were used. They were divided equally into 3 groups. The animals in Group I received the normal diets bought from the Animal Food Suppliers, Postogola, Dhaka. The animals in Group II received the LSS only and the animals in Group III received LSS plus sufficient amount (15mg/day/guinea pig) of vitamin C. The animals of each group received their prescribed diets for 35 days. After this period the animals were sacrificed by decapitation and the blood, liver, kidney, heart, adrenal, small intestine, spleen, lung, cerebral cortex, cerebellum, caudate nucleus, brain stem, hypothalamus and colliculi were collected and washed with saline, if necessary, and the serum was collected from the blood. The samples were stored at -20°C until use.

The peripheral tissues were homogenized (10–fold dilution) with 0.25M sucrose (1 part tissue plus 9 Vol. 0.25M sucrose) in a Potter glass homogenizer. The brain tissues were homogenized in the same way but with 0.32M sucrose.

2.3 *Experimental Procedures*

i) Assay or AADC with 5–HTP as substrate AADC activity with L–5–HTP as substrate (5–HTP decarboxylase) was measured by fluorometry based on the native fluorescence of 5–HT. The standard incubation mixture for 5–HTP decarboxylase contained (total volume 400$\mu\ell$, final pH8.3): 0.1M sodium phosphate buffer (pH9.0), 1.0mM pyridoxal–5–phosphate, 1.0mM pargyline HCl, 1.0mM L–5–HTP (or D–5–HTP plus N.S.D.1015 for the blank) and the enzyme. The incubation was done at 37°C for 90 minutes and the reaction was stopped by adding 80$\mu\ell$ of 3M trichloracetic acid. After 10 minutes, 1.92$m\ell$ of water were added and the mixtures were centrifuged at 3000

r.p.m. for 10 minutes at 4°C. The supernatant fractions were passed through a column (packed Vol.0.5$m\ell$) of Amberlite CG–50–Na^+ equilibrated with 0.1M potassium phosphate buffer (pH6.5). The resin was washed twice with 4.0$m\ell$ of the buffer and 500$\mu\ell$ of 1M HCl. The 5–HT was eluated with 1.2$m\ell$ of 1M HCl. Then the eluate was measured spectrofluorometrically with a Shimadzu spectrofluorometer RF–500, with excitation wavelength at 295nm and emission at 334nm.

ii) Assay of dipeptidyl–amino–peptidase IV activity. The experimental tube contained 75μmol of glycine–Na OH buffer (pH8.7), 1.5μmol of glycyl–prodine–p–nitroanilide tosylate, and enzyme plus water to 1.05$m\ell$. The blank and standard tubes contained water and p–nitroaniline (750μmol), instead of enzymes, respectively. The control tube contained no enzyme. Then all the tubes were incubated for 30 min. at 37°C. After this period the reaction was stopped by adding 3.0$m\ell$ of 1M acetate buffer, pH4.2. The same amount of enzyme was added to the control, after stopping the reaction. The change in the absorbance of the experimental, control and standard were measured in a Shimadzu Double–Beam Spectrophotometer at 385nm in a 1–cm cuvette as a function of DAP–IV activity. One unit of tissue enzyme was expressed as 1μmol of p–nitroaniline liberated per min. per g wet weight of tissue and the serum enzyme as that liberated per min. per $m\ell$ of serum.

3. RESULTS AND DISCUSSION

Tissue distribution of AADC activity, using L–5–HTP as substrate, in eleven tissues including peripheral and brain regions of normal, LSS-treated and LSS plus vitamin C treated-guinea pigs is shown in Table I. The tissues are arranged in the order from the highest to the lowest L–5–HTP decarboxylase activity in the peripheral and the brain tissues of the healthy group. In the peripheral tissues of healthy guinea pigs liver had the highest activity of 101.15 units, followed by adrenal glands 1005, kidney 66.63, spleen 59.79, heart 55.61 and lung 53.14 units. Among the brain tissues caudate nucleus had the highest activity of 67.48 units followed by colliculi 63.23, brain stem 62.49, cerebral cortex 57.00 and hypothalamus 46.30 units. Cerebellum had the lowest enzyme activity of 43.23 units. The data for the cerebral cortex were not available.

The activity of AADC in serum was very low [10] which was the limit of the sensitivity of the present spectrofluoremetric assay method.

AADC activity was low in all the central and peripheral tissues of the LSS treated guinea pigs, as compared to the respective tissue activity of the control guinea pigs. In this group, heart had the highest activity of 35.80 units, followed by colliculi 34.01, liver 30.48, kidney 30.10, adrenal gland 27.95, caudate nucleus 21.96, hypothalamus 15.52 and brain stem 13.95 units. The lowest activity was observed in the spleen.

It was observed that the enzyme activity had the tendency to recover completely or partially when the animals were treated with LSS plus vitamin C. The caudate nucleus recovered its activity to 94% followed by liver 85%, colliculi 82%, adrenal 81%, hypothalamus 78%, heart 68%, lung 39%, cerebellum 39%, brain stem 25% and spleen 20%.

Table II shows the DAP–IV activity, using glycylprolyl–p–nitroanilide tosylate as the substrate, in thirteen tissues, peripheral and brain regions, of LSS treated and LSS plus vitamin C treated guinea pigs. The tissues were arranged from the highest to the lowest of the DAP–IV activity, in the healthy guinea pigs. Kidney had the highest activity of 30.53 units, followed by liver 29.74 and lung 23.50 units. The activity in spleen, adrenal and heart was almost similar, 4.79, 4.54 and 3.26 units, respectively, but the activity in the brain tissues was much lower than that in the peripheral tissues. Among the brain tissues, caudate nucleus had the highest activity of 1.84 units, followed by cerebral cortex 0.97, hypothalamus 0.79, brain stem 0.71 and colliculi 0.63 units. Cerebellum had the lowest enzyme activity. The serum enzyme activity was found to be 1.06 units.

DAP–IV activity was low in all the pheripheral tissues of LSS treated and LSS plus vitamin C treated guinea pigs. In the LSS treated guinea pigs, kidney had the highest enzyme activity of 12.39 units, followed by liver 12.0, lung 10.94, adrenal 4.02, spleen 3.48 and heart 2.34 units. In the LSS plus vitamin C treated guinea pigs, again, kidney had the highest DAP–IV activity of 14.83 units followed by liver 14.70, lung 14.20, adrenal 4.41, spleen 4.12 and heart 2.88 units. DAP–IV activity did not decrease in the brain tissues of the LSS treated group. In this group, the caudate nucleus had the highest activity of 1.88 units, followed by cerebral cortex 1.70, hypothalamus 1.41, colliculi 89, and brain stem 87 units. Cerebellum had the lowest enzyme activity in this group. But treatment with vitamin C has the tendency to increase the DAP–IV activity in the brain tissues. In this group, colliculi had the highest enzyme activity of 1.67 units, followed by caudate nucleus 1.50, cerebellum 1.29, hypothalamus 1.29 and brain stem 1.25 units. Cerebral cortex had the lowest enzyme activity of 1.12 units.

It was discovered in the United States of America that L–glutamic acid or monosodium glutamate (MSG) when administered intraperitoneally produces lesions in the brain in very young mice [18], They were similar to those found in treatment with the LSS–toxin, ODAP, in very young animals like chicks. It has also been reported [2] that neurolathyrism could be experimentally produced in adult guinea pigs by feeding LSS and the symptoms of the disease can be removed by the administration of high doses of vitamin C with the diet. The activity of AADC, an important neurotransmitters–mediating enzyme, producing two of the most important neurotransmitters, namely, dopamine and seretonin, was significantly low in all of the central and peripheral tissues of guinea pigs treated with the LSS. This may be due to the possibility that LSS–toxin may affect some binding sites of this enzyme and ultimately affecting the enzyme activity. But the vitamin C administration with the LSS reversed the effect in most of the tissues; vitamin C might have some protective action and effect on the recepter site of the enzyme, so that the toxin is not effective at the receptor site of this enzyme. The enzyme, DAP–IV is not a neurotransmitter–mediating enzyme, but it is a newly discovered enzyme which showed different effects compared to that of AADC on the administration of LSS or LSS plus vitamin C to the guinea pigs.

Acknowledgments

The authors wish to thank Drs. M. Aftabuddin and A.F. Naved for their help during the experiments. One of the authors (M.K.R.) would also like to thank Professor Abdus Salam, the International Atomic Energy Agency and UNESCO for hospitality at the International Centre for Theoretical Physics, Trieste. This work was partly supported by Grant No.BC–87–33–BD–2 from the Third World Academy of Sciences (TWAS), Trieste, to M.K. Rahman.

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Table I

Aromatic L-amino acid decarboxylase (AADC) activity in central and peripheral tissues of normal healthy guinea pigs and in guinea pigs treated with *Lathyrus Sativus* and *Lathyrus Sativus* seeds plus vitamin C.

Name of Tissues	Number of Samples	AADC activity with L-5-HTP as substrate (nmole/min/g wet weight of tissues) Mean ± S.E.M.			
		Normal Healthy Group	Lathyrus Sativus treated group	P-values (two-tailed test)	Lathyrus Sativus plus vitamin C treated group
A. Peripheral Tissues					
Liver	7	101.15 ± 16.51	30.48 ± 2.60	P<0.01	85.92 ± 12.24
Adrenal	7	100.58 ± 44.04	27.95 ± 11.76	P<0.01	81.48 ± 5.70
Kidney	7	66.63 ± 10.78	30.10 ± 3.28	P≤0.01	47.93 ± 25.85
Spleen	7	59.79 ± 14.91	7.79 ± 1.68	P<0.01	12.20 ± 3.80
Heart	7	55.61 ± 16.13	35.80 ± 2.56	P<0.01	37.77 ± 5.55
Lung	7	53.14 ± 17.63	16.72 ± 11.96	P<0.01	20.81 ± 11.57
B. Brain Tissues					
Caudate nucleus	7	67.48 ± 26.05	21.96 ± 1.58	P<0.01	63.44 ± 1.93
Colliculi	7	63.23 ± 26.55	34.01 ± 1.80	P<0.01	52.13 ± 1.82
Brain Stem	7	62.49 ± 19.65	13.95 ± 1.90	P<0.01	16.06 ± 1.00
Hypothalamus	7	46.30 ± 12.27	15.52 ± 2.09	P≤0.01	36.44 ± 2.20
Cerebellum	7	43.24 ± 17.88	16.01 ± 01.87	P≤0.01	16.89 ± 0.38

The assay was done as described under Materials and Methods.

Table II

Dipeptidyl aminopeptidase IV (DAP–IV) activity in central and peripheral tissues and serum of normal healthy guinea pigs and in guinea pigs treated with *Lathyrus Sativus* seeds and *Lathyrus Sativus* plus vitamin C.

Name of Tissues	Number of Samples	DAP–IV activity (μM/min/g wet weight tissue) Mean ± S.E.M.			
		Normal Healthy Group	Lathyrus Sativus treated group	P–values (two–tailed test)	Lathyrus Sativus plus vitamin C treated group
A. Peripheral Tissues					
Kidney	7	30.53 ± 2.79	12.39 ± 0.65	P<0.01	14.83 ± 1.00
Liver	7	29.74 ± 2.73	12.00 ± 1.07	P<0.01	14.70 ± 0.90
Lung	7	23.50 ± 1.26	10.94 ± 0.51	P≤0.01	14.20 ± 1.43
Spleen	7	4.79 ± 1.45	3.48 ± 0.34	P≤0.01	4,12 ± 1.81
Adrenal	7	4.54 ± 1.17	4.02 ± 0.65	P<0.01	4.41 ± 0.89
Heart	7	3.26 ± 0.91	2.34 ± 0.34	P>0.01	2.88 ± 0.87
B. Brain Tissue					
Caudate nucleus	7	1.84 ± 0.61	1.88 ± 0.23	P>0.01	1.50 ± 0.88
Cerebral cortex	7	0.97 ± 0.45	1.70 ± 0.18	P>0.01	1.12 ± 0.69
Hypothalamus	7	0.79 ± 0.36	1.41 ± 0.14	P>0.01	1.29 ± 0.51
Brain Stem	7	0.71 ± 0.59	0.87 ± 0.14	P>0.01	1.25 ± 0.69
Colliculi	7	0.63 ± 0.21	0.89 ± 0.19	P>0.01	1.67 ± 0.15
Cerebellum	7	0.39 ± 0.07	0.81 ± 0.06	P>0.01	1.29 ± 0.32
Serum*	7	1.06 ± 0.06	0.71 ± 0.10	P>0.01	0.64 ± 0.05

* Units are expressed in μM/min/ml Serum.
The assay was done as described under Materials and Methods.