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L-ARGININE MITIGATES HEAVY METAL INHIBITED NITRIC OXIDE SYNTHASE ACTIVITY IN TISSUES OF THE ALBINO RAT

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ABSTRACT
Nitric oxide (NO) is synthesized endogenously by the enzymes NO synthase (NOS) and NO is well known to act as physiologic messenger in the body. Heavy metals induce number of toxic effects in animals including man. Keeping in view the important biologic roles played by NO and toxic effects exerted by heavy metals, the *in vitro* effects of selected heavy metals like mercury (Hg), lead and the amino acid L-arginine separately and in combination was studied on the rat tissue NOS activity. The results indicated, Hg^{2+} and Pb^{2+} in concentrations of 10-1000 μM inhibited rat major tissues cNOS and iNOS activities and that L-arginine at selected concentration of 10-20 μM appeared to reverse the IC_{50} conc. Of heavy metal inhibited rat tissue NOS activity levels *in vitro*. It is reported that L-arginine as it acts as a substrate to NO production, its supplementation may improve the NOS disorders in experimental animals.

INTRODUCTION

Nitric oxide (NO) is made by nitric oxide synthase (NOS) in an unusual reaction that converts arginine and oxygen into citrulline and NO (White and Marletta 1992). All NO synthase isoforms are homologous and are broadly divided into two categories with different regulation and activities (Sessa *et al.,* 1992). The Constitutive isoforms in neuronal or endothelial cells are always present and these are calcium/calmodulin (Ca^{2+}/CaM) - dependent ones and the inducible NOS is Ca^{2+}/CaM- independent (iNOS) (Cho *et al.,* 1992). Oral and/or parental administration of L-arginine is known to increase NO synthesis from the endothelial cells which could be useful for therapeutic purpose (Angidin *et al.,* 2001; Carrier *et al.,* 2002). Heavy metals impairing the normal functioning of NO is well established (Wang *et al.,* 2002).

In view of the varied physiologic roles assigned to NO and toxic effects exerted by heavy metals, present study is designed to investigate the *in vitro* effect of selected heavy metals like lead (Pb^{2+}) and mercury (Hg^{2+}) and L-arginine separately and combination on rat brain, heart, lung and kidney based cNOS activity levels.

MATERIALS AND MATHODS

Albino rats of the weight range 125 ± 5grm were selected for the present study. They were maintained at 20 ± 5°C temperature and the humidity being 70%. They were fed *ad libitum* with commercial diet supplied by Sri Kamadhenu Agencies, Bangalore.

All the chemicals used were of either sigma, St. Louis, USA or BDH, India. Stock solutions of either mercury or lead were prepared in sterile water. (1gr/2mL) and stock solutions of L-arginine (1mM) was also prepared in sterile water. Proper dilutions of heavy metals (10-1000 μM) and 10μM of L-arginine were prepared by diluting stock solutions with sterile water.

The NOS activity of 100000xg soluble fractions of rat brain, heart, liver and kidney was measured by the procedure as given by Bredt and Snyder (1990) and as modified by Rao *et al.* (1997).

RESULTS AND DISCUSSION

The two heavy metals Hg^{2+} and Pb^{2+} in tested concentrations of 10-1000μM inhibited rat brain, heart, liver and kidney based cNOS activity levels *in vitro* and all the changes were found to be statistically significant ($p < 0.001$) over the control ones (Table 1 and 2). More percent inhibition was observed for 500 or 1000μM of heavy metals tested in this study.

The mammalian cNOS is well known to be dependent on Ca^{2+}/CaM. Vig *et al.* (1989) showed metals like vanadium, cadmium, mercury, aluminium, lead and magnesium as to interact with calmodulin regulated Ca^{2+}-ATPase. The experiments of Vig *et al.* (1991) demonstrated heavy metals as to impair Ca^{2+}- homeostasis including inactivation of CaM activity of rat synaptosomes. The present observed trend of inhibition of rat tissue based cNOS activity by the two heavy metals tested may be due to interaction of Pb^{2+} and Hg^{2+} with rat tissue based Ca^{2+}/CaM mediated events on which the cNOS is known to be dependent. Similar reasons were also reported from our laboratory for toxicants like insecticides (Rao *et al.,* 1997) and for heavy metals (Neelakantam, 2007).

Table 1: ffect of methyl mercury on the cNOS activity (P mol citrulline/mg protein/min) levels in Various tissues of the rat *in vitro*

Name of the Tissue	Control	Concentration of heavy metals tested 10μ mol	100 μ mol	500 μ mol	1000μ mol	IC_{50}	Control + IC_{50} dose of Hg^{2+}	Control + IC_{50} Conc of Hg^{+} + 10 μ mol L-arginine	Control + IC_{50} Con. of Hg^{2+} + 20 μ mol L-arginine
Brain									
AV	120.08	115.62*	110.79*	72.42*	60.56*	135.9	90.16*	98.82*	110.52*
SD	±3.16	±3.16	±1.22	±0.89	±0.72		±1.25	±3.67	±0.92
PC +		-3.714	-7.74	-39.69	-49.57		-24.91	9.6	23.02
Heart									
AV	80.14	72.22*	70.15*	61.91*	40.07*	51.02	75.05*	62.36*	78.94*
SD	±0.14	±1.49	±2.16	±0.54	±0.42		±3.67	±	±1.23
PC +		-9.882	-12.46	-22.74	-50		-6.76	8.14	22.92
Liver									
AV	85.62	62.05*	57.65*	50.21*	41.52*	18.18	54.19*	62.00*	68.01*
SD	±1.25	±2.26	±1.65	±0.89	±0.58		±2.16	±0.62	±1.45
PC +		-27.52	-32.66	-41.35	-51.5		-31.64	14.41	25.48
Kidney									
AV	43.52	38.68*	33.19*	32.36*	30.05*	45.04	24.24*	32.11*	36.60*
SD	±0.49	±1.22	±0.95	±0.88	±0.62		±1.36	±0.51	±0.74
PC +		-11.12	-23.73	-25.64	-39.5		-32.46	35.08	50.99

Each Value is the mean ± SD of 5 samples assayed in duplicate; AV = Average, SD = Standard Deviation, PC = Percent change over control/IC_{50} dose Hg^{+} in habited ones.

Table 2: Effect of lead on the cNOS activity (P mol citrullin/mg protein/min) levels in the major tissues of rat *in vitro*

Name of the Tissue	Control	Concentration of heavy metals tested 10μ mol	100 μ mol	500 μ mol	1000μ mol	IC_{50}	Control + IC_{50} dose of Pb^{2+}	Control + IC_{50} Con. of Pb^{+} + 10 μ mol L-arginine	Control + IC_{50} Con. of Pb^{2+} + 20 μ mol L-arginine
Brain									
AV	118.05	110.21	102.46*	68.29*	79.14*	54.08	76.35*	85.42*	91.85*
SD	±0.16	±0.012	±0.096	±3.16	±1.94		±1.05	±3.98	±1.22
PC +		6.64 NS	-13.2	-42.15	-60.29		-35.32	11.87	20.3
Heart									
AV	82.08	78.62	72.70*	43.55*	40.68*	480.36	75.05*	71.67*	74.78*
SD	±0.80	±0.52	±0.98	±2.11	±0.63		±3.67	±1.91	±0.96
PC +		-4.125 NS	-11.42	-46.94	-50.43		-6.76	5.14	9.71
Liver									
AV	79.14	73.04	71.0*	40.99*	33.16*	520.36	54.19*	52.47*	60.28*
SD	±1.02	±0.23	±3.61	±2.43	±0.75		±2.16	±1.07	±2.40
PC +		-7.707 NS	-10.28	-48.2	-58.09		-40.73	37.35	57.82
Kidney									
AV	45.05	45.22	32.11*	28.06*	20.62*	520.39	24.24*	29.11*	37.33*
SD	±0.98	±0.89	±0.22	±1.21	±0.36		±1.36	±0.41	±1.36
PC +		-0.377 NS	-28.72	-37.71	-54.22		-32.46	17.52	50.7

Each value is the mean ± SD of 5 samples assayed in duplicate; AV = Average, SD = Standard Deviation, PC = Percent change over control/IC_{50} dose Pb^{+} or HG^{+} inhibited ones.
*$p < 0.01$

IC_{50} value is indicative of 50% inhibition of any metabolite/ enzyme. Based on the IC_{50} value the potency of any agent to inhibit any enzymatic activity can be determined. In the current study based on the calculated IC_{50} values (Table 1) Hg^{2+} appeared to be more potent than Pb^{2+} and the observed trends are in agreement with reports of the early authors for heavy metals (Vig *et al.,* 1997: Neelakantam, 2007).

Further the authors of the present investigation tested the *in vitro* effect of 10μMor 20μM concentrations of L-arginine and checked whether L-arginine can normalize the IC_{50} dose, Pb^{2+} or Hg^{2+} inhibited rat tissues cNOS activity. As expected, 10 or 20μM concentration of L-arginine appeared to normalize IC_{50} dose heavy metal inhibited rat tissues cNOS activity (percent changes are presented in Table 1 and 2). To some extent these results are in agreement with report of earlier authors where oral and/or parental administration of L-arginine will

increase NO induced pathological conditions of endothelial cells and this could be useful for therapeutic purpose (Angidin *et al.,* 2001 and Carrier *et al.,* 2002). Similar reasons might be responsible for the observed trend of reversal of Pb^{2+}/Hg^{2+} inhibited cNOS activity levels of rat major tissues in the present study.

Based on the overall study, it is reported that L-arginine supplementation inpart may reduce the toxic effects of heavy metals with reference to NO pathway in experimental animals.

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