Int J Pharm Bio Sci 2014 Jan; 5(1): (B) 856 - 859

Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

IMPACT OF ALCOHOL ON RAT HEART AND ENDOTHELIAL CELLULAR NITRIC OXIDE SYNTHASE ACTIVITY *IN VITRO AND IN VIVO*

DR. M. YUGANDHAR* AND M. RAJESWARA RAO

Department of Zoology, S.V. University, Tirupati – 517502, A.P.

ABSTRACT

Nitric oxide (NO) has been implicated in the regulation of heart contractility, platelet aggregation in the cardio vascular system of mammals. Multiple authors reported alcohol as to interact with cardio vascular NO pathway parameters. The impact of 20% ethanol (w/v) at selected doses of 5gm/per kg over 5 or 10 weeks *in vivo* and 100-1000 µ litre *in vitro* on the heart and endothelial (ET) cellular nitric oxide synthase (NOS) activity was reported. Alcohol at the employed doses in both *in vitro and in vivo* appeared to inhibit the rat heart and ET cellular 100000 x g soluble fractions NOS activity and this was reported as due to interaction of alcohol with rat heart and ET cellular based calcium / Calmodulin – dependent events on which NOS activity is known to be dependent.

KEYWORDS: **Nitric Oxide Synthase (NOS), Endothelial cells, Heart, Alcohol.**

DR. M. YUGANDHAR
Department of Zoology, S.V. University, Tirupati – 517502, A.P.

INTRODUCTION

Several mammalian tissues and cells were known to express NOS activity (Stuehr and Griffith, 1992). The tissue distribution of cNOS has been characterized in rat brain and other tissues including heart (Bredt *et al;* 1990). Besides, cells like endothelial (ET), macrophages (MAC), lymphocytes, myocytes and hepatocytes have also been reported as to exhibit NOS activity (Rao *et al;* 1998). Early studies with brain, ET cells and macrophages showed that NOS was localized exclusively in the soluble fraction (Marletta *et al;* 1988; Palmer and Moncada, 1989; Schmidt *et al;* 1989; Stuehr *et al;* 1989; Bredt and Snyder, 1990). Alcohol interfering with animal models NO functioning has been reported by very many authors [Uzbay et al., 1997, Spangel et al., (2002); Epstein (1997); Uzen et al., (2005)]. These authors demonstrated alcohol consumption as to affect various organs of animals including heart. Since, NO is involved in the heart contractility and platelet aggregation and as the main enzyme that mediates the conversion of L- arginine to citrulline and NO, the author of the present study made an attempt to investigate the *in vitro and in vivo* effect of alcohol primarily on the iNOS and cNOS activity levels of rat cardio vascular system..

MATERIALS AND METHODS

Albino rats of the weight range 125 $\pm$ 5 gm were selected for the current study. Animals were maintained at a constant temperature of 15 $\pm$ 5^0c. The rats were divided into three groups of seven each and were maintained in separate cages. They were fed *ad libitum* with commercial rat feed supplied by Kamadhenu Agencies, Bangalore, India.

TREATMENT OF ANIMALS

For *in vitro* study 20% of ethanol in the concentration range of 100 – 1000 µl was selected. Group one rats acted as untreated control ones. For *in vivo* studies group two and three rats were gavaged with 20mg/kg wt of 20% ethanol over 5 or 10 weeks period (weekly doses). After treatment of animals, they were anesthetized with chloroform and were dissected. The tissues like heart and arterioles from the control and experimental animals were quickly removed, blotted on a filter paper and were subjected for the study of constitutes nitric oxide synthase (cNOS) and inducible nitric oxide synthase activity following the procedure as given by Bredt and Snyder (1990) and Knowles et al; (1990). The separation of endothelial (ET) cells from the arterioles the procedure followed was that of Rao et al; (1997).

STATISTICAL ANALYSIS OF THE DATA

For each parameter, the mean of individual observations (for both control and experimental groups were taken into consideration). Statistical significance of the data was analysed through two way ANOVA (analysis of Variance); SNK (Student – Newman - Keuls) test and regression analysis.

RESULTS AND DISCUSSION

The present study investigated the impact of alcohol on the cardiovascular rat iNOS and cNOS activities *in vitro* and *in vivo*. The results presented in Table: 1 and 2 shows that alcohol (20%) in the concentration range of 100 – 1000 µl *in vitro* and 5 gr / kg wt over 5 or 10 weeks *in vivo* inhibited rat 100000 x g heart and E T cellular soluble fractions based cNOS and iNOS activities and the change were found to be statistically significant ($P<0.01$) Basically the cNOS activity in mammals is calcium / calmodulin (Ca^{2+}/CaM) – dependent one and the other one the iNOS activity is Ca^{2+}/CaM-independent one (Bredt and Synder, 1990) It is now established that number of agents inhibiting either the cNOS or the iNOS activity in experimental animals interact with Ca^{2+}/CaM – Mediated events and thus inhibit the NOS activity, the same reason has been cited by many authors as responsible for the inhibition of rat/mice cNOS, iNOS activities by alcohol (Itshak *et al.,* 1998; Itshak and Martin, 2000; Spanagel *et al.,* 2002; Uzun *et al.,* 2005). From our laboratory, we reported the same reasons for drugs like cyclosporin A, organic insecticides, heavy metals, CCl_4,etc, as to inhibit NOS activity in rat tissues by way of

interacting with rat tissue based Ca^{2+}/ CaM-mediated events (Rao and Bhaskar, 2000; Sudhakar Reddy *et al.,* 2005; Hulcheson *et al.,* 1995; Rao *et al.,* 1997; Neelakantam, 2008). In the presents study identical reasons might be responsible for inhibition of cNOS and iNOS activity by alcohol both *in vitro* and *in vivo.*

Table 1

Impact of alcohol on rat heart and endothelial cellular cNOS and iNOS activities in vitro (Values expressed as counts / minutes).

	cNOS activity				**iNOS activity**			
	Concentration of alcohol				**Concentration of alcohol**			
Name of the tissue / cellular preparation	Control	Control + 100 µl of 20% alcohol	Control + 500 µl of 20% alcohol	Control + 1000 µl of 20% alcohol	Control	Control + 100 µl of 20% alcohol	Control + 500 µl of 20% alcohol	Control + 1000 µl of 20% alcohol
Heart soluble factions								
A V	415.00	346.57	325.86	232.27	470.429	294.429	294.429	226.286
S D	+5.85	±4.20	±3.49	±1.79	2.78	1.90	1.38	0.65
P C		-16.49	-21.48	-44.63		-5.19	-37.41	-51.89
t-test		*	*	*		*	*	*
Endothelial cellular soluble fractions								
A V	354.000	334.571	213.14	155.00	442.857	375.42	220.57	193.143
S D	3.76	1.38	0.64	0.24	2.92	1.45	0.81	0.35
P C		-5.448	-39.790	-56.215		-15.22	-56.38	-50.19
t-test		*	*	*		*	*	*

AV : average
SD : Standard deviation
PC : Percent Change over control
NS : Not Significant
**** p< 0.001***

Table 2

Effect of alcohol on rat heart and endothelial cellular soluble fractions of cNOS and iNOS activities in vivo (Values expressed as counts / minutes).

	cNOS activity			**iNOS activity**		
	Concentration of alcohol			**Concentration of alcohol**		
Name of the tissue / cellular preparation	Control	Alcohol treated ones		Control	Alcohol Treated ones	
		5 weeks	10 weeks		5 weeks	10 weeks
Heart soluble factions						
A V	348.71	322.56	219.86	482.56	405.00	214.56
S D	±2.36	±1.49	±3.61	±4.71	±2.39	±1.27
P C		-7.49	-36.94		-16.07	-55.63
t-test		*	*		*	*
Endothelial cellular soluble fractions						
A V	285.86	233.14	144.29	464.14	373.14	216.86
S D	±2.19	±0.74	±2.14	±3.14	±1.91	±0.75
P C		-18.48	-45.52		-19.61	-53.28
t-test		*	*		*	*

Each value is the mean ± SD of 7 samples
AV : Average
SD; Standard variation
PC: Percent change over the control
**** P < :0.01***

REFERENCES

1. Bhaskar, LVKS and Rao MR. Inhibition of rat brain nitric oxide synthase activity by phosaline in vitro, pure. A;ll.Sci. 19 (A) (no.2): 117-119, (2000).
2. Bredt, DS. and Snyder, SH. Isolation of nitric oxide synthase, a calmodulin-requiring enzyme. Proc. Natl. Acad.Sci.USA. 57: 682-687,(1990).
3. Harish Babu K: Reversal of CCl_4 induced toxicity in rat liver by alpha tocopherol. Ph.D. Dissertation, S.V. University, Tirupati, (2011).
4. Hutcheson, A., Rao M.R., Olinda, K. and Markov, A.K.. Myocardial toxicity of cyclosporine – A inhibition of calcium ATPase and nitric oxide synthase activities and their reversal by fructose 1,6 diphosphate in vitro. Res. Common.Pathil. Pharmacol. 89: 17-26, (1995).
5. Itshak, Y. and Martic, J.L. Blockage of alcohol-induced locomotor sensitization and conditioned place preference in DBA mice by 7-nitromindazole. Brain Res. 858:402-407,(2000).
6. Itshak, Y. Ali, S.F., Martin, J.L., Black, M.D and Huang, P.L. Resistance of neuronal nitric oxide synthase – deficient mice to Cocaine – induced locomotors sensitization. Psychopharmacology. 154 : 269-270,(1998).
7. Knowles, R.G., Marret, M., Saths, M. and Moncador, S. Differential induction of brain, lung and liver nitric oxide synthase by endotoxin in rat. Biochem.j. 270: 833-836,(1990).
8. Marletta, M.A., Yoo, P.S., Lyengar, R., Leaft, C.D. and Wishnok, J.S. Macrophage oxidation of L-arginine to nitrite and nitrate: Nitric Oxide is an intermediate. Biochemistry. 24: 8706-8711, (1988).
9. Neelekantam, C. Impact of selected heavy metals on the nitric oxide pathway in rats. Ph.D. thesis, S.V. University, Tiruapti, (2007).
10. Palmer, M.J. and Moncada, S.A. A Novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. Biochem. Res. Commun. 158: 348-352, (1989).
11. Rao, M.R., Kanji, K.V., Dass, P.J., Vijaya, P. and Bharathi, P.V. Fructose – 1,6-diphosphate mitigates CCl_4, suppressed nitric oxide synthase activity in rats. Ind. J. Expt. Bio. 39: 68-69, (2001).
12. Rao. M.R., Kurt, D., Glinde and Markov, A.K. Protection from Amphotericin-B-induced lipid peroxidation in rats by fructose -1, 6-diphosphate. Res. Common. Mo. Pathol. Pharmacol, 95:217-220, (1997).
13. Rao. M.R., Olinde, K.D. and Markov, A.K.. In vitro induction of nitric oxide by fructose -1,6 – diphosphate in the cardiovascular system of rats,. Mol.Cell. Biochem. 185: 171-175,(1998).
14. Schmidt, H.H.H.W., Wilke, P., Evers, B. and Bohme, E. Enzymatic formation of nitrogen oxides by vascular endothelial cells. Biochem. BIophys. Res.Commun. 165: 284-291,(1989).
15. Spanagel R, Siegmund S, Cowen M, et al., The neuronal nitric oxide synthase gene is critically involved in neurobehavioral effects of alcohol. J Neurosci; 22: 8676-83,(2002).
16. Stuehr, D.J., Kwon, N.S., Gross, S.S., Thiel, B.A., Levi, R. and Nathan, C.F. Synthesis of nitrogen oxides from L-arginine by macrophage cytosol: Requirement for inducible and constitutive components. Biochem. Biophys.REs. Commun. 161: 420-426, (1989).
17. Stuehr, J.D. and Griffith, W.O. Mammalian Nitric Oxide synthases. Advances in Enzymology. 287-346, (1992).
18. Tayfun Uzbay, B. Faruk Erden, E.Erbu Tepangist and S. Oguz kalyalo, Nitric oxide synthase inhibition attennates signs of ethanol withdrawl in rats vol.61. p 2197-2209,(1997).
19. Uzun H. Zengin K. Taskin M. Aydin S. Simsek G. Dariyerli N. Changes in leptin. plasminogen activator factor and oxidative stress in morbidly obese patients following open and laparoscopic Swedish adjustable gastric banding. Obes Surg.; 14: 659-665 ,(2004).