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Nitric oxide production - impairment and disruption in learning behaviour and memory of rats exposed to aflatoxin B_1

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Effect of aflatoxin B_1 on avoidance learning and memory in rats was studied by observing their prformance in Y-maze. It was found that the rats treatment with aflatoxin B_1 at 760 mg/kg body weight experienced a significant deficit in the avoidance learning and memory and also showed an inhibition in nitric oxide synthesis. However, the untreated rats showed their ability to learn the avoidance response and also exhibited the uninhibited production of nitric oxide. These indicate that the impairment in avoidance learning and memory in rats is related to the suppression of neuronal nitric oxide production brought about by aflatoxin B_1.

Key words : Nitric oxide, learning behavoour, memory, aflatoxin B_1, rat

INTRODUCTION

It is well established that aflatoxin B_1 has a role in neuro-degenerative disorders. In our laboratory, we have observed an intriguing fact that the experimental rats when fed on AFB_1 - contaminated feed developed apathy for explorative behaviour and also failed to reach to the correct food source (Chatterjee and Mukherjeee, 1993). However, no work has hitherto been done to identify the role (if any) of AFB_1 in learned behaviour. Although, evidence is accumulating that nitric oxide (NO), which serves as a neurotransmitter in the peripheral and central nervous system, participates in longterm potentiation (LTP) in the hippocampus, which is a form of persistnt increase in synaptic plasticity and strength implicated in learning and memory (Emily, 1997). Activation of N-methy 1-D-aspartate receptor by glutamate and influx of Ca^{2+} is required to induce LTP and Ca^{2+} influx activates neuronal nitric oxide synthase (nNOS) in brain. Further more, NO causes a rise in the frequency of spontaneous miniature excitatory post-synaptic current in cultivated hippocampal neurons (Schuman and Madison, 1991; Son *et al,* 1996). Although, the role of NO in certain form of learning and memory has been reported, its role in avoidance learning has not yet been studied.

Therefore, to understand the effect of AFB_1, we examined the performance in a learning task together with neuronal NO synthesis in AFB_1 - fed rats. This paper reports a link between AFB_1 - induced inhibition in neuronal NO synthesis and a disruption in avoidance learning and memory.

MATERIALS AND METHODS

Aflatoxin B_1 treatment

Aflatoxin B_1, at the dose of 75, 355 and 760 µg/kg body wt., dissolved in 0.3 ml corn oil was given orally to the male rats (Norvegicus strain) on alternate days for a period of 28 days. Control rats were treated with the corn oil only.

Learning task

A sensitive learning task that represent a well-characterized complex form of learning was performed. Untreated and AFB_1-treated rats of the same age group and sex were examined separately for their performance in a Y-maze apparatus which consists of three trough-shaped arms at 45° angles to each other, and each arm is equipped with a sliding door. The normal exploratory behaviour of rat was observed for 10 minutes during the initial day for testing and the number of entries in the arm by each rat were recorded. The next day, on a trial, the rats are freely allowed to enter either arm of the maze and the choice was thought to be as preferred arm and was termed as incorrect on further trials. Five trials were made per day and the trials had a intertrial interval of one minute. Altogether seven days were given for training. At the beginning of each trial the door was kept open and the rat was allowed 20 seconds to enter the correct arm of the maze. But, if the rat failed to have the correct choice (or if moved into the incorrect arm), all parts of the maze excepting the correct arm was given a footshock of 0.4 mA as long as they could not enter the correct arm. Each rat was kept confined to the correct arm for 15 seconds after they entered it. Performance of entering the correct arm before footshock was scored as "correct avoidance". However, moving into the correct arm after of otshock is given was scored as "escape". But, entering the wrong arm was scored as "error". Shock sensitivity in rats was tested for controlling any alteration in somatosensory reactivity to shock.

Nitric oxide synthesis

Brain tissue were homogenized in ice-cold buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA containing phenyl methyl sulfonyl fluoride (100 µg/ml) and centrifuged at 100,000 x g for 1 h. Then the supernatant, in triplicate, was removed for the assay of NO_2 (an indicator of NO production) by the Griess reactin following Stauehr and Nathan (1989). Culture samples (50 ml) were combined in plate with a 1/1 mixture of 1% sulfanilamide in 2.5% H_3PO_4. Plates were incubated at room temperature. for 10 min and absorbance was determined at 550 nm. NO_2 concentration was measured in triplicate using a standard curve of sodium nitrite from 125 to 1 µM prepared in culture medium.

Statistical Analysis

Statistical significance was determined by Analysis of Variance (ANOVA) and Duncan multiple range test.

RESULTS AND DISCUSSION

This avoidance task measures the ability to initiate a response to avoid a negative outcome.

The results suggest a relationship between learned behaviour, nitric oxide synthesis and AFB_1 exposure. The results suggest that AFB_1 impairs the avoidance learning and memory. The untreated rats showed their ability to learn the avoidance response (Table 1). However, the rats treated with AFB_1 experienced a considerable deficit in the avoidance learning. This AFB_1- induced decrease in learning ability was found to be prominent at the level of 760 µg/kg body wt. (Table 1). Such deficit in avoidance learning accounts for an alteration in the associative factors required for learning this task and was not the consequence of inhibition of somatosensory functions as was revealed by the shock sensitivity threshold which remained the same both in the AFB_1 treated and the untreated rats. The untreated rats which were able to learn the avoidance task exhibited inhibited synthesis of neuronal nitric oxide (Table 2). However, nitric oxide production was inhibited in the AFB_1 - treated rats that showed disrupted learning ability (Table 2). The results indicate that AFB_1, possibly through its neuro-degenerative action, leads to the suppression of neuronal NO synthesis in hippocampus with the resultant impairment in avoidance learning and momory.

Table 1 : Number of entries (out of total 35 trials) in the arms by 10 rats before and after AFB_1 treatment

Rats	AFB_1	Entries Correct	Error	Escape
1	-	30	2	3
	+	4	26	5
2	-	34	0	1
	+	2	28	0
3	-	31	0	4
	+	5	25	5
4	-	35	0	0
	+	3	31	1
5	-	28	0	7
	+	0	33	2
6	-	32	0	3
	+	6	29	0
7	-	30	1	4
	+	5	27	3
8	-	26	4	5
	+	0	35	0
9	-	34	1	0
	+	6	27	2
10	-	29	0	6
	+	4	28	3

Table 2 : Effect of aflatoxin treatment on nitric oxide productin in rats

Number of studies	AFB_1 concentration (µg/kg body wt.)	NO production (µM)
1	0	26
2	0	30
3.	0	27
1	75	28
2	75	20
3	75	25
1	355	10
2	355	18
3	355	13
1	760	6
2	760	4
3	760	0

REFERENCES

Chatterjee, D. and Mukherjeee, S. K. (1993). Destruction of phagocytosis - suppressing activity of aflatoxin B_1 by ozone. *Letters in Applied Microbiology* **17** : 52-54.

Emily, P. H. (1997). Synaptic Plasticity - A role for NO in LTP. *Current Advaces in Biology* R141 - R143.

Stauehr, D. and Nathan, C.F. (1989). Nitric Oxide, a macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. *Journal of Experimental Medicine* **167** : 1534-1546.

Schuman, E. M., and Madison, D.V. (1991). A requirement for the intracellular messenger Nitic Oxide in long term potentiation. *Science,* **254 :** 1503-1506.

Son, H., Hawkins, R.D., Martin, K., Kiebler, M., Huang, P.L., Fishman, M.C., and Kandel, E.R. (1996). Long-term potentiation is reduced in mice that are doubly mutant in endothelial and neuronal Nitric oxide synthase. *Cell,* **87** : 1018-1023.

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