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Modulatory Effects of L-Tyrosine on Neurobehavioural Consequences of Combat Stress in Rats

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ABSTRACT

The paper presents the results of a study conducted to elucidate the potentiality of tyrosine, a precursor of catecholaminergic neurotransmitters, against combat stress-associated behavioural changes and brain catecholamine status in an animal model. The results obtained showed that stress impaired the performance on Morris water maze (MWM) in saline-injected rats and concurrently lowered norepinephrine (NE) levels in brain. This could be due to decreased dopamine β-hydroxylase (DBH) activity and increased monoamine oxidase (MAO) activity. On the contrary, there was a significant improvement in post-stress performance in MWM test in animals receiving tyrosine. Stress-induced increase in catecholamine metabolites, homovanillic acid and vanillylmandelic acid in brain was prevented by treatment with tyrosine. The present findings imply that precursor availability could be a limiting factor to sustain intensified catecholamine synthesis during stress and hence supplementation of L-tyrosine could be beneficial to improve performance during stress.

NOMENCLATURE

CA	Catecholamine
DBH	Dopamine β hydroxylase
DA	Dopamine
HVA	Homovanillic acid
5-HT	5-Hydroxytryptamine/Serotonin
5-HIAA	5-Hydroxyindoleacetic acid
MAO	Monoamine oxidase
MWM	Morris water maze
NE	Norepinephrine
N	Normal
S	Stress
TY	Tyrosine
VMA	Vanillylmandelic acid

1. INTRODUCTION

Soldiers facing the vagaries of war at certain times succumb to extremes of environmental, physiological and emotional stresses. This may lead to decline in the performance of some of them and create behavioural and psychological problems. Therefore, an effective psychological and pharmacological therapy is highly desirable to keep the soldiers bodily and mentally fighting fit. The present investigation was aimed at identifying neurochemical markers of the stress response to the so-called combat stress in an animal model and provide the biochemical assertion that tyrosine administration could be useful as a non-pharmacological countermeasure against stress.

2. MATERIALS & METHODS

Male Wister young adult rats weighing approx. 180 g each (50-day old) housed in polypropylene cages (6 rats/cage) were used. They were fed pelleted diet and water *ad-libitum*. After a

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habituation period of one week, the rats (n = 24) were trained on Morris water maze (MWM), three trials at a time, twice a day for three consecutive days and then randomly divided into two groups. One group was administered L-tyrosine (25 mg/kg bw, ip) and another, normal saline daily for six consecutive days. Tyrosine-treated (TY) and saline-treated (N) rats were redivided into two groups of six rats each. One group from both TY and N was subjected to combat stress comprising prerecorded gun noise (90-103 dBA), vibration (40 CPS on an angular platform at the angle of 15 °) light flash (6 sessions of 5 bursts of 10,000 lux, each lasting 5 s) electric shock (3 sessions of 5 min each, 5 Hz 0.24 amp.) and immobilisation 30 min daily, for 6 consecutive days and christened as TY+S and N+S group, respectively. Acquisition scores, i.e., duration of time to reach the hidden platform were recorded daily prior to and after exposure to combat stress. All the stressed rats were sacrificed immediately after 30 min of stress on 7th day of stress exposure along with their respective controls and their brains were immediately removed, frozen in liquid nitrogen and stored at -65 °C for subsequent estimation of catecholamines, their metabolites and metabolic enzymes.

Brain neurotransmitters, NE, DA and 5-HT, and metabolites, HVA and HIAA were extracted and measured spectrofluorometrically, as described by Haubrich and Denzer[1]. VMA was assayed by spectrophotometric method of Pisano, *et al*[2]. DBH activity in brain homogenates prepared in 0.3M sucrose was measured by the method of Nagatsu and Udenfriend[3]. MAO in brain was assayed by a colorimetric method using 1-[(4-amino-methylphenyl) azo]-2-naphthol hydrochloride as substrate and 1-[(4-formylphenyl) azo]-2-naphthol as standard[4]. Data were analysed statistically by one way ANOVA and student's t-test.

3. RESULTS

The effect of tyrosine administration and stress exposure on acquisition response is depicted in Fig.1. An overall ANOVA showed significant differences between groups in the time taken to locate the platform. Exposure to stress increased the latencies of acquisition response in control rats ($p < 05$), but in tyrosine-treated animals subjected to combat stress, there was 20 per cent decrease in mean acquisition scores as compared to the scores prior to stress exposure and 36.6 per cent improvement as compared to post-stress scores of N+S group.

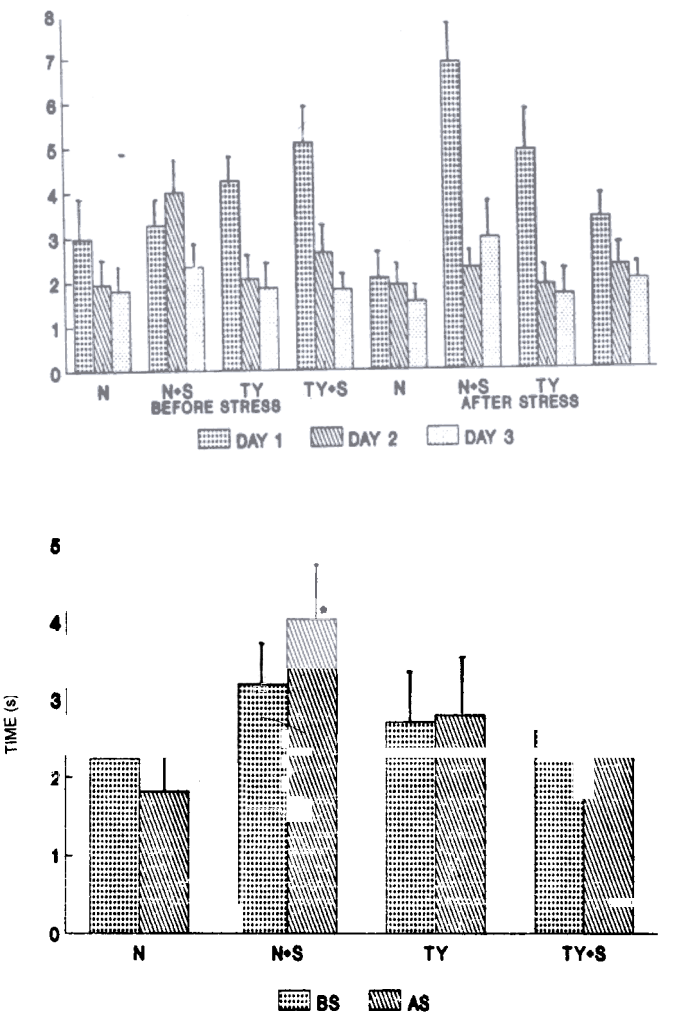

Figure 1. Effect of L-tyrosine on spatial location scores of rats exposed to combat stress. Experimental groups: N-saline injected stressed rats, N+S - saline injected stressed rats TY+S-tyrosine injected stressed rats. BS-before stress, AS-after stress. Significance $p < 0.05$ in comparison to BS.

Exposure to stress significantly decreased brain NE levels in rats which received saline ($p < 05$). Tyrosine pretreatment caused no change in NE levels in unstressed animals, but when

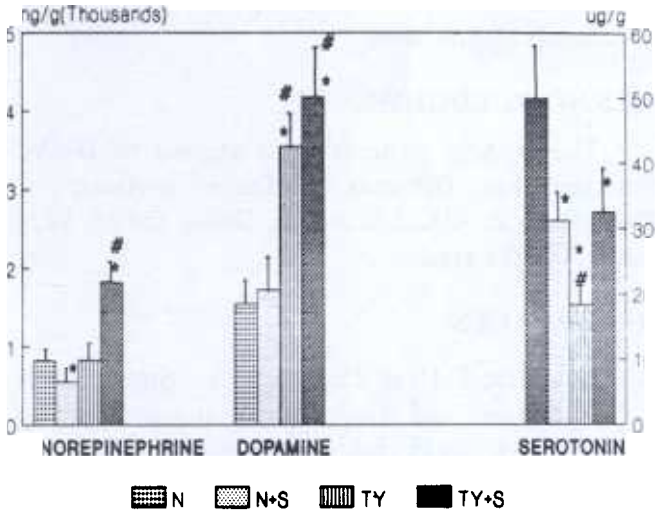

Figure 2. Effect of tyrosine on brain catecholamine levels in stressed and nonstressed rats. Experimental groups as in Fig. 1. Significance $p < 0.05$ as compared to N*, N+S #.

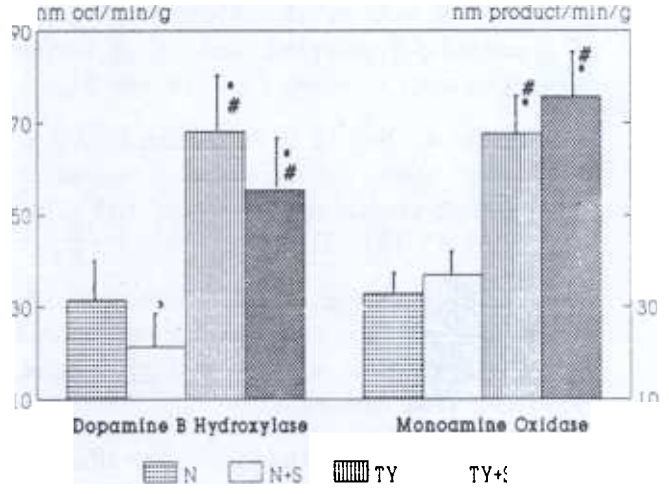

Figure 3. Effect of tyrosine on combat stress brain catecholamine metabolism. Symbols as in Figs 1 and 2.

combined with stress, exogenous tyrosine elevated NE levels significantly relative to all the groups. Rise in DA levels in response to stress failed to reach statistical significance in saline-treated rats, but were significantly higher in both stressed and non-stressed tyrosine administered rats (Fig. 2).

The changes in brain DBH and MAO activity in stressed and non-stressed rats treated with tyrosine are depicted in Fig. 3. Decrease in DBH activity and increase in MAO activity were

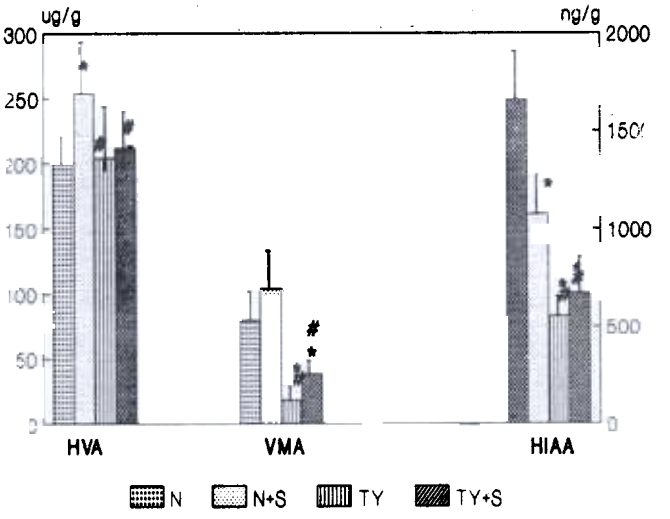

Figure 4. Effect of tyrosine on brain catecholamine metabolites in stressed and nonstressed rats. Experimental groups and symbols as in Figs 1 and 2.

observed in control rats exposed to combat stress, but tyrosine elevated the levels of both the enzymes. Levels of 5-HT were lower than in control rats in all the treatment groups and so were the levels of its metabolite, HIAA (Fig. 4). Stress exposure increased HVA levels and VMA levels in control rats. Tyrosine pretreatment prevented rise in HVA in both stressed and unstressed rats and lowered VMA levels significantly.

4. DISCUSSION

The results of this study indicate that acute intense stress impaired the performance of rats on a spatial location task and concurrently led to lower NE levels in brain. On the other hand, exogenous tyrosine restored NE levels and simultaneously improved the latency scores. This implies that NE might play a role in learning and performance on a task involving motor activity. Increase in DA levels in stressed animals could be due to its slower conversion to NE due to impaired DBH activity, thereby causing substrate accumulation.

Tyrosine being the precursor amino acid for CA, its exogenous supply increased the level of both NE and DA in brains of stressed rats. Increase in both synthesising enzyme DBH and degrading enzyme MAO indicates enhanced turnover of these neurotransmitters. Lack of any effect of tyrosine pretreatment on accumulation of DA metabolite,

HVA in brain could probably be due to rapid reuptake of DA, so that less of it is made available for metabolism.

Data regarding behavioural depression accompanied by NE depletion in whole brain consequent to stress is consistent with those obtained in numerous other studies utilising various other procedures of stress exposure, such as immobilisation[5,6], shock[7], oscillation[8], cold [9,10], hypoxia[11], vibration and noise[12]. In these studies, behavioural depression and/or decrease in NE content of brain was found to be proportional to the intensity of stress, viz., decrease in temperature from 37° to 30°, increase[10] in vibration acceleration from 0.4 to 5.0 g and sound pressure level[12] 60-80 dBA, shock frequency and duration[13] and the ability to escape the aversive stimuli[14], thus indicating that magnitude rather than nature of stress is important for altered CA metabolism. Involvement of serotonergic system (5-HT) in stress is inconsistent[5,7,15]. In this study, a decrease in the level of 5-HT and its metabolite HIAA has been found in response to stress as well as tyrosine treatment.

The observation that in experimental animals exposed to combat stress, behavioural abnormalities and NE depletion can be prevented by its replenishment by exogenous supply of its precursor amino acid L-tyrosine in this and previous studies [10,16,17] raises the possibility of its use in humans exposed to severe stress. Some human studies on this aspect also support the use of TY as a performance enhancing agent[17,18]. However, the key issues regarding dosage, schedule, duration and periodicity of TY administration and its beneficial effect in human subjects must be addressed before its incorporation in food to attenuate the physical as well as psychological stress.

5. CONCLUSION

The findings of the present study imply that increased CA turnover in brain under combat stress could cause a localised limitation of precursors for the synthesis process and thereby lead to CA deficiency and hence decline in performance. Restoration of CA status achieved by supplementation of TY improves the performance of animals during stress.

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