REPORT

5-HT-1A RECEPTOR RESPONSIVENESS FOLLOWING SUBCHRONIC ADMINISTRATION OF BUSPIRONE

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ABSTRACT

Anxiety and its disorders have long been known to be familial. Anxiety levels are associated with low social connectedness and high environmental threats. Studies provide evidence that anxiety disorders may be link to malfunctioning of serotonin neurotransmission. The present study is designed to monitor serotonin-1A (5-HT-1A) receptor responsiveness following subchronic administration of a serotonergic anxiolytic buspirone. Administration of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) at a dose of 0.25 mg/kg produced comparable syndrome in repeated saline and repeated buspirone injected rats. Cage crossings were significantly lower in repeatedly buspirone injected rats. Decreases in 5-hydroxytryptamine (5-HT) and 5- hydroxyindoleacetic acid (5-HIAA) levels were higher in saline than buspirone injected rats. Result suggests that following long term administration of buspirone somatodendritic and postsynaptic 5-HT-1A receptors are desensitized. Role of serotonin 1 A receptors in the treatment of anxiety is discussed.

Keywords: 8-OH-DPAT buspirone 5-HT syndrome, somatodendritic 5-HT1A receptors, postsynaptic 5-HT1A receptors.

INTRODUCTION

Anxiety has its origin in a complex interaction of environmental, psychological, biological events and processes. Polymorphism in the serotonergic system is believed to play a role in the etiology and treatment of different psychiatric disorders. Serotonin (5-hydroxytryptamine; 5-HT) is the important candidate for anxiety and depression. Buspirone is known to be partial agonist at 5-HT1A postsynaptic receptors, but a full agonist at 5-HT1A presynaptic receptors (Hjorth and Carlsson, 1982) and is used to treat anxiety (VanderMaelen *et al.*, 1986, Sprouse and Aghajanian, 1987). The basis of the anxiolytic action of buspirone seems to be attributable to its effects on 5-HT system (Tunicliff, 1991). In clinical studies, the anxiolytic effects of buspirone manifest themselves after a treatment period of more than 2 weeks (Feighner *et al* 1982, Rickels *et al* 1982, Jacobson *et al* 1985) suggesting that the therapeutic effects of buspirone are probably not due to its immediate actions on the 5-HT system but rather to adaptive changes occurring after prolong treatment.

Stimulation of 5-HT1A somatodendritic receptors (autoreceptors) by selective 5-HT1A agonist such as 8-OH-DPAT inhibits 5-HT cell firing (Hutson *et al*, 1990). This implies a net decrease of 5-HT neurotransmission. 8-OH-DPAT induced decrease of 5-HT synthesis give a measure of presynaptic receptor responsiveness (Haleem, 1999). Stimulation of postsynaptic 5-HT1A receptors by 8-OH-DPAT elicits hyperactivity syndrome that is taken as a measure of postsynaptic receptor responsiveness (Haleem, 1992).

Because of the postulated role of 5-HT1A autoreceptors in the onset of clinical effect of anxiolytic treatment. We have investigated the 5-HT1A receptor dependent responses following subchronic administration of the anxiolytic buspirone.

MATERIALS AND METHODS

Animals

Locally bred male albino Wistar rats weighing 200-250gm purchased from The Agha Khan University, Pakistan were housed individually with free access to standard rodent diet and water 3 days before experimentation.

Drugs

Buspirone hydrochloride and 8-OH-DPAT purchased from Research Biochemicals (RBI, USA) were used in this study. Buspirone dissolved in saline (0.9% NaCl) and given i.p. at a dose 0.5mg/kg of body weight. 8-OH-DPAT solution of dose 0.25 mg/kg was prepared in the same manner. In the control animals vehicle (0.9% NaCl) was administered in volumes of 1ml/kg.

Experimental protocol

24 rats were randomly assigned to control & test group.

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Buspirone (0.5mg/kg/ml) was injected repeatedly to test animals for 2 weeks while control animals received vehicle (1ml/kg). 72 hours after the drug withdrawal the animals were injected with saline or 8-OH-DPAT.

Postsynaptic 5-HT1A receptor dependent responses were monitored as the intensity of 5-HT syndrome elicited by 8-OH-DPAT. Components of the syndrome were scored for 20 minutes starting 5 minutes post injection.

Presynaptic 5-HT1A receptor dependent responses were monitored as the decrease of 5-HT synthesis following the administration of 8-OH-DPAT. The animals were sacrificed 1 hour after the injection of saline or 8-OH-DPAT to collect midbrain and hippocampus as described before (Haleem & Perveen, 1994). The samples were stored at -70 C for the HPLC-EC estimation of 5-HIAA and 5-HT.

8-OH-DPAT elicited 5-HT syndrome

Saline or buspirone injected rats were transferred to square Perspex activity cages (26 x26 x 26 cm) with sawdust-covered floor 15 minutes before injecting saline or 8-OH-DPAT. Forepaw treading, flat body posture & locomotion elicited by 8-OH-DPAT were scored as described before (Haleem & Khan, 2003).

HPLC-EC determination of 5-HIAA and 5-HT

A 5µ shim-pack ODS separation column of 4.5mm internal diameter and 15 cm length was used. The mobile phase was 0.1 M sodium phosphate buffer (pH 2.9) containing 14% HPLC grade methanol, 0.023% OSS, 0.05% EDTA. Electrochemical detection was done at an operating potential of 0.8 V (glassy carbon electrode Vs Ag/AgCl reference electrode).

STATISTICAL ANALYSIS

The results are presented as means ± S.D. Behavioral data was analyzed by two-tailed t-test. Neurochemical data was analyzed by Two-way ANOVA. Post-hoc analysis was done by Newman-Keuls test. p values < 0.05 were taken as significant.

Table 1: 8-OH-DPAT induced forepaw treading, hyper-locomotion and flat body posture in repeatedly saline and repeatedly buspirone injected rats.

Parameters	Repeated Saline + 8-OH-DPAT	Repeated Buspirone + 8-OH-DPAT
Forepaw Treading	26.6±3.50	23.6±1.50
Hyperlocomotion	31.6±2.58	28.16±1.60*
Flat Body Posture	2.7±0.9	3.4±0.4

Values are means ± S.D (n=6). *p<0.05 from respective saline injected rats.

RESULTS

Figure 1 shows the intensity of 5-HT syndrome elicited by 0.25mg/kg 8-OH-DPAT in rats preinjected with saline or buspirone (0.5mg/kg). Mean values of forepaw treading but not flatbody posture were smaller in repeated buspirone than saline preinjected rats. Differences by t-test were not significant (p>0.05). The locomotive effects of the drug monitored as the number of cage crossings were smaller (p<0.05) in repeated buspirone than repeated saline injected rats.

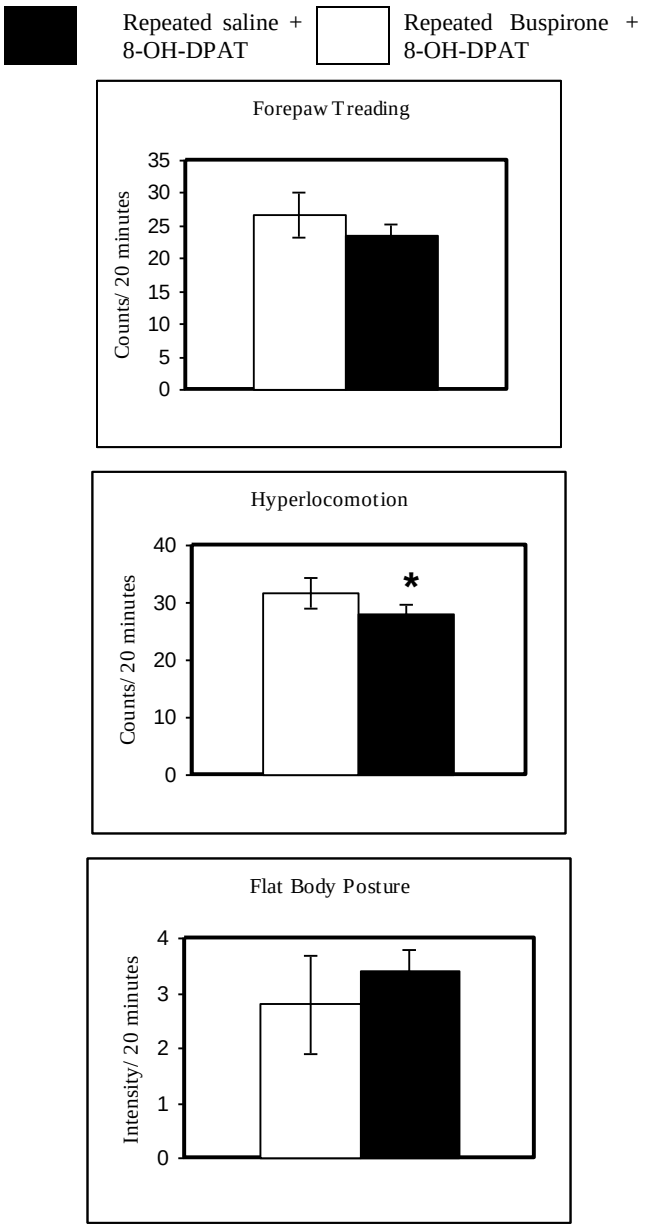

Fig. 1: 8-OH-DPAT induced forepaw treading, hyperlo-comotion and flatbody posture in repeatedly saline and repeatedly buspirone injected rats. Values are means ± S.D. (n=6). *p<0.05 from respective saline injected rats.

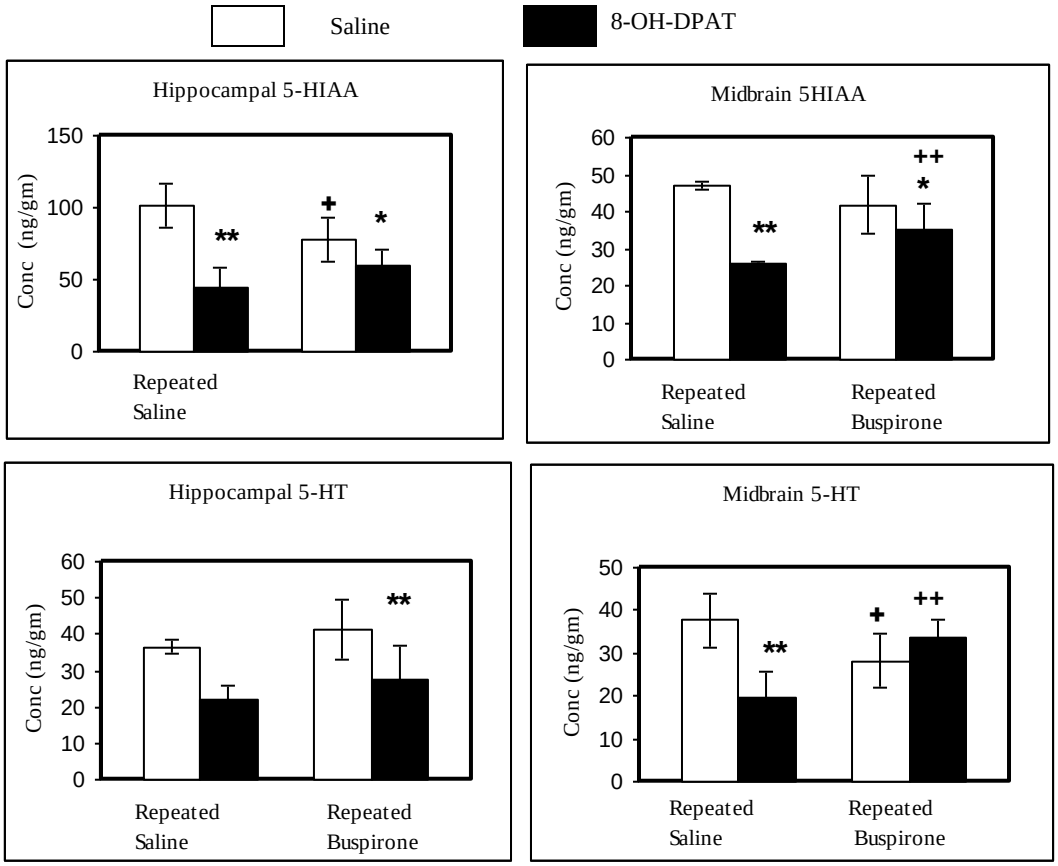

Fig. 2: Effects of 8-OH-DPAT on 5-HIAA and 5-HT levels in hippocampus and midbrain of repeatedly saline and repeatedly buspirone injected rats. Values are means + S.D.(n=6). *p<0.05, **p<0.01 in 8-OH-DPAT injected rats. +p<0.05, ++p<0.01 in repeated buspirone injected rats from respective repeated saline injected rats following Two-Way ANOVA.

Table 2: Effects of 8-OH-DPAT on 5-HIAA and 5-HT levels in hippocampus and midbrain of repeatedly saline and repeatedly buspirone injected rats.

Concentration (ng/gm)	Repeated Saline (1 ml/kg)		Repeated Buspirone (0.5 mg/kg)	
	Saline	8-OH-DPAT	Saline	8-OH-DPAT
Hippocampal 5-HIAA	101.25 ± 15.25	43.9 ± 14.49**	77.60 ± 15.77 +	59.40 ± 11.29*
Hippocampal 5- HT	36.57 ± 2.17	22.21± 3.74**	41.23 ± 8.21	27.78 ± 9.05**
Midbrain 5-HIAA	46.92 ± 1.17	26.08 ± 0.64**	41.81 ± 7.98	35.16 ± 7.22* ++
Midbrain 5-HT	37.62 ± 6.10	19.5 ± 6.10**	28.17 ± 6.24+	33.87± 2.58** ++

Values are means ± S.D. (n=6). *p<0.05, **p<0.01 in 8-OH-DPAT injected rats. +p<0.05, ++p<0.01 in repeated buspirone injected rats from respective repeated saline injected rats following Two-Way ANOVA.

Figure 2 shows the effects of 8-OH-DPAT on the levels of 5-HIAA and 5-HT in the hippocampus and midbrain of repeatedly saline and repeatedly buspirone injected rats. Two way ANOVA (repeated measure design) showed insignificant effects of buspirone injections on hippocampal 5-HIAA (F=0.49 df 1,20 p>0.01), 5-HT (F=3.73 df 1,20 p>0.01), Midbrain 5-HIAA (F=0.804 df 1,20 p>0.01) & 5-HT (F=1.26 df 1,20 p>0.01). Effects of 8-OH-DPAT injections were significant for hippocampal 5-HIAA (F=41.76 df 1,20 p<0.01), 5-HT (F= 27.59 df 1,20 p<0.01), midbrain 5-HIAA (F= 38.50 df 1,20 p<0.01) and 5-HT (F=7.55 df 1,20 p<0.01). Interaction between two factors were significant for hippocampal 5-HIAA (F=11.19 df 1,20 p<0.01), midbrain 5-HIAA (F=10.27 df 1,20 p<0.01) and

midbrain 5-HT ($F=28.70$ df 1,20 $p<0.01$). Interaction was not significant for hippocampal 5-HT ($F=0.029$ df 1,20 $p>0.01$). Newman Keuls test showed that 8-OH-DPAT decreased hippocampal 5-HIAA, 5-HT and midbrain 5-HIAA levels of repeated saline and repeated buspirone but not 5-HT levels in midbrain of repeated buspirone injected rats. 8-OH-DPAT induced decreases in midbrain 5-HIAA and 5-HT were smaller in repeated buspirone injected rats. Repeated buspirone plus saline injected rats exhibited decreased levels of 5-HIAA in hippocampus and 5-HT in midbrain compared to their repeated saline plus saline injected counterparts.

DISCUSSION

5-HT syndrome and hypothermia are two responses that have been widely used to study 5-HT1A receptor activation in intact rats. Direct stimulation of 5-HT1A receptors or manipulations that increase the synaptic concentration of 5-HT accounts for the induction of 5-HT syndrome in rats (Trulson and Jacob 1976, Hjorth etal 1982). The 5-HT syndrome is a collection of behaviors that include increase in motor activity, flat body posture, forepaw treading and head weaving (Tricklebank 1985, Haleem 1992). It has been reported previously that 8-OH-DPAT induced locomotion and head weaving were blocked by haloperidol and reserpine (Tricklebank *et al* 1985a) except flat body posture and forepaw treading (Tricklebank *et al.,* 1985 a,b). This suggests that flat body posture and forepaw treading are most clearly linked to 5-HT1A receptor activation. The behavior is independent of presynaptic machinery as it was not blocked by the inhibition of 5-HT synthesis (Middlemiss and Fozard, 1983). Therefore 5-HT1A receptors mediating the syndrome appear to be postsynaptic (Weiland *et al.,* 1989, Middlemiss and Fozard, 1983, Haleem, 1990). In the present study subchronic administration of buspirone desensitizes postsynaptic 5-HT1A receptors as the intensity of 8-OH-DPAT elicited syndrome was higher in repeated saline than repeated buspirone injected rats (fig. 1).

We also monitored serotonin metabolism following subchronic buspirone treatment in the midbrain and hippocampus of rats (fig 2). The precise mechanism that underlies the therapeutic action of buspirone may involve desensitization of 5-HT1A autoreceptors. The effect of 5-HT1A agonists on 5-HT synthesis is considered to be mediated predominantly by somatodendritic 5-HT1A autoreceptors rather than postsynaptic 5-HT1A (Invernizzi *et al*, 1991). The present study showed that in the hippocampus and midbrain, subchronic administration of buspirone decreased 5-HIAA and 5-HT levels, respectively (table 2). Since the hippocampus receives its innervation from the median raphe nucleus (Jacob and Azmita, 1992), it is possible that via activation of somatodendritic 5-HT1A autoreceptors in the median raphe nucleus, buspirone inhibits 5-HT cell firing (Sprouse and Aghajanian, 1987). However, several lines of evidence now indicate that the activity of 5-HT neurons can also be modulated by a long feed back loop involving 5-HT1A receptors in fore brain structures (Ceci *et al.,* 1994, Bosker *et al.,* 1997, Dong *et al.,* 1997). The unaltered decrease in 5-HT levels observed using an acute challenge with 5-HT1A agonists after chronic administration of 5-HT1A agonists in the present study and in the microdialysis studies (Larrson *et al.,* 1990, Sehechter *et al.,* 1990, sharp *et al.,* 1993) is explainable in terms of long feed back loop mediating via postsynaptic 5-HT1A receptors.

Stimulation of somatodendritic 5-HT1A receptors by 8-OH-DPAT produce greater feed back effects in the midbrain region of repeated buspirone than repeated saline treated animals. This is considered to be due to desensitization of somatodendritic 5-HT1A receptors (Blier and de Montigny, 1987) in repeated buspirone treated animals.

CONCLUSION

In the present study subchronic administration of buspirone desensitizes postsynaptic 5-HT1A receptors as the intensity of 8-OH-DPAT elicited syndrome was higher in repeated saline than repeated buspirone injected rats. The desensitization of somatodendritic 5HT1A receptors as indicated by the levels of 5-HIAA and 5-HT in the midbrain region was not seen in the hippocampus suggesting that postsynaptic 5-HT receptors or some other neurotransmitters are also involved in the control of 5-HT levels particularly in the hippocampus.

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REPORT

THE PROTECTIVE EFFECT OF ALOE VERA JUICE ON LINDANE INDUCED HEPATOTOXICITY AND GENOTOXICITY

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ABSTRACT

The protective effect of fresh aloe vera (AV) leaves extract on lindane (LD) – induced hepatoxicity and genotoxicity was studied. Serum levels of hepatic enzyme markers: glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) were

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