Pharmacological Analysis of Novel Serotonin Analogues

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ABSTRACT

Serotonin (5-HT) is a biogenic amine and modulatory neurotransmitter that acts throughout the brain. It has been shown to influence a wide spectrum of physiological systems and behavioural functions, and has been implicated in the mediation of sensory-motor response, via the acoustic startle response (ASR). 8-hydroxy-2-(di-n-propylamino)tetrinalin (10 mg/kg), a selective 5-HT₁A receptor agonist, increased the magnitude of startle response, while ketanserin tartrate (10 mg/kg), a 5-HT₂ antagonist, decreased the magnitude of startle response. 5-HT has also been implicated in inducing fatigue during prolonged exercise. Pharmacological manipulation of brain serotonergic activity affects run time to exhaustion in rat. Evidences suggested that increases and decreases in brain 5-HT activity during prolonged exercise hasten and delay fatigue respectively. 1-(3-trifluromethyphenyl)piperazine (5 mg/kg), a 5-HT₂C receptors agonist, decreased the run time to fatigue, while ketanserin tartrate (5mg/kg) increased the run time to fatigue on treadmill test. Using the results of the known antagonists and agonists as guidelines, four novel 5-HT analogues were tested: LBT3007 (10 mg/kg) and LBT3008 (10 mg/kg) were studied using ASR and yielded no significant variation in the magnitude of startle response; and similarly, LBT3004 (5 mg/kg) and LBT3009 (5 mg/kg) did not significantly affect the performance in rat during prolonged exercise.

INTRODUCTION

Serotonin [5-hydroxytryptamine (5-HT)] is a biogenic amine and modulatory neurotransmitter that acts throughout the brain. It has been shown to influence a wide spectrum of physiological systems and behavioural functions. Evidences implicated that alteration in the firing patterns of 5-HT in the Central Nervous System (CNS) could be correlated with depression. Novel 5-HT analogues compounds were thus designed in hope of creating a new class of anti-psychotic agent. This study aims to examine serotonergic properties of four novel 5-HT analogues via two preliminary in vivo animal behavioral tests: acoustic startle response (ASR) and treadmill test.

The ASR is an unconditioned behavior widely used to index pharmacological manipulations and pinpoint neurobiologic sites of drug action in rats. CNS 5-HT mediates sensory-motor responsiveness, and have been implicated 5-HT in the mediation of sensory-motor response via ASR. Under appropriate experimental conditions, the startle response magnitude has a non-zero baseline and can be enhanced or attenuated.

The modulatory role of 5-HT on ASR was studied in the present study using 5-HT receptor agonists and antagonists to reaffirm those reported observations.

5-HT has also been implicated in modulating physical fatigue, originating within the CNS, known as central fatigue. The involvement of 5-HT in central fatigue was presented in studies where administration of a 5-HT agonist and antagonist to rats impaired and

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enhanced running performance, respectively (Bailey et al. 1992, 1993). These observations suggested that enhanced serotonergic neurotransmission might give rise to central fatigue. Two known serotonergic drugs were used to examine the effects of 5-HT activity on fatigue via pharmacological manipulation of 5-HT activity in rats.

Using the results of the known antagonists and antagonists as guidelines, four novel 5-HT analogues were subsequently investigated via ASR and treadmill test, to determine their seotonergic properties.

**MATERIALS AND METHODS**

*Drugs*  Four to seven Sprague-Dawley rats (200 – 350 g) received either the pharmacological agent or vehicle prior to placement into the testing apparatus of ASR. 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) hydrobromide (Sigma Chemical Co., St Louis, USA), ketanserin tartrate (KT) (TOCRIS, Ellisville, USA), LBT3007 (Lynk Biotechnologies Pte. Ltd., Science Park II, Singapore) and LBT3008 (Lynk Biotechnologies) were administered in a single dose of 10 mg/kg, iv, 15 min prior to testing. Four to five Sprague-Dawley rats (230 – 300 g) received either the pharmacological agent or vehicle or saline control prior to placement into the testing apparatus of treadmill test. 1-(3-trifluromethyphenyl)piperazine (TFMPP) hydrochloride (TOCRIS), LBT3004 (Lynk Biotechnologies), LBT3009 (Lynk Biotechnologies) and KT were administered in a single dose of 5 mg/kg, ip, immediately before the test. All drugs were dissolved in 50% DMSO solution, vortexed and diluted with 50% of total volume of isotonic saline.

*Acoustic startle response*  Startle reflexes was measured in startle response system (SR-LAB, San Diego Instruments, San Diego CA, USA) where 25 startle stimuli at 120dB were presented per subject and the signal were transduced and recorded by a computer and interface assembly. A startle response was defined as the largest response within 200 ms from the onset of the stimulus.

*Treadmill Accommodation*  Subjects were divided into groups of six and were trained everyday for a period of seven days. Each rat was to run 30 min for each training session, starting at the speed of 15.0 m/min. The treadmill speed was increased by 2.5 m/min daily, to a maximum speed of 30.0 m/min on the seventh day. An electric grid placed at the rear of the treadmill was used to motivate rats to run.

*Treadmill Test*  Five rats from each group were to exercise till exhaustion on a motorized treadmill three days after the completion of the accommodation regime. The treadmill speed is increasing at 2.5 m/min for every 5 minutes, from 15.0m/min to 30.0m/min. The grade of the treadmill was kept at 15%. Exhaustion was defined as the refusal of the subjects to continue the exercise after 15 or more consecutive seconds on the electric grid.

**RESULTS & DISCUSSIONS**

*Acoustic Startle Response*  In order to facilitate comparisons between the experiments, data in figure 1 are presented as the percent change from each respective vehicle control. The administration of 8-OH-DPAT to rats produces a marked increase in the acoustic startle response, especially at 15 min and 2 h after drug administration (P<0.05, one way ANOVA test). Conversely, the administration of KT produced a marked decrease in the acoustic startle response.
response, especially at 2h after drug administration (P<0.05, one way ANOVA test). The administration of the two 5-HT analogues, LBT3007 and LBT3008, however, did not produce any marked differences in the acoustic startle response with respect to their respective vehicle control.

![Relative Change in Magnitude of Acoustic Startle Response of LBT3007 and LBT3008](image)

**Fig. 1** The effects of LBT3007 (Δ) and LBT3008 (●) on the magnitude of the acoustic startle response in the rat. Data are mean percent of vehicle control for each compound ± SEM. Each drug was given at single dose of 10 mg/kg, iv, 15 min before startle measurement. The effects of 10 mg/kg 8-OH-DPAT (■) and 10 mg/kg KT (●) are included to contrast the known drugs from the novel drugs.

The results of the present experiments supported previous conclusions that 5-HT-containing systems played a modulatory role in the mediation of the acoustic startle reflex (Davis et al. 1980). Hence, it could be safely concluded that enhancement of 5-HT activity enhanced the ASR, and vice versa. On the other hand, since the two 5-HT analogues, LBT3007 and LBT3008, did not cause any statistically significant variation in ASR magnitude with respect to their respective vehicle controls, the serotonergic properties of these analogues remained inconclusive.

The inconclusive results could be attributed to a few factors: (1) the small sample size (n = 5 – 7) may yield unreliable results; (2) the dosage of the compound might not be sufficient to elicit any effects on ASR; (3) the 5-HT analogues, administered iv, may not be able to cross the blood-brain barrier to mediate a startle response. Due to time constraint, the investigations of the 5-HT analogues remained incomplete in the present study. Further studies looking into the dosage-dependent effects of the 5-HT analogues on ASR is required. Additional histological work is also required elucidate the precise anatomical localization of the 5-HT analogues, to determine whether these compounds do indeed pass through the blood-brain barrier.

**Treadmill Test**

Exhaustion occurred within ~46 min for the drug vehicle rats. This time was decreased ~66% and increased ~43% in TFMPP and KT rats, respectively (P<0.05; Fig. 2). However, the administration of the two 5-HT analogues, LBT3004 and LBT3009, did not produce any marked differences in the mean run time to fatigue, with respect to both drug vehicle and saline control.
Effects of 0.9% saline, drug vehicle, TFMPP (5 mg/kg), KT (5 mg/kg), LBT3004 (5 mg/kg) and LBT3009 (5 mg/kg) on the run time to exhaustion. α indicates a significant difference from 0.9% saline (P<0.05, one way ANOVA test). β indicates a significant difference from drug vehicle (P<0.05, one way ANOVA test). Each drug was given at single dose of 5 mg/kg, ip, immediately prior to the treadmill test.

The effects of increased (TFMPP) and decreased (KT) 5-HT activity on run time to exhaustion are similar to those previously reported (Bailey et al. 1992). Consequently, it is reasonable to assume that the changes in run time to exhaustion in this study can be attributed to changes in 5-HT activity.

From the present study, it can be safely concluded that increase and decrease brain 5-HT activity via pharmacological manipulation would negatively and positively affect run time to exhaustion in the rat, respectively. Based on this conclusion, the serotonergic properties of the two 5-HT analogues tested – LBT 3004 and LBT 3009 – in the present study remained inconclusive. The inconclusive results could be due to the following: (1) the dosage might not be sufficient to affect the performance of prolonged exercise in rats; (2) The two novel analogues might not bind to receptors that initiate central fatigue; (3) The possible error in the modification of treadmill accommodation regime. Further studies which adopt a longer treadmill accommodation regime and investigating the dosage-dependent effects of the 5-HT analogues on treadmill test is required. Additional biochemical work is also required to determine the affinity of these 5-HT analogues to the different 5-HT receptors.

REFERENCES