ORIGINAL ARTICLE

INCREASED SEROTONERGIC FUNCTIONS FOLLOWING ADMINSTRATION OF 1-(1-NAPHTHYL) PIPERAZINE IN PROPRANOLOL INJECTED RATS

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ABSTRACT

Propranolol a β adrenergic antagonist, binds with 5-HT_1 receptor. 1-(1-naphthyl) piperazine (1-NP) a derivative of quipazine has serotonin antagonist activity at 5-HT_2 and agonist activity at 5-HT_1 site. In the present study neurochemical and behavioral effects of 1-NP was monitored in saline and propranolol injected rats. 1NP increased locomotor activity in saline as well as in propranolol injected rats. Administration of propranolol also increased locomotor activity and these increases were more enhanced following 1-NP administration. Levels of 5-HT were not altered following the administration of 1NP in saline as well as propranolol injected rats. 5-HT turnover however decreased by the administration of propranolol Adminstration of 1NP decreased 5-HT turnover in saline but not in propranolol injected rats.

Keywords: Serotonin, propranolol, 1-(1–naphthyl) piperazine, locomotion.

INTRODUCTION

5-HT play an important role in the modulation of various behaviors such as mood, nociception, motor behavior, endocrine secretion, memory and appetite (Haleem *et al*., 2002, Haleem 1992, Saima *et al*., 2006). It exerts its effect via multiciplicity of sites, these have been classified as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C},5-HT_{1D}, 5-HT_2, 5-HT_3 and5-HT_4 (Glennon, 1990; Hoyer, 1988; Hoyer and Martin, 1996). Among these subsites 5-HT_{1A} receptors are of particular interest. 5-HT_{1A} receptors are present on the soma and dendrites of 5-HT neuron and on postsynaptic site (Verge *et al*., 1985).

Studies shows that 5-HT_{1A} agonist possess anxiolytic, (Glesson *et al*., 1989; Noreen *et al*., 2005) antidepressive (Blier and Montigny, 1990) and antihypertensive (Hartog and Wouters, 1988) properties. 1-(1-napthyl)piperazine (1-NP) is a derivative of quipazine. Evidence show that quipazine decreases the rate of 5-HT turnover in brain and stimulates central serotonergic receptors (Samanin *et al*. 1997,Fuller *et al*. 1986). 1-NP is a derivative of quipazine with high affinity for 5-HT_1 substype of serotonin binding site and acts as 5-HT_{1A} partial agonist (McKenamey, 1989; Moret and Briley, 1995). Samotodentritic 5-HT_{1A} autoreceptors increase the release of 5-HT and thus are beneficial in the treatment of hyposerotonergic state such as depression (Blier and Montigny 1990). Arylpiperazine derivatives act as 5-HT_{1A} partial agonist and are known as serotonin normalizers, therefore these derivatives are expected to have anxiolytic as well as antidepressant effect (Murasaki and Miura, 1992). Propranolol is β adrenergic antagonist and binds in a stereoselective manner at 5-HT_{1A} and 5-HT_{1B} receptors (Hoyer *et al*., 1985; Glieter and Deckert, 1996). β adrenergic antagonist propranolol and pindolol has ability to antagonize the hypothermic effects (Goodwin *et al*., 1987) and serotonin syndrome produced by various 5-HT agonist (Tricklebank *et al*., 1985). Previous studies shows that propranolol inhibits 5-HT induced behavioral changes (Goodwin *et al*., 1987; Tricklebank *et al.,* 1985). In view of these findings present study is designed to investigate the neurochemical and behavioral effects of 1NP in saline and propranolol injected rats.

MATRIALS AND METHODS

Animals

Locally bred male albino wistar rats weighing 200-250 gm were used for the experiment. The rats were housed individually in plastic cages under 12h light and dark cycle with free access to cubes of standard rodent diet and water before starting the experiment. All experiments were performed according to a protocol approved by the local Animals Care Committee.

Drugs

Propranolol (ICI, Maceles-Field, UK). 1-(1-Naphthyl) piperazine HCl (RBI research Biochemical; USA)

Experimental protocol

Animals were randomly assigned into two groups (control and test animals). A single dose of propranolol (5mg/Kg body wt.) was administrated intraperitoneally to test animals while control animals received an equal volume of saline (0.9% NaCl) by the same route. 30 minutes following the

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proprapanolol administration, a group of saline and a group of propranolol injected rats were injected with 1-(1-naphthyl) piperazine (1mg/Kg body wt.) and the rats from the other group received an equal volume of saline (0.9% NaCl). Immediately after the administration of 1-NP home cage activity was monitored. Animals were decapitated 2hr following the first injection. Brain was removed immediately after the decapitation. Fresh brain were dipped in chilled saline (0.9% w/v) and stored at low temperature -70^{o}c for the determination of 5-HT and 5-HIAA in the brain by HPLC-EC.

Home cage activity

Transparent perpex cages (26x26x26 cm) with sawdust cover floor were used to monitor the activity in familiar environment. 15 minutes before 1-NP/saline injection, rats were placed individually in these cages to get them familiarized with the environment, and number cage crossing were counted for 10 minutes.

HPLC analysis

5-HT and 5-HIAA from brain samples were extracted as described previously (Haleem and Perveen 1994). A 5µ ODS (ECHPHERE) separation column 4mm i.d. and 250mm length was used. The solvent system was methanol (14%), octyl sodium sulhate (ODS; 0.023%) and EDTA (0.05%) in 0.1M phosphate buffer of pH 2.9. Electrochemical detection of brain 5-HT and 5-HIAA was achived on Shimadzu L-EC 6A detector at an operating potentional of 0.8V (Haleem *et al*., 2002).

STATISTICAL ANALYSIS

The results are presented as mean $\pm$ S.D. Behavioral data on the effect of 1-NP on home cage activity in saline and propranolol injected rats was analyzed by Mann-Whitney U-test. Statistical analysis of neurochemical data were performed by Two-Way analysis of variance (ANOVA). Post-hoc comparison were done by Newman-Keuls test. Values $p<0.05$ were considered statistically significant.

RESULTS

Fig. 1 shows the effect of 1-NP on locomotor activity in saline and propranolol injected rats. Data analyzed by Mann-Whitney U-test showed that home cage locomotion (monitored as number of cage crossing) per 10 minutes significantly increased $p<0.01$ by 1-NP in saline as well as propranolol injected rats. Prior administration of propranolol also shows a significant increase $p<0.05$ in locomotor activity. Increased in locomotor activity by 1-NP was more in propranolol injected rats as compare to saline injected rats.

Fig. 2 shows the effect of 1-NP on brain 5-HT and 5-HIAA levels in saline and propranolol injected rats.

Two way ANOVA (df=1, 20) performed on brain levels of 5-HT shows an insignificant effect of propranolol ($F=0.213$), and insignificant effect of 1-NP ($F=0.59$) and a significant ($F=4.83, p<0.05$) interaction between the two factors.

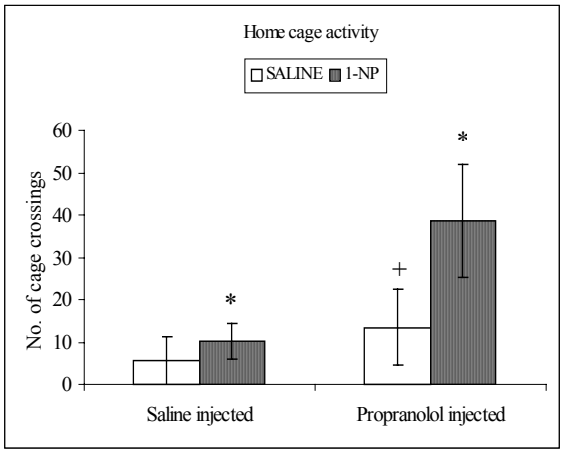
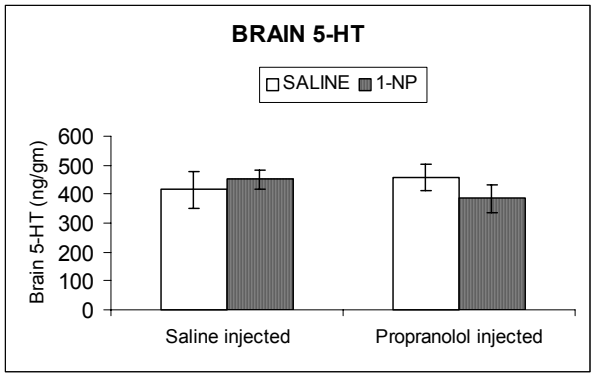


Fig. 1: Values are means ± S.D. (n=6). Significant difference by Mann-Whitney U-Test. *p<0.05, **P<0.01 from respective control, +P<0.05 from saline injected control.



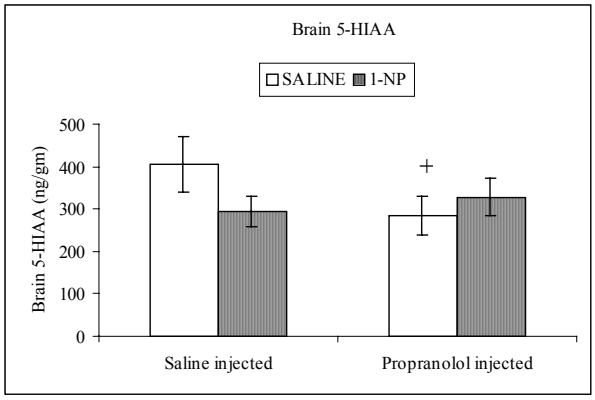


Fig. 2: Values are means ± S.D. (n=6). Significant difference by Newman-Keuls test * P<0.01 from respective controls and + P<0.01 from saline injected controls.

Two Way ANOVA (df=1, 20) performed on 5-HIAA levels revealed an insignificant effect of both propranolol (F=4.11) and 1-NP (F=2.32) and a significant interaction (F=12.78, $p<0.01$) between the two factors. Post-hoc analysis by Newman-Keuls test shows that 1-NP and propranolol significantly ($p<0.01$) decreased 5-HIAA levels in saline injected rats.

DISCUSSION

Behavioral effects of 1-NP

Behavioral effects of 1-NP were monitored in saline and propranolol injected rats. Present study shows that administration of 1-NP produced an increase in motor activity in saline as well as in propranolol injected rats. An increase in motor activity following the administration of 1-NP has been also reported previously (Simansky and Shechter, 1988; Haleem *et al*., 2002; Farhat *et al*., 2000). These behavioral symptoms were also observed following the administration of pyridinyl indole 5 methoxy-3-(1, 2, 3, 6 tetra pyridinyl) indole (RU 24969) (Green *et al*., 1984; O'Neil and Parameswaren, 1997) a drug that binds with both 5-HT_{1A} and 5-HT_{1B} receptors sites (Perioutka 1986). Prpranolol is β adrenoceptor antagonist possess high affinity for 5-HT1 sites (Engel *et al*., 1986; Glieter and Deckert *et al.,* 1996). Propranolol has agonist activity at 5-HT1B receptor sites (Hjroth and Magnusson, 1988; Maura *et al*., 1987). In the present study it is observed that 1-NP increases the locomotor activity both in saline and propranolol injected rats however this effect is greater in saline injected rats than propranolol injected rats. Increase in the locomotor activity following the administration of 1-NP has been reported previously (Simansky, 1988; Farhat *et al*., 2000; Haleem *et al*., 2002). Attenuation of stress induced behavioral deficits by buspirone and propranolol have also been reported previously (Noreen *et al*., 2005). Co-administration of the selective 5-HT_{1A} agonist 8-OH-DPAT and 5-HT_{1B} agonist anpirotoline also produce an increase in locomotor activity (O'Neil and Parameswaren, 1997). An increase in the locomotor activity following the coadminstration of 5-HT_{1A} agonist 1-NP and 5-HT_{1B} agonist propranolol is also observed in the present study. A synergistic effect of stimulation of 5-HT_{1A} and 5-HT_{1B} receptors may be involved in the elicitation of this behavior.

Neurochemical effects of 1-NP

Decreased turnover of serotonin indicated by reduced steady state concentration of serotonin metabolite 5-HIAA and by reduced accumulation of 5-hydroxy tryptophan (5-HTP) after decarboxylase inhibition is a well characterized effect of centrally acting serotonin agonist (Haleem and Perveen, 1994). The synthesis and release of 5-HT is under the control of feed back mechanism involving the stimulation of 5-HT1A autoreceptor that are located on the dendrites and/or soma of the dorsal raphe (Verge *et al*., 1985) produced an inhibition of nerve impulse flow within serotonergic neuron (Blier *et al*., 1987). A decrease in 5-HT turnover and release occurs in the brain regions where serotonergic neuron projects (Hjorth and Magnusson, 1988; Hutson *et al*., 1989; Haleem *et al*., 1990). A decrease in brain 5-HT metabolism following the administration of 1-NP has been also reported previously (Fuller *et al*., 1986; Haleem *et al*., 2002). 1-NP has good affinity for 5-HT1A autoreceptors (Glennon *et al*., 1991) and decreases 5-HT synthesis in rats through the stimulation of these receptors (Moret and Briley, 1995). In the present study a significant decrease in brain 5-HIAA concentration was also observed following the administration of 1-NP.

The release of 5-HT from the nerve terminal is under the control of inhibitory 5-HT autoreceptors. These 5-HT autoreceptors have been suggested to be 5-HT_{1B} type in rodents (Engel *et al*., 1986, Knobelman *et al*., 2000) and 5-HT_{1D} type in other species (Hoyer and Middlemiss, 1989 and Limberger *et al*., 1991). The β blocker propranolol is a 5-HT antagonist with some selectivity towards 5-HT1A receptors (Gliter and Deckert, 1996), and agonist activity towards 5-HT_{1B} receptor (Hjorth and Magnusson, 1988; Maura *et al*., 1987). A selective antagonist at terminal autoreceptors having no effect on somatodendritic autoreceptors would be expected to increase 5-HT release. Turnover of 5-HT to 5-HIAA will also increase. Present results show a decrease of 5-HIAA concentration following the administration of 1-NP in saline injected rats but not in propranolol injected rats. Lack of any affect on 5-HIAA concentration by 1-NP in propranolol injected rats may be due to the antagonist activity of prapranolol towards 5-HT1A receptors.

CONCLUSION

Co-administration of 5-HT_{1A} agonist 1-NP and 5HT_{1B} agonist propranolol increases the locomotor activity in rats. However the insignificant effect on brain 5-HIAA concentration by 1-NP in propranolol injected rats may be due to antagonist activity of propranolol towards 5-HT_{1A} receptor.

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