ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of DEVELOPMENT RESEARCH

International Journal of Development Research
Vol. 5, Issue, 03, pp. 3721-3728, March, 2015

Full Length Research Article

A NOVEL ANTIANXIETY- LIKE ACTIVITY OF AMINO GUANIDINE IN STRESSED MICE: EVIDENCE OF REGULATION BY NO: CGMP MODULATION

Shalini Yadav, Shakti Goel and *Neeraj Gilhotra

Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak – 124 001, Haryana, India

ARTICLE INFO

Article History:

Received 09th December, 2014
Received in revised form
13th January, 2015
Accepted 18th February, 2015
Published online 17th March, 2015

Key words:

8-Br-cGMP;
Amino guanidine;
Anxiety;
Bay-60-7550;
Methylene Blue;
Nitric oxide

ABSTRACT

An investigation on possible regulation of antianxiety- like activity of amino guanidine in stressed mice by nitric oxide: cyclic guanosine monophosphate (NO: cGMP) modulation was made. Stressed mice were administered with amino guanidine and tested on elevated plus maze and light/dark box. NO: cGMP modulators (L-arginine, Methylene blue, Bay-607550, 8-Br-cGMP) were administered as pre-treatments in the separate groups of amino guanidine- treated stressed mice and modulation of the noted effect of amino guanidine in stressed mice was observed. Diazepam (2 mg/kg, i.p.) was employed as comparator drug. Amino guanidine (50 mg/kg, i.p.) produced a significant antianxiety- like activity in stressed mice ($p<0.05$). The noted antianxiety-like activity of amino guanidine in stressed mice was accompanied by a significant decrease in plasma nitrite levels ($p<0.05$). L-Arginine (100 mg/kg, i.p.), a nitric oxide (NO) donor, significantly attenuated the noted antianxiety- like activity of amino guanidine in stressed mice ($p<0.05$). This attenuation of antianxiety- like activity of amino guanidine in stressed mice was accompanied by a significant increase in plasma nitrite levels in stressed mice ($p<0.05$). Methylene blue (1mg/kg, i.p.), a soluble gunylate cyclase (sGC) inhibitor, served to significantly enhance the noted antianxiety- like activity of amino guanidine in stressed mice ($p<0.05$). Pre-treatment with a cGMP facilitator (Bay-607550*;* 3 mg/kg, i.p.) and a cGMP analogue (8-Br-cGMP; 1mM, i.p.) failed to bring any significant change in the noted antianxiety- like activity of amino guanidine in stressed mice. It is noteworthy that no significant increase ($p<0.05$) was observed in plasma cGMP levels in stressed mice, treated with combination of amino guanidine and Bay-607550. The results of the present study strengthen the possibility of a novel antianxiety-like activity of amino guanidine in stressed mice and serve to provide an evidence of possible regulation of the antianxiety- like activity of amino guanidine in stressed mice by NO: cGMP modulation.

INTRODUCTION

Stress has long been observed to play a role in the etiology of neurodegenerative diseases and mental disorders (McEwen, 2000; Esch *et al*., 2002). Exposure to different stressors like forced swim (Britton *et al*., 1992), surgical stress (Adamec *et al*., 1991), social defeat (Heinrichs *et al*., 1992) and immobilization (Alobnetti and Farabellini, 1992) has been shown to result in anxiogenic behaviour (Hata *et al*., 2001). Immobilization stress has been reported to increase the production of nitric oxide (NO) and result in an anxiogenic-like behavior in rodents (Tsuchiya *et al*., 1997; Sevgi *et al*., 2006). The reported immobilization stress- induced deregulation of nitriergic system is manifested by an increase in an expression of inducible form of nitric oxide synthase (iNOS) (Olivenza, 2000; Madrigal *et al*., 2002; Jang *et al*., 2008). The exaggerated anxiety behaviour, derived from production of NO, is reported to be influenced by different modulators of nitric oxide: cyclic guano sine monophosphate (NO: cGMP) cascade as evident by such reports; (a) L-Arginine (L-arg), a NO donor, serves to enhance the anxiogenic effect of sildenafil (Kurt *et al*., 2004); (b) Methylene blue (MB), an inhibitor of soluble gunylate cyclase (sGC), produced an antianxiety– like activity (Eroglu and Caglayan, 1997) and (c) augmentation of anxiety behaviour by sildenafil that enhances the levels of cGMP (Kurt *et al*., 2004). Pharmacological inhibition of PDE2 activity, resulting in an increase in cGMP levels, influenced stress- related anxiety via cGMP-PKG signalling (Werner *et al*., 2004).

Corresponding author: Neeraj Gilhotra
Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak – 124 001, Haryana, India

Bay- 607550 is a selective inhibitor of PDE2 (Boess *et al*., 2004; Beavo, 2006). 8-Br-cGMP is a cGMP analogue that is reported to decrease GABAergic currents (Lee, 2009). Amino guanidine (AG), a selective inhibitor of iNOS is well used in the treatment of disease states characterized by the pathological overproduction of nitric oxide (Misko *et al*., 1993; Southan and Szabo, 1996). AG decreased indices of NO production and cGMP levels in a dose- dependent manner (Griffiths *et al*., 1995). AG has received much attention as an inhibitor of NOS due to its selectivity towards iNOS, low acute toxicity and potential clinical usefulness (Southan and Szabo, 1996).

Recently, AG has also been reported to show an antianxiety–like activity in stressed mice (Gilhotra and Dhingra, 2009). Diazepam is a clinically utilized anxiolytic drug (Schwartz *et al*., 2005). As evident from literature, the noted bolstering effect among NO facilitator (L-Arginine) and cGMP facilitator (Sildenafil), as well as, among NO suppressor (amino guanidine) and cGMP suppressor (methylene blue), clearly signifies the importance of role of NO-cGMP pathway in modulation of anxiety behaviour. In our knowledge, there is no report in literature that employs co-administration of methylene blue, L-Arginine, Bay-60-7550 and 8-Br-cGMP with amino guanidine in area of anxiety research. Further, there is only a single study in literature till date, that suggests a possible antianxiety- like activity of AG in stressed mice (Gilhotra and Dhingra, 2009).

The report indicated a reversal by AG of actions, mediated through one of components of NO: cGMP pathway i.e. cGMP; reversal of anxiogenic activity of sildenafil by AG. This report points that actions, possibly mediated by cGMP, may be prevented by inhibition of a NOS enzyme, in this case, iNOS. Therefore, the present study was designed to explore the possible regulation (if any) of reported antianxiety- like activity of amino guanidine in stressed mice by modulators of all the three components of NO-cGMP pathway, not employed till date with AG i.e. NO (L-arg), sGC (MB) and cGMP (Bay–607550, 8-Br-cGMP). Nonetheless, such design also served to strengthen the earlier observations on AG (Gilhotra and Dhingra, 2009). Biochemical estimations of NO and cGMP levels were also carried out to find possible biochemical regulation of the observed behaviour.

MATERIALS AND METHODS

Animals

Swiss albino mice (male; 20-30g) were employed in the present study. Animals were kept in polypropylene boxes covered with sawdust, at controlled temperature, with 12h light/dark cycles. Animals were allowed to habituate to the housing facilities for at least one week before the experiments began. Behavioral studies were conducted in a quiet room. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Drugs and treatments

Amino guanidine, Bay60-7550, 8-Br-cGMP (Genetix Biotech Asia Pvt. Ltd.), Diazepam (Ranbaxy Laboratories Ltd.), Methylene blue (Loba Chemie Chemicals), L-Arginine (CDH Ltd.) were employed in the present study. Normal saline (0.9% sodium chloride) was used as vehicle for all drugs except for Bay 60-7550, for which the vehicle was 50%, dim ethyl sulfoxide. All drugs were administered intraperitoneally (i.p.) in a volume of 0.1 ml per 10g body weight of mice.

Elevated plus maze test

The elevated plus maze was a wooden maze, comprising two open arms (16 cm × 5 cm), and two closed arms (16 cm × 5 cm × 12 cm), connected to a central platform (5 × 5 cm). The maze was elevated to a height of 25 cm above the floor. Each mouse was placed separately at the centre of EPM with its head facing towards any one open arm and observed for 5 min to record the time spent in open arm. In the EPM test, percent time spent on the open arms was determined as follows:

$$\textbf{Percent time spent in open arms} = 100 \times \frac{\text{Number of seconds spent on open arms}}{\text{300 total seconds}}$$

Light and Dark Box test

The apparatus consisted a rectangular box (45 cm × 27 cm × 27 cm), partitioned into two compartments with the black/dark section comprising one-third and the white/light section comprising the remaining two-thirds of the chamber connected by a 7.5 cm × 7.5 cm opening in the wall between compartments. An animal was placed into the centre of the light compartment and was observed for 5 min for the time spent in the open (white / light) compartment. Percent time spent in the light compartment was determined as follows:

$$\textbf{Percent Time Spent} = 100 \times \frac{\text{Number of seconds spent in light compartment}}{\text{300 total seconds}}$$

Plasma Corticosterone Estimation

The quantitative estimation of plasma corticosterone level was performed by the method of Bartos and Pesez, 1979.

Plasma Nitrite Estimation

For nitrite estimation, plasma was collected using cooling centrifuge at 2500 rpm and 4^0C for 10 min. The plasma was stored in a refrigerator for estimation of nitrite content within 24h. Plasma nitrite was measured by a spectrophotometric assay based on the Griess reaction (Green *et al*., 1982). Briefly, plasma was mixed with equal volume of Gris reagent (1% sulphanilamide + 0.1% naphthalene ethylenediamine dihydrochloride + 2.5% ortho-phosphoric acid) and incubated at room temperature for 10 min to yield chromophore. The absorbance was read at 545 nm.

cGMP level estimation

Effect of selected drug treatment on plasma cGMP levels was measured, using Cayman Chemical cyclic GMP Assay kit was used according to manufacturer's protocol instructions.

Experimental Protocol

Experimental animal groups employed in the present study consisted of six mice each (n = 6). Stress was induced in mice by immobilizing them for 6h by taping all their four limbs and trunk on a wooden board. Mice subjected to immobilization stress were called as stressed mice and mentioned accordingly in the manuscript. Mice administered with vehicle(s) were exposed to elevated plus maze and light/dark test for normal duration (5 min), sufficient to assess the anxiety levels in rodents (Calatayud *et al*., 2004). Such mice were called as unstressed mice. Behavioural tests were performed in independent groups of mice. In case of stressed mice, behavioural testing was started 10 min after setting the animals free from immobilization. Doses employed in the present study were selected based on the earlier reports. The experimental protocol was subjected to animal reduction ethics; hence, only stressed mice were treated with drug treatments with a strictly defined objective to answer the research question in hand. Biochemical estimation of cGMP was conducted only in the selected groups that served to substantiate its role in testing the hypothesis of the present study. The dosage schedule in the stressed group assures that the treatment(s) employed inhibited any change(s) occurring immediately after and during immobilization, thereby producing the net change in behavior or biochemical parameter of mice under investigation. The experimental protocol was approved by institutional animal ethics committee.

Locomotor activity

The effects of various treatments on spontaneous locomotor activity of animals were measured by using an actophotometer. The locomotor activity scores for each animal were recorded for a period of 10 min before and after drug treatments.

Statistical Analysis

The Statistical analysis was performed by using one way ANOVA followed by Tukey's Post hoc test by using the software Graph pad Instat; version 3.05 (Graph Pad Software, San Diego, CA, USA).

RESULTS

Effect of immobilization on mice behaviour

6h immobilization served to enhance an anxiety-like behaviour in mice as compared to vehicle- treated unstressed mice (Fig. 1 and 2).

Effect of amino guanidine on behaviour of stressed mice

Amino guanidine (50 mg/kg, i.p.) produced a significant antianxiety- like activity as compared to immobilized mice (Fig. 1 and 2).

Modulation of antianxiety- like activity of amino guanidine by NO-cGMP modulators (L-Arginine, Methylene blue, BAY-607550 and 8-Br-cGMP)

Pre- treatment with L-Arginine (100 mg/kg, i.p.), NO donor, significantly attenuated the antianxiety- like activity of amino guanidine (50 mg/kg, i.p.). Pre- treatment with methylene blue (1 mg/kg, i.p.), a direct NOS inhibitor as well as a sGC inhibitor significantly enhanced the antianxiety- like activity of amino guanidine (50 mg/kg, i.p.). Pre- treatment with *BAY-607550* (3mg/kg, i.p.), a selective PDE-2 inhibitor and cGMP facilitator, failed to bring any significant change in the noted antianxiety- like activity of amino guanidine (50 mg/kg, i.p.). Pre- treatment with 8-Br-cGMP (1mM, i.p.), *a cGMP* analogue, also failed to bring any significant change in the noted antianxiety- like activity of amino guanidine (50 mg/kg, i.p.) (Fig. 1 and 2).

Effect of Immobilization on plasma corticosterone levels

Stressed mice were observed to show a significant rise in their plasma corticosterone levels.

Effect of amino guanidine and L-Arginine on plasma nitrite levels

Stressed mice showed a significant increase in plasma nitrite levels. A significant decrease in plasma nitrite level was produced by amino guanidine (50 mg/kg, i.p.). A significant increase in plasma nitrite levels was observed in stressed mice treated with combination of L-Arg (100 mg/kg, i.p.) and amino guanidine (50 mg/kg, i.p.) (Fig. 3).

Effect of Immobilization, amino guanidine and NO-cGMP modulators (L-Arginine, Methylene blue, Bay-60-7550) on plasma cGMP levels

Stressed mice showed a significant increase in cGMP levels. A significant decrease in cGMP levels was produced by amino guanidine (50 mg/kg, i.p.). A significant increase in cGMP levels was observed in stressed mice, treated with combination of amino guanidine (50 mg/kg, i.p.) and L-Arginine (100 mg/kg, i.p.), a NO donor. A significant decrease in cGMP levels was observed in stressed mice, treated with combination of amino guanidine (50 mg/kg, i.p.) and methylene blue (1 mg/kg, i.p.), a NO donor. *Bay- 607550* (3mg/kg, i.p.) failed to produce any significant change in cGMP levels in amino guanidine (50 mg/kg, i.p.)- treated stressed mice (Fig. 4).

Effect of drug treatments on spontaneous locomotor activity

Drug treatments used in the present study did not produce any significant change in the spontaneous loco motor activity of mice**.**

DISCUSSION

Stress- potentiated behaviour is a relevant and physiologically close pathological feature of anxiety in humans (Korte, 2003). Forced immobilization is the best explored models of stress in rodents that combines emotional stress (escape reaction) and physiological stress (muscle work), resulting in both restricted mobility and aggression. As painful stimuli are not directly involved in restraint stress, this form of stress is probably more akin to physiological stress (Bhattacharya and Bhattacharya, 1982).

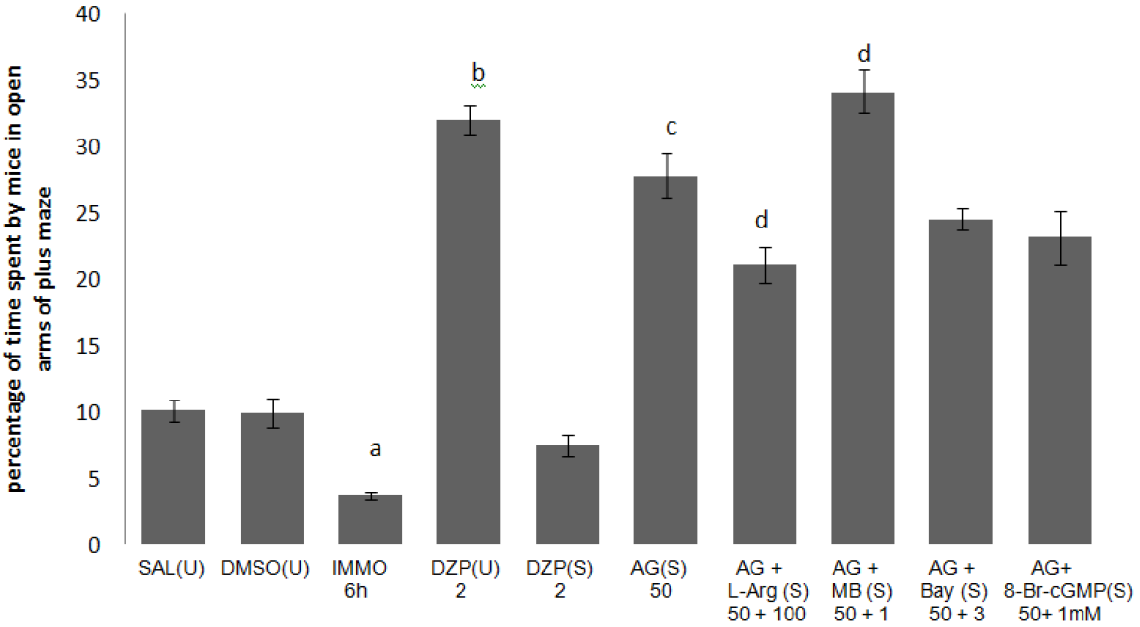

Fig.1. Effect of different treatments on percentage of time spent in open arm of elevated plus maze. n=6 in each group

Values are expressed as mean ± S.E. Data was analyzed by one-way ANOVA followed by Tukey's Post hoc Test, F (9,50) =73.53; p<0.0001, a = p<0.05significant difference from saline treated control group (unstressed mice) and significant difference from DMSO treated control group (unstressed mice), b = p<0.001significant difference from saline treated control group (unstressed mice) and significant difference from DMSO treated control group (unstressed mice), c = p<0.001 significant difference from immobilization treated stressed mice. and d = p<0.05 significant difference from aminoguanidine treated stressed mice. **SAL(U)**: normal saline (unstressed mice); **DMSO(U)**: dimethyl sulfoxide (unstressed mice); **IMMO**: stressed mice; **DZP (U)**: diazepam (unstressed mice); **DZP(S)**: diazepam (stressed mice); **AG(S)**: aminoguanidine (stressed mice); **L-Arg(S)**: L-Arginine (stressed mice); **MB(S)**: methylene blue (stressed mice); **Bay(S):** Bay-607550 (stressed mice); **8-Br-cGMP(S)**: 8-bromo-cGMP (stressed mice). Values mentioned are doses in mg/kg, except for 8-bromo-cGMP, which is in micromolar.

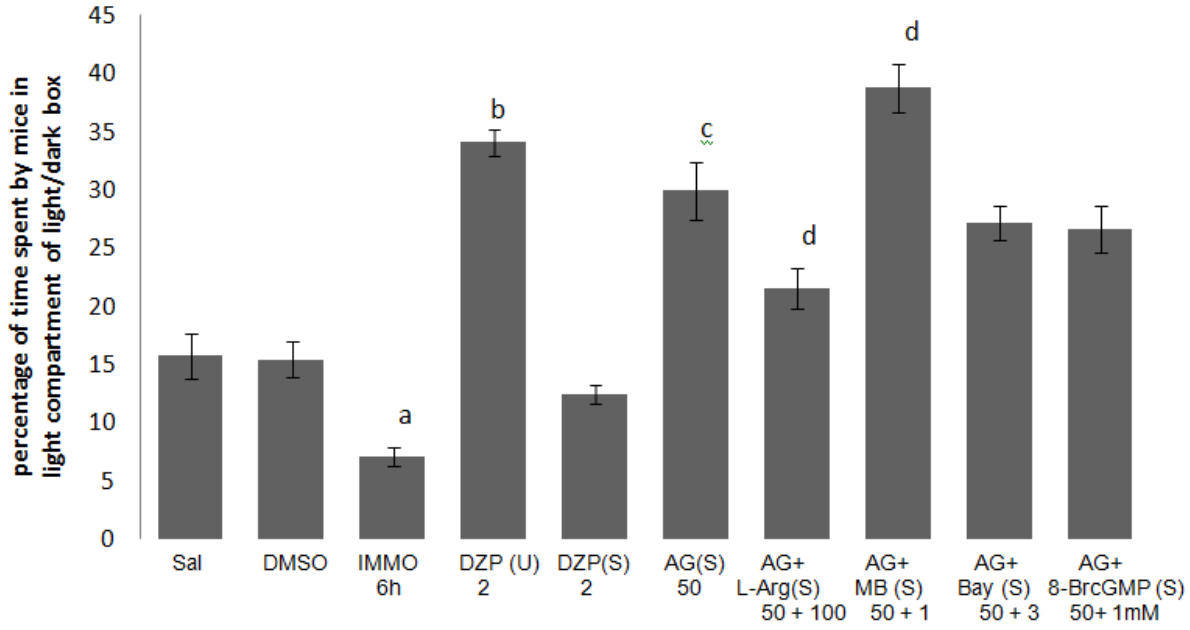

Fig.2. Effect of different treatments on percentage of time spent in light compartment of light/dark box. n=6 in each group

Values are expressed as mean ± S.E. Data was analyzed by one-way ANOVA followed by Tukey's Post hoc Test. F(9,50) = 36.12; p<0.0001, a = p<0.05significant difference from saline treated control group (unstressed mice) and significant difference from DMSO treated control group (unstressed mice), b = p<0.001 significant difference from saline treated control group (unstressed mice) and significant difference from DMSO treated control group (unstressed mice) c = p<0.001 significant difference from immobilization treated stressed mice. and d = p<0.05 significant difference from aminoguanidine treated stressed mice. **SAL(U)**: normal saline (unstressed mice); **DMSO(U)**: dimethyl sulfoxide (unstressed mice); **IMMO**: stressed mice; **DZP (U)**: diazepam (unstressed mice); **DZP(S)**: diazepam (stressed mice); **AG(S)**: aminoguanidine (stressed mice); **L-Arg(S)**: L-Arginine (stressed mice); **MB(S)**: methylene blue (stressed mice); **Bay(S):** Bay-607550 (stressed mice); **8-Br-cGMP(S)**: 8-bromo-cGMP (stressed mice). Values mentioned are doses in mg/kg, except for 8-bromo-cGMP, which is in micromolar.

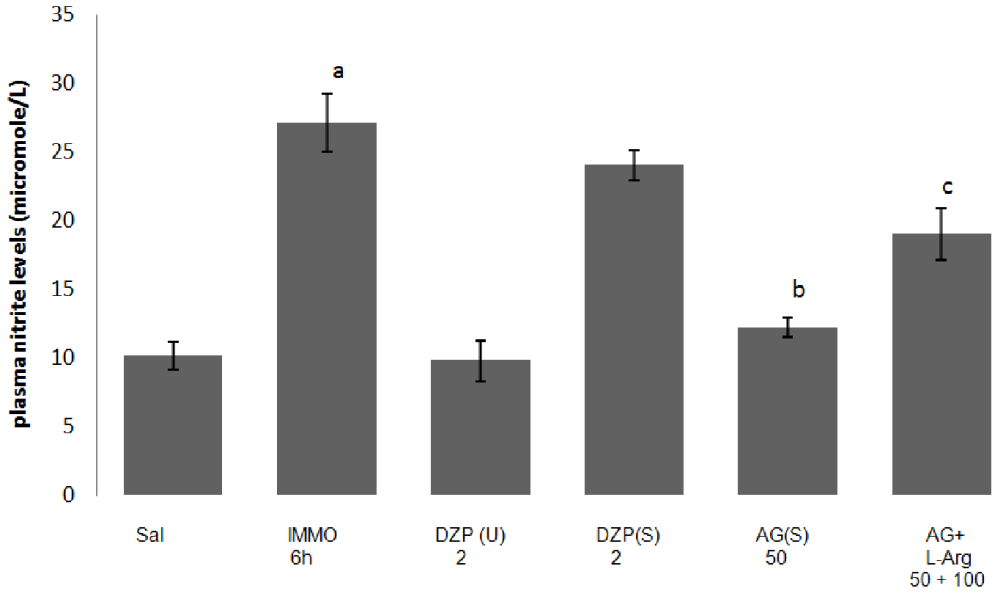

Fig.3. Effect of different treatments on plasma nitrite levels (micromol/L)

n = 6 in each group. Values are expressed as mean ± S.E. Data was analyzed by one-way ANOVA followed by Tukey's Post hoc Test, F (5, 30) = 25.95; p<0.0001, a = p<0.001 significant difference from vehicle treated control group (unstressed mice), b =p<0.001 significant difference from immobilization treated stressed group, c =p<0.05 significant difference from aminoguanidine treated stressed group. **SAL(U)**: normal saline (unstressed mice); **DMSO(U)**: dimethyl sulfoxide (unstressed mice); **IMMO**: stressed mice; **DZP (U)**: diazepam (unstressed mice); **DZP(S)**: diazepam (stressed mice); **AG(S)**: aminoguanidine (stressed mice); **L-Arg(S)**: L-Arginine (stressed mice). Values mentioned are doses in mg/kg.

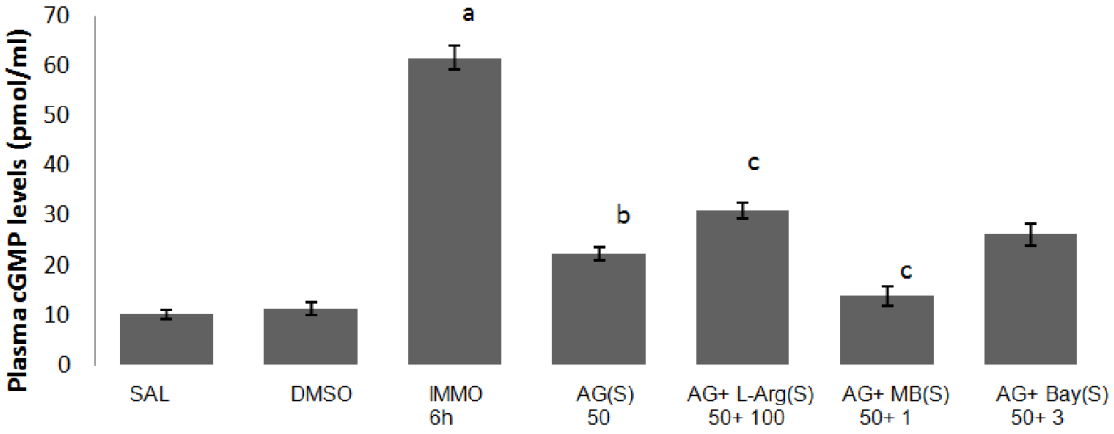

Fig. 4. Effect of different treatments on plasma cGMP levels (pmol/ml)

Data are expressed as mean ± S.E.M. a = p < 0.001 significant difference from unstressed mice, b = p < 0.001 significant difference from stressed mice, c = p < 0.05 significant difference from aminoguanidine treated stressed mice. **SAL**: Normal Saline; **DMSO**: Dimethyl Sulfoxide **IMMO**: Immobilization; **6h:** Six hours; **AG(S)**: Aminoguanidine (stressed mice); **L-Arg**: L-arginine; **MB**: methylene blue; **Bay:** Bay-60-7550. Doses mentioned are in mg/kg.

The present study has also employed physical immobilization for 6h as stressor for mice and found that mice subjected to 6h immobilization stress were more anxious in their behaviour as compared to unstressed mice. Belzung and Griebel, 2001 have also reported that continuous exposure to different stressors result in enhancement of normal anxiety. Immobilization stress has also been reported to induce a rise in plasma corticosterone level (Ida *et al*., 1984; Iukhananov *et al*., 1990). Increased corticosterone level has been linked with anxiety-like behaviour (Rodgers *et al*., 1999; Callahan *et al*., 2013). We have also observed a significant increase in plasma corticosterone levels of mice subjected to 6h immobilization stress. Immobilization stress for as short as 2h has been reported to activate NOS and enhance anxiety behaviour in rodents (Sevgi *et al*., 2006). The present study has employed 6h immobilization stress that is reported to increase expression of iNOS and production of the stable nitric oxide metabolites (nitrite and nitrate) in both plasma and brain (Madrigal *et al*., 2002; Lee *et al*., 2007). We have also observed a significant increase in anxiety behaviour in stressed mice. Our observations on behaviour of stressed mice were accompanied by a significant increase in plasma nitrite levels. These observations further strengthen the suggested role of 6h immobilization stress and NO as anxiogenic mediators (Volke

et al., 1997; Faria *et al*., 1997; Dunn *et al*., 1998; Sevgi *et al*., 2006). In several pharmacological reports, amino guanidine has been observed to show anti-ageing property (Klandorf *et al*., 1996), help alleviate or prevent senile cataracts, thickening of the arteries, kidney failure, thinning bones, osteoarthritis, improve overall heart and arterial condition (Lee *et al*., 1997), stabilize the metabolism of glucose, and help prevent and treat adult onset diabetes. Here, in present study, amino guanidine has produced a significant anxiolytic activity in stressed mice. This observation is in accordance with earlier report by Gilhotra and Dhingra, 2009. Involvement of iNOS-NO-cGMP pathway may be indicated on the basis of observations that amino guanidine has significantly decreased the plasma cGMP levels in the stressed mice. Amino guanidine has also been reported to be an inhibitor of guanylyl cyclase which is the working link between nitric oxide and cGMP, as NO endogenously produced by NO synthases or released from exogenously applied NO donors activates NO-sensitive guanylyl cyclase and leads to increased synthesis of cGMP (Friebe and Koesling, 2003).

Amino guanidine, a selective inhibitor of iNOS, reduced the plasma nitrite levels in mice previously subjected to immobilization stress, which further supports the observed anti-anxiety effect of amino guanidine. Our results are in accordance with the earlier reported role of nitric oxide as anxiogenic mediator (Faria *et al*., 1997; Volke *et al*., 1997). Augmentation of the NO-cGMP cascade induces anxiogenic-like effect in mice (Volke *et al*., 2003; Kurt *et al*., 2004). Further, L-Arginine (100 mg/kg, i.p.) is assumed to increase the synthesis of NO, and has been able to block the anxiolytic-like effect of diazepam (Volke *et al*., 1998). In the present study, L-Arginine (100 mg/kg, i.p.) significantly attenuated the noted anxiolytic- like activity of amino guanidine (50 mg/kg, i.p.) in stressed mice. L-Arg (100 mg/kg, i.p.) reversed the protective effect of withaferin (Khan and Ghosh, 2011), which is reported to exert its action by inhibition of NOS under conditions of immobilization stress, as utilized in the present study. Moreover, L-Arginine, at even lower dose (50 mg/kg, i.p.) served to reverse the anxiolytic activity of hesperidine, which is also observed to show its activity through nitriergic inhibition under similar conditions of 6h immobilization stress as utilized in the present study (Vishwanatha *et al*., 2012).

This attenuation of anxiolytic- like activity of amino guanidine is accompanied by a significant increased in the plasma cGMP levels in stressed mice, treated with combinations of AG and L-Arg. cGMP is involved as a mediator of NO induced anxiety (Eroglu and Caglayan, 1997). An increase in intracellular cGMP results in anxiogenic like effect. It is noteworthy that L-Arginine (50 mg/kg, *i.p.*) augments the anxiogenic activity of sildenafil, a cGMP facilitator by inhibiting the PDE5 activity. The anxiogenic-like activity of this combination can be explained by its influence on NO-cGMP signalling pathway, which has been held responsible for alternation behaviour in hippocampus, an important area involved in anxiety (Kurt *et al*., 2004; Milman and Arnold, 2002). In our study, MB, an inhibitor of sGC; an enzyme responsible for cGMP synthesis, significantly enhanced the antianxiety- like activity of amino guanidine (50 mg/kg, i.p.), an inhibitor of iNOS. This observation is supported by earlier observations that MB serve to potentiate the antianxiety- like activity of thymoquinone, another inhibitor of iNOS expression (Gilhotra and Dhingra, 2011). Furthermore, both methylene blue (Kurt *et al*., 2004) and amino guanidine (Gilhotra and Dhingra, 2009) are reported to reverse the anxiogenic effect of Sildenafil (1mg/kg). This enhancement of anxiolytic- like activity of amino guanidine by MB is accompanied by a significant decrease in the plasma cGMP levels in stressed mice, treated with combinations of AG and MB. Administration of Bay - 607550, a selective inhibitor of PDE2 (3mg/kg) increased cGMP levels (Masood *et al*., 2008). In the present study, Bay-60-7550 failed to produce any significant change in the noted antianxiety- like activity of amino guanidine. Similar results were obtained with sildenafil, where amino guanidine suppressed the anxiogenic activity of sildenafil, another facilitator of cGMP (Gilhotra and Dhingra, 2009). 8-Br-cGMP, a cell permeable cGMP analogue mimic the effects of NO donors and exert an anxiogenic activity (Baretta *et al*., 2001). In the present study, amino guanidine, an agent working at the levels of synthetic enzyme of NO i.e. iNOS, has been observed to suppress anxiogenic behaviour of 8-Br-cGMP, known to be mediated via cGMP signalling. These observations with amino guanidine and cGMP modulators are supported by an evidence that effects of SNAP, a NO donor and known to reduce GABAergic current frequency via NO-cGMP pathway, are mimicked by 8-Br-cGMP (a cGMP analogue) and are blocked by a NO scavenger (Lee, 2009).

Conclusions

These results suggest that manifestation of anxiety behaviour in stressed mice is mediated by iNOS-NO-cGMP pathway. Further, it may be concluded that amino guanidine may attenuate stress- induced anxiety not only by iNOS inhibition, but is also capable of modulating iNOS-NO-cGMP cascade.

Abbreviations

MB: Methylene blue; **PDE**: phosphodiesterase; **8-Br-cGMP:** 8-bromo-cGMP; **NOS**: nitric-oxide synthase

REFERENCES

Adamec, R. E. Sayin, U. And Brown, A. 1991. The effects of corticotrohic releasing factor (CRF) and handling stress on behavior in elevated plus maze test of anxiety. *J Psychopharmacol,* 5, 175-86.

Alobnetti, M. E. and Farabollini, P. 1992. Behavioral responses to a single and repeated restraint in male and female rats. *Behav Proc,* 28, 97-110.

Baretta, I. P., Assreuy, J. and De Lima, T. C. M. 2001. Nitric oxide involvement in the anxiogenic-like effect of substance P. *Behav Brain Res,* 121, 199–205.

Bartos, J. and Pesez, M. 1979. Colorimetric and fluorimetric determination of steroids. *Pure Appl Chem,* 51, 2157–69.

Belzung, C. and Griebel, G. 2001. Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res*, 125, 141–9.

Bender, A. T. and Beavo, J. A. 2006. Cyclic Nuclotides Phosphodiesterases: molecular regulation to clinical use. *Pharm Rev,* 58, 488-520.

Bhattacharya, S. K. and Bhattachatyya, D. 1982. Effect of restraint stress on rat brain serotonin. *J Biosci,* 4, 269–74.

Boess, F. G., Hendrix, M., van der Staay, F. J., Erb, C., Schreiber, R., van Staveren, W., de Vente, J., Prickaerts, J., Blokland, A. and Koenig, G. 2004. Inhibition of phosphodiesterase 2 increases neuronal cGMP, synaptic plasticity and memory performance. *Neuropharmacology,* 47, 1081–92.

Britton, K. T., McLeod, K. G. F. and Hauger, R. 1992. Pregnane steroid alphaxelone attenuates anxiogenic behavioral effects of corticotrophic releasing factor and stress. *Pharmacol Biochem Behav,* 41, 399-403.

Calatayud, F., Belzung, C. and Aubert, A. 2004. Ethological validation and the assessment of anxiety-like behaviours : methodological comparison of classical analyses and structural approaches. *Behav Proc,* 67, 195–206.

Callahan, L. B. Tschetter, K. E and Ronan, P. J. 2013. Inhibition of corticotropin releasing factor expression in the central nucleus of the amygdale attenuates stress-induced behavioural and endocrine responses. *Front Neurosci,* 7, 1-11.

Dunn, R. W., Reed, T. A., Copeland, P. D. and Frye, C. A. 1998. The nitric oxide synthase inhibitor 7 nitroindazole displays enhanced anxiolytic efficacy without tolerance in rats following subchronic administration. *Neuropharmacology*, 37, 899-904.

Eroglu, L. and Caglayan, B. 1997. Anxiolytic and antidepressant properties of methylene blue in animal models. *Pharmacol Res,* 36, 381–5.

Esch, T., Stefano, G. B., Fricchione, G. L. and Benson, H. 2002. The role of stress in neurodegenerative diseases and mental disorders. *Neuro Endocrinol Lett,* 23, 199–208.

Faria, M. S., Muscará, M. N., Moreno Júnior, H., Teixeira, S. A., Dias, H. B., De Oliveira, B., Graeff, F. G. and De Nucci, G. 1997. Acute inhibition of nitric oxide synthesis induces anxiolysis in the plus maze test. *Eur J Pharmacol*, 323, 37-43.

Friebe, A. and Koesling, D. 2003. Regulation of nitric oxide–sensitive guanylyl cyclase. *Circ Res,* 93, 96-105.

Gilhotra, N. and Dhingra, D. 2009. Involvement of NO-cGMP pathway in anti-anxiety effect of aminoguanidine in stressed mice. *Prog Neuropsychopharmacol Biol Psychiatry,* 33, 1502–7.

Gilhotra, N. and Dhingra, D. 2011. Thymoquinone produced antianxiety-like effects in mice through modulation of GABA and NO levels. *Pharmacol Rep,* 63, 660-9.

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnock, J. S. and Tannenbaum, S. R. 1982. Analysis of nitrate, nitrite, and [N–15N]-labelled nitrate in biological fluids. *Anal Biochem,* 126, 131–8.

Griffiths, M. J., Messent, M., Curzen, N. P. and Evans, T. W. 1995. Aminoguanidine selectively decreases cyclic GMP levels produced by inducible nitric oxide synthase. *Am J Respir Crit Care Med,* 152, 1599–1604.

Hata, T., Nishikawa, H., Itoh, E. and Funakami, Y. 2001. Anxiety like behavior in elevated plus maze test in repeatedly cold stressed mice. *Jpn J Pharmacol,* 85, 189-96.

Heinrichs, S. C., Pich, E. M., Miczeck, K. A., Britton, K. T. and Koob, G. F. 1992. Corticotrophic releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. *Brain Res,* 581, 190-7.

Ida, Y., Tanaka, M., Tsuda, A., Kohno, Y., Hoaki, Y., Nakagawa, R., Iimori, K. and Nagasaki, N. 1984. Recovery of stress-induced increases in noradrenaline turnover is delayed in specific brain regions of old rats. *Life Sci*, 34, 2357-63.

Iukhananov, R., Rozhanets, V. V., Mikhaleva, I. I. and Maĭskiĭ, A. I. 1990. Analysis of the mechanism of the stress-protective action of delta sleep-inducing peptide. *Bull Eksp Biol Med*, 109, 46-7.

Jang, S., Suh, S. H., Yoo, H. S., Lee, Y. M. and Oh, S. 2008. Changes in iNOS, GFAP and NR1 expression in various brain regions and elevation of sphingosine-1-phosphate in serum after immobilized stress. *Neurochem Res,* 33, 842–51.

Klandorf , H., Zhou, Q. and Sams, A. R. 1996. Inhibition by aminoguanidine of glucose-derived collagen cross-linking in skeletal muscle of broiler breeder hens. *Poult Sci*, 75, 432-7.

Korte, S. M. and De Boer, S. F. 2003. A robust animal model of state anxiety: fear potentiated behaviour in the elevated plus maze. *Eur J Pharmacol,* 463, 163-75.

Kurt, M., Bilge, S. S., Aksoz, E., Kukula, O., Celik, S. and Kesim, Y. 2004. Effect of sildenafill on anxiety in the plus-maze test in mice. *Pol J Pharmacol,* 56, 353–57.

Lee, C. Y., Cheng, H. M. and Sim, S. M. 2007. Mulberry leaves protect rat tissues from immobilization stress-induced inflammation. *Biofactors*; 31:25–33.

Lee, F. Y., Wang, S. S., Tsai, Y. T., Lin, H. J., Lin, H. C., Chu, C. J., Wu, S. L., Tai, C. C. and Lee, S. D. 1997. Aminoguanidine corrects hyperdynamic circulation without ameliorating portal hypertension and portal hypertensive gastropathy in anesthetized portal hypertensive rats. *J Hepatol,* 26, 687-93.

Lee, J. J. 2009. Nitric oxide modulation of GABAergic synaptic transmission in mechanically isolated rat auditory cortical neurons. *Korean J Physiol Pharmacol,* 13, 461-7.

Madrigal, J. L. M., Olivia, H. O., Moro, M. A., Lizasoain, I., Lorenzo, P. and Castrillo, A. 2002. The increase in TNF α Levels is implicated in NF-κB activation and inducible nitric oxide synthase expression in brain cortex after immobilization stress. *Neuropsychopharmacology,* 26, 155–63.

Masood, A., Nadeem, A., Mustafa, S. J. and O'Donnell, J. M. 2008. Reversal of oxidative stress-induced anxiety by inhibition of phosphodiesterase-2 in mice. *J Pharmacol Exp Ther,* 326, 369–79.

McEwen, B. S. 2000. Allostasis and allostatic load: Implication for neuropsychopharmacology. *Neuropsychopharmacology,* 22, 108-24.

Milman, H. A. and Arnold, S. B. 2002. Neurologic, psychological, and aggressive disturbances with sildenafil. *Ann Pharmacother,* 36, 1129–34.

Misko, T. P., Moore, W. M., Kasten, T. P., Nickols, G. A., Corbett, J. A. and Tilton, R. G. 1993. Selective inhibition of the inducible nitric oxide synthase by aminoguanidine. *Eur J Pharmacol,* 233, 119–25.

Nagao, K., Takenaka, S., Yamaji, R., Inui, H. and Nakano, Y. 2003. Nitric oxide synthase induction, cGMP elevation, and biopterin synthesis in vascular smooth muscle cells stimulated with interleukin-1beta in hypoxia. *J Biochem,* 133, 501–5.

Olivenza, R., Moro, M. A., Lizasoain, L., Lorenzo, P., Fernández, A. P. and Rodrigo, J. 2000. Chronic stress

induces the expression of inducible nitric oxide synthase in rat brain cortex. *J Neurochem,* 74, 785–91.

Rodgers, R. J., Haller, J., Holmes, A., Halasz, J., Walton, T. J. and Brain, P. F. 1999. Corticosterone response to the plus-maze: high correlation with risk assessment in rats and mice. *Physiol Behav*, 68, 47-53.

Schwartz TL, Nihalani N, Simionescu M, Hopkins G. History repeats itself: pharmacodynamic trends in the treatment of
anxiety disorders. Curr Pharm Des. 2005;11:255–63.

Sevgi, S., Ozek, M. and Eroglu, L. 2006. L-NAME prevents anxietylike and depression-like behavior in rats exposed to restraint stress. *Methods Find ExpClinPharmacol,* 28,95-9.

Snyder, S. H. and Bredt, D. S. 1997. Nitric oxide as a neural messenger. *Trends Pharmacol Sci,* 12, 125–8.

Southan, J. G. and Szabo, C. 1996. Selective pharmacological inhibition of distinct nitric oxide synthase isoforms. *Biochem Pharmacol,* 51, 383–94.

Tsuchiya, T., Kishimoto, J., Koyama, J. and Ozawa, T. 1997. Modulatory effect of L-NAME, a specific nitric oxide synthase (NOS) inhibitor, on stress- induced changes in plasma adrenocorticotropic hormone and corticosterone levels in rats: physiological significance of stress- induced NOS activation in hypothalamic–pituitary–adrenal axis. *Brain Res,* 776, 68–74.

Viswanatha, G. L., Shylaja, H., Rao, K. S. S., Kumar, V. R. S. and Jagadeesh, M. 2012. Hesperidin ameliorates immobilization-stress-induced behavioral and biochemical alterations and mitochondrial dysfunction in mice by modulating nitrergic pathway. *ISRN Pharmacology,* 2012, 1-8.

Volke, V., Soosar, A., Koks, S., Bourin, M., Mannisto, P. T. and Vasar, E. 1997. 7-Nitroindazole, a nitric oxide synthase inhibitor, has anxiolytic-like properties in exploratory models of anxiety. *Psychopharmacology,* 131, 399–405.

Volke, V., Wegener, G., Bourin, M. and Vasar, E. 2003. Antidepressant- and anxiolytic-like effects of selective neuronal NOS inhibitor 1-(2-trifluoromethylphenyl)-imidazole in mice. *Behav Brain Res,* 140, 141–7.

Volke, V., Soosaar, A., Kõks, S., Vasar, E. and Männistö, P. T. 1998. L-arginine abolishes the anxiolytic-like effect of diazepam in the elevated plus-maze test in rats. *Eur J Pharmacol*, 351, 287-90.

Werner, C., Raivich, G., Cowen, M., Strekalova, T., Sillaber, I., Buters, J. T., Spanagel, R. and Hofmann, F. 2004. Importance of NO/cGMP signalling via cGMP-dependent protein kinase II for controlling emotionality and neurobehavioural effects of alcohol. *Eur J Neurosci,* 20, 3498–3506.

****