

Short communication

Effect of restraint stress on nociceptive responses in rats: role of the histaminergic system

***Ibironke G F and Mordi N E**

Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Summary: Stress induced analgesia (SIA) is well known, but the reverse phenomenon, hyperalgesia is poorly documented. This study investigated the role of the histaminergic system in restraint stress hyperalgesia in rats, using thermal stimulation method (hot plate and tail flick tests). Paw licking and tail withdrawal latencies were taken before and after restraint for about one hour. Significant decreases ($p < 0.05$) were obtained in these latencies after the restraint in both tests. Administration of H1 and H2 receptor blockers, chlorpheniramine and cimetidine respectively 30 mins before the restraint still resulted in significant ($p < 0.05$) reductions in these latencies, connoting the persistence of hyperalgesia, showing that histamine H1 and H2 receptors did not participate in the mechanism of restraint stress hyperalgesia. We therefore suggest a histaminergic independent mechanism for restraint stress induced hyperalgesia.

Keywords: Restraint stress, Thermal stimulation, Hyperalgesia, Histamine.

©Physiological Society of Nigeria

*Address for correspondence: gibironk@yahoo.com

Manuscript Accepted: July, 2011

INTRODUCTION

Stress can have bilateral effects on pain related phenomena. Firstly, acute stress can produce analgesia in humans and animals (Amit and Galina, 1986). Secondly, stress has also been reported to produce hyperalgesia (increased sensitivity to painful stimulus) or allodynia (pain triggered by innocuous stimuli). It appears that the repetitive nature of the stress seems to favour the induction of hyperalgesia/allodynia in rodents (Fishbain et al, 2006). Hence the repeated exposure to loud sounds, cold environment, restraint, swim, or sleep deprivation potentiates painful perception in rats. However, acute stress produced by non-noxious stimuli (handholding, vibration, novel environment, etc) can also trigger hyperalgesia (Imbe et al, 2006). In humans too, exposure to chronic stress may increase pain sensitivity and reduce pain threshold (Caceres and Burns, 1997).

In laboratory studies (Caceres and Burns, 1997) reported that human volunteers exposed to stress before a cold pressor test had lower pain tolerance and a decrease in pain threshold. At the clinical level, stress has a major impact on major pathologies, for example patients suffering from chronic back pain, or cancer induced arthritic pains are known to report

higher levels of suffering during stressful episodes (Conrad et al, 2007, Fishbain et al, 2006). Numerous pre-clinical models have been developed to reproduce various pain modalities that are encountered in the clinic, however, animal studies assessing the interrelationship between stress and hyperalgesia/allodynia are rather scanty, despite the close relationship between stress and pain observed in the clinic and even worse still, the mechanism by which stress exerts its hyperalgesic effect is still largely unknown. This study therefore, evaluated in rats, the impact of the histaminergic mechanism on stress induced hyperalgesia using the restraint method.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (150-220g) purchased from the pre-clinical animal house of the College of Medicine, University of Ibadan were used for the study. They were housed in a room where light was maintained on a 12-h light, 12-h dark cycle with food and water *ad libitum*. The rats were allowed to acclimatize to the laboratory environment for about two weeks before the commencement of the study.

Drugs

Chlorpheniramine (0.15mg/kg) and cimetidine (0.15mg/kg) purchased from Crown Chemist a local pharmaceutical outfit in the city of Ibadan, Nigeria were administered intraperitoneally (i p).

Hot plate test

The original method of Eddy and Leimbach (1953) as modified by Ibironke et al (2004) was used. The hot plate temperature was maintained at $52 \pm 2.0^{\circ}\text{C}$ and a cut off time of 60 seconds was imposed to avoid tissue damage. Pain sensitivity was evaluated by the response latency for paw licking on the hot plate. The latency was measured twice at 15 minutes interval and the average calculated.

Tail flick test

Nociception was assessed by a modification of D'Amour and Smith (1941)'s tail flick method. The test is highly sensitive and reproducible. Each animal was gently hand-held with the terminal 3cm of the tail immersed in water maintained at $50 \pm 1^{\circ}\text{C}$. The time taken for the animal to flick its tail out of water (tail withdrawal latency) was noted and recorded.

Restraint test

The animals were restrained in a perspex tube measuring 18cm long and 5cm radius with a wire mesh at both ends for adequate ventilation.

The first group of animals (n=6) had their tail withdrawal and paw licking latencies measured. They were then restrained for one hour immediately after which the tail withdrawal and paw licking latencies were again measured.

The second and third groups of animals (n=6each) went through the same procedure except that they received 0.15mg/kg chlorpheniramine and 0.15 mg /kg cimetidine (Oyadeyi et al ,2006) respectively 30 mins before they were restrained after which the paw licking and tail withdrawal latencies were measured.

Statistical analysis

Results were presented as mean $\pm$ SEM, differences were evaluated using the student's t-test, a value of $p < 0.05$ was regarded as significant.

RESULTS

The results are as shown in tables 1 and 2

Hot plate test Table 1

There was a significant fall in the paw licking latency in the control group after the restraint. This denotes hyperalgesia. Also in the two groups treated with chlorpheniramine and cimetidine before restraint, the

hyperalgesia still persisted after the restraint showing that the histamine receptor blockers have no effect.

Tail flick test

The same trend was observed as in the hot plate test i.e. persistence of hyperalgesia despite pre-treatment with the histamine receptor blockers

Table 1.

Effect of restraint stress on paw licking latencies in the hot plate test

	Before restraint	After restraint
A	11.3 $\pm$ 0.86	5.33 $\pm$ 0.78*
B	10.70 $\pm$ 0.27	6.01 $\pm$ 0.033*
C	12.09 $\pm$ 0.67	8.33 $\pm$ 0.50*

Values are means $\pm$ SEM, n = 6; A, control; B, chlorpheniramine; C, cimetidine; * $P < 0.05$ (before vs. after restraint)

Table 2.

Effect of restraint stress on tail withdrawal latencies in the tail flick test

	Before restraint	after restraint
A	3.83 $\pm$ 0.19	1.19 $\pm$ 0.10*
B	3.49 $\pm$ 0.16	1.21 $\pm$ 0.16*
C	2.44 $\pm$ 0.29	1.01 $\pm$ 0.15*

Values are means $\pm$ SEM, n = 6; A, control; B, chlorpheniramine; C, cimetidine; * $P < 0.05$ (before vs. after restraint)

DISCUSSION

This study is to our knowledge, the first to assess the role of the histaminergic system in restraint stress hyperalgesia. Our findings have ruled out the possibility of any role for histamine H1 and H2 receptors; however these findings could not be compared with any previous study as stated earlier for obvious reasons.

Our results using the thermal test on restraint stress hyperalgesia is in agreement with the observations of Pilcher et al (1983) using the paw pressure test. The possibility of the result being test dependent is therefore made invalid.

However, our findings on thermal hyperalgesia is in contrast with some other reports (Da Silver et al, 2003; Gamaro et al, 1998) using rats submitted to a restraint stress and tested in a tail flick procedure. These later studies used a light source to trigger hyperalgesia instead of hot water used in this study. This difference in protocol might play a primary role in the outcome of stress on this type of nociception, it might be that the more localized nociceptive stimulus produced by a light source presents a higher sensitivity to the impact of stress.

Also, in our own protocol, rats were gently hand held and the tail immersed in water, whereas in the study of Da Silva et al (2003), the rats were wrapped in a

towel and the tail placed under the light source. It is possible that wrapping produced an additional type of restraint stress that might further account for the differences.

Further studies are still warranted to shed more light on the exact mechanism by which restraint stress causes hyperalgesia.

Concluding, this study showed that restraint stress causes hyperalgesia the mechanism of which might not be histaminergic dependent.

REFERENCES

- Amitz Z, Galina H (1986). Stress induced analgesia: adaptive pain suppression *Physiol Rev* 66: 1091-1120.
- Caceres C, Burns JW (1997). Cardiovascular reactivity to psychological stress may enhance subsequent pain sensitivity. *Pain*; 69: 237-44.
- Conrad R, Schilling G, Bausch C, Nadstewak J, Watenbery HC, Wegener I (2007). Temperament and character personality profiles and personality disorders in Chronic pain patients. *Pain* 133:197-209.
- D'Amour F E, Smith DA (1941). A method for determining loss of pain sensation. *J Pharmacology and Exper Ther* .72:149-151.
- Da Silva T H, Bonan C D, Crema L, De Leone N M, Batastini A M, Sarkis H (2003). Long lasting delayed hyperalgesia after chronic restraint stress in rats: Effects of morphine administration *Neurosci Res* 2003; 45 :277-83
- Eddy N B, Leimbach D (1953). Synthetic analgesics II: Diathianyl and Diathienylbutylamines. *J Pharmacol* 107; 385 -389
- Gamaro C D, Xavier M L, Denardin J D, Pilger J A, Ely D R, Ferreira M B (1998). Effects of acute and repeated restraint stress on the nociceptive response in rats. *Physiol Behav.*;63: 693-97.
- Ibrinke G F, Saba O J, Olopade F O (2004). Glycemic control and pain threshold in alloxan diabetic rats. *Afr J. Biomed. Res* 7: 149-151.
- Imbe H, Iwai-Liao Y, Semba E (2006). Stress induced hyperalgesia: animal models and putative mechanisms. *Front Biosci*, 11:2179-92.
- Oyadeyi AS, Ajao F O, Ibrinke G F, Afolabi AO (2006). Acute restraint stress induces hyperalgesia via nonadrenergic mechanism in rats. *Afr J Biochem Res* vol 8, 123-125.
- Pilcher C W, Brow J L (1983). Effect of naloxone and Mr1452 on stress induced changes in nociception of different stimuli in rats. *Life Sci* 33:697-700