Linear Relationship Exists Between Plasma Testosterone Level And Acute Pain Reaction Time In Male Rat

O Oyekunle, G Ibironke, S Ige, U Udoh, A Okojie

Citation

O Oyekunle, G Ibironke, S Ige, U Udoh, A Okojie. *Linear Relationship Exists Between Plasma Testosterone Level And Acute Pain Reaction Time In Male Rat*. The Internet Journal of Pain, Symptom Control and Palliative Care. 2009 Volume 8 Number 1.

Abstract

The choice of experimental animal in pain research over the years has been based on weight and in most cases taken little cognizance of the role played by sex hormones and reproductive stage of the experimental animal. This study was carried out to know the ideal quality upon which the choice of experimental animal in pain research should be based whether weight age or sex and the role played by sex/gonadal hormones in the perception of pain. Eight male prepubertal (about 4 weeks old) rats of Wistar strain were used to evaluate pain reaction time using both tail flick and hot plate model of evaluation and blood samples taken to determine the plasma testosterone concentration in all the animals before the tests. The same sets of animals were kept for the next 4 weeks under the same conditions. The experiment was repeated and pain reaction time was also evaluated in the pubertal 8 weeks old rats with blood samples collected to determine the plasma testosterone level. The results showed a significant ($p<.01$) increase in pain reaction time with corresponding significant ($p<.01$) increase in plasma testosterone level in both models of evaluation. The pubertal rats tolerated well the stimuli and subsequent increase in pain reaction time. Therefore age and sex remained important factors of consideration in the choice of animal in pain experiments.

INTRODUCTION

Expanding literatures indicate that gender is an important influence on the experience of pain. Authors who have reviewed studies of clinical and laboratory pain [1, 2, 3, 4] have generally concluded that females and males differ in their perception and experience of pain. Reviewers, such as Rollman et al [3] have also concluded that female typically report greater sensitivity to and less tolerance for experimentally induced noxious stimulation than males do. Experimental and clinical data demonstrate that gonadal hormones affect pain-induced response [4, 5, 6]. There could be several reasons for the differences recorded in pain reactivity between male and females, from genes to hormonal and cultural influences. These gonadal hormones have their receptors present in many brain areas including some involved in pain transmission and modulation. Testosterone as a major gonadal hormone in mature males has been known to play a protective role such as in adjuvant-induced arthritis [7] and in chronic pain stimulation [8]. Male rats with physiological level of testosterone recovered better than gonadectomized males with very low testosterone levels.

Exogenous testosterone injection (testosterone propionate) in male and female rats was found to modulate behavioral responses differently in both sexes when evaluated with chronic model of pain. It has been reported to influence all the organization of behaviour, decreasing the entropy of the behaviour which is connected with a precipitation of the process of inhibition of the exploratory activity and emotional reactivity, simultaneously changing the dynamics of the behavioural entropy [9]

In this study we investigated the relationship between the plasma testosterone concentration and pain reaction time in both immature and mature male rats using two experimental models of acute pain i.e hot plate test and tail flick test.

MATERIALS AND METHODS

ANIMALS

Prepubertal male rats (4 wks old) were used for the study, 8 in number. Animals were housed in the preclinical animal house of physiology department Ladoke Akintola University of Technology which was maintained at the temperature of about 25□□□□□1c with an alternating 12h light-dark cycle. Food and water were made available ad libitum and all

experiments were carried out between 9:00 and 12:00h.

HOT PLATE

Rats were placed on a hot plate maintained at 55.5□□□□0.5c of temperature according to the procedure described by Eddy and Liemback [10]. The reaction time(s) was measure by either jumping off the plate or hind paw licking. The cut-off imposed was 60s to avoid tissue damage [11]. Each rat was tested twice and the reaction times were averaged to obtain a baseline.

TAIL FLICK

The tail-flick was evoked by a source of radiant heat which was focused on the dorsal surface of the tail according to the procedure described by D' Amour and Smith [12] and modified by Dewey et al [13] each rat was tested twice and the reaction time was averaged to obtain a baseline. The intensity of the heat stimulus was adjusted so that the rat flicked its tail within 10 second. This cut-off time of 10 seconds was imposed to prevent tissue damage.

TESTOSTERONE DETERMINATION

Blood was collected from the rats from the middle vain in their ear. The blood was centrifuged at 3000rmp for 3 to 5minutes. Enzyme linked immunosorbent assay, Microwell method was used to determine testosterone (Cat.No.K00234, Dialab, Austria). Procedure Take 25UL of calibrator into wells, take 25UL of sample in their wells also. Add 100UL of enzyme conjugate to both calibrators and samples, mix and incubate at 37 degree Celsius for 1 hour. Wash 3 times after incubation with distilled water. Add 100UL of substrate to both calibrator and sample wells. Mix and incubate in the dark for 15 minutes at 20 to 25 degree Celsius. Stop reaction by adding 100UL of stop solution. Read optical density at 450nm against reagent blank.

DATA ANALYSIS

The data were expressed as mean deviation and tested by paired student's t-test. Statistical significance was considered as p-value < 0.01 for all tests.

RESULTS

TAIL FLICK

Figure 1

Table 1

	4wks	8wks	d	Mean d	S.E d
Average reaction time (Seconds)	37.73	74.62	-36.89	4.61	0.23*
Plasma testosterone (nmol/L)	11.20	39.10	-27.90	3.49	0.20*

* P<0.01

HOT PLATE

	4wks	8wks	d	Mean d	S.E d
Average reaction time (Seconds)	37.73	186.9	-149.17	18.65	0.65*
Plasma testosterone (nmol/L)	9.50	9.70	-10.20	1.30	0.23*

* P<0.01

Pain reaction time and plasma testosterone concentration in immature and matured male rats.

Table 1 showed the summary statistics of S.E (d) using paired student t- test for tail flick procedure. The plasma testosterone observed in the prepubertal 4 week old was due to its secretion from other source other than the testis. There is a significant difference ($p<0.01$) in both plasma testosterone and pain reaction time between prepubertal and pubertal rat when both were compared. As the animal increases in age, reproductive organs matures as they transit from immature to matured rat and subsequent increase in plasma testosterone level, pain reaction time also increase in a linear fashion.

Table 2 showed the summary statistics obtained from hot plate test. Also there is a significant ($p<0.01$) increase in pain reaction as plasma testosterone concentration also increased significantly ($p<0.01$). Therefore, as the animal transit from immature to mature stage (4 to 8 wks) plasma testosterone concentration increases and pain reaction time increases as well in linear fashion indicating a kind of inhibition or tolerance as a result of the presence of increased plasma testosterone.

DISCUSSION AND CONCLUSION

Experimental and clinical data have implicated the presence of sex hormone in most parameters affected by painful stimulation [14]. Their have been increasing evidences that the brain not only respond to hormones produced by the

reproductive system, but that these hormones the so-called 'female hormone' estrogen and progestin and the 'male' androgen such as testosterone play important roles in the perception and brain modulation of pain [15]. Our investigation focuses on the differences in the perception of pain or pain reaction time in both immature and mature stage of same male rat. The initial little insignificant testosterone observed in immature rats in our study indicates testosterone from other some other than the test such as the adrenal [16]. The matured male rats well tolerated the stimuli from both models of evaluation thereby increasing the pain reaction time recorded from them [17]. The presence of testosterone in a significant amount offered this inhibition or tolerance in matured males. The observation that testosterone has a protective role is supported by the previous demonstration that testosterone can reduce the intensity of nociception by inhibiting its inflammatory component [18]. Our results also agree with the results obtained by Aloisi and colleague [19] and Hau and colleagues [20] who showed a protective role for testosterone in their various studies. From our studies we observed a linear relationship between plasma testosterone concentration and pain reaction time which is agreement with earlier studies [20] except that our data are collected in same animal at different stage of sexual development. Also testosterone seems to be responsible for an 'habituation' capacity in intact males during repetitive nociceptive stimulations that is lost in castrated males [8], this is in agreement with our results which were obtained naturally from the animals.

Furthermore, it is important to point out that the role of testosterone in pain has too often been neglected, our result support the crucial role for testosterone in the reduction of nociceptive responses observed in intact animals, some studies already show that testosterone is responsible for the reduction of some clinical pain in both men [21,22] and women[5].

Finally, this study highlights the importance of taken into account the hormonal status of experimental animals when evaluating pain perception and/or pain inhibition. Therefore the choice of experimental animals for pain experiment should be based on age rather than weight which have become a norm over the years having seen clearly the role played by sex hormones in pain perception. Therefore using immature animals will be most appropriate.

References

1. Fillingim, R. B. Sex, Gender and Pain: Progress in pain research and Management (2000) Seattle: International Association for the study of pain.
2. Riley, J. L., Robinson, M. F., Wise, E. A., Myers, C. D., and Fillingim, R. B. Sex Differences in the perception of noxious experimental stimuli. A meta-analysis. Pain, 74 (1998) 181-187.
3. Rollman G. B., Lautenbacher, S., and Jones K. S; Sex and Gender differences in responses to experimentally induced pain. In R. B. Fillingim (Ed) sex, gender and pain, progress in pain research and management (2000) pp. 165-190 Seattle; International Association for the study of pain.
4. Unruh A. M; Gender Variations in Clinical Pain Experience. Pain 65, (1996) 123-167
5. Desstein P.H, Shipton E. A, Doffe B. I, Hadebe D. P., Stanwix A. E, Van der Merwe B. A; Hyper secretion of Adrenal androgens and the relation of serum adrenal steroids, serotonine and insulin-like growth factor 1 to clinical feature in women with fibromyaglia. Pain 83 (1999) 313-319.
6. Peduckar J. R; Mulgonker V. K, Role of Testosterone on pain threshold in rats. Indian J. Physiology pharmacol 39 (1995) 423-424
7. Harbuz M. S., Perveen-Gill S. L, Lightman D. S., Jessop A. Protective role of testosterone in adjuvant - induced arthritis. Br. J. Rheumatol 34 (1994) 1117-1122.
8. Ceccarelli, I., Scaramizzino C., Massafra C, Aloisi A.M. The behavioural and neuronal effects induced by repetitive nociceptive stimulation are affected by gonadal hormones in male rats. Pain 104 (2003) 35-47.
9. Lambadjieva N. D., Influence of testosterone on some behavioral reactions of male immature rats. Methods Find. Exp. Clin. Pharmacol (1998), 20 (8) pp 673
10. Eddy N. B. and Liemback D. (1953) Synthetic Anagesic II Dithenylbatenyl and dithienylbutylamines. J. Pharmacol. 221:171-174
11. Forreira S. H., Duarte I. D. G and Lorezentti B.B; The Molecular Mechanism of action of peripheral morphine analysis: Stimulation of the GMP system via metric oxide release. Eur. J. Pharmacol 201: (1991) 74-79
12. D' amour F.E. and Smith,D.L.; A method for determination of pain sensation. J. Pharmacol. Exp. Ther. 72 (1941) 72-79
13. Dewey, W. L, Harris L. S, Howes J. F, Nuite J. A. The effect of various neurohumoral modulations on the activity of morphine and the narcotic antagonists in the tail flick and phenylquinone tests. J. Pharmacol Exp. Ther 175 (1970) 435-442
14. Berkely K. J., Sex Difference in Pain. Behav.Brain Sc. 20(1997) 371-380.
15. Stoffel E. C., Ullibanri C.M and Craft Q. M; Gonadal Steriod hormone modulation of nociception, morphine antinociception and reproductive indices in male and female rats. Pain 103 (2003) pp 285-302
16. Ganoong, W.F; Review of Medical Physiology, 18th Edition, pp 402-403.
17. Hau, M., Dominguez O. A. and Evrand H. C; Testosterone reduces responsiveness to nociceptive stimuli in a wild bird Horm. Behav. 46 (2004) pp 165-170.
18. Dasilva J. A. P., Peers S. H., Perretti M and Willoughby D. A., Sex steroids affect glucocorticoidd response to chronic inflammation and to interleukin-1, J. Endocrinol 136 (1993) pp 389-397
19. Aloisi A. M., Ceccarelli I., Fiorenzani P., De Padova A. M. and Massafra, C; Testosterone affects formalin-induced responses differently in male and female rats. Neurosci Lett 361 (2004) pp 262-264.
20. Mogil J. S., Chester E. J., Wilson S. G. Jurasaka J. M.

and Sternberg W. G; Sex differences in thermal nociception and morphine antinociception in rodent depends on genotype. Neurosci Biobeva Rev. 24 (2000) pp 375-389
21. Isaacs W. A. Effiong C. E. and Ayeni O., Steriod treatment in the prevention of painful episode in sickle-cell disease, Lancet 1 (1972) Pp 570-571
22. English K. M., Steeds R. P., Jones T. H., Dive M. F and Channer, K.S.; Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: a randomized, double-blind,placebo-controlled study, Circulation 102 (2000), pp1906-1911.

Author Information

OA Oyekunle
Department of physiology, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

GF Ibironke
Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan Oyo State, Nigeria

SF Ige
Department of physiology, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

US Udoh
Department of physiology, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

AK Okojie
Department of Physiology, Faculty of Basic Medical Sciences, University of Benin, Edo State, Nigeria.