UDC 577.17+612.884

O. A. OYEKUNLE[1], G. FIBIRONKE[2], S. F. IGE[1], U. S. UDOH[1], AND A. K. OKOJIE[1]

RELATIONSHIP BETWEEN THE PLASMA TESTOSTERONE LEVEL AND PAIN REACTION TIMES IN MALE RATS

Received 10.01.09

Male prepubertal (about 4 weeks old) Wistar rats were used to estimate the pain reaction times using the tail-flick and hot-plate models; the testosterone concentration in all the animals before the tests in the blood plasma was measured. The same sets of animals were kept for the next 4 weeks under standard conditions; the experiment was repeated, and pain reaction times were also evaluated in the 8-week-old rats with blood samples collected to determine the plasma testosterone level. The results showed significant ($P < 0.01$) increases in the pain reaction times in both pain models in pubertal animals observed in a parallel manner with a corresponding significant ($P < 0.01$) increase in the plasma testosterone level. Therefore, age and sex are important factors in the choice of animals in pain experiments.

Keywords: tail flick, hot plate, testosterone, pain reaction time.

INTRODUCTION

Expanding literature indicates that gender is an important factor influencing the experience of pain. Clinical and laboratory observations [1-4] led to a general conclusion that females and males noticeably differ from each other in their perception and experience of pain; females typically showed a greater sensitivity to and less tolerance for experimentally induced noxious stimulation than males did. Experimental and clinical data demonstrated that gonadal hormones significantly affect pain-induced responses [4-6]. There could be a number of reasons for the differences recorded in the pain reactivity between males and females, from dissimilar genes to hormonal and cultural (in humans) influences. Gonadal hormones possess the respective 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 manifested, e.g., in adjuvant-induced arthritis [7] and in chronic pain stimulation [8]. Male rats with a physiological level of testosterone recovered better than gonadectomized males with very low testosterone levels.

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

In this study, we examined the relationship between the testosterone concentration in the blood plasma and pain reaction times using two pain behavioral tests (hot plate and tail flick) in immature and pubertal male rats.

METHODS

Two groups, including 8 prepubertal male rats (4 weeks old) each, were used in the study. Animals were housed under standard vivarium conditions at about 25 ± ± 1°C with an alternating 12 h/12 h light-dark cycle. Food and water were made available *ad libitum*; all experimental manipulations were carried out between 9.00 and 12.00. The same groups of the animals were examined 4 weeks later, i.e., when the animals reached the pubertal age.

Hot Plate. Rats were placed on a hot plate maintained at 55.5 ± 0.5°C according to the described procedure

[1] Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[2] Faculty of Basic Medical Sciences, University of Ibadan, Ibadan. Nigeria.
Correspondence should be addressed to A. K. Okojie
(e-mail: pintos4live@yahoo.com).

[10]. The reaction time was measured from the moment of placing until either jumping off the plate or licking of the hind paw(s). The cut-off imposed for heating was 60 sec, to avoid tissue damage [11]. Each rat was tested twice, with a 20-min-long interval, and the reaction times were averaged.

Tail Flick. The tail-flick reaction was evoked by a source of radiant heat 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 (with a sufficient interval), and the reaction times were also averaged to obtain a baseline. The cut-off time of 10 sec for heating was imposed to prevent tissue damage.

Measuring of the Testosterone Level. Blood samples were collected from the middle vein of the ear and centrifuged at 3000 rev. min for 3 to 5 min. The testosterone level in the blood plasma was measured using standard enzyme-linked immunosorbent assay (Microwell Method, Dialab, Austria) with parallel measurements in the respective calibrators attached to the kit.

RESULTS

In the group of 4-week-old rats subjected to the hot-plate tests, the level of testosterone in the blood plasma was 9.50 ± 0.65 nM, on average. On the 8th week, this index increased more than twofold and reached 19.70 ± 1.40 nM ($P < 0.01$). The mean delay in the reaction to noxious thermostimulation in 4-week-old animals subjected to the above test was 37.73 ± 1.80 sec. In rats reaching the age of pubertation (8 weeks), the mean reaction time in this test was 186.90 ± 8.90 sec, i.e., it was nearly five times longer than in the first measurement ($P < 0.01$).

The observation made in the tail-flick group were quite comparable. The results of testosterone measurements were qualitatively the same but somewhat differed from the quantitative aspect. In the tail-flick group, the mean testosterone level in 4-week-old animals was 11.20 ± 0.56 nM, i.e., it was nearly the same as in the hot-plate group. At 8 weeks, this index increased to 39.10 ± 1.90 nM, i.e. an increase was noticeably greater than in the former group (more than threefold; $P < 0.01$). Yet, such a difference was not unexpected because there was no pre-experimental standardization of the animals according to their secretion capabilities, and individual variability influencing the intergroup mean was quite natural. In the tail-flick test, the average reaction time of 4-week-old animals was 37.75 ± 0.65 sec. In 8-week rats, this time increased twofold (74.62 ± 1.25 sec; $P < 0.01$).

DISCUSSION

Experimental and clinical data have implicated the presence of a sex-hormone factor in most parameters affected by painful stimulation [14]. There has been increasing evidence that the brain not only respond to hormones produced by the reproductive system, but that the levels of these hormones, the so-called "female hormone" estrogen and progestin and the "male" androgen, such as testosterone, play important roles in the perception of pain and its modulation [15]. Our investigation focuses on the differences in the perception of pain (or pain reaction time) in immature and (relatively) mature stages of ontogenesis of the same male rats. The initial rather low (nonetheless, quite noticeable) testosterone concentrations observed in the blood plasma of prepubertal rats in our study are related to testosterone produced within this period mostly by some other sources than the testes, such as the adrenals [16].

The matured male rats well tolerated the stimuli in both experimental models of evaluation thereby increasing the delays of pain behavioral reactions recorded from them [17]. The presence of testosterone in a much more significant amount should be considered one of the main crucial factors responsible for this inhibition (or tolerance) in matured males. The observation that testosterone has a protective role is supported by earlier demonstrations that this hormone can reduce the intensity of nociception by inhibiting its inflammation-related component [18]. Our results also agree with the results obtained by Aloisi et al. [19] and Hau et al. [20] who showed a protective role for testosterone in their various studies. In our experiments, we observed a quite obvious and close relationship between the plasma testosterone concentration and the pain reaction times, which is in agreement with earlier studies [20], except that our data were collected in same animals at different stages of sexual development. Also testosterone seems to be responsible for a habituation capacity in intact males during repetitive nociceptive stimulations, which is lost in castrated males [8]. This is also in agreement with our results.

Of course, the role of other factors influencing changes in the pain sensitivity in maturating animals should not be underestimated. First of all, this is an increase in the mass of the animals and the respective increase in the thickness of the skin on the pads and tail (accompanied by the improvement of thermoisolation

properties). It should, however be taken into account that growth of a maturating animal and changes in the properties of its skin (to say nothing on the peculiarities of the composition and density of somatic receptors of different modalities in these tissues) are also interlinked hormone-dependent phenomena. Different environmental factors and seasonal changes also influence, to a certain extent, the parameters of pain reactions (both experimental and observed in clinics). Nonetheless, the state of the system of the mail sex hormone looks as one of the most important factors influencing the brain systems of nociception and antinociception. As is known, there is abundant distribution of testosterone receptors in the brain. The roles of endogenous mediators of nociception in relation to the testosterone concentration and activation of testosterone receptors may be an urgent circle of questions for further studies.

Furthermore, it is important to point out that the role of the hormonal background (in our case, testosterone) in pain reception has too often been neglected. It was reported that testosterone can provide the reduction of some clinical pain in both men [21, 22] and women [5].

Finally, our observations highlight the importance of taking into account the hormonal status of experimental animals when evaluating pain perception and/or pain inhibition. Therefore, the choice of experimental animals for pain experiments should be based on their age and sex rather than on their weight. It should be recognized that just the latter aspect has become a norm over the years, while the role played by sex hormones in pain perception was underestimated. Therefore, in some cases using immature animals in such experimental studies will be more appropriate.

О. А. Ойєкунле[1], Г. Фібіронке[2], С. Ф. Іге[1], Ю. С. Уго[1], А. К. Окойє[1]

ЗАЛЕЖНІСТЬ МІЖ РІВНЕМ ТЕСТОСТЕРОНУ В ПЛАЗМІ КРОВІ ТА ЛАТЕНТНИМИ ПЕРІОДАМИ БОЛЬОВИХ РЕАКЦИЙ У САМЦІВ ЩУРІВ

[1] Факультет медичних наук, Технологічний університет Ладоке Акінтола, Огбомозо (Нігерія).
[2] Факультет медичних наук, Ібаданський університет, Ібадан (Нігерія).

Р е з ю м е

У щурів-самців препубертатного віку (чотири тижні) вимірювали латентні періоди больових реакцій в умовах тестів «відсмикування хвоста» та «гарячої пластинки»; у всіх тварин перед тестами вимірювалася концентрація тестостерону в плазмі крові. Ці ж самі групи тварин утримувалися протягом чотирьох тижнів у стандартних умовах, після чого експеримент повторювали на восьмитижневих щурах (вимірювали час больових реакцій та рівень тестостерону). Для тварин, що досягли віку статевої зрілості, було характерне істотне ($P < 0.01$) збільшення латентних періодів больових реакцій в обох використаних моделях, що відбувалося паралельно з відповідним вірогідним ($P < 0.01$) збільшенням рівня тестостерону в плазмі. Отже, вік і стать є найважливішими факторами при відборі тварин для проведення експериментів з больовою стимуляцією.

REFERENCES

1. R. B. Fillingim, *Gender and Pain (Progress in Pain Research and Management*, Vol. 17), International Association for the Study of Pain, IASP Press, Seattle (2000).
2. J. L. Riley, M. F. Robinson, E. A. Wise, et al., "Sex differences in the perception of noxious experimental stimuli. A meta-analysis," *Pain*, **74** 181-187 (1998).
3. G. B. Rollman, S. Lautenbacher, and K. S. Jones, "Sex and Gender differences in responses to experimentally induced pain in humans," in: *Sex, Gender, and Pain (Progress in Pain Research and Management*, Vol. 17), International Association for the Study of Pain, R. B. Fillingim (ed.), IASP Press, Seattle (2000), pp. 165-190.
4. A. M. Unruh, "Gender variations in clinical pain experience," *Pain*, **65**, 123-167 (1996).
5. P. H. Desstein, E. A. Shipton, B. I. Doffe, et al., "Hyper secretion of adrenal androgens and the relation of serum adrenal steroids, serotonin and insulin-like growth factor 1 to clinical feature in women with fibromyaglia," *Pain*, **83**, 313-319 (1999).
6. J. R. Pednekar and V. K. Mulgaonker, "Role of Testosterone on pain threshold in rats," *Indian J. Physiol. Pharmacol*., **39**(4), 423-424 (1995)
7. M. S. Harbuz, S. L. Z. Perveen-Gill, D. S. Lightman, and A. Jessop, "Protective role for testosterone in adjuvant-induced arthritis," *Br. J. Rheumatol*., **34**, No. 12, 1117-1122 (1995).
8. I. Ceccarelli, C. Scaramuzzino, A. Massafra, and A. M. Aloisi, "The behavioral and neuronal effects induced by repetitive nociceptive stimulation are affected by gonadal hormones in male rats," *Pain*, **104**, 35-47 (2003).
9. N. D. Lambadjieva, "Influence of testosterone on some behavioral reactions of male immature rats," *Methods Find. Exp. Clin. Pharmacol*., **20** (8), 673 (1998).
10. N. B. Eddy and D. Leimbach, "Synthetic Analgesic. II. Dithienylbutenyl- and dithienylbutylamines," *J. Pharmacol. Exp. Ther*., **221**, 171-174 (1953).
11. S. H. Forreira, I. D. G. Duarte, and B. B. Lorezentti, "The molecular mechanism of action of peripheral morphine analysis: Stimulation of the GMP system via metric oxide release," *Eur. J. Pharmacol*., **201**, 74-79 (1991)
12. F. E. D'amour and D. L. Smith, "A method for determining loss of pain sensation," *J. Pharmacol. Exp. Ther*., **72**, 72-79 (1941).

13. W. L. Dewey, L. S. Harris, J. F. Howes, and J. A. Nuite, "The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests," *J. Pharmacol. Exp. Ther.*, **175**, 435-442 (1970).
14. K. J. Berkley, "Sex difference in pain," *Behav. Brain Sci.*, **20**, 371-380 (1997).
15. E. C. Stoffel, C. M. Ulibarri, and R. M. Craft, "Gonadal steriod hormone modulation of nociception, morphine antinociception and reproductive indices in male and female rats," *Pain*, 103, **285**-302 (2003).
16. W. F. Ganoong, *Review of Medical Physiology*, 19th ed. McGraw Hill Publ. (2000), pp. 402-403.
17. M. Hau, O. A. Dominguez, and H. C. Evrand, "Testosterone reduces responsiveness to nociceptive stimuli in a wild bird," *Horm. Behav.*, **46**, No. 2, 165-170 (2004).
18. J. A. P. Dasilva, S. H. Peers, M. Perretti, and D. A. Willoughby, "Sex steroids affect glucocorticoid response to chronic inflammation and to interleukin-1," *J. Endocrinol.*, **136**, 389-397 (1993).
19. A. M. Aloisi, I. Ceccarelli, P. Fiorenzani, et al., "Testosterone affects formalin-induced responses differently in male and female rats," *Neurosci. Lett.*, **361**, 262-264 (2004).
20. J. S. Mogil, E. J. Chester, S. G. Wilson, et al., "Sex differences in thermal nociception and morphine antinociception in rodents depend on genotype," *Neurosci. Biobehav. Rev.*, **24**, 375-389 (2000).
21. W. A. Isaacs, C. E. Effiong, and O. Ayeni, "Steriod treatment in the prevention of painful episode in sickle-cell disease," *Lancet*, **1**, 570-571 (1972).
22. K. M. English, R. P. Steeds, T. H. Jones, et al., "Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: A randomized, double-blind, placebo-controlled study," *Circulation*, **102**, 1906-1911 (2000).