Nociceptive Sensitivity and Lymphocytic Index of Peripheral Blood in Rats with Different Behavioral Activity in Model of Inflammatory Pain Provoked by Injection of Freund's Complete Adjuvant and Bovine Serum Albumin

E. V. Nikenina*,, A. Yu. Abramova*, and A. Yu. Kozlov***

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 5, pp. 582-584, May, 2012
Original article submitted March 5, 2011

Nociceptive thresholds decreased in rats at the early stage of inflammatory reaction induced by subcutaneous injection of BSA and complete Freund's adjuvant. At the later stage of this reaction, there was a trend of restoring nociceptive parameters in behaviorally passive rats in contrast to active animals, which demonstrated further decrease in the nociceptive thresholds. During the late inflammatory period, the lymphocytic index (by Shaganin) changed unidirectionally in the rats with different behavioral parameters. Probably, the changes in nociceptive thresholds were not triggered by the shift in lymphocyte/segmented neutrophil ratio, but resulted from production of yet not established biologically active agents with proalgesic and analgesic nature.

Key Words: *nociceptive sensitivity; lymphocytic index; inflammatory pain model; behaviorally active and passive rats*

Experimental studies in animals demonstrated the development of hyperalgesia at the early inflammation stage followed by analgesia characteristic of the later period after local injection of the proinflammatory agents [8-10]. Outside the inflammatory area, the nociceptive thresholds can also vary. The important role in provoking the proalgesic or analgesic processes in the affected location is given to inflammation mediators such as cytokines TNF-α, IL-1β, IL-6, NGF, IL-4 and IL-10, prostaglandins, and migrating leukocytic cells that produce these biologically active substances [4]. However, the processes underlying the nociceptive changes outside the inflammation area were little studied.

There are sound data that the animals with various behavioral activity also differ in the blood content of cytokines [3,6]. The leukocytes producing the immunomodulating cytokine play the key role at various stages of inflammatory pain [10].

Our aim was to examine nociceptive sensitivity and leukocytic profile of peripheral blood in rats with different behavioral activity at the early and late stages of inflammation provoked with combined injections of bovine serum albumin (BSA) and Freund's complete adjuvant (FCA).

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats (n=36) weighing 250-300 g with strict adherence to the Directive "Guidance to works involving animals in experiments" approved by the Ethics Committee of P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences (Protocol No.1, 3.09.2005), Regulations of World Society for Protection of Animals (WSPA), and European Convention for

*P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences; **I. M. Sechenov First Moscow State Medical University; Moscow, Russia. ***Address for correspondence:*** nikenina@mail.ru. E. V. Nikenina

the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123).

Behavioral activity was tested using the open field test [5]. The following parameters were recorded: latent periods (LP) of the first movement and visit to arena center and the number of crossed peripheral and central sectors. Motor activity index (MAI) was calculated by the formula:

$$MAI=\Sigma G(i)/\Sigma LP(i),$$

where ΣG(i) is total horizontal motor activity (the number of crossed segments at the periphery and in the center of the open field), ΣLP(i) is the sum of latent periods for the first movement and visits to arena center.

Based on test results, the animals were subdivided into three behavioral groups: passive (MAI<0.8), ambivalent (MAI=0.8–1.2), and active (MAI>1.2). Behaviorally active and passive animals were used in further experiments.

Four equal groups (n=9) have been formed: passive rats with inflammatory pain (group 1), control passive rats (group 2), active rats with inflammatory pain (group 3), and control active rats (group 4).

Inflammatory pain was modeled with injection of suspension of bovine serum albumin (BSA) in CFA. To this end, BSA (2 mg/kg body weight in 25 ml physiological saline) was mixed with 0.25 ml CFA. This suspension was injected subcutaneously in three spinal locations [10]. Control animals received injection of physiological saline via the same route.

The nociceptive thresholds were measured outside the inflammation areas according to vocalization thresholds in response to electrocutaneous stimulation of the tail (pulse duration 0.5 msec, frequency rate 10 Hz) performed with an electrical stimulator (Nihon Kohden). Current strength was gradually increased from the starting value of 0.1 mA to the level provoking vocalization [2]. The nociceptive thresholds were measured on postinjection days 1 and 7.

The peripheral blood was drawn from the caudal vein before injections and on postinjection day 7. The blood films were stained according to Romanowsky–Giemsa technique and examined under a light microscope (Biomed) at ×10. The blood lymphocytic index (LI) was calculated according to Shaganin [7]:

$$LI=L/N,$$

where L is the relative number of lymphocytes (%), and N is the relative number of segmented neutrophils (%).

The data were analyzed statistically by Student's t test and presented as M±SEM. Due to LI variability, it was normalized to initial value and measured in percentage.

RESULTS

The initial vocalization threshold varied from 0.90±0.08 to 1.00±0.13 mA in passive rats and from 0.88±0.13 to 1.00±0.09 mA in active animals (Fig. 1).

On postinjection day 1, the experimental passive and active rats (injected with BSA and CFA) significantly decreased vocalization threshold to 0.43±0.07 and 0.70±0.08 mA, respectively, relatively to the initial values (p<0.01; Fig. 1, *II*). These changes probably indicate the development of general hyperalgesia. The revealed decrease of vocalization threshold in active and passive rats suggests that enhancement of noxious sensitivity outside the inflammation area in behaviorally different animals is mediated by similar mechanisms.

On postinjection day 7 (injected with BSA and CFA), passive experimental rats demonstrated a pronounced elevation in vocalization threshold in comparison with postinjection day 1 (up to 0.59±0.06 mA, p<0.05), although it remained below the initial level (p<0.01, Fig. 1, *II*). By contrast, the vocalization threshold in active experimental animals measured on postinjection day 7 was lower than on day 1 or initial value (p<0.05, Fig. 1, *II*). These data suggest that the changes in nociceptive sensitivity outside the inflammation area observed at the late stage of the inflammatory response provoked by BSA and CFA are mediated by different mechanisms in behaviorally active and passive animals. This conclusion agrees with our previous study, which revealed strong correlations between behavioral parameters of rats with their nociceptive thresholds and immune responsiveness [1].

We hypothesized that the opposite changes in vocalization threshold in experimental active and passive rats on day 7 postinjection are somewhat related

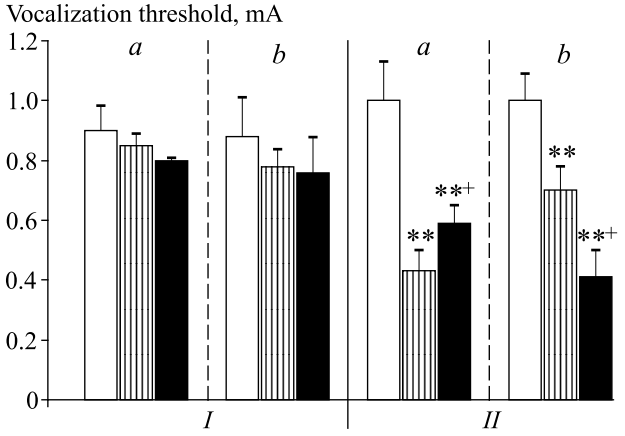

Fig. 1. Effect of subcutaneous injection of physiological saline (*I*) or inflammatory suspension BSA+CFA (*II*) on vocalization thresholds in behaviorally passive (*a*) and active (*b*) rats. Open bars: initial state; dashed bars: postinjection day 1; dark bars: postinjection day 7. **p<0.01 in comparison with initial values, +p<0.005 in comparison with postinjection day 1.

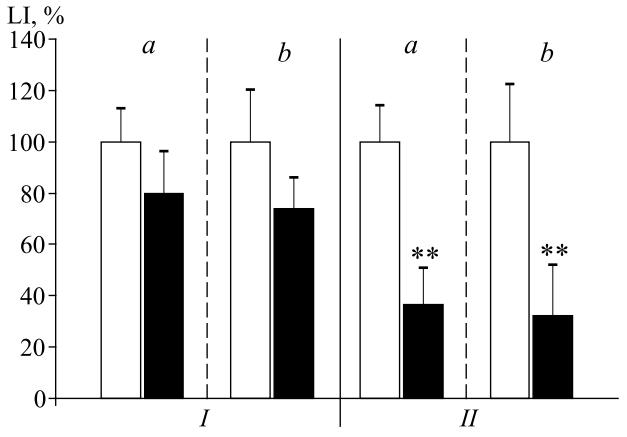

Fig. 2. Effect of subcutaneous injection of physiological saline (*I*) or inflammatory suspension BSA+CFA (*II*) on LI in peripheral blood in behaviorally passive (*a*) and active (*b*) rats. Open bars, initial state; closed bars, postinjection day 7. $^{**}p<0.01$ in comparison with initial values.

to peculiarities of involvement of peripheral blood leucocytes in the development of the late stage of the inflammation response in both types of animals. Both groups of control rats demonstrated no significant changes in blood LI during examination period (Fig. 2, *I*). In contrast, LI significantly decreased on postinjection day 7 in both passive and active rats by more than 2-fold in comparison with the initial values (to 36.7±13.9 and 32.1±20.0%, respectively, Fig. 2, *II*). These changes attest to an increase in the relative number of segmented neutrophils and a decrease in lymphocyte count in the blood.

These findings suggest that changes in nociceptive sensitivity in rats with different behavioral activity were not caused by changes in proportion between segmented neutrophils and lymphocytes in the blood, but were related to production of some unexplored active proalgesic and analgesic substances.

REFERENCES

1. A. Yu. Abramova and Yu. B. Abramov, *Byull. Eksp. Biol. Med.,* **152**, No. 8, 124-127 (2011).
2. Yu. B. Abramov, A. Yu. Kozlov, O. S. Sinel'shchikova, and G. V. Torgovanova, *Ross. Fiziol. Zh.,* No. 6, 699-705 (2002).
3. Yu. B. Abramov, *Bol'*, No. 4, 2-8 (2009).
4. A. S. Simbirtsev, *Tsitokin. Vosp.,* **2**, No. 3, 16-22 (2004).
5. E. V. Koplik, *Vestn. Nov. Med. Tekhnol.,* **9**, No. 1, 16-18 (2002).
6. S. S. Pertsov, E. V. Koplik, V. L. Stepanyuk, and A. S. Simbirtsev, *Byull. Eksp. Biol. Med.,* **148**, No. 8, 161-165 (2009).
7. N. I. Yabluchansky, V. A. Pilipenko, and P. G. Kondratenko, *Lab. Delo*, No. 1, 60-61 (1983).
8. F. L. Chen, Y. L. Dong, Z. J. Zhang, *et al., Brain Res. Bull*., **87**, No. 1, 60-66 (2012).
9. F. Q. Cunha and S. H. Ferreira, *Adv. Exp. Med. Biol*., **521**, 22-39 (2003).
10. H. L. Rittner, H. Machelska, and C. Stein, *J. Leukoc. Biol*., **78**, No. 6, 1215-1222 (2005).