PHYSIOLOGY

Nociceptive Thresholds and Indexes of Hyperthermia in Rats Treated with Lipopolysaccharide Pyrogenal

A. Yu. Abramova and Yu. B. Abramov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No. 8, pp. 124-127, August 2011
Original article submitted June 24, 2010

The relationships between open field behavior, initial nociceptive thresholds, and immunoreactivity assessed according to the development of hyperthermia triggered by LPS pyrogenal were examined in Wistar rats. Behaviorally active animals demonstrated higher nociceptive thresholds and greater immunoreactivity in comparison with passive rats.

Key Words: *nociception; immunity; pyrogenal; hyperthermia*

Numerous autoimmune diseases such as neurodegenerative processes in CNS, neuropathies, myasthenia, tumor diseases, AIDS, *etc*.) are accompanied by the pain syndrome of unclear etiology. The researches especially focus on the diseases related to the primary immune deficiency, which explains actuality of the works aimed to examine the immune-dependent nociceptive mechanisms.

Under normal physiological conditions, the immune status determines the nociceptive thresholds in mammals. Specifically, low nociceptive thresholds in intact mice correlate with a certain state of their immunity: the immunodeficient animals demonstrate higher nociceptive thresholds in comparison with the normal mice [2]. We previously showed that immunomodulator Imunofan changes the nociceptive thresholds in intact rats [1].

Pyrogenal, bacterial LPS, is a typical microbial antigen inducing systemic immune reaction (hyperthermia), activates cells of the macrophage-monocyte system, stimulates secretion of cytokines, up-regulates functional activity of cellular and humoral immune response, and modulates the hypothalamic thermoregulatory centers [4,5]. Analysis of the dynamics of body temperature makes it possible to assess quantitatively the immune reactivity in animals.

This study was designed to reveal correlations and interplay between the nociceptive parameters and development of pyrogenal-provoked hyperthermia in rats with different behavioral profiles.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (n=19) weighing 300-320 g. All experiments were performed in strict adherence to Directive "Guidance to Works Involving Animals in Experiments" approved by Ethics Committee of P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences (Protocol No.1, 3.09.2005), to the Regulations of World Society for the Protection of Animals (WSPA), and to European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123). The individual typological peculiarities of rats were examined in the open field test and animal activity index was calculated [3]. The experiments were performed on rats demonstrating extreme activity indices: active rats scored 1.4-4.9 and passive ones scaled merely 0.5-1.2.

P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** nansy71@mail.ru. A. Yu. Abramova

0007-4888/11/1522-0165

Pyrogenal was injected intraperitoneally (30 μg/kg). Rectal temperature was measured with a TPEM-1 medical electrical thermometer by inserting the transducer into the rectum to a depth of 1.0-1.5 cm. Temperature was measured immediately after determining the nociceptive thresholds and then repeatedly every 5 minutes until thermal stabilization. After pyrogenal injection and fixation of the temperature transducer, the rectal temperature was measured every 10-20 min until it returned to the baseline value. Typical dynamics of rectal temperature is shown in Figure 1.

Perceptual component of the nociceptive reaction was assessed by the latency of the tail flick response (TFR) to radiant heat in a tail flick apparatus (nocimeter) DS20 (Ugo Basile). The mean TFR latency was established for each rat subjected to 6 successive thermal stimuli presented every 5-6 min.

The emotional component of nociception was determined according to the vocalization threshold (VT) assessed by the strength of transcutaneous electric pulses (mA) applied to the tail with a Nihon Kohden electrical stimulator (pulse duration 0.5 msec, repetition rate 10 Hz). Current strength was gradually increased (starting from 0.1 mA) until evoking the vocalization response.

The data were processed statistically using Student's *t* test.

RESULTS

The study was focused on revealing the relationships between activity index, baseline thresholds of nociceptive reactions, and pyrogenal-induced hyperthermia.

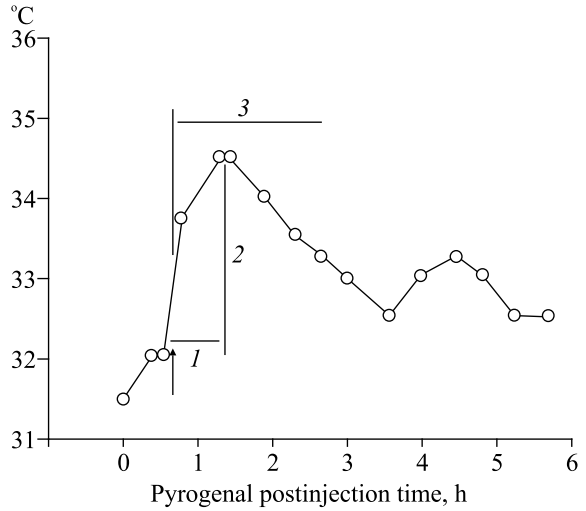

Fig. 1. Parameters of typical rectal hyperthermic reaction induced in rat by pyrogenal (arrow). *1*) LP assessed by the time needed to attain maximum temperature; *2*) hyperthermia amplitude assessed by maximum temperature; 3) duration of hyperthermia assessed at a half of its maximum.

The baseline rectal temperature was 32.0±0.3°C. Behaviorally active rats demonstrated longer TFR latency (3.7±0.1 sec) and maximum postinjection temperature increment (2.1±0.3°C in comparison with the baseline). In contrast, passive rats had shorter TFR latency (3.3±0.1 sec) and smaller postinjection temperature increment (1.2±0.2°C in comparison with the baseline). The differences between TFR latency in active and passive rats were significant (Fig. 2). In respect to VT, these groups of rats demonstrated only a pronounced differential trend. In active rats, VT was 1.1-1.8 mA with the temperature increment of 1.7±0.3°C. In passive animals, the corresponding values were 0.1-0.7 mA and 1.1±0.3°C (Fig. 3). LP and duration of hyperthermia did not significantly differ in active and passive rats.

Thus, active rats are characterized by higher tolerance to noxious stimulation and simultaneously higher immune reactivity than passive animals.

A number of reasons can underlie the above differences of nociceptive reactions in rats demonstrating different open field behavior. The open field environment and noxious stimulation are stress factors provoking negative emotions. The open field test was invented to score the level of fear or emotional excitation in rats. Probably, under the described experimental conditions the passive rats feel pronounced anxiety in contrast to active animals. This state is accompanied by enhanced emotional excitation in response to noxious stimulation. The revealed correlation between behavioral activity and hyperthermia in rats is a rather unexpected new fact. It can be concluded that rat behavior under alarming conditions is closely related to individual immune reactivity or initial immune status.

The most significant correlations were revealed between animal activity index, nociceptive thresholds, and the amplitude of hyperthermia. The importance of this fact should be stressed because after ranging the rats by TFR latency and VT, but without taking into consideration their activity, no significant correlations with hyperthermia amplitude were detected. There was only a trend to greater temperature increment (up to 1.8±0.3°C) in rats with short TFR latency ranging from 2.9 to 3.7 sec in comparison with the temperature increment of 1.4±0.3°C in rats with long TFR latency (3.5-6.7 sec).

The immune reactions are not exclusively manifested by fever; moreover, they can be chronic in character. Other data on the involvement of immune processes in nociception were obtained by comparing nociceptive thresholds recorded immediately after termination of hyperthermia with the corresponding baseline values.

It was established that termination of hyperthermia can be accompanied by either increment or decre-

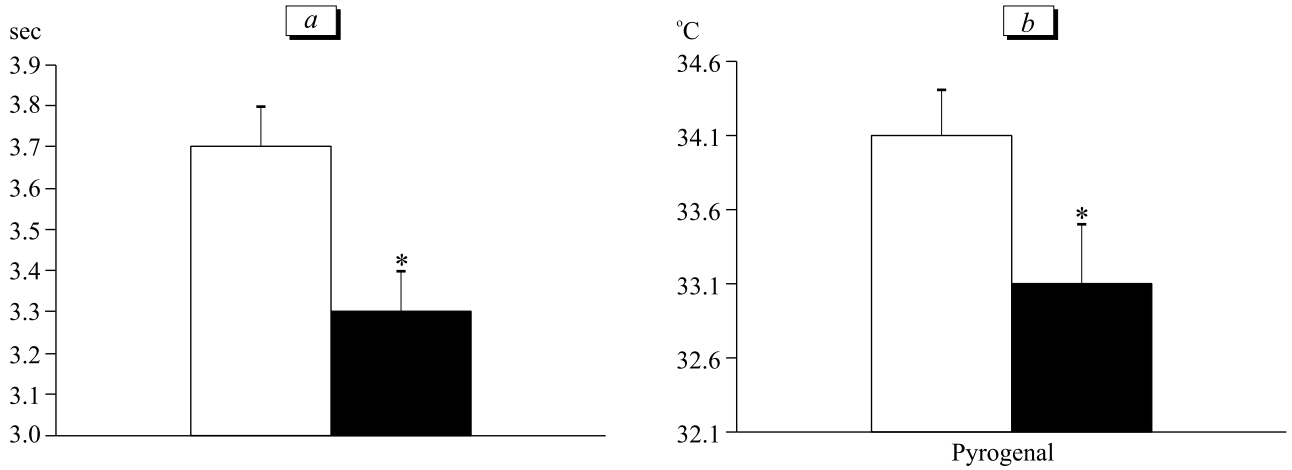

Fig. 2. Latency of TFR (baseline value, *a*) and the amplitude of pyrogenal–induced hyperthermia (*b*) in active (open bars) and passive (closed bars) rats. Here and in Fig. 3: the baseline rectal temperature was 32.0±0.3°C. *$p<0.05$ in comparison with active rats.

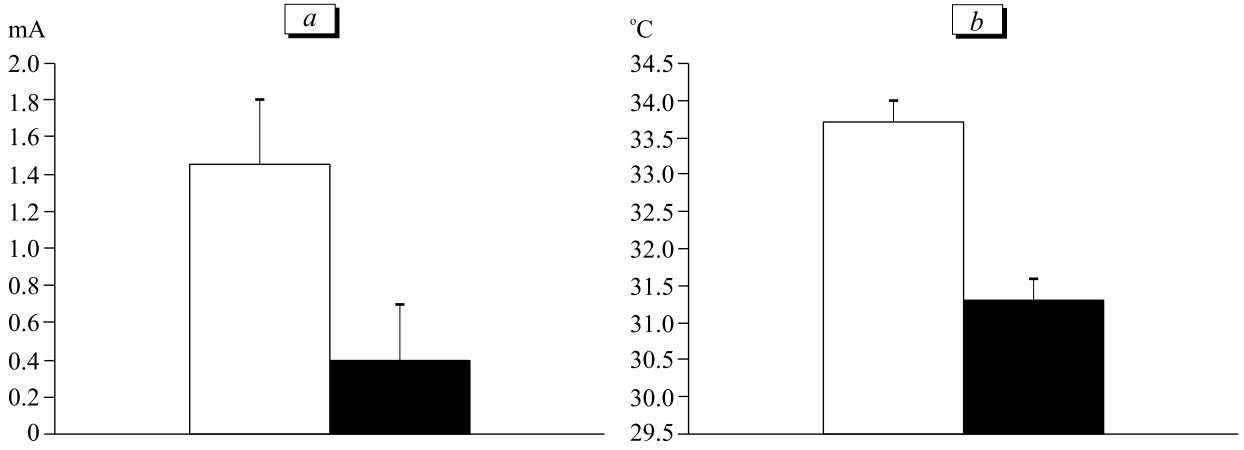

Fig. 3. Baseline VT (*a*) and the amplitude of pyrogenal–induced hyperthermia (*b*) in active (open bars) and passive (closed bars) rats.

ment of VT and TFR latency induces. The directivity of these changes did not depend on the duration of hyperthermia. Of 19 rats, TFR latency increased in 7 and decreased in 12 animals; VT increased in 8 and decreased in 11 rats. The changes in VT (% from baseline values) were more pronounced than the changes in TFR latency. In further analysis, the rats were subdivided into the groups depending on the increase or decrease in VT and TFR latency. The groups with decrease or increase in TFR latency differed significantly by hyperthermia amplitude: in the group with TFR latency decrease, hyperthermia was 0.2-1.3°C, while in the group with TFR latency increase it was 0.4-2.1°C. When the rats were subdivided according to changes in VT, they demonstrated somewhat an opposite trend: the group with decreased VT had insignificantly greater hyperthermia than the group with increased VT.

After injection of pyrogenal, the directivity in the changes in nociceptive threshold significantly depended also on the initial thresholds. Elevation in TFR latency relatively to the baseline values was observed in rats with short LP (3.2±0.8 sec), while decrease in PL of this reaction was characteristic of the rats with long LP (3.9±0.2 sec, $p<0.01$). Similar regularities were found also for VT: the postinjection increase in this parameter was observed in rats with low baseline VT (0.5±0.1 mA), while a decrease in VT was characteristic of the rats with a high baseline VT (2.4±0.9 mA, $p<0.05$).

Interpretation of the data obtained during repeated measurements of the nociceptive thresholds is rather complicated due to methodical peculiarities of the experiments. The rats were immobilized for a long time, which is a stress stimulation *per se*. Injection of LPS additionally affects nociception. There are numerous data attesting to decrease of nociceptive thresholds and pain sensitization during activation of the immune processes [6,8]. Similar effect was quite expected after pyrogenal injection. However, some types of stress stimulation moderate the nociceptive reactions; this effect is known as stress-induced analgesia mediated via opiate and immune mechanisms [6,7]. It can be hy-

pothesized that contribution of each interplaying factor determines directivity in changes of the nociceptive thresholds. Comparison of the numbers of rats with hypo- and hyperalgesia at the time of termination of hyperthermia showed that the pyrogenal-induced immune reactions dominate over the systemic response to immobilization. Analysis of these relationships needs further studies. It can be concluded that directivity in the changes of nociceptive thresholds after injection of pyrogenal is determined by the amplitude of hyperthermia and by the baseline nociceptive thresholds. Therefore, irrespective to the character of hyperthermia, pyrogenal eliminates the individual peculiarities in nociceptive indices.

The biological importance of accentuation or inhibition of the nociceptive reactions by pyrogenal seems to be as follows. Leveling of the nociceptive thresholds can be considered as manifestation of homeostasis, which eliminates the differences between the nociceptive indices among the rats. We observed namely this effect during pyrogenal-induced activation of immunity. Otherwise, the rats with low baseline nociceptive thresholds LPS could provoke the pain syndrome, while in the rats with elevated threshold it could suppress the nociceptive reactions thereby masking the major symptom of infectious attack and inflammation.

The revealed correlation between behavioral activity, nociception, and immune reactivity showed the character of involvement of the immune processes in the control of pain sensitivity in animals with different individual typological peculiarities. Experimental study of interrelations between nociception, behavior, and immune reactivity can be fundamental for elaboration of the strategy and methods to eliminate pain with due account for immune status of the organism.

REFERENCES

1. Yu. B. Abramov, A. Yu. Kozlov, O. S. Sinel'shchikova, G. V. Torgovanova, *Ross. Fiziol. Zh*., No. 6, 699-705 (2002).
2. A. M. Vasilenko, O. G. Yanovskii, O. V. Koptelov, *et al*., *Byull. Eksp. Biol. Med.,* **119**, No. 4, 405-408 (1995).
3. E. V. Koplik, *Vestn. Nov. Med. Tekhnol*., **9**, No. 1, 16-18 (2002).
4. *Drugs,* Ed. M. A. Klyuev [in Russian], Moscow (2002).
5. *Drug Guide*, Ed. M. D. Mashkovskii [in Russian], Moscow (2004).
6. Y. Kawasaki, L. Zhang, J. K. Cheng, and R. R. Ji, *J. Neurosci*. **28**, No. 20, 5189-5194 (2008).
7. J. W. Lewis, J. T. Cannon, and J. C. Liebeskind, *Science,* **208**, 623-625 (1980).
8. F. Marchand, M. Perretti, and S. B. McMahon, *Nat. Rev. Neurosci*., **6**, No. 7, 521-532 (2005).