Effect of Antiserotonin Antibodies on Pain Sensitivity in Rats with Adjuvant-Induced Arthritis

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Active immunization with a serotonin—protein conjugate inducing the formation of antiserotonin antibodies exerts an analgesic effect in rats with adjuvant-induced arthritis and inhibits the development of arthritis in early stages after injection of Freund's complete adjuvant.

Key Words: *pathological pain; adjuvant-induced arthritis; serotonin; antiserotonin antibodies; nociceptors*

It is well established that serotonin (5-hydroxytryptamine, 5-HT) and other mediators of inflammation play an essential role in peripheral mechanisms of pain through participating in sensitization and activation of nociceptors [7,8]. Controlled reduction of the content of 5-HT in inflammatory pain syndromes is used for the development of new analgesic drugs. During the last decade a new immunological approach has been developed for the regulation of endogenous biologically active substances based on the induction of specific antibodies against these substances [2,4-6].

It has been shown that induction of specific antibodies against neurotransmitters, in particular, against serotonin, reduces alcohol intake in experimental alcoholism [4], prevents the development of abstinent symptoms in experimental morphinism and alcoholism [1, 5,6], and reduces abstinent morphine-induced hyperalgesia in nociceptive thermal injury [3]. In light of this, it seems interesting to use artificial induction of anti-5-HT antibodies for producing an analgesic effect in inflammatory pain syndromes. The aim of the present study was to evaluate the role of anti-5-HT antibodies in the mechanisms of pain syndrome in rats with adjuvant-induced arthritis.

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MATERIALS AND METHODS

Experiments were carried out on random-bred male rats with an initial weight of 180-200 g.

Anti-5-HT antibodies were induced by active immunization with 5-HT-protein conjugate synthesized using the bifunctional agent p-aminophenylalanine [9] and bovine serum albumin (BSA) as a carrier. Thus synthesized conjugate, judging from spectrophotometrical analysis, contained 8-10 molecules of 5-HT per one protein molecule, which ensures the maximum immune response to the hapten. The animals were immunized according to the following scheme: the first immunization with 2 mg/kg 5-HT—BSA conjugate with Freund's complete adjuvant (FCA) (multiple subcutaneous injections into the back). Two weeks later the animals were intraperitoneally immunized with 5 mg/kg conjugate in 0.5 ml physiological saline without FCA (second immunization), and after 1 week they were intraperitoneally injected with 10 mg/kg conjugate without FCA (third immunization). Control rats received injections of physiological saline with or without FCA according to the same scheme. After completion of this schedule, anti-5-HT antibodies in the serum were determined by enzyme-linked immunosorbent assay using a Mini-Reader device (Dynateck) and 5-HT—horse γ-globulin conjugate as the test antigen.

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After immunization, adjuvant-induced arthritis was modeled by injecting 0.1 ml FCA containing 2 mg/ml BCG vaccine into the right hinder paw. The severity of arthritis was assessed by the degree of inflammatory changes in affected joint, body weight loss, and involvement of other extremities into the pathological process. Inflammatory changes were evaluated by the index of edema calculated from the formula: $(A—B)/B\times100$, where A and B are diameters of the joint after and before FCA injection (mm).

Mechanical and thermal nociceptive sensitivity was assessed using Ugo Basile devices. Pain sensitivity to thermal nociceptive stimulation was determined using the hot-plate test (Hot Plate Device): rat was placed on a plate heated to 55°C (for no more than 60 sec), and the latency of pain reaction (licking of the hind paw or a jump) was measured. Pain reaction to mechanical nociceptive stimulation was evaluated by paw withdrawal after application of a load on an Analgesy-Metry apparatus. Moreover, in control and experimental animals the pain reaction was assessed by the number of vocalizations in response to 5-fold passive flexion of the affected talocrural joint and expressed by index of vocalizations $V=n/5\times100\%$, where n is the number of vocalizations.

The severity of adjuvant-induced arthritis and mechanical and thermal pain sensitivity in rats were determined on days 3, 7, 14, and 21 after injection of FCA.

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

RESULTS

Immunization with the 5-HT—BSA conjugate induced production of anti-5-HT antibodies (serum

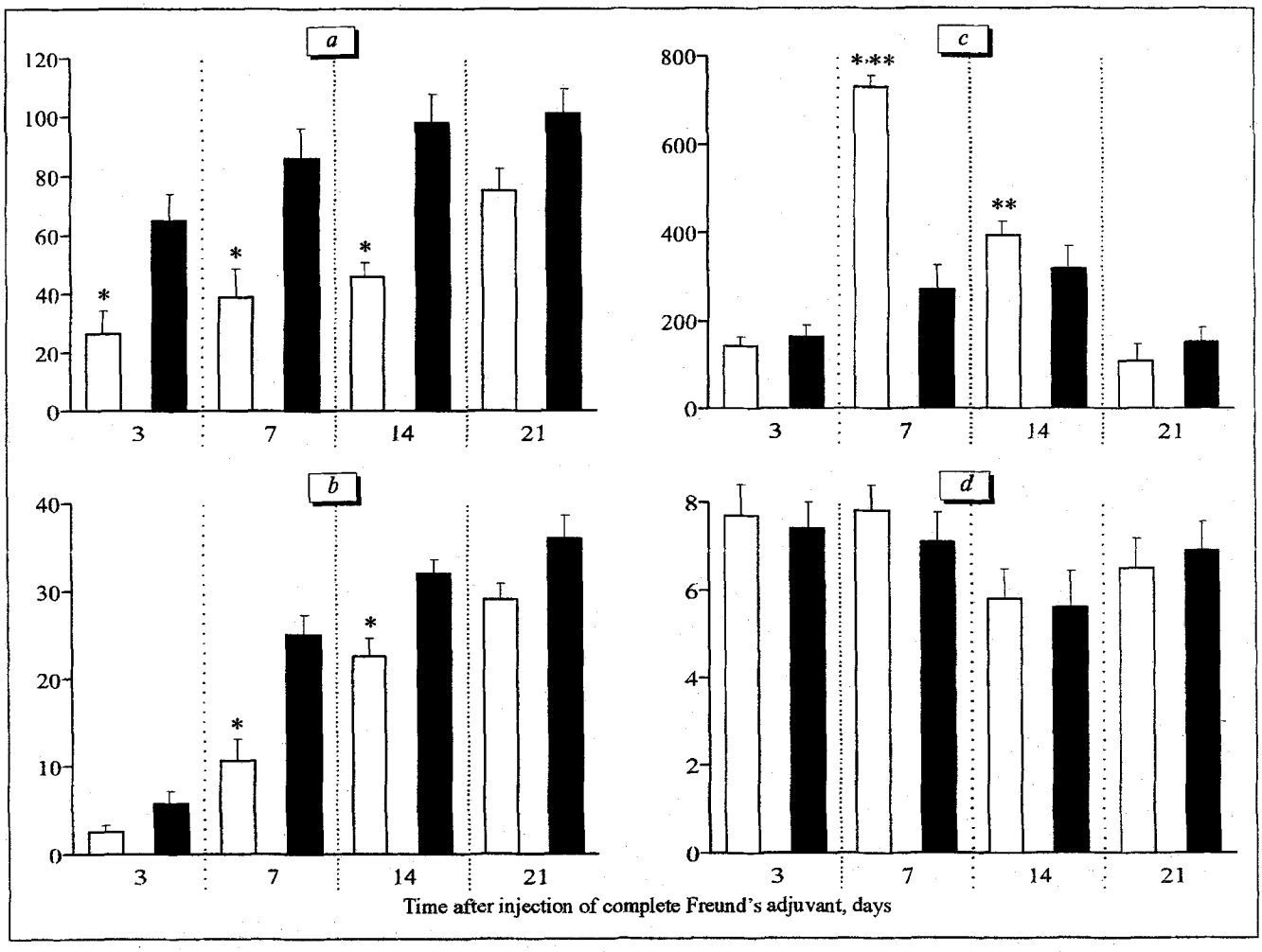

Fig. 1. Effect of active immunization with serotonin—bovine serum albumin conjugate on pain sensitivity and development of adjuvant-induced arthritis in rats. Ordinate: *a*) index of edema, %; *b*) loss of body weight, g; *c*) mechanical nociceptive reaction measured by paw withdrawal in response to applied load, g; *d*) latency of thermal nociceptive reaction, sec. Open bars: experimental rats with adjuvant-induced arthritis immunized with 5-HT—BSA conjugate, dark bars: control rats with adjuvant-induced arthritis. $p<0.001$: *between the control and experimental groups, **compared with the initial level.

TABLE 1. Effect of Active Immunization with 5-HT—BSA Conjugate on Pain Reaction upon Flexion of the Talocrural Joint in Rats with Adjuvant-Induced Arthritis

Group	Index of vocalizations, %		
	days after injection of FCA		
	3	7	14
Rats with adjuvant-induced arthritis immunized with 5-HT—BSA conjugate (n=8)	15.0±7.9	20.0±8.1	72.5±6.1
Rats with adjuvant-induced arthritis (n=8)	94.2±2.9**	97.1±2.0**	100.0±0*

Note. *p<0.05, **p<0.001 intergroup differences.

titers 1:128-1:1000). Adjuvant-induced arthritis was less severe in immunized rats: inflammatory changes in affected joint assessed from the index of edema and the loss of body weight (Fig. 1, *a*, *b*) were less pronounced in experimental animals in comparison with the control. Similarly, the involvement of the fore paws was less frequently observed in the experimental than in the control group. For instance, arthritis of the fore paws on day 14 after injection of FCA developed in 1 out of 8 immunized rats (12.5%) and in 4 out of 8 control rats (50%).

Changes in mechanical and thermal pain sensitivity in rats immunized with 5-HT—BSA conjugate are presented in Fig. 1, *c* and *d*. Immunization induced the development of analgesic effect in mechanical nociceptive stimulation at early stages after FCA injection. For instance, on days 3 and 7 after injection of FCA, in experimental animals with high titers of serum anti-5-HT antibodies we observed reduced pain sensitivity to mechanical stimulation (load) in comparison with its initial level measured before FCA injection. Simultaneously, the index of vocalization upon passive flexion of the affected talocrural joint in control animals was considerably higher than in immunized animals (Table 1). No analgesic effect of anti-5-HT antibodies in thermal nociceptive stimulation was found, and the latency of pain reaction to thermal stimulation in immunized rats was the same as in the control animals throughout the observation period.

Thus, our findings suggest that in rats with adjuvant-induced arthritis, a preliminary active induction of anti-5-HT antibodies reduces pain reaction to mechanical nociceptive stimulation and has no effect on thermal nociceptive stimulation. In rats with adjuvant-induced arthritis, the analgesic effect of anti-5-HT antibodies in mechanical stimulation is most likely due to the binding of 5-HT in the blood and inflammatory focuses (joints), which reduces the sensitizing and excitatory effect of this substance on mechanoreceptors of groups III and VI afferent fibers, which are sensitive not only to the proper mechanical stimuli, but also to inflammatory transmitters [7].

In rats with adjuvant-induced arthritis, active production of anti-5-HT antibodies was attended by reduction of inflammatory changes in affected joints, which manifests itself in decreased index of edema. This can also suppress activation of mechanoreceptors due to reduction of mechanical pressure and thus alleviating pain syndrome.

Since the development of neuropathological syndromes is accompanied by enhanced production of autoantibodies against neurotransmitters [3,4,6], anti-5-HT antibodies can be regarded as a new class of natural peripheral analgesics.

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REFERENCES

1. L. A. Basharova, V. A. Evseev, L. A. Vetrile, *et al.*, *Byull. Eksp. Biol. Med.*, **115**, No. 5, 469-471 (1993).
2. R. A. Danilova and I. P. Ashmarin, *Usp. Fiziol. Nauk*, **25**, No. 1, 3-23 (1994).
3. V. A. Evseev, L. A. Basharova, L. A. Vetrile, *et al.*, *Byull. Eksp. Biol. Med.*, **122**, No. 8, 189-192 (1996).
4. V. A. Evseev, T. V. Davydova, V. G. Fomina, *et al.*, *Ibid.*, **112**, No. 7, 333-334 (1991).
5. V. A. Evseev, T. V. Davydova, V. G. Fomina, *et al.*, *Ibid.*, **116**, No. 12, 582-584 (1993).
6. V. A. Evseev, N. A. Trekova, L. A. Basharova, *et al.*, *Ibid.*, **119**, No. 5, 526-529 (1995).
7. G. J. Birrell, D. S. McQueen, A. Iggo, and B. D. Grubb, *Br. J. Pharmacol.*, **101**, 715-721 (1990).
8. H. Ollat, in: *Serotonin and Pain*, J.-M. Besson (Ed.), New York - Paris - London (1990), pp. 17-30.
9. B. Pescar and S. Spector, *Science*, **480**, 1340-1341 (1973).