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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

The Effect of Catecholamine and Serotonin Antibodies on Pain Sensitivity and on the Development of Morphine Tolerance in Experimental Narcomania

L. A. Vetrile, L. A. Basharova, S. I. Igon'kina, N. A. Trekova, and V. A. Evseev

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Antibodies (AB) against the neurotransmitters serotonin (5-HT), dopamine (DA), and norepinephrine (NE) [2,6,8,9,12] are found in different forms of CNS pathology (parkinsonism, alcoholism, narcomania, PKU) and in hypertension, and possess a broad spectrum of biological activity, namely, they change behavior reactions of animals [1,16], inhibit alcohol motivation [4,7], and may provoke (AB against DA) or attenuate (AB against 5-HT) the development of Parkinson's syndrome when locally administered to brain structures [11,13]. Chronic morphinization of animals induces the production of autoAB against neurotransmitters (5-HT and cathecholamines), as was demonstrated previously [3]. AutoAB against neurotransmitters were also found in the blood serum of patients with different types of narcotic dependence [2]. Published data attest to the participation of the catecholamine- and 5-HT-ergic systems in the correction of pain and in the mechanisms of the analgesic effects of morphine [10,19,22,23], as well as in the development of morphine tolerance [5].

Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow. (Presented by G. N. Kryzhanovskii, Member of the Russian Academy of Medical Sciences)

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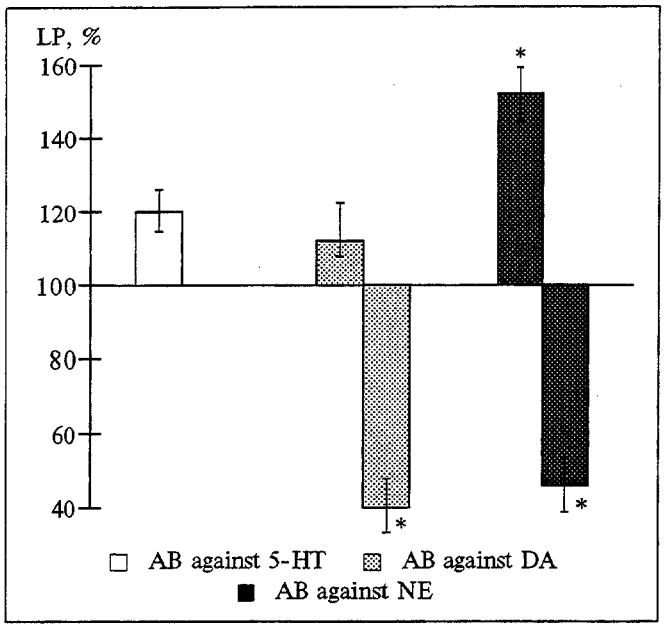

Fig. 1. Effect of AB against neurotransmitters for active immunization on LP of reaction induced by nociceptive stimulation (LP in control group is taken as 100%). An asterisk means $p<0.05$ relative to control.

The present investigation was undertaken to study the effect of AB against neurotransmitters (5-HT, DA, and NE) on pain sensitivity and on the development of morphine tolerance.

MATERIALS AND METHODS

Experiments were carried out on 56 male Wistar rats weighing 250-300 g. Two experimental series were performed. In the first series the effect of AB against neurotransmitters for active immunization with the corresponding conjugated antigen on physiological pain was studied in rats. In the first experimental series animals were divided into 4 groups, with 14 rats in each: the first group was the control, and the rats of the 2nd-4th groups were immunized with conjugates 5-HT-BSA, DA-BSA, and NE-BSA, respectively. The second series was performed to examine the effect of AB on the development of morphine tolerance for chronic morphinization of animals. Conjugated antigens 5-HT-bovine serum albumin (BSA), DA-BSA, and NE-BSA were synthesized according to the methods described elsewhere [20,21]. The conjugates contained 5, 13, and 22 moles of neurotransmitter per protein molecule, respectively, according to the spectrophotometric analysis. Animals were immunized according to the following scheme: the first immunization with conjugates in a dose of 2 mg/kg s.c. at numerous points in the back region with complete Freund adjuvant. The second immunization was performed two weeks later with conjugates (5 mg/kg) i.p. in 0.5 ml saline (0.9% NaCl) without adjuvant, and after one more week the third immunization with a conjugate dose of 10 mg/kg i.p. without adjuvant was performed. Control rats were injected in the same periods and with the same volume of 0.9% NaCl with and without complete adjuvant. Serum AB of immunized animals were determined using the method of solid-phase immunoenzyme analysis (IEA). Test antigens were synthesized using an analogous technique on heterologous protein support material, namely, horse γ-globulin, to determine AB and their immunochemical characteristics. Pain sensitivity was assessed by the latent period (LP) of reaction of licking of one of the hind paws in response to a thermal nociceptive stimulation for testing with the "hot plate" method using Ugo Basil equipment. The maximal time of exposure to the plate was 60 sec. Morphinization of animals with i.p. injections of graduated doses of morphine hydrochloride was performed with the participation of S. I. Tronnikov following a described scheme [15]. The development of tolerance was indicated by a change of pain sensitivity after administration of the test dose of morphine (10 mg/kg i.p.). Morphine tolerance (T) was expressed in percent and determined using the formula

$$T = \frac{LP_2 - LP_1}{LP_1} \times 100\%,$$

where LP_1 is the latent period before morphinization and LP_2 the latent period after morphinization.

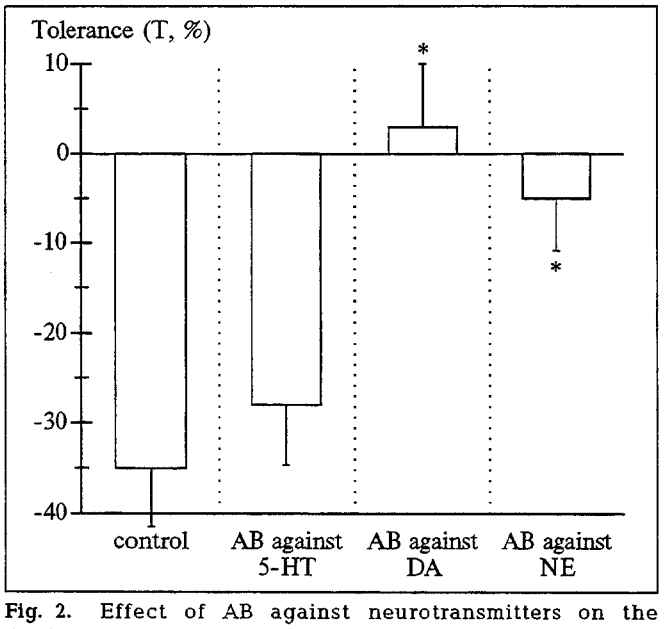

Fig. 2. Effect of AB against neurotransmitters on the development of morphine tolerance for chronic morphinization of rats. An asterisk means $p<0.05$ relative to morphinized control.

TABLE 1. Effect of AB against Neurotransmitters on the Development of Morphine Tolerance in Rats Subjected to Chronic Morphinization

Group	Number of animals	Latent period (LP), sec ($M\pm m$)	
		before morphinization (after immunization)	after morphinization
Control	14	16.5±2.2	9.2±0.9*
Immunized with 5-HT-BSA	8	17.0±2.5	10.2±1.7*
Immunized with DA-BSA	7	11.8±0.8	12.0±2.1
Immunized with NE-BSA	9	12.2±0.8	11.7±1.9

Note. Asterisk means $p<0.05$ relative to LP before immunization.

RESULTS

Immunization with conjugates of neurotransmitter-BSA in the first series resulted in an induction of AB against 5-HT, DA, and NE in high titers (1:16-1:512). The specificity of AB was confirmed in the reaction of concurrent inhibition of corresponding neurotransmitter in IEA.

The effect of AB on pain sensitivity was assessed by the change of LP before and after the conclusion of immunization. The initial pain sensitivity did not differ significantly among the groups. The data obtained (Fig. 1) tend to an increase of LP in rats immunized with 5-HT conjugate ($p<0.2$). The effect of catecholamine AB on pain sensitivity was ambiguous. The rats immunized with conjugated DA-BSA and NE-BSA were divided into two equal groups according to their response to pain stimulation (Fig. 1). In one half of the animals AB against DA did not cause changes of LP in comparison with the control. The others exhibited a reliable 1.7-fold decrease of LP which testifies to the development of hyperalgesia for thermal pain stimulation. Induction of AB against NE produced a marked analgesic effect in half of the rats. In comparison with the control LP was increased 1.4-fold. In the other 7 animals immunization with NE-BSA caused hyperalgesia and the LP dropped more than 2-fold. The ambiguous effect of AB against DA and NE on pain sensitivity is not associated with the level of AB in the serum of immunized rats because the mean titer of AB in these groups was identical.

In the second experimental series all rats were morphinized after the cycle of immunization for a study of the effect of immunization with conjugated neurotransmitter-BSA on the development of tolerance to morphine.

The experiments performed (Table 1 and Fig. 2) revealed that the chronic morphinization led to the development of morphine tolerance in the rats of the control group. 5-HT AB did not affect the development of morphine tolerance as compared to the control (Fig. 2). The effect of catecholamine AB was fundamentally different. According to the mean values, a preventive immunization of animals with DA-BSA and NE-BSA conjugates inhibited the development of morphine tolerance for chronic administration. The analgesic effect of the test dose of morphine was at the same level as before morphinization. However, an individual analysis of the response to pain stimulation before and after chronic morphinization showed that in some rats ($n=4$) AB against NE enhanced significantly ($p<0.05$) the analgesic effect of the test dose of morphine. The latent period after immunization and narcotization in these animals was 20.2±4.5 sec vs. 12.2±0.8 sec before morphinization and 9.2±4.5 sec in morphinized rats without immunization.

Four rats among the animals immunized with DA-BSA conjugate tended to exhibit an enhanced analgesic effect of the test dose of morphine after chronic narcotization.

The findings testify to the participation of AB against catecholamines in the modulation of pain sensitivity. Of particular interest are the data attesting to an inhibition of the tolerogenic effect of morphine for induction of the formation of AB against catecholamines. The effect of these AB on pain sensitivity and on the development of morphine tolerance mediated via the CNS may be related to a change in the neurotransmitter status of the animals or to a change in the sensitivity of brain receptors for immunization, as was shown in our previous studies [1,17]. Furthermore, it may be assumed that the effect of AB on pain sensitivity and on the development of morphine tolerance is mediated by transmitters of the immune system (interleukin-1, interferon, tumor necrosis factor), whose neurotropic effect, including the effect on pain sensitivity, is proven [14,18].

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Effect of Adaptation to Stress-Inducing Electrical Stimulation on the Reactivity of the Isolated Resistive Artery

F. Z. Meerson, S. Yu. Mashina, E. B. Manukhina, E. Ya. Vorontsova, and S. A. Radzievskii

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Recent studies have shown that adaptation to repeated moderate stress induces the activation of the so-called stress-limiting systems [1] and simultaneously improves the organism's resistance to stress-induced [1] and ischemic [2,5] damage, and global hypoxia [3]. It is now proven that these protective effects of adaptation may be achieved through course of mild transauricular electrostimulation (ES), which during its first presentation induces a stress reaction, but then suppresses it while at the same time activating the stress-limiting systems [4]. The cardioprotective effects of a course of EC have now been studied in detail, yet there are still no data on the effect of a course of ES on the tonus or adreno- and cholinoreactivity of blood vessels.

Research Institute of General Pathology and Pathophysiology, Academy of Medical Sciences, Moscow. (Presented by G. N. Kryzhanovskii, Member of the Russian Academy of Medical Sciences)

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