Induction of Autoantibodies to Serotonin and Catecholamines in Chronically Morphinized Rats with the Manifestation of Withdrawal Symptoms

L. A. Basharova, V. A. Evseev, L. A. Vetrile, N. A. Trekova, N. B. Gamaleya, A. G. Veretinskaya, and S. I. Tronnikov

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It is known that the serotonin and catecholamine systems of the organism take part in the realization of the morphine effect [1,2,5]. Morphine changes the metabolism of neurotransmitters such as dopamine (DA), norepinephrine (NE), and serotonin (5-HT) [6,8], raises the levels of endogenous tryptophan and 5-oxyindoleacetic acid [13], and accelerates the circulation of serotonin in the animal brain [12]. There are also experimental data which testify to the influence of the catecholamine and serotonin systems on the bechavioral reactions and basic manifestation of withdrawal symptoms of animals under morphine injection [2].

As was shown by our previous results, disturbances of neurotransmitter metabolism can lead to changes in the organism's immune status, in particular, to the induction of autoantibodies to the neurotransmitters, which may be considered to be one of the compensatory mechanisms for the maintenance of homeostasis [3,4].

The aim of the present work was to study the possibility of inducing autoantibodies to neurotransmitters (5-HT, NE, DA) in experimental morphine addiction and of changing the levels of the corresponding neurotransmitters in the hypothalamus and blood of mice in the presence of withdrawal symptoms.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 250-300 g. Morphine hydrochloride in 0.5 ml isotonic solution (IS) was injected into the rats of the test group (n=8) intraperitoneally twice a day at 12-hour intervals and the dose was increased from 10 to 50 mg/kg over 14 days. IS was injected into the control group of animals (n=10).

The morphine dependence was evaluated according to the expression of withdrawal symptoms, elicited by naloxone injection (1 mg/kg intraperitoneally) under testing in the "open field" (OF), and according to the increase in the tolerance of the morphine

TABLE 1. Variation of Threshold of Rat Pain Sensitivity under Chronic Morphinization ($M \pm m$)

Initial latent period of the paw withdrawal (threshold of pain sensitivity), T_1, sec	35.3±4.5
Latent period of paw withdrawal at end of morphinization, T_2, sec	15.0±1.5
Index of drop of threshold of pain sensitivity (rise of tolerance), $(T_2-T_1)/T_1 \times 100$ (%)	−48.3±11.7

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

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TABLE 2. Main Parameters of Behavior of Chronic Morphinized Rats with Withdrawal Symptoms ($M \pm m$)

Parameter	Group	
	control (n=10)	morphinized animals (n=8)
Horizontal motor activity (over 4 min)	4.40±0.70	13.75±6.19 (312%)
Vertical motor activity (over 4 min)	1.00±0.36	4.00±1.30 (400%)
Gritting of teeth (over 10 min)	0	5.75±1.81
Hyperactivity (over 10 min)	0	3.75±1.81

Note. Data in % in comparison with the control are shown in parentheses.

test-dose during investigation of the pain sensitivity threshold in the "hot-plate" test. Two hours after the reproduction of withdrawal symptoms the animals were decapitated and blood was taken for investigation. Antibodies to neurotransmitters were determined by the method of enzyme-linked immunosorbent assay (ELISA) on polystyrene plates sensitized with the corresponding test antigens; NE-horse gamma-globulin (NE-HGG), DA-GGL, and 5-HT-GGL in 0.3 μg/well in 0.05 M carbonate-bicarbonate buffer, pH 9.5. 5-HT-GGL and DA-GGL conjugates were synthesized by the reaction of a transmitter with diazotized protein, using a modified method [11]. NE-GGL-conjugate was obtained by the utilization of glutaraldehyde as a reagent [10]. The obtained conjugates, according to the data of spectrophotometric analysis, contained 33 molecules of 5-HT,

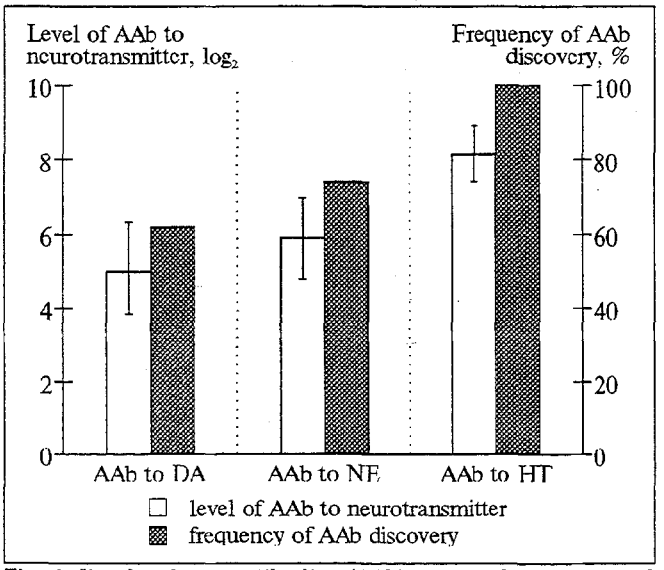

Fig. 1. Levels of autoantibodies (AAb) to catecholamines and serotonin in chronically morphinized rats under conditions of withdrawal syndrome.

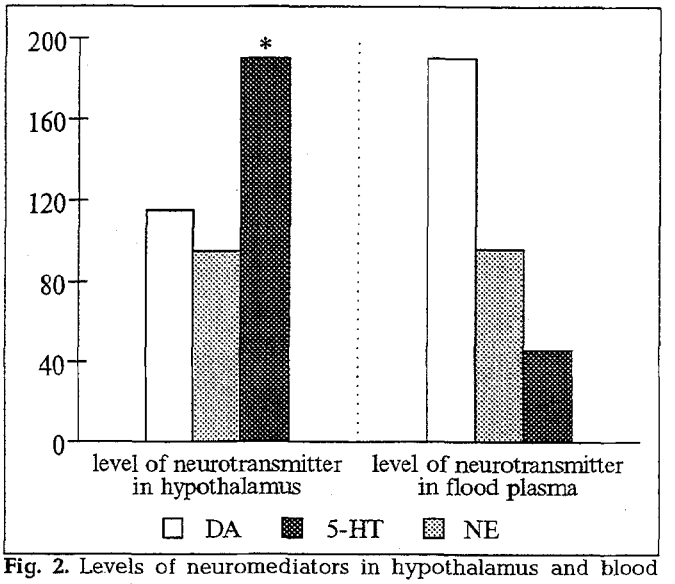

Fig. 2. Levels of neuromediators in hypothalamus and blood plasma in chronically morphinized rats under conditions of withdrawal syndrome. Asterisk: $p<0.01$.

8 molecules of DA, and 26 molecules of NE in one molecule of protein. Antibodies against rat gamma-globulins, labeled with horseradish peroxidase (Gamaleya Institute), were used as the secondary antibodies and the dye was orthophenildiamine (Sigma). The antibody titer to neurotransmitters was determined relative to the corresponding optical indexes of the control animal sera. The antibody specificity was determined in the competitive inhibitory reaction test. The percentage of antibody binding inhibition in the immunoenzyme analysis was determined by the formula: 100-(Ax/Ao)× ×100 (in %), where Ax is the optical density (OD, nm) of the studied serum with the substance for inhibition and Ao is the OD (nm) of the studied serum without the substance for inhibition (control).

The meurotransmitter content in the brain tissues and blood was measured by the method of high-performance liquid chromatography with electrochemical detection [7,9].

RESULTS

The results showed that the chronic morphine injections led to a drop of the threshold of pain sensitivity, evidence of an increased tolerance of the morphine test dose in the analgetic effect (Table 1). The blockade of the opioid receptors led to the appearance of withdrawal symptoms (Table 2), expressed in an intensification of horizontal motor activity, an increase of the number of vertical movements, and the appearance of specific withdrawal symptoms such as hyperactivity and gritting of the teeth.

It was shown in the analysis of animal sera, that autoantibodies to DA, NE, and 5-HT were induced in high levels (1:256-1:512) (Fig. 1). Their specificity,

TABLE 3. Specificity of Autoantibodies to Transmitters Revealed in Morphinized Rats.

Substance introduced in the reaction of antibody competitive inhibition (ELISA)	Test antigen	Concentration of substance for inhibition, M	OD, 492 nm (Ax/Ao)×100, %	% inhibition
Serotonin	5-HT-GGL	2.5×10^{-7}	54.67	45.33
		2.5×10^{-8}	92.16	7.8
Control (without substance)		–	100	–
Dopamine	DA-GGL	5.0×10^{-7}	44.4	55.6
		5.0×10^{-8}	88.9	11.1
Control		–	100	–
Norepinephrine	NE-GGL	3.0×10^{-7}	35.1	63.9
		3.0×10^{-8}	58.0	42.0
Control		–	100	–

determined in the test of competitive inhibition, is shown in Table 3. The presence of these antibodies testifies that there are disturbances of catecholamine and serotonin metabolism in experimental morphine addiction against the background of withdrawal symptoms, as is confirmed by biochemical data on the neurotransmitter content in the brain tissue and blood plasma. It is obvious that the revealed antibodies can be the markers of disturbed neurotransmitter metabolism.

As is evident from the data presented (Fig. 2), there is a rise in the level of DA in the blood and a still greater change in the content of 5-HT: a significant decrease it in the blood and an increased level in the brain tissue (hypothalamus), two times higher than in the controls. It is to be noted that the most pronounced alterations, according to the immunological results, were also revealed for 5-HT (Fig. 1).

Thus, it is shown for the first time that chronic animal morphinization, which induces the development of dependence, leads to the induction of antibodies to DA, NE, and 5-HT. The phenomenon of the synthesis of autoantibodies to neurotransmitters under chronic morphinization has important significance for the study of the pathogenesis of addiction.

The obtained data are of substantial importance for the study of the mechanisms of dependence and the development of tolerance in the case of morphine addiction and point the way to further research in this direction.

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