ANTIBODY FORMATION TO MORPHINE AND NEUROTRANSMITTERS IN MORPHINIZED RATS

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According to data in the literature, in animal experiments morphine has an inhibitory action on the cellular and humoral immune response [5, 12]. On the other hand, a number of investigations have shown that chronic morphine poisoning induces the appearance of antibodies capable of binding specifically with radioactively labeled morphine in the blood of animals [8, 10]. These antibodies prolonged the half-life of the drug in the blood and prevented its rapid penetration into the brain [9], a fact which may probably be linked with the weakening of the pharmacologic effects of morphine in their presence [10, 11].

More and more evidence has recently accumulated in the literature on the possibility of induction of antibodies against other low-molecular-weight substances also, including endogenous biologically active compounds [4]. The presence of antibodies to beta-endorphine [6, 15], Met- and Leu-enkephalins and dermorphine [6], and neurotransmitters [1, 3], has been demonstrated. These antibodies are capable of modulating functional activity of the corresponding biologically active compounds. It has been shown, for instance, that antibodies to neurotransmitters modify the content of biogenic amines in the brain and the behavioral reactions of animals, especially their attitude toward alcohol [2].

Accordingly the aim of the investigation described below was to study the effect of chronic morphinization of animals on the levels of antibodies to morphine and to neurotransmitters, and also the possible role of these antibodies in the pathogenesis of morphine dependence.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g. The animals were given an intraperitoneal injection of morphine hydrochloride twice a day, with an interval of 12 h between injections, in increasing doses. One group of rats received morphine injections for 2 weeks, starting with 10 and ending with a dose of 50 mg/kg. Another group of rats was morphinized for 4 weeks in doses graduaLly increasing from 10 to 70 mg/kg. The control group included intact animals. The severity of the withdrawal syndrome was assessed in the open field test (diameter 1 m) 3 days before the end of a period of 2 and 4 weeks of morphine administration, 24 h after the morning injection of morphine, and the integral parameter of abstinence (IPA) was determined on the basis of the existence of specific and general behavioral signs [16]. Tolerance to morphine was judged from the threshold of nociceptive sensitivity in the hot plate test before the beginning of morphinization and 3 h before its end, on the injection of a test dose of morphine (10 mg/kg intraperitoneally). The rats were decapitated 3 h after the last injection of morphine and blood was taken for investigation.

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TABLE 1. Antibody Levels (optical density units) to Hapten–Protein Antigens (M ± m)

Group of rats	Antigen			
	M-P	NA-P	DA-P	5-HT-P
Group of rats	0.41±0.03 n=13	0.31±0.05 n=13	0.37±0.04 n=13	0.34±0.04 n=13
Morphine				
2 weeks	0.62±0.03* n=21	0.32±0.02 n=17	0.37±0.04 n=17	0.31±0.01 n=17
4 weeks	0.62±0.04* n=22	0.61±0.08* n=18	0.55±0.06* n=18	0.55±0.07* n=18

Legend. Here and in Table 2, asterisk indicates values for which $p < 0.05$ compared with control. M) Morphine, P) protein.

TABLE 2. Blood Levels of Biogenic Amines in Morphinized Rats (M ± m)

Group of rats	Number of rats	Neurochemical parameters		
		NA, pg/ml	DA, pg/ml	5-HT, ng/ml
Control	20	699.3±55.2	120.4±24.6	97.6±14.5
Morphine				
2 weeks	18	1287.4±133.7*	99.3±11.3	109.5±19.0
4 weeks	18	551.6±91.3	325.6±61.7*	105.1±13.1

Plasma levels of free forms of neurotransmitters were determined by high-performance liquid chromatography with electrochemical detection, by the methods described previously for catecholamines and serotonin [13, 14]. Levels of antibodies to morphine and to neurotransmitters were determined by ELISA, using conjugates of the corresponding haptens with protein as antigens for parallel comparison with the reactions of the sera to the protein carrier. The reaction was carried out with the aid of antispecific IgG, labeled with horseradish peroxidase ("Sigma"). Optical density was recorded on a "Minireader II" photometer for planchets ("Dynatech"), at a wavelength of 492 nm.

EXPERIMENTAL RESULTS

Chronic morphinization of the rats for 2 and 4 weeks led to the development of physical dependence. The IPA was 5.94 ± 0.51 for rats after 2 weeks of morphinization and 6.25 ± 0.74 after 4 weeks of morphinization. Hence, as regards the intensity of the manifestations, there was no significant difference between rats given morphine for 2 and 4 weeks. In the process of morphinization there was a significant ($p < 0.001$) shortening of the latent period of withdrawal from the hot plate from 38.2 ± 3.9 in the background tests to 8.9 ± 0.6 sec after 2 weeks and 12.7 ± 1.4 sec after 4 weeks, evidence of the development of tolerance to morphine. Partial recovery of sensitivity to morphine, estimated from the reduction of sensitivity to thermal pain in the hot plate test, in rats morphinized for 4 weeks compared with those receiving morphine for 2 weeks ($p < 0.02$), could be the result of induction of antibodies to morphine. Activation of synthesis of antimorphine antibodies led previously to an increase in specific binding of ^{3}H-morphine by opiate receptors of the animal brain [7].

In fact, a significant increase in the level of antibodies to morphine compared with the control group ($p < 0.001$) was discovered in the blood serum of animals morphinized for 2 and 4 weeks (Table 1). Correlation analysis of antibody levels to morphine antigen and the duration of the nociceptive response in the hot plate test of rats after morphinization for 2 weeks did not reveal any significant correlation. Significant positive correlation ($r = 0.561$, $n = 18$, $p < 0.05$) between these parameters was established in the group of rats after morphinization for 4 weeks. The presence of this correlation is evidence of the activation of mechanisms, toward the end of the 4-week period of morphinization, which evidently led to sensitization of opiate receptors.

Chronic morphinization led to changes in plasma levels of free forms of catecholamines, and differences were discovered between the groups of rats receiving morphine for 2 and 4 weeks (Table 2). Meanwhile chronic morphinization was accompanied by a rise of antibody levels to the neurotransmitters studied (Table 1). Considering data in the literature on the modulating effect of antibodies to mediators on levels of the corresponding neurotransmitters in brain structures and on animal behavior [2], a role of raised antibody levels to noradrenalin (NA) by 97 ± 4% in the normalization of the state of the noradrenergic system toward the end of morphinization for 4 weeks cannot be ruled out. In support of this view there is evidence of negative correlation between blood levels of NA and antibodies to NA ($r = -0.363$, $n = 33$, $p < 0.05$). Despite an increase in levels of antibodies to dopamine (DA, by 49 ± 12%) toward the end of a 4-week period of morphinization, the blood DA level was raised, possible evidence of inadequate induction of antibodies to DA for binding with the sharply increased quantity of DA. It is interesting to note that in the case of serotonin (5-HT) the concentration of the neurotransmitter remained normal after 2 and 4 weeks of morphinization. In the 4-week rats significant positive correlation was found between the neurotransmitter concentration and the level of antiserotonin antibodies ($r = 0.499$, $n = 18$, $p < 0.05$), indicating the involvement of these antibodies in the maintenance of a physiological 5-HT level.

These results are evidence of the participation of anti-morphine antibodies, induced during a 4-week period of morphinization of rats, in the mechanisms of development of tolerance to morphine, bearing features of an adaptive nature.

REFERENCES

1. I. P. Anokhina, N. B. Gamaleya, and T. P. Nebarakova, Byull. Éksp. Biol. Med., No. 10, 460 (1987).
2. L. A. Basharova, L. A. Vetrile, V. A. Evseev, et al., Fiziol. Zh. Rossii, No. 10, 1367 (1988).
3. A. S. Zaks and A. A. Bykova, Patol. Fiziol., No. 4, 23 (1979).
4. I. E. Kovalev and O. Yu. Polevaya, Biochemical Bases of Immunity to Low-Molecular-Weight Compounds [in Russian], Moscow (1985).
5. K. V. Pletsity and T. V. Davydova, Progress in Science and Technology. Series: Immunology [in Russian], Vol. 20, Moscow (1989), pp. 48-120.
6. O. Yu. Polevaya, V. A. Samsonov, M. A. Lyakhova, et al., Immunologiya, No. 2, 42 (1991).
7. V. A. Tomilin, MS No. 6033-V89 (1989).
8. J. T. Beranek, L. De Cato, and F. L. Adler, Int. Arch. Allergy, **51**, 298 (1976).
9. B. Berkowitz and S. Spector, Science, **178**, 1290 (1972).
10. B. A. Berkowitz, K. V. Ceretta, and S. Spector, Life Sci., **15**, 1017 (1974).
11. K. F. Bonese, B. H. Wainer, F. W. Fitch, et al., Nature, **252**, 708 (1986).
12. H. U. Bryant and R. E. Roudebuch, J. Pharmacol. Exp. Ther., **255**, 410 (1990).
13. A. M. Krstulovic, Advances in Chromatography, ed. by A. Zlatkis, Boston (1981), pp. 561-575.
14. E. F. Marchall and N. N. Kennedy, Biochem. Med. Metab. Biol., **37**, 81 (1987).
15. B. F. Roy, J. W. Rose, H. F. McFarland, et al., Proc. Nat. Acad. Sci. USA, **83**, 8739 (1986).
16. S. K. Sudakov, M. A. Konstantinopolsky, L. A. Surkova, et al., Drug Alcohol Depend., **29**, 69 (1991).