Antimorphine Antibodies as an Indicator of Chronic Morphine Intoxication and Impaired Immunological Reactivity

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Chronic intoxication of rats with morphine during 2- and 4-week periods resulted in the emergence of antimorphine antibodies in the blood of 50 and 80% of the animals, respectively, in hemagglutination titers 1:20 and higher. Antibodies were not detected in the control animals (not treated with morphine) but were detected in 17% of rats given alcohol during a 3-month period. The animals with a high titer of antimorphine antibodies displayed a lower level of humoral reactivity in response to immunization with a thymus-dependent antigen (sheep erythrocytes) compared with the animals with a low titer of the antibodies.

Key Words: *antimorphine antibodies; chronic morphine intoxication; immunological reactivity*

According to data in the literature, chronic morphine intoxication (CMI) has a damaging effect on the lymphoid organs and immune cells (T and B cells, natural killer cells, and phagocytes), which lowers their resistance to infectious and carcinogenic agents [4,10]. On the other hand, CMI induces the production of antibodies capable of specific binding with radiolabeled morphine [7,9]. These antibodies prolong the half-life of morphine and prevents its entry into the brain [8], which may account for the lower intensity of the pharmacological effect of morphine in their presence [9,10]. In our previous studies we detected antimorphine antibodies in the sera of rats chronically intoxicated with morphine in the hemagglutination test [1] and ELISA [6,12].

The objective of this study was to evaluate the diagnostic significance of antimorphine antibodies as an indicator of CMI and impaired immunological reactivity.

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MATERIALS AND METHODS

Chronic morphinization was achieved by intraperitoneally injecting morphine in increasing doses (10-70 mg/kg) twice a day at 12-h intervals during 2- and 4-week periods. Antimorphine antibodies were identified in the reaction of indirect hemagglutination in a Takacci microtitrator, using tannin- and formalin-treated sheep erythrocytes (SE) optimally loaded with the antigen (morphine-protein conjugate) [1]. Blood sera were pretreated with erythrocytes loaded with the corresponding carrier protein and titrated at 2-fold dilutions starting from a dilution of 1:20 or 1:2. Immunological reactivity was assessed by the ability of the animals to develop the immune response manifested in the emergence of antibody-producing cells (APC) in the spleen and hemagglutinating antibod-

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ies in the serum on day 5 after a single immunization with 0.5 ml of a 15% SE suspension. The number of APC was determined by local hemolysis according to the method of Erne [2]. The phagocyte activity was evaluated by the ability of these cells to phagocytize baker's yeast cells and expressed as the phagocytosis number (% of leukocytes that had phagocytized yeast cells, counted per 200 leukocytes) and phagocytosis index (the number of yeast cells phagocytized by one leukocyte) [5].

RESULTS

The occurrence of antibodies to morphine (the morphine-protein conjugate adsorbed on SE) in the sera of morphinized rats in a titer 1:20 and higher is given in Table 1. For comparison we also investigated control rats that had been injected with normal saline in the same time periods and rats chronically intoxicated with alcohol (forced consumption of a 15% ethanol solution during a 3-month period). The specificity of the revealed antibodies was assessed by inhibition of indirect hemagglutination (IHA) in the presence of morphine and other inhibitors (serotonin and dopamine). Table 2 shows the inhibition (%) of hemagglutination when the inhibitors were added in a high concentration (10^{-3} M) to sera from two rats. The IHA titer of antimorphine antibodies was 1:128 in both sera. The morphine concentration inducing a 50% decrease in the antibody titer (IC_{50}) was 10^{-3} M in the first serum and 10^{-4} M and 10^{-6} M in the second. The constant of the antibody affinity determined as inverse IC_{50} [3] was 10^3 M^{-1} for the first serum and 10^4 and 10^6 M^{-1} for the second. These results indicate that the sera of morphinized rats contained low-affinity antimorphine antibodies.

From these results we concluded that it is possible to use high titers of antimorphine antibodies (in this study 1:20 and higher) as an indicator of CMI, since the occurrence of these antibodies in alcoholized animals was much lower and the antibodies were not detected in the control sera.

In another experimental series, rats treated with morphine during a 3-week period were divided into two groups according to the titer of antimorphine antibodies: group I with a high titer (1:16 and higher) and group II with a low titer (1:2-1:8). Group I consisted of 11 animals. The mean titer ($-\log_2$ Tab) in this group was 4.6±0.2. The second group included 14 animals, $-\log_2$ Tab was 1.8±0.4. The difference was highly significant ($p<0.001$).

Immunological reactivity of the animals in relation to the presence or absence of the antibodies was evaluated by the character of the humoral response to immunization with SE (Fig. 1). Immunization of the control animals ($n=12$) with SE led to a significant increase ($p<0.001$) in the number of APC in the spleen compared with that in nonimmune control rats ($n=6$). Antibody-producing cells have surface receptors for SE and induce local hemolysis of SE in the presence of complement. There was a simultaneous significant increase ($p<0.001$) in the hemagglutinin titer. Immunization of rats chronically intoxicated with morphine led to a considerably lesser induction of APC in the spleen, which is consistent with published data [13,14].

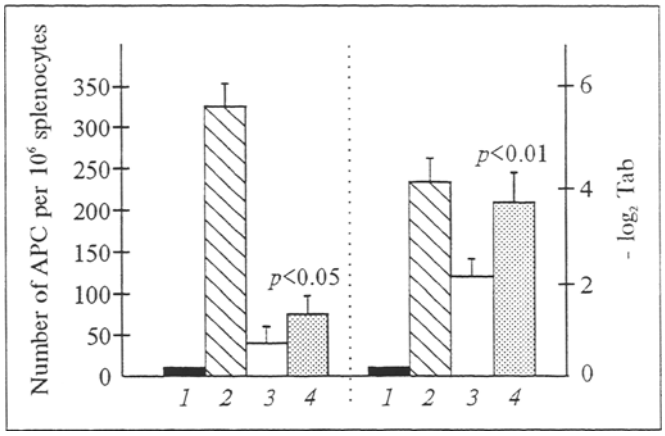

Fig. 1. Immune response to SE in morphinized rats with high (AbM+) and low (AbM−) levels of antimorphine antibodies. *1*) control group; *2*) control immunized group; *3*) immune rats with AbM+; *4*) immune rats with AbM−. The significance of intergroup differences between AbM+ and AbM− rats is given.

We were the first to demonstrate that animals with high levels of antimorphine antibodies are characterized by a weaker humoral response to im-

TABLE 1. Occurrence of Antimorphine Antibodies (%) in Rat Serum in Titers 1:20 and Higher (M±s)

Group of rats	Number of animals	Occurrence of antibodies
Control	20	0.0±0.0
Morphinized		
2 weeks	10	50.0±18.9 $p<0.001$*
4 weeks	8	80.0±13.3 $p<0.001$*
1 month after last morphine injection	8	50.0±18.9 $p<0.001$*
3-month alcoholization	18	16.7±9.1 $p<0.05$* $p<0.01$**

Note. Comparison of two random samples with Fisher f-transform: with the control group (one asterisk) and with the 4-week morphinization group (two asterisks).

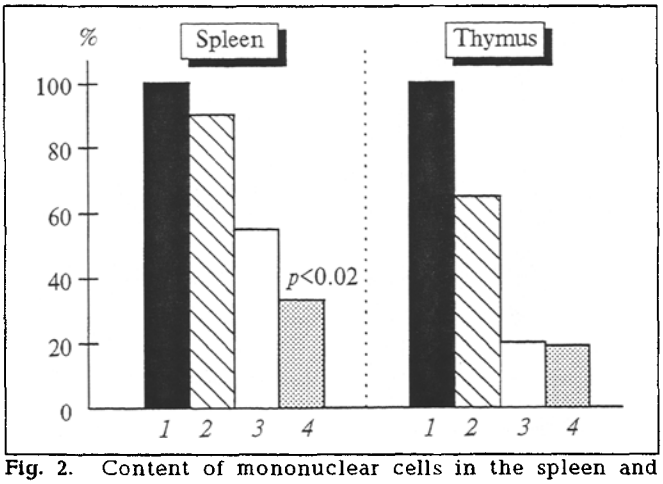

Fig. 2. Content of mononuclear cells in the spleen and thymus of morphinized rats with high (AbM+) and low (AbM−) levels of antimorphine antibodies (% of control).

munization. A significant inverse correlation between -$\log_2$ Tab, on the one hand, and the number of APC per 10^6 splenocytes (r=-0.46, p<0.05) and the titers of anti-SE antibodies (r=-0.63, p<0.01), on the other, was established in the group of rats chronically intoxicated with morphine (n=25).

The mechanisms underlying this phenomenon are unclear. Light could be shed by an investigation of the state of immunocompetent cells in morphinized rats. SE are a T-dependent antigen, and both T and B cells are involved in the immune response to it. Figure 2 shows the number of mononuclear cells in the spleen and thymus of rats of the studied groups. In the groups with high and low levels of antimorphine antibodies, chronic morphinization led to a pronounced decrease in the number of lymphoid cells in the spleen and, particularly, in the thymus. The level of thymic T cells was equally reduced in the two groups, whereas the number of B cells in the spleen was considerably higher in the rats with a high level of antimorphine antibodies.

From the comparison of the results shown in Figs. 1 and 2 it can be concluded that chronic morphinization of animals not accompanied by the production of high levels of antimorphine antibodies does not lead to any significant decrease in the titer of hemagglutinin in response to immunization with SE, despite a considerable decrease in the number of lymphoid cells in the thymus and spleen. By contrast, the induction of antimorphine antibodies during morphinization is accompanied by a marked inhibition of the immune response to a T-dependent antigen (SE). The latter may be due to a particularly strong inhibition of the functional activity of T cells in rats producing increased amounts of antimorphine antibodies.

TABLE 2. Inhibition of Antibody Binding to Erythrocyte Morphine Antigen in the Presence of 10^{-3} M Inhibitor

Inhibitor	Inhibition (%)	
	serum № 1	serum № 2
No inhibitor	0	0
Morphine	60	50
Serotonin	20	33
Dopamine	20	33

Since phagocytosis is a factor influencing the intensity of the immune response to antigen, we assessed the phagocytizing activity of leukocytes in this study. The concentration of phagocytizing cells (phagocytosis number) was significantly (p<0.01) increased in the blood of rats injected with morphine during a 3-week period without any substantial changes in their phagocytizing activity (phagocytosis index). There were no significant differences in the phagocytosis number in rats producing and not producing antimorphine antibodies (50.1±3.9 and 48.3±4.0%, respectively). In immune nonmorphinized rats this parameter was 31.7±3.2%. These findings indicate that phagocytosis does not play any significant role in the lowering of rat immunological reactivity to SE.

Our results show that increased production of antimorphine antibodies can serve not only as an indicator of CMI but also as an indicator of impaired immunological reactivity, specifically, of decreased functional activity of T cells.

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