ACTION OF MORPHINE ON REPRODUCTION AND IMMUNOLOGICAL STATUS IN MICE

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The course of embryogenesis is disturbed in CBA mice during prolonged administration of morphine. There is a parallel decrease in the level of antibody-synthesizing cells and a significant fall in cooperative interaction between T- and B-lymphocytes in response to injection of sheep's red blood cells. Meanwhile stimulation of bone marrow cells and specific sensitization of producers of migration inhibition factor to antigen from the brain of mice treated with morphine are observed.

KEY WORDS: reproduction; immunological status.

States of narcotic dependence are accompanied by disturbance of the reproductive function in man. According to modern views of the pathogenesis of addiction to narcotics and, in particular, to opium, complex relations exist between the opiate receptors of the synaptic membrane and endogenous peptides with morphine-like activity (endorphines and enkephalins) [15, 16]. It has been shown that narcotics inhibit the functional activity of the gonads in the male and female indirectly through this system, by lowering the gonadotrophin, testosterone, and estrogen levels [1, 5]. An important role in the pathogenesis of addiction to narcotics is undoubtedly played by immune mechanisms also. This suggestion is based on the results of previous investigations by the present writers [3, 4] and others [14], which showed that the hormonal activity of estrogens is directly dependent on the immune response. There is also evidence [2] that prolonged morphinization is accompanied by the development of immunological reactions such as association of the xenobiotic with albumins and γ-globulins [6], synthesis of antibodies and autoantibodies [9], and also the development of skin hypersensitivity reactions [13].

The object of the present investigation was to compare reproductive functions with certain nonspecific and specific indices of the immune response of CBA mice during chronic administration of morphine.

EXPERIMENTAL METHOD

A model of chronic morphinization was produced by administration of unconjugated morphine-HCl in physiological saline to sexually mature female CBA mice in accordance with the scheme described in [13]. The narcotic was applied subcutaneously in increasing doses from 4 to 40 mg/kg body weight, in four courses, each lasting 10 days, with intervals of 10 days between them. Reproductive function was assessed by mating the morphinized females with intact males. The number of implantation sites, the number of embryos, and the number of pregnant animals as a percentage of the total in the experimental and intact groups were determined. Several immunological indices also were studied. To estimate the number of stem cells, 10^5 viable bone marrow cells from 10 experimental and 10 intact donors were transplanted into syngeneic recipients irradiated in a dose of 850 R. On the 8th day the number of colony-forming units (CFU) in their spleens was counted. The antibody-synthesizing function was estimated after preliminary intravenous injection of sheep's red blood cells (SRBC) in a dose of 5×10^8 cells per mouse. Cooperative interaction between T- and B-lymphocytes was determined from the number of antibody-synthesizing cells appearing against SRBC after simultaneous intravenous injection of 2×10^7 thymocytes and 1×10^7 bone marrow cells into syngeneic supralethally irradiated re-

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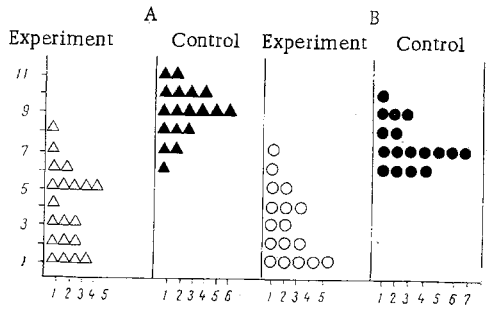

Fig. 1. Reproductive function in female CBA mice taking morphine. Abscissa, ratio distribution of animals among groups; ordinate: A) number of implantation sites, B) number of embryos.

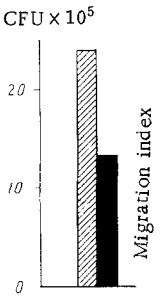

Fig. 2

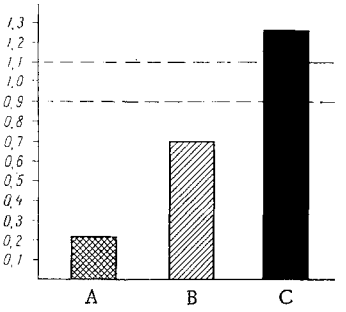

Fig. 3

Fig. 2. Number of colonies of stem cells after transplantation of 10^5 syngeneic bone marrow cells. Obliquely shaded column, experiment; black column, control.

Fig. 3. Specificity of cellular response to injection of morphine into CBA mice. Test systems: A) with antigen from brain of morphinized mice, B) with antigen from liver of morphinized mice, C) response of intact mice to antigen from brain of morphinized animals.

cipients. The level of specific cellular reactivity was determined by the macrophage migration inhibition test from capillary tubes [7]. Saline extracts obtained after centrifugation of the brain and liver tissue of mice treated with morphine and of intact animals at 20,000 g were used as the antigens.

EXPERIMENTAL RESULTS

Analysis of the reproductive function of the groups of control and experimental animals showed that fertility was considerably reduced during chronic morphine administration. In particular, the number of implantation sites in the experimental mice was not more than 52.2% of that in the intact controls. Morphine sharply disturbed embryonic development. In the group of morphinized females this parameter was 41.2%, but did not exceed 10% in the control. The results of one experiment to study the number of implantation sites and the number of embryos in 20 females taking morphine and 20 control animals are given in Fig. 1. Clearly the level of these indices in the experimental series was significantly lower than in the control.

The study of the immune response to the model chosen demonstrated that morphine acts on various stages of the immunocompetent system, starting with the stem cell, Chronic morphinization of mice was accompanied by stimulation of stem cells (Fig. 2). The number of CFU in this group of animals was 23.8×10^5 bone marrow cells, whereas in the control it did not exceed 12.1×10^5. A significant decrease in the humoral response also was recorded: the number of antibody-synthesizing cells in the spleens of the female mice receiving morphine was 47×10^6, compared with 221×10^6 in the control. Similar results also were obtained when cooperative activity of T- and B-lymphocytes in response to injection of SRBC was estimated. In the morphinized mice the immune response was depressed by one-third compared with that in intact animals.

The results are very interesting for they reveal specific sensitization of T-lymphocytes producing the factor inhibiting macrophage migration in mice exposed to the chronic action of morphine. In a system in which peritoneal exudate from mice receiving morphine and antigen isolated from their brain were used the migration index was 0.19. This result indicates a high level of sensitization to the primary target cells for morphine. The specificity of the response was confirmed by two controls: the reaction with antigen from liver of morphinized animals, when the migration index was 0.68, and the reaction of morphine-treated mice to antigen obtained from the brain of intact females (migration index 1.17).

Prolonged morphinization is thus accompanied by considerable changes in several immunological indices and by depression of reproductive functions in females. Morphine exhibits its strongest effect on brain tissue, the antigenic properties of which are changed. These observations are indirectly confirmed by results obtained by other workers who found changes in protein synthesis in the brain under the influence of morphine [8, 11]. When the effects of morphine on the immune system are assessed, in connection with the facts described above the presence of a common θ-antigen in the thymus and brain tissue should be noted. In this connection the possibility of direct involvement of the thymus at a particular stage in the pathogenesis of opium addiction, with subsequent disturbance of immune and reproductive functions, cannot be ruled out.

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