Effect of chronic administration of morphine on primary immune response in mice

M. Güngör, E. Genç, H. Sağduyu, L. Eroğlu and H. Koyuncuoğlu

Department of Pharmacology and Clinical Pharmacology, Istanbul Medical Faculty, Istanbul University, Capa, Istanbul (Turkey), 10 March 1980

Summary. The effect of 3 different doses of chronically-administered morphine on the primary immune response was studied in mice by estimating spleen/body weight ratio and serum hemolysin production against sheep red blood cells (SRBC). It was observed that morphine exerted a dose-dependent inhibitory effect on the immune response which was antagonized by the concomitant administration of naloxone. The findings suggest that the inhibitory effect of morphine is specific.

The relative incidence of various pathological states among opiate addicts is much higher and more dangerous[1-3]. It has generally been believed that this high incidence is due to non-hygienic methods of administration or to mistakes in the dilution of the administered drugs[4]. On the other hand, the susceptibility of addicts to pathogens has been observed, and a parallelism between this susceptibility and the duration of drug abuse has been proposed[3,4]. Moreover, it has been shown that morphine has an inhibitory effect on some immune phenomena in man and animals[5-8], and immune response to infection agents is a determining factor in the clinical manifestations and the outcome of infections. Therefore, it was considered of interest to investigate the effect of chronic morphine administration on the primary immune response to SRBC and to determine whether naloxone, a selective morphine antagonist, could antagonize the probable inhibitory effect in mice.

Materials and methods. Inbred white mice (obtained from the Center of the Turkish Scientific and Technical Research Council) weighing 20–25 g, fed on a pellet diet and tap water ad libitum were used throughout. Daily fluid and food intake and weight gain were measured during the course of the experiment. Morphine hydrochloride (McFarlan Smith Co, Edinburgh) and naloxone hydrochloride (Endo Lab. USA) were dissolved in saline. Fresh SRBC, obtained from the healthy animals in Alsever's solution, were washed 3 times by centrifugation with phosphate buffered (pH 7.3) saline (PBS). A 10% suspension of the SRBC in PBS was used to produce immunization in mice and a 1% suspension of SRBC to determine the titration of serum hemolysin. All treatments were administered i.p.

Mice were divided into 7 groups. The 1st group (control) was injected with 0.1 ml/10 g of 0.9% saline twice a day for 15 days. The 2nd group (morphine-I) was given increasing doses of morphine 10, 20, 30, 40, 50, 70, 90, 110, 130, 150, 160, 170, 180, 190, 200 mg/kg twice a day for 15 days. The 3rd (morphine-II) and 4th (morphine-III) groups were given morphine, the dose being $1/3$ and $1/4$ of that of the 2nd group's, respectively, throughout the experiment. The 5th group (morphine-I + naloxone) was subjected to the same procedure as the 2nd group but received additionally naloxone at $1/10$ the dose of morphine given on those days. The 6th group (naloxone) received 5 mg/kg of naloxone twice a day for 15 days. A single 0.25-ml injection of a 10% suspension of SRBC was administered to the groups 1–6 on the 1st day. The last group received no injections, serving as a baseline or background control. On the 1st, 10th and 15th days of the experiment, 2 h after the morning injection, at least 5 mice taken at random from each group were decapitated and their blood was collected and then the spleen/body weight ratio of each animal determined. After 30 min at room temperature the clotted blood was loosened from the sides of the tubes and kept at 4 °C for 30 min. Following centrifugation at 3000 rpm for 2 min, the serum was removed and incubated at 56 °C for 30 min to destroy endogenous complement. Serial dilutions of serum were prepared with PBS in microtitration plates (Cooke Engineering Co, Arlington, USA). 0.1 ml of serial dilution was mixed with 0.1 ml of 1% suspension of SRBC and a standard amount of fresh guinea-pig serum was added as complement. The mixture was incubated for 1 h at 37 °C. Wells were observed and the reciprocal of the highest serum dilution which gave a detectable hemolysis was used as the titer[9]. All the titers were expressed as the $\log_2$ of the dilution. Results were given as the mean $\pm$ SE. Statistical evaluation was performed using Student's t-test.

Results. No differences in fluid and food intake and weight gain were observed among control, morphine and morphine-naloxone groups.

The serum hemolysin titers which were determined in mice immunized with SRBC and injected with saline twice a day throughout the experiment are shown in figure 1. On the 1st day no hemolysin was detected in the serum. On the 5th day the titration was 6.83 ± 0.94. It gradually increased to 11.00 ± 0.73 and 14.87 ± 1.48 on the 10th and 15th days respectively. The immunization also resulted in a progressive increase in the spleen/body weight ratios of the immunized mice during the period of experiment, as shown in figure 1. No significant changes were observed in spleen/body weight ratio of the background control group.

Gradually increasing serum hemolysin formation and spleen/body weight ratio increase were also observed in all chronically morphine treated groups (figure 2). However, the mean values of these 2 parameters in the morphine-treated groups were lower than those of the control groups. Namely, the mean values of serum hemolysin titers on the 5th, 10th and 15th, and the spleen/body weight ratios on the 10th and 15th days of experiment in the morphine-I group were significantly lower than those of the immunized control group. On the other hand, the mean serum hemolysin titers and the spleen/body weight ratios of the morphine-II and morphine-III groups were not significantly different from the corresponding control values except for the serum hemolysin titer and the spleen/body weight ratio of the morphine-II group on the 10th day of experiment.

Naloxone had no effect on either serum hemolysin formation or spleen/body weight ratio (figure 3). However, when naloxone was administered 5 min before morphine at $1/10$ of the morphine doses, it antagonized the inhibitory effect of morphine on both parameters.

Discussion. In the present experiment SRBC induced rapidly and progressively increasing serum hemolysin formation, and produced a marked increase in spleen/body weight ratio of mice during the period of experiment[10].

The primary immune response seen in the immunized group was not blocked completely by the chronic administration of morphine. Thus, a progressive increase in serum hemolysin levels and spleen enlargement were found in morphine-treated groups as well as in immunized control mice. Nevertheless, when the mean values of serum hemolysin titers and spleen/body weight ratios of the immunized control group and morphine-treated groups were compared, statistically significant low levels of both parameters

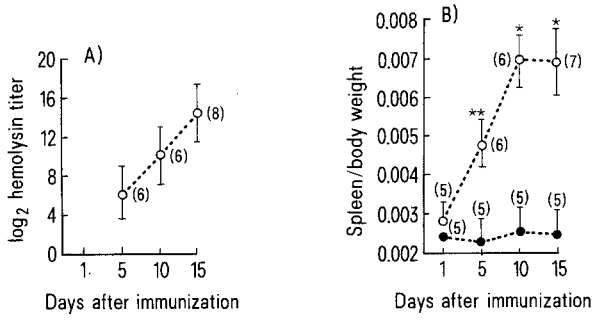

Fig. 1. Immunization induced serum hemolysin formation (A) and spleen/body weight ratio (B) increase. Immune mice received 0.25 ml of 10% of SRBC, i.p., on day 1; ○, immune; ●, nonimmune. Each point and vertical bar represent mean ± SE. Figures within the parenthesis indicate number of animals. Mean values of immune groups were compared to corresponding nonimmune values using t-test. * $p < 0.01$, ** $p < 0.001$.

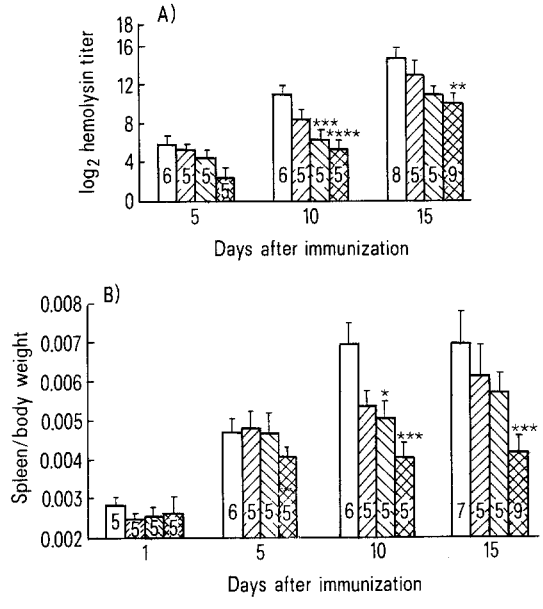

Fig. 2. Effect of chronic administration of 3 different morphine doses on serum hemolysin formation (A) and spleen/body weight ratio (B) of immunized mice. 0.25 ml of 10% SRBC were injected on 1st day for immunization. □, control (0.1 ml/10 g saline); ⊠, morphine-I (10–200 mg/kg); ▨, morphine-II (5–100 mg/kg); ▨, morphine-III (2.5–50 mg/kg). Each bar represents mean ± SE. Figures within the bars indicate number of animals. The mean values of morphine-treated groups were compared to that of the control group on the same day using t-test. * $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$, **** $p < 0.001$.

were found. In other words, morphine exerted a marked inhibitory effect on serum hemolysin formation and spleen enlargement. As shown in figure 2, this inhibitory effect was dose dependent.

The inhibitory effect of morphine on the primary immune response was unlikely to be due to a general toxicity since there was no significant difference in weight gain among the groups.

The occurrence of an immune response requires a rapid proliferation of lymphocytes and other related cells. Additionally, morphine and other opioids have an inhibitory effect on phospholipid[11–13], protein, RNA and DNA synthesis[14–16]. Therefore, the inhibitory effect of morphine on

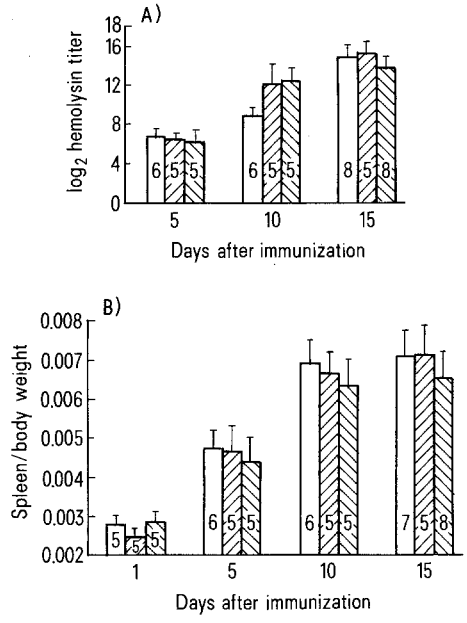

Fig. 3. The naloxone antagonism of inhibitory effect of morphine on serum hemolysin formation (A) and spleen/body weight ratio (B) of immunized mice. □, control; ▨, naloxone (5 mg/kg twice a day i.p.); ▨, morphine + naloxone (10–200 mg/kg morphine twice a day i.p. + preceded by naloxone injection which was 1/10 the dose of morphine). Each bar represents mean ± SE. Figures within the bars indicate the number of animals. The mean values of naloxone and morphine + naloxone groups were compared to value of control group on the same day using t-test.

primary immune response might be due to the inhibition of biosynthesis of these macromolecules which are to some extent essential for cell proliferation. Administration of naloxone together with morphine seemed to have an antagonistic effect on the inhibitory effect of morphine on primary immune response. This finding suggests that on the primary immune response the inhibitory effect of morphine might be a receptor mediated effect, since it is postulated that naloxone is a blocker of morphine and related narcotic drugs receptors[17].

1 B. Eiseman, R.C. Lam and B. Rush, Ann. Surg. *159*, 748 (1964).
2 C.E. Cherubin, Ann. intern. Med. *67*, 23 (1967).
3 D.B. Louria, T. Hensle and J. Rose, Ann. intern. Med. *67*, 1 (1967).
4 D.B. Louria, Arch. intern. Med. *123*, 82 (1969).
5 T.H. Stanley, G.E. Hill, M.R. Portas, N.A. Hogan and H.R. Hill, Anesth. Analg. *55*, 668 (1976).
6 C.Y. Hung, S.S. Lefkowitz and W.F. Geber, Proc. Soc. exp. Biol. Med. *142*, 106 (1973).
7 W.F. Geber, S.S. Lefkowitz and C.Y. Hung, Archs int. Pharmacodyn. *214*, 322 (1975).
8 S.S. Lefkowitz and C.Y. Chiang, Life Sci. *17*, 1763 (1975).
9 N.R.St.C. Sinclair and E.V. Elliott, J. Immunol *101*, 251 (1968).
10 M.L. Lukic, A. Janezic and L. Popeskovic, Immunology *34*, 791 (1978).
11 S.J. Mule, Biochem. Pharmac. *19*, 581 (1970).
12 N. Wurster, P. Elsbach, J. Rand and E.J. Simon, Biochim. biophys. Acta *248*, 282 (1971).
13 W.P. Dole and E.J. Simon, Biochim. biophys. Acta *248*, 282 (1974).
14 D.H. Clouet and M. Ratner, Brain Res. *4*, 33 (1967).
15 R.K. Datta and W. Antopol, Toxic. appl. Pharmac. *23*, 75 (1972).
16 R.K. Datta and W. Antopol, Brain Res. *53*, 373 (1973).
17 W.R. Martin, Pharmac. Rev. *19*, 463 (1967).