PHARMACOLOGY AND TOXICOLOGY

Effect of Rat Immunization with Antimorphine Antibodies on Morphine Sensitivity and Predisposition to Dependence Formation

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Active immunization of rats with mouse antibodies to morphine leads to the formation of antiidiotypic antibodies. This is paralleled by an increase in animal sensitivity to the analgesic and positive reinforcing effects of morphine, slower development of tolerance and formation of craving. These data indicate the possibility of active immunization against antibodies to morphine/heroin for the prevention of opium addiction relapses.

Key Words: *analgesia; positive reinforcement; antiidiotypic antibodies; autoinfusion of morphine; tolerance*

Treatment of patients with opium addiction is difficult because of the absence of effective pathogenetic drug therapy. The priority problems are arrest of pathological addiction and prevention of relapses after short-term therapy. Today "antagonistic therapy" is used with this aim: the patient receives opioid receptor antagonists suppressing the sensitivity to the positive reinforcing effect of opiates. Another trend of "antagonistic therapy" is the use of antibodies to the narcotic, which leads to its neutralization in the body and suppression of the euphoric effect. The development of this latter method is now in progress. Anticocaine vaccines were created [3-5], but the main drawback of antinarcotic vaccines is incomplete neutralization of the narcotic during vaccination: increasing the dose leads to development of euphoria, and narcotic consumption increases.

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Injection of mouse antibodies to morphine to rats will promote the production of secondary antibodies. These antibodies (antiidiotypic antibodies) specifically bind to peripheral opioid receptors, which theoretically can modulate not only the peripheral opioid system, but also the function of the cerebral opioid system. *In vitro* studies showed that antiidiotypic antibodies to morphine exhibited morphine-like effects and bound to opioid receptors in a dose-dependent manner [6].

We studied the effects of antiidiotypic antibodies to morphine produced in rats on the sensitivity of animals to analgesic and positive reinforcing effects of morphine and on the development of tolerance and predisposition to dependence formation.

MATERIALS AND METHODS

Experiments were carried out on 20 Fischer-344 rats. Half of animals received solution (1 ml/kg) of murine monoclonal antibodies to morphine (Fitzgerald, clone M9942910) with complete Freund

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adjuvant (1 ml/kg) on days 1, 14, and 21 of the experiment (subcutaneously on the back). Other animals served as controls and were injected with complete Freund adjuvant (1 ml/kg).

During immunization the rats were trained instrumental alimentary behavior: after 48 h food deprivation the rats were placed for 50 min into an instrumental cage (Lafayette Instruments Inc.), where they could obtain a fodder granule (45 mg; P.S. Noyes Company Inc.) after pressing a lever. The appearance of fodder in the feed box was followed by a 17-sec latent period (LP) during which the animal received no fodder after lever pressing. LP was associated with darkness (the light was switched off). During the first 2 days of training the rats received a fodder granule after a single lever pressing, during the next 2 days the lever was to be pressed twice. During the next 3 days the fodder was delivered after 3-fold lever pressing, and during the subsequent 5 days the lever was to be pressed five times. Hence, stage I was over after 12 days, when all rats developed stable food acquisition behavior. At this stage the animals received 12 g fodder during the time free from the experiment.

On day 20 after the start of immunization the animals were implanted (under ketamine narcosis, 100 mg/kg) two-component synthetic catheters through an orifice in the jugular vein. The inner part of the catheter was a silicone tube (Dow Corning Corp.) with the outer diameter 1.2 mm, 25 mm long. The tip of the catheter was in the vena cava superior. The remaining part of the catheter was made from a vinyl tube (Dural Plastic and Engineering; outer diameter 1.0 mm, length 55 mm), connected on one side through a special adapter (Small Parts Inc.) to the intravenous part of the catheter, while the other side was fixed to the skin on the neck.

After one-week rest (on day 28 after the start of immunization) during which the rats were kept in individual boxes with free access to water and food, the sensitivity to analgesic effect of morphine was evaluated. The latency of tail flick from hot (56°C) water before and after injection of morphine (5 mg/kg) was measured.

On days 29-38 the rats were daily placed for 50 min into experimental cages (in which they formed food acquisition behavior) and the characteristics of intravenous autoinjection of morphine were studied. Free end of implanted catheter was connected (through a liquid rotating contact) to a Harvard Apparatus precision pump. After the lever was pressed 5 times, 100 μg morphine hydrochloride dissolved in 0.05 ml isotonic NaCl was infused through implanted catheter into vena cava superior. The animals were placed into the experimental chambers for 9 days. During this period all animals formed stable consumption of morphine. On days 39, 40, and 41 the rats received 50, 100, and 150 μg morphine, respectively, after pressing the lever 5 times.

On day 42 the formation of physical dependence was started by injecting ascending doses of morphine from 5 to 60 mg/kg (over 8 days). On day 48 of the experiment the intensity of the analgesic effect produced by 55 mg/kg morphine (*i. e.* tolerance development) was evaluated. On day 49 six hours after the last injection of morphine the animals were intraperitoneally injected with 1 mg/kg naloxone, after which specific abstinence reactions (shaking, respiratory disorders, ptosis, piloerection, gnashing, cramps) were evaluated in an open field. The total abstinence score (sum of all signs) and incidence of individual signs were evaluated for each rat.

After the end of the experiment the blood was collected for enzyme immunoassay of antibodies to antimorphine antibodies. In subsequent analysis only animals of the experimental (immunized) group with antibody titers at least 1:64 were used (8 of 10 rats).

The data were statistically processed using Student's *t* test.

RESULTS

No appreciable differences in the food-procuring behavior between immunized and nonimmunized rats were detected. On day 1 of the experiment control and immunized animals consumed 25.55± 4.01 and 28.23±4.45 fodder granules, respectively.

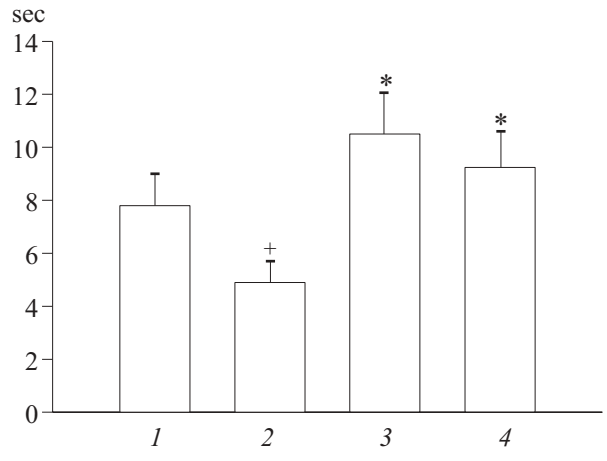

Fig. 1. Analgesic effect of morphine in intact controls (*1*), tolerant controls (*2*), immunized intact (*3*), and immunized tolerant (*4*) rats. Intact animals were injected with 5 mg/kg, tolerant ones with 55 mg/kg morphine. Ordinate: tail flick latency. $p<0.05$ compared to *controls; +intact animals.

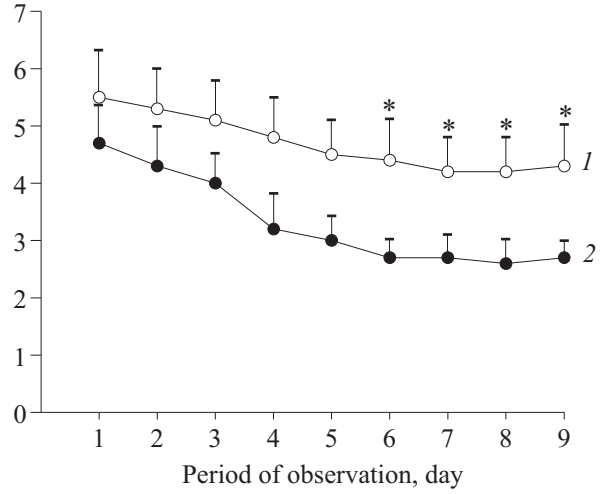

Fig. 2. Intravenous autoinjections of morphine by control (*1*) and immunized (*2*) rats. Here and in Fig. 3: ordinate: number of infusions per session. *$p<0.05$ compared to immunized animals.

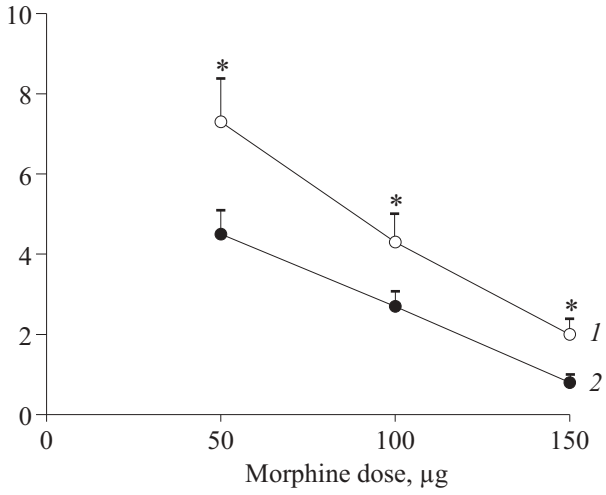

Fig. 3. Intravenous autoinfusion of different morphine doses by control (*1*) and immunized (*2*) rats.

On day 12 controls consumed 74.52±3.21 granules, immunized animals consumed 75.38±5.33 granules. The time course of lever pressing frequency during the sessions was also virtually the same in experimental and control animals.

Immunization appreciably increased animal sensitivity to the analgesic effect of morphine. This manifested in longer tail flick latency in immunized rats injected with 5 mg/kg morphine (Fig. 1).

Control and immunized animals easily learned to get morphine instead of food support. On day 1 of food replacement controls pressed the lever 27.3± 4.55 times, immunized animals pressed it 23.3± 4.15 times. The frequency of lever pressing decreased in both groups on subsequent days, morphine consumption decreasing more rapidly in immunized animals (Fig. 2). Reduction of morphine dose to 50 μg per injection increased the number of injections, while increasing the dose to 150 μg, consequently, decreased the number of lever pressings by immunized and control animals. However, the number of morphine infusions (50, 100, and 150 μg) was significantly lower in immunized rats compared to controls (Fig. 3).

The analgesic effect of morphine in a dose of 55 mg/kg in morphine dependent/tolerant immunized rats was appreciably higher than in controls. However, in tolerant control rats the analgesic effect of morphine in a dose of 55 mg/kg was significantly lower than the effect of 5 mg/kg morphine in intact controls, while in immunized animals no difference was observed. This can indicate slower formation of tolerance in these animals (Fig. 1). However, the degree of physical dependence of experimental animals and controls was virtually the same.

Hence, antibodies to antimorphine antibodies (antiidiotypic antibodies) in the blood of experimental animals modified morphine sensitivity. We previously showed that Fischer-344 rats were highly sensitive to the analgesic and positive reinforcing effects of morphine. The density of cerebral opioid receptors was decreased in these animals [1,2,7]. Presumably, the presence of antiidiotypic antibodies in the blood through its direct agonistic effect on the peripheral opioid receptors leads to "reflected" effects in the CNS, which can result in increased sensitivity to the analgesic and positive reinforcing effects of opiates. Active immunization with antimorphine antibodies can reduce liability to the formation of opium narcomania by suppressing pathological craving to opiates, which manifested in rats in decreased frequency of morphine autoinjections.

The data on the decrease of pathological opiate craving paralleled by higher sensitivity to them can indicate the possibility of using active immunization against morphine/heroin antibodies for preventing relapses in patients with opium narcomania.

REFERENCES

1. S. K. Sudakov, D. Yu. Rusakov, I. V. Tyurina, and E. V. Borisova, *Vopr. Narkol.*, No. 1, 40-45 (1995).
2. S. K. Sudakov, I. B. Figurina, O. F. Medvedeva, and I. V. Rusakova, *Byull Eksp. Biol. Med.*, **131**, No. 10, 364-367 (2001).
3. M. R. Carrera, J. A. Ashley, B. Zhou, *et al.*, *Proc. Natl. Acad. Sci. USA*, **97**, 6202-6202 (2000).
4. B. S. Fox, *Drug Alcohol Depend.*, **48**, 153-158 (1997).
5. K. M. Kantak, S. L. Collins, E. G. Lipman, *et al.*, *Psychopharmacology* (Berl.), **148**, 251-262 (2000).
6. D. S. Ng and G. E. Isom, *Biochem. Pharmacol.*, **34**, 2853-2858 (1985).
7. S. K. Sudakov, S. R. Goldberg, E. V. Borisova, *et al.*, *Psychopharmacology* (Berl.), **112**, 183-188 (1993).