Immunological Correlation between Morphine Tolerance and the Effect of Naloxone in Rats of Different Age

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In 3-6-week-old morphine-sensitive rats, in which morphine injection produced an analgetic effect, the serum titer of antimorphine antibodies 24 h postinjection is less than half that observed in morphine-resistant animals. Administration of naloxone to morphine-sensitive rats induces hyperalgesia and considerably raises the serum titer of antimorphine antibodies. Chronic injections of the same dose of morphine, which cause its analgetic effect to disappear, increase the titer of antibodies in morphine-sensitive rats 2-fold. In morphine-resistant rats naloxone produces an analgetic effect followed by its gradual decay and disappearance in the course of chronic administration. Subsequent administration of morphine induces analgesia, raises the titer of antimorphine antibodies, and lowers the titer of antiidiotypic antibodies.

Key Words: *morphine; tolerance; naloxone; antimorphine antibodies; antiidiotypes*

Although the immune system is known to play a role in the formation of morphine tolerance - the disappearance of the effect of the initial dose of the narcotic, which develops especially rapidly in children [13] - this role remains poorly understood [12]. It has been found that antimorphine antibodies (AMAB) are produced in animals and man in response to morphine injection [4]. However, there are morphine-resistant individuals [1,14], in whom the reactions of the immune system have remained virtually unstudied. Recently, special interest has been paid to the detection of not only idiotypic but also antiidiotypic antibodies [2], in particular AMAB (AIAMAB). However, so far there is no clear evidence that these antibodies play a part in the mechanisms of morphine tolerance.

Moreover, administration of naloxone to morphine-resistant animals has been previously found to induce an analgetic rather than hyperalgetic (as in controls) effect and to abolish morphine tolerance [1]. It also reverses the reactions of intestinal muscles and *vas deferens*, a feature attributed to the production of AMAB-reacting substances [10].

The aim of the present study was to investigate the dynamics of formation of AMAB and AIAMAB in 3-6-week-old rats with morphine resistance and in the course of tolerance development and naloxone treatment.

MATERIALS AND METHODS

The experiments were carried out on 3-6-week-old male albino Wistar rats weighing 50-75 g. Changes in nociception were evaluated on an Ugo Basile automatic analgesimeter by the latency of the tail flick (LTF) in response to 10 presentations of a thermal nociceptive stimulus every 1.5-2 min (tail-flick test). The device switches off automatically after 40 sec to avoid skin burn.

Morphine hydrochloride (2 mg/kg) and naloxone (0.3 mg/kg, Sigma) were injected subcutaneously and intraperitoneally, respectively.

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The results were processed statistically using the unpaired Student's test.

The animals were decapitated and the blood was sampled 24 hours after the injection of morphine or naloxone in the case of chronic administration (one injection per day) and 30 min postinjection in the experiments with a single naloxone injection. In the serum 50% AMAB (T_{50} AMAB) and AIAMAB were measured by enzyme-linked immunosorbent assay (standard direct method) using anti-rat peroxidase conjugates and o-phenylenediamine as the substrate [3]. AMAB were determined using morphine conjugated with bovine serum albumin as described earlier [6]. AIAMAB were measured using 0.1% IgG isolated from serum of rats hyperimmunized with a standard morphine conjugate. IgG fractions were purified by affinity chromatography on protein A-Sepharose 4B [8].

RESULTS

In ten 3-week-old intact rats the mean LTF was 13.2± ±0.8 sec (Fig. 1, *a*) and T_{50} AMAB ranged from 1:4 to 1:8.

Morphine injection in 9 rats with an initial LTF of 11.9±0.5 sec produced an analgetic effect (no tail flick reaction over a 40-sec nociceptive thermal stimulation, Fig. 1, *b*). This effect was observed 10-15 min postinjection, lasted for 60-90 min, and was followed 90-100 min later by restoration of the reaction with a latency of 13.1±0.6 sec. T_{50} AMAB in these morphine-sensitive animals was 1:16.

In 6 rats with an initial LTF of 16.6±0.4 sec morphine injection did not reliably change this parameter (16.8±0.4 sec, Fig. 1, *c*) and T_{50} AMAB in these animals ranged from 1:32 to 1:64.

A single injection of naloxone to five 6-week-old morphine-sensitive rats produced hyperalgesia (LTF dropped to 9.3±0.4 sec, $p<0.001$, Fig. 1, *d*). T_{50} AMAB in these animals was 1:64-1:128.

Eight 3-week-old rats were chronically injected with morphine (Fig. 1, *e*). The first injection increased LTF from 14.3±0.5 to 33.3±2.0 sec ($p<0.001$). The subsequent injections gradually reduced the analgetic effect and after 6 injections LTF did not differ reliably from the initial value (15.4±0.9 *vs.* 17.0±0.5 sec), indicating the formation of morphine tolerance. T_{50} AMAB in this group ranged from 1:32 to 1:64 and AIAMAB was 1:4.

In the next experimental series 7 rats were chronically injected with morphine until tolerance was attained and then received a single injection of naloxone, which produced an analgetic effect and increased LTF from 17.4±1.0 to 26.3±1.0 sec ($p<0.01$, Fig. 1, *f*). T_{50} AMAB and AIAMAB in these animals were 1:16 and 1:40, respectively.

In the last series ten 3-6-week-old rats, in which tolerance to morphine had been induced by its chronic administration, were chronically injected with naloxone until the analgetic effect disappeared (Fig. 1, *g*). T_{50} AMAB and AIAMAB in these animals were 1:5 and 1:20-1:40, respectively. After the analgetic effect of naloxone had disappeared, 2 rats from this series were again injected once with morphine, which increased LTF from 18.1±0.6 to 27.3±1.1 sec ($p<0.01$), i.e., produced an analgetic effect (Fig. 1, *h*), T_{50} AMAB and AIAMAB being 1:10-1:20 and 1:5-1:8.

Thus, the experiments demonstrated that AMAB are virtually absent in the serum of intact animals. A single injection of morphine caused an analgetic effect in the majority of animals (morphine-sensitive rats) but had no effect in some of them (morphine-resistant rats). AMAB were detected in all morphine-treated animals, their titer being about twice as high in morphine-resistant rats. At the same time, when morphine tolerance was induced in morphine-sensitive rats, the AMAB titer in these animals increased 2-fold and did not differ from that in morphine-resistant rats. This may indicate that the mechanisms of morphine tolerance and resistance are similar. In this context it should be noted that in comparison with morphine-sensitive animals, morphine-resistant and morphine-tolerant animals have a lower content of endogenous opioids [7,14], which are known to mediate the analgetic effect of morphine [9]. This may result from either inhibition of their synthesis, or elevation of endopeptidase activity [7], as was demonstrated earlier in experiments with chronic administration of morphine [11]. On the other hand, it has been found that endopeptidase 24.11 can act as an immunomodulator [5] and its activation evidently not only accelerates proteolysis of opioids but also stimulates production of AMAB. Given the fact that AMAB bind with morphine more effectively than do morphine receptors [15], it may be assumed that stimulation of AMAB production is a key component in the formation of morphine resistance and tolerance.

Our experiments demonstrated that naloxone administered to morphine-sensitive rats raised the titer of AMAB to a level even surpassing that in morphine-resistant animals. In view of this it should be noted that naloxone blocks morphine-induced analgesia and lowers the content of endogenous opioids in the central nervous system of morphine-sensitive rats [7], i.e., these animals become morphine-resistant over the time of action of naloxone. The increased content of AMAB evidently represents an additional and essential immune component of the blockade of morphine-induced analgesia. Moreover, injection of naloxone in these experiments induced a hyperalgetic effect. This may point to an accentuation of the mechanisms blocking the analgetic effect of the opioids, which is probably mediated through the higher level of AMAB.

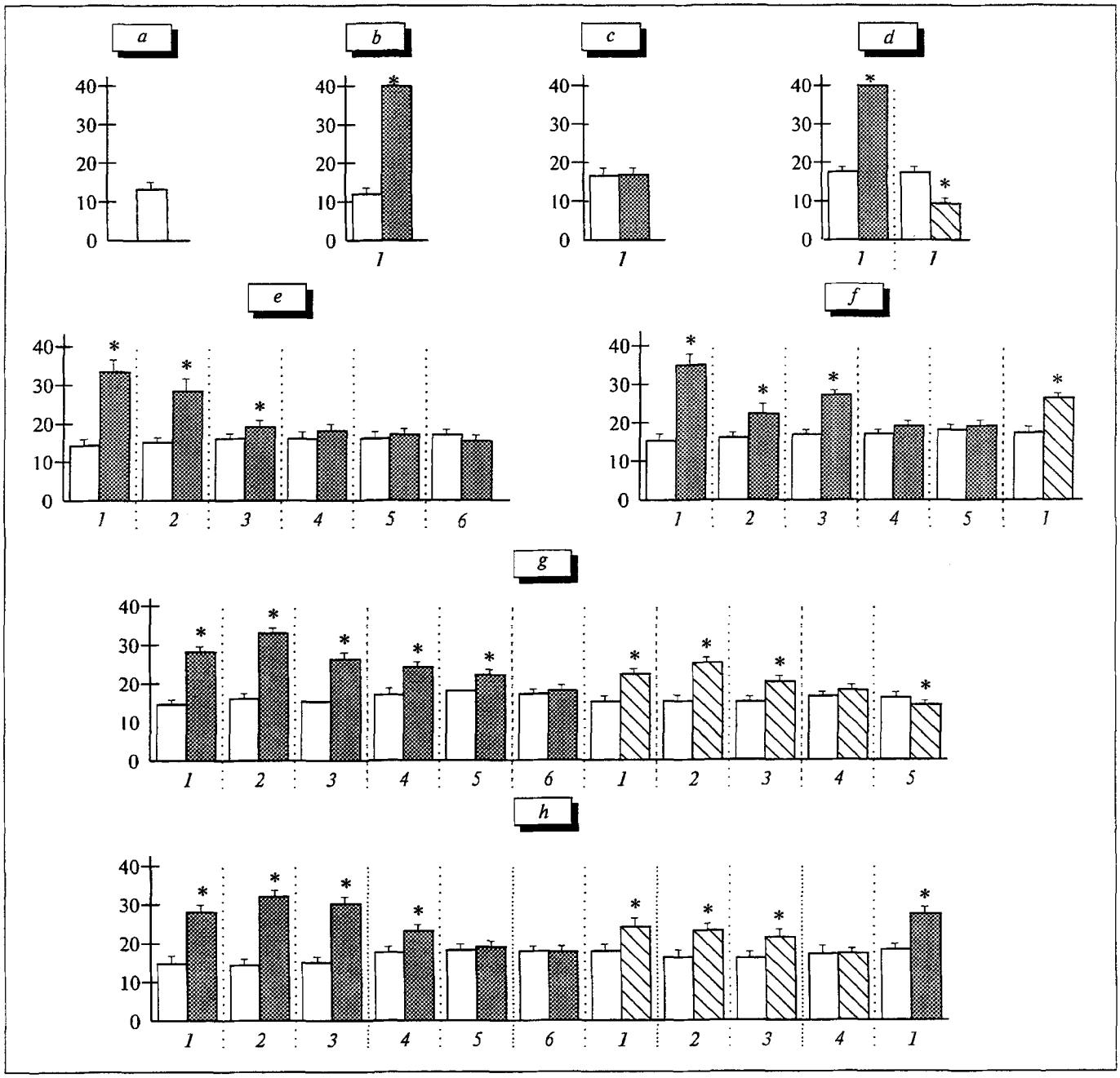

Fig. 1. Changes in LTF in intact (*a*), morphine-sensitive (*b*), and morphine-resistant (*c*) rats; in morphine-sensitive rats after a single naloxone injection (*d*), after chronic morphinization (*e*) and subsequent single injection of naloxone (*f*), and after chronic morphinization and subsequent chronic administration of naloxone (*g*), followed by a single injection of morphine (*h*). Ordinate: LTF, sec. Light bars: initial LTF; dark bars: LTF after injection of morphine; hatched bars: LTF after injection of naloxone. *$p<0.001$.

On the other hand, we found that in tolerant rats the same dose of naloxone produced an analgetic effect accompanied by a drop of the content of AMAB to the level observed in intact animals, together with a marked increase in AIAMAB. This confirms previous data on the opposite effects of naloxone in morphine-sensitive and morphine-tolerant animals [1,10] and on the involvement of AMAB [10]. Moreover, a single injection of naloxone to tolerant rats is known to induce a short-term, and chronic administration a long-term, recovery of the analgetic effect of morphine in these animals [1]. A similar effect has been produced by D-phenylalanine, an inhibitor of endopeptidase 24.11 [14]. Our findings suggest that the naloxone-stimulated production of AIAMAB reducing the content of AMAB plays a key role in restoring the analgetic effect of morphine in morphine-tolerant animals, i.e., in abolishing morphine tolerance.

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