Enkephalinase Mechanisms of Resistance and Tolerance to the Analgesic Action of Morphine in Rats. II. Differential Effects of Naloxone in Morphine-Sensitive, Morphine-Resistant, and Morphine-Tolerant Rats

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Naloxone is known as a specific antagonist of opioids [8]. It blocks morphine-induced [10] and acupuncture-induced [3] analgesias which are both associated with the release of endogenous opioids [8]. When administered alone, naloxone either did not alter or even increased pain sensitivity [2]. In contrast, its administration to acupuncture-resistant rats, which are also morphine-resistant [7], did produce algesia [3]; moreover, the effects of naloxone on intestinal muscle (ileum) from morphine-tolerant guinea-pigs and on the vas deferens from morphine-tolerant mice were opposite to those on the ileum and vas deferens from morphine-untreated ("naive") animals, and the authors did not associate the mechanisms of these effects with the opioid-antagonizing properties of naloxone [4,11]. These findings suggest that both the mechanism of action and the effects of naloxone in morphine-resistant or morphine-tolerant animals are other than those based on its antagonism toward opioids.

The purpose of this study was to compare the effects of naloxone in morphine-sensitive, morphine-resistant, and morphine-tolerant rats.

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MATERIALS AND METHODS

The experiments were conducted on male Wistar rats in which latencies of the tail-flick response (TFR) to a nociceptive thermal stimulus were recorded as described in a preceding article [1].

Morphine hydrochloride was injected subcutaneously at 1.5 mg/kg and naloxone hydrochloride (Sigma) intraperitoneally in various doses.

RESULTS

As shown in the preceding paper [1], morphine in a dose of 1.5 mg/kg produced a significant analgesic effect (increased TFR latency) in 18 out of 26 rats (classified as morphine-sensitive) and failed to do so in the other 8 (morphine-resistant rats).

Of 10 morphine-sensitive rats with a mean baseline TFR latency of 20.1±1.0 sec, in which morphine increased the TFR latency to 172±7% compared to its baseline value, naloxone at 0.3 mg/kg significantly reduced TFR latency (produced hyperalgesia) in 5 animals and virtually did not alter it in the other 5 (Fig. 1, *a*). In the group of 8 morphine-resistant rats with a mean baseline TFR latency of 21.5±0.5 sec, in which morphine had not significantly changed TFR latency, naloxone exerted analgesic effects of varying degrees in doses of 0.2 (n=3), 0.3 (n=8), 0.5 (n=5), and

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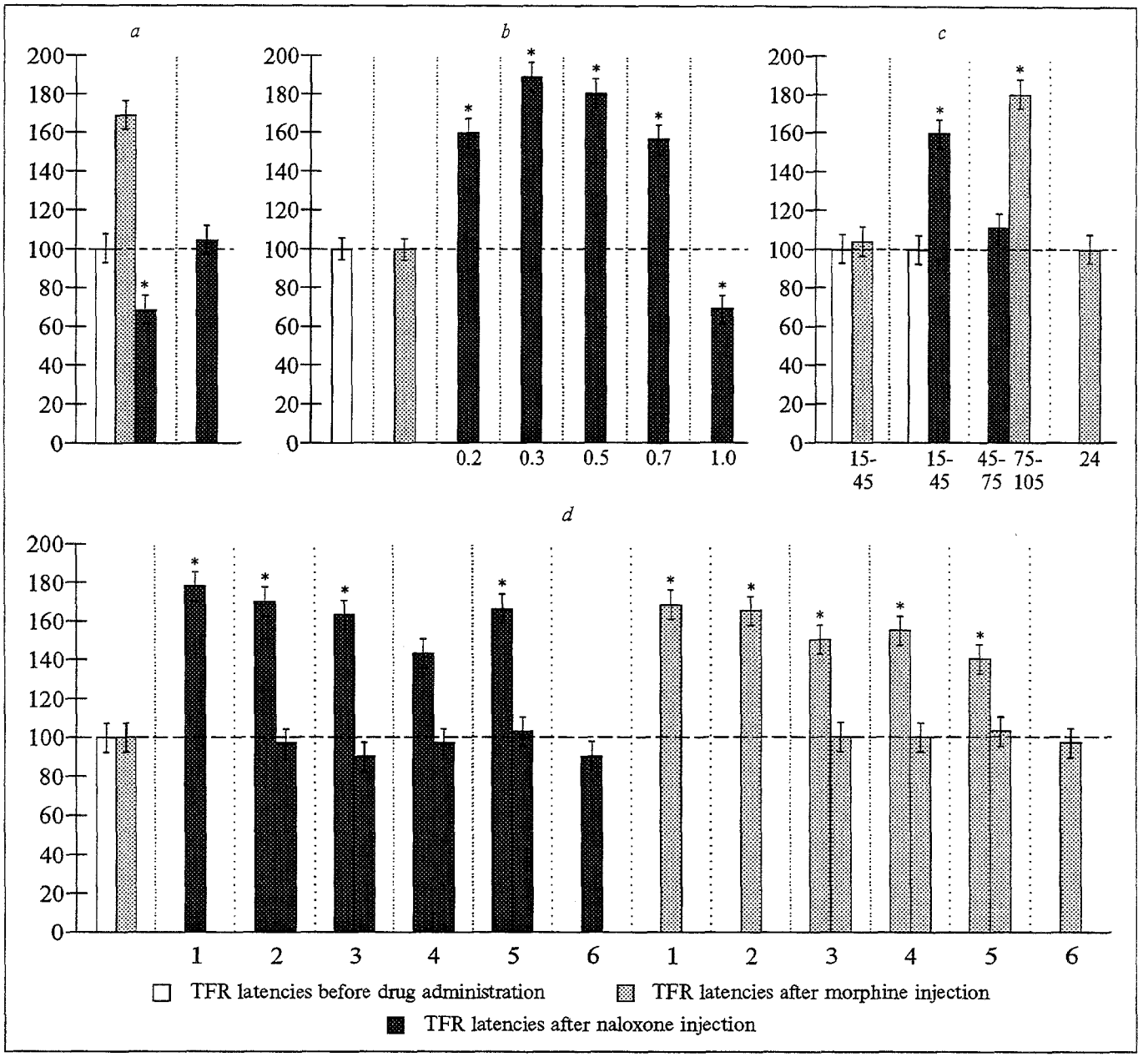

Fig. 1. Latencies of tail-flick response (TFR), expressed as percentages of their baseline values (before drug administration) taken as 100%, in morphine-sensitive (*a*) and morphine-resistant (*b*, *c*, and *d*) rats after morphine or naloxone injection. Abscissa: number of rats (*n*) in *a*; naloxone doses (mg/kg) in *b*; time of TFR recording (min) after naloxone or morphine injection in *c*; and number of animals (*n*) and ordinal numbers of naloxone and morphine injections in the course of their chronic administration in *d*. Ordinate: % changes in TFR latency. Asterisks mark statistically significant differences.

0.7 (n=3) mg/kg but produced hyperalgesia when injected at 1.0 mg/kg (n=5) (Fig. 1, *b*).

In the 6 morphine-resistant rats (Fig. 1, *c*), with a mean baseline TFR latency of 20.6±0.9 sec, in which TFR latency had not been affected by morphine but had been increased significantly during the 15- to 45-minute period after naloxone injection at 0.3 mg/kg followed by its decrease during the next 30 min, morphine injected 60 min after naloxone significantly increased latencies during the 15- to 45-minute period postinjection, i.e., it exerted an analgesic effect; when injected on the following day (n=5), morphine did not alter TFR latency significantly (Fig. 1, *c*).

In the group of 8 morphine-insensitive rats (Fig. 1, *d*) with a mean baseline TFR latency of 19.3±0.8 sec, in which morphine had increased TFR latency to only 101±5% of its baseline value, whereas naloxone at 0.3 mg/kg had increased it significantly to 180±11%, repeated administration of naloxone in this dose (1 injection per day) led to a decline of its analgesic effect in

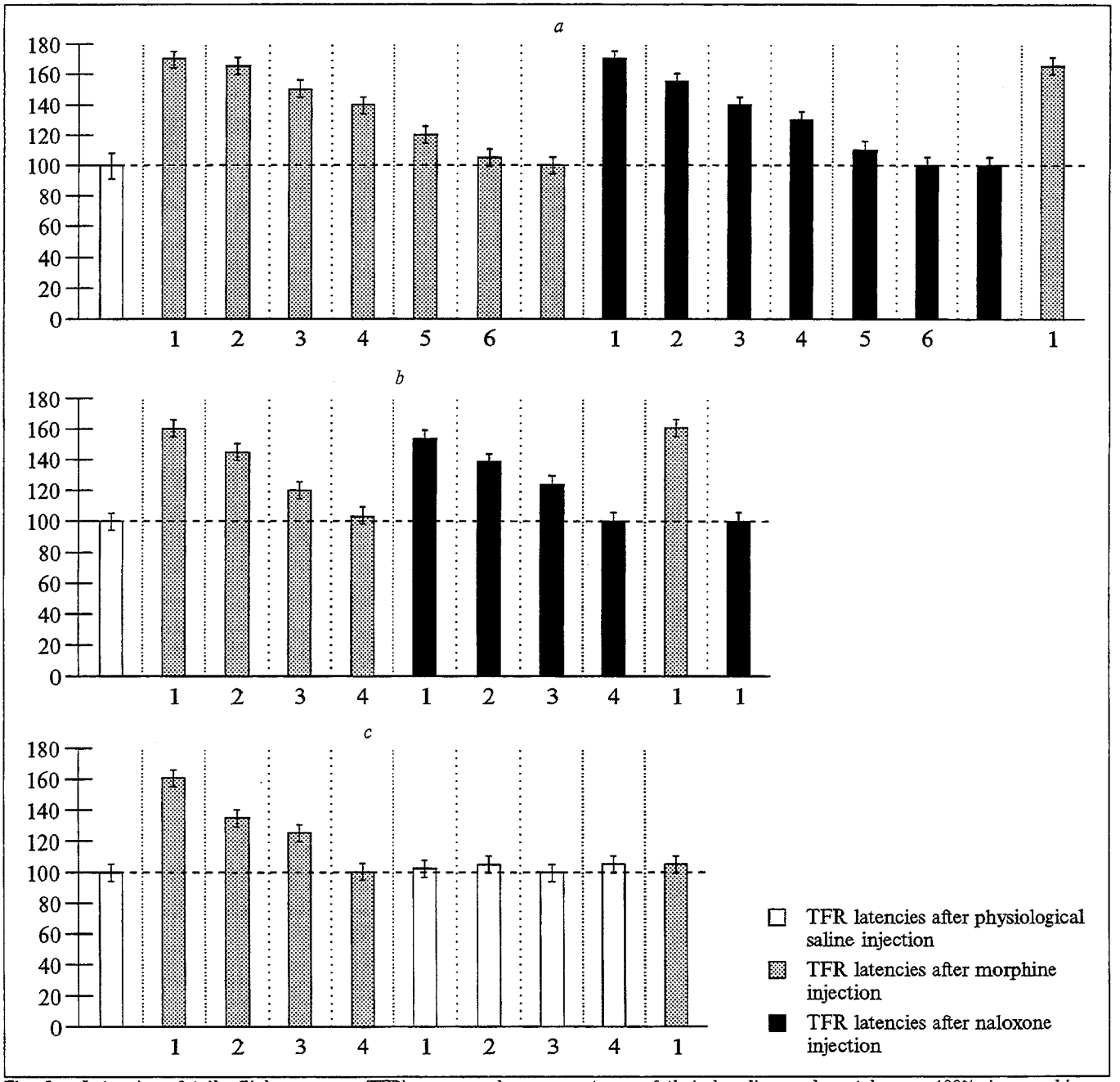

Fig. 2. Latencies of tail-flick response (TFR), expressed as percentages of their baselines values taken as 100%, in morphine-sensitive rats during chronic morphine administration (*a*, *b*, and *c*) and subsequent chronic administration of naloxone (*a* and *b*) or of physiological saline (*c*). Other designations as in Fig. 1.

all rats (Fig. 1, *d*), with its complete disappearance after the 2nd naloxone injection in 1 rat, after the 3rd in 3, after the 4th in 2, and after the 4th and 5th in the other 2, respectively. After the last injection, the mean TFR latency in this group was 19.6±1.7 sec (102±9%).

When the same 8 rats were injected with morphine on the following day, the mean TFR latency increased to 172±3% of its baseline value, i.e., analgesia was produced (Fig. 1, *d*); it declined progressively, however, with further morphine injections, to disappear completely after the 3rd injection in 2 rats, after the 4th in 3, after the 5th in 1, and after the 6th in 2. After the last morphine injection, the mean TFR latency in this group was 19.8±0.9 sec (102.5%). It should be mentioned that no definite relationship was observed between the durations of naloxone- and morphine-produced analgesias in this group.

Two of the rats in which morphine failed to produce analgesia for repeated (chronic) administration (morphine-tolerant rats) again received a

single naloxone injection, which elicited a significant analgesic effect (the mean TFR latency rose to 156±25% of its baseline value), although the effect was less marked than after the first naloxone injection (209±11%).

In the aforementioned 10 morphine-sensitive rats with a mean TFR latency of 20.1±1.0 sec, in which morphine had increased it to 171±4%, while naloxone had produced hyperalgesia or had not changed the TFR latency significantly (Fig. 1, *a*), repeated injections of morphine led to a progressive decline of its analgesic effect (Fig. 2, *a*), with its complete disappearance after the 3rd injection in 2 rats, after the 4th in 4, after the 5th in 2, and after the 6th also in 2. After the last morphine injection, the mean TFR latency in this group was 19.4±1.4 sec (Fig. 2, *a*).

When this group of 10 rats that had developed morphine tolerance again received naloxone on a single occasion, the mean TFR latency rose to 170±5% but then decreased progressively with further naloxone injections, to disappear after the 3rd injection in 1 rat, after the 4th in 4, after the 5th in 3, and after the 6th in 2 (Fig. 2, *a*). After the last naloxone injection the mean TFR latency in this group was 19.1±1.0 sec, i.e., very close to its baseline value, but increased to 168±5% when morphine was injected on the following day (Fig. 2, *a*).

In this group of 10 rats, the 4 rats with a mean TFR latency of 18.4±1.6 sec, in which the analgesic effects of morphine and then of naloxone had previously disappeared after the 4th injection of each, were again injected with morphine; the mean TFR latency increased significantly to 167±8% but was not altered significantly by naloxone injected on the following day (Fig. 2, *b*). Repeated administration of morphine to these rats led to the disappearance of its analgesic effect after the 4th injection; when the same 4 rats subsequently received 4 injections of physiological saline instead of naloxone and were then injected with morphine, no analgesic effect was observed (Fig. 2, *c*).

In the experiments described here, naloxone in a dose of 0.3 mg/kg, which blocks morphine-produced analgesia in rats in the tail-flick test [10], either exerted a hyperalgesic effect or did not alter nociception in morphine-sensitive rats, which agrees with the results reported by other scientists [2]. On the other hand, naloxone produced an analgesic effect in morphine-tolerant rats in the 0.3 mg/kg dose and in morphine-resistant rats in doses of 0.2 to 0.7 mg/kg, and it also exerted such effects in acupuncture-resistant rats [1], which have also been shown to be morphine resistant [7]. Morphine-resistant rats became morphine responsive immediately after the disappearance of naloxone-induced analgesia.

Thus, naloxone acted as an opioid antagonist in morphine-sensitive rats in the 0.3 mg/kg dose while exerting an analgesic effect similar to that of an enkephalinase inhibitor [1] in morphine-tolerant rats and, in the 0.2-0.7 mg/kg dose range, also in morphine-resistant rats, in which it induced hyperalgesia only in a dose as high as 1.0 mg/kg. This suggests that in these last rats naloxone in the 0.2-0.7 mg/kg dose range exhibits properties of enkephalinase inhibitor - a suggestion supported by the findings that the responses to naloxone of intestinal muscle (ileum) from morphine-tolerant guinea-pigs [4] and of the vas deferens from morphine-tolerant mice [11] were opposite to those of the ileum and vas deferens from morphine-untreated ("naive") animals, which was attributed by the authors to the naloxone-induced release of substances inhibiting the antimorphine mechanism rather than to its opioid-antagonizing properties.

Naloxone has also been shown to elicit analgesic effects in humans [5] and animals [9,11] in very low doses, presumably by blocking inhibitory effects on the release of endogenous opioids [9]. It is therefore likely that the affinity of naloxone for enkephalinase is higher than for opiate receptors so that it manifests its inhibitory properties with respect to enkephalinase in low doses and its antagonistic properties with respect to opioids in high doses. Where enkephalinase activity is high, as it is in morphine-resistant and morphine-tolerant organisms [6], this dose ratio shifts to the right in the sense that naloxone manifests its inhibitory properties at higher doses and its antagonistic properties at still higher ones.

As found in the present experiments, in morphine-resistant and morphine-tolerant rats chronically administered naloxone at 0.3 mg/kg, the analgesic effect wanes to disappear completely. This may be explained by a progressive decline of enkephalinase activity, which results in multiple manifestations of analgesic morphine activity, i.e., in the disappearance of both morphine resistance and morphine tolerance.

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Effect of Activation and Blocking of the Dopaminergic System of the Neostriatum on Hyperkinesia Caused by Intrastriatal Injections of Picrotoxin in Rats

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With the aid of chronic selective blocking of the GABA-ergic system of the neostriatum by picrotoxin in rats, we succeeded in reproducing a model of choreomyoclonic hyperkinesia of the paws and the head [4]. An important role in the pathogenesis of hyperkinesias is traditionally attributed to the dopaminergic system of the brain. In this study, the influence of chronic activation and blocking of the dopaminergic system of the neostriatum on the development of the above-mentioned neuromotor dyskinesias is investigated.

MATERIALS AND METHODS

The experiments were carried out on 32 male Wistar rats weighing 250-280 g. Stereotaxically, under hexenal, two polyethylene cannulas were bilaterally implanted into the rostral neostriatum (via a single opening on each side of the skull). One of them was filled with a solution of picrotoxin (Sigma, USA), and the second one contained the preparation acting upon the dopaminergic system. The rats were divided into 6 groups (5-6 animals in each). In group 1, the effect of picrotoxin (dose of one microinjection 5 μg) was studied against the background of preliminary microinjections of physiological saline in the neostriatum, and in groups 2, 3, and 4 its effect was investigated against the background of haloperidol (Gedeon Richter, Hungary, 5 μg), metoclopramide (Germed, Germany, 5 μg), and phenamine (Russian-made, 15 μg), respectively. In group 5, the order was reversed: picrotoxin was injected first and phenamine afterwards; in group 6, both preparations were injected simultaneously. The microinjection technique was described earlier [2,3]; the volume of microinjections was 1.0 μl; the second preparation was administered 10-15 min after the first.

The experimental conditions were the same as those previously described [3,4]. The experiments were carried out over 3 weeks; microinjections were performed practically daily. At the end of the ex-

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