PHARMACOLOGY

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

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It is a well-established fact that chronic administration of morphine in analgesic doses elicits a state of tolerance to this drug so that higher doses need to be administered to relieve pain [11]. One mechanism of morphine tolerance is thought to be reduced release of endogenous opioids [8]. This view is supported by the findings that concentrations of endogenous opioids are low in morphine-tolerant animals [5] and that morphine-insensitive individuals with low levels of these compounds also exist within various animal species [10], including the rat [10], rabbit [2], and mouse [3].

It is now well documented that morphine elicits increased release of endogenous opioids [7]. Although the mechanism of their reuptake remains unknown, the levels of endogenous opioids in the body have been shown to be regulated by the activity of peptidases that degrade them [1]. Hence the low levels of these opioids found in morphine-insensitive and morphine-tolerant animals may be thought to result from high activities of the specific peptidases responsible for the rapid degradation of opioids, so that the analgesic effect of morphine, which is mediated through the release of endogenous opioids, is suppressed [7]. An inhibitor of enkephalinase is therefore likely to affect the analgesic effect of morphine both in morphine-tolerant and morphine-insensitive animals.

The considerations outlined above led us to undertake a comparative study to see how D-phenylalanine (D-Pha), an enkephalinase inhibitor [6], might affect the analgesic action of morphine in animals sensitive, resistant, or tolerant to this drug.

MATERIALS AND METHODS

Male Wistar rats weighing 180-250 g were used. Before the tests, they were kept in cages, 2-3 rats in each, and allowed to consume food and water *ad libitum*. The lighting schedule was light from 07:00 to 19:00 h followed by 12 hours of darkness. The temperature was kept within 22° to 24°C. During the test periods, the rats were kept in individual cages that restricted body movements while permitting free movements of the tail.

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As the index of nociception, we used the tail-flick response (TFR), recorded by means of an automatic analgesimeter (Ugo Basile, Italy), to a hot beam of light focused on the tail 15-20 mm from its tip. The latency between the start of thermal stimulation and the TFR was also recorded automatically. If the latency reached 45 sec, the thermal stimulus was immediately switched off to prevent burning of the tail. Each rat was tested 10 times at 2- to 3-minute intervals. The latencies of the TFR before injection of the drugs were taken as 100% (baseline values) and the subsequent changes in TFR latency were expressed as percentages of these baseline values.

Morphine hydrochloride (1% solution) was injected subcutaneously at 1.5 mg/kg body weight. D-phenylalanine (Nova Biochem.) was injected intraperitoneally at 600 mg/kg or 300 mg/kg in aqueous solution.

The results were treated statistically by the unpaired Student test.

RESULTS

Morphine increased the TFR latency significantly from 20.1±0.4 sec (100±2%) to 37.8±1.0 sec (188±5%) in 18 out of 26 rats (69.4%), i.e., it produced an analgesic effect (Fig. 1, *a*), which was recorded for 30-40 min starting from minute 15 postinjection; in the other 8 rats (30.7%), the TFR latency increased insignificantly (Fig. 1, *b*) - from 19.3±0.5 sec (100±3%) to 19.5±0.6 sec (100±3%).

D-Pha injected into morphine-sensitive rats at 300 mg/kg (n=5) or 600 mg/kg (n=5) did not elicit significant changes in TFR latency (Fig. 1, *c*) during the 90-minute period of recording, although a dose-dependent tendency toward increases in the latency of this response was observed. In morphine-resistant rats (n=5), D-Pha at 300 mg/kg produced an analgesic effect for 45 min (Fig. 1, *d*), with greatest increases in TFR latency between minutes 15 and 30 postinjection (Fig. 2, *a*). At 600 mg/kg (n=6), it caused significant increases in TFR latency during 75 min postinjection, with greatest increases between minutes 30 and 45 postinjection (Figs. 1, *d* and 2, *b*).

In 5 morphine-insensitive rats with a mean TFR latency of 18.4±0.2 sec (100±4%), in which morphine had failed to elicit significant changes in the latency (105±5%), while D-Pha at 300 mg/kg had elicited analgesic effects for 45 min, the subsequent morphine injection 40 to 45 min after D-Pha did increase the TFR latency significantly between minutes 15 and 45 postinjection, i.e., between the 60th and 90th min after D-Pha (Fig. 2, *a*). However, morphine injected into the same rats (n=3) on the following day, did not cause significant increases in TFR latency.

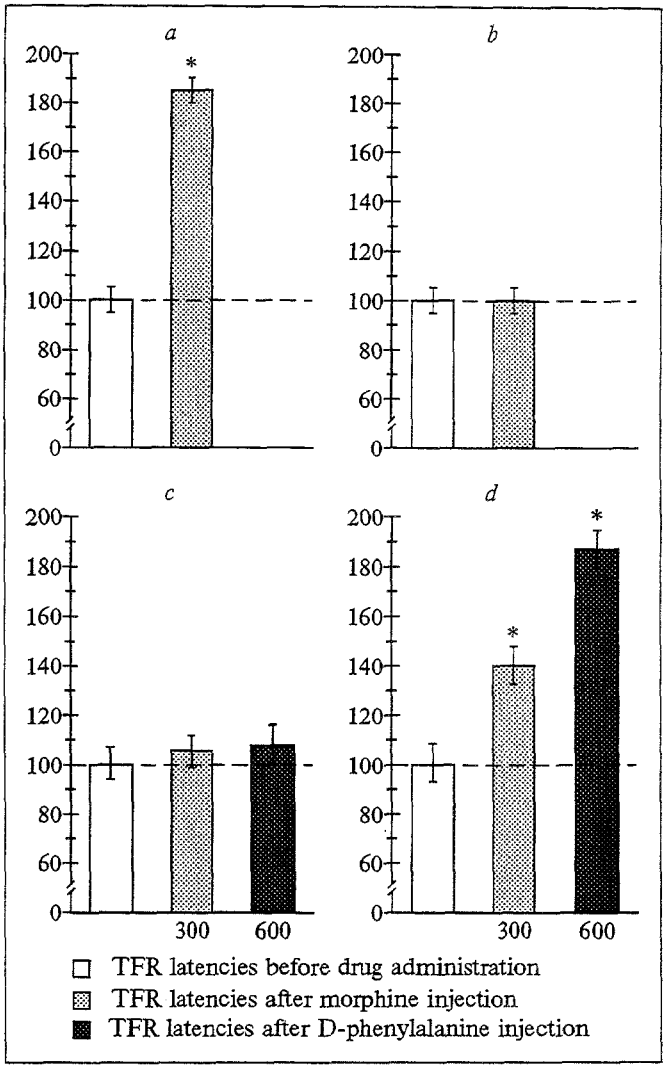

Fig. 1. Latencies of the tail-flick response (TFR), expressed as percentages of their baseline values (before drug administration) taken as 100%, in morphine-sensitive (*a* and *c*) and morphine-resistant (*b* and *d*) rats after morphine injection (*a* and *b*) and after D-phenylalanine injection (*c* and *d*). Abscissa: number of rats (n) in *a* and *b* and drug dose in *c* and *d*; ordinate: % changes in TFR latency. Asterisks mark statistically significant changes (p<0.01).

In 6 morphine-insensitive rats with a mean TFR latency of 20.0±0.6 sec (100±3%), in which morphine had not altered TFR latency, while D-Pha at 600 mg/kg had increased it significantly during 75 min postinjection (Fig. 2, *b*), the subsequent morphine injection at 70th to 80th min after D-Pha resulted in significantly increased TFR latencies during the 15- to 45-minute period after morphine, i.e., at 90 to 120 min after D-Pha (Fig. 2, *b*). Morphine in the same dose on the following day (n=4) also caused a significant in-

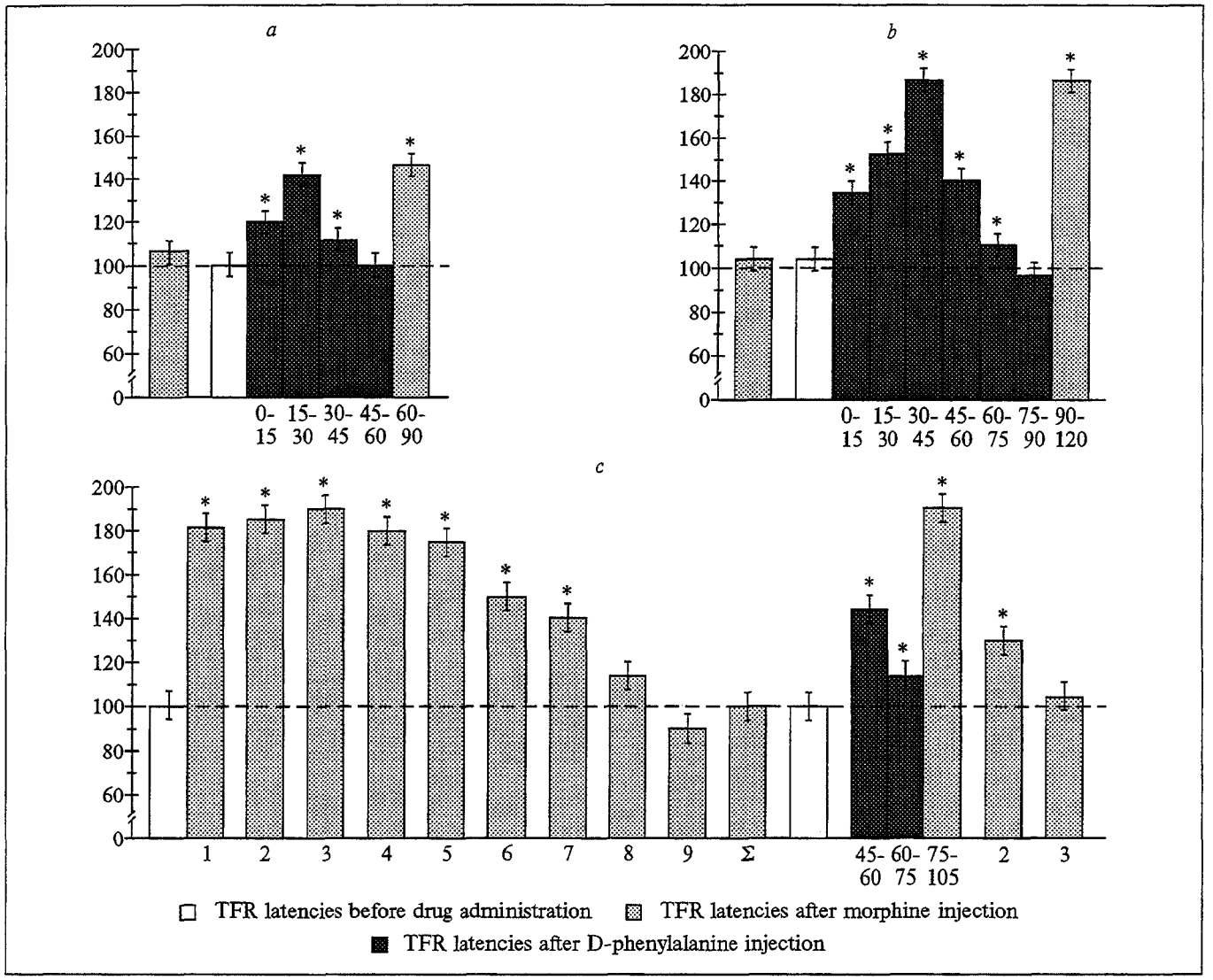

Fig. 2. Latencies of the tail-flick response (TFR), expressed as percentages of their baseline values taken as 100%, in morphine-resistant (*a* and *b*) and morphine-sensitive (*c*) rats injected with D-phenylalanine and morphine. Abscissa: time in minutes after D-phenylalanine injection (in *a*, *b*, and *c*) and ordinal numbers of morphine injections in the course of chronic administration (in *c*). Other designations as in Fig. 1.

crease in TFR latency, though to a lesser extent (126±6%) than before, but failed to increase it significantly the day after.

In 6 of the 8 morphine-sensitive rats that were exposed to morphine chronically in the dose used (1.5 mg/kg), the analgesic effect progressively declined after the 3rd injection and then disappeared at different times in different rats: already after the 4th injection in 1, after the 5th also in 1, after the 8th in 3, and after the 9th in 1 (Fig. 2, *c*). In the remaining 2 rats, more prolonged (lasting to the 18th and 19th injections) and fluctuating manifestations of the analgesic effect were noted. In these 8 morphine-sensitive rats, the mean TFR latency was 17.6±0.6 sec (100±3%) at the last stage and 18.3±0.7 sec (104±4%) after morphine injection, i.e., these animals became morphine-tolerant.

When 6 of those 8 rats were injected with DPha at 600 mg/kg, significant increases in TFR latency to 144±6% were recorded between minutes 45 and 60 postinjection, with decreases in the latency to 110±10% in the next 15 min. When the same 6 rats were injected with morphine 60 min after D-Pha, significant increases in TFR latency to 188±12% were recorded between minutes 15 and 45 after morphine injection (Fig. 2, *c*). Morphine given to 3 of those 6 rats on the following day also elicited significant, though smaller, increases in TFR latency (to 124±2%) while failing to do so the day after (Fig. 2, *c*).

Thus, in these experiments, morphine did not exert its analgesic effect in about 30% of rats exposed to a nociceptive thermal stimulus. D-Pha, an enkephalinase blocker [6], failed to alter noci-

ception in morphine-sensitive rats but did produce an analgesic effect in morphine-insensitive ones; a similar observation was made by us for acupuncture-resistant rats [2], which have also been shown to be morphine resistant [10]. This indicates that enkephalinase activity is higher in morphine-resistant than in morphine-sensitive animals, for inhibition of this activity results in elevated concentrations of endogenous opioids [4], which enables D-Pha to exert its analgesic effect. However, when enkephalinase activity is progressively restored after its inhibition by D-Pha, the analgesic effect of the latter disappears but will emerge again if morphine is administered because the level of enkephalinase activity at that time appears to be sufficiently high to deactivate the endogenous opioids present before morphine administration but too low to deactivate the large amounts of them released under the action of morphine [7]; the level of enkephalinase activity during that period probably corresponds to that in morphine-sensitive animals.

The absence of an analgesic effect in morphine-sensitive rats given D-Pha at 300 or 600 mg/kg is an indication of lowered enkephalinase activity in these rats. However, the tendency of morphine-sensitive rats to perform the TFR with longer latencies following their injection with D-Pha (in the doses used in this study) indicates that enkephalinase activity is present at some level in such rats, especially since D-Pha was found to inhibit enkephalinase activity by only 50% [6], so that this drug may be expected to act as an analgesic in morphine-sensitive animals in higher doses.

On the other hand, as found in these experiments, in those morphine-sensitive rats that had developed tolerance to morphine as a result of its chronic administration, D-Pha injected in the same dose as before produced an analgesic effect and led to a subsequent manifestation of the analgesic action of morphine - just as was the case in morphine-resistant rats. This indicates that chronic morphine administration results in elevated peptidase activities (as has also been established by biochemical methods [9]), probably because of the need for accelerated degradation of the chronic excess of endogenous opioids, whose release is stepped up following morphine administration [7].

In conclusion, it appears that morphine-resistant animals have high enkephalinase activity congenitally, whereas morphine-tolerant ones acquire such activity, with the result that their endogenous opioids are rapidly degraded and the analgesic effect of morphine is suppressed.

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