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Cross-Tolerance Between Morphine and Swim Analgesia in Mice Selectively Bred for High and Low Stress-Induced Analgesia

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SADOWSKI, B. AND I. PANOCKA. *Cross-tolerance between morphine and swim analgesia in mice selectively bred for high and low stress-induced analgesia.* PHARMACOL BIOCHEM BEHAV **45**(3) 527–531, 1993.—Mice selectively bred for high (HA) and for low analgesia (LA) induced by 3-min swimming at 20°C and unselected controls (C) were injected three times daily for 3 days with 20 mg/kg morphine HCl. The analgesic effect of 10 mg/kg morphine in nontolerant mice differed between the lines in the rank order of HA > C > LA and significantly decreased after repeated treatment with morphine, as revealed by the hotplate test (56°C). The tolerance to morphine analgesia was more pronounced in HA than in C mice but did not develop at all in LA mice. Similarly, the magnitude of swim-induced analgesia in morphine tolerant mice decreased to a greater degree in the HA than the C line but did not change in LA mice. Naloxone HCl (1 and 10 mg/kg) attenuated swim analgesia more in nontolerant HA than C mice but had no effect in morphine-tolerant HA and C and in all LA mice. The differential degree of morphine tolerance and cross-tolerance with swim analgesia suggests that the strategy of selective breeding toward divergent magnitudes of stress-induced analgesia has differentiated opioid involvement in endogenous pain inhibition in the selected lines.

Cross-tolerance Mouse lines Morphine tolerance Naloxone Pain mechanisms Stress-induced analgesia Swim stress

A multiplicity of experimental stressors cause pronounced suppression of nociceptive reflexes in laboratory animals and humans. Based upon antagonism of this stress-induced analgesia by the prototypic opiate antagonist, naloxone, two forms of stress-induced analgesia are commonly distinguished: opioid and nonopioid (2,9,10,13,37). Because altering parameters of the stressing agent (i.e., duration and intensity of electric footshock or water temperature and duration or schedule of swimming) can elicit either form of analgesia, it is recognized that a single stressor, depending upon its severity and/or temporal pattern, can trigger neurochemically different pain inhibitory mechanisms.

There is good evidence that the occurrence of opioid or nonopioid forms of analgesia after exposure to the same stressor may depend upon the animal strain (11,12,36). Of particular interest are recombinant mouse strains in which the form of analgesia is related to opioid receptors density. Thus, CXBK mice, which are deficient in brain opioid receptors (1), show virtually no opioid-mediated stress analgesia, whereas CXBH mice, rich in opioid receptors, manifest naloxone-reversible analgesia after the same stressors (19,21).

Since 1983, we have been selectively breeding mice toward high and low swim-induced analgesia (SIA). We have developed a high-analgesia (HA) line, in which SIA is greater in magnitude and also lasts longer than in randomly bred control mice (C), and a low-analgesia (LA) line, in which SIA is significantly lower and of shorter duration (25). Interestingly, the HA and LA lines not only manifest divergent magnitudes of SIA but also differ with respect to opioid involvement in SIA. Because naloxone antagonizes SIA in the HA but not in the LA line, and the analgesic dose of morphine is roughly a hundred times lower in the HA than in the LA line, we suggested that our selective breeding has produced a genetic differentiation of opioid involvement in endogenous pain inhibition (26). This hypothesis was recently supported by finding that D-amino acids (assumed to retard biodegradation of enkephalins) potentiate SIA, in a naloxone-sensitive manner, in the HA but not in LA line (28). Also, HA mice display low-threshold, naloxone-sensitive antinociception, whereas LA mice display high-threshold, naloxone-resistant antinociception resulting from electric stimulation of the periaqueductal gray matter (20).

Other criteria for assessment of opioid involvement in stress analgesia are the demonstration of tolerance following

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repeated exposures to the stressor and of cross-tolerance with morphine or other forms of opioid-mediated analgesia, in contrast to a lack of cross-tolerance in the case of nonopioid analgesia (3,4-7,10,14,31,34). We recently reported that repeated swimming produced a higher degree of both tolerance to SIA and cross-tolerance with morphine in the HA than the C line but did not in LA mice (27). The purpose of the present study was to examine the development of cross-tolerance in HA, C, and LA mice in an opposite direction, that is, to compare SIA in animals rendered tolerant to morphine.

METHOD

Animals

Subjects were male and female Swiss-Webster mice from our colony that had been selectively bred for 22 generations for divergent magnitude of swim-induced analgesia. Details of the procedure were described earlier (25). Briefly, mice taken from an outbred stock were tested for hindpaw flick latencies on a hotplate (56°C) following 3-min swim at 20°C. Those falling in the right and the left tail of postswim latencies distribution (i.e., manifesting latencies longer than 50 s or shorter than 10 s) were qualified for further reproduction to create the HA and LA lines, respectively. The same principle was applied to each subsequent offspring generation except that in the HA and LA lines only subjects manifesting the highest and lowest degree of swim analgesia, respectively, were mated. The unselected C line was concurrently bred throughout an equal number of generations without regard to hotplate latencies.

Mice were maintained on a natural daylight cycle, five to a cage, and were fed and watered ad lib. All experimental procedures were done in the same room where animals were housed. Each mouse was used only once.

Algesiometry

To assess pain sensitivity, each mouse was placed on a metal surface heated with water at 56°C and the latency of a characteristic hindpaw flick reflex measured with a stopwatch by an experienced observer unaware of the drug treatment. Animals not responding within 60 s were removed from the plate to avoid tissue damage.

Procedures

HA, C, and LA mice were injected three times daily (at 0700-0800, 1100-1200, and 1500-1600 h) during 3 consecutive days with morphine HCl (Polfa, Poland, 20 mg/kg in 10 ml/kg physiological saline, SC) or saline. On day 4, 18-20 h after the last injection, some morphine-tolerant and -nontolerant animals were injected with morphine HCl (10 mg/kg, IP) or saline, whereas others were exposed to 3-min swim in a tank filled with water at 20°C. Thirty minutes before the swim, mice were injected with saline or naloxone HCl (Endo Labs, Garden City, NY, 1 or 10 mg/kg, IP).

Baseline hotplate latencies in animals tested for morphine analgesia were measured immediately before injection and in swum animals immediately before swimming. Morphine analgesia was assessed 30 min after injection and SIA was assessed 2 min after completion of swim, during which time animals remained in a drying box lined with gauze.

Statistics

The difference between postmorphine or postswim and baseline hotplate latencies was defined as the measure of analgesia. The results were analyzed using a multiway analysis of variance (ANOVA) followed by posthoc and individual comparisons between cells according to Winer et al. (38). Student's *t*-test was applied where appropriate.

RESULTS

Two-way ANOVA revealed that morphine analgesia (Fig. 1) differed between the genetic lines, $F(2, 114) = 14.47$, $p < 0.001$. Newman-Keuls comparisons showed that the magnitude of morphine analgesia in mice previously treated with saline was higher in the HA line than in other lines ($p < 0.001$) but did not significantly differ between the C and LA lines. Repeated treatment with morphine resulted in significant tolerance, $F(1, 114) = 37.43$. The degree of tolerance was line dependent as shown by a significant line × treatment interaction, $F(2, 114) = 17.24$, $p < 0.001$. Newman-Keuls comparison of this interaction revealed that tolerance was more pronounced in HA than in C mice ($p < 0.001$) whereas no tolerance developed in LA mice. In all animals receiving repeated treatment with morphine, postmorphine hotplate latencies still significantly exceeded baseline ($p < 0.05$ or better, Student's *t*-test). This analgesia was not caused by the injection and/or testing procedure per se because no significant increase in hotplate latencies was seen in a parallel group of HA, C, and LA mice 30 min after IP administration of saline, $F(1, 80) = 2.57$, n.s. Baseline latencies differed between lines both in animals receiving saline [10.8 ± 1.16 s in HA, 5.8 ± 0.44 s in C, and 4.7 ± 0.39 s (SEM) in LA lines] and morphine (11.4 ± 1.17 s in HA, 5.8 ± 0.49 s in C, and 4.0 s ± 0.38 s in LA lines), $F(2, 114) = 24.78$, $p < 0.001$, two-way ANOVA, but not between treatments, $F(1, 114) = 0.01$, n.s.

Swimming produced a significant increase in hotplate latencies in all animal subgroups ($p < 0.05$ or better, Student's *t*-test). The magnitude of SIA (Fig. 2) differed between mouse lines as shown by three-way ANOVA, $F(2, 342) = 246.31$, $p < 0.001$. Newman-Keuls test revealed significant differences in SIA, in the rank order of HA > C> LA, between nontol-

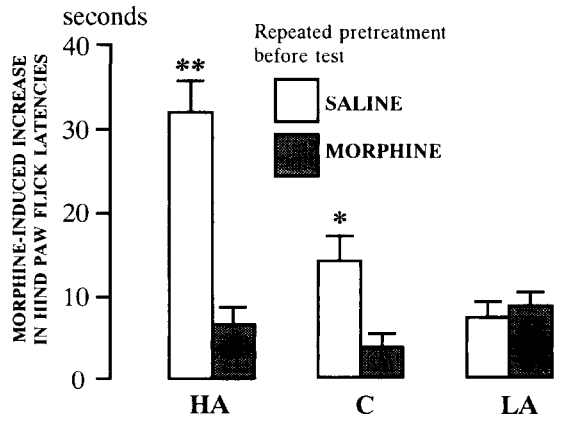

FIG. 1. Increase in hind paw flick latencies above baseline ± SEM after IP injection of 10 mg/kg of morphine hydrochloride in high analgesia (HA), control (C) and low analgesia (LA) mouse lines. Mice were treated SC 3 × daily during 3 days preceding the test with 10 ml/kg of saline or 20 mg/kg of morphine hydrochloride. $^{**}p < 0.001$ and $^{*}p < 0.01$—significantly different from morphine-tolerant animals of the same genetic line. Number of animals in each subgroup: 20 ± 1.

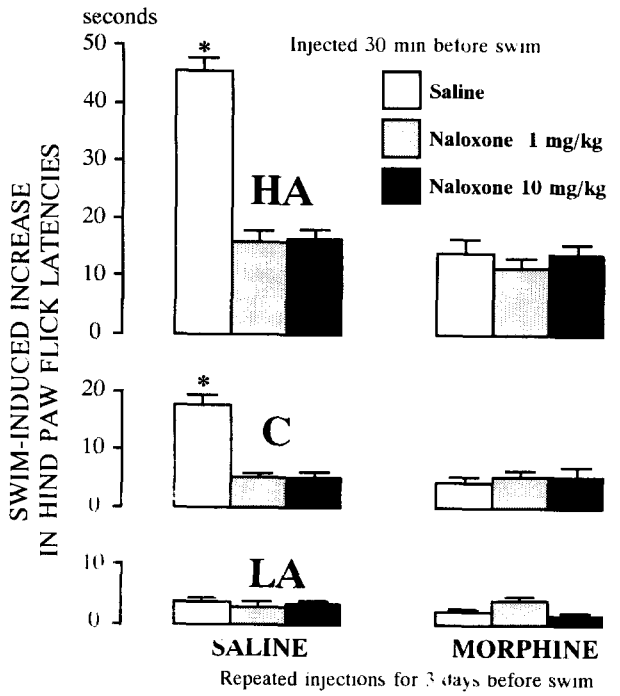

FIG. 2. Increase in hind paw flick latencies above baseline ± SEM after 3 min/20°C swim in high analgesia (HA), control (C) and low analgesia (LA) mice treated during 3 days preceding the swim with saline or morphine as described in Fig. 1. Thirty minutes before the swim mice received saline or naloxone. *Significantly different from all other nontolerant and morphine-tolerant subgroups of the same mouse line, $p < 0.001$. Number of animals in each subgroup: 20 ± 1.

erant animal subgroups that were not given naloxone before the swim ($p < 0.001$). Morphine-tolerant animals displayed significantly lower SIA than saline controls, $F(1, 342) = 87.16$. The degree of cross-tolerance differed between mouse lines, $F(2, 342) = 30.83$, line × repeated treatment interaction, $p < 0.001$. Newman-Keuls analysis of this interaction revealed that the decrease in SIA was greater in morphine-tolerant HA than C mice ($p < 0.001$). The magnitude of SIA did not change in LA mice repeatedly given morphine as compared to the LA subgroup receiving saline.

Naloxone produced a significant antagonism of SIA, $F(2, 342) = 51.55$, which was genotype and treatment dependent as revealed by a significant line × treatment × naloxone interaction, $F(4, 342) = 16.78, p < 0.001$. Individual comparisons revealed naloxone antagonism of SIA in nontolerant HA and C mice ($p < 0.001$) but not in morphine-tolerant HA and C mice and not in LA mice at all. No difference was seen between the effectiveness of 1 and 10 mg/kg naloxone.

DISCUSSION

The main finding of this study is a line-dependent cross-tolerance between morphine analgesia and SIA in mice selectively bred for high and low stress-induced analgesia. HA mice rendered tolerant to morphine manifested a higher degree of cross-tolerance than C mice, whereas no tolerance developed in LA mice. The difference in the degree of cross-tolerance between HA and C mice might be even greater than seen in this study because throughout the entire experiment an arbitrary 60-s cutoff was imposed, which gives an artificial "ceiling" effect masking the real level of analgesia and tolerance. In fact, 13 of 20 nontolerant HA mice, but only 1 of 20 morphine-tolerant HA mice, displayed cutoff hotplate latency after swimming.

Because naloxone at the two doses did not further attenuate SIA in mice rendered tolerant to morphine, we assume that repeated injections of morphine caused complete tolerance of the opioid substrate of SIA, leaving unaltered its nonopioid component. This result is congruent with the nature of 20°C swimming analgesia used in our selection procedure. This form of SIA has a pronounced nonopioid, NMDA receptor-mediated component (18), which may explain why HA mice of the 22nd generation manifest more naloxone-insensitive SIA than C mice.

In our previous study (27), two different schedules of repeated swimming, either daily for 14 days or at 2-h intervals during 2 days, were equally effective in producing higher degrees of tolerance to SIA in HA than C mice, but only the more frequent schedule produced line-dependent cross-tolerance with morphine. We proposed two possible explanations of this phenomenon. First, more frequent repetition of stress may cause more frequent occupations of opioid receptors, leading to their desensitization to both endogenous ligands and to morphine. Alternatively, different chronic stress procedures may trigger the release of endogenous opioids binding to different subpopulations of opioid receptors, and the condition adequate to produce tolerance to SIA may be insufficient for a cross-tolerance with morphine. In the present experiment, repeated morphine injections completely blocked the opioid component of SIA but produced only a partial tolerance to morphine analgesia. This finding is in agreement with those of other investigators using similar dose/temporal injection schedules in the mouse (4,5,15). This result, together with our previous findings, suggests that neurochemical substrates of SIA and morphine analgesia overlap but are not identical.

The demonstration of a two-way line-dependent cross-tolerance between morphine analgesia and SIA provides further evidence that our selective breeding led to genetic differentiation of opioid activity between the HA and LA lines. In addition, the results of the present study permit the intriguing suggestion that susceptibility to morphine tolerance development, as the magnitude of morphine analgesia, may depend upon the genetic make-up of animal. Such an assumption is consistent with earlier reports of strain differences in morphine tolerance, dependence, and addiction (8,23,27,29,30). In fact, HA, C, and LA mice, which in our earlier studies consistently differed in the magnitude of morphine analgesia (18,26,27), in this study also manifested a pronounced difference in the amount of morphine tolerance. However, interpretation of the genetic differences in tolerance to morphine is difficult because of complex and not fully understood mechanisms underlying tolerance, such as the involvement of associative and nonassociative components (16) or the important contribution of nonopioid factors (17,35). For the same reason, the apparent deficit in morphine tolerance in LA mice, despite low morphine analgesia in this line, is difficult to explain. We can only speculate that the breeding strategy might differentiate the distribution of opioid receptors subtypes in our lines so that the low morphine analgesia in the LA line may be mediated through other receptors than those mediating the high morphine analgesia in the HA line. Therefore, the repeated occupation of these receptors by morphine is insufficient to produce tolerance. This possibility is justified

by a finding that in mice treated with β-funaltrexamine (β-FNA), an irreversible μ-receptor antagonist, morphine analgesia is mediated through δ- or κ-receptors (32). Alternatively, the difference in morphine tolerance could be due to a different kind of interaction in HA and LA mice between supraspinal and spinal sites to produce morphine analgesia. Such a difference has been reported in outbred mouse strains differing in the amount of tolerance after implantation of morphine pellets (30).

An interesting finding is that our genetic lines, apart from differences in stress and opiate analgesia, manifest differential baseline nociceptive thresholds. This may suggest that the selection has modified the physiological mechanism of pain sensitivity at all, not necessarily restricted to environmentally or pharmacologically elicited pain inhibition. However, we do not have enough data to interpret this phenomenon in terms of opioid vs. nonopioid involvement.

In conclusion, the data of the present study support the hypothesis that our strategy of selective breeding toward high and low stress-induced analgesia has not only changed the overall level of SIA in HA and LA mice but also led to different contributions of the opioid and nonopioid components to SIA in the selected lines.

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