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

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PANOCKA, I., P. MAREK AND B. SADOWSKI. *Tolerance and cross-tolerance with morphine in mice selectively bred for high and low stress-induced analgesia.* PHARMACOL BIOCHEM BEHAV **40**(2) 283–286, 1991.—Mice selectively bred for high (HA) and low (LA) swim-induced analgesia were exposed to two different stress paradigms; one consisting of a 3-min swim at 20°C daily for 14 days, and the other consisting of 3-min swims repeated at 2-h intervals for 48 h. Both forms of chronic stress resulted in the development of tolerance to swim-induced antinociception to a greater degree in the HA mice than in control (C) mice, but were both ineffective at inducing tolerance in LA mice. Swimming repeated at 2-h intervals for 48 h resulted in cross-tolerance with morphine in HA and C mice. Naloxone (1 and 10 mg/kg, IP) failed to antagonize swim-induced analgesia in mice that had experienced chronic swimming in the 2-h/48-h paradigm. The daily swimming paradigm failed to produce cross-tolerance with morphine analgesia in any line. Differential degree of tolerance in three lines supports a hypothesis that selective breeding for high and low stress-induced analgesia has modified the degree of opioid involvement in the endogenous analgesia mechanisms.

Chronic stress Morphine Mouse lines Naloxone Pain Selective breeding Swim-induced analgesia Tolerance

THE exposure of rodents to a variety of stressors can produce pronounced analgesia, and this phenomenon has been termed stress-induced analgesia (SIA). Based on naloxone antagonism, at least two kinds of SIA have been proposed: opioid and nonopioid (20). The appearance of either form depends on the kind of stressor used and its parameters (20), as well as on the genetic make-up of the strain employed. For example, CXBK mice which are deficient in brain opiate receptors show virtually no opioid-mediated SIA, whereas opiate-receptor rich CXBH mice display opioid-mediated SIA after the same stressor (7, 8, 10). Moreover, within outbred populations, some individuals respond to the same stressor with naloxone-sensitive analgesia, and the others with naloxone-resistant analgesia (3,13).

Recently, using the strategy of selective breeding we have developed mouse lines displaying differential magnitudes of SIA. These lines were selectively bred toward high analgesia (HA) and low analgesia (LA) induced by a 3-min forced swimming at 20°C. The resulting SIA is greater in magnitude, and also lasts longer in HA than LA mice (11). Naloxone antagonizes SIA in the HA but not in the LA line, and the HA line was found to be a hundred times more sensitive to the analgesic action of morphine (12). We have suggested that selective breeding has produced genetic differentiation of opioid involvement in endogenous pain inhibition. This assumption is supported by our recent demonstration that D-amino acids produce a naloxone-sensitive potentiation of swim-induced analgesia in HA and, but not in LA mice (14), and by finding a difference in the stimulation-produced analgesia from the periaqueductal gray matter between these lines (9).

The purpose of the present study was to compare the tolerance resulting from repetitive exposure to stress and its cross-tolerance with morphine in HA and LA lines.

EXPERIMENT I

Stress repeated daily has been shown to cause attenuation of opioid, but not nonopioid SIA (1, 2, 16–19). In mice, tolerance to chronic stress occurs after two weeks of swimming (3 min daily at 32°C) (4). In this experiment, the development of tolerance was assessed in HA, LA and control (C) mice following repeated swimming.

METHOD

Animals

The subjects were 6-week-old male and female Swiss-Webster mice, selectively bred for 11 generations toward high (HA) and low (LA) SIA (11). Briefly, selective breeding involved testing mice for swim-induced analgesia (3 min swimming in

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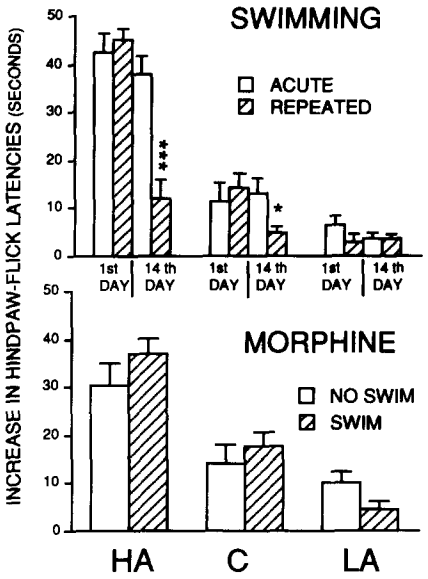

FIG. 1. Increase in hind paw flick latencies ±SEM in high analgesia (HA) and low analgesia (LA) genetic mouse lines and in unselected controls (C) after 3 min swimming at 20°C (top panel) and after 10 mg/kg of morphine hydrochloride (bottom panel); ACUTE—mice subjected to swimming only on days 1 and 14 of the experiment, REPEATED—mice exposed to swimming for two weeks daily and tested for analgesia on days 1 and 14, NO SWIM—morphine analgesia in naive mice, SWIM—morphine analgesia in mice exposed to 13 daily swims. ***$p<0.001$, *$p<0.05$ compared to other subgroups (Newman-Keuls). Number of mice in each subgroup: 13±3.

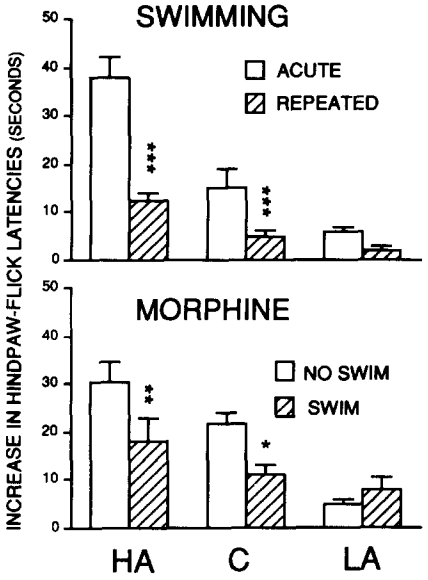

FIG. 2. Explanation as in Fig. 1 except that animals from REPEATED group were subjected to swimming every 2 h during 48 h and tested for analgesia after last swim. Mice from the ACUTE group (top panel) swam only once. ***$p<0.001$, **$p<0.01$, *$p<0.05$ with respect to ACUTE (top panel) or NO SWIM (bottom panel) group. Number of mice in each subgroup: 16±2.

20°C water) in the hot-plate test (56°C). In each generation mice displaying hot-plate latencies longer than 50 s after swimming were interbred as the HA line and animals displaying latencies shorter than 10 s were interbred as the LA line. Mice randomly mated for an equal number of generations served as unselected controls (C). The animals were housed 5 to a cage under a natural daylight cycle, and given ad lib access to food and water.

Procedures

Animals from each line were assigned to one of two groups: one to study the effect of repeated stress on SIA, and the other to test the development of cross-tolerance with morphine.

To examine tolerance to repeated stress mice were individually swum for 3 min daily at 20°C for 14 days. A control group of acutely stressed mice was swum only twice, on the first and on the 14th day. Pain sensitivity was assessed on the first and on the last day. Each mouse was placed on a hot plate maintained at 56°C to measure the latency of a characteristic hindpaw flick. Pain testing was done by an observer unaware of the animal line and treatment. After assessment of baseline pain sensitivity the animals were swum for 3 min at 20°C, placed for 2 min in a drying-box lined with gauze, and tested again for hot-plate latencies. Mice not responding within 60 s were removed from the plate to avoid tissue damage.

The morphine-treated group was exposed to 13 daily swims, and on day 14 was tested for baseline pain sensitivity, injected with morphine hydrochloride (Polfa, Poland, 10 mg/kg, IP, dissolved in physiological saline) and tested again 30 min later. A control group consisting of unswum animals received the same treatment.

Statistics

The difference between postswim or postinjection and baseline latencies was accepted as a measure of analgesia in each mouse. The results were evaluated statistically with multi-way analysis of variance (ANOVA) followed by post hoc comparisons using the Newman-Keuls test (24). The Student t-test was applied where appropriate.

RESULTS

Mice assigned to the acute and repeated stress manifested the same magnitude of SIA on the day 1 [$F(1,69)=0.16$, nonsignificant (NS), Fig. 1, top panel] that significantly differed between the lines, $F(2,69)=120.25$, $p<0.01$, in the rank order of HA > C > LA (all between group differences significant). In acutely swum animals SIA did not differ between the first and the fourteenth day, $F(1,52)=1.17$, NS. In chronically swum animals, however, there was a significant difference between SIA on day 1 and day 14, indicating the development of tolerance, $F(1,69)=38.16$, $p<0.001$. The degree of tolerance differed between the lines as revealed by significant mouse line × repeated stress interaction, $F(2,69)=15.54$, $p<0.001$. Individual comparisons showed that repeated stress attenuated swim-induced analgesia in the HA line, $t(69)=7.96$, NS. The tolerance was more pronounced in HA than in C mice ($p<0.001$). In neither line was the tolerance complete, as postswim latencies in all repeatedly swum mice were higher than baseline ($p<0.05$ for the LA line, $p<0.001$ for the other lines, Student t-test). The magnitude of SIA after repeated swimming still differed between the lines in rank order HA > C > LA ($p<0.001$).

The analgesic effect of morphine (Fig. 1, bottom panel) differed between mouse lines, $F(2,87)=38.4$, $p<0.001$, in the rank order HA > C > LA (all between-group differences significant). Morphine analgesia was unchanged after chronic swimming,

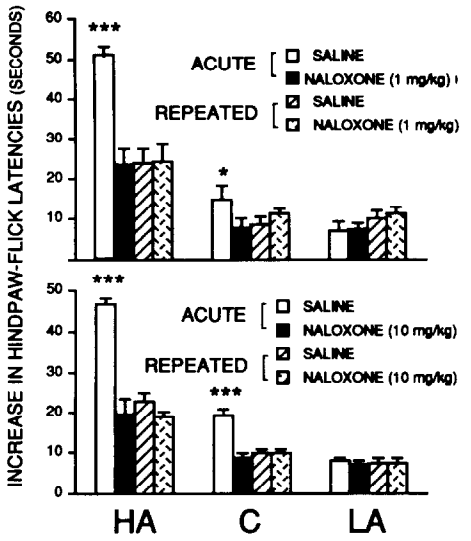

FIG. 3. Increase in hind paw flick latencies ±SEM after single 3 min swim at 20°C (ACUTE) or after a swim preceded by repeated swimming every 2 h during 48 h (REPEATED). Saline or naloxone was administered IP 30 min before the single or the last swim. Pain sensitivity was assessed just before and 2 min after this swim. ***$p<0.001$, *$p<0.05$, Newman-Keuls comparisons with other subgroups of the high-analgesia (HA), control (C) and low-analgesia (LA) lines. Number of mice in each subgroup: 14±2.

$F(1,87)=0.23$, NS, indicating that no cross-tolerance had developed.

EXPERIMENT II

In Experiment I no cross-tolerance with morphine was seen after 13 days of daily swims. This finding is consistent with the report that daily swimming in mice is a relatively weak stressor that causes tolerance of SIA, but does not modify morphine analgesia (4). Therefore, in Experiment II we adopted a more severe schedule reported to produce signs of physical dependence (5) and cross-tolerance with morphine in mice (6).

METHOD

The procedures were the same as in Experiment I except that mice exposed to repeated stress swam for 3 min every 2 h for 48 h. During the dark phase of the circadian cycle a dim light was switched on for approximately 10 min in order to prepare for and complete each swim. Sensitivity to pain was determined before and after the last swim. A control group of mice not exposed to repeated swimming was kept in the testing room for the duration of the experiment in order to experience comparable amount of environmental disturbance. These mice swam only once at the end of this period. Hot-plate latencies were measured before and again 2 min after the swim.

The morphine group swam on the same 48-h schedule, except that instead of final swim this group was injected with morphine (10 mg/kg, IP), and 30 min later tested for morphine analgesia. The nonswum control group was similarly tested for morphine analgesia.

RESULTS

Chronic stress attenuated SIA and morphine analgesia in HA and C mice, but not in the LA mice (Fig. 2, top panel). A two-way ANOVA revealed that the magnitude of SIA differed between mouse lines, $F(2,86)=49.55$, $p<0.001$, in the order HA > C > LA (all between-line differences significant). Mice exposed to repeated swimming manifested significantly less SIA than unswum controls, $F(1,86)=45.86$, $p<0.001$. The degree of tolerance differed between the lines as reflected by a significant mouse line × repeated swimming interaction, $F(1,86)=13.27$, $p<0.01$. Individual comparisons showed that chronic stress attenuated SIA in HA, $t(86)=7.69$, $p<0.01$, and C mice, $t(86)=3.62$, $p<0.001$, and this attenuation was more pronounced in the HA line ($p<0.001$). No change in the magnitude of SIA was observed in the LA line, $t(86)=0.42$, NS. The tolerance appeared incomplete, because mean postswim hot-plate latencies in chronically swum animals were significantly longer than baseline ($p<0.05$ in the LA line and $p<0.001$ in the other lines, Student t-test). As in Experiment I, the magnitude of SIA after repeated swimming still differed between the lines in the order HA > C > LA ($p<0.001$).

The magnitude of morphine analgesia (Fig. 2, bottom panel) differed between the lines, $F(2,91)=16.93$, $p<0.001$, in the order HA > C > LA (all between-line differences significant) and was significantly lower following chronic swimming [$F(1,91)=7.89$, $p<0.01$, in a line-dependent manner, $F(2,91)=4.32$, $p<0.05$, mouse line × chronic swimming]. Sensitivity to morphine decreased equally in chronically swum HA and C mice, but unchanged in the LA line.

EXPERIMENT III

Chronic swimming daily for 14 days (Experiment I) or at 2-h intervals for 2 days (Experiment II) decreased SIA more in the HA line than in C line, but had no effect in LA mice. The attenuation of SIA by chronic stress was incomplete, which is quite similar to the partial and line-dependent attenuation of SIA by naloxone (14,15). The purpose of Experiment III was to examine the effect of naloxone on SIA in repeatedly swum and unswum mice.

METHOD

The subjects were HA and LA mice bred for 16 generations toward divergent magnitude of swim SIA, and unselected C mice from the same generation. Mice were exposed to swim stress as in Experiment II on an every 2 h for 48-h schedule. Thirty min before the last swim some animals were injected with naloxone hydrochloride (1 or 10 mg/kg, IP, dissolved in saline), and others with an equal volume of saline (10 ml/kg). Hot-plate latencies were measured immediately before and 2 min after the last swim. Acutely stressed mice received naloxone or saline before a single swim, which occurred at the same time as the chronically swum mice were exposed to their last swim.

Statistical analyses were the same as described in Experiment I, except that baseline latencies of the hindpaw-flick were also analyzed.

RESULTS

Naloxone antagonized postswim analgesia only in acutely stressed HA and C mice (Fig. 3). Analgesia in repeatedly swum HA and C mice, and all LA mice was insensitive to naloxone. ANOVA revealed a significant effect of mouse line, $F(2,311)=150.92$, chronic swimming, $F(1,311)=18.77$, and naloxone, $F(1,311)=36.62$, and significant interaction of these three factors, $F(2,311)=12.35$, $ps<0.001$. No difference was seen between the effects 1 and 10 mg/kg of naloxone, $F(1,311)=0.35$, NS.

Post hoc comparisons showed that the attenuating action of repeated stress and naloxone on postswim analgesia was more pronounced in the HA line than in unselected controls ($p<0.001$). Nevertheless, the magnitude of SIA in HA mice remained higher than LA and C mice ($p<0.001$).

Baseline hot-plate latencies [9.95 ± 0.81 s (SEM) in the HA; 7.42 ± 0.56 s in the C and 6.15 ± 0.47 s in the LA line] differed between lines, $F(2,311) = 9.36$, $p<0.001$, but did not change after chronic swimming and/or naloxone.

GENERAL DISCUSSION

Tolerance has been found to follow chronic administration of a variety of stressors producing opioid, but not nonopioid forms of SIA (1, 2, 4, 6, 16–19). In the present study repeated swimming administered on two different time schedules led to tolerance that was more pronounced in HA than in C mice, but did not develop in LA mice. It is recognized that repetitive activation of opioid pain-inhibitory mechanisms by stress causes the release of endogenous opioid ligands leading to development of tolerance (6,21). Thus our results support the hypothesis that the selection has modified the distribution of genes controlling the amount of opioids released following stress and/or genes controlling opioid receptor density (12, 14, 15).

Although both the 14-day and 2-h/48-h swimming paradigms produced tolerance, only the latter schedule led to development of cross-tolerance with morphine. This finding is in agreement with the demonstration that only more frequent exposure to swim stress leads to cross-tolerance with morphine (6) and naloxone precipitated abstinence syndrome in the mouse (5). A possible explanation for this discrepancy is that more frequent repetition of stress causes more frequent occupation of opioid receptors by released ligands, and leads to desensitization of these receptors to morphine. An alternative interpretation is that different chronic stress procedures trigger the release of endogenous opioids binding to different receptor subtypes, and therefore the condition necessary for the development of tolerance may be insufficient to promote concomitant cross-tolerance with morphine.

Repeated swimming and naloxone produced identical, genotype-dependent attenuation of SIA. Since there was no difference between the effect of 1 and 10 mg/kg of naloxone, and naloxone did not affect SIA in repeatedly swum mice, we assume that chronic swimming and the lower dose of naloxone fully inactivated the opioid mechanism of SIA leaving unchanged its nonopioid component.

After suppression of the opioid component pharmacologically or by chronic swimming the magnitude of residual naloxone-insensitive SIA still remained higher in HA than in the other lines. This finding supports our earlier suggestions (9) that both opioid and nonopioid pain-inhibitory processes were influenced by selective breeding. This dual effect most likely results from the characteristic of the stress paradigm used throughout the breeding process (12). A 20°C swim induces analgesia mediated by both opioid and nonopioid mechanism in the C (Swiss-Webster) mice that constituted the parental stock; selection process based on the magnitude of analgesia and not discriminating its pharmacological basis may have changed both opioid and nonopioid components of SIA.

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