

CORRELATION BETWEEN MAGNITUDE AND OPIOID MEDIATION OF STRESS-INDUCED ANALGESIA: INDIVIDUAL DIFFERENCES AND THE EFFECT OF SELECTIVE BREEDING

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Abstract. Two experiments were made showing that opioid involvement coincides with the magnitude of stress-induced analgesia. In Experiment I rats subjected to cold water swims were screened for jump threshold levels on electrified grid and divided into high, medium and low threshold responders' groups. Later on the three groups were given 90 s forepaw footshock. Tail-flick latencies rose highest in the high threshold, and lowest in the low threshold responders. This decrease in nociception was counteracted by naloxone more effectively in high than in medium threshold responders, and not all in low threshold responders. In Experiment II mice selectively bred for high (HA) and low (LA) post-stress analgesia swam at 20 and 2°C. Both stressors were followed by an increase in tail-flick latencies in the order of magnitude HA > unselected controls > LA line. Naloxone attenuated analgesia after both stressors in the HA line, but was ineffective in LA mice. In unselected controls swimming at 20°C caused naloxone-sensitive, and cold water swims naloxone-resistant analgesia. It is concluded that apart from the kind of stressor, inborn properties of an individual are essential for the development of opioid vs. non-opioid form of post-stress analgesia.

INTRODUCTION

Several stressors produce a conspicuous decrease in responsiveness to painful stimuli in rodents. This phenomenon termed stress induced

analgesia, is not unique regarding its physiological and/or neurochemical mechanism. Commonly two kinds of analgesia are distinguished, an opioid form which is prevented, and a non-opioid form which is not altered when naloxone, a opiate antagonist, is administered prior to stressing. Which of the two forms will develop in the particular situation, depends not only on the kind of the stressing stimulus, but also on its intensity and duration. With respect to swim stress, usually low temperature of water or prolonged duration of swimming cause non-opioid analgesia, whereas swimming at 20°C or in warm water elicits the opioid mediated form (2, 13, 18, 24). The situation is more complicated with electric footshock, because in addition to stimulus intensity, its continuous or intermittent administration and duration also the region of the body exposed to shocks is critical for the development of particular form of analgesia. For example, brief forepaw shocks were described to produce an opioid but hindpaw shocks a non-opioid analgesia in the rat (22, 23).

Individual differences between members of the population subjected to a stressing procedure may also account for the form of post-stress analgesia. High magnitude analgesia is usually more counteracted by naloxone than a low decrease in nociception following the administration of a single stressor. Thus, in some rats even post-cold water swim analgesia, regarded as non-opioid in nature, was partially reversed by naloxone if its level attained a particularly high value (3).

The present study consists of two experiments which demonstrate that the role of opioid component in stress analgesia may depend on an inborn factor, irrespective of the kind of the stressing agent.

EXPERIMENT I

The purpose of Experiment I was to create groups of rats manifesting differential magnitudes of post-stress decrease in nociception, and to find out whether these groups will also differ with the degree of opioid involvement in the mechanism of stress-induced analgesia. The animals were assigned to appropriate groups according to threshold currents eliciting a jump on electrified grid after a cold water swim. Later on, rats responding within a high, medium and low threshold range were subjected to forepaw footshock and tested for tail-flick latencies to radiant heat to see whether similar differences in post-stress antinociception will also occur after another stressor and upon another algometric test. Since forepaw shocks are known to produce an opioid-mediated analgesia (23, 24), part of the subjects were preinjected with na-

lozone in order to find out whether the animal groups differing in the magnitude of stress analgesia are also differentially sensitive to this opioid antagonist.

Material and Methods

Ninety two male adult Wistar rats weighing 250 - 300 g were screened for the magnitude of jump threshold to electric nociceptive stimulus. The measurements were made in a cage with transparent walls and a grid floor connected to a shock generator. The stimulation lasted 2 s at each current intensity which was increased in a step-wise manner until the animal performed a jump strong enough to remove both hind paws from the grid. At this moment the stimulation was interrupted and restarted after 30 s break. The between-step difference was 50 μA for currents below 800 μA , 100 μA for currents from 0.8 to 1 mA and 400 μA for higher currents. A mean from four measurements was accepted as the jump threshold.

Following the determination of baseline nociceptive sensitivity each rat swam in water at 2°C for 3.5 min and remained 30 min in a box lined with gauze to dry, after which was retested for jump threshold as before swimming.

On the basis of post-swim responsiveness to electric current the animals were incorporated into two groups, one consisting of high threshold responders which jumped at threshold currents above 1,500 μA (22 rats), and the other composed of low threshold responders sensitive to currents below 850 μA (21 rats). Out of the remaining animals which performed between the above limits, 19 rats chosen at random formed a control group of medium threshold responders.

A week later the three groups were given 90 s forepaw foot-shock in a cage equipped with a grid floor. The hind part of the body and the hind paws were prevented from electric contact with the grid by suspending the animal so that the forepaws only touched the floor. Prior and immediately after the footshock the animals were tested for tail-flick latencies. Each rat was kept gently by the experimenter and heat from 100 W electric bulb placed in the focus of a parabolic mirror was concentrated on the tail until a clear flick response. The latency of tail-flick was measured with a stop watch. Four measurements were made at 15 s intervals with illuminating each time a different place of the tail. Fifteen s cut-off was adopted in the case of non-responding to prevent burning of the skin. A mean of these four measurements was accepted as a measure of the animal's sensitivity to noxious heat. Determination of tail-flick latencies was repeated every 5 min up to 30 min after the footshock. Twelve other rats with intermediate jump thresholds

were subjected to identical procedure, except that footshock was not administered.

Seven days later the same rats were again footshocked and tested up to 15 min for post-stress tail-flick latencies. Half of the animals were preinjected intraperitoneally with 10 mg/kg b. w. of naloxone hydrochloride, others with equal volume (10 ml/kg) of 0.9% saline 30 min prior to the footshock.

The data were analyzed with CPC 6128 Amstrad-Schneider computer equipped with statistical programs for multifactor analysis of variance followed by analysis of simple effects, and a priori and post-hoc comparisons. The results were treated as repeated measures if obtained from the same subject, otherwise as independent measures (25).

Results

Figure 1 shows frequency distribution of jump thresholds after swimming in cold water for the whole population of 92 rats, and the ranges of current values according to which the animals were assigned to appropriate responders' groups. Pre-swim thresholds varied between 300 and 400 μ A.

Mean tail-flick latencies before and after forepaw footshock are pre-

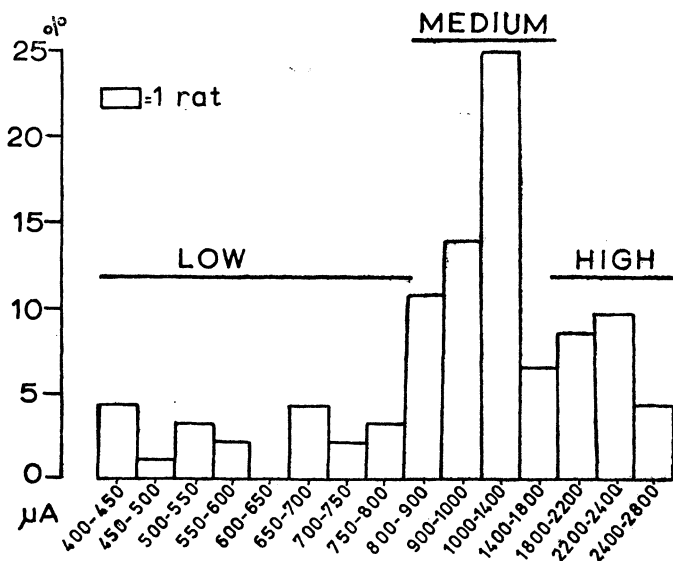


Fig. 1. Frequency distribution of jump thresholds on electrified grid in a population of 92 Wistar rats after swimming in cold water. Low, high and medium threshold responders' groups were formed of subjects jumping within the indicated ranges of the current level.

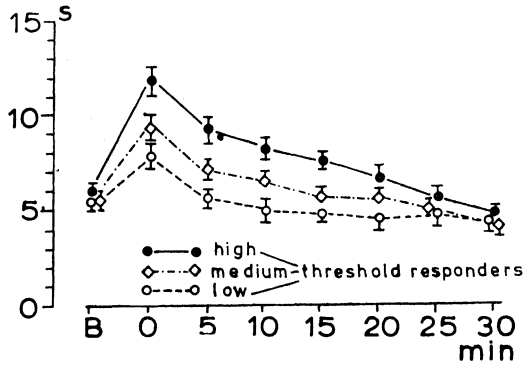


Fig. 2. Mean tail-flick latencies \pm SE in three groups of rats before (B) and 0-30 min after 90 s forepaw footshock. The groups consisted of subjects displaying high, medium and low jump thresholds on electrified grid after cold water swims.

sented in Fig. 2. The highest and prolonged analgesia ensued in rats which had been qualified in the preliminary screening as high threshold responders. In other groups the analgesia did not outlast the immediate post-shock test and attained the lowest level in the low threshold responders. Between-group difference in the magnitude of analgesia was confirmed statistically by significant main effect of the animal groups factor [$F(2,59)=12.16$, $p<0.001$], and the group-dependent difference in the duration of analgesia by significant animal groups \times tests interaction [$F(14,397)=5.69$, $p<0.001$]. The duration of analgesia was further examined in detail by checking the simple effect of animal groups at successive time points. Significant between-group difference persisted until 20 min after forepaw shocks ($p<0.001$). Post-hoc Newman-Keuls comparisons showed that tail-flick latencies in high threshold responders significantly exceeded baseline during 20 post-stress minutes, whereas in the other two groups the elevation of pain threshold was limited to the first test after the termination of footshock ($p<0.001$).

Tail-flick latencies did not change in unstressed rats tested for pain sensitivity at the same time schedule.

The effect of naloxone on post-footshock analgesia is summarized in Fig. 3. Overall ANOVA revealed significant effect of treatment [$F(1,56)=27.9$, $p<0.001$], animal groups [$F(2,56)=11.42$, $p<0.001$] and tests [$F(4,224)=106.71$, $p<0.001$], and significant treatment \times animal groups \times tests interaction [$F(8,224)=3.08$, $p<0.01$]. Two-way ANOVA applied separately to each of the groups demonstrated significant attenuation of analgesia in the high threshold [$F(1,20)=31.71$, $p<0.001$] and medium threshold [$F(1,17)=7.12$, $p<0.05$], but not in the low threshold group [$F(1,19)=0.1$, (NS)].

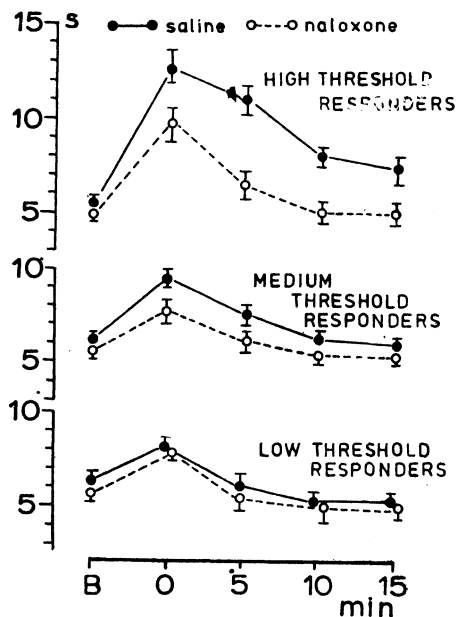


Fig. 3. Effect of 10 mg/kg of naloxone hydrochloride on tail-flick latencies before (B) and 0-15 min after 90 s fore-paw footshock in the same groups of rats as in Fig. 2.

Basal latencies neither differed between animal groups nor were modified by naloxone.

Discussion

The results show that individual differences in the magnitude of post-stress analgesia depended neither on the kind of the stressor nor on the method of measuring pain. Rats manifesting high, medium and low nociceptive thresholds after cold water swimming also displayed long, intermediate and short tail-flick latencies, respectively after fore-paw shocks. The post-shock analgesia in the high threshold group endured for about 20 min, whereas in low threshold responders was detectable only immediately after the administration of the stressor.

The differential response of the animal groups to naloxone permits to assume that the magnitude and duration of analgesia correlate with the degree of opioid involvement in its mechanism. In fact, the post-forepaw shock analgesia appeared more sensitive to naloxone in high than in medium threshold responders, but was not reversed by naloxone in rats displaying low jump thresholds after cold water swims and short tail-flick latencies after forepaw shocks. Thus, the opioid mediation of analgesia does not appear to be an absolute attribute of the stressor used, but may differ between particular members of a nonselected population.

This conclusion was further verified in mouse lines with inherited

property to exhibit low and high stress analgesia which coincide, respectively, with depressed and augmented endorphin system(s) activity.

EXPERIMENT II

For several years we have been breeding mice toward divergent magnitudes of analgesia caused by swimming at 20°C. As a result of this work two genetic lines were obtained, one displaying high, and the other low increase in response thresholds to noxious heat after stress (15). The high analgesia in the former line is more reversed by naloxone than the medium level antinociception in control randomly bred mice, whereas the low decrease in nociception in the third line is not altered after administration of the opioid antagonist (14).

The purpose of the subsequent research was to compare, in our mouse lines, two forms of analgesia, a non-opioid one caused by cold water swims (13) and an opioid mediated form following swimming at 20°C (24), with regard to their magnitude and sensitivity to naloxone. Our particular interest was to see whether the former stressor will promote opioid analgesia in the line exhibiting signs of increased endorphin activity.

Material and Methods

The subjects were Swiss mice of both sexes at the age of 6 weeks — members of the 19th generation selectively bred for high and low analgesia after swims at 20° C (for details of the selection see (15)). Sixty mice swam for 3 min at 20° C and 57 mice for 90 s at 2° C. Half of the mice were preinjected ip 30 min prior to swimming with 1 mg/kg of naloxone hydrochloride dissolved in physiological saline. The other half received an equal volume (10 ml/kg) of saline. After the swim, the mice were allowed to dry during 2 min. Tail-flick latencies were measured before the swim, immediately after drying and 5 min later according to the same procedure as in rats. The cut-off was 7 s for 20° C and 10 s for 2° C swimmers.

Results

The selected mouse lines in the 19th generation were highly differentiated with respect to post-swim nociceptive thresholds, which were shifted toward low and high values in the low analgesia (LA) and high analgesia (HA) lines (Fig. 4), whereas in unselected controls (C) the distribution frequency of these thresholds resembled the pattern seen in the outcome parental generation (15).

Mice assigned to swim at the two temperatures did not differ with pre-swim tail-flick latencies [$F(1,225)=0.79$, NS]. A slight difference

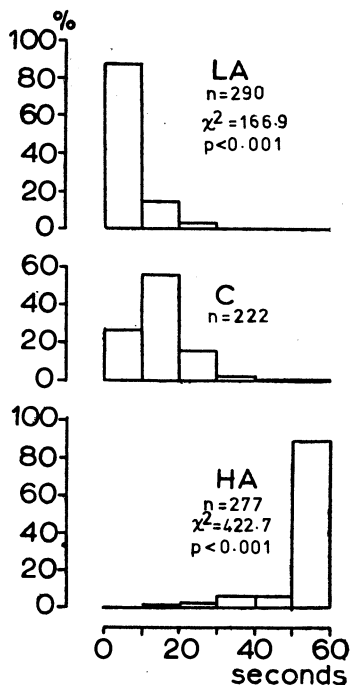


Fig. 4. Frequency distribution of post- 20°C swim latencies of hind paw flick response on a hot plate (56°C) in the 19th generation of mice selectively bred for high (HA) and low (LA) stressinduced analgesia. The χ^2 and corresponding p values show statistical significance with respect to unselected controls (C) upon Kolmogorov-Smirnov goodness of fit test.

was found between the lines [$F(2,225)=3.42$, $p < 0.05$]. Naloxone failed to modify basal nociception [$F(1,225)=1.31$, NS].

Figure 5 (top panel) shows tail-flick latencies in mice subjected to swims at 20°C. Overall ANOVA revealed a significant difference between lines [$F(2,114)=102.22$, $p < 0.001$], treatments [$F(1,114)=74.9$, $p < 0.001$] and tests [$F(4,228)=680.66$, $p < 0.001$], and significant mouse lines \times treatment \times tests interaction [$F(4,228)=17.72$, $p < 0.001$] which indicates that naloxone attenuated analgesia to a different degree depending on the line. According to Newman-Keuls comparisons, tail-flick latencies immediately and 5 minutes after swimming differed between mouse lines in the rank order of HA>C>LA ($p < 0.01$ or better). The magnitude of analgesia remained unchanged for 5 minutes in HA mice, but significantly decreased at the last measurement in the other lines ($p < 0.001$).

Two-way ANOVA applied separately to each line showed that naloxone failed to influence analgesia in the LA line [$F(1,38)=1.12$, NS], but was effective in two other lines as evidenced by the significant effect of treatment [$F(1,38)=90.67$ and 19.1 for the HA and C lines, respectively, $p < 0.001$].

Tail-flick latencies in mice swimming at 2°C (Fig. 5, bottom panel)

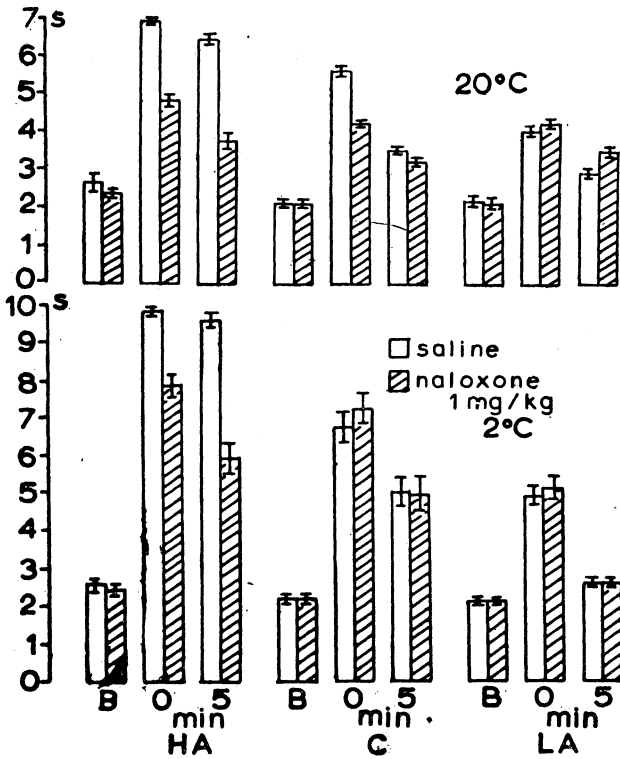


Fig. 5. Mean tail-flick latencies \pm SE in mice belonging to the 19th generation selectively bred for high (HA) and low (LA) stress-induced analgesia; C, unselected controls. B, basal sensitivity, 0 and 5, minutes after 3 min swim at 20°C (top) and 90 s swim at 2°C (bottom) followed by 2 min of drying. Saline or naloxone hydrochloride was administered ip 30 min before swimming.

showed essentially similar differences between mouse lines [$F(2,111)=118.18$], treatments [$F(1,111)=11.96$] and tests [$F(2,222)=679.85$], and significant mouse \times treatment \times tests interaction [$F(4,222)=10.55$, all $p<0.001$] as at 20°C. Post-swim latencies differed between mouse lines at the same order of magnitudes ($p<0.001$) as after 20°C swims, except that after 5 min the analgesia diminished, though slightly ($p<0.05$), also in the HA line in addition to a more pronounced decrease in the other lines ($p<0.001$).

Post-cold water swim analgesia was found insensitive to naloxone, besides the LA line [$F(1,39)=0.22$, NS], also in unselected controls [$F(1,36)=0.16$, NS]. On the contrary the opiate antagonist significantly attenuated this analgesia in the HA line [$F(1,36)=59.51$, $p<0.001$ (two-way ANOVA)].

Discussion

The temperature-dependent effect of naloxone on post-swim pain thresholds in unselected mice is consistent with the findings that swimming at 20 and 2° C in the mouse causes, respectively, an opioid and non-opioid form of analgesia (13, 24). This conclusion, however, is not valid for the selected lines, because the low level of analgesia did not change whereas the high analgesia was reversed by naloxone irrespective of swim temperature. The results demonstrate that in addition to the kind of stressor inherited properties of the animals are no less important for the generation of the particular form of post-stress analgesia.

GENERAL DISCUSSION

Several mouse and rat strains are characterized by genetic variations of the endorphin system(s) activity, such as differences in central opioid binding sites (1) or elevated pituitary β -endorphin level (10). These in-born factors account for the variability in basal nociception (5, 11, 17), sensitivity to morphine (5), acupuncture analgesia (16) and analgesic action of D-amino acids (4).

It was discovered that also the character of stress-induced analgesia is genetically determined (21). Moskovitz et al. (12) described that opioid-mediated footshock analgesia develops in C57BL/6BY mouse strain abundant in opioid receptors, but not in CXBK known to be deficient in central opioid binding sites. On the contrary a non-opioid form could be easily established in both strains. Marek et al. (8) administered 20° C swim stress (known to produce opioid analgesia) to four inbred mouse strains differing in opioid receptor density, and found that the post-swim elevation of hot-plate latencies was highest and naloxone-reversible in opioid-abundant CXBH mice, as opposed to CXBK mice in which the level of analgesia was low and not modified by naloxone. Differences in the magnitude and biochemical basis of stress analgesia were also noted in outbred populations of deer mice (7). All these findings claim that the involvement of opioid pain inhibitory mechanism in post-stress analgesia is genetically determined.

Selective breeding of animals for the desired biochemical or behavioural property permits to study physiological mechanism of the trait for which the selection has been conducted. Our procedure focused on the magnitude of post-stress analgesia, consisted in mating mice which displayed particularly long (close to cut-off) and particularly short (not exceeding 10 s) post-swim latencies of a nociceptive reflex on a hot plate. The two genetic lines obtained differ from the outcome parental gene-

ration and from concurrently bred randomly mated controls in a shifted frequency distribution of post-stress latencies toward extreme high and low values, respectively.

Several data claim that opioid mediation of stress-induced analgesia is augmented in the high analgesia line, and reduced in the low analgesia line, so that these lines resemble inbred mouse strains abundant and deficient, respectively, in opioid receptors. Thus, the antinociceptive effect of swimming and footshock is naloxone-reversible in the former, but resistant to naloxone in the latter line (14). Post-swim analgesia in the high analgesia line is subject to autotolerance after repetitive swimings, but not in the other line. The enhancement of antinociceptive effect of swimming by D-aminoacids is pronounced in high, but absent in low analgesia mice (to be published). Mice of the former line are more sensitive and of the latter line less sensitive to analgesic action of morphine as compared to unselected controls. Finally, the threshold for brain-stimulation produced antinociception, with electrodes in the periaqueductal grey, was found lower in HA than in LA mice; naloxone raised this threshold in the former, but was ineffective in the latter line (9). Since stimulation-produced analgesia (SPA) and stress-induced analgesia share a common mechanism (19, 20), the above difference in SPA thresholds and in their susceptibility to naloxone may reflect augmented, in the HA line, and decreased, in the LA line, activity of the endorphin system(s).

In the present study analgesia caused by cold water swims was found naloxone-sensitive in high analgesia line; on the contrary naloxone pre-injected low analgesia mice did not differ from those obtaining saline with respect to the antinociceptive action either of 2° C or 20° C swims. This finding additionally supports the hypothesis about differentiation of the opioid system(s) activity between the two lines (14). Besides it demonstrates that not only the kind of the stressor, but also inherited properties of the individual account for the development of opioid mediated or non-opioid form of analgesia.

During selective breeding the degree of opioid involvement in stress-induced pain inhibition increased in the HA line, and decreased in the LA line together with the corresponding change in the magnitude of analgesia. Experiment I revealed that also in a nonselected population a certain number of individuals respond to the same stressor with a high or low analgesia, the high level coinciding with prevalence of an opioid and the low one with manifestation of a non-opioid mechanism. This result is consistent with the reports of other authors (8, 12), who perceive a positive correlation between the level of analgesia and the degree of opioid involvement in this process. Thus, the magnitude of an

antinociceptive response to stress may be a reliable criterion to distinguish individuals with high and low endorphin system(s) activity.

In conclusion, the classification of commonly used stressing agents according to opioid vs. non-opioid background of the provoked analgesic response reflects averaged genetically determined properties of the animal population tested, and does not recognize individual differences which may account for unexpected sensitivity or resistance of stress analgesia to opioid antagonists in particular subjects.

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