Pharmacology Biochemistry & Behavior, Vol. 37, pp. 593–596. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Potentiation of Swim Analgesia by D-Amino Acids in Mice Is Genotype Dependent

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Received 2 April 1990

PANOCKA, I. AND B. SADOWSKI. *Potentiation of swim analgesia by D-amino acids in mice is genotype dependent.* PHARMACOL BIOCHEM BEHAV **37**(4) 593–596, 1990.—The effect of combined treatment with 125 mg/kg of D-phenylalanine plus 125 mg/kg of D-leucine (IP) on magnitude and duration of analgesia caused by 3 min swim at 20°C was studied in mouse lines selectively bred for 20 generations toward high and low level of stress-induced analgesia. The D-amino acids administered 30 min prior to swimming increased postswim tail-flick latencies and prolonged antinociception more in the high analgesia line (HA) than in concomitantly bred unselected controls, but were not effective in the low analgesia line (LA). The potentiation of swim analgesia by D-amino acids was prevented by simultaneous administration of 1 mg/kg of naloxone hydrochloride which, given alone, antagonized the analgesia more in the HA line than in controls, but not in the LA line. The results are interpreted in terms of genetic differentiation of opioidergic transmission in the selectively bred mouse lines.

D-Amino acids D-Phenylalanine D-Leucine Enkephalins Genetic mouse lines Naloxone Opioids Selective breeding

A wide range of environmental stimuli cause a decrease in pain sensitivity in rodents. This phenomenon, termed stress-induced analgesia (SIA), relies on complex pain inhibitory systems, opioid and nonopioid in nature. Activation of either system may depend on the kind of stressor and on its intensity or temporal parameters (29, 30, 32). In addition, as the level of endorphin activity is genetically determined (2,26), the degree of opioid involvement in SIA differs between species [e.g., mice and rats (18)], strains (13, 15, 20, 31) or even individuals (4,24).

Taking into account that animals selected for different expression of SIA might be a good model to study the nature of its variability, we developed two genetic mouse lines exhibiting divergent magnitudes of stress analgesia (22). In one line the poststress elevation of nociceptive thresholds lasts longer, attains high values and is counteracted by naloxone, whereas in the other line a low and short-lasting SIA appears naloxone-resistant. The former, high analgesia (HA) line is more, whereas the concurrent low analgesia (LA) line is less sensitive, in comparison with unselected control mice (C), to analgesic action of morphine (23). Also, basal nociception is higher in the LA than in the HA line. This divergent response to stress as well as to the opiate agonist and antagonist led us to assume that the two lines differ with respect to the level of endorphin system activity and so with the neurochemical mechanism of SIA. Such divergence could be a consequence of variability in opioid receptor density (2, 6, 15, 19, 27) or, especially in relation to basal nociception and the duration of SIA, of different pituitary opioid levels (6,16).

There is evidence that D-amino acids decrease pain sensation in mice and humans (1,10), as well as potentiate opioid-mediated acupuncture analgesia in mice (7). The analgesic effect of D-phenylalanine and D-leucine was found to vary between opioid abundant and opioid-deficient inbred mouse strains (6). The present report describes unequal effect of D-amino acids on the magnitude and duration of postswim analgesia in our mouse lines. Since some data argue that these compounds prolong the lifetime of enkephalins (1,5), such result is an additional argument for genetic differentiation of the endorphin system activity achieved through the procedure of selective breeding.

METHOD

Animals

The subjects were 6-week-old Swiss mice of either sex from our colony which had been selectively bred for 20 generations, as earlier described (22), toward long (the HA line) and short (the LA line) latencies of hind paw flick reflex on a hot plate (56°C) measured 2 min following 3 min swim at 20°C. Simultaneously bred unselected mice served as controls (a C line). The animals were kept under natural daylight cycle, fed and watered ad lib.

Treatments

Mice belonging to the HA, C and LA lines were randomly assigned to four subgroups one of which was given physiological

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saline, the second 1 mg/kg of naloxone hydrochloride (Endo Laboratories, USA), the third 125 mg/kg of D-phenylalanine and 125 mg/kg of D-leucine purchased from Reanal, Hungary, and the last one a combined treatment with the above doses of D-amino acids plus naloxone. All compounds were dissolved in 0.9% NaCl and injected IP in a volume of 10 ml/kg.

Procedures

Half an hour after the injection each mouse was tested for basal pain sensitivity with a tail-flick method as previously described (22). Four points were marked on the tail and successively illuminated at 30-s intervals from 100-W projector bulb positioned in the focus of a parabolic mirror. Tail-flick latencies were measured with a stop-watch by an experienced observer unaware of the genetics and pharmacological treatment of the animal. The maximum heating time (cut-off) was set at 7 s to avoid skin burns. A mean from these measurements was accepted as the latency of the reflex. The intensity of the light was earlier adjusted so as to elicit, in unselected mice, tail-flick at latencies between 2 and 3 s.

After the test each mouse swam single 3 min in 30 cm deep tank filled with tap water at 20°C, and then remained 2 min in a box lined with gauze to dry. Postswim tail-flick latencies were checked with the same method starting 2, 7, 12, 17, 27, 37, 52 and 67 min after the end of swimming.

Statistics

The results were analyzed with CPC 6128 Amstrad Schneider computer equipped with statistical programs for multiway analysis of variance (ANOVA) followed by a priori and post hoc cross comparisons between mouse lines and treatments. The data were regarded as repeated measures if obtained from the same mouse, otherwise as independent measures (34).

RESULTS

The magnitude of swim analgesia differed between mouse lines in the rank order of HA>C>LA ($p<0.001$). Similar differences were seen with respect to its duration. D-Amino acids (DAA) augmented and/or prolonged, whereas naloxone attenuated the analgesia in HA and C, but not in LA mice (Fig. 1).

Four-way ANOVA of pre- and 2-min postswim data revealed significant effect of swimming, $F(1,127)=874.19$, $p<0.001$, animal lines, $F(2,127)=64.37$, $p<0.001$, DAA, $F(1,127)=6.78$, $p<0.05$, and naloxone, $F(1,127)=58.97$, $p<0.001$. Significant lines × DAA × swimming and lines × naloxone × swimming interactions, $F(2,127)=3.35$, $p<0.05$, and 46.48, $p<0.001$, respectively, confirm that the augmenting action of DAA and the attenuating action of naloxone on swim-induced analgesia depended on the selection.

Newman-Keuls comparisons of consecutive postswim vs. preswim tail-flick latencies (following one-way ANOVA applied to each animal subgroup) demonstrated that in NaCl- and DAA-injected mice of the HA line the nociceptive thresholds remained significantly elevated throughout the whole testing period. In unselected controls receiving DAA the analgesia persisted for 37 minutes, whereas after NaCl did not outlast 7 min after the swim. In LA mice the increase in tail-flick latencies was limited to the first postswim test, irrespective of the injection.

The duration of swim analgesia was further analyzed separately in each mouse line by means of two-way ANOVA. Significant difference between treatments was found in the HA and C lines, $F(3,42)=45.1$ and 22.9, respectively, $ps<0.001$, but not in the LA line, $F(3,43)=1.8$, nonsignificant (NS). The difference

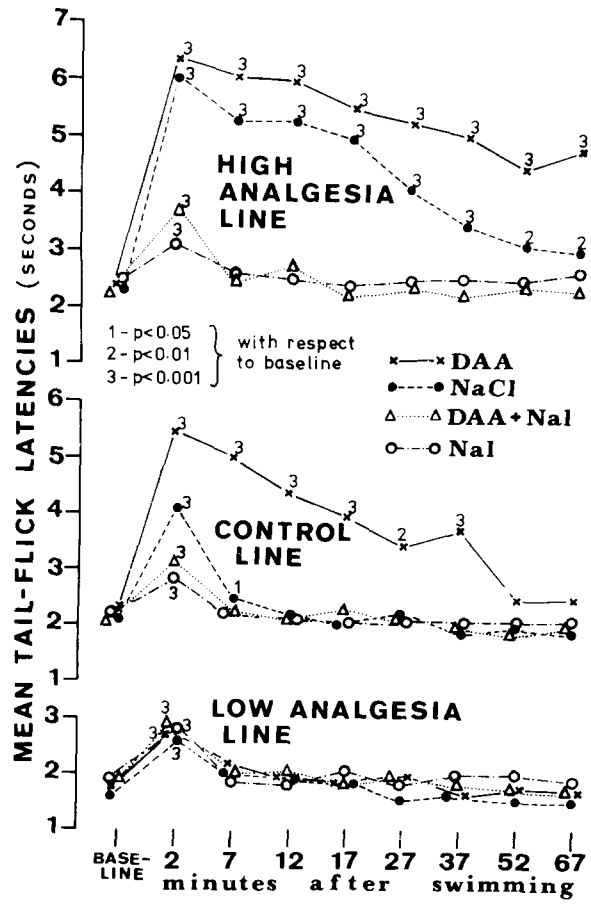

FIG. 1. Mean tail-flick latencies before and after swim stress in subgroups of 11 or 12 mice preinjected with D-phenylalanine + D-leucine (DAA), naloxone (Nal), D-phenylalanine + D-leucine + naloxone (DAA+Nal) and saline (NaCl). Standard errors (not shown) were 6.44 ± 2.54% (SD) of the means.

between high analgesia after DAA and its lower level after NaCl in HA mice (Fig. 1, top panel) was not confirmed statistically, upon Newman-Keuls comparisons at consecutive time points, until 17 minutes after swimming. Later on, the DAA-treated subgroup displayed significantly higher nociceptive thresholds ($p<0.01$ or better) because of more rapid decay of insensitivity to pain in NaCl-injected counterparts. In C mice significant difference ($p<0.01$ or better) in pain thresholds compared to NaCl lasted for 37 minutes due to higher and prolonged analgesia after DAA (Fig. 1, middle panel).

Naloxone reduced and shortened the analgesia in HA and C mice, so that significant elevation of tail-flick latencies upon Newman-Keuls comparisons with baseline ($p<0.001$) was detected, as in the LA line, at the first postswim test only. Three-way ANOVA of pre- and 2-min postswim data from mice treated with naloxone alone revealed no difference with respect to the subjects given the opiate antagonist together with DAA, $F(1,63)=0.42$, NS. Significant main effect of the line factor, $F(2,63)=6.87$, $p<0.01$, resulted from residual difference in the magnitude of analgesia between mouse lines after DAA + naloxone, $F(2,63)=7.46$, $p<0.001$, but not after naloxone alone [$F(2,63)=0.9$, NS, simple effects of the lines at postswim latencies after respective treatments].

Preswim latencies examined separately by two-way ANOVA differed between mouse lines, $F(2,127)=7.19$, $p<0.01$, but not between treatments, $F(3,127)=0.98$, NS.

DISCUSSION

Swimming in water at 20°C which produces opioid-mediated analgesia in mice (33) increased tail-flick latencies to a different degree depending on the selected line. The highest and prolonged analgesia ensued in HA and the lowest, short-lasting one in the LA mice, whereas in unselected controls the magnitude and duration of analgesia assumed intermediate values. Naloxone counteracted this effect of swim stress more in the HA line than in controls, but not in the LA line. These results obtained from mice which had been selectively bred for 20 generations toward divergent magnitudes of swim analgesia are consistent with our previous finding at earlier stage of the breeding procedure (22,23) and confirm that the selection led to an enhancement of opioid mediation of postswim analgesia in HA mice, and to prevalence of a nonopioid mechanism in the LA line.

D-Amino acids augmented and/or prolonged postswim analgesia more in HA than C mice, and this potentiation was reversed by naloxone. This observation is consistent with a report on naloxone-reversible potentiation of footshock analgesia by 250 mg/kg of D-phenylalanine in the rat (5). On the contrary, either DAA or naloxone or combined injection of these compounds failed to modify tail-flick latencies in the LA line. So all mice pretreated with naloxone exhibited a similar low level of swim analgesia, and only minor line-dependent difference in the magnitude of the latter was present after simultaneous administration of DAA. As our previous studies (23–25) revealed that opioidergic mechanisms are enhanced in the HA line and suppressed in LA mice, the present results are in good agreement with the data showing a straight correlation between analgesic effect of the combined dosage of 125 mg/kg of D-phenylalanine plus 125 mg/kg of D-leucine and the development of the endorphin systems in the mouse (6).

Attenuation of pain sensation by D-phenylalanine and D-leucine was ascribed to their inhibitory action on carboxypeptidase A (12) and leucine aminopeptidase (9), respectively assumed to cleave enkephalins to inactive fragments. Although this interpretation is not tenable in the light of enzyme studies (28), some indirect data support the notion that D-amino acids may potentiate opioid-mediated events by prolonging the lifetime of enkephalins. Thus, D-phenylalanine or D-leucine (at a concentration of 80 μg/ml in the bath) were found to augment Met-enkephalin inhibition on guinea pig ileum (5), and mice pretreated with 250 mg/kg of D-phenylalanine manifested enduring increase in Met-enkephalin levels in the periaqueductal grey, cerebral cortex and spinal cord (1). Finally, the action of D-amino acids on pain sensation resembles that of thiorphan, a specific enkephalinase inhibitor which potentiates opioid-mediated but does not change (8, 11, 21) or even antagonizes (3) the nonopioid form of stress analgesia. This similarity, taken together with the above-mentioned findings, additionally justifies the assumption that potentiation of swim analgesia in our HA and C mice could occur due to increased level of brain enkephalins after DAA treatment.

Differences in the magnitude and duration of opioid-mediated forms of analgesia may depend on opioid receptor density (2, 15, 20) and on the availability of active opioids in pain inhibitory centers. Since intraventricular injections of bacitracin and thiorphan produce naloxone-reversible analgesia on some pain tests, it is suggested that preventing enzymatic degradation of enkephalins increases central opioidergic transmission (17,28). The level and time course of analgesia in our unselected mice given DAA were fairly similar to those seen in the HA line after NaCl. The analgesic effect of swimming was prolonged by DAA more in HA mice than in controls, but within the former line the magnitude of analgesia during 17 postswim minutes did not significantly differ between DAA- and NaCl-injected subgroups. Prolongation of SIA, with little pronounced augmentation of its magnitude might result from longer occupation of opioid receptors by the released opioids in HA mice. On the other hand, in DAA-treated controls, the analgesia was not only prolonged, but also significantly higher during 37 minutes compared to NaCl-injected counterparts. This may suggest that in unselected mice the amount of normally released opioids is not sufficient to saturate the available pool of binding sites, therefore their increased level after DAA could account for greater number of opioid receptors occupied. We then assume that our selection, apart from possible differentiation of opioid receptor density, could result in differential release of opioids in HA and LA mice under the conditions of stress, as was suggested in our earlier report on differential potentiation by adrenalectomy and reversal by dexamethasone of swim analgesia in these mouse lines (25).

Neither did D-amino acids or naloxone modify preswim tail-flick latencies (which differed between mouse lines). According to several data in the literature the analgesic effect of D-amino acids was evidenced with complex hot-plate, jump threshold or vocalization techniques (1, 6, 7, 10), but not with tail-flick method (5,31) which relies on a relatively simple spinal reflex. The same is apparently valid for naloxone which lowers nociceptive thresholds only upon pain tests requiring long exposure of the subject to a noxious stimulus (14).

ACKNOWLEDGEMENTS

This study was financially supported by the Polish Academy of Sciences, project CPBP 10.4/6.13. Naloxone hydrochloride was a gift of Endo Laboratories, USA. Technical assistance of I. Gasiorkiewicz and B. Sobolewska is appreciated.

REFERENCES

1. Balagot, R. C.; Ehrenpreis, S.; Kubota, S.; Greenberg, J. Analgesia in mice and humans by D-phenylalanine: relation to inhibition of enkephalin degradation and enkephalin levels. In: Bonica, J.; Lindblom, U.; Iggo, A., eds. Advances in pain research and therapy, vol. 5. Proceedings of the Third World Congress on Pain. New York: Raven Press; 1983:289–293.
2. Baran, A.; Shuster, L.; Eleftheriou, B. E.; Bailey, D. W. Opiate receptors in mice: genetic differences. Life Sci. 17:633–640; 1975.
3. Bodnar, R. J.; Lattner, M.; Wallace, M. M. Antagonism of stress-induced analgesia by D-phenylalanine, an anti-enkephalinase. Pharmacol. Biochem. Behav. 13:829–833; 1980.
4. Bodnar, R.; Szikorsky, V. Naloxone and cold-water swim analgesia: parametric considerations and individual differences. Learn. Motiv. 14:223–237; 1983.
5. Carenzi, A.; Biasini, I.; Frigeni, V.; Della Bella, D. On the enzymatic degradation of enkephalins: pharmacological implications. In: Costa, E.; Trabucchi, M., eds. Advances in biochemical psychopharmacology, vol. 22. Opioid peptides and neural communication. New York: Raven Press; 1980:237–246.
6. Cheng, R. S. S.; Pomeranz, B. Correlation of genetic differences in endorphin systems with analgesic effects of D-amino acids in mice. Brain Res. 177:583–587; 1979.
7. Cheng, R. S. S.; Pomeranz, B. A combined treatment with D-amino acids and electroacupuncture produces a greater analgesia than either treatment alone; naloxone reverses these effects. Pain 8:231–236; 1980.
8. Chipkin, R. E.; Latranyi, M. B.; Iorio, L. C. Potentiation of stress-induced analgesia (SIA) by thiorphan and its block by naloxone. Life Sci. 31:1189–1192; 1982.
9. DeLange, R. J.; Smith, E. L. Leucine aminopeptidase and other N-terminal exopeptides. In: Boyer, P. D., ed. Enzymes, vol. 3, 3rd ed. New York: Academic Press; 1971:81–118.

10. Ehrenpreis, S.; Balagot, R. C.; Comaty, J. E.; Myles, S. M. Naloxone reversible analgesia in mice produced by D-phenylalanine and hydrocinnamic acid, inhibitors of carboxypeptidase A. In: Bonica, J.; Liebeskind, J. C.; Albe-Fessard, D. G., eds. Advances in pain research and therapy, vol. 3. Proceedings of the Second World Congress on Pain. New York: Raven Press; 1979:479–488.
11. Greenberg, A.; O'Keefe, E. H. Thiorphan potentiation of stress-induced analgesia in the mouse. Life Sci. 31:1185–1188; 1982.
12. Hartsuck, J. A.; Lipscomb, W. N. Carboxypeptidase A. In: Boyer, P. D., ed. Enzymes, vol. 3, 3rd ed. New York: Academic Press; 1971:1–56.
13. Jacob, J. J.; Nicola, M.-A.; Michaud, G.; Vidal, C.; Proudhomme, N. Genetic modulations of stress-induced analgesia in mice. Ann. NY Acad. Sci. 467:104–115; 1986.
14. Jacob, J. J.; Tremblay, E. C.; Colombel, M.-C. Facilitation de réactions nociceptives par la naloxone chez le souris et chez le rat. Psychopharmacologia 37:217–233; 1974.
15. Marek, P.; Yirmiya, Y.; Liebeskind, J. C. Strain differences in the magnitude of swimming induced analgesia correlate with brain opiate receptor concentration. Brain Res. 447:188–190; 1988.
16. Margules, D. L.; Beatriz, M.; Lewis, M. J.; Shibua, H.; Pert, C. B. Beta-endorphin is associated with overeating in genetically obese mice (Ob/Ob) and rats (fa/fa). Science 202:988–991; 1978.
17. Michael-Titus, A.; Dourmap, N.; Caline, H.; Costentin, J.; Schwartz, J. C. Role of endogenous enkephalins in locomotion and nociception studied with peptidase inhibitors in two inbred strains of mice (C57BL/6J and DBA/2J). Neuropharmacology 28:117–122; 1989.
18. Millan, M. J. Stress and endogenous opioid peptides: a review. Modern Prob. Pharmacopsychiatry 17:49–67; 1981.
19. Moskowitz, A. S.; Terman, G. W.; Carter, K. R.; Morgan, M. J.; Liebeskind, J. C. Analgesic, locomotor and lethal effects of morphine in the mouse: strain comparisons. Brain Res. 361:46–51; 1985.
20. Moskowitz, A. S.; Terman, G. W.; Liebeskind, J. C. Stress-induced analgesia in mice: strain comparisons. Pain 23:67–72; 1985.
21. O'Connor, P.; Chipkin, R. E. Comparisons between warm and cold water swim stress in mice. Life Sci. 35:631–639; 1984.
22. Panocka, I.; Marek, P.; Sadowski, B. Inheritance of stress-induced analgesia in mice: selective breeding study. Brain Res. 397:152–155; 1986.
23. Panocka, I.; Marek, P.; Sadowski, B. Differentiation of neurochemical basis of stress-induced analgesia by selective breeding. Brain Res. 397:156–160; 1986.
24. Panocka, I.; Sadowski, B. Correlation between magnitude and opioid mediation of stress-induced analgesia: individual differences and the effect of selective breeding. Acta Neurobiol. Exp., in press; 1990.
25. Panocka, I.; Sadowski, B.; Marek, P. Adrenalectomy and dexamethasone differentially affect postswim antinociception in mice selectively bred for high and low stress-induced analgesia. Physiol. Behav. 40:597–601; 1987.
26. Peets, J. M.; Pomeranz, B. CXBK mice deficient in opiate receptors show poor electroacupuncture analgesia. Nature 273:675–676; 1978.
27. Ramabadran, K.; Michaud, G.; Jacob, J. J. C. Genetic influences on the control of nociceptive responses and precipitated abstinence in mice. Ind. J. Exp. Biol. 20:74–76; 1982.
28. Simmons, W. H.; Ritzman, R. F. An inhibitor of opioid peptide degradation produces analgesia in mice. Pharmacol. Biochem. Behav. 13:715–718; 1980.
29. Terman, G. W.; Morgan, M. J.; Liebeskind, J. C. Opioid and nonopioid stress analgesia from cold water swim: importance of stress severity. Brain Res. 372:167–171; 1986.
30. Terman, G. W.; Liebeskind, J. C. Relation of stress-induced analgesia to stimulation-produced analgesia. Ann. NY Acad. Sci. 467:300–308; 1986.
31. Urca, G.; Segev, S.; Sarne, Y. Footshock-induced analgesia: its opioid nature depends on the strain of rat. Brain Res. 329:109–116; 1985.
32. Watkins, L. R.; Mayer, D. J. Organization of opiate and nonopiate pain control systems. Science 208:1185–1192; 1986.
33. Willow, M.; Carmody, J.; Carrol, P. The effect of swimming in mice on pain perception and sleeping time in relation to hypnotic drugs. Life Sci. 26:219–224; 1980.
34. Winer, B. J. Statistical principles in experimental design. New York: McGraw-Hill; 1970:77–85, 175–178 and 298–351.