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Antinociceptive Effect of D-Aspartic Acid in Mice

F. ONAT, F. TOKER, N. ASLAN, Ş. OKTAY AND K. BERKMAN[1]

Department of Pharmacology, Marmara University School of Medicine, Haydarpaşa, 81326, Istanbul, Turkey

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ONAT, F., F. TOKER, N. ASLAN, Ş. OKTAY AND K. BERKMAN. *Antinociceptive effect of D-aspartic acid in mice.* PHARMACOL BIOCHEM BEHAV **51**(4) 715–719, 1995.—The effects of D- and L-aspartic acids on the nociceptive tail flick reflex in mice were investigated. D-Aspartic acid (115–230 mg/kg, IP) was found to increase tail flick latency significantly. Naloxone (0.1 mg/kg) abolished the analgesic effect of D-aspartic acid (115 mg/kg). Morphine and D-aspartic acid, when combined at their nonanalgesic doses, led to significant analgesia. It may be concluded that the opioid system is involved in the antinociceptive effect of D-aspartic acid. Both morphine and D-aspartic acid were previously reported to inhibit L-aspartic acid production via blockade of L-asparaginase. L-Aspartic acid, which was ineffective alone, significantly inhibited the antinociceptive effects of both D-aspartic acid and morphine.

Aspartic acid Analgesia Morphine Naloxone

ENDOGENOUS opioid peptides are known to be antinociceptive in various species. On the other hand, the NMDA subclass of excitatory amino acid receptors, which is stimulated by L-glutamate and L-aspartate, has been implicated in the processing of afferent nociceptive information at synapses in the spinal cord dorsal horn (1,4,8,22,30). Some workers have reported that the analgesia produced by opiates and NMDA receptor antagonists is similar, based on findings that naloxone reverses the analgesic effects of the NMDA antagonist, ketamine (11). However, several other investigators have reported that the analgesic effect of ketamine was not mediated via opioid mechanisms (20,26,28).

On the other hand, D-isomers of those amino acid neurotransmitters have distinct pharmacological profiles (e.g., D-aspartic acid has been shown to exert some morphine-like effects, such as decrease in food and fluid intake, and decrease in body temperature) (14,17). These morphine-like effects of D-aspartic acid were significantly antagonized by its L-isomer (L-aspartic acid), which is an NMDA receptor agonist (14,17). Interestingly, the systemic administration of some D-amino acids, D-phenylalanine and D-leucine, has been reported to produce naloxone-reversible analgesia (5,10), indicating an opioid mechanism of action.

In the present study, the effects of D- and L-aspartic acids on the nociceptive tail flick reflex in mice were investigated. The main aim was to study whether D-aspartic acid also has a morphine-like effect on nociception. We also planned to determine whether L-aspartic acid has an antagonistic acitivity on the analgesic effects of both morphine and D-aspartic acid.

METHOD

Animals

Inbred albino BALB/C mice of both sexes weighing 25–40 g were used. Food and water were available ad lib. Animals were used only once in all the experiments.

Assessment of Analgesia

Analgesia was determined by the tail flick analgesia meter (Harvard). The intensity of the heat stimulus was adjusted so that the normal animal flicked its tail in 3.0–4.5 s. The cutoff time was set at 10 s. Tail flick latencies (TFL) were measured before and after the drug injection. The inhibition of the tail flick response was expressed as "change in TFL" in seconds. Statistical analysis was done by Student's *t*-test.

Experimental Protocol

Saline, morphine (2.5, 5, 10, and 15 mg/kg), naloxone (0.1 mg/kg), D-aspartic acid (55, 115, and 230 mg/kg), or

[1] Requests for reprints should be addressed to Kemal Berkman, Marmara Üniversitesi, Tip Fakültesi, Farmakoloji, Anabilim Dali, Haydarpaşa, 81326, Istanbul, Turkey.

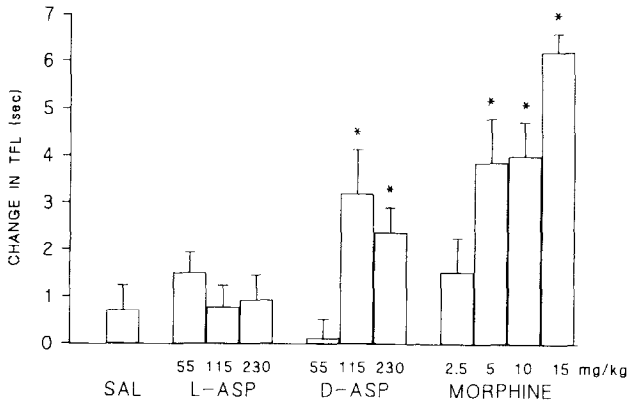

FIG. 1. Change in tail flick latencies (TFL, mean + SEM) from pretreatment values in saline-, L-aspartic acid-, D-aspartic acid-, and morphine-treated groups 60 min after the injection. *Significantly different from saline-treated rats ($p < 0.05$).

L-aspartic acid (55, 115, and 230 mg/kg) were injected intraperitoneally (IP) in a volume of 0.1 ml/10 g body weight and TFLs were measured 30, 45, 60, 90, and 120 min after the injection. Because the maximal analgesic effects of morphine and D-aspartic acid were observed at 60 min, only TFLs measured at this time point were evaluated in the second part of the study.

If D-aspartic acid has antinociceptive activity via opioid mechanisms, synergistic interaction is expected between D-aspartic acid and morphine in this respect. Furthermore, because L-aspartic acid was reported to antagonize some effects of morphine, it is expected to antagonize antinociceptive effects of both morphine and D-aspartic acid, if they share the same mechanisms of action. Morphine + D-aspartic acid, morphine + L-aspartic acid, or D-aspartic acid + L-aspartic acid were injected simultaneously to investigate their interactions. Naloxone pretreatment was done in seperate groups of animals 10 min before the injection of morphine or D-aspartic acid.

Drugs

The drugs used in the present study were morphine sulfate (TMO, Turkey), D-aspartic acid (Sigma), L-aspartic acid (Sigma), and naloxone (Sigma). Morphine and naloxone were dissolved in saline. D- and L-Aspartic acids were dissolved in distilled water and pH was adjusted to 7.4 using 1 N NaOH.

RESULTS

Saline, L-aspartic acid (55, 115, and 230 mg/kg), and naloxone (0.1 mg/kg) alone did not cause any antinociceptive effect. Morphine increased TFL dose dependently and naloxone (0.1 mg/kg) significantly antagonized its antinociceptive effect (Figs. 1-3). D-Aspartic acid was ineffective at 55 mg/kg, but it was analgesic at higher doses (Figs. 1 and 2). The antinociceptive effect of D-aspartic acid was rapid in onset and lasted at least 120 min; it was very similar to morphine in this respect (Fig. 2). Naloxone (0.1 mg/kg) abolished the analgesia due to D-aspartic acid (115 mg/kg) (Fig. 3).

When morphine and D-aspartic acid were combined at their nonanalgesic doses (2.5 mg/kg and 55 mg/kg, respectively), TFLs increased significantly (Fig. 3). L-Aspartic acid (230 mg/kg) significantly inhibited the antinociceptive effects of D-

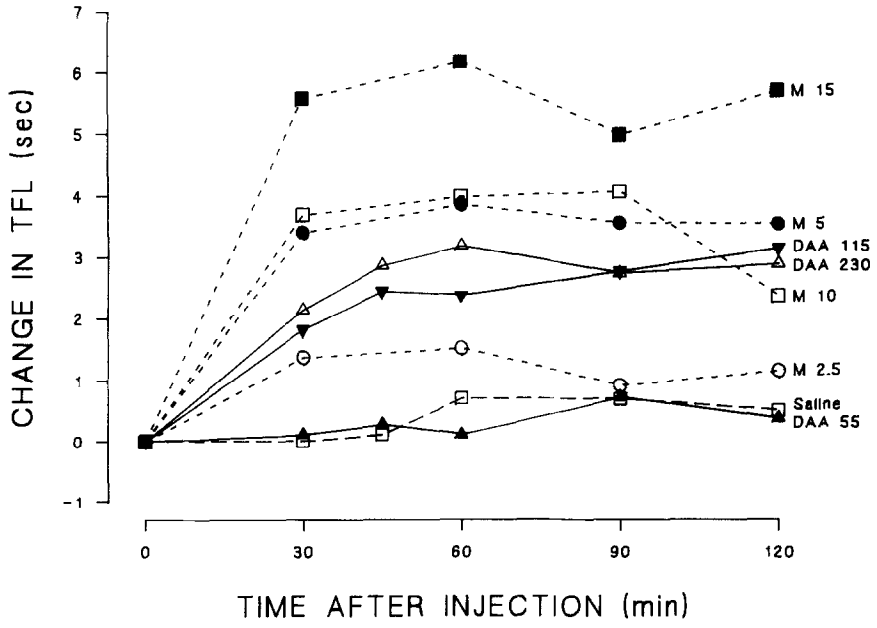

FIG. 2. Change in tail flick latencies (TFL) from pretreatment values (time zero) in saline (□), morphine (M, ○ 2.5, ● 5, □ 10, and ■ 15 mg/kg, respectively) and D-aspartic acid (DAA) (▲ 55, ▼ 115, and △ 230 mg/kg, respectively) treated groups. All points represent the mean value of 10–15 mice. Standard error bars are omitted for clarity.

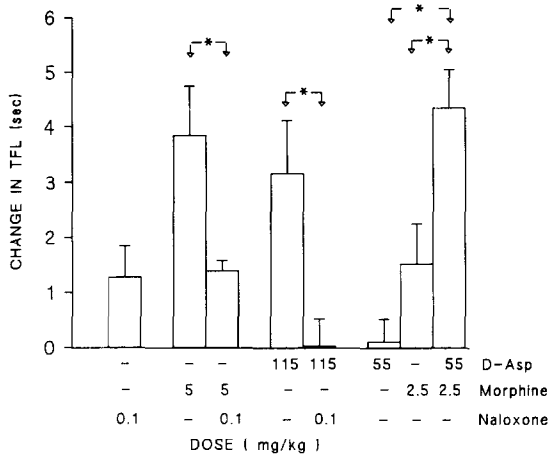

FIG. 3. Change in tail flick latencies (TFL, mean + SEM) from pretreatment values in mice receiving D-aspartic acid, morphine, and naloxone alone and in combination. n = 6–11. *Significantly different from each other ($p < 0.05$).

aspartic acid (115 mg/kg) and shortened the duration of morphine's effect (5 mg/kg) (Fig. 4).

DISCUSSION

In the present study, D-aspartic acid was shown to have an analgesic effect in mice that was synergistic with morphine. The analgesic effect of D-aspartic acid was antagonized by naloxone. L-Aspartic acid antagonized the analgesic effects of both morphine and D-aspartic acid.

The excitatory amino acids (EAA), L-glutamate and L-aspartate, are putative neurotransmitters at sites widely distributed throughout the mammalian central nervous system (1,3,7,27). At least three subtypes of EAA receptors exist in the mammalian central nervous system, which are relatively selectively stimulated by *N*-methyl-D-aspartic acid (NMDA), quisqualate, and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (6,21). A number of observations suggest that the NMDA receptors may play a role in processing nociceptive input to the spinal dorsal horn. It was reported that acute noxious stimulation induced an increase in L-aspartate and L-glutamate concentration in the dorsal lumbar spinal cord of freely moving rats (25). L-Glutamate, L-aspartate, or NMDA produces excitatory responses from spinal dorsal horn nociceptive neurons (2,9,12,23,24,29). Intrathecal administration of NMDA to conscious mice produces an hyperalgesic effect in the tail flick and hot plate tests (1), whereas intrathecal NMDA receptor antagonists have been shown to inhibit responses to noxious stimuli (4).

On the other hand, the D-amino acids, D-phenylalanine (D-Phe) and D-leucine (D-leu), which are carboxypeptidase A and leucine aminopeptidase inhibitors, respectively, cause analgesia in man and mice (5,10). D-Phe- and D-Leu-induced analgesia is naloxone reversible and cross-tolerance has been shown with morphine. In the present study, D-aspartic acid was demonstrated to have an antinociceptive effect in conscious mice. When nonanalgesic doses of D-aspartic acid and morphine were combined, a synergistic interaction was observed. Naloxone antagonized the analgesic effects of both D-aspartic acid and morphine, indicating that the endogenous opioid system is involved in D-aspartic acid-induced analgesia.

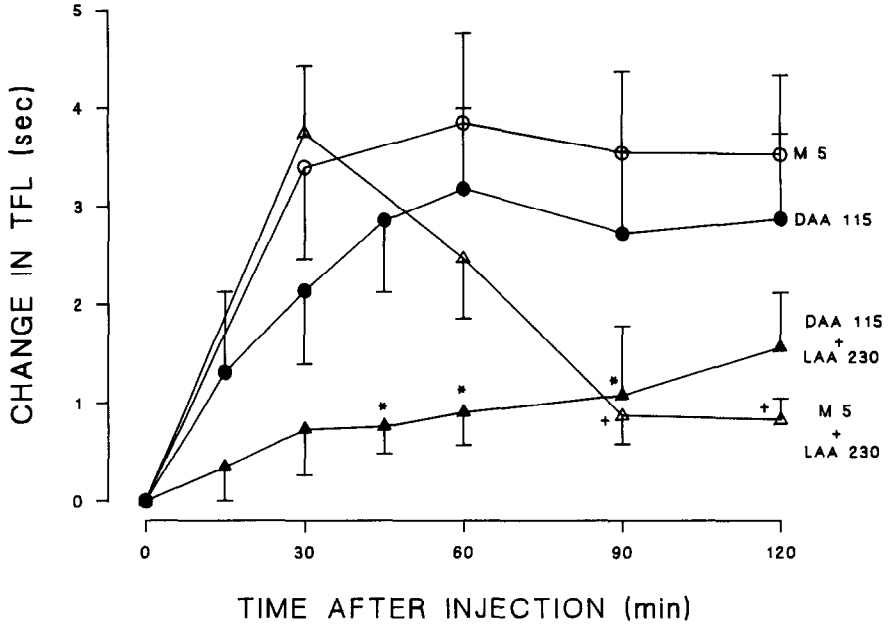

FIG. 4. Change in tail flick latencies (TFL, mean + SEM) from pretreatment values (time zero) in (○) morphine (M, 5 mg/kg), (△) morphine and L-aspartic acid (LAA, 5 and 230 mg/kg, respectively), (●) D-aspartic acid (DAA, 115 mg/kg), and (▲) D- and L-aspartic acid (115 and 230 mg/kg, respectively) treated groups. *Significantly different from the D-aspartic acid ($p < 0.05$); +significantly different from the morphine group ($p < 0.05$).

D-Aspartic acid, which was reported previously to exert some morphine-like effects such as decrease in food and fluid intake and decrease in body temperature (14,16,17), has been shown in our study to have an analgesic effect mediated via opioid mechanisms. Therefore, the affinity of D-aspartic acid for opioid receptors must be investigated to be able to explain the similarity between the pharmacological effect profiles of morphine and D-aspartic acid.

On the other hand, all morphine-like effects of D-aspartic acid were shown to be antagonized by L-aspartic acid (14, 16,17). The analgesic effect of D-aspartic acid was also shown here to be significantly inhibited by L-aspartic acid. Endogenous L-aspartic acid production is known to be reduced by the inhibition of L-asparaginase, the enzyme that converts L-asparagine into L-aspartic acid. Both morphine and D-aspartic acid inhibit L-asparaginase, resulting in reduced L-aspartic acid formation (18). Because L-aspartic acid antagonized both morphine- and D-aspartic acid-induced antinociception, the inhibition of L-aspartate synthesis may serve as a common mechanism of analgesic action. On the contrary, L-aspartic acid did not cause hyperalgesia, indicating that it does not have any tonic influence on the pain perception, so that the inhibition of L-aspartic acid synthesis by morphine and D-aspartic acid seems unlikely to cause analgesia.

Finally, evidence have been reported suggesting that morphine may act as an NMDA receptor antagonist (13,15,19). The antagonism between morphine and L-aspartic acid may be a pharmacological antagonism at the level of NMDA receptors. If this explanation is correct, D-aspartic acid may also act as an NMDA receptor antagonist because it has morphine-like effects that are antagonized by naloxone.

In conclusion, it is presented here that D-aspartic acid has significant analgesic activity. Although further studies are required to clarify the mechanisms of action, another aspect may be the development of more potent and more stable analogues of D-aspartic acid that could be used as analgesic drugs in humans.

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