INTERACTION OF DIFFERENT DOSES OF ASPARTAME WITH MORPHINE-INDUCED ANTINOCICEPTION IN THE PRESENCE OF MK-801 A NMDA ANTAGONIST

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ABSTRACT

This study was designed to investigate the relative role of sweetness and comparative effects of different taste sensation of the non-caloric sweetener, aspartame on pain and its interaction with MK-801 as a non-selective NMDA antagonist by formalin-test in mice. The formalin-test was chosen because it measures the response to a long-lasting nociceptive stimulus and closely resembles to the clinical pain. Morphine induced a dose dependent antinociception in the early and late phases of formalin test. Twelve days pretreatment of animals by aspartame (0.08%, 0.16%, 0.32%) significantly potentiated morphine-induced (1.5-9 mg/kg) analgesia in the early phase but significantly antagonized its analgesic effect in the late phase, dose dependently. Aspartame (0.16%) alone showed a reduction in pain response. Naloxone (0.4 mg/kg) significantly antagonized the antinociceptive effect of morphine in the presence of aspartame (0-0.32%) in the early phase. Increasing the dose of aspartame decreased effects of naloxone. MK-801 (0.1 mg/kg) as an N-Methyl-D-Aspartate (NMDA) antagonist significantly potentiated the effect of aspartame on morphine-induced antinociception in the early phase. In the late phase, naloxone (0.4 mg/kg) increased pain response but MK-801 (0.1 mg/kg) induced antiinflammatory effect significantly. Treatment of animals with MK-801 alone, significantly induced analgesia in both phases of formalin-test. This effect was potentiated with aspartame dose-dependently. Possible interaction of aspartame with NMDA receptors and its role to facilitate endogenous opioid system are proposed mechanisms of aspartame in modulating morphine-induced antinociception. Furthermore, the resulting association between morphine and aspartame chronic consumption may be explained as an interactive action rather than simple dose combination of both drugs.

Keywords: Antinociception, Formalin test, Morphine, Aspartame, Sweetening agents, NMDA

INTRODUCTION

The effect of sweet tasting substances including aspartame in moderating morphine-induced analgesia has been reported previously (1-6). However, intake of sweet substances does not always produce the same alterations in morphine-induced analgesia. Some investigations showed that intake of sweet solutions reduce sensitivity to morphine's analgesic properties (1,4,7), whereas others showed sweet intake increases sensitivity to the pain relieving effects of morphine (3,5,8,9,10). Also, there are reports indicating that aspartame (L-aspartyl-L-phenyl-alanine methyl ester) as a non-caloric sweetening agent could alter the effect of morphine in nociception and inflammation induced by formalin (6,11). There are evidences suggesting that aspartate derivatives produce both analgesic and hyperalgesic effects (12,13). The formalin-test was selected in this study because it measures the response to a long-lasting nociceptive stimulus and thus may closely resemble clinical pain (14,15). It has been proposed that excitatory aminoacid N-Methyl-D-Aspartate (NMDA) receptors within midbrain periaqueductal gray (PAG) are involved in the perception of tonic, inescapable pain as measured in the formalin test (16). Injection of NMDA into subarachnoid space of rats could produce both analgesic and hyperalgesic effects (17). Aspartame in the gastrointestinal is hydrolyzed to aspartyl phenylalanine and subsequently; aspartate and phenylalanine enter into the normal metabolic paths for the aminoacids (18,19). There

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is evidence that some effects of aspartame may be mediated via activation of NMDA receptor (20). It is also proposed that aspartame may cause some neurological effects which are observed with glutamates (18).

The present study was mainly undertaken to investigate the hypothesis that aspartame might possess antinociceptive or nociceptive properties and to determine its correlation with the analgesic activity of morphine in the presence of NMDA antagonist in more detail. In general, the present experiment investigated the roles of aspartame on the effect of morphine by the formalin test in mice.

MATERIALS AND METHODS

Chemicals:

Morphine sulphate, naloxone and aspartame were purchased from Sigma Chemical Co. (UK). Formalin was supplied from Merck Chemical Co. (Germany) and MK-801 from Tocris Co. (USA).

Animals:

Male albino mice weighing 22-27g were used in the experiments. The animals were housed in conditions of constant temperature (22±2°C) and light controlled room (12h, 12h).

Treatment:

Mice were randomly distributed in groups of 9 as control and tests. Initially the dose response antinociceptions by morphine sulphate (1.5,3,6,9 mg/kg) and MK-801 (0.05,0.1,0.2,0.4 mg/kg) (21) were determined. Morphine sulphate was dissolved in 0.9% saline and administered subcutaneously (sc) 30 minutes before formalin injection. MK-801 was dissolved in saline and administered intraperitoneally (ip) 30 minutes before formalin (21). Controls received equal volume of saline. All injection solutions were prepared in a volume of 10 ml/kg.

In the next step, 4 groups of animals were used. Animals in the first group were assigned as control and received only tap water for 12 days. The remained three groups of animals were treated orally by aspartame solutions of 0.08%, 0.16% and 0.32% w/v respectively for 12 days. All animals had access to food and water throughout the experiments. After 12 days treatment of animals by aspartame and tap water, the antinociception recording was done on day 13. Morphine sulphate (1.5,3,6,9 mg/kg) was administered subcutaneously (sc) 30 minutes before formalin injection. Naloxone solution (0.4 mg/kg) in normal saline was administered (ip) 5 minutes before morphine. MK-801 (0.1 mg/kg) was administered (ip) 15 minutes before morphine.

Antinociception recording:

Mice were allowed to acclimatise for 30 minutes before any injection. Twenty five µl of formalin (0.5%) was injected subcutaneously into the dorsal surface of the right hind paw of mouse using a microsyringe with a 26-gauge needle. Immediately after formalin injection, animals were placed individually in glass cylinder (20-cm wide, 25-cm length) on a flat glass floor and a mirror was arranged in a 45° angle under the cylinder to allow clear observation of the paws of the animals. The total time (seconds) spent licking and biting responses of the injected paw during periods of 0-5 minutes (early phase) and 10-30 minutes (late phase) were measured as an indicator of pain and inflammatory response.

Statistical analysis:

Comparison between groups were made by one-way ANOVA and then Newman-Keuls tests. Differences with $P<0.05$ between experimental groups of each point were considered statistically significant.

RESULTS

Antinociception induced by different doses of morphine in formalin-test in mice:

Effects of morphine on antinociception in formalin test have been shown in figure 1. There is a significant difference between animals that were treated sc with different doses of morphine in the early (1.5-9 mg/kg) and late phases (1.5-9 mg/kg) with saline.

Antinociception induced by different doses of MK-801 in formalin-test in mice:

Effects of MK-801 on antinociception in formalin test have been shown in figure 2. There is a significant difference between animals that were treated ip with different doses of MK-801 in the early (0.05-0.4 mg/kg) and late phases (0.05-0.4 mg/kg) with saline.

Effects of twelve days pretreatment of animals by aspartame (0.08%, 0.16%, and 0.32%) in morphine-induced antinociception:

In the early phase, aspartame significantly potentiated morphine-induced analgesia in all doses (1.5-9 mg/kg) in a dose-dependent manner. In the late

phase, pretreatment of animals with three doses of aspartame significantly antagonized the analgesic effects of morphine (1.5-9.0 mg/kg) dose-dependently. Treatment of mice by aspartame (0.16) alone reduced pain response in both phases of formalin test.

Influence of naloxone on effects of twelve days regimen of different concentrations of aspartame on morphine-induced antinociception:
In the early phase, naloxone (0.4 mg/kg) significantly antagonized the antinociceptive effect of morphine in the presence of aspartame (0-0.32%). Increasing the dose of aspartame decreased the effect of naloxone (figure 4).

Influence of MK-801 (0.1 mg/kg) on effects of twelve days regimen of different concentrations of aspartame in morphine-induced anti-nociception:
In the early phase, MK-801 (0.1 mg/kg) potentiated the effect of aspartame on morphine-induced antinociception significantly.
In the late phase, MK-801 (0.1 mg/kg) induced antiinflammatory effects (figure 5).

DISCUSSION

Results of this study show that aspartame acts differentially on morphine-induced anti-nociception in two phases of the formalin test. In the early phase, aspartame potentiated but in late phase antagonized the antinociceptive effects of morphine. These effects were antagonized by naloxone in both phases of formalin test especially in the presence of low doses of aspartame. On the other hand, MK-801 (0.1 mg/kg) potentiated these synergistic actions of morphine and aspartame in both phases of formalin test. Overall, these results demonstrate that aspartame plays a significant role in morphine-induced antinociception and has an interaction with NMDA receptors. The initial pain in formalin test (early phase) is explained as a direct stimulation of nociceptors and the late phase is thought to be secondary to the inflammatory reactions (22-24). It has been demonstrated that central changes induced by the early phase may contribute to the development of the late phase, indicating that mechanisms other than inflammation may take part (25). There is evidence that consumption of palatable solutions can store the endogenous opioid systems. For example, intake of sweet solutions increases the release of beta-endorphin in the hypothalamus (26,27) and chronic consumption of sweet solutions attenuates the analgesic effect of small doses of morphine (1,28,29). However the fact that naloxone was able to block the analgesic potentiation effect of aspartame in the present study suggest the involvement of endogenous opioid systems. The synergistic action of aspartame and morphine in the early phase of formalin test supports the hypothesis that aspartame may facilitate release of endogenous opioid peptides from neurons which may ultimately be responsible for the observed antinociceptive effect. It is suggested that aspartame at high doses affects some additional pathways that are not clear yet but some explanations are proposed.
Aspartame has been described as unabsorbed and completely metabolized agent (30). Following hydrolysis of aspartame by esterases in the gastrointestinal tract, aspartate and phenylalanine enter into the normal metabolic paths for the aminoacids (18,19). There are also some concerns that aspartame may cause some of the neurologic effects seen with glutamates (18). Aspartate and glutamate are the predominant neurotransmitters in the mammalian spinal cord (31) and various parts of the brain (12).
It has been proposed that the sensory neurons subserved by these excitatory aminoacids posses large diameter axons participating in monosynaptic reflex arcs and coursing within the dorsal columns (32). However, there is evidence suggesting release of the excitatory aminoacids from small diameter primary afferents involved in nociception (13). It has been reported that subcutaneous injection of formalin produces an immediate release of glutamate and aspartate in the spinal cord (33) and administration of NMDA receptor antagonists before formalin injection reduces pain during the late phase of formalin test (34,35). Supporting of this hypothesis is our results that show MK-801 as a NMDA antagonist has significant antinociception in the formalin test and antagonize combined effects of aspartame and morphine-induced antinociception in the late phase. Inhibition of this effect in the presence of MK-801 shown in this study supports this hypothesis and indicates that aspartame can act on NMDA receptors. These results suggest that the late phase of the formalin test depends on NMDA activity immediately after formalin injection. Antagonistic action of aspartame on the late phase of morphine-induced antinociception might result from its effect on NMDA receptors that could lead to hyperalgesia.

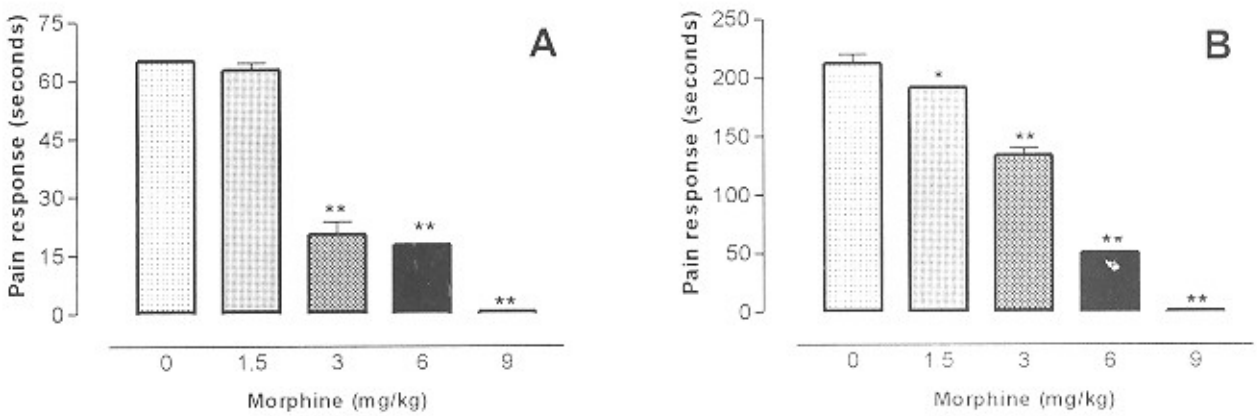

Figure 1. Effect of morphine on formalin-test in mice.
Saline (0) or different doses of morphine 1.5, 3, 6, 9 mg/kg were administered sc 30 minutes before formalin to mice. Antinociception was recorded 0-5 (early phase, A) and 10-30 (late phase, B) minutes after formalin injection. Each point is the mean±SE of 9 animals. Difference between control and treated groups is significant at *P<0.05 or **P<0.01.

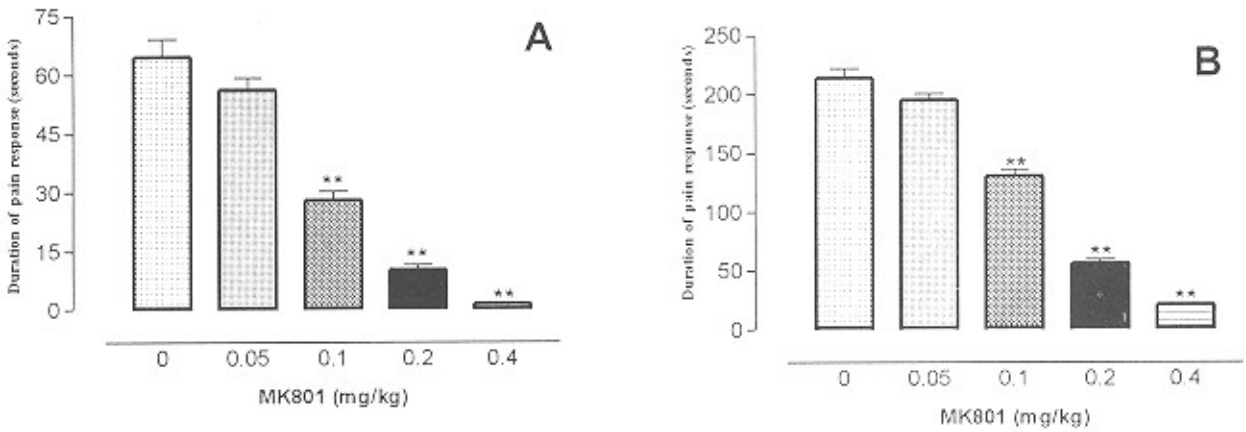

Figure 2. Effect of MK-801 on formalin-test in mice.
Saline (0) or different doses of MK-801 (0.05, 0.1, 0.2, 0.4 mg/kg) were administered ip 30 minutes before formalin to mice. Antinociception was recorded 0-5 (early phase, A) and 10-30 (late phase, B) minutes after formalin injection. Each point is the mean±SE of 9 animals. Difference between control and treated groups is significant at **P<0.01.

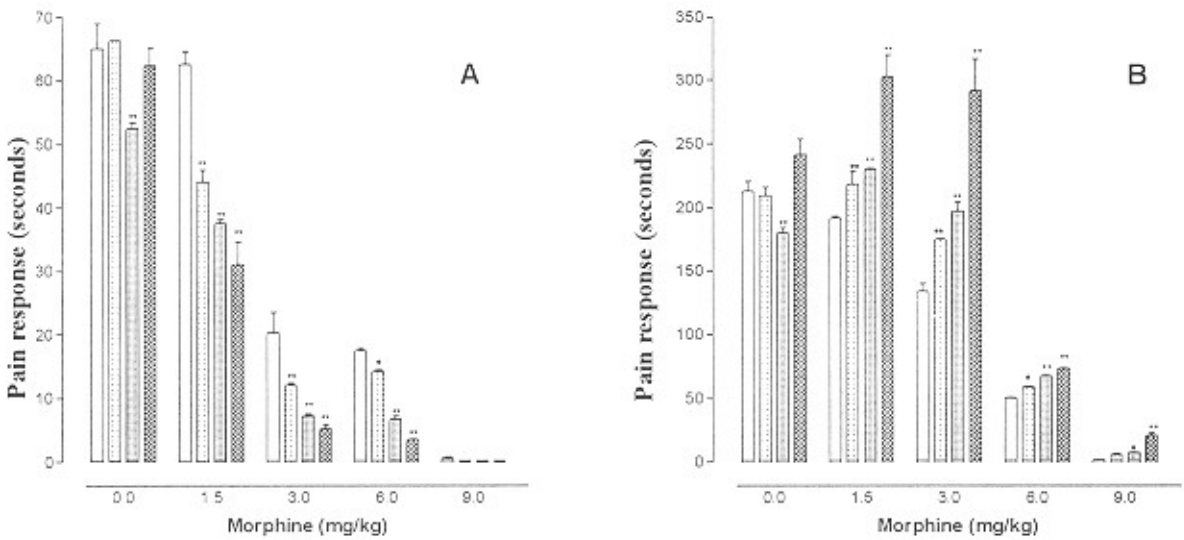

Figure 3. Effects of twelve days water or aspartame (0.08%, 0.16% and 0.32%) treatment on different doses of morphine (1.5, 3, 6, 9 mg/kg) induced antinociception.
Morphine was administered 30 minutes before formalin injection. Antinociception was recorded 0-5 (early phase, A) and 10-30 (late phase, B) minutes after formalin injection. Each point is the mean±SE of 9 animals. Each group of bar (4) represents water, aspartame 0.08%, aspartame 0.16% and aspartame 0.32% respectively from left to right. Difference between control and treated groups is significant at *P<0.05 or **P<0.01.

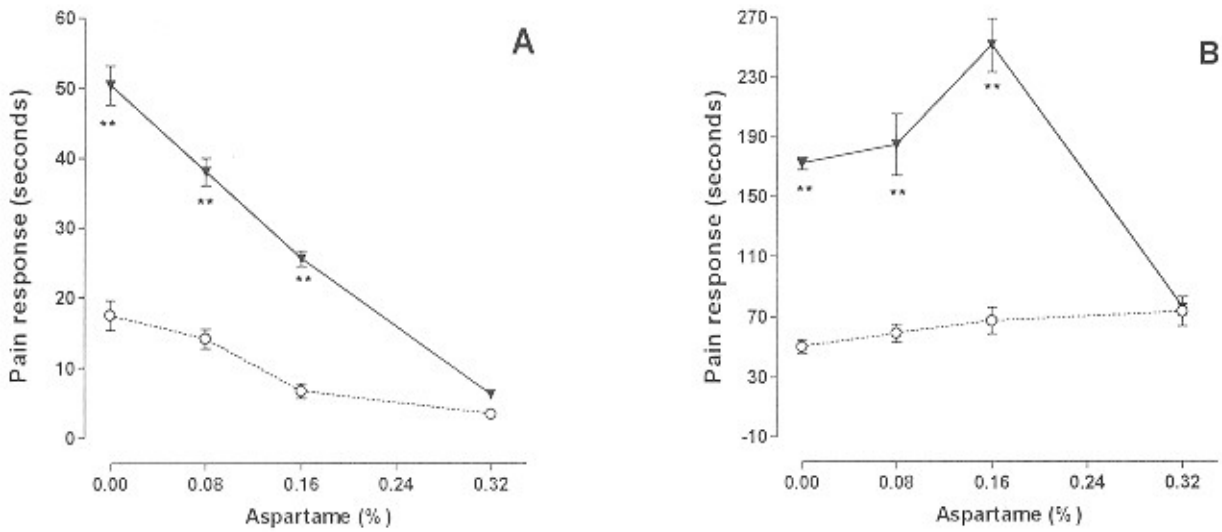

Figure 4. Effect of naloxone on morphine-induced antinociception affected by twelve days pretreatment by aspartame (0.08%, 0.16%, and 0.32%).
Morphine (6 mg/kg) was administered 30 minutes before formalin injection. Naloxone (0.4 mg/kg) was administered 5 minutes before morphine. Antinociception was recorded 0-5 (early phase, A) and 10-30 (late phase, B) minutes after formalin injection. Each point is the mean±SE of 9 animals. Difference between data of morphine (○) and that of naloxone+morphine (▼) treated groups is significant at **P<0.01.

This stimulus action of aspartame on NMDA receptors seems to be greater than its property to facilitate endogenous opioid peptide system. In the early phase it is supposed that aspartame's facilitization effect on endogenous opioids is more noticeable than its agonistic action on NMDA receptors and as a result it potentiates antinociceptive effects of morphine. On the other hand, several mediators such as prostaglandins, kinins or serotonin and calcium accumulation in the cells are responsible for the inflammation and nociception induced by various noxious stimuli (36). These mediators take part in the inflammatory response and are able to stimulate nociceptors and thus induce pain (37). All these mediators are also known to exert their effects by calcium dependent mechanisms (38,39).

Our previous investigations illustrated that coadministration of calcium channel blockers and aspartame before morphine injection alters its effects in both phases of the formalin test (11). Calcium serves a vital role in the integration of cellular activities and the resultant physiological sequel (40). Thus it is not surprising that manipulating the ratio of extracellular to intracellular calcium can modulate the pharmacological actions of opiates levels. Activation of NMDA receptor leads to calcium influx (41), increased production of the intracellular messengers, inositol triphosphate and diacylglycerol (42), release of calcium from intracellular stores (43), increased expression of protooncogenesis such as C-fos and C-jun, and increased production of nitric oxide (44). These combined events could lead to prolonged changes in activation threshold of dorsal horn neurons and augmented release of excitatory neurotransmitters from primary afferent terminals (42,45). Thus another possible reason for observed effects of aspartame might be its interation with calcium channels (11).

Another possibility would be that the differential responses are due to the differential stimulation of different opioid receptor subtypes (46), other neurotransmitters, receptors and second messengers.

However, it is important to consider the fact that naloxone was not able to reverse the effect of high dose of aspartame on morphine (figure 4). It is suggested that aspartame at high doses acts via pathways rather than opioid system or NMDA and therefore its effects cannot be elimination by morphine antagonists.

Based on these findings, the association between morphine and aspartame chronic regimen may be explained as an interactive action rather than simple dose combination of both drugs. Additional investigations are required to find the biochemical way of pain modulation in order to describe the role of aspartame regimen on opioid receptor type and its interaction with NMDA system to alter pain sensitivity and analgesia in formalin test.

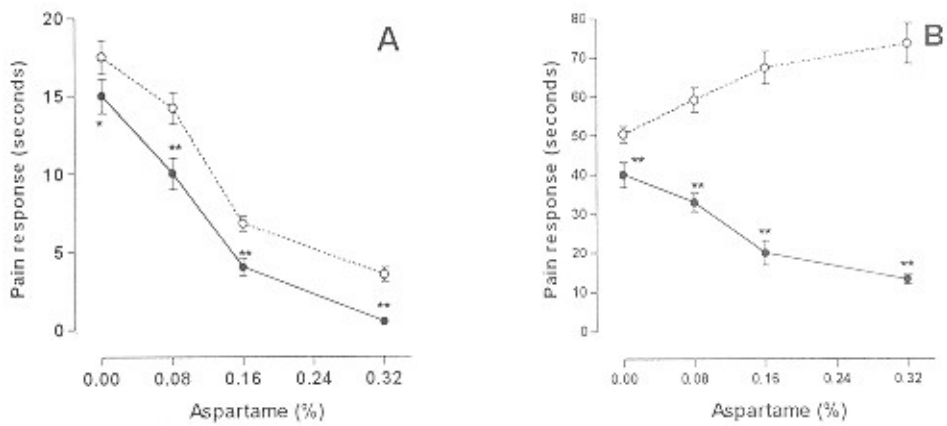

Figure 5. Effect of MK-801 (0.1 mg/kg) on morphine-induced antinociception affected by twelve days pretreatment by aspartame (0.08%, 0.16%, and 0.32%).

Morphine (6 mg/kg) was administered 30 minutes before formalin injection. MK-801 was administered 15 minutes before morphine. Antinociception was recorded 0-5 (early phase, A) and 10-30 (late phase, B) minutes after formalin injection. Each point is the mean±SE of 9 animals. Differences between data of morphine (o) and that of MK-801+morphine (•) treated groups are significant at *P<0.05 and **P<0.01.

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