Antinociception induced by stimulating amygdaloid nuclei in rats: changes produced by systemically administered antagonists

M.A. Oliveira and W.A. Prado

Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

Abstract

The antinociceptive effects of stimulating the medial (ME) and central (CE) nuclei of the amygdala in rats were evaluated by the changes in the latency for the tail withdrawal reflex to noxious heating of the skin. A 30-s period of sine-wave stimulation of the ME or CE produced a significant and short increase in the duration of tail flick latency. A 15-s period of stimulation was ineffective. Repeated stimulation of these nuclei at 48-h intervals produced progressively smaller effects. The antinociception evoked from the ME was significantly reduced by the previous systemic administration of naloxone, methysergide, atropine, phenoxybenzamine, and propranolol, but not by mecamylamine, all given at the dose of 1.0 mg/kg. Previous systemic administration of naloxone, atropine, and propranolol, but not methysergide, phenoxybenzamine, or mecamylamine, was effective against the effects of stimulating the CE. We conclude that the antinociceptive effects of stimulating the ME involve at least opioid, serotonergic, adrenergic, and muscarinic cholinergic descending mechanisms. The effects of stimulating the CE involve at least opioid, β-adrenergic, and muscarinic cholinergic descending mechanisms.

Key words
- Antinociception
- Amygdala
- Medial nucleus of the amygdala
- Central nucleus of the amygdala
- Tail flick test
- Stimulation-produced antinociception

Introduction

Behavioral and electrophysiological studies have demonstrated that at many sites in the brain electrical or chemical stimulation produces analgesia by activating centrifugal pathways that act to inhibit sensory neurons in the spinal cord (see Ref. 1). Special attention has been given to the mesencephalic periaqueductal gray (PAG)/dorsal raphe nucleus (DRN) and nucleus raphe magnus (NRM) (see Ref. 2), but evidence exists for the involvement of more rostral structures, including the amygdala, in this central pain control mechanism (see Ref. 3).

The amygdala is a subcortical complex of nuclei considered to be an important site for the induction of morphine analgesia (4), in addition to playing a role in the mediation of emotionality (see Ref. 5). The amygdala also seems to be critical in processing the aspect of noxious stimulation that results in aversive conditioning (6). Most of the information in favor of the involvement of the amygdala in pain control mechanisms derives from experiments on amygdaloid-lesioned animals.
or from the observation that the microinjection of some agonists into the amygdala can evoke antinociception. Lesions of the amygdala, mainly at its basolateral and central (CE) nuclei, attenuate several forms of environmentally induced antinociception (7-10). Bilateral lesions of the CE abolish the antinociceptive effects of low doses of systemically administered morphine in both the rat tail flick (11) and formalin (12) tests. Microinjection of aκ-opioid agonist (13) or neurotensin (14) into the amygdala evokes antinociception. A similar effect was demonstrated in the rat tail flick test following microinjection of carbachol into various amygdaloid nuclei, including the CE and the medial (ME) nuclei (15,16). Microinjection of morphine into the corticomedial subdivision of the amygdala is effective in the flinch-jump (17,18) and hot plate (19) tests. Microinjection of opioids into the CE (9,20-25) or of serotonin (26) into the basomedial part of the amygdala also induces antinociception.

Few studies, however, have been conducted on the effects of electrical stimulation of the amygdala on nociceptive responses. Early studies have demonstrated that stimulation of the lateral region of the amygdala elicits antinociceptive-like effects while the medial region yields a painful response pattern (27). However, Abbott and Melzack (28) did not obtain immediate antinociception in rats following stimulation of the amygdala. More recently, reduction of visceral pain in cats has been reported to occur after electrical stimulation of the CE (29). Unilateral stimulation of the basolateral nucleus, CE, or ME did not affect the threshold for the tail withdrawal response evoked by electric shock but increased the tail flick latency (TL) to noxious heat, reduced the tonic phase of the animal response to formalin, and elevated the threshold for vocalization during and after the application of an electric shock to the tail skin (30).

The present study was undertaken to examine the effects of stimulating the CE or the ME on the tail flick response evoked by noxious heating of the skin in rats. We demonstrate that a brief (30 s) stimulus applied to either nucleus evokes antinociception. In addition, we demonstrate that previous systemic administration of methysergide, naloxone, propranolol, phenoxybenzamine or atropine, but not mecamylamine, is effective in inhibiting the antinociception produced by ME stimulation. Moreover, systemic naloxone, atropine and phenoxybenzamine, but not propranolol, methysergide, or mecamylamine, are effective against CE stimulation-produced antinociception.

Material and Methods

Subjects and surgery

The experiments were conducted on male Wistar rats (140-160 g) housed two to a cage with free access to food and water and maintained at an average ambient temperature of 24°C with a 12-h light-dark cycle before and after surgery. The proposals of the Committee for Research and Ethical Issues of IASP (31) were followed throughout the experiments. Each animal was anesthetized with sodium thiopentone (50 mg/kg, ip) and a Teflon-insulated monopolar electrode (OD = 0.007") was stereotaxically implanted into the skull to lie in the CE or ME nuclei. The coordinates used were: AP = +5.8, L = 3.5, and H = -3.2 mm, for the ME, and AP = +5.8, L = 4.4, and H = -5.5 mm, for the CE, as proposed elsewhere (32). The electrode was then fixed to the skull with two steel screws and dental cement. One of these screws was used as the indifferent electrode. After receiving penicillin (50 mg/kg, im) the animal was allowed to recover for at least one week before the experiments.

Tail flick test

The animal was introduced into a ventilated glass tube for a period of up to 20 s,
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with the tail laid across a small wire that was at room temperature (23 ± 2°C). The coil temperature was then raised by the passage of electric current, which was previously adjusted to ensure a tail withdrawal reflex within 2.5-3.5 s. A cut-off time of 6 s was established to minimize the probability of skin damage. Tail flick latencies were measured at 10-min intervals until a stable baseline (BL) was obtained over three consecutive trials. Only rats showing a stable BL after six trials were used in each experiment. Each TL was normalized by an index of antinociception (IA) using the formula IA = (TL - average BL)/(6 - average BL).

Stimulation procedures

Immediately after BL determination the animal was placed inside a glass-walled box (20 x 30 x 35 cm), a 60-Hz sine-wave alternating current was applied to the electrode for 15 or 30 s and the TL determined within 10 s and the procedure was repeated at 10-min intervals over a period of 30 min. During the stimulation period the drop in voltage across a 1-kΩ resistor in series with the electrode was continuously monitored on an oscilloscope. No attempt was made to test for the presence of antinociception during the stimulation. Two groups of 5 animals each with electrodes implanted in the ME or CE were used as sham-stimulated rats.

A group of 18 rats with electrodes implanted in the ME or CE was preliminarily used for the determination of the CI50, i.e., the current intensity producing an antinociceptive effect in 50% of the animals in the experimental group. For calculation, antinociception was arbitrarily considered to occur whenever IA ≥0.5 was obtained. Immediately after BL determination each animal received 30 s of brain stimulation, and the TL was determined up to 10 s later. During this first stimulation period the lowest current of 1.4 µA root mean square (rms) was used and then increased to 3.5 µA in a second test 5 min later and to a maximum of 35 µA in a stepped sequence of 1.4, 3.5, 7.0, 10.0, 14.0, 21.0, 35.0 µA rms. The animals were spared further stimulation whenever IA = 1.0 was obtained.

Histology

At the end of the experiment the animal was killed with an overdose of sodium thiopentone and perfused through the heart with formalin. Electrode tracks were localized on 50-µm serial coronal sections stained with neutral red, and identified on diagrams from the atlas of König and Klippel (32).

Statistical analysis

The CI50 was calculated by the method of Litchfield and Wilcoxon (33). The results of the remaining studies are reported as graphs of averaged IA (± SEM) values against time of reading for a group of rats. The effects of different treatments on IA were analyzed statistically by multivariate analysis of variance (MANOVA) with repeated measures to compare the groups over all times. The factors analyzed in the experiments of ME or CE stimulation were treatments, time and treatment x time interaction. In the case of significant treatment x time interactions a one-way ANOVA followed by the Duncan test was performed for each time. The analysis was performed using the statistical software package SPSS/PC+, version 3.0, and the level of significance was set at P<0.05.

Drugs

A range of antagonists were administered intraperitoneally. Naloxone hydrochloride and phenoxybenzamine hydrochloride were from RBI (Research Biochemicals International, Natick, MA), atropine sulfate, mecamylamine hydrochloride, and propranolol hydrochloride were from Sigma Chemical Co. (Saint Louis, MO), and methysergide.
bimaleate was from Sandoz (Basel, Switzerland). The antagonists were all dissolved in saline and given at the dose of 1.0 mg/kg, 10 min (naloxone, atropine, and propranolol), 15 min (methysergide and mecamylamine), or 3 h (phenoxycbenzamine) before intracerebral stimulation. A longer interval had to be used between phenoxybenzamine administration and brain stimulation because the central effects of this drug develop slowly (34).

Results

Determination of the current intensity applied to the ME and CE for the production of antinociception

The latency for the tail flick reflex was increased by electrical stimulation of ME or CE at the sites depicted in Figure 1A. The current intensity for the maximal possible effect in the test (IA = 1.0), however, varied widely. One of eleven animals stimulated in the ME with a current intensity of 1.4 µA rms yielded IA = 1.0, whereas other rats showed full antinociception after stimulation at current intensities of 3.5 (2 animals), and 7.0, 10.0, 14.0, and 21.0 µA rms (1 animal at each intensity). Four animals did not show a full effect even after stimulation at 35 µA. Similar results were obtained for 7 rats stimulated in the CE. Full antinociception was obtained at current intensities of 1.4 and 10.0 µA rms (2 animals each) and at 3.5 and 7.0 µA rms (1 animal each). The remaining animal did not show a full effect even at the current intensity of 35.0 µA. The CI50 were 10.11 (confidence limits = 7.99 and 11.87) and 5.16 (3.74 and 6.29) µA rms for the ME and CE, respectively. We therefore decided to stimulate systematically these nuclei with 21.0 µA rms.

Time-course of the effects of stimulating the ME and CE. Influence of duration of the stimulation and repeated stimulation

Stimulation of the ME (Figure 1B) or CE (Figure 1C) with 21.0 µA over a period of 15 s produced a slight increase in the index of antinociception (26% and 35% for ME and CE stimulation, respectively) and the effects...
did not change significantly for experiments repeated 48 or 96 h later. The effects were stronger and of short duration following stimulation of the ME (Figure 1D) or CE (Figure 1E) with the same current intensity applied over a period of 30 s (58% and 54% for ME and CE stimulation, respectively), but the effects were smaller when the stimulation was repeated 48 and 96 h later. The curves in Figure 1B and C did not differ significantly regarding the different occasions of stimulation ($F_{3,16} = 0.97, P = 0.43$, and $F_{3,16} = 1.15, P = 0.36$, respectively) nor did they show significant effect x time interactions ($F_{21,112} = 0.96, P = 0.52$, and $F_{21,112} = 1.01, P = 0.46$, respectively). The curves in Figure 1D did not differ when the different occasions of stimulation were compared ($F_{3,13} = 1.73, P = 0.21$) and showed no significant effect x time interaction ($F_{21,91} = 1.10, P = 0.35$). The curves in Figure 1E were significantly different ($F_{3,16} = 3.89, P = 0.029$) and showed a significant effect x time interaction ($F_{21,112} = 2.42, P = 0.002$). The effects obtained for animals stimulated in the CE on the first occasion were significantly different from those obtained for sham-stimulated rats at times 0 and 2 min (ANOVA followed by the Duncan test). The subsequent experiments were then conducted on animals stimulated only once with a current intensity of 21.0 µA rms applied to each nucleus over a period of 30 s.

Some rats stimulated in the CE presented aversive-like behaviors during stimulation, including vocalization, masticatory movements and attempts to escape from the box. Apparently, the frequency of behaviors evoked by stimulating these nuclei was not changed by increasing the duration of the stimulation. Escape was also observed in some rats stimulated in the ME. These behaviors were more frequent during longer periods of stimulation. After the end of stimulation, no gross motor disturbance was detected. The animals walked and responded normally to innocuous stimuli. No attempt was made to quantify these behaviors in the present study.

Effects of ip administration of antagonists on the antinociception induced by stimulation of the ME

Six groups of rats were treated by ip administration of antagonists, 10 min (naloxone, atropine, and propranolol), 15 min (methysergide and mecamylamine) or 3 h (phenoxybenzamine) before intracerebral stimulation. All drugs were given at the dose of 1 mg/kg. A group of rats treated with saline (0.1 ml/kg, ip) was used as control.

Naloxone (Figure 2A), methysergide (Figure 2B), phenoxybenzamine (Figure 2C), atropine (Figure 2D) and propranolol (Figure 2E), but not mecamylamine (Figure 2F), significantly inhibited the antinociceptive effects of stimulating the ME. The curves in Figure 2 did not differ significantly regarding treatments ($F_{6,39} = 2.33, P = 0.05$) but showed a significant treatment x time interaction ($F_{42,273} = 2.44, P<0.001$). On the other hand, naloxone (Figure 3A), atropine (Figure 3B), and propranolol (Figure 3C), but not methysergide (Figure 3D), mecamylamine (Figure 3E), or phenoxybenzamine (Figure 3F), were effective against the antinociception induced by stimulation of the CE. The curves in Figure 3 differed significantly regarding treatments ($F_{6,38} = 5.10, P = 0.001$) and showed a significant treatment x time interaction ($F_{42,266} = 2.31, P<0.001$). The antagonists alone had no significant effect on tail flick latency.

Discussion

The present study demonstrated that stimulation of the ME or CE in rats produced antinociception in the tail flick test. Behavioral changes such as vocalization, masticatory movements and attempts to escape from the restraining box were occasionally observed during the stimulation period. Masi-
catory movements during stimulation of the CE have also been reported elsewhere (35).

The antinociceptive effects of stimulating the ME or the CE were dependent on the pattern of electrical stimulation. The current intensity required for a full antinociceptive effect was variable, the CE being more sensitive than the ME. Electrical stimulation of these nuclei was more effective when applied for 30 s than when applied for 15 s. The small monopolar electrodes used in these experiments reduce the risk of tissue lesion and the occurrence of edema at the site reached by the electrode tip. Moreover, the biphasic alternating current applied to monopolar electrodes allows a more focal stimulation of the target structure (36).

We have also shown that the antinociception evoked from the ME or CE was progressively weaker when the stimulation was repeated at 48-h intervals. The repeated stimulation of the amygdala may somehow cause irreversible or long-lasting functional changes at the site of stimulation. Repeated stimulation of the amygdala may produce kindling, a phenomenon that may change the animal’s responsiveness to pain (37). An alternative explanation for the phenomenon could be the development of tolerance to the stimulation. Similar changes induced by repeated stimulation of the PAG have been previously demonstrated, and probably involve the participation of endogenous opioid modulation (38). In fact, opioid mechanisms may participate in the antinociception evoked by amygdaloid stimulation. The ME and CE express mRNA for \( \mu \) and \( \kappa \)-opioid receptors (39, 40). Fibers and terminals immunoreactive to \( \beta \)-endorphins (41) or enkephalins (42, 43) were demonstrated in the ME and CE, respectively. Our data, however, do not allow us to conclude about the mechanism involved in the reduced effectiveness of repeated stimulation of the ME or CE.

The antinociceptive effects of stimulating the ME were significantly inhibited by the previous systemic administration of naloxone (an opioid receptor antagonist), methysergide (a 5-HT receptor antagonist), atropine (a muscarinic cholinergic receptor antagonist), phenoxybenzamine (an \( \alpha \)-adrenoceptor antagonist), and propranolol (a \( \beta \)-adrenoceptor antagonist), but not by mecamylamine (a nicotinic cholinergic re-

**Figure 2 - Effects of intraperitoneal administration (arrow 1) of saline (SAL; 0.1 ml/kg) or antagonists (1 mg/kg) on the antinociception induced by electrical stimulation (arrow 2) of the medial nucleus of the amygdala of rats.**

- **A**, Effect of naloxone (NLX);
- **B**, effect of methysergide (MET);
- **C**, effect of phenoxybenzamine (PBZ);
- **D**, effect of atropine (ATR);
- **E**, effect of propranolol (PRO);
- **F**, effect of mecamylamine (MEC). The number of rats per curve is given in parentheses. Data are reported as mean ± SEM for each group of rats. *P<0.05 compared to drug-treated animals (Duncan test).
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ceptor antagonist). Naloxone, atropine, and propranolol, but not methysergide, phenoxybenzamine, or mecamylamine, were significantly effective against the antinociception induced by stimulating the CE. These antagonists were all used at doses already known to be effective against similar effects induced by the stimulation of other brain structures known to participate in the descending control of pain (44-48). The effectiveness of propranolol against the stimulation-produced antinociception from the ME or CE is indicative that ß-adrenergic mechanisms may be involved in the phenomenon. Propranolol exhibits local anesthetic properties and has affinity also for a range of serotonergic receptor subtypes (49). A local anesthetic effect of propranolol seems to depend on higher drug concentrations (see 50) and is, therefore, unlikely to be the reason for its inhibitory effect found in this study. The present results do not allow us to exclude that the effectiveness of propranolol against the stimulation-produced antinociception from the ME derives from its 5-HT antagonist property. However, the nonspecific 5-HT antagonist methysergide was effective against the effect of stimulating the ME, but not the CE. Thus, ß-adrenergic mechanisms may also be involved in the descending mechanism activated from the CE. The different profiles of effectiveness of the antagonists used in this study provide evidence that the ME and CE function separately to produce inhibition of the tail flick reflex.

The tail flick escape from noxious heat is a spinal reflex (51) and its inhibition by stimulating supraspinal structures indicates that this action may somehow inhibit spinal mechanisms. Motor impairment produced by intracerebral stimulation could be one reason for the inhibition of the tail flick reflex. Objective tests for motor changes were not conducted in the present study. However, no gross motor disturbance was detected throughout experiments. The animals walked normally after the stimulation period and responded to innocuous stimuli.

Few reports are available regarding direct projections from the amygdala to the spinal cord. A sparse population of CE neurons in monkeys (52) and cats (53) projects...
to the cervical spinal cord. Alternatively, anatomical studies have demonstrated direct reciprocal projections between the amygdala and the PAG (54-59). The CE (56,60) and ME (55,56,61) have direct and indirect (via the hypothalamus) connections with the PAG. The CE also sends projections to the parabrachial nucleus (62,63) and locus coeruleus (60,64), which are structures also known to exert antinociceptive effects when electrically stimulated (65,66). Thus, the depression of the tail flick reflex by stimulating the ME or CE probably involves activation of descending pathways that utilize relay stations before reaching the spinal cord.

In summary, this study demonstrates that brief electrical stimulation of the ME and CE amygdaloid nuclei increases the tail flick response latency. The effect obtained from the ME involves at least opioid, serotonergic, adrenergic, and muscarinic cholinergic mechanisms. The effect from the CE seems to depend on at least opioid, ß-adrenergic, and muscarinic cholinergic mechanisms.

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References

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