Korean J Physiol Pharmacol
Vol 2: 687–693, December, 1998

Microinjection of Glutamate into the Amygdala Modulates Nociceptive and Cardiovascular Response in Freely Moving Rats

Dong-Kuk Ahn, Yun-Sook Kim, and Jae-Sik Park[1]

Department of Oral Physiology, School of Dentistry & [1]Department of Physiology, School of Medicine, Kyungpook National University, Taegu 700–422, Korea

This study was performed to examine the mean arterial pressure and nociceptive jaw opening reflex after microinjection of glutamate into the amygdala in freely moving rats, and to investigate the mechanisms of antinociceptive action of amygdala. Animals were anesthetized with pentobarbital sodium (40 mg/kg, ip). A stainless steel guide cannula (26 gauge) was implanted in the amygdala and lateral ventricle. Stimulating and recording electrodes were implanted into each of the incisor pulp and anterior digastric muscle. Electrodes were led subcutaneously to the miniature cranial connector sealed on the top of the skull with acrylic resin. After 48 hours of recovery from surgery, mean arterial pressure and digastric electromyogram (dEMG) were monitored in freely moving rats. Electrical shocks (200 μsec duration, 0.5~2 mA intensity) were delivered at 0.5 Hz to the dental pulp every 2 minutes. After injection of 0.35 M glutamate into the amygdala, mean arterial pressure was increased by 8±2 mmHg and dEMG was suppressed to 71±5% of the control. Injection of 0.7 M glutamate elevated mean arterial pressure by 25±5 mmHg and suppressed dEMG to 20±7% of the control. The suppression of dEMG were maintained for 30 minutes. Naloxone, an opioid receptor antagonist, inhibited the suppression of dEMG elicited by amygdaloid injection of glutamate from 28±4 to 68±5% of the control. Methysergide, a serotonin receptor antagonist, also inhibited the suppression of dEMG from 33±5 to 79±4% of the control. However, phentolamine, an α-adrenergic receptor antagonist, did not affect the suppression of dEMG. These results suggest that the amygdala can modulate both cardiovascular and nociceptive responses and that the antinociception of amygdala seems to be attributed to an augmentation of descending inhibitory influences on nociceptive pathways via serotonergic and opioid pathways.

Key Words: Amygdala, Glutamate, Jaw opening reflex, Antinociception, Freely moving rats

INTRODUCTION

Both human and animal studies have been shown amygdala as important region regulating emotional response such as anxiety or fear (Hilton & Zbrozyna, 1963; Weiskrantz, 1956). Recently, the amygdala has been demonstrated to play a critical role in the expression of conditioned antinociceptive responses in the tail-flick (Helmstetter & Bellgowan, 1993; Watkins et al, 1993) and formalin tests (Helmstetter, 1992). There are other strong evidences for the role of amygdala in antinociceptive response. The amygdaloid complex contains high levels of opiate receptors and enkephalin which are involved in antinociception of the central nervous system (Kuhar et al, 1973). It also has direct afferent connections with the periaqueductal gray (PAG), nucleus raphe magus (NRM), and locus coeruleus (LC) (Hopkins & Holstege, 1978; Price & Amaral, 1981) which are involved in the descending control of the nociceptive transmission (Basbaum & Fields, 1984; Carsteins, 1987; Fields & Besson, 1988). Moreover, the microinjection of neurotensin or morphine into the central amygdala

Corresponding to: Dong-Kuk Ahn, Department of Oral Physiology, School of Dentistry, Kyungpook National University, Taegu 700-422, Korea. (Tel) 053-420-6995 (Fax) 053-421-4077, E-mail: dkahn@ kyungpook.ac.kr

produced a significant increase in the nociceptive threshold (Rodger, 1978; Kalivas et al, 1982). Recently, Kowada et al (1991) reported that stimulation of central amygdala suppressed the jaw opening reflex. These results suggest that the amygdala seems to modulate the processing of the pain information in the orofacial area. Much work, however, has been done to elucidate the mechanism of central analgesic action of amygdala, but limited data are available concerning mechanisms of antinociceptive action from amygdala in the orofacial area.

On the other hand, it is well known that the amygdala forms dense neuronal connections with the hypothalamus and brainstem areas which are known to play an important role in cardiovascular regulation (Dampney, 1994). The amygdala has been demonstrated to contain acetylcholine and acetylcholinestrase (Hoover et al, 1978) and muscarinic binding sites (Rotter et al, 1979). Further, the pressor response evoked by the carbachol (icv) was suppressed by electrolytic lesions of the central nucleus of the amygdaloid complex (Özkutlu et al, 1995). These results indicate that the amygdala is also involved in cardiovascular regulation.

The primary objective of this study was to help clarify the role of the amygdala on cardiovascular and nociceptive regulation. To achieve this purpose, we monitored mean arterial pressure and nociceptive jaw opening reflex after administration of glutamate into the amygdala in freely moving rats. The secondary objective of this study was to investigate the mechanisms of antinociception from amygdala.

METHODS

Animals and surgery

Experiments were carried out in 26 male Sprague-Dawley rats (400~450 gm) which had stimulating electrodes implanted in the dental pulp, recording electrodes inserted into the anterior digastric muscle, and indwelling cannula implanted in amygdala and lateral ventricle. Surgical procedures were performed under pentobarbital sodium (40 mg/kg). Anesthetized rats were mounted on a stereotaxic frame (1404, David Kopf Instruments). A stainless steel guide cannula (26 gauge) was implanted in the amygdala. Coordinates were as follows, with flat skull position and bregma as the reference: A/P 2.5 mm, M/L 4.2 mm, and D/V 8.0 mm. We also implanted stainless steel guide cannula (23 gauge) into the lateral cerebral ventricle. Coordinates were 0.8 mm posterior to bregma, 1.5 mm lateral from midline, and 4.0 mm ventral from the surface of the skull. Left femoral artery was cannulated by a polyethylene tube (PE50) to record arterial blood pressure using a pressure transducer connected to a polygraph (CyberAmp 380, Axon). A stainless steel cannula and polyethylene tube were secured in place by means of stainless steel screws and acrylic resin on the skull. A 48h recovery period from surgery was allowed before starting the recording sessions.

Noxious assessment

A pair of stimulating electrodes (150 μm in diameter) was inserted bilaterally into the lower incisor pulp. The electrodes were secured in place with dental acrylic resin. Electrical shocks (200 μsec, 0.5~2 mA intensity) were delivered at 0.5 Hz to the dental pulp. Intensity of stimulation was adjusted at 2~3 times thresholds for evoked digastric electromyogram (dEMG). At this range of stimulation and frequency, no consistent behavior responses apart from a jaw opening reflex arise. Electromyograms (dEMGs) were recorded from the digastric muscle using a pair of recording electrodes inserted into the anterior belly muscle. Stimulating and recording electrodes were led to subcutaneously to a miniature cranial connector sealed on the top of the skull with acrylic dental resin. Electromyographic reflex responses were amplified (DAM 80, WPI) and fed to a computerized system for on-line digitization (CED 1401, CED). This procedure allowed reflex responses to be expressed as percentage of the control values. The amplitude of the reflex response following drug injection was expressed as percentage of the control values. For each trial, control responses were determined throughout the 3 min preceding the test period (60 stimulation). The mean control value was then calculated, and each individual reflex response was expressed as percentage of this mean.

Administration of drugs

We examined the mean arterial pressure and dEMG after injection of 0.35 M or 0.7 M glutamate into the amygdala. Drugs were given in a volume of 0.5 μl in one minute through a 31 gauge stainless

steel injector that extended 1 mm beyond the end of the guide cannula. To investigate the mechanisms of antinociception, we also examined changes in dEMG after intracerebroventricular injection of drugs. The drugs (30 μg/ 7 μl) employed were naloxone, an opioid receptor antagonist, methysergide, a serotonin receptor antagonist, and phentolamine, an nonselective α-adrenergic receptor antagonist, and each of these was followed by an 8 μl flush of aCSF. Fifteen minutes later, the nociceptive jaw opening reflex (JOR) was redetermined to assess whether these agents had altered the nociceptive response. Drugs dissolved in an artificial cerebrospinal fluid (aCSF) were employed. The aCSF solution contained (in mM) NaCl 128, KCl 3, $CaCl_2$ 1.2, $MgCl_2$ 0.8, NaH_2PO_4 0.25, $NaHCO_3$ 20, and glucose 3.4 (pH 7.4).

Histology and statistics

At the end of each experiment, the injection site was marked with 0.5 μl of 2% pontamine sky blue solution. Rats were perfused transcardially with 10% formaldehyde in phosphate-buffered saline, and then the brain was removed and placed in 10% formaldehyde solution over 24 hours. The brain was sectioned 60 μm on a vibratome. The serial sections were stained with cresylviolet and histologically examined under light microscopy. Only data from rats with injection sites clearly within the central nucleus of amygdala were used for final analysis (Fig. 1). All data were statistically evaluated by analysis of variance and student t-test. All data are represented as mean ±standard error.

RESULTS

The results of the present study showed that administration of glutamate into the central nuclus of amygdala elevated mean arterial blood pressure and suppressed dEMG in response to noxious electrical stimulation in the incisor (Table 1, Fig. 2). Basal mean arterial pressure was 108±2 mmHg. aCSF had no effects on the mean arterial pressure and the

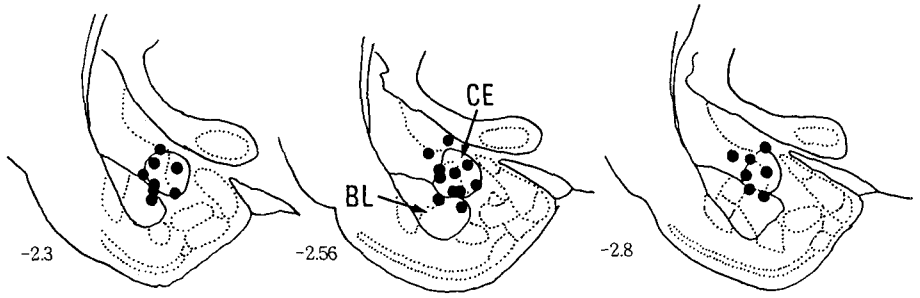

Fig. 1. Anatomical mapping of injection sites where glutamate was administered into the amygdala. The sections were taken from the atlas of Paxinos and Watson (1986) at the AP level indicated. Injection sites are scattered around the central nucleus of the amygdala. Central nucleus of amygdala consists of central and lateral part. CE, central nucleus of amygdala; BL, basolateral mucleus of amygdala.

Table 1. The changes in mean arterial pressure (MAP, mmHg) amygdaloid injection of glutamate

Time (min)	2	4	6	8	10	15	20	25	30	35	40
aCSF	1±2	2±2	4±3	3±3	3±3	2±4	3±2	1±4	2±3	2±4	1±4
0.35 M*	8±2	6±1	6±2	7±2	6±2	8±3	8±2	6±4	6±2	4±2	2±2
0.7 M**	14±2	19±7	21±6	25±5	19±5	17±4	13±5	8±4	5±3	4±1	3±1

Values are mean±S.E.
Number of experiments were 8. Basal mean arterial pressure was 108±2 mmHg. Amygdaloid glutamate produced a significant elevated the mean arterial pressure as compared to basal values(ANOVA, *$p<0.05$, **$p<0.01$).

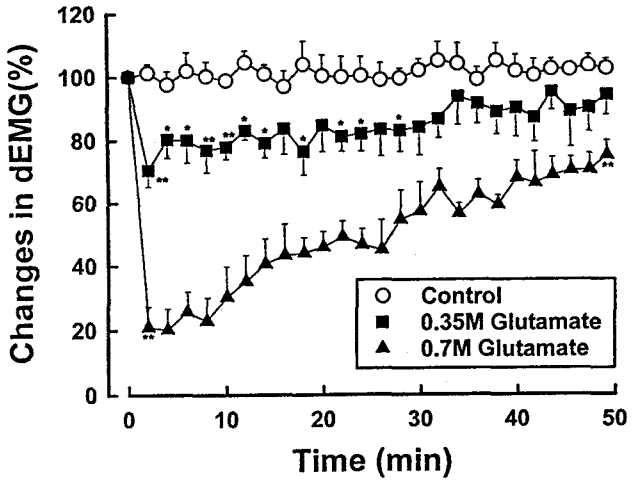

Fig. 2. The responses of digastric electromyogram (dEMG) produced by noxious electrical stimulation in the incisor to amygdaloid injection of glutamate. Number of experiments were 8. Stars represent a significant inhibition of dEMG as compared to rest values (*$p<0.05$, **$p<0.01$). In 0.7 M glutamate injection experiments, all points between two identical symbols have the same level of significance.

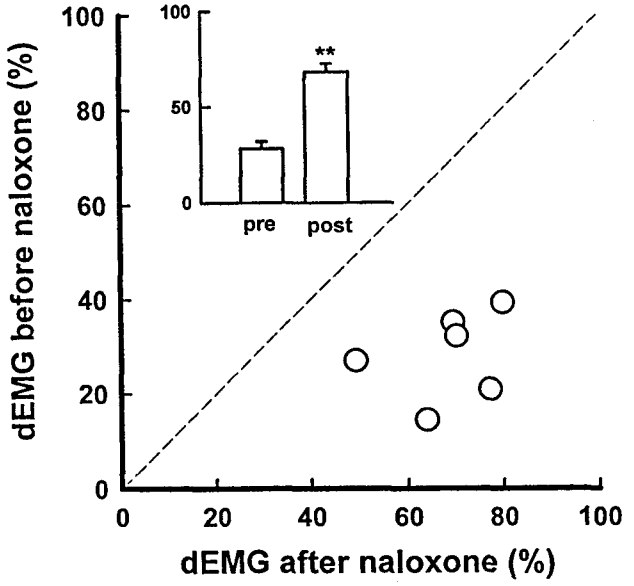

Fig. 3. Maximum effects of naloxone (30 μg, icv) on the suppression of dEMG in response to amygdaloid injection of 0.7 M glutamate. **$p<0.01$, vs. before naloxone. Each point in the scatter diagram depicts dEMG response (expressed as a percentage of the control) to injection of glutamate before and 15 minutes after naloxone in the same animal. Dashed diagonal indicates equal inhibition. The inset in scatter diagram portrays the mean percentage of dEMG changes before (pre) and after (post) the administration of naloxone.

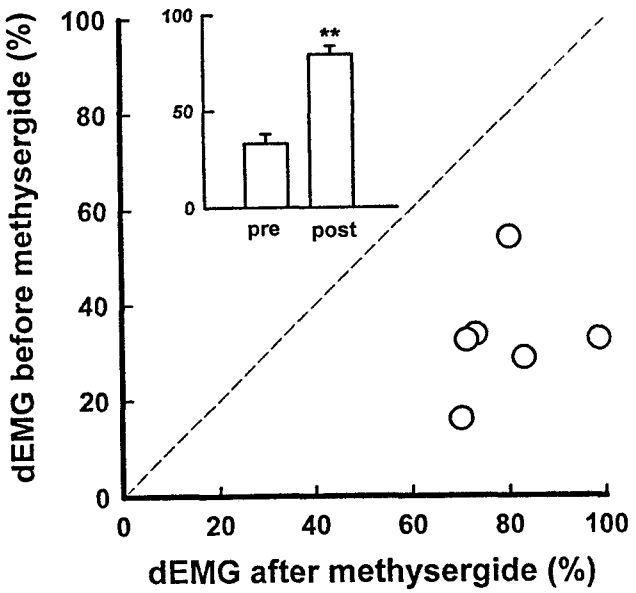

Fig. 4. Maximum effects of methysergide (30 μg, icv) on the suppression of dEMG in response to amygdaloid injection of 0.7 M glutamate. **$p<0.01$, vs. before methysergide. Each point in the scatter diagram depicts dEMG response (expressed as a percentage of the control) to injection of glutamate before and 15 minutes after methysergide in the same animal. Dashed diagonal indicates equal inhibition. The inset in scatter diagram portrays the mean percentage of dEMG changes begore (pre) and after (post) the administration of methysergide.

dEMG. After amygdaloid injection of 0.35 M glutamate, mean arterial pressure was increased by 8 ± 2 mmHg and dEMG was suppressed to 71 ± 5% of the control. Amygdaloid injection of 0.7 M glutamate elevated mean arterial pressure by 25 ± 5 mmHg and suppressed dEMG to 20 ± 7% of the control. The suppression of dEMG were maintained for 30 minutes.

To investigate the mechanisms of antinociception of amygdala, we examined dEMG avtivity after intracerebroventricular injection of naloxone, methysergide, or phentolamine (Fig. 3, 4, 5). All drugs did not affect the basal dEMG activity at the dose applied. Each point in the scatter diagram represents the value obtained from one experimental animal, and the mean effect is summarized in the inset of the scatter diagram. Naloxone, an opioid receptor antagonist, inhibited the suppressive effect of glutamate on dEMG from 28 ± 4 to 68 ± 5% of the control. Methysergide, a serotonin receptor antagonist, also inhibited the suppression of dEMG from 33 ± 5 to 79 ± 4% of the

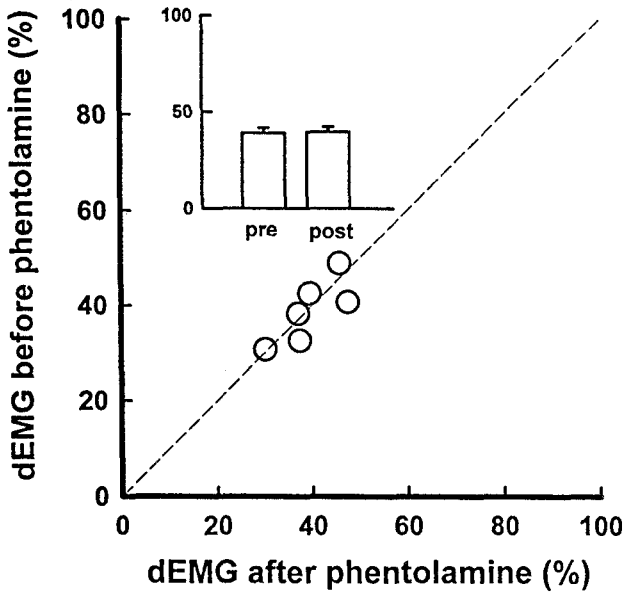

Fig. 5. Maximum effects of phentolamine (30 μg, icv) on the suppression of dEMG in response to amygdaloid injection of 0.7 M glutamate. **$p < 0.01$, vs. before phentolamine. Each point in the scatter diagram depicts dEMG response (expressed as a percentage of the control) to injection of glutamate before and 15 minutes after phentolamine in the same animal. Dashed diagonal indicates equal inhibition. The inset in scatter diagram portrays the mean percentage of dEMG changes before (pre) and after (post) the administration of phentolamine.

control. However, phentolamine, an α-adrenergic receptor antagonist, did not affect the suppression of dEMG.

DISCUSSION

Jaw opening reflex, one of the withdrawal reflex in response to noxious stimuli, has been used as a pain assessment method. Generally, jaw opening reflex is elicited by noxious stimulation in the orofacial area and is quantified by estimating the magnitude of electromyogram recorded from anterior belly of digastric muscles in anesthetized animals (Ahn, 1991, 1995, 1996). Anesthetic agents might influence the results of pain assessment experiments. Therefore, jaw opening reflex have to be evaluated in conscious or freely moving rats (Ahn & Kim 1998; Ahn et al, 1998). In the present study, microinjection of glutamate into the amygdala increased blood pressure and suppressed dEMG.

The amygdala consists of several distinct nuclei, including the lateral, basolateral, basomedial, and central amygdaloid nuclei in the rats (Brodal, 1947; Krettek & Price, 1978). Anatomical and behavioral evidence indicates that these nuclei are components of two distinct subsystems within the amygdala (LeDoux, 1995). The first subsystem of the amygdala consists of the lateral, basolateral, and basomedial nuclei. This complex deals with the primary sensory interface of the amygdala. The second subsystem of the amygdala consists of the central nucleus, and it provides the fear response systems such as a significant regulator of the autonomic and neuroendocrine outputs. The present study demonstrated that microinjection of glutamate into the central nucleus of amygdala increased mean arterial pressure. This result indicates that the amygdala plays an important role in cardiovascular regulation as an autonomic component of amygdala. This result is supported by others' studies that chemostimulation of the central amygdala with cholinergic drugs or glutamate resulted in pressor response with bradycardia or tachycardia (Iwata et al, 1987; Ohta et al, 1991).

On the other hand, there are several evidences for a role of amygdala in processing the nociceptive information. Microinjection of opioid agonist into the basolateral or central amygdala showed the antinociception in the rat (Helmstetter et al, 1993; Manning & Mayer, 1995). In the present study, the microinjection of glutamate into the central nucleus of amygdala suppressed dEMG in response to noxious stimulation applied to the incisor. This result suggests that amygdala has an important role on the processing of pain in the orofacial area.

It has been well established that a number of brain stem nuclei, including the periaqueductal gray matter (PAG), nucleus raphe magnus (NRM), nucleus paragigantocellularis, and locus coeruleus (LC), play an important role in the stimulation-produced analgesia (Basbaum & Fields, 1984; Carstens, 1987; Jensen & Gebhart, 1984). Monoamines and opiates have been demonstrated to be involved in the modulation of spinal nociceptive transmission. Intrathecal administration of norepinephrine, serotonin or enkephalin produced profound antinociception (Reddy et al, 1980; Yaksh & Wilson, 1979; Yaksh et al, 1977). In addition, intrathecal administration of noradrenergic, serotonin, and opioid receptor antagonist attenuated the analgesia induced by electrical stimulation on the brainstem nuclei (Hammond & Yaksh, 1984). There

were several evidences to show that serotonin (5-HT) was involved in the function of amygdala. Serotonin facilitated the anxiety of the amygdala while inhibited panic of the PAG (Graeff, 1994). Moreover, microinjection of 5-HT receptor antagonist in the basolatral amygdala produced anxiolytic effects, whereas 5-HT receptor agonist enhanced behavioral suppression determined by punishment (Petersen & Scheel-Kruger, 1985; Hodges et al, 1987). Recently, antinociception elicited following microinjection of morphine into amygdala was mediated by anatomical connections between the amygdala and PAG (Tamara et al, 1995). These results imply that there is an intimate interrelationship between amygdala and brainstem nuclei which have a potent analgesic effect. In the present study, the antinociceptive response of amygdala was blocked by intracerebroventricular injection of opioid and serotonergic receptor antagonists. These results indicate that antinociception of amygdala is mediated by serotonergic or opioid pathways involved in the descending antinociceptive mechanisms in brainstem.

In summary, the microinjection of glutamate into the amygdala increased mean arterial pressure and suppressed dEMG, simultaneously. The antinociception of amygdala seems to be attributed to an augmentation of descending inhibitory influences on the nociceptive pathways via serotonergic and opioid pathway.

ACKNOWLEDGMENT

This paper was financially supported by Kyungpook National University Research Fund (1996) and the Medical Research Institute Fund of Kyungpook National University Hospital (1997).

REFERENCES

Ahn DK. Antinociceptive and cardiovascular response to chemical stimulation in the periaqueductal gray matter. *J Oral Biol* 19: 13−18, 1995

Ahn DK, Kim KH, Yang EK, Kim HJ, Park JS, Lee WJ. Nucleus tractus solitarius-induced suppression of the jaw opening reflex and a possible interrelationship between antinociceptive and cardiovascular responses. *J Oral Biol* 20: 57−63, 1996

Ahn DK, Kim YS. Intracisternal antidepressants suppressed the nociceptive jaw opening reflex in freely moving rats. *Kor J Physiol Pharmacol* 2: 301−305, 1998

Ahn DK, Kim YS, Park JS. Central NO is involved in the antinociceptive action of intracisternal antidepressants in freely moving rats. *Neurosci Lett* 243: 105−108, 1998

Ahn DK, Sung JH, Park JS, Lee WJ, Choo YE. Modulation of the nociceptive jaw opening reflex by injection of enkephalin, glutamate and GABA in PAG. *J Oral Biol* 15: 54−61, 1991

Basbaum AI, Fields HL. Endogenous pain control system: brainstem spinal pathways and endorphin circuitry. *Ann Rev Neurosci* 7: 309−338, 1984

Brodal A. The amygdaloid nucleus in the rat. *J Comp Neurol* 87: 1−16, 1947

Carstens EE. Endogenous pain suppression mechanisms. *JAVMA* 191: 1203−1206, 1987

Dampney PAL. Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev* 74: 323−364, 1994

Field HL, Besson JM. Pain modulation. *Progress in brain research*, Elsevier, Amsterdam, 77: 454−464, 1988

Graeff FG. Neuroanatomy and neurotransmitter regulation of defensive behaviors and related emotions in mammals. *Brazilian J Med Biol Res* 27: 811−829, 1994

Hammond, DL, Yaksh TL. Antagonism of stimulation-produced antinociception by intrathecal administration of methysergide or phentolamine. *Brain Res* 298: 329−337, 1984

Helmstetter FJ. The amygdala is essential for the expression of conditional hypoalgesia. *Behav Neurosci* 106: 518−528, 1992

Helmstetter FJ, Bellgowan PS, Tershner SA. Inhibition of the tail flick reflex following microinjection of morphine into the amygdala. *Neuroreport* 4: 471−474, 1993

Hilton SM, Zbrozyna AW. Amygdaloid region for defense reaction and its efferent pathway to the brainstem. *J Physiol* 165: 160−173, 1963

Hodges H, Green S, Glenn B. Evidence that the amygdala is involved in benzodiazepine and serotonergic effects on punished responding but not in discrimination. *Psychopharmacology* 92: 491−504, 1987

Hoover DB, Muth EA, Jocobowitz DM. Mapping of the distribution of acetylcholine and choline acetyltransferase and acetylcholinestrase in discrete areas of rat brain. *Brain Res* 153: 295−306, 1978

Hopkins DA, Holstege G. Amygdaloid projections to the mesencephalon, pons and medulla oblongata in the cat. *Exp Brain Res* 32: 529−549, 1978

Iwata J, Chida K, LeDoux JE. Cardiovascular responses elicited by stimulation of neurons in the central amyg-

daloid nucleus in awake but not anesthetized rats resemble conditional responses. *Brain Res* 418: 183–188, 1987

Jensen TS, Gebhart GF. General anatomy of antinociceptive systems. In: J Olesen & L Edvinsson eds. *Basic mechanisms of headache,* pp. 189-200, Elsevier Science Publishers, 1984

Kalivas PW, Gau BA, Nemeroff CB, Prange AJ. Antinociception after microinjection of neurotensin into the amygdaloid nucleus of the rat. *Brain Res* 243: 279–286, 1982

Kowada K, Kawarada K, Matsumoto N, Ooe M, Suzuki TA. Inhibition of jaw opening reflex by stimulation of central amygdaloid nucleus in the cat. *Jap J Physiol* 41: 513–520, 1991

Krettek JE, Price JL. A description of the amygdaloid complex in the rat and cat with observations on intra-amygdaloid axonal connection. *J Comp Neurol* 178, 225–279, 1978

Kuhar MJ, Pert CB, Snyder SH. Regional distribution of opiate receptor binding in monkey and human brain. *Nature* 245: 447–450, 1973

LeDoux JE. Emotion: clue from the brain. *Ann Rev Psych* 46: 209–235, 1995

Manning BH, Mayer DJ. The central nucleus of the amygdala contributes to the production of morphine antinociception in the formalin test. *Pain* 63: 141–152, 1995

Ohta H, Watanabe S, Ueki S. Cardiovascular changes induced by chemical stimulation of amygdala in the rats. *Brain Res Bull* 28: 575–581, 1991

Özkutlu U, Coskun T, Onat F, Yegen BC, Oktay S. Cardiovascular effects of centrally active cholinomimetics in conscious and anesthetized rats: The role of amygdala. *Brain Res Bull* 37: 569–573, 1995

Paxinos G, Watson C. The rat brain in stereotaxic coordinations second edition, Academic Press, 1986

Petersen EN, Scheel-Kruger J. Anticonflict effect of 5-HT antagonists by intraamygdaloid injection. *Abstarcts of 14th CINP Congress,* 654, 1984

Price JL, Amaral DG. An autoradiographic study of the projections of the central nucleus of the monkey amygdala. *J Neurosci* 1: 1242–1259, 1981

Reddy SVR, Maderdrut JL, Yaksh TL. Spinal cord pharmacology of adrenergic-mediated antinociception. *J Pharmacol Exp Ther* 213: 525–533, 1980

Rodgers RJ. Influence of intraamygdaloid opiate injection on shock thresholds, tail flick latencies and open field behavior in rats. *Brain Res* 153: 211–216, 1978

Rotter A, Birdsall NJM, Burgen ASV, Field PM, Hulme EC, Raisman G. Muscarinic receptors in the central nervous system of the rat I. Technique for autoradiographic localization of binding of H^3 propylbenzilycholine mustard and its distribution in the forebrain. *Brain Res* 180: 141–165, 1979

Tamara M, Gomez DC, Behbehani MM. An electrophysiological charaterization of the projection from the central nucleus of the amygdala to the periaqueductal gray of the rat: th erole of opioid receptors. *Brain Res* 689: 21–31, 1995

Watkins LR, Wiertelak EP, Maier SF. The amygdala is necessary for the expression of conditioned but not unconditioned analgesia. *Behav Neurosci* 107: 402–405, 1993

Weiskrantz L. Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *J Comp Physiol Psychol* 4: 381–391, 1956

Yaksh TL, Wilson PR. Spinal serotonin terminal system mediates antinociception. *J Pharmacol Exp Ther* 208: 446–453, 1979

Yaks AL, Luang SP, Ruddy TA. The direct and specific opiate like effect of met-enkephalin and analogues on the spinal cord. *Neuroscience* 2: 593–598, 1977