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THE EFFECT OF LOCUS CERULEUS LESIONING ON TONIC AND PHASIC PAIN

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ABSTRACT

Bulbospinal noradrenergic pathways are shown to have an important role in descending inhibition of pain sensation. Locus ceruleus (LC), as a rich noradrenergic nucleus in the brain stem which has projections into the dorsal horn of the spinal cord, was evaluated for antinociceptive activity by using tonic and phasic pain models in the rat. LC-lesioned rats demonstrated moderate increase in both phases of the formalin test, but did not show any effect to thermal noxious stimuli, applied in the tail-flick test. These results indicate the relative involvement of LC in inhibition of tonic, but not phasic, pain.

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Keywords: Locus Ceruleus, Formalin, Nociception, Tail flick, Norepinephrine, Tonic pain, Phasic pain.

INTRODUCTION

It has been shown that neurons originating from brain stem regions mediate descending inhibition through the release of noradrenaline and other substances at spinal terminals.[19,26] Locus ceruleus (LC) is a noradrenaline-containing bilateral pontine structure with widespread projections to numerous forebrain,[22] brainstem and spinal areas[11,12] and in primates accounts for about 70% of brain norepinephrine.[20,7,21] The activation of LC neurons in animals is known to be correlated with vigilance and arousal, and responses associated with noxious stimuli.

Noxious stimuli such as footshock, as well as electrical stimulation of the LC are associated with an increase in LC discharge[5] and accelerated norepinephrine turnover in the cerebral cortex; the effect of the former can be blocked by LC lesions.[16,18] Lesions of the LC decrease the amplitude of the startle reponse of rats, and abolish the fearful response of monkeys to previous fear-producing stimuli.[25] LC lesions increase the nociceptive threshold to mechanical stimulation of the rats.[4]

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MATERIALS AND METHODS

To study and compare the role of LC in phasic and tonic pain, we have examined the effect of thermal (radiant heat) and chemical (1% formalin) noxious stimuli in LC-lesioned rats. 22 male Wistar rats weighing 200-300 g, were housed four per cage and had free access to food and water. They were kept in an animal room which was maintained at 22±1°C with a 12-hr light-dark cycle for at least one week before the experiments. The rats were not tested more than once and testing took place between 9:00 A.M. and 12:00

A.M.

The rats were assigned to two groups. As the control group, nociceptive responses to a noxious chemical stimulus were examined in 10 rats, using modification of the formalin test of Dubuisson and Dennis.[8] Subcutaneous injection of dilute formalin has been employed as a model of chemogenic nociception in rats, cats[8,23] mice[15] and primates.[2]

After a 15-min period of acclimatization to the test chamber (a clear plexiglas box of 30 cm ×30cm×30cm positioned over a mirror angled at 45°, to allow an unobstructed view of the formalin injected paws by the observer), each rat was given a subcutaneous injection of 50 µl of 1% formalin into the plantar surface of the left hind paw, using a 27 gauge syringe needle. The animals were then immediately returned to the test chamber. Observations to determine nociceptive responses began upon placing the rat into the box and continued at least for the next 60 min. When hind paws were used in the formalin test and measurements were taken visually, there were no pain related behaviors prior to formalin injection due to normal grooming. Therefore, pain score measurements were not taken before injection of formalin. A nociceptive score was determined for each 5 mins block during that period by measuring the amount of time spent in each of four behavioral categories: 0, the injected hind paw was held and treated normally; 1, the injected paw had little or no weight placed on it; 2, the injected paw was elevated and was not in contact with any surface; 3, the injected paw was licked, bitten or shaken. Then a weighted nociceptive score, ranging from 0 to 3 was calculated by multiplying the time spent in each category by the category weight, summing these products, and dividing by the total time for each 5 min. block of time. Formalin injections produce a characteristic biphasic response; following formalin administration, pain behavior starts immediately and lasts for 5-6 minutes and then diminishes, and again it increases after 15-20 min. to a steady level which lasts an additional 40-50 minutes.

The second group of 12 rats were anesthetized with sodium pentobarbital (50mg/kg) and their body temperatures were maintained during the operation at 37.5±0.5°C by a homeothermic blanket. The rats were placed in a stereotaxic frame and 0.2 mm stainless steel electrodes, insulated with epoxy resin, with the tip bared for 0.1 mm, were lowered towards the LC, under the following coordinates: 6mm posterior from Bregma; 1.2mm lateral to the midline; and a depth of 7 mm from dura with a 60° angle, according to the stereotaxic atlas.[24] Cathodic current from a standard lesion-maker was passed between the electrode and an anode, clipped to the edge of the scalp skin, and 1.5 milliamperes of D.C. current was applied for 6 seconds. The formalin test experiments were conducted 1 week after surgery. None of the animals showed any motor dysfunction due to the surgical procedure. Sham-operated control rats were prepared by using an identical procedure, except that the electrode was not lowered in place. In this group, the skull was drilled, the dura pierced, and the skin sutured when the bleeding had stopped. For assessing the effect of LC lesioning on phasic pain we used the tail-flick response to a noxious thermal stimulus (a high intensity focused beam of light), applied to the dorsal surface of the distal one -third portion of the rat's tails. Each rat was subjected to 3 successive trials of thermal noxious stimuli at 5 minute intervals. The average time interval between the onset of light stimuli and the tail-flick response was measured and defined as tail-flick latency. Tail-flick latencies (TFL) were measured electronically, and the cut-off time for preventing tissue damage was 14 seconds. The TFL of all experimental rats were measured before and one week after LC lesioning. Following testing, the rats were overdosed with sodium pentobarbital and their brains were removed and stored in formalin for 1 day. The lesioned regions were demonstrated by preparing slices 100 microns thick, using a vibroslice microtome (Campden, England). Data were analyzed by two-way analysis of variance (ANOVA) using a computer and commercial software. This was followed by Student's t-test. The P-values less than 0.05 were considered significant.

RESULTS

The histological checking showed that only 7 rats out of 12 had received bilateral, symmetrical LC lesions, and the pain scoring data of this group was compared with the control group (Fig. 1). The results obtained showed a significant increase in nociceptive response in both phases of pain expression ($P<0.05$), as compared with the control group (Fig.2, Table I). The painless gap period between the two phases was also elevated with accordance to the peaks. The results of the tail-flick test showed no significant difference between control and lesioned groups (Fig. 3).

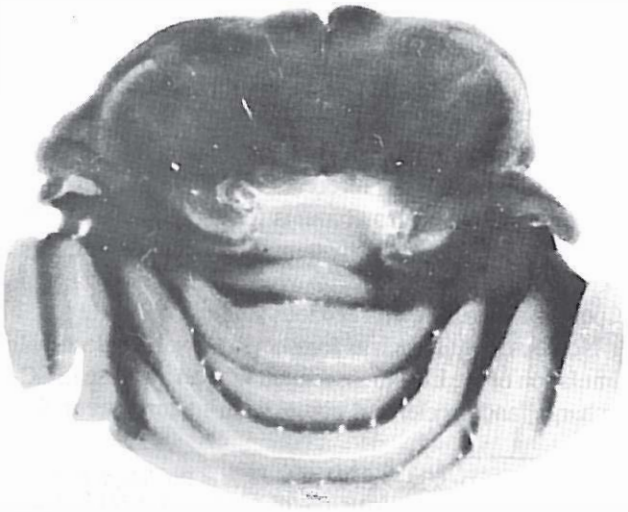

Fig.1. A representative coronal section from a LC-lesioned subject.

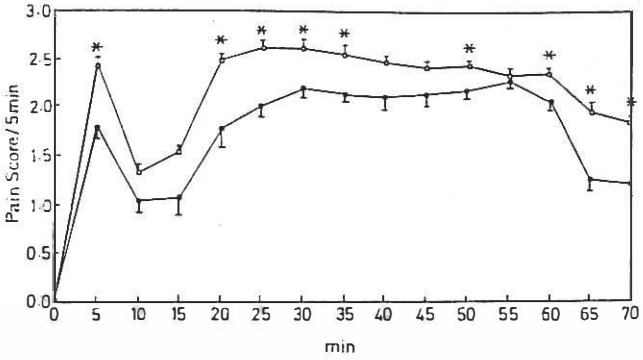

Fig.2. The effectiveness of LC lesioning in nociception measured by the formalin test. Control animals (●) illustrate the effects of subcutaneous formalin (n=10). LC lesioned rats (○) were more sensitive to formalin pain (n=7).
*significant difference from control values (P<0.05).

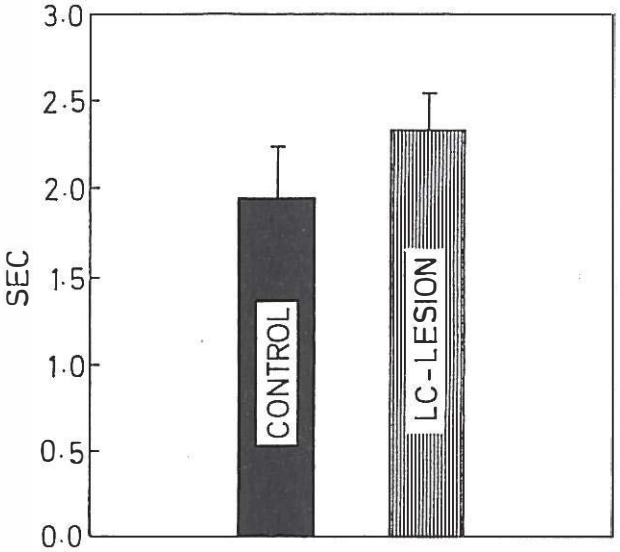

Fig.3. The effect of LC-lesioning on tail-flick latency. The histograms show mean tail-flick latencies in control (n=10), and LC-lesioned (n=7) groups.

DISCUSSION

The locus ceruleus is a small, compact nucleus located in the pons, near the lateral edge of the fourth ventricle. In rodents and primates it appears to be composed almost exclusively of noradrenaline (NA)-containing neurons.[7] The efferents of this nucleus project extensively throughout the brain and into the spinal cord.[26] LC neurons exhibit pronounced excitation following painful or polymodal non-noxious stimuli (in waking animals), suggesting that the LC is strongly regulated by afferents that integrate sensory information across modalities such as nucleus paragigantocellularis.[10]

Table I: Mean pain scores (+SEM)of control and test groups for critical time blocks.

mins	0-5	15-20	20-25	35-40
control	1.8±0.07	1.75±0.19	2.01±0.1	2.13±0.07
test	2.46±0.05	2.49±0.06	2.62±0.06	2.59±0.08

In single-cell recording studies it has been found that the entire population of LC neurons, even in deeply anesthetized animals respond to noxious stimuli with a burst of activity followed by a quiescent interval[5]. LC has been shown to project monosynaptic connections to large marginal zone neurons in the dorsal horn of the spinal cord[6] which participate in the relaying of nociceptive information.[29]

The pathways that mediate periaqueductal gray-evoked, norepinephrine-mediated analgesia (PAGNA) and blockade of dorsal horn nociceptive responses have not been thoroughly identified. Our findings agree with the results indicating that the physiological influence of PAG on LC neurons is only weak to moderate.[9] This pathway suggests the existence of one possible circuit that may mediate part of the PAG-evoked, NA-mediated analgesia. Our results concerning phasic pain are in accordance with findings which indicate that centrally-produced antinociception is not influenced by neurotoxin-produced lesions of the dorsolateral tegmentum, including LC,[1] but the ones focusing on tonic pain disagrees with this conclusion. The reason for the difference in these findings may be the kind of noxious stimulus and the type of analgesiometer used.

Focal injection of tracers into PAG showed only occasional, isolated fibers in LC proper. Consistent with these anatomical results, focal electrical stimulation of LC antidromically activated only a few PAG neurons. Finally, activation of PAG produced primarily weak synaptic activation of some LC neurons.

Another possible circuit concerning descending inhibition through LC is the PAG-nucleus paragigantocellularis-LC pathway. It is shown that PAG projects strongly to nucleus paragigantocellularis,[28] which by itself is a major afferent and potent excitatory input to LC.[3]

Many investigators have demonstrated a dissociation between the early and late phases of formalin pain[27] and it is suggested that they represent two independent processes which are mediated by separate neural systems. LC lesioning did not induce any significant differences between the first and the late phases of pain elevation in the formalin test.

Recently, using an *in vivo* voltammeter, it was shown that exposure of rats to mechanical and chemical stimuli (formalin) increases LC noradrenergic activity, but thermal stimuli (55°C water) have no such effect.[13] Our studies, using different behavioral analgesiometer tests showed the

saine results.

The present results indicate the moderate effect of the locus ceruleus on controlling pain induced by chemical, but not thermal, stimuli.

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REFERENCES

1. Aimone LD, Bauer CA, Gebhart GF: Brain stem relays mediating stimulation-produced antinociception from the lateral hypothalamus in the rat. J Neurosci 8: 2652-2663, 1988.
2. Alreja M, Mutalik PG, Nayar U, Manchanda SK: The formalin test: a tonic pain model in the primate. Pain 20: 97-105, 1984.
3. Aston-Jones G, Ennis M, Peribone VA, Nickell WT, Shipley MT: The brain nucleus ceruleus: Restricted afferent control of a broad efferent network. Science 234: 734-737, 1886.
4. Bodnar RJ, Ackerman RF, Kelley DD, Glusman M: Elevations in nociceptive threshold following locus ceruleus lesions. Brain Res Bull 3: 125-130, 1978.
5. Cedarbaum JM, Aghajanian GK: Activation of the locus ceruleus neurons by peripheral stimuli: modulation hy a collateral inhibitory mechanism. Life Sci 23: 1383-1392, 1978.
6. Cedarbaum JM, Aghajanian GK: Afferent projections to the rat locus ceruleus as determined by a retrograde tracing technique. J Comp Neurol 178: 1-16, 1978.
7. Dahlstrom A, Fuxe L: Evidence forthe existenceof monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurones. Acta Physiol Scand 62: (Suppl. 232): 1-55, 1964.
8. Dubuisson D, Dennis SG: The formalin test: a quantitative study of the analgesic effects of morphine, meperidine and brain stem stimulation in rats and cats. Pain 4: 161-174, 1977.
9. Ennis M, Behbehani M, Shipley MT, Van Bockstaele EJ, Aston-Jones G: Projections from the PAG to the rostromedial pericerulear region and nucleus locus ceruleus. Anatomic and Physiologic studies. J Comp Neurol 306: 480-494, 1991.
10. Foote SL, Bloom FE, Aston-Jones G: Nucleus locus ceruleus: new evidence of anatomical and physiological specificity. Physiol Res 63: 844, 1988.
11. Fritchy JM, Grazanna R: Demonstration of two separate descending noradrenergic pathways to the rat spinal cord: evidence for an intragriseal trajectory of locus ceruleus axons in the superficial layers of the dorsal horn. J Comp Neurol 291: 533-582, 1990.
12. Fritchy JM. Grazanna R: Distribution of locus ceruleus axons within the rat brainstem demonstrated by Phaseolus vulgaris leucoagglutination anterograde tracing in combination with dopamine-β-hydroxylase immunofluorescence. J Comp Neurol 293: 616-631, 1990.
13. Hong M, Milin B, Loomis CW, Jhamandas K: In vivo catechol activity in the rat locusceruleus following different nociceptive stimuli and naloxone. Can J Pharmacol 70: 1082-1089. 1992.
14. Jones BE, Moore RV: Ascending projections of the locus ceruleus in the rat. II Autoradiographic study. Brain Res 127: 23-53, 1977.
15. Hunskaar S, Berge OG, Hole K: Dissociation between antinociceptive and anti-inflammatory effects of acetylsalicylic acid and indomethacin in the formalin test. Pain 25: 125-132. 1986.
16. Krof J, Aghajanian GK, Roth RH: Increased turnover of norepinephrine in the rat cerebral cortex during stress. Neuropharmacol 12: 933-938, 1973.
17. Korf J, Roth RH, Aghajanian GK: Alterations in turnover and endogenous levels of norepinephrine in cerebral cortex following electrical stimulation and acute axotomy of cerebral noradrenergic pathways. Eur J Pharmacol 23: 276-282, 1973.
18. Korf J, Aghajanian GK, Roth RH: Stimulation and destruction of the locus ceruleus: opposite effects on 3-methoxy-4-hydroxyphenylglycol sulfate levels in the rat cerebral cortex. Eur J Pharmacol 21: 305-310, 1973.
19. Kuraishi Y, Harda Y, Takaga H: Noradrenaline regulation of pain transmission in the spinal cord mediated by α-adrenoreceptors. Brain Res 174: 333-337, 1979.
20. Lu CC, Tseng CJ, Wan FJ, Yin TH, Tung CS: Role of Locus ceruleus and serotonergic drug actions on schedule-induced polydipsia. Pharmacol Biochem Behavior 43: 255-261, 1992.
21. Moore RY, Bloom FE: Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. Annu Rev Neurosci 2: 113-168. 1979.
22. Moore RY, Card JP: Noradrenaline containing neuron systems. In: Bjorklund A and Hokfelt T. Handbook of Chemical Neuroanatomy, vol 2. Elsevier, Amsterdam. 123-156. 1984.
23. O'Keefe J: Spinal cord mechanisms subserving pain perception. Masters Thesis, McGill University. Montreal, 1964.
24. Paxinos G, Watson C: The Rat Brain Stereotaxic Coordinates. Academic Press, New York, 1986.
25. Hanin I, Usdin E (eds): Animal Models in Psychiatry and Neurology. (Eds. I. Hanin and E. Usdin), Pergammon Press, New York, pp. 293-304, 1978.
26. Tyce GM, Yaksh TL: The release of serotonin and noradrenaline from cat spinal cord *in vivo* by somatosensory stimulation: description of an intrinsic modulatory system. J Physiol, 341: 513-527, London, 1981.
27. Vaccarino AL, Tasker RAR, Melzack R: Analgesia produced by normal doses of opioid antagonists alone and in combination with morphine. Pain 36: 103-110, 1989.
28. Van Bockstaele EJ, Peribone VA, Aston-Jones G: Diverse afferent converge on the nucleus paragigantocellularis in the rat ventrolateral medulla: Retrograde and anterograde tracing studies. J Comp Neurol 290: 561-584, 1989.
29. Westlund KN, Bowker RM, Coutler JD: Origins of spinal noradrenergic pathways demonstrated by retrograde transport antibody to dopamine-beta-hydroxylase. Neurosci Lett 25: 243-249, 1981.