Indian J Physiol Pharmacol 2003; 47 (3): 311–317

ENKEPHALINERGIC INVOLVEMENT IN SUBSTANTIA NIGRA IN THE MODULATION OF HYPOTHALAMICALLY-INDUCED PREDATORY ATTACK BEHAVIOR

S. N. SAHA, S. C. BHATIA* AND U. NAYAR

Department of Physiology,
All India Institute of Medical Sciences,
New Delhi – 110 029

(Received of March 24, 2003)

Abstract : The present study was carried out in five cats which did not attack the rats spontaneously. Predatory attack on an anaesthetized rat was elicited by electrical stimulation of lateral hypothalamus at a mean current strength of 650 µA. The attack was accompanied by minimal affective display and culminated in neck biting. Microinfusions of DAME (delta-alanine methionine enkephaline) in 500 ng dose in substantia nigra facilitated the predatory attack and there was a significant reduction in the threshold current strength for affective display as well as somatomotor components. Microinfusions of naloxone, an opioid antagonist in 1.0 µg dose when DAME effect was at its peak reversed the facilitatory effects and the threshold returned to the control levels within 10 minutes of naloxone infusion at the same locus. Microinfusions of naloxone alone in similar dosage completely blocked the predatory attack response as indicated by an increase in the threshold current strength for somatomotor as well as affective display components. The somatomotor were completely inhibited and could not be elicited even when the current strength was increased to 1000 µA. Control injections of saline in similar volumes (0.5 µl) failed to produce any response Microinfusions of naloxone in lower dose (250 ng) failed to produce any blocking effect. These findings indicate that hypothalamically elicited predatory attack is facilitated by enkephalinergic mechanisms operating at the midbrain level.

Key words: predatory attack, naloxone, delta-alanine methionine enkephaline, neck biting, substantia nigra, lateral hypothalamus

INTRODUCTION

It is well established that predatory attack behaviour can be elicited by electrical stimulation of extreme lateral regions of hypothalamus in cats (1, 2). The neural pathways from hypothalamus to substantia nigra have already been traced (3). It has been reported that hypothalamically elicited predatory quiet biting attack can be

*Corresponding Author

controlled by electrical stimulation of substantia nigra sites in the midbrain (4). It has also been reported that neurones in substantia nigra possess nerve terminals and neuronal membrane, which are immunoreactive to opioids (5). Recent studies indicate the involvement of enkephalinergic mechanisms in the modulation of predatory attack in various midbrain sites namely dPAG, locus ceruleus, and ventral tegmental regions (6–8). However, there is no report indicating the involvement of enkephalinergic mechanisms at the substantia nigra level in the modulation of predatory attack even though the presence of enkephalines as well as receptors has been well demonstrated in this region by a number of workers (9, 10). Therefore, the present study was undertaken to investigate the role of enkephalinergic mechanisms in these midbrain regions in the modulation of hypothalamically elicited predatory attack behavior. This study indicates that predatory attack as elicited by hypothalamic stimulation is facilitated by prior microinfusions of DAME in substantia nigra while it is completely blocked by microinfusion of naloxone in this region.

METHODS

Selection of the animals : The present study was conducted in five cats of either sex weighing between 2.5 and 3.0 kg. The cats were tamed and adjusted to the behavioral cage for a period of about two weeks in order to stabilize their behavior. The tamed cats were very friendly and were not suspicious of their surroundings. These animals were fed ad libitum and only those cats, which did not bite the rats, were chosen for the present study. Animals, which did not display this behavior, were not used for the present study.

Experimental design : The general design of the experiment was to implant bipolar concentric electrodes in the lateral hypothalamus for electrical stimulation and chemitrodes in substantia nigra for chemical manipulation. The details of the construction of chemitrodes and electrodes have been given previously (11).

Implantation of electrodes and chemitrodes: Sterilized bipolar electrodes were implanted in the LHA using pentobarbitone sodium (35–45 mg/kg body weight) as an anesthetic agent. The stereotaxic coordinates as worked out from the atlas of Jasper and Ajmore-Marssen (12) were found to be A 12.5–14.0 mm, L2.5–3.5 mm, V-3.0–5.0 mm. Sterilized chemitrodes were implanted in substantia nigra which had the coordinates A3.0–5.0 mm, L3.5–4.5 mm, V-5.0–6.0 mm (13). While implanting the electrodes and chemitrodes, hypothalamic and midbrain loci were stimulated electrically to elicit some of the affective components like pupillary dilatation, respiratory excitation and acceleration of the heart rate and only then the electrodes were fixed at these loci. Benzathine pencilline was administrated to prevent any infection. The animals were allowed a post recovery period of 7 days before conducting any study.

Behavioral recording : The hypothalamic sites were stimulated electrically and the responses were recorded in an already prepared protocol. All behavioral recordings were done in the behavioral cage (1 m × 1 m × 1 m) with a sliding for entrance and exit of the animal. The cage had a smoked glass for one way viewing while the

other side had a clear glass for photography. Graded electrical stimulation using current strength between 300–800 µA was repeated on successive days with ten ascending and descending trials with a gap of half an hour for each trial and also a gap of five minutes for each electrical stimulation. These electrical stimulations were repeated on successive days to check the reproducibility of the responses. Subsequently, microinfusions of DAME and naloxone, an opioid antagonist were carried out in the substantia nigra and electrical stimulations were repeated to check any change in stimulation strengths. Microinfusions of normal saline in 0.5 µl volume served as control. Electrical stimulated consisted of square wave pulses, having a duration of 1 ms and a frequency of 60 Hz. The current strength as measured by voltage drop technique was within 300–800 µA.

Histological localization : Histological localization of LHA and midbrain sites was done by passing an anodal d.c. current of 2 mA for 10 seconds at the site of stimulation. After lesioning, the brain was fixed by perfusing transcardially with 10% formal saline dissolved in 2% potassium ferrocyanide solution and sections were stained with haematoxylin and eosin.

Statistical analysis : Statistical analysis of the data was carried out using Wilcoxon's signed rank test.

RESULTS

In the present study each animal served as its control producing a goal-directed attack by electrical stimulation on an anaesthetized rat at a mean current strength of 350 to 700 µA. Predatory attack as indicated by minimal affective display was produced by electrical stimulation of lateral hypothalamus. During the development of this response the animal slowly moved towards the rat with an extended neck and finally a full-fledged attack on the rat was performed which culminated in neck biting, often to kill the rat on the first bite. The cat dropped the rat as soon as the stimulation was switched off. The predatory attack components included motor components of attack like extended neck, unsheathing of claws, neck biting and sometimes striking and holding the prey with paws, just before the final lethal neck bite. The affective display components included the autonomic responses such as alertness, pupillary dilatation, respiratory excitation, ear flatness and slight piloerection. Sometimes, growling

TABLE I

S.no	*Observation*	*Behavioral scoring precentage value*
	A. Somatomotor components of predatory attack	
1.	Extended neck	25%
2.	Unsheathing of claws	25%
3.	Striking with paws	25%
4.	Biting	25%
		100%
	B. Affective components of Predatory attck	
1.	Hissing	15%
2.	Growling	15%
3.	Showing of teeth	15%
4.	Piloerection	15%
5.	Alertness with movements	6%
6.	Puillary dilatation	6%
7.	Respiratory acceleration	6%
8.	Salivation	6%
9.	Ear flattening	6%
10.	Urination	5%
11.	Defecation	5%
		100%

was exhibited with the neck biting attack. These somatomotor and affective components along with the respective scores assigned to each component are tabulated in Table I. It was observed that on stimulation with lower current strengths (350–400 µA) alertness, pupillary dilatation with extended neck and stalking posture, searching for the prey was initiated. Increasing the current strength (500–600 µA) at these loci produced salivation, piloerection, growling and finally neck bite was produced on a rat, thus showing a full blown predatory attack at a mean current strength of 700 µA. It was observed that microinfusions of DAME in 500 ng dose in substantia nigra facilitated the predatory response as indicated by a decrease in the threshold current strength for both somatomotor and affective components. There was a significant reduction in the current strength for the elicitation of predatory attack as a whole.

Microinfusions of naloxone in 1.0 µg dose at these sites when DAME effect was at its peak reversed the facilitatory effect within 10 minutes of microinfusions and the thresholds returned to control level. It was observed that naloxone when infused alone at these sites completely blocked the predatory attack as indicated by an increase in the threshold current strength for the affective components. The somatomotor components were completely blocked and could not be elicited even when the current strength was raised to 1000 µA. However this current strength was never utilized in the present study as this could lead to the development of seizure. Table II and III give the exact changes in the current strength

TABLE II: Data showing changes in current strength for elicitation of somatomotor components of predatory attack from LHA.

Group	*Behavioral components*			
	Extended neck	*Unsheathing of claws*	*Striking with paws*	*Neck biting*
Control	300±0	600±63	660±102	680±75
DAME 500 ng in SN	100±0	100±0	400±0	460±49
Naloxone 1 µg in SN	580±75	inhibited	inhibited	inhibited

Each cat served as its own control.
Numerals written below behavior scorings represent mean current strengths in µA.
Values are shown as Mean±SD. SN, substantia nigra.

TABLE III: Data showing changes in current strength for elicitation of affecive display components of predatory attack from LHA.

Group	*Behavioral components*					
	Pupil. dialation	*Resp. accel.*	*Ear flatness*	*Alertness*	*Salivation*	*Pilo-erection*
Control	300±0	300±0	300±0	300±0	580±75	660±80
DAME 500 ng in SN	100±0	100±0	100±0	100±0	420±40	480±40
Naloxone 1 µg in SN	560±49	560±49	560±49	560±49	inhibited	inhibited

Each cat served as its own control.
Numerals written below behavior scorings represent mean current strengths in µA.
Values are shown as Mean±SD. SN, substantia nigra.

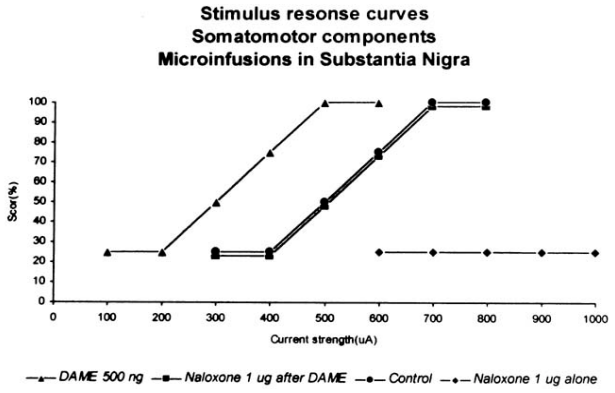

Fig. 1: Effects of microinfusions of DAME and naloxone in midbrain loci on the somatomotor components the predatory attack. These components were completely blocked and could not be elicited even when the current strength was increased to 1000 µA.

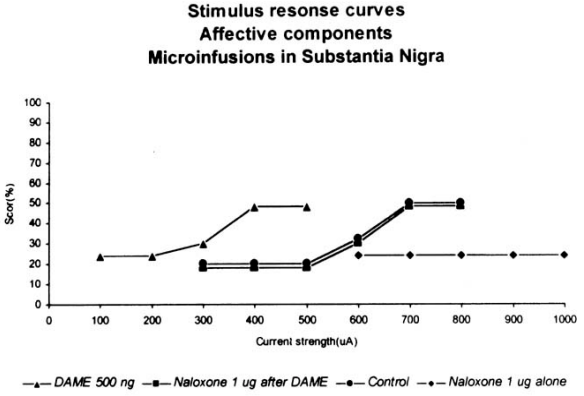

Fig. 2: Effects of microinfusions of DAME and naloxone in midbrain loci on the affective components the stimulus response curves. The shifting of the curves to the right indicates that much higher current strength was required to elicit the same components.

following the microinfusions of DAME and naloxone. Figs 1 and 2 give the stimulus response curves as obtained by plotting the respective scores of affective display and somatic components against the current strength. Ten hypothalamic sites and ten loci in the midbrain region were confirmed. These midbrain sites in substantia nigra gave successful modulatory response when DAME and naloxone were microinfused at these sites. The facilitatory effects of DAME and inhibitory effects of naloxone were found to be significant at $P<0.01$ and $P<0.05$ respectively. The exact localization of the substantia nigra sites is depicted in Fig. 3.

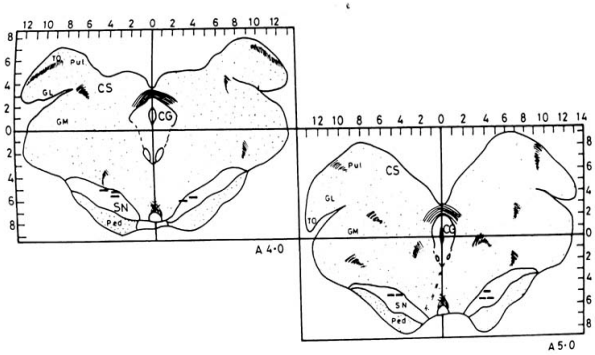

Fig. 3: Morphological reconstruction of the midbrain sites in coronal sections at which microinfusions of various drugs and normal saline was performed.

DISCUSSION

The results of the present study indicate that hypothlamically elicited predatory attack behavior can be facilitated by microinfusions of DAME in substantia nigra and this facilitatory effect of DAME could be reversed by microinjections of naloxone at these sites. Naloxone, an opioid antagonist when injected alone at these sites completely blocked the predatory attack response and there was a significant increase in the threshold current strength for affective components and the somatomotor components were completely blocked and could not be elicited even the current strength was raised to 1000 µA. Ascending and descending connections between hypothalamus and substantia nigra have been reported (13). Although the

involvement of cholinergic and adrenergic mechanisms at this level has already been reported (14, 15), there is no report indicating the involvement of enkephalinergic mechanisms at this level in the modulation of predatory attack response. Recent studies indicate that the enkephaline are involved in the modulation of predatory attack behaviour at various midbrain sites such as dPAG, locus cereleus as well as ventral tegmental sites (6–8). It has been reported that predatory attack as elicited by hypothalamic stimulation was inhibited by DAME infusion in dPAG and ventral tegmental area while it was facilitated by similar infusions in locus ceruleus (7). It has also been reported that the blocking and facilitatory effects of DAME are reversed by naloxone infusion at these sites According to Pert et al (9, 10) there is a sizeable population of all types of opioid receptors in the substantia nigra. Enkephaline is an overall inhibitor of neuronal action in brain except in hippocampus and spinal cord. Stimulation by enkephaline in substantia nigra leads to hyperpolarization of the neurons, thus leading to a decrease in their discharge rate (18). It has already been reported that stimulation of locus ceruleus and substantia nigra regions can lead to the development of analgesia (19). It is likely that DAME infusion may lead to similar action. In fact, enkephaline involvement in the development of anesthesia has been documented by a number of workers (20, 21). It is likely that DAME may be exerting some inhibitory action on the somatomotor and affective neurones mediating predatory aggression. It has been suggested that enkephalines mediate their action by disinhibition as well as direct excitation (22). The facilitatory effect of DAME in substantia nigra may be attributed to disinhibition. It has been suggested by Duggan (23) that the enkephalines can tonically activate adjacent inhibitory neurones rather than acting on the excitatory neurones and have made a strong case in favour of disinhibition, which in turn can lead to the excitatory effect of enkephalines. It is thus likely that DAME, which is secreted in the interneurones, may be causing disinhibition, thus leading to excitation by releasing norepinephrine, which is known to facilitate aggression (24). Atweh and Kuher (25) have reported that the receptors, associated with the sensory system subserving pain are μ-receptors while those associated with the limbic system are predominantly δ-receptors. Aggression is a function of the limbic system and the involvement of the δ-receptors in the facilitation of predatory attack behaviour is therefore, understandable. We additionally report that the facilitatory effect induced by DAME infusion could be reversed by naloxone infusion, an opioid antagonist. We also report that microinjections of naloxone in similar dosage in these regions, after the effects of DAME had waned off, blocked the predatory attack behavior.

REFERENCES

1. Bandler RJ. Predatory attack elicited in the cat by lower brainstem and hypothalamic stimulation. *Brain Behav Evol* 1977; 14: 440–460.

2. Wasman M, Flynn JP. Directed attack elicited from hypothalamus. *Arch Neurol* 1962; 6: 220–227.

3. Kostowski W, Treskowsha E, Jerlicz M, Bidjinski A, Hauptman M. Effects of the lesions of the ventral noradrenergic bundle on aggressive behaviour in rat. *Physiol Behav* 1980; 24: 429–433.

4. Piazza P, Crescimanno G, Berigno A, Amato A. The control exercised by substantia nigra on the quiet biting attack elicited by hypothalamic stimulation in the cat. *Neuroscie Lett* 1985; 53: 75–79.

5. Akil H, Watson SJ, Young E, Michael EL, Khachauturiaxn H, Walker M. Endogenous opiods: Biology and function. *Ann Rev Neuroscie* 1984; 7: 223–255.

6. Weiner S, Shaikh MS, Shaikh AB, Seigel A. Enkephalinergic involvement in periaqueductal gray control of hypothalamically elicited predatory attack in the cat. *Physiol Behav* 1991; 49: 1099–1105.

7. Manchanda SK. Poddar A, Saga S. Bhatia SC, Mohan Kumar V, Nayar U. Predatory aggression induced by hypothalamic stimulation: modulation by periaqueductal gray (PAG). *Neurobilogy* 1995; 3: 405–417.

8. Bhatia SC, Saha S, Manchanda SK, Nayar U. Role of midbrain ventrolateral (VTA) enkephalinergic mechanisms in the facilitation of hypothalamically–induced predatory attack behaviour. *Indian J Physiol Pharmacol* 1997; 41: 116–122.

9. Pert CB, Kuhar MJ, Snyder SH. Autoradiographic localization of the opiate receptors in the rat brain. *Life Scie* 1975; 16: 1849–1854.

10. Pert CB, Kuhar MJ, Snyder SH. The opiate receptors: Audioradiographic localization in the rat brain. *Proc Natl Acad Sci* 1976; 73: 3729–3733.

11. Saha S, Manchanda SK, Bhatia SC, Nayar U. Midbrain adrenergic mechanism modulating predatory attack behaviour induced by hypothalamic stimulation. *Indian J Physiol Pharmacol* 1991; 37: 121–126.

12. Jasper HH, Ajmone–Marsan C. Stereotaxic atlas of the diencephalon of the cat. *National Res Concil Canada* 1955.

13. Berman AL. The brainstem of the cat: A cytoarchetectonic atlas with stereotaxic coordinates: Wisconsin, (1968).

14. Fuchs SAG, Dalsass M, Siegal HE, Siegal A. The neural pathways mediating quiet biting attack behaviour from the hypothalamus in the cat: A functional autoradiography study. *Aggressive Behav* 1981; 7: 51–68.

15. Bandler RJ. Cholinergic synapses in the lateral phypothalamus for the control of predatory aggression in the rat. *Brain Res* 1970; 20: 409–424.

16. Kostowski W, Jarlicz K, Bidzinski A, Hauptmann M. Evidence for existence two opposite noradrenergic brainstem systems controlling behavior. *Psychopharmacoly* 1978; 59: 311–322.

17. Saha SN. Midbrain adrenergic and enkephalinergic mechanisms in the modulation of aggressive behaviour elicited on hypothalamic stimulation. *Ph. D. Thesis AIIMS New Delhi,* 1994.

18. Bohnar RJ, Paul D, Pasternik J. Synergistic analgesic interactions between the periaqueductal gray and the locus coeruleus. *Brain Res* 1991; 558: 224–230.

19. Lee JH, Beitz AJ. The distribution of brainstem and spinal cord nuclei associated with different frequencies of electroacupuncture analgesia. *Pain* 1993; 52: 11–28.

20. Fardin V, Olivers JL, Besson JM. A reinvestigation of the analegesic effects induced by stimulation of PAG matter in the rat brain II. Differential characteristics of analgesia induced by ventral and dorsal PAG stimulation. *Brain Res* 1984; 306: 125–129.

21. Lipp J. Possible mechanism of morphine analgesia. *Clin Neuropharmacol* 1991; 14: 131–147.

22. Handerson G. Electrophysiological action of opioid action. *Br Med Bull* 1983; 39: 59–64.

23. Duggan AW. Electrophysiology of opioid peptides and sensory system. *Brit Med Bull* 1983; 39: 65–70.

24. Starke K. Presynaptic receptors and the control of noradrenaline release. *Trends in pharmacol Sciences* 1980; 2: 268–271.

25. Atweh SF, Kuhar MJ. Autoradiographic localization of opiate receptors in the rat brain III. The telencephalon. *Brian Res* 1977; 134: 393–405.