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ROLE OF MIDBRAIN VENTRO-LATERAL TEGMENTAL AREA (VTA) ENKEHALINERGIC MECHANISMS IN THE FACILITATION OF HYPOTHALAMICALLY-INDUCED PREDATORY ATTACK BEHAVIOUR

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Abstract: Bipolar concentric electrodes were implanted in five cats in extreme lateral regions of hypothalamus. These sites were electrically stimulated using biphasic square wave pulses at a current strength ranging from 300-800 μA to evoke predatory attack on an anaesthetized but live rat. At lower current strength (300 μA) only alertness with pupillary dilatation was prodcuced. Gradual increase in the current strength led to the recruitment of somatic and affective components and a predatory attack was exihibited at a mean current strength of 700 μA. A scoring system allowed the construction of stimulus response curves, which remained fairly constant when repeated over a period of 3-4 weeks. Bilateral microinjections of delta-alanine methoinine enkephaline (DAME) (500 ng in 0.5 μl saline) in ventrolateral tegmental area (VTA) elevated the mean threshold current strength for affective components while somatomotor components were totally inhibited. The blocking effect of DAME persisted for 1 hour. Microinjections of naloxone (1 μg) in similar volumes facilitated the response as indicated by a reduction in threshold current strength for somatomotor and affective components. Microinjections of naloxone (1 μg) in similar volumes facilitated the response as indicated by a reduction in threshold current strength for somatomotor and affective components. Microinjections of naloxone (1 μg) also reversed the blocking effect of DAME and the thresholds returned to the control level within 10 min while microinjection of normal saline as control had no effect. The excitatory effects of naloxone and inhibitory effects of DAME were statistically significant at $P < 0.01$ and $P < 0.05$ respectively with Wilcoxon's signed rank test. The present study indicates that enkephalinergic as well as opioidergic mechanisms operating at the midbrain (VTA) level are involved in the inhibition of predatory attack as elicited from lateral hypothalamus.

Key words : lateral hypothalamus, predatory attack, naloxone, chemitrodes, ventrolateral tegmental area, delta-alanine methionine enkephaline, bipolar concentric electrodes

INTRODUCTION

Elicitation of quiet biting attack behaviour from lateral hypothalamus is a well established fact (1, 2). The neural pathways from hypothalamus to midbrain subserving predatory attack behaviour have already been traced (3). Manchanda et al (4) have reported that lesions in dPAG facilitate the hypothalamically-induced

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predatory attack behaviour while similar lesions in VTA blocked the predatory attack behaviour (5). We have earlier reported that dPAG adrenergic mechanisms facilitate hypothalamically-induced predatory attack behaviour (6) while Bandler (7) has reported the involvement of cholinoceptive machanisms in hypothalamus in the modulation of predatory attack behaviour. Stimulation of VTA regions is known to elicit predatory attack behaviour as reported by Shaikh et al (8) and Piaza et al (9). Bandler (10) has also suggested that everage current required for the elicitation of predatory attack from VTA sites is much lower as compared to that required for the elicitation of this response from the lateral hypothalamus. Recently Pott et al (11) have demonstrated the involvement of enkephalinergic machanisms in dPAG in the facilitation as well as suppression of aggression while we have reported the suppresion of predatory attack response by microinjections of DAME in rostral aspect of dPAG as induced by hypothalamic stiumulatioon (6). From the ongoing studies it is clear that there is no report indicating the involvement of midbrain enkephalinergic machanisms at VTA level, in the modulation of predatory attack as elicited by hypothalamic stimulation although the presence of enkephalinergic receptors has been well demonstrated in this region by a number of workers (12, 13). Therefore, the present study was undertaken to investigate the role of midbrain VTA enkephalinergic mechanisms in the modulation of predatory attack as elicited by electrical stimulation of lateral hypothalamus.

METHODS

Selection of the animals: The present study was conducted on five cats of either sex weighing between 2.5 and 4.0 kg. The cats were tamed and adjusted to the behavioural cage for a period of about two weeks in order to stabilize their behaviour. The tamed cats were friendly and were not suspicious of their sorroundings. These cats were fed *ad libitum* and only those cats which did not bite rats were chosen for the present study. Animals which did not display this behaviour 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 ventrolateral tegmental area for chemical manipulation. The details of the construction of chemitrodes and electrodes have already been dealt in details in our initial paper (6).

Implantation of electrodes and chemitrodes: Sterilized bipolar electrodes were implanted in the LHA using pentobarbitone sodium (35-45 mg/kg, body weight) as an anaesthetic agent. The stereotaxic coordinates as worked out from the altas of Jasper and Ajmone-Marssen (14) were found to be A12.5-14.0, L 3.5-3.7, V-3.7mm. Sterilized chemitrodes were implanted in ventolateral tegmental area which had the coordinates A3.0 mm–4.0 mm, L3.0–4.0 mm, V-2.5 mm-3.5 mm. While implanting the electrodes and chemitrodes, hypothalamic and midbrain loci were stimulated electrically to elicit some of the affective components like pupillary dilation, respiratory excitation and acceleration of the heart rate and only then the electrodes were fixed at these loci. Benzathine Penicillin was administered as an antibiotic to prevent infection. The animals were allowed post-operative recovery for 7 days before conducting any experimental study.

Behavioural recording: The hypothalamic sites were stimulated electrically and the responses were recorded in an already prepared protocol. All behavioural recordings were done in the behavioural cage (1m × lm × 1m) with a sliding door 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 varying between 300-800 uA 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, microinjections of DAME and naloxone were carried out in the VTA and electrical stimulations were repeated to check any change in stimulation strengths. Microinjections of normal saline (pH 7.4) in similar volume served as control. Electrical stimulation consisted of biphasic 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 uA.

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

RESULTS

The present study was carried out on 7 animals. Each animal served as its own control producing a goal-directed attack on an anaesthetized rat at a mean current strength of 600 to 700 uA. A typical silent, crawling posture indicating the stalking attack posture was produced on electrical stimulation of LHA. The cat moved slowly towards the rat, showing minimum affective signs and finally a full fledged attack on a rat was performed which culminated in a lethal neck bite often of kill the rat on the first bite. The cat dropped the rat from the mouth as soon as the stimulation was put off. The predatory attack behaviour consisted of the somatomotor and affective components. The somatomotor components included the motor components of attack like extended neck with crawling posture, unsheathing of claws, neck biting and sometimes holding the prey with paw, just before the lethal neck bite. The affective display component comprised of the autonomic responses which included

TABLE I

Sr. No.	*Observation*	*Behavioural scoring % age 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 attack	
1.	Hissing	15%
2.	Growling	15%
3.	Showing of teeth	15%
4.	Piloerection	15%
5.	Alertness with movements	6%
6.	Pupillary dialation	6%
7.	Respiratry acceleration	6%
8.	Salivation	6%
9.	Ear flattening	6%
10.	Urination	5%
11.	Defaecation	5%
		100%

alertness, pupillary dilatation, respiratory acceleration, ear flattening, piloerection etc. Occasionally, growling was found to be accompained with the neck biting attack. These somatomotor and affective components are tabulated in Table I and the respective score assigned according to the work of Saha et al (6). At a lower current strength between 300 to 400 µA, alertness, pupillary dilatation with extended neck, stalking posture, searching for the prey for a typical predatory attack was initiated, whereas with higher current strength between 500 to 600 µA salivation and piloerection was produced. There was no vocal manifestation but growling was occasionally exihibited. A full blown predatory attack was produced at a mean current strength of 700 µA by electrical stimulation of lateral hypothalamus. The maximum affective display score never exceded 60%.

Effects of delta-alanine methionine enkephalin (DAME) and Naloxone: In this study it was observed that microinjections of DAME (500 ng in 0.5 µl saline) into the VTA regions, elevated the current strength for elicitation of somatomotor and affective components of quiet biting attack. Microinjections of naloxone alone (1 µg in 0.5 µl saline, pH 7.4) into the VTA region facilitated the quiet biting attack by lowering the stimulus strengths for the somatomotor and affective components of predatory attack within 10 minutes, shifting the stimulus response curve to the left. Fig. 1 and 2 depicts the shifting of stimulus response curves following microinjections of naloxone and DAME into the VTA sites. At each of these loci, microinjections of naloxone (1 µg) was also performed when DAME effect was at its peak. Within 10 minutes of microinjection the blocking effect of DAME was reversed and the thresholds for both somatomotor and affective components returned to the control levels. Effects of DAME appeared within 20 min of microinjection and persisted for about one hour after which the thresholds returned to control levels. Table II and III indicate the changes in the mean value of current strength for somatomotor and affective components

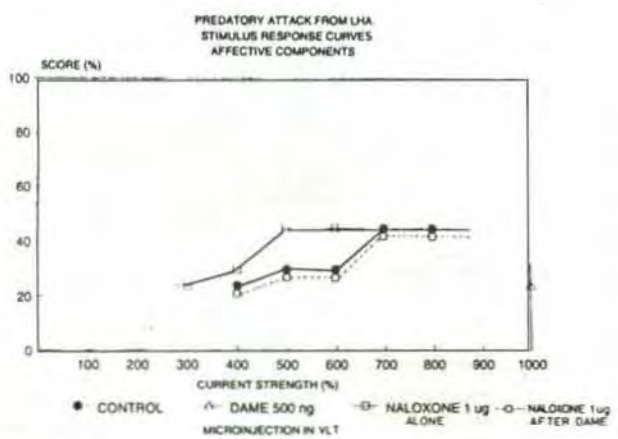

Fig. 1: Stimulus response curves of affective components following microinjections of DAME and Naloxone in ventrolateral tegmental area.

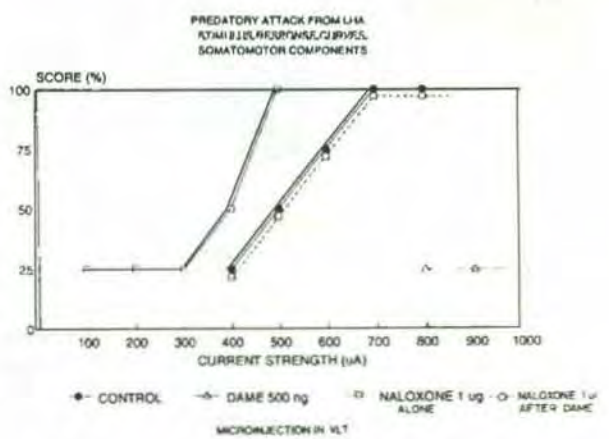

Fig. 2 : Stimulus response curves of somatomotor components following micoinjection of DAME and Naloxone in ventrolateral tegmental area.

respectively following microinjections of DAME and naloxone in VTA sites. The peak period of the blocking effect of DAME was exihibited within 10-15 min of microinjection. Microinjections of normal saline in similar volume as control did not affect the response. Ten hypothalamic and ten midbrain VTA sites were histologically confirmed. These ten loci in midbrain VTA region gave successful modulatory response when manipulated with DAME and naloxone. The anatomical reconstruction of the hypothalamic and midbrain loci is depicted in Fig. 3 and 4. Statistical analysis was carried out by comparing the effects of DAME as well as naloxone with their change in current strengths by applying Wilcoxon's signed rank test. The inhibitory effects of DAME and the facilitatory effects of naloxone were found to be statistically significant at the respective dosages at $P<0.01$ and $P< 0.05$ respectively.

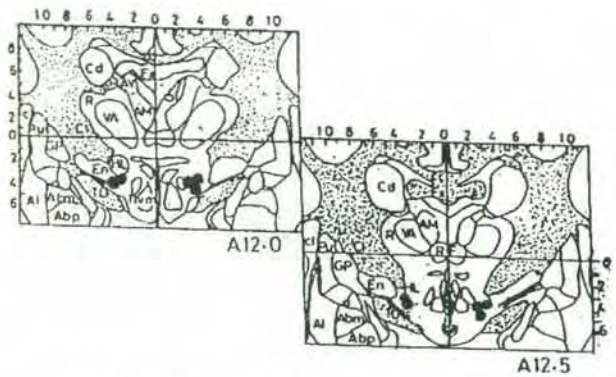

Fig. 3 : Morphological reconstruction of hypothalamic sites from where predatory attack response was obtained on electrical stimulation. Abbreviations: AM: Medical Nucleus of Amygdala, AL: Lateral Nucleus of Amygdala, Abp: Basal Nucleas of Amygdala, Cd: Caudate Nucleus CI Internal Capsule, En: Endopeduncular Nucleus, Fx: Fornix GP: Globus Pallidus, HL: Lateral Hypothalamus, HVm: Ventromedial Hypothalamus, TO: Optic Tract, VA: Ventralis anterior Nucleus of Thalamus.

DISCUSSION

The present study indicates that the hypothalamically induced predatory attack can be inhibited by DAME and facilitated by naloxone by microinjections into VTA sites. Ascending and descending connections between hypothalamus and various midbrain regions concerned with quiet biting attack have already been reported (3,15,16). Manchanda et al (4) and Poddar (12) have reported that hypothalamically induced quiet biting attack could be inhibited by lesions in VTA. The involvement of enkephalinergic mechanism in dPAG in the modulation of predatory attack behaviour has been reported in our laboratory (5) as well as by Weiner et al (17). However there is no report indicating the involvement of enkephalinergic mechanism operating at the VTA level in the modulation of predatory attack behaviour as induced by hypothalamic stimulation even though the presence of enkephalinergic terminals and opioid receptors has been well demonstrated

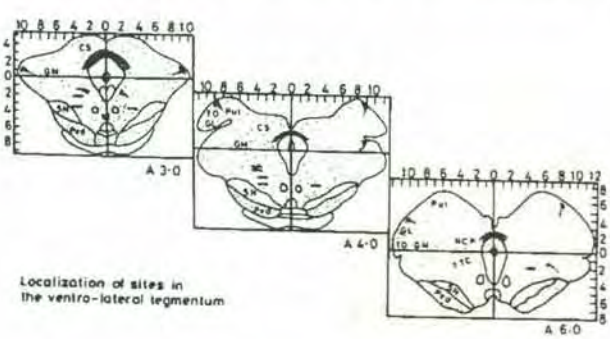

Fig. 4 : Morphological reconstruction of ventrolateral tegmental areas sites which were manipulated chemically by NE, clonidine & yohimbine. Abbreviations: CG: Central Gray. CS: Superior Colliculus. IP: Interpeduncular Nucleus. GM: Medial Geniculate. NR: Red Nucleus. Ped: Peduncle. SN: Substantia Nigra. VTA: Ventral tegmental area.

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

Group		Behavioural scoring			
		Extended neck (25%)	*Unsheathing of claws (25%)*	*Striking with paws (25%)*	*Neck biting (25%)*
Control:	M	345	460	590	685
	SD	±50	±50	±65	±45
DAME					
500 ng :	M	655	inhibited	inhibited	inhibited
in VTA.	SD	±105			
Naloxone					
(1 μg) :	M	100	285	375	400
in VTA.	SD	±0	±35	±45	±55

Numerals written below behaviour scorings represent mean current strengths in uA.
M = Mean and SD = Standard Deviation.
Each cat served as its own control.

in this region by a number of workers (12,13). The present study clearly indicates that hypothalamically induced predatory attack behaviour is inhibited by DAME and the inhibitory effect of DAME is completely reversed by naloxone. Microinjection of naloxone alone facilitates the predatory response as indicated by a significant reduction in the threshold current strength for both somatomotor and affective components as well as predatory attack behaviour as a whole. It can be concluded from the present study that enkephalnergic neurones are involved in the inhibition of predatory attack response. Atweh and Kuhar (12) have speculated that the receptors associated with limbic structures are predominantly delta receptors while those associated with sensory system are predominantly μ-receptor. Aggression is a function of limbic system and the involvement of delta-receptors in inhibiting aggression is therefore clealy understable. Facilitation of response by naloxone, an opioid antagonist clearly demonstrates that enkephalinergic receptors are involved in

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

Group		Behavioural scoring					
		Pupil. dialation (6%)	*Resp. Accel. (6%)*	*Ear flatness (6%)*	*Alert-ness (6%)*	*Saliva-tion (6%)*	*Pilo-erection (15%)*
Control :	M	345	345	345	345	515	630
	SD	±50	±50	±50	±50	±65	±70
DAME							
500 ng:	M	645	645	645	645	inhibited	inhibited
in VTA	SD	±45	±45	±45	±45		
Naloxone							
(1 μg) :	M	100	100	100	100	360	415
in VTA	SD	±0	±0	±0	±0	±50	±35

Numerals written below behaviour scorings represent mean current strengths in uA.
M = Mean and SD = Standard Deviation.
Each cat served as its own control.

inhibition of predatory aggression. Taube et al (18) have shown the endogenous opioid receptor ligands may cause presynaptic inhibition of central noradrenergic transmission. It is thus possible that DAME may be bloking the release of norepinephrine, thus inhibiting the predatory aggression as the involvement of norepinephrine in the facilitation of predatory attack in VLT has recently been reported in our laboratory (19). It may be concluded from the present study then enkephalines inhibit the predatory aggression possibly by blocking the release of norepinephrine. This is as far as we know the first report in which direct involvement of enkephaline in the inhibition of predatory attack has been demonstrated in ventrolateral tegmental area.

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