Indian J Physiol Pharmacol 1993; 37(2) : 121-126

MIDBRAIN ADRENERGIC MECHANISM MODULATING PREDATORY ATTACK BEHAVIOUR INDUCED BY HYPOTHALAMIC STIMULATION

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(Received on August 10, 1992)

Abstract: The present study was carried out in ten cats which did not attack the rats spontaneously. Predatory attack on a rat was produced by lateral hypothalamic stimulation using mean current strength of 340-690uA. This attack was accompanied by minimal affective display and culminated in neck biting. It was found that norepinephrine (NE) when microinjected into dorsal periaqueductal gray (dPAG) region in doses of 2, 4 and 10ug significantly lowered the mean current strength required for the elicitation of predatory attack by hypothalamic stimulation. Microinjection of propranolol (Prop), a beta-blocker, within the same region in similar doses significantly blocked the response as indicated by the increase in current strength required to produce the response. Control injections of normal saline and propylene glycol failed to produce any change. These findings indicate that hypothalamically induced aggressive responses involves beta adrenocepotive mechanisms located in the dPAG.

Key words: predatory attack, dorsal PAG, propranolol, clonidine, neck biting, voltage drop technique, lateral hypothalamic area

INTRODUCTION

It is well established that predatory attack behaviour or quiet biting attack behaviour can be elicited by activating specific loci of lateral hypothalamic area (LHA) in cats (4). The projections related to biting attack from hypothalamus to midbrain have been traced (8, 10). Manchanda et al (16) have reported that lesions in dPAG have facilitated the hypothalamically induced predatory attack. It is also established that cholinoceptive mechanisms located in the periaqueductal gray (10) and hypothalamus (2, 3, 7) are involved in the elicitation of attack behaviour of various types. Barrett et al (5) have indicated the adrenergic involvement in the elicitation of aggressive behaviour. The existence of noradrenergic terminals in dPAG is well known (9, 13). Recently the involvement of propranolol (a beta blocker) in the antiaversive effect from midbrain (1) and in the reduction of anger (22) was reported. However, there is still no report in the literature in which the involvement of midbrain dorsal periaqueductal gray region concerned with adrenergic mechanisms in the elicitation of aggressive behaviour has been cited. Therefore, the present study was undertaken to investigate the role of midbrain adrenergic mechanisms in the elicitation of hypothalamically induced predatory attack. This study indicates that hypothalamically induced predatory attack is facilitated by prior microinjection of NE in the midbrain dPAG region and blocked by propranolol from the same region.

METHODS

(a) Selection of animals: The present study was carried out on ten cats of either sex weighing 2.5 to 4.0 kg. These cats were tamed and adjusted to the behavioural cage for a period of two weeks in order to stabilize their behaviour. The tamed cats were friendly and became pet and were not suspicious of their surroundings. These animals were fed *ad libitum* and only those cats which did not bite rats were chosen for the experiment. Animals which did not display this behaviour were not used for the present study.

(b) Experimental design: The general design of the experiment was to implant bipolar concentric electrodes in the lateral hypothalamus for electrical stimulation and chemitrodes in dPAG for chemical

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manipulation. Bipolar concentric electrodes were made out of 23G stainless hypodermic needle which had 37G prestraightened stainless steel wire as the inner electrode. Both these were insulated with araldite (epoxylite resin) except at the tip (0.25 mm) through which electrical stimulation was performed at the site. Both these electrodes were assembled in such a way that the outer inactive electrode tip (0.5 mm) and active inner electrode (0.5 mm) were separated by a distance of 0.25 mm. These electrodes were implanted at the desired locus. The chemitrodes as constructed in our laboratory were made from 23G stainless steel hypodermic needle, pre-insulated with araldite except at the tip, through which an inner capillary of 32G could easily slide pass and reach only the bare tip of the outer capillary. The inner capillary was in turn connected to the 2 ul Hamilton microsyringe which only delivered drugs in volumes of 0.5 ul. A stylet remained snugly fitted into the outer capillary for most of the time. This arrangement allowed a continued patency of the outer tube and also prevented the infection.

(c) Implantation of electrodes and chemitrodes: Sterilized bipolar electrodes were implanted in the (LHA) using pentobarbitone sodium (35-45 mg/kg body wt) as an anaesthetic agent. The stereotaxic coordinates as worked out from the atlas of cat by Jasper & Ajmone-Marssen (1954) were found to be A12.0-12.5, L3.5-3.75, V-3.75 mm. Sterlized chemitrodes were implanted in dPAG which had the coordinates A3.5-4.0, L0-0.5, V+1.0 to +1.5mm. While implanting the electrodes and chemitrodes, hypothalamic and midbrain loci were stimulated electrically in order to test some of the affective components like pupillary dilatation, respiratory excitation, and acceleration of heart rate and only then the electrodes were fixed at these loci. Benzathine Penicillin was administered intramuscularly as an antibiotic which had its long lasting effect for 7 days, to prevent infection. The animals were allowed a post-operative recovery for 7 days before studies were conducted on these animals.

(d) Behaviour recording: The hypothalamic sites were tested with electrical stimulations and the responses were recorded in an already prepared protocol for recording visual observations (7). All behaviour recordings were done in the behaviour box (1m × 1m × 1m) with a sliding door for exit and entrance of the animal. The cage was constructed in such a manner that one side had a smoked glass, while the other side had a clear glass for one way viewing, video-recording and camera photography. Graded electrical stimulation of current strengths varying between 300-800 uA were 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 graded electrical stimulations. These stimulations were repeated on successive days in order to check the reproducibility of the responses which occurred with graded electrical stimulations. Subsequently, microinjections of noradrenergic agonists (norepinephrine and clonidine) and antagonists (propranolol, practolol, yohimbine, prazosin, and phenoxybenzamine) were carried out in the dPAG area and electrical stimulations were repeated to check any change in stimulus strengths. Microinjections of normal saline and propylene glycol (PH 7.4) in volumes of 0.5ul served as controls. Electrical stimulations were carried out at the tip of the electrodes by means of a Grass stimulator of S4E type through a Grass isolation stimulation unit of SIU-4A model no: 768. Electrical stimulations consisted of biphasic square wave pulses at 60 Hz and 1 ms duration and the current strengths as measured by voltage drop technique was within 300-800 uA. The current strengths were measured by peak to peak deflections from a dual beam Tektronics oscilloscope of type 502.

(e) Histological localisations: Histological locations of LHA and dPAG loci were done by application of 2 mA d.c anodal lesioning current for 10 sec. After lesioning, the brian was fixed by perfusing transcardially, heparinised 10% formol saline dissolved in 2 gm% potassium ferrocyanide solution and sections were stained by haematoxylin and eosin.

RESULTS

The present study was carried out in ten animals. Each animal served as its own control producing goal directed predatory attack on a rat between mean current strengths of 340uA to 690uA. A typical silent, crawling posture called the stalking attack posture was produced, on LHA stimulation where the cat moved silently towards the rat, showing minimum affective signs and finally a full fledged attack on the rat was performed,

which culminated in a lethal neck bite, often to 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 behaviour consisted of the somatomotor and the affective components. The somatomotor components included motor components of attack like, extended neck with crawling posture, unsheathing of claws, neck biting and sometimes striking and holding the prey with paw, just before the final lethal neck bite. The affective display components consisted of the autonomic responses which included alertness, pupillary dilatation, respiratory acceleration, ear flattenning, piloerection etc. Occasionally, growling was found to be accompanied with the neck biting attack. These somatomotor and affective components are tabulated in table 1 and 2 and the respective scores assigned to the work of Bhatia (7) and Poddar (19). During stimulations under low mean current strengths (340-360 uA), alertness, pupillary dilatation with extended neck and stalking posture, searching and sniffing for the prey for a typical predatory attack was initiated, whereas with higher current strengths between 540 to 690 uA, salivation, piloerection, growling and finally a lethal quiet neck bite was produced, showing a full blown, nature of predatory attack behaviour at around mean current strength 690 uA.

In this study it was well observed that NE and Prop in a dose dependent manner, when microinjected in dPAG region modulated quiet biting attack response elicited on electrical stimulation of LHA. The study was conducted on ten cats, where NE in doses of 2, 4, and 10 ug (in 0.5 ul saline, pH 7.4), were microinjected in dPAG. It was observed that NE in each of these doses lowered stimulus strengths to elicit each of the somatomotor and affective components of predatory attack elicited from LHA within 3-5 min, shifting the stimulus response curve towards the left (Tables I and

TABLE I: Stimulus response data for somatomotor components of predatory attack behaviour.

Group		*Behaviour scorings*			
		Extended neck 25%	*Unsheathing of claws 25%*	*Striking with paws 25%*	*Neck biting 25%*
Control :	M	340	600	620	690
	SD	± 51.63	± 97.18	± 78.88	± 87.55
Norepinephrine:					
2 μg	M	280	390	430	550
	SD	± 63.24	± 110.05	± 105.93	± 70.71
4 μg	M	150	350	370	460
	SD	± 70.71	± 97.18	± 94.86	96.60
10 μg	M	115	280	310	370
	SD	± 66.87	± 122.92	± 110.05	± 125.16
Propranolol:					
2 μg	M	520	690	790	inhibited
	SD	± 161.93	± 137.03	± 137.03	
4 μg	M	710	860	———	inhibited———
	SD	± 198.60	± 126.49		
10 μg	M	820	inhibited	———	inhibited———
	SD	± 200.45			

Total no of animals done, N=10.
Numericals written below the behaviour scorings represent the mean current strengths in uA. M = Mean and SD = Standard deviation.

TABLE II : Stimulus reponse data for affective display components of predatory attack behaviour.

Group		*Behaviour scorings*						
		Pupil. dilatation 6%	*Alertness* 6%	*Ear flatness* 6%	*Resp. accel.* 6%	*Salivation* 6%	*Piloerction* 6%	*Growling* 6%
Control:	M	340	340	340	390	540	600	620*
	SD	± 51.63	± 51.63	± 51.63	± 73.78	± 117.37	± 133.33	± 164.31
Norepinephrine:								
2 µg	M	210	210	210	270	390	450	440*
	SD	± 73.78	± 73.78	± 73.78	82.32	± 137.03	± 135.40	± 114.01
4 µg	M	130	160	160	200	330	380	340*
	SD	± 67.49	± 69.92	± 69.92	± 47.14	± 125.16	± 122.92	± 89.44
10 µg	M	120	120	120	120	270	320	320*
	SD	± 63.24	± 63.24	± 63.24	± 63.24	± 125.16	± 113.52	± 44.72
Propranolol:								
2 µg	M	510	520	520	590	710	770	inhibited
	SD	± 166.33	± 161.93	± 161.93	± 137.03	± 128.66	± 149.44	
4 µg	M	590	610	610	680	810	890	inhibted
	SD	± 191.19	± 179.19	± 179.19	± 161.93	± 137.03	+±159.5	
10 µg	M	700	710	710	800	——inhibited——		
	SD	± 200	± 196.12	± 196.12	± 200			

Total no of animals done, N = 10.
Numericals written below behaviour scorings represent mean current strengths in uA. M = Mean and SD = Standard deviation.
*Growling was obtained in five fnimals, hence the mean current strengths in uA are of 5 animals.

II and Figs 1 and 2). At each of these loci Propranolol was microinjected in similar gradedi doses of 2,4, and 10 µg. The electrical stimulation was carried out after one hour. It was observed that somatomotor and affective components required much higher strength to elicit the attack response, thus shifting the curve to the right. The effects of Propranolol remained upto 12 hrs after which they gradually came back to their control levels. Growling, piloerection remained fairly inhibited during this period when LHA was stimulated to check the reproducibility of this type of attack response within 800uA.

Ten hypothalamic and ten dPAG sites were histologically confirmed. These ten loci in dPAG gave successful modulatory response when manipulated with NE and prop on LHA stimulation (Fig.3). Statistical analysis was carried out by comparing the doses of NE and Propranolol with their change in current strengths by applying Wilcoxan's signed rank test.

The facilitatory and inhibitory effects of NE and propranolol were found to be statistically significant at all dosages, at the P<0.01 amd {<0.5 levels, respectively.

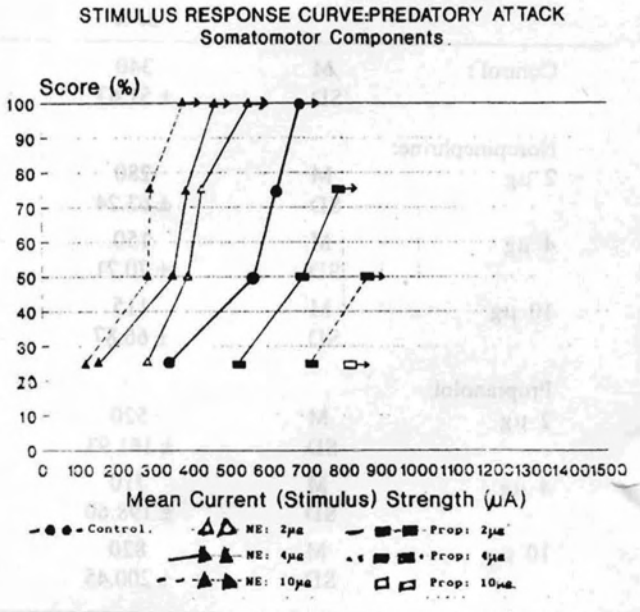

Fig. 1 : The diagram shows the stimulus response shifting towards the left with the microinjection of norepinephrine (NE) and towards the right with miroinjection of Propranolol (Prop) in graded doses.

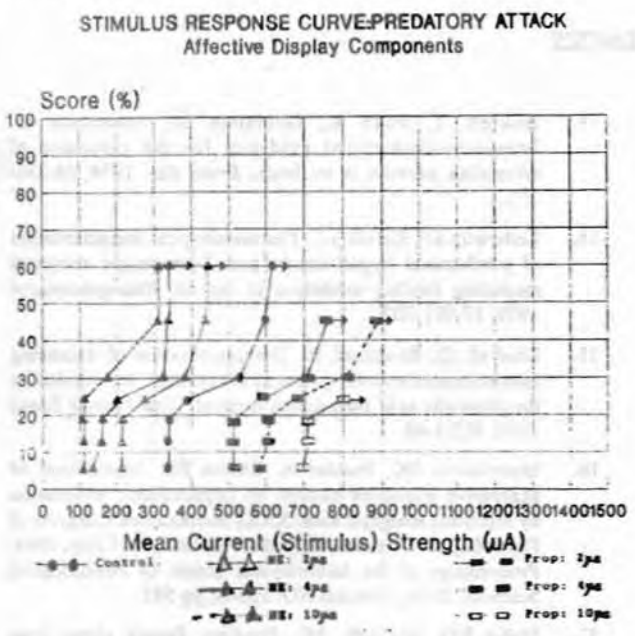

Fig. 2: The diagram shows that the stimulus response shifting to the left with Norepinephrine (NE) microinjection and to the right with Propranolol (Prop) microinjection in graded doses.

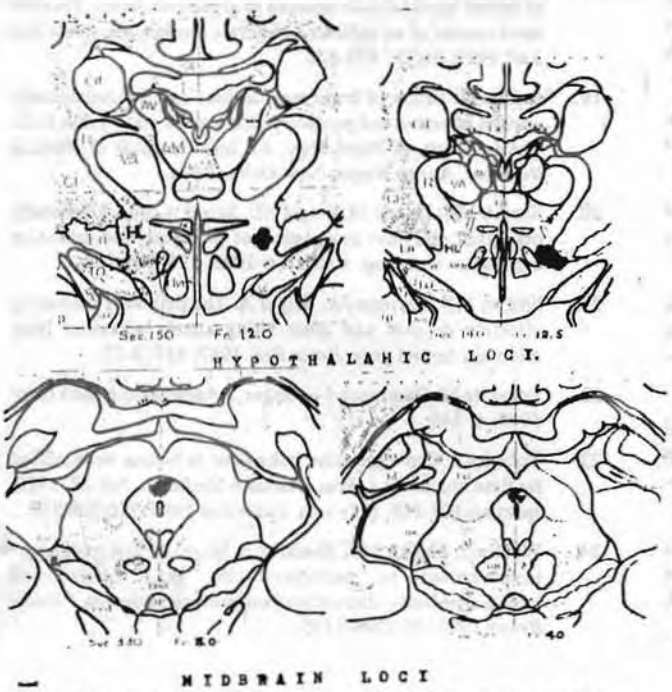

Fig. 3: Morphological reconstructions of hypothalamic sites (●) showing the loci from where predatory attack was produced by electrical stimulation and midbrain sites (▼) showing the loci from where modulation of predatory attack was obtained, when chemically manipulated by norepinephrine and propranolol.

DISCUSSION

The results of the present study indicate tht hypothalamically induced quiet biting attack can be facilitated by NE microinjection in dPAG area blocked by Propranolol from the same midbrain region. Ascending and decending connections between hypothalamus and midbrain concerned with quiet biting attack have already been reported (5, 7, 11). Manchanda et al (16) have recently reported that hypothalamically induced quiet biting attack could be facilitated by lesions in the PAG. Although the involvement of cholinergic (2,3) and enkephalinergic (24) mechanisms in the modulation of the predatory attack has been reported, there is no data indicating the involvement of adrenergic mechanisms operating at the dPAG level in the elicitation of the predatory attack. Our results show that hypothalamically induced quiet biting attack could be blocked by Propranolol. It was facilitated by NE, thus indicating the specificity of the involvement of beta adrenoceptors operating at the midbrain level. The involvement of propranolol in reduction of anger (12, 21, 22) and in the mediation of antiaversive effect (1) has been recently reported. The involvement of beta adrenoceptors in the feeding mechanisms at the hypothalamic level has also been recently reported (14, 18). Since, feeding (17) and predatory attack (8, 20, 21) have a common element of biting involving the movements of jaws, it is not surprising that both these behaviours might involve the same beta adrenoceptor mechanism. It can be concluded from the present study that hypothalamically elicited quiet biting attack involves the beta-adrenoceptive pathways operating at the dPAG level. Possibly this is the first report in which the involvement of beta-adrenoceptive mechanisms operating at the dPAG level in the modulation of hypothalamically elicited quiet biting attack has been demonstrated.

ACKNOWLEDGEMENTS

We thank the Indian Council of Medical Research for their financial assistance.

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