Indian J Physiol Pharmacol 2004; 48 (1) : 51–58

ADRENERGIC INVOLVEMENT IN THE LOCUS CERULEUS AND ADJOINING REGIONS IN THE FACILITATION OF PREDATORY ATTACK BEHAVIOR AS INDUCED BY HYPOTHALAMIC STIMULATION IN CATS

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(Received on September, 5, 2000)

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 extreme lateral regions of hypothalamus. These sites were stimulated at a current strength from 300–700 µa to evoke a predatory attack on an anaesthetized rat. The attack was accompanied by minimal affective display such as alertness, pupillary dilatation, and culminated in beck biting at higher current strength. A scoring system allowed the construction of stimulus response curves, which remained fairly constant when repeated over a period of 3–4 weeks. Microinfusions of norepineprine and clonidine in 4.0 and 5.0 µg dose respectively in locus ceruleus and adjoining tegmental fields facilitated the predatory attack and there was a significant reduction in the threshold current strength for the elicitation of affective and somatomotor components. Microinfusions of yohimbine, an α-2 blocker, in 5 µg dose completely blocked the predatory attach response as indicated by an increase in the threshold current strength for the affective components. The somatomotor components were completely inhibited and could not be elicited even when the current strength was increased to 1000 µA. The predatory attack behavior remained completely inhibited for almost two hours following microinfusion of yohimbine. During this period, the animal was extremely drowsy and reacted very slowly even to a painful stimulus such as pinching of tail. Microinfusions of propranalol (β-blocker), practalol (β-1 blocker), prazosin (α-1 antagonist), propylene glycol as well as saline in similar volumes (0.5 µl) as control failed to produce any blocking effect, thus indicating the involvement of α-2 adrenoceptive mechanisms in the modulation of predatory attack in this region of midbrain. The facilitatory effects of norepinephrine and clonidine were significant at $P<0.01$ and $P<0.05$ respectively with Wilcoxon's signed rank test. The inhibitory effects of yohimbine were significant at $P<0.05$. The present study indicates the involvement of α-2 adrenoceptive mechanisms in the facilitation of hypothalamically elicited predatory attack.

Key words : predatory attack locus ceruleus norepinephrine clonidine yohimbine lateral hypothalamus neck biting

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INTRODUCTION

It is well established that predatory attack behavior can be elicited by electrical stimulation of extreme lateral regions of hypothalamus in cats (1, 2). The neural pathways subserving predatory attack behavior from hypothalamus to locus ceruleus have already been traced (3). It has been reported that locus ceruleus neurones increase their discharge rate during defense reaction and these neurones are adrenergic in nature (4). It has also been reported that these regions possess nerve terminals and neuronal membranes which are immunoreactive to opioids (5). The involvement of cholinergic mechanisms in the facilitation of predatory attack in midbrain and hypothalamus has been reported by a number of workers (6, 7). It has also been reported that α-2 adrenergic mechanisms are involved in the modulation of hypothalamically elicited aggressive behavior (8). In our initial study we have also reported the involvement of β-adrenoceptive mechanisms in dPAG, substantia nigra, and α-2 adreceptive mechanisms in the ventral tegmental area in the modulation of hypothalamically elicited predatory attack (9–11). Facilitation of muricidal behavior in rats by lesions in locus ceruleus has also been documented (12) while another study indicates that this excitatory effect is mediated by α-2 adrenergic mechanisms (13). The pathways related to biting attack from hypothalamus to various midbrain sites including locus ceruleus have been already traced (14, 15). Locus ceruleus is the site from where ascending and descending adrenergic pathways originate and traverse to lateral hypothalamus (16). However, there is no report indicating the involvement of adrenergic mechanisms in this region in the modulation of predatory attack even though the presence of adrenergic terminals and adrenergic receptors has been demonstrated in this region (17). Therefore, the present study was undertaken to investigate the role of adrenergic mechanisms in this midbrain region in the modulation of hypothalamically elicited predatory attack behavior. This study indicates that predatory attack as induced by hypothalamic stimulation is facilitated by prior microinfusions of norepinephrine, and its agonist clonidine in locus ceruleus while it is completely blocked by microinfusion of yohimbine, and α-2 antagonist 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 extremely friendly and were not suspicious of their surroundings. These animals were fed ad libitum and only those cats, which did not attack or 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 lateral hypothalamus for electrical stimulation and chemitrodes in locus ceruleus for chemical manipulation. The details of the construction of chemitrodes and electrodes have already been dealt in details (9–11).

Implantation of electrodes and chemitrodes: Sterilized bipolar electrodes were the LHA using pentobarbitone sodium (35–45 mg/kg body weight) as an anaesthetic agent. The stereotaxic coordinates as worked out from the atlas of Jasper and Ajmone-Marssen (18) were found to be A 12.0–14.0 mm, L 3.0–4.0 mm, V–4.0–5 mm. Sterilized chemitrodes were implanted in locus ceruleus and adjoining tegmental regions, which had the coordinates A3.0–5.0 mm, L 3.0–4.0 mm. V–.5.0–6.0 mm (19). While implanting the electrodes and chemitrodes, hypothalamic and midbrain loci were stimulated electrically to elicit some of the affective components like papillary dilatation, respiratory excitation and increase in the heart rate and only then the electrodes were fixed at these loci. Benzathine pencilline was administered to prevent any infection. The animals were allowed a postoperative recovery period of seven days before conducting and 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 of cage 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 norepinephrine, clonidine and yohimbine as well as other agonists and antagonists were carried out in the locus ceruleus and electrical stimulations were repeated to check any change in threshold current strength. Microinfusions of normal saline and propylene glycol in 0.5 µl volume served as control. Electrical stimulation 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 100–800 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% formol saline dissolved in 2% potassium ferrocyanide solution and sections were stained with haematoxylin and eosin.

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

RESULTS

The present study was carried out in five cats. Each animal served as its control, producing a goal-directed attack on an anaesthetized rat at a mean current strength of 600 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 a rat was performed. The attack

culminated in lethal 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 consisted of somatomotor as well as affective components. The somatomotor components included motor components of predatory attack like extended neck with crawling posture, unsheathing of claws, neck biting and sometimes striking and holding the prey with paws just before the final neck biting attack. The affective components comprised of the autonomic responses as alertness, pupillary dilatation, respiratory acceleration, and minimal piloerection. The somatomotor and affective components are tabulated in Table I and the respective scores assigned as in our initial work (9–11). It was observed that on stimulation with lower current strengths (300–400 μA) alertness, pupillary dilatation with extended neck and stalking posture, searching for the prey was exhibited while with higher current strength (500–600 μA), more pertinent affective components of predatory attack as salivation and piloerection could be observed. Increasing the current strength (700–800 μA) at these loci produced a full blown predatory attack on an anaesthetized rat. There was no vocal display except occasional growling during neck biting on the rat. Microinfusion of norepinephrine in 4 μg and clonidine in 5 μg dose into the locus ceruleus and adjoining tegmental regions, facilitated the predatory attack response as indicated by a significant reduction in the threshold current strength for the elicitation of somatomotor and affective components. The facilitatory effects of norepinephrine appeared within 3–5 minutes of microinfusion while the effects of clonidine appeared after a gap of 20 minutes of microinfusion and persisted for almost 6 hours. Microinfusions of yohimbine, an α-2 blocker in 5.0 μg dose at the same site, completely blocked the predatory attack response as indicated by an increase in the threshold current for affective components. The animal was in a stuporous state for almost 2 hours. There was a tremendous reduction in the muscle tone and the animal was extremely slow in reacting even to a painful stimulus such as pinching of the tail. The somatomotor components were completely blocked and could not be elicited even when the current strength was raised to as high as 1000 μA in some animals. However, such a high current strength was never utilized in the present study as this could lead to the development of seizure. Microinfusions of propranalol (β-blocker), practalol (β-1 blocker), prazosin (α-1 blocker), propylene glycol as well as saline

TABLE I:

Sr. No.	*Observation*	*Behavioral scoring percentage 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 dialatation	6%
7.	Respiratory acceleration	6%
8.	Salivation	6%
9.	Ear flattening	6%
10.	Urination	5%
11.	Defaecation	5%
		100%

TABLE II: Data depicting 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 bitting*
Control	300±40	620±75	640±102	660±80
NE 4 µg in LC	120±40	440±49	460±80	460±80
Clonidine 5 µg in LC	100±0	440±49	440±49	460±80
Yohimbine 5 µg in LC	700±49	inhibited	inhibited	inhibited

Each cat served as its own control.
Numerals written below behaviour scorings represent mean current strength in µA.
Values are shown as Mean ± SD. LC: Locus cereleus.

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

Group	*Behavioral components*					
	Pupil. dialation	*Resp. accel.*	*Ear flatness*	*Alertness*	*salivation*	*Pilo-erection*
Control	340±49	340±49	340±49	340±49	560±102	660±49
NE 4 µg in LC	120±40	120±40	120±40	120±49	460±49	460±49
Clonidine 5 µg in LC	100±0	100±0	100±0	100±0	460±49	460±49
Yohimbine 5 µg in LC	700±90	700±90	700±90	700±90	inhibited	inhibited

Each cat served as its control.
Numerals written below behaviour scorings represent current strength in µA.
Values are shown as Mean ± SD. LC: Locus cereleus.

as control in similar volumes failed to produce any blocking or facilitatory effect and there was no change in the threshold current strength for elicitation of both affective and somatomotor components. Table II shows the exact changes in the current strength following changes following microinfusion of yohimibine in locus ceruleus. Figs. 1 & 2 give the stimulus response curves as obtained by plotting the respective scores of affective display and ten loci in the locus ceruleus region were confirmed. These midbrain sites gave successful modulatory response when microinfusions of various agonists and antagonists were performed at these sites. The facilitatory effects of norepinephrine and clonidine were significant at $P<0.01$ and

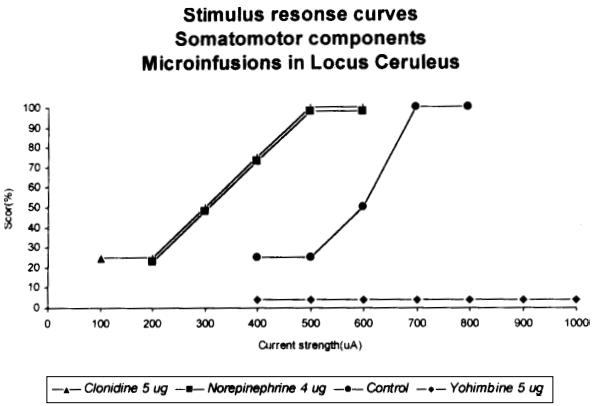

Fig. 1: Shows the effects of microinjections of norepinephrine and clonidine in midbrain loci on the somatomotor components of stimulus response curve. The somatomotor components could be elicited at much lower current strength following microinfusion and the curves shifted to the left while microinfusion of propranalol shifted the curves to the right and only one component of predatory attack in the form of extended neck could be observed at a much higher current strength, thus indicating the complete abolition of predatory attack.

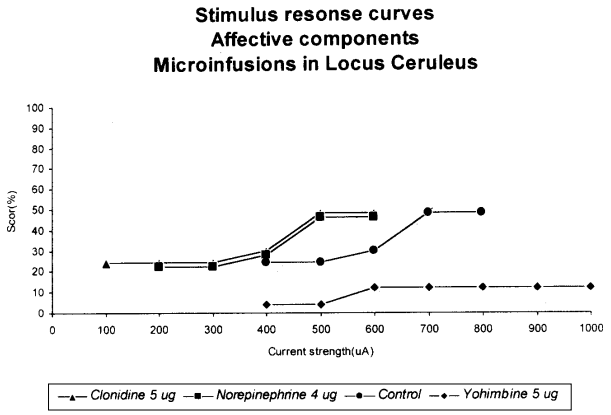

Fig. 2: Shows the effects of microinfusion of norepinephrine and clonodine into midbrain loci on the affective components of the stimulus response curves. The shifting of the curves to the left indicates that much lower current strength was required to elicit the same components Microinfusions of yohimbine blocked the affective components and the curves shifted to the right as indicated by an increase in the threshold current strength.

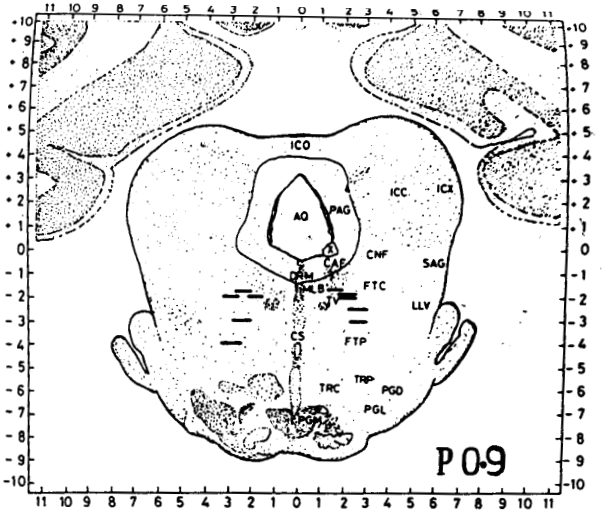

Fig. 3: Shows the morphological reconstruction of the midbrain sites in coronal sections at which microinfusions of various drugs and saline was performed.

P<0.05 respectively while the inhibitory effects of yohimbine were significant at P<0.05. The exact localization of these midbrain sites is depicted in Fig. 3.

DISCUSSION

The results of the present study indicate that hypothalamically elicited predatory attack behavior can be facilitated by microinfusions of norepinephrine and its antagonist clonidine in locus ceruleus and adjoining tegmental regions of the midbrain and this facilitatory effect could be blocked by prior microinfusion of yohimbine at these sites Ascending and descending connections between hypothalamus and locus ceruleus have been reported (17). Although the involvement of cholinergic mechanisms at this level has already been reported (6), there is hardly any report indicating the involvement of adrenergic mechanisms at this level in the modulation of predatory attack response. Recent studies indicate the involvement of norepinephrine in the facilitation of predatory attack behavior at various midbrain sites such as ventral tegmental area, substantia nigra and d PAG (10, 11, 20). It has been reported that predatory attack behavior as elicited by hypothalamic stimulation is blocked by infusion of yohimbine in ventral tegmental area, thus indicating the involvement of α-2 adrenergic mechanisms in this midbrain region (11). Affective defense response from anterior hypothalamus is also mediated through α-2 adrenoceptive mechanisms (8). However, there is no report indicating the involvement of adrenoceptive mechanisms in the modulation of predatory attack behavior in the locus ceruleus, even though the neural connections between lateral hypothalamus and this region have already been demonstrated (3). The presence of adrenergic receptors in this region has also been reported (17). It is also the region from where ascending and descending adrenergic

pathways originate and traverse to the higher central regions as well as to the spinal cord (21). The excitatory response of locus ceruleus is mediated by α-2 adrenoceptive mechanisms (20) Development of stupor like state following microinfusions of yohimbine indicates that this response is mediated by α-2 adrenergic mechanisms. The drowsiness observed in our animals was due to the blockage of adrenergic fibers, which could lead to a state of sleep. Another study indicates that the hyponotic action observed on administration of dexmetomidine in the locus ceruleus of rats was blocked by equally specific antagonist, antipemazole (22). These observations seem contrary to the findings that locus ceruleus neurones increase their firing rate during arousal (13). Since α-2 receptors are present on both presynaptic and postsynaptic membranes (23), it is likely that yohimbine may be acting on the presynaptic receptors to cause inhibition, which in turn could lead to decrease in the release of norepinephrine at the postsynaptic level. In the present study it seems that clonidine acts on the receptors on the post-synaptic membrane, thus facilitating the predatory aggression. Since yohimbine, an, α-2 antagonist was the only blocker to block the predatory attack response and induce a stupor like state, it can be concluded from the present studies that only α-2 adrenoceptive mechanisms facilitate the quiet biting attack response as induced by hypothalamic stimulation. This is possibly the first study indicating the involvement α-2 adrenoceptive mechanisms in the facilitation of predatory attack behavior in the locus ceruleus region.

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