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MIDBRAIN ADRENERGIC MECHANISMS MODULATING FLIGHT BEHAVIOUR INDUCED BY HYPOTHALAMIC STIMULATION

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Abstract: The present study was carried out in ten cats of either sex. Flight response was obtained by electrical stimulation of dorsomedial regions of preoptic area (A13-14.5, L3.5 V-3.5 to–3.7) and lateral hypothalamic regions (A12.5, L2.5-3.5, V–3.7). It consisted of a goal directed attempt to get out of the cage with a vigorous leaping to foot. Norepinephrine when microinjected in 10 μg doses into pretectal area of midbrain (A3.5, 3.0, V+1.0 to + 1.5mm) significantly lowered the mean current strength from 640uA to 420uA; clonidine, an alpha-2 agonist in 5 μg dose when microinjected into the same locus also significantly lowered the mean current strength to the same level. On the other hand yohimbine, an alpha-2 blocker in 5 μg dose when microinjected in to the same locus significantly increased the mean current strength from 640 to 970 uA. These results indicate that hypothalamically induced flight response is mediated via the alpha-2 adrenoceptive mechamism operating at the midbrain level. Control microinjection of normal saline and propylene glycol in similar volumes failed to produce any changes in current strength.

Key words: flight behaviour lateral hypothalamic area preoptic area pretectal area clonidine yohimbine norepinephrine

INTRODUCTION

Flight response, an important component of defence behaviour can easily be obtained by electrical stimulation of dorsomedial region of the anterior hypothalamus (5). In this response the animal makes a goal-directed attempt to get out of the cage after locating the escape route. It is accompanied by characteristic affective display components like piloerection, growling, and respiratory excitation etc. The involvement of cholinoceptive mechanism in the elicitation of flight response from anterior hypothalamus and preoptic area has already been shown in our laboratory (4), however, there is no report indicating the involvement of adrenergic mechanisms operating at the hypothalamic or midbrain level in the modulation of flight response elicited by ypothalamic stimulation. Recently Barrett et al (2) ve shown that intrahypothalamic microinjections of repinephrine into the anterior hypothalamus ificantly lowered the thresholds for elicitation of attack response while microinjection of clonidine at the some locus reversed this response, thus indicating the involvement of adrenergic mechanism in the regulation of affective defense behaviour. Bell and Hepper (3) have also recently reported that norepinephrine microinjections in cats facilitated the predatory attack behaviour thus highlighting the importance of noradrenergic mechanism in the elicitation of different types of aggressive behaviour in hypothalamus. Recently the presence of alpha-2-adrenoceptors in the midbrain pretectal area was reported (8). Therefore the present study was undertaken to investigate whether the noradrenergic mechanism operating at the midbrain level are in any way involved in the modulation of hypothalamically induced flight response. Our results indicate that flight response induced by hypothalamic stimulation can be blocked by prior microinjection of Yohimbine, an alpha-2 adrenoceptor blocker in the pretectal area, and facilitated by norepinephrine microinjected at the same midbrain locus.

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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 were not suspicious of their surroundings. They never showed any signs of fear or made any attempt to get out of the cage. Animals which did not display this behaviour at the end of two weeks were not used.

(b) Experimental design : The general design of the experiment was to implant bipolar concentric electrodes in the lateral hypothalamus and preoptic area for electrical stimulation and chemitrodes in the pretectal area for chemical manipulation. Bipolar concentric electrodes were made out of 23 G stainless hypodermic needle which had 37 G 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, preinsulated with aradite except at the tip, through which an inner capillary of 32 G could easily slide pass and reach the bare tip of the outer capillary. The inner capillary was in turn connected to the 2 microlitre Hamilton syringe through a polyethylene tube for microinjection of the drug, otherwise a stylet snugly fitted into the outer capillary remained fixed for most of the time. This arrangement allowed a continued patency of the outer tube and also prevented infection from occuring.

(c) Implantation of electrodes and chemitrodes : Bipolar electrodes were implanted in the lateral hypothalamus (LHA) and preoptic area (POA) 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 A12.5, L3.5, V-3.75 and A13.5–14.5, , L3.5, V-3.5 to –3.70. The chemitrodes were implanted in pretectal area which had the coordinates A3.5–4.0, L3.0, V + 1.0 to +1.5 mm. While implanting the electrodes and chemitrodes the hypothalamic and midbrain loci were stimulated electrically in order to test some of the affective components like pupillary dialation, respiratory excitation, and acceleration of heart rate and only then were the electrodes fixed at these loci. Benzathine Penicillin, for its long lasting effect was administered intramuscularly as an antibiotic 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 (4). Graded electrical stimulation of current strengths varying between 300-800 microamperes were repeated on successive days with ten ascending and descending trials with a gap of half an hour for each trials and also a gap of five minutes for each graded electrical stimulation. 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 pretectal area and electrical stimulation was repeated. Microinjections of normal saline and propylene glycol (pH 7.4) in the 0.5ul volumes served as a control. 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.

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 smoked glass fo one way viewing and the other side had clear glass fo viewing, video-recording and camera photography. T cage had a hole at the top which allowed the animal escape with a goal directed leaping to foot.

Histological confirmations of the hypothalamic and midbrain sites were done by lesion studies. A d.c. anodal lesion using 2 mA current strength for 10 sec. both in the stimulation and microinjection site was made to locate the loci. After the lesions were done the brain was transcardially perfused with a solution of heparinised 10% formol saline, dissolved in 2 gm% potassium ferrocyanide. Haematoxylin and eosin staining was carried on with the brain sections to decipher the exact sites.

RESULTS

The present study was carried out in ten animals. Each animal served as its own control producing flight reaction between mean current strengths of 310 μA to 640 μA. The flight response consisted of two components namely somatomotor and affective components. The somatomotor components included motor components of flight, like searching glances, going to corner, staring at the escape route, wild running in the cage and leaping to foot. The affective display components consisted of the autonomic responses accompanying the rage response. These included alertness, pupillary dilatation, piloerection, growling, hissing with bare teeth, etc. These components are given under Fig. 1 and 2, and scores assigned according to the work of Bhatia (4). During stimulations under low mean current strengths (300±10 μA) alertness, pupillary dilatation, with searching glances, and going to corner for escape were initiated, whereas with higher current strengths (530 μA–640 μA), wild running, leaping to foot, hissing, growling, piloerection and showing of teeth were the prominent components observed.

Each observation was recorded in an already prepared protocol. It was observed that norepinephrine (10 μg in 0.5 μl of saline, pH 7.4) and clonidine (5 μg in 0.5 μl of propylene glycol, pH 7.4), microinjected into the pretectal area lowered the mean current strengths required for elicitation of each component of hypothalamically induced flight response. Microinjections of yohimbine (5 μg in 0.5 μl of propylene glycol, pH 7.4) at the same locus was able to block the flight response. The effects of norepinephrine persisted for 3-5 min following microinjection and gradually waned off within 10 min. The effects of clonidine started within half an hour, and reached its peak response between 3-6 hrs, then gradually declined and the effect was over after 12 hrs. The effects of yohimbine appeared after 2 hrs of microinjections and reached its peak response between 3-8 hrs. During this period flight response with its both somatomotor and affective display components remained fairly inhibited with its mean current strengths increased upto almost 600 μA to initiate initial components of flight response and almost 1000 μA to elicit a full flight response (Fig. 1 & 2). Yohimbine, appreciably inhibited growling, hissing, and showing of teeth, which returned to control levels with a gradual decay of time i.e. within 48 hours.

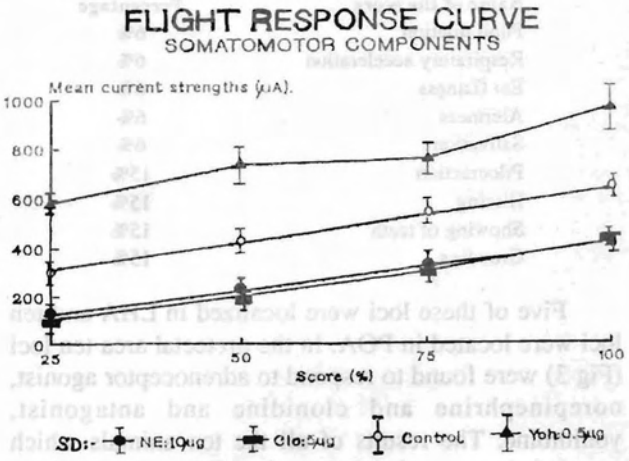

Fig. 1. Stimulus response curve of flight response showing the effects of control and the effects of NE, clo and yoh in the modulation of current strengths required to elicit somatomotor components of flight response.

Name of the score	Percentage
Searching glances	25%
Going to corner	25%
Wild running	25%
Leaping to foot	25%

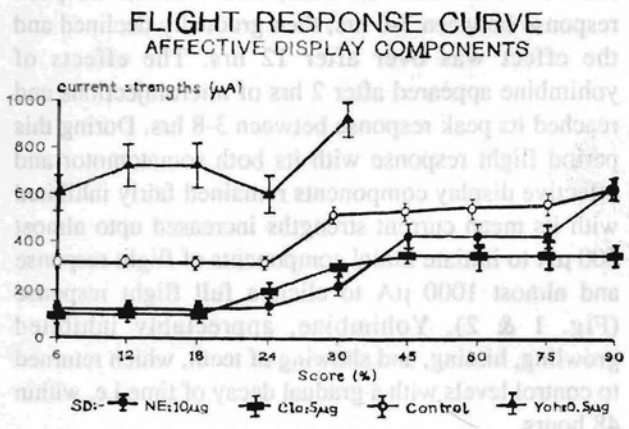

Fig. 2 : Stimulus response curve of flight response showing the effects of control and the effects of NE, clo and yoh in the modulation of current strengths required to elicit affective display components of flight response.

Name of the score	Percentage
Pupil dilation	6%
Respiratory acceleration	6%
Ear flatness	6%
Alertness	6%
Salivation	6%
Piloerection	15%
Hissing	15%
Showing of teeth	15%
Growling	15%

Five of these loci were localized in LHA and ten loci were located in POA. In the pretectal area ten loci (Fig 3) were found to respond to adrenoceptor agonist, norepinephrine and clonidine and antagonist, yohimbine. The results of all the ten animals which responded to norepinephrine, clonidine and yohimbine were analysed by Wilcoxan's rank correlation test and were found to be statistically significant at $P<0.05$ and $P<0.01$ respectively.

DISCUSSION

Flight is one of the essential components of defence response which aims at the survival of self. It is a goal-directed behaviour which can be produced by electrical stimulation of lateral hypothalamus and dorsomedial region of preoptic area (7). Pathways for this response have been traced fom hypothalamus to midbrain and subsequently to motor nuclei of the spinal cord (5, 7, 9, 13). Initial studies by Bhatia (4) had indicated the involvement of cholinoceptive mechanisms in the elicitation of hypothalamically induced flight response. In the present study, our aim was to study the role of adrenergic mechanisms in the modulation of flight response produced by electrical stimulation of the hypothalamus. Our studies indicate that norepinephrine (DL) when microinjected into the pretectal area, considerably reduced the mean current strength required for producing both the somatomotor and affective components while yohimbine, an alpha-2 adrenoceptor blocker significantly increased the mean current strength for the somatomotor components as well as affective components. Again, clonidine, an alpha-2 adrenoceptor agonist, when microinjected into the pretectal area, also significantly lowered the mean current strength, thus indicating the involvement of alpha-2 adrenoceptor mechanisms in the elicitation of flight response. Recently the presence of alpha-2 receptors in the pretectal area has been demonstrated (8). These alpha-2 receptors are involved in the elicitation of analgesic effect at the spinal cord level (10). It can be concluded that alpha-2 receptor mechanism by causing presynaptic inhibition (11, 12) at the midbrain level may be responsible for the termination of the flight response. Since no other blocker, namely propranolol (Beta-blocker), practolol (Beta-1 blocker), prazosin (alpha-1 blocker), phenoxybenzamine (alpha-blocker), was able to block this response, there is a strong possibility that only alpha-2 adrenoceptor mechamisms are operating at the midbrain level in the pretectal area in the elicitation of flight response. Although alpha-2 receptor involvement at the hypothalamic level in the elicitation of affective defence from anterior hypothalamus has been reported (2), there is no study indicating the involvement of these receptors at the midbrain level. This is possibly the first report in which we have been able to show the involvement of midbrain alpha-2 adrenergic mechanisms in the elicitation of flight response. The parallel reduction in threshold for flight response produced by clonidine and norepinephrine is best understood as resulting from the actions of these agents on post-junctional alpha-2 adrenoceptors (6).

ACKNOWLEDGEMENTS

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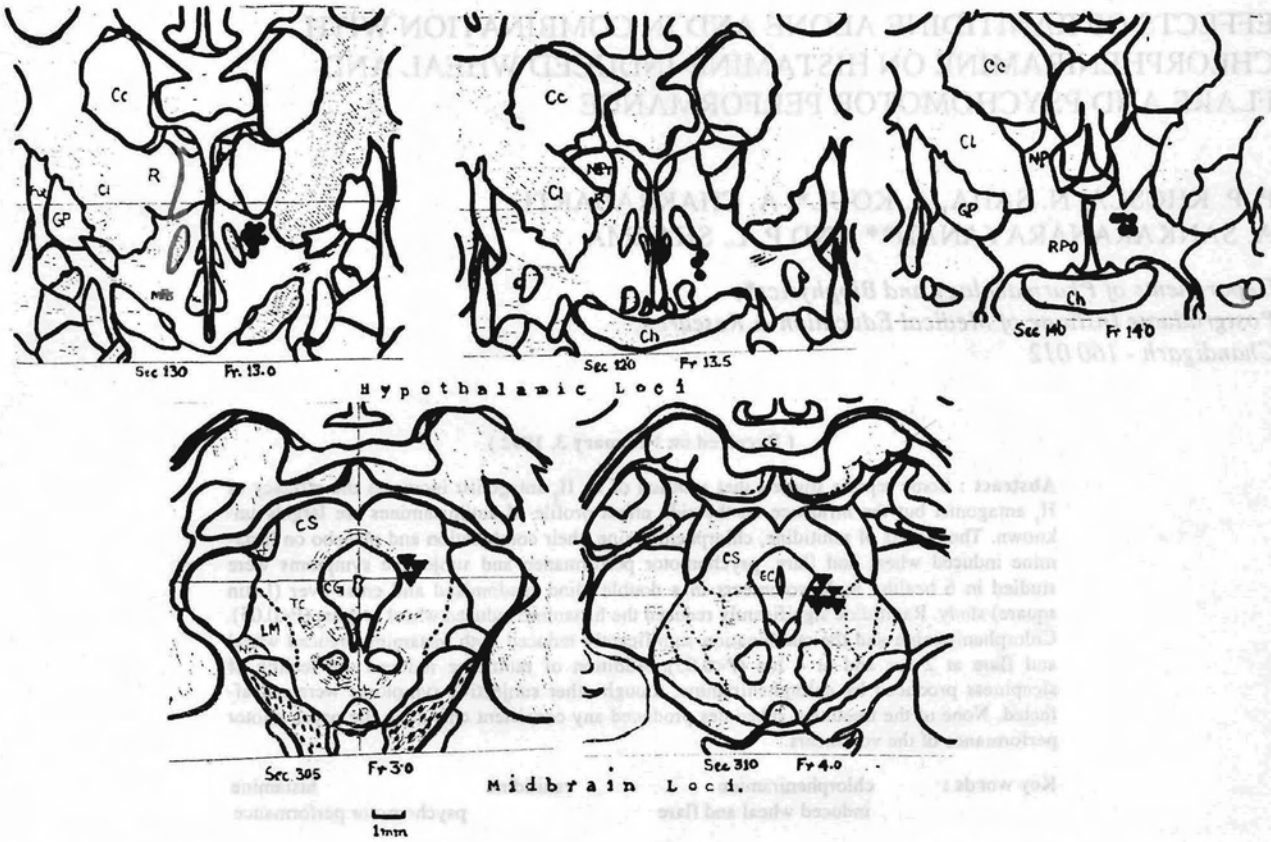

Fig. 3 : Morphological reconstructions of hypothalamic sites (●) showing the loci from where flight response was produced by electrical stimulation and midbrain sites (▼) showing the loci from where modulation of flight response was obtained, when chemically manipulated by NE, clo and yoh.

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