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HYPOTHALAMO-LIMBIC INVOLVEMENT IN MODULATION OF TOOTH-PUMP STIMULATION EVOKED NOCICEPTIVE RESPONSE IN RATS

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Abstract : The hypothalamo-limbic system has been implicated in recognizing the affective significance of pain and elicitation of related emotional responses. Several evidences from different studies support a role of these areas in endogenous analgesic mechanisms for pain modulation as elucidated by different pain tests in more than one animal model. In the above context, the aim of this study was to investigate the relative effectiveness of the pain modulatory action of hypothalamic and limbic structures in rat using similar stimulation parameters, and studying the effect on tooth pulp stimulation evoked jaw opening reflex (TP-JOR). To achieve the objective, unilateral stimulation of hypothalamic (lateral=LH; ventromedial=VMN; anterior=AH) and limbic areas (amygdala=AMYG; hippocampus=HIPP) was done on the TP-JOR test. A significant reduction in the amplitude of EMG recorded from the digastric muscle (dEMG) as a result of tooth-pulp stimulation was observed on stimulation of LH, VMN, AMYG and HIPP but not from AH. Also, the magnitude of this effect was almost similar from these areas. The results suggest that these areas (except AH) have an antinociceptive role in tooth-pulp stimulation evoked pain response.

Key words : antinociception hypothalamus amygdala hippocampus tooth pulp jaw opening reflex

INTRODUCTION

Several reports from animal and human experimentation provide behavioural, electrophysiolgical and anatomical evidences, that direct electrical stimulation to some parts of the diencephalon and midbrain can attenuate

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or abolish noxious properties of a painful stimulation.

The role of hypothalamus in regulation of nociceptive sensitivity has been studied using various experimental approaches, mostly in rats and cats as animal models. Nociceptive afferents entering this structure, apparently activating the population of antinociceptive neurons by a negative feedback mechanism, forms the hypothalamic control of nociceptive sensitivity (1). Stimulation of lateral and medial hypothalamic areas has been shown to prdouce a marked analgesic effect (1–3). The investigation of neuronal mechanism of hypothalamic antinociception has revealed that stimulation of different structures of this region suppresses the nociceptive reaction of the spinal cord dorsal horn neurons (2, 4). Immunohistochemical data demonstrating a high content of endogenous morphine-like oligopeptide, endorphins and enkephalins in the hypothalamic neurons point towards its important role in regulation of nociception (5).

The amygdaloid complex, one of the components of the limbic system also contains high levels of opioid and enkephalin receptors that modulate nociception. The central and corticomedial nuclei of the amygdala have been implicated in the modulation of pain whereas the lateral nucleus is not effective in pain modulation (6). An analgesic effect of stimulation of amygdala has been demonstrated in cat and rat models using various pain tests (6, 7).

From the functional point of view, the limbic structures and hypothalamus have important interactions in the control of emotional and motivational behaviour such as feeding, drinking and aggression as well as mnemonic and cognitive functions. The hypothalamic anti-nociceptive system is thought to be a component of the functional system of affect (1). Since the limbic system components, apart from the hyothalamus, are of major importance for shaping the intracerebral mechanism of "reward" and emotionally-motivated response of the organism, a comparative investigation into the anti-nociceptive effects of these regions was undertaken. In addition, the relative effectiveness of the antinociceptive action of hypothalamic and limbic structures has not been established till date. This is important in the context of prominent hypothalamo-limbic connections which might mediate the emotional-affective component of pain. To achieve these objectives, certain hypothalamo-limbic structures were stimulated electrically using same stimulus parameters, utilising a single pain test, that is tooth pulp stimulation evoked jaw opening reflex (TP-JOR).

Therefore, initial part of the present study elucidates the threshold current for stimulation of tooth-pulp at which the dEMG amplitude appears. This provides a basis for determination of the current required to obtain basal dEMG amplitidue for further study. The latter part of the study provides a direct comparison of the effect of stimulation of different pain modulatory areas of the hypothalamo-limbic system on TP-JOR.

METHODS

Animals: Adult male Wistar rats weighing between 200 to 300 gms, bred and reared in

the central experimental animal facility of All India Institure of Medical Sciences, New Delhi, were used in this study. They were housed in separate cages and maintained under 14 : 10 light-dark period at an ambient temperatue of 26 ± 2°C. Food pellets and water was provides ad libitum. Pain tests were carried out in accordance with the ethical guidelines formulated by the International Association for the Study of Pain (8).

Implantation of TP & brain stimulation electrodes: The animals were anesthetised with urethane (1.2 g/kg, i.p.) and placed in the supine position with head fixed in the stereotaxic apparatus for convenience in surgery. For selective intrapulpal nerve stimulation (9, 10) two small holes were drilled into the dentine of the incisor with the help of dental drill. The holes were drilled 10-15 mm from the tip of the incisor at the labial surface of the tooth crown, till the redness of the pulp was clearly visible. Two stimulation electrodes (tip bared to 0.2 mm, etched to 0.1 mm) were inserted with the interpolar distance of 2 to 3 mm. Thereafter, the surface of the tooth was carefully dried and the holes were filled with dental cement to avoid any short-circuiting in the presence of saliva and for fixation of the electrodes in position respectively. The wires leading from the electrodes were then connected to the stimulation set up. Records of the TP evoked dEMG were obtained by implanting bipolar steel needle electrodes (inter electrode distance 3-4 mm) in the rostral aspect of the anterior belly of the digastric muscle in rats ipsilateral in relation to the tooth chosen for implanting brain electrodes. Thereafter, the animal's head was fixed in the stereotaxic head holder with the skull upwards and electrodes were implanted ipsilaterally at LH (A-1 to –3.3, L1.5 to 2, V 9), AH (A –1.8, L 0.6, V 9), Amygdala (A –2.5, L 3.5, V 8) and Hippocampus (A –3, L 2.5, V 3.5) following the stereotaxic atlas of Paxincs and Watson (11). A rectal probe was introduced and left in place to monitor core temperature which was maintained at 37 ± 1°C.

Stimulation and recording of TP-JOR : The jaw-opening reflex (JOR) was elicited by tooth-pulp stimulation (TP) and quantified by recording the electromyogram from the anterior belly of the digastric muscle in rats (12, 13).

Tooth-pulp was stimulated using Nihon Kohden Stimulatior Model SEN 3301 and Isolation Unit Model SS 201J with rectangular pulses of frequency 0.5 Hz, duration 0.3 msec and intensity 1.5 to 3.0 times the threshold (9, 12) in order to produce a dEMG response for basal recording which varied between 50-75% of the maximal amplitude. Amplification of the dEMG signal was achieved by using a biophysical amplifier (Nihon Kohden Model AVB-11A) and displayed on a memory oscilloscope (Nihon Kohden Model VC-11). The magnitude of the JOR was estimated by averaging (Nihon Kohden Averager Model QC-111J) the peak to peak amplitude of sixteen superimposed dEMGs and recorded on a heat writing recorder for permanent copy. The threshold intensity for eliciting TP-JOR was determined by gradually increasing the intensity of the stimulus until the first response could be detected in the amplified signal which was also confirmed with an observable opening of the jaw of the animal. Three successive

averages of sixteen responses each, were then recorded and an overall variation in amplitude within ± 5% was considered as stable. The procedure was repeated two times and if the responses remained identical then that stimulus strength (current) was considered as the threshold current for eliciting dEMG response. The amplitude of dEMG obtained on stimulation of the plup at 1.5 to 3.0 times the threshold intensity was taken as basal (Fig 1).

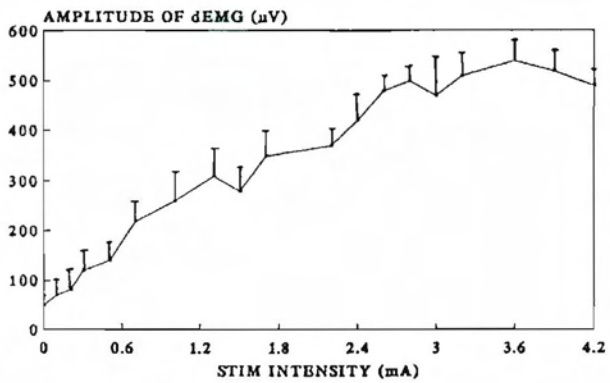

Fig. 1 : The effect of tooth pulp stimulation on dEMG amplitude with increasing intensities.

At least four such averaged responses were recorded as basal after which the brain area was stimulated ipsilaterally at 300μA current strength, 60 Hz frequency for a period of 10 seconds with concentric bipolar electrodes while the tooth-pulp was stimulated with the lowest current strength. The resultant dEMG was recoreded till the response came back to the basal. The above process was repeated for higher strengths of stimulation. Subsequently, the stimulation sites were lesioned for verification of the sites, followed by sacrifice of the animal and histological processing of the brain.

Analysis of data : All the values in the phasic pain test have been represented as percent change in comparison to the basal. The basal was taken as zero due to a large variation in the basal EMG from animal to animal. However, there was insignificant variation within one animal as revealed by one-way analysis of variance (ANOVA). Thus, a positive change observed depicts an increase in amplitude of dEMG and hence more pain, and vice versa. The connotations PS1 through PS3 given in "results" section, indicate successsive post-stimulatory periods of 37 second each. Each post-stimulatory period is the average of 16 responses. A response is repeated after 2 seconds and additional 5 seconds are required for recording, which accounts for the 37 seconds per post-stimulatory period. Therefore, PS1, PS2 and PS3, correspond to 37, 74, and 111 sec (i.e. multiples of 37) respectively, considering the end of stimulation time as zero. Effect of stimulation of different hypothalamic and limbic regions and their modulation of pain was compared with the basal at each strength of stimulation using Friendman's Test (Two-way ANOVA). All values were expressed as mean ± SD and the minimum level of significance was 5% ($p<0.05$).

RESULTS

I. Determination of threshold of TP-JOR

The left lower incisor tooth was stimulated in eight rats in a graded fashion and the resulting change in the amplitude of EMG was recorded from the ipsilateral digastric muscle. Amplitude of dEMG before tooth-pulp stimulation (at rest) which was 50 ± 10.2 uV in eight rats, increased to 80 ± 22.3 uV on stimulation of the pulp at

0.2 mA. The threshold intensity for appearance of tooth-pulp (TP) evoked response ranged from 0.2 mA to 0.5 mA. Increase in the amplitude of dEMG was almost proportional to the intensity of pulpal stimulation upto a stimulation strength of 3.6 mA where it was 540 ± 61.5 μV in amplitude. No further increase in the amplitude was observed although the current strength was increased upto 4.2 mA. The amplitude at this current strength was 490 ± 43.3 μV (Fig. 1). The intensity of tooth-pulp stimulation which was used to obtain baseline data varied from 1.5-3.0 times the threshold (12). In addition, the opening of the jaw on stimulation of TP can be appreciated when the reflex response was visually confirmed by increasing the intensity of stimulation to more than two times the threshold value. The latency of the response varied from 8.2 msec to 12.8 msec.

II. Comparison of pain modulation by hypothalamic and limbic areas:

Stimulation of Lateral Hypothalamus in 10 rats resulted in a decrease in dEMG amplitude. Reduction in amplitude of dEMG was 67.94±13.17%, 66.27±18.44%, and 63.44±17.85% in PS1, PS2 and PS3 respectively. This reduction was statistically significant (P<0.01) (Fig. 2a).

Stimulation of the Ventromedial nucleus in 12 rats resulted in significant reduction (P<0.05) in all the post

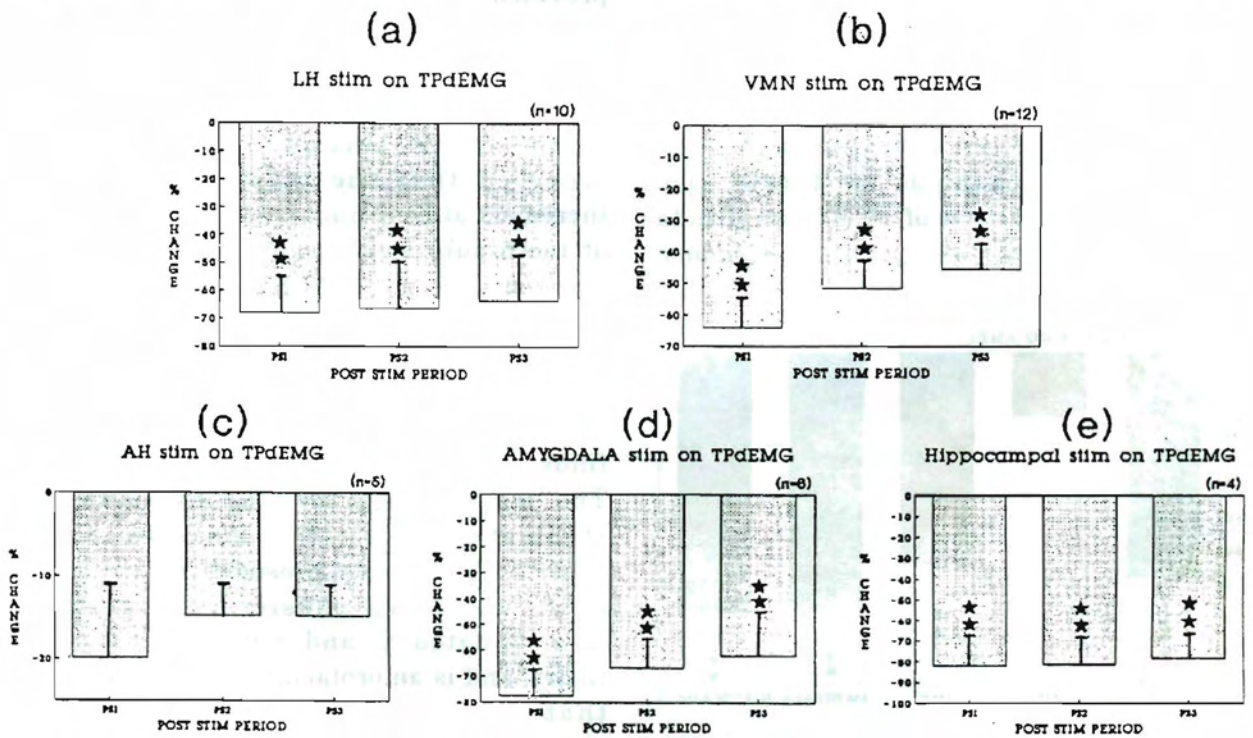

Fig. 2 : The effect of stimulation of (a) LH; (b) VMN; (c) AH; (d) anygdala and (e) Hippocampus on percent change in the amplitude of dEMG response with basal taken as zero. The values are expressed as mean ± SD. **P<0.01.

stimulatory periods i.e. PS1 to PS3 (Fig 2b).

Anterior Hypothalamus (AH) stimulation decreased the dEMG amplitude which was much less as compared to other hypothalamic areas (Fig 2c) and was statistically not significant.

Different nuclei of the amygdaloid complex when stimulated in 6 rats using the same parameters resulted in a significant reduction in the amplitude of dEMG ($P<0.01$) (Fig 2d). However, there was no qualitative or quantitative difference observed in the reduction of the amplitude of dEMG when different nuclei of the complex were considered. The results were pooled together, showing a maximum reduction of 77. 39 ± 9.12% at PS1 which became 62.01 ± 16.26% in PS3.

Stimulation of the CA3 and CA4 regions of Hippocampus in 4 rats also resulted in a reduction of dEMG amplitude with no regional difference in the response (Fig. 2e). This reduction was of the magnitude of 82.35 ± 10.98% in the first post stimulatory period which subsequently became 75.63 ± 9.17% in PS3. This decrease was found to be statistically significant ($P<0.01$).

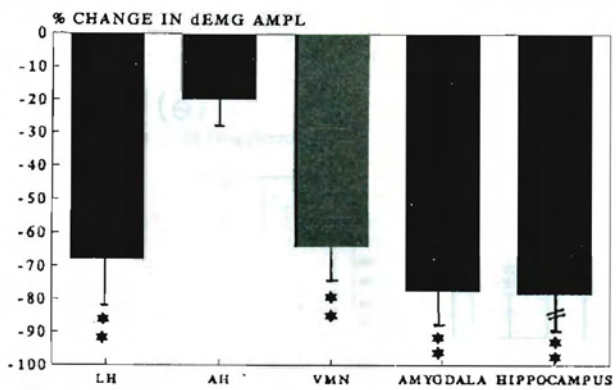

Fig. 3 : Comparison of the effect of stimulation of LH, AH, VMN, amygdala and hippocampus on TP-JOR response, during PS1. The values are expressed as Mean ± SD. **P<0.01.

Thus, stimulation of Lateral and Ventromedial nucleus of Hypothalamus produced significant reduction ($P<0.01$) in the amplitude of dEMG whereas stimulation of Anterior Hypothalamus did not produce any marked effect. Similarly, stimulation of the limbic areas i.e. Amygdala and Hippocampus produced significant reduction in tooth-pulp evoked dEMG response. A comparative figure depicting the aforesaid stimulatory effect during PS1 has been provided (Fig. 3).

DISCUSSION

In the initial part of the study, it was observed that the amplitude of dEMG increased almost linearly with the intensity of tooth-pulp stimulation till the intensity reached upto 3.6 mA. Subsequent increase in the intensity of stimulation did not raise the amplitude of dEMG any further. This direct correlation between the stimulus intensity and magnitide of dEMG response, finds support from the earlier reports (12). The jaw opening induced by tooth-pulp stimulation is a reflex measure of pain sensitivity (7) and represents the trigeminal pain pathway, consisting mainly of unmyelinated C and myelinated A-delta fibers, and is an orofacial, masticatory reflex that can be elicited by the pulpal stimulation and is quantified by measuring the amplitude of digastric electromyogram (dEMG). This test is considered to be a

reliable nociceptive assay system since a) stimulation of the tooth-pulp with any modality produces pain sensation in a subject (14-16); b) within physiological range of pulpal stimulation, the intensity of pain is highly correlated with the amplitude of the digastric electromyogram (12), c) at any level of stimulation, there is no habituation or adaptation of response with repeated application of nociceptive stimuli (17, 18) and d) the JOR is suppressed by stimulation of endogenous antinociceptive brain regions such as the PAG and certain raphe nuclei and/or administration of analgesic drugs but not by stimulation of non-antinociceptive structures (10, 19).

The study showed that, among the hypothalamic areas studied, stimulation of LH and VMN produced significant antinociceptive response whereas stimulatoion of AH did not produce any sigificant change.

The fact that LH and VMN stimulation has given rise to significant analgesia is in agreement with earlier reports (1,20-22) using different pain tests such as the tail-flick and formalin test. Stimulation of LH in rats caused significant analgesia in the TP-JOR test as measured by the decrease in amplitude of dEMG. This is supported by a study in cats where tetanic stimulation of posterior and medial hypothalamus at a frequency of 100 Hz and intensity of 20v resulted in a decreased TP-JOR response (1). The effect of hypothalamic stimulation can act at different levels, upto the spinal level (2, 4). LH can have either a direct inhibitory action on one or more of the trigeminal nuclei in this nociceptive reflex pathway (1, 23) or an indirect action mediated through structures in the brainstem or higher centres (1). Similarly, anatomical evidences of a descending connection from the VMN to trigeminal nucleus exist which is indirect through midbrain reticular formation (24).

Although many reports indicate that the antinociception producing structures are mostly located in the paraventricular region of the medial hypothalamus, we did not obtain appreciable antinociceptive responses from stimulation of AH. One reason for this observation might be that unlike the aforesaid areas, this region sparsely contains opioidergic neurons or, the threshold for activation of neurons in this region is higher than the others.

Of the different limbic structures, we found that both amygdala and hippocampus produced significant analgesic response. This response is supported by earlier reports which used different types of pain tests which included tests such as the tail-flick and formalin test while the TP-JOR test has been employed in one study using conditioning stimuli of very high frequency (330 Hz) as compared to our study (7). Besides, the stimulation sites at amygdala consisted mainly of central and medial nuclei which contains predominantly enkephalinergic neurons which accounts for the intense inhibitory effects (25-27). This antinociceptive effect of amygdala might operate directly through transhypothalamic pathways connecting amygdala with the brain stem structures involved in pain modulation, as well as, via hypothalamic structures (28-30). Similarly, the hippocampal regions stimulated in our study contains opioid and enkephalinergic receptors as reviewed earlier.

Therefore, the present study provides an insight into the analgesic efficacies of different hypothalamo-limbic structures in direct comparison with each other, using a common and sensitive pain test and same parameters of stimulation.

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