Effect of microinjections of 5-hydroxytryptamine and adrenaline in central grey on pain responsiveness during acute food deprivation in conscious rats

Snehasis Bhunia, M.S. Bharambe, R. Singh, J. Premendran & S. Pande
Departments of Physiology, Community Medicine and Pharmacology
Mahatma Gandhi Institute of Medical Sciences, Sevagram 442 102, India
Received 9 October 1998; revised 29 November 1999

To study the effects of microinjections of 5-hydroxytryptamine and adrenaline in central grey on pain responsiveness during acute food deprivation, experiments were conducted in nine male rats. Microinjections of 5-HT (10 μg/μl) and adrenaline (10 μg/μl) were given in central grey before and at the end of 6, 12, 18 and 24 hr food deprivation and the effects on pain threshold, cardiorespiratory parameters and body temperature were noted. Observations showed that 5-HT increased the pain threshold (antinociception) significantly (P<0.05) with no change in cardiorespiratory response and body temperature, adrenaline did not alter pain threshold with no change in cardiorespiratory response and body temperature. The observations suggest the possible existence of two types of monoaminergic receptors or pathways in the central grey.

Acute food deprivation is a common stressful condition where we find a lot of changes in body's homeostasis such as the improvement of the body immune systems, modulation of endogenous opiate production, and readjustment in the metabolic activities. Pain threshold is also altered i.e. analgesia during the early periods and algesia during the longer periods, which would be the principal factors to protect our survival.

By means of a sensitive fluorescence method it has been shown that catecholamines (adrenaline and noradrenaline) and 5-HT in the mammalian central nervous system are accumulated in very high concentration in synaptic terminals belonging to special systems of neurons (serotonergic, catecholaminergic) which are monoaminergic. Nerve cells of the catecholamine type (A8-A10) and 5HT type (B7-B9) are present in central grey area of mesencephalon of rat brain and powerful antinociceptive effects are produced by these neurons in rats exposed to any type of stressful conditions. It is postulated that analgesia from central grey (periaqueductal grey) could be due to monoamines when microinjected, mediated by activating descending inhibitory systems which block the transmission of nociceptive messages at the spinal level. The numerous studies performed in rat clearly indicate that analgesic effects can be induced by stimulation or microinjections of area widely spread over the whole of the central grey area. There is an evidence of more involvement of serotonergic mechanism having involvement of serotonin receptors, than adrenergic, not having plenty of receptors for adrenaline in stress induced analgesia (SIA). There is no information about the role of monoamines on pain responsiveness during different durations of food deprivation stress. Since there is a direct involvement of central grey area by microinjections of monoamines on pain sensitivity and the variable pain responsiveness during different durations of food deprivation specially during the longer periods, the present study was undertaken to investigate the effects of central microinjections of adrenaline and 5-HT in central grey on pain responsiveness during different durations of acute food deprivation.

Materials and Methods

Experimental animals—Experiments were performed on nine albino male rats of Wistar strain weighing 250 g aged 90 days. They were maintained under 14 hr:10 hr light and dark conditions and were given food (20 g per day) and water (20-35 ml per day) ad libitum. Feed consisted of protein (23.4%), fat (4.5%) and balanced with carbohydrate, fibers, vitamins and minerals. They were observed carefully for changes in water intake, food intake, body weight general behaviour and mortality.

Experimental protocol—Experiments were performed in three steps. In each step all the animals (9 rats) were used. Rats were anesthetized with sodium pentobarbitone (40 mg/kg, ip). Cannulated steel and
insulated electrodes of 23 G were prepared from needles. The electrodes were stereotaxically implanted into the central grey area (central nucleus - posterior to the bregma (AP) = -6.3; lateral to the midline (ML) = 0.7; ventral to the dura (DV) = 5.5), according to the rat brain atlas by Paxinos and Watson. The anchoring screws were driven into the skull with dental cement. After 4-5 postoperative days, different pain tests according to the ethical guidelines were performed on these rats of different (normal control, adrenaline treated and 5HT treated) groups. Each animal was housed individually in a polypropylene cage. The number of animals in each group was nine.

In first step, microinjection of normal saline in central grey was done approximately 30 min before the start of the experiment (0 hr food deprivation). The body temperature and all the pain related parameters (tailflick response (TFL in sec and TFL in volts), vocalization (SV in volts), heart rate (HR) in beats / min and respiratory rate (RR) in cycles / min) were measured in all nine rats kept with food and water (normal control during 0 hr food deprivation). After recording, all the animals were kept without food for 6, 12, 18 and 24 hr. Microinjection of normal saline was conducted in central grey 30 min before the mentioned food deprivation periods (6, 12, 18 and 24 hr). All the above pain related parameters and body temperature were recorded just after 6, 12, 18 and 24 hr of food deprivation periods i.e. in between 6 and 6.15, 12 and 12.15, 18 and 18.15 and 24 and 24.15 to have basal control value of pain threshold during different durations of food deprivation.

In second step, after 7 days of resting period, adrenaline was microinjected in central grey 30 min before the start of the experiment (0 hr of food deprivation) in all the rats. After recording all the above mentioned parameters, animals were kept without food for 6, 12, 18 and 24 hr. Microinjection of adrenaline was done 30 min before of every mentioned (6, 12, 18 and 24 hr) time of food deprivation periods. At the end of 6, 12, 18 and 24 hr of food deprivation, all the above pain related parameters were recorded.

Similarly, in third step, after keeping all the animals for 7 days in rest, 5 HT was microinjected in central grey 30 min before the start of the experiment (0 hr of food deprivation). After recording all the above mentioned parameters, animals were kept without food for 6, 12, 18 and 24 hr. Microinjection of 5HT was done 30 min before of every mentioned (6, 12, 18 and 24 hr) time of food deprivation period. At the end of 6, 12, 18 and 24 hr of food deprivation, all the above pain related parameters were recorded.

Recording of HR, RR and body temperature—“Student physiograph” (INCO, Ambala, India) was used to record ECG (lead-III), respiratory rate and body temperature. Biopotential ECG coupler, respiration coupler and temperature coupler were used. Heart rate was calculated from ECG records.

Experimental pattern to determine emotional component of pain (TFL and SV in volts)—It consisted of restraining the animals for 30 min in a talcum powder tin cut longitudinally so as to accommodate the animal with head and tail being kept out. Two short needles were inserted as cannula subcutaneously in the middle of the tail at 2 mm depth and a distance of 2 cm between the two was maintained. Both the needle electrodes were fastened with adhesive tape. Rats were conditioned for 30 min, following the needle insertion. The electrical stimulation consisted of a train of one second duration with pulse width of 1 m sec at a frequency of 10/sec. The voltage was progressively increased in step by 0.1 V. and interval of 5 min was kept between two electrical shock in the same animal. Two types of pain threshold were determined as below: (1) A low intensity stimulation producing motor responses - tail withdrawal (TFL in volts) and (2) A higher voltage producing vocalization (SV in volts).

Experimental pattern to determine phasic pain (TFL in sec)—Phasic pain was induced by noxious heat to the tail of the rats and the tail-flick latency (TFL) was measured by tail-flick analgesia monitor. Each rat was conditioned for 30 min in the restrainer before starting the experiment. The rat’s tail was cleaned with spirit and placed on the heating coil which was 4 mm below the tail. The cut off time was set to 30 sec to avoid tissue damage. The response time was forced on the display when the tail interrupted the infra-red beam. Heat was applied to the tail thrice, at intervals of 5 min and the basal TFL was measured by taking mean of these 3 observations.

Histology—At the end of experiment, the tip of cannula was tested. Brains were perfused with saline and 10% formalin solution transcardially. The brain was isolated and was put in a container filled with 10% formalin for using freezing microtome. Sections (40 μm thick) were cut and stained with cresyl violet staining procedure (NISSL staining) for determining the site of the tip of the cannula.
The statistical significance of the results was calculated by analysis of variance followed by Student's t test. Significant difference were those with P < 0.05.

Results

Pain sensitivity to microinjection of normal saline—Pain threshold (increased TFL in sec and volts, increased SV in volts, decreased HR) was increased (antinociception) during early periods (6 hr) but the same was reduced (decreased TFL in sec and volts, decreased SV in volts, increased HR and RR) significantly (P<0.05) during the longer periods (24 hr) of food deprivation as compared to the pain sensitivity during 0 hr of food deprivation (Fig. 1). The percentage change of TFL in sec and volts, HR were significant during 18 hr and 24 hr and RR during 24 hr of food deprivation as compared to normal control condition of 0 hr food deprivation (Fig. 2).

Pain sensitivity to microinjection of adrenaline—The pain threshold (TFL in sec and volts, SV in volts) HR, RR were not changed significantly during 0 hr of food deprivation to microinjection of adrenaline in central grey as compared to normal control conditions of 0 hr of food deprivation.

Microinjection of adrenaline in central grey did not alter pain threshold significantly (P>0.05) with no significant change in HR and RR (Fig. 1), during different periods (6, 12, 18, and 24hr) of food deprivation as compared to normal control condition of 0, 6, 12, 18 and 24hr food deprivation. The percentage change was also not significant (Fig 2) during all the periods of food deprivation when compared to the normal control of 0, 6, 12, 18 and 24hr food deprivation.

Pain sensitivity to microinjection of 5 HT—The pain threshold (TFL in sec, SV in volts) was increased significantly (P < 0.05) during 0 hr of food deprivation to microinjection of 5 HT in central grey as compared to normal control conditions of 0 hr of food deprivation.

Microinjection of 5 HT in central grey increased pain threshold significantly (P>0.05) with no significant change in HR and RR (Fig. 1), during the longer periods (18, and 24 hr) of food deprivation as compared to normal control condition of 0, 18 and 24 hr food deprivation. The percentage change was also significant during the longer periods (18, 24 hr) of food deprivation when compared to the normal control of 0, 18, 24 hr (Fig. 2) food deprivation. Body temperature was not altered (Fig. 1) before (0 hr) and during different periods of food deprivation in normal

Fig. 1—Effects of central microinfusion of adrenaline and 5 hydroxytryptamine (SHT) on body temperature (A), TFL in sec (B), TFL in volts (C), SV in volts (D), HR (E), RR (F) during 0, 6, 12, 18 and 24hr, of food deprivation in male rats 90 days age of normal control, adrenaline treated and SHT treated groups. Significant difference between mean values of two groups was determined by Student's 't' test and judged significant if P < 0.05. * Compared with normal control of 0 hr food deprivation, a and b Compared with normal control group during 18 and 24 hr of food deprivation respectively.
Adrenaline judged significant if control group during 18 and 24 hr of food deprivation as compared to normal control, adrenaline treated and 5HT treated groups. Significant difference between mean values of two groups was determined by Student's 't' test and the different form of SIA, (3) changes in the sensitivity of the receptors (monoaminergic) with the change of brain tissue metabolism and (4) the variation in blood metabolites during the different durations of food deprivation stress.

Noradrenaline and serotonin (5HT) are the principal candidates for activating descending systems by activating receptors followed by nerve cells of A8-A10 and B7-B9 present at central grey of mesencephalon area. The noradrenergic and serotoninergic terminals in the spinal cord are well known to have their cell bodies in the mesencephalon areas. Significant increase in pain threshold to microinjections of 5HT during 0 hr of food deprivation would be due to powerful antinociceptive effect of central grey area by involving descending serotoninergic system as it was reported earlier. No significant change on pain threshold by microinjection of adrenaline before and during the different durations of food deprivation would be due to the absence of descending adrenergic

Discussion

It is well known that the descending inhibitory systems play an important role in pain modulation and analgesia. These systems participate in morphine analgesia, stimulation produced analgesia SPA and SIA and exert a tonic inhibition from the central grey to spinal cord on nociceptive processes during SIA. Variable pain responsiveness (analgesia during the early period and algesia during the longer periods) might be due to (1) the alterations in tissue opiates (brain, spleen, liver, plasma, adrenals) (2) the different form of SIA, (3) changes in the sensitivity of the receptors (monoaminergic) with the change of brain tissue metabolism and (4) the variation in blood metabolites during the different durations of food deprivation stress.

Noradrenaline and serotonin (5HT) are the principal candidates for activating descending systems by activating receptors followed by nerve cells of A8-A10 and B7-B9 present at central grey of mesencephalon area. The noradrenergic and serotoninergic terminals in the spinal cord are well known to have their cell bodies in the mesencephalon areas. Significant increase in pain threshold to microinjections of 5HT during 0 hr of food deprivation would be due to powerful antinociceptive effect of central grey area by involving descending serotoninergic system as it was reported earlier. No significant change on pain threshold by microinjection of adrenaline before and during the different durations of food deprivation would be due to the absence of descending adrenergic

![Graphs showing percentage change on TFL in sec (A), TFL in volts (B), SV in volts (C), HR (D), RR (E) during 6, 12, 18 and 24 hr food deprivation as compared to normal control of 0 hr food deprivation in rats of normal control, adrenaline treated and 5HT treated groups. Significant difference between mean values of two groups was determined by Student's 't' test and judged significant if P < 0.05. * Compared with normal control of 0 hr food deprivation, † and ‡ Compared with normal control group during 18 and 24 hr of food deprivation respectively.](image-url)
systems which could modulate / influence the spinal mechanisms mediating antinociception. There is no such evidence of antinociceptive effects of adrenaline injected in the different regions of the brain15.

No significant change on HR and RR to microinjection of both adrenaline and 5HT before and during the different duration of food deprivation would prove the ineffectiveness of the receptors of central grey area on the cardiorespiratory centers or due to non involvement of these neurons (adrenergic and serotonergic) on the activity of vasomotors centres. Significant changes on HR during 18 and 24 hr and on RR during the longer period (24 hr) of food deprivation observed, would be due to the stimulation of sympathetic nervous system12,27. No change in body temperature before and during the different durations of food deprivation in all normal control, 5HT treated and adrenaline treated as reported earlier, could be due to the presence of compensatory mechanisms operated during the stressful condition23.

In conclusion, we may suggest that there could be an involvement of two types of receptors / pathways on the modulation of nociception to microinjections of 5HT and adrenaline in central grey during different periods of food deprivation. We need much more information about their relative involvement and their functional significance. We intend to follow up these preliminary findings with detailed explanations of large numbers of rats exposed to different duration of acute food deprivation.

References
3 Sodeman AW & Sodeman JM, Pathologic physiology (Souders Company; Philadelphia) 1979, 981.
8 Bhunia S, Central grey catecholaminergic involvement on pain responsiveness during food deprivation in conscious rats, paper presented to the symposium on 13th National Conference of Parasitology, Bangalore, India,24th to 26th February 1998.
15 Besson JM, Chauch A, in Neurotransmitters and pain control, edited by Aki H & Lewis JW (Karger, New Delhi), 1987, 64.
23 News letter (Central Drug Research Institute, Lucknow), 1994, 6.