Gender differences in predator induced pain perception in rats

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Received 25 October 2002; revised 24 December 2002

Pain is an unpleasant sensation. It warns the living being about the impending damage to the tissues. The perception of pain is influenced by physical and psychological factors. The impact of chronic intermittent psychological stress on pain perception and the differences in antinociceptive responses have been studied in male and anestrous female albino rats. Fifteen rats in each group were subjected to psychological stress, by exposing them to their natural predator—cat, for a duration of 20 min daily for 12 consecutive days. Tail flick response latency to radiant heat was used as a measure to evaluate pain perception. It was observed that both the groups had a relatively high pain threshold at the beginning of exposure schedule due to the modulation of opioid analgesic system by the higher level of circulating testosterone in males and low level of estrogen in anestrous females. However, the threshold for pain perception showed a gradually declining trend in both the groups over the next 11 days to reach the control values. This increase in sensitivity to pain or decreased pain threshold could be attributed to the phenomenon of habituation.

Pain is produced due to varying degrees of tissue damage. Sex steroids, physical and psychological stressors are demonstrated to modulate the perception of complex pain sensation. Further, a variety of biological factors like reproductive status, species, strain, behavioral and physical conditions can affect the manifestation of stress-induced analgesia. The odor released by the stressed conspecific or dominant male is known to produce analgesic response. There appears to be a difference in the type of perception of pain for a single exposure and for repeated exposure to a stressor inducing psychological stress. Further, it has been reported that there is a gender difference in the sensitivity to pain which predominantly depends on the presence and the level of circulating sex steroids. The antinociceptive response produced by physical or psychological stress is due to the modulation of endogenous opioid analgesic system by the sex steroids. The female animals exhibit a greater anxiety reaction resulting in non opioid analgesia on being exposed to physical stress of short duration when compared to their male counterparts. The anxiety response causes the release of 5 HT. However, the nature of stress-induced analgesia depends on the parameters of the stressor. Most of the earlier studies have reported the effect of physical stress or short-term exposure to psychological stress on pain perception. In the present study, efforts are made to assess the pattern of pain perception in male and female anestrous rats exposed to chronic intermittent psychological stress.

Methods

Fifteen laboratory bred male albino rats aged between 4 and 5 months and weighing about 175-200 g constituted group I. Equal numbers of female rats with similar characters kept in small groups totally isolated from males formed the anestrous group (group II). It is established that female rats kept totally in isolation from the males become anestrous. Each cage housed 3 rats and natural day and night cycle was maintained. The animals had free access to food and water. All the recordings were carried out during the same part of the day. Rats belonging to group I and group II were subjected to similar experimental protocol. The degree of pain perception was evaluated by measuring the tail flick response latency to radiant heat by using an analgesiometer (INCO). The experimental animal was kept for a duration of 20 min in the modified Plexiglas rat restrainer, which provided a good front view and better ventilation as compared to the conventional metallic restrainer. The duration of exposure was standardised by serially exposing different groups of rats to its natural predator for varying periods ranging from 5 to 30 min. It was observed that the duration of exposure of 20 min produced the maximum tail flick response. The instrument was calibrated to deliver radiant heat.
at the base of the rat’s tail. The necessary temperature of 42°C at the base of the rat’s tail was maintained by heating the Nichrome wire to 60°C. The control tail flick response was recorded as a measure of pain perception without the stressor. The maximum duration of exposure of the tail to heat was restricted to 2 min to avoid heat necrosis. Subsequently, to evaluate the impact of psychological stress, the same animals were kept in the rat restrainer the following day and exposed to its natural predator-cat for a period of 20 min. Care was taken to ensure that the rat could get a good view of the cat. The rats received the auditory and olfactory cues in addition to visual cues from its predator. At the end of 20 min of exposure, the tail flick response latency was recorded. This procedure of recording the tail flick latency after exposure to the psychological stressor was continued till the values returned to control levels.

**Statistical analysis**

The results are presented as mean ± SD. Data were analysed by using two way repeated measure ANOVA, with days of experiment as within subjects factor and group (male/female) as between subjects factor.

The control tail flick latency (day 0) for the male and anestrous female rats were 29.07±3.20 sec and 25.07±4.40 sec respectively. On the first day of exposure (day 1), the male rats had a tail flick response of 117.67±4.75 sec and the anestrous female 66±8.03 sec. On the subsequent days, both the groups showed a declining trend in the analgesic response till they returned to the control values on 12th day. However, throughout the duration of the experiment, males showed a longer tail flick response time and hence, a higher pain threshold when compared to the anestrous female rats.

Tail flick latency data from day 0 to day 12 are presented in Fig. 1. There was a significant main effect for days (F= 74.869, df = 12,336, P<0.001) indicating that tail flick latencies were overall significant compared to day 0. The days X groups interaction was also significant (F= 8.722, df = 12,336, P<0.001) indicating that the pattern of change in tail flick latency across days differed significantly between male and anestrous female groups.

The results clearly establish that the male and anestrous female rats had a high pain threshold on the first day of exposure to their natural predator. Watson et al.12 have reported the presence of opioid system in both brain and pituitary glands. According to Lewis8 the system supposed to mediate pain inhibition includes the hypothalamus, pituitary adrenal axis. The removal of pituitary gland reduced the opioid mediated stress induced analgesia. Amit et al.5 have reported that β endorphins could mediate the analgesic response. The pituitary β endorphins, dynorphins, metencephalins are lower in females and these peripheral peptides are believed to have a role in the analgesic process13. Kepler et al.14 are of the opinion that the gender difference in central morphine analgesia could be due to the interaction between central opiates and gonadal steroid receptors. Further, corticosterone or one of its metabolites plays a permissive role in interacting with the opioid system to inhibit pain15. The olfactory cues originating from the aggressive or conspecific predator has an important role in activating the endogenous analgesic system16.

It has been demonstrated that the reproductively intact male after gonadectomy showed a decrease in analgesic response. However, injection of testosterone improved antinociceptive response in these rats13,18. A lower analgesic response has been reported in castrated males and the levels of pain inhibition were comparable to anestrous females. The breeding males have a greater opioid and non-opioid stress induced analgesia when compared to non-breeding males18. Hammer19 has documented a higher level of opioid receptors in males. These experimental evidences suggest the definite role of testosterone in the stress induced analgesic response in males. The greater analgesic response in male rat is attributable to higher levels of circulating androgens, which are known to potentiate the opioid analgesic system.

Earlier studies have indicated the presence of non-opioid and opioid pain inhibitory systems in female rats. Short-term exposure to physical stress is known to activate the non-opioid analgesic system and anxi-
The effect of possibly due to the phenomenon of habituation. The culminating estrogens. There reduced level of estrogen or calculations of subsequent days of exposure the pain threshold and its metabolites are known to desensitize or down regulate the opioid receptors, reduce the binding in the opioid system and decrease the electro physiological actions of μ opioids. The higher pain threshold in anestrous females could be due to lower levels of circulating estrogens. The reduced level of estrogen or the disappearance of its metabolites could increase the number of receptors and leave them in a highly sensitive state. The significant difference between the male and female anestrous rats for perception of pain was more evident on the first day of exposure. On the subsequent days of exposure the pain threshold showed a gradually declining trend in both the groups, possibly due to the phenomenon of habituation. The pain threshold for males remains consistently higher throughout the duration of exposure. These changes observed in males are attributable to the potentiating effect of circulating androgens.

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