Hypothalamic γ-glutamyl transpeptidase activity in rats following morphine and naloxone treatment

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Hypothalamic γ-glutamyl (γ-GT) transpeptidase activity was measured in pubertal and sexually mature male rats. Rats were given subcutaneous injection of morphine and naloxone and the enzyme activity was measured at different time intervals. Morphine had an inhibitory effect on the enzyme activity in both the age groups. Naloxone, an opioid receptor antagonist, had a stimulatory effect on γ-GT activity in pubertal rats while it had no effect in sexually mature rats. This suggests that opiates exert an inhibitory effect on glutathione utilization through γ-GT cycle. Physiological significance of this finding with reference to pituitary function appears to be the suppression of gonadotropin secretion.

GAMMA-glutamyl transpeptidase (γ-GT) is a widely distributed enzyme that functions in the utilization of glutathione by transferring the γ-GT moiety of the tripeptide to amino acid or peptide acceptors. This enzyme together with others plays a major role in the synthesis and degradation of glutathione via the γ-GT cycle. Gamma-GT is a membrane-bound enzyme present in abundance in epithelia of many organs. The substrates for the enzyme are glutathione (GSH), oxidized glutathione (GSSG), S-substituted glutathione and other γ-GT compounds. Glutathione’s function is to maintain protein thiol groups which may be required for catalysis and is involved in protein assembly and degradation. Glutathione also helps in protecting protein and cell membranes against peroxides and free radicals. Glutathione has also been reported to play a modulatory role on anterior pituitary hormone release. It is probable that glutathione interacts with neuropeptides such as GnRH, opioid peptides and other regulatory elements at the hypothalamic level to elicit an endocrine response. The present study was designed to evaluate whether hypothalamic γ-GT transpeptidase activity responds to treatment with morphine sulphate – an opiate agonist – and naloxone – an opioid receptor antagonist – and thereby affects glutathione metabolism in pubertal and sexually mature rats.

All the experiments were done in colony-bred rats, derived from Wistar strain, purchased from the Animal Facility of Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry, and Kind Institute of Preventive Medicine, Madras. Forty (pubertal) and 60 (sexually mature) days old male rats were used in this study. They were maintained under controlled conditions of light (14 h light and 10 h dark) and temperature with free access to drinking water and standard rat pellets.

Morphine sulphate, γ-GT paranitroanilide, glycylglycine and naloxone were obtained from Sigma Chemical Co., St Louis, USA. Naloxone (2 mg/kg body weight, dissolved in 0.9% saline), morphine sulphate (2 mg/kg body weight dissolved in 0.9% saline) were injected subcutaneously. All the doses employed are known to produce physiological responses. Rats were sacrificed at 1 and 4 h after injection and hypothalamus was quickly dissected out and processed for estimation of γ-GT transpeptidase. The enzyme activity was estimated according to the method of Tate and Meister as standardized by Pasha and Sadasivudu. This method has been widely used to measure the enzyme activity in
choroid plexus and other areas of brain. The assay mixture consisted of 80 μmol of Tris-HCl buffer (pH 9.0), 150 μmol of NaCl, 40 μmol of glycerol glycine, 5 μmol of γ-GT paranitroanilide and 0.4 ml of 2% homogenate (0.25 M sucrose). After 30 min incubation at 37°C the reaction was terminated by the addition of 2 ml of 10% acetic acid. After centrifugation at 5000 rpm for 20 min the absorbance of the clear supernatant was read at 410 nm. The enzyme activity was expressed as μmol of paranitroanilide liberated per gram weight of tissue per hour with reference to a standard graph plotted using different concentrations of paranitroanilide and treating it with acetic acid.

Statistical analysis was done using analysis of variance, followed by Duncan’s new multiple range test.

γ-GT activity ranged from 44.14 ± 0.64 in 40-day-old to 52.96 ± 1.91 μmol p-nitroaniline released/g wt tissue/h in 60-day-old rats. Administration of morphine to 40-day-old rats did not modify the enzyme activity at 1 h, whereas the activity was reduced at 4 h (P < 0.05) after injection. In contrast, naloxone treatment produced an increase in the γ-GT activity at 1 h (P < 0.05) while there was no change at 4 h (Table 1). Morphine treatment to 60-day-old rats reduced hypothalamic γ-GT activity significantly both at 1 h (P < 0.01) and at 4 h (P < 0.05) after injection. Naloxone, however, caused no change in the enzyme activity either at 1 or 4 h in 60-day-old rats (Table 2).

The present results show that morphine, a natural opiate agonist and naloxone, an opiate receptor antagonist, produce changes in hypothalamic γ-GT transpeptidase activity. Though morphine sulphate failed to modify γ-GT at 1 h, it produced significant reduction at 4 h in 40-day-old rats. Administration of naloxone, on the other hand, produced an opposite effect. Earlier studies showed that glutathione content in hypothalamus reached a peak at puberty. Further, intraventricular administration of glutathione resulted in a significant increase in plasma FSH and a reduction in LH levels in ovarioctomized oestrogen-progesterone primed rats. Such an effect of glutathione on gonadotropin levels together with the observation of pubertal peak in glutathione content suggested an interaction of glutathione with the hypothalamic-pituitary axis. The increase in γ-GT activity in pubertal rats is consistent with the above observation. Further, it is significant in consonance with the recently held view that attenuation of opioid inhibition at the time of puberty in male and female rats is one mechanism for the onset of puberty. Increased utilization of glutathione through γ-GT cycle maybe one mechanism for increased secretion of gonadotropins. In sexually mature rats naloxone did not modify γ-GT activity, suggesting noninvolvement of glutathione utilization in endogenous opioid-mediated hormone response in these rats. Morphine sulphate had a marked inhibitory effect on hypothalamic γ-GT activity in both 40- and 60-day-old rats. Administration of morphine is reported to be inhibitory to gonadotropin secretion. Available evidences suggest that glutathione utilization by the γ-GT cycle is involved in gonadotropin release. A decrease in γ-GT activity following morphine treatment suggests that this may be one of the mechanisms of morphine-induced suppression of gonadotropin secretion. Morphine is known to regulate release of other pituitary hormones as well. It may do so by altering the release of neurotransmitters at the hypothalamic level. The physiological significance of the effect of morphine on glutathione utilization through the γ-GT cycle on pituitary hormone release is not clear. The formation of γ-GT derivatives such as γ-GT dopamine may have functional significance as dopamine is known to influence release of a number of hormones from the pituitary.

Since opiates of other classes (prodynorphine and proenkephalin) are less potent in eliciting a response on gonadotropin release as compared to morphine, they were not used in the present study. However, the present results suggest that hypothalamic γ-GT transpeptidase activity is altered under the influence of morphine and reversed by opioid receptor antagonist naloxone. The exact mechanism and physiological significance of this response is yet to be understood.

The data presented suggest that opioid peptides, have an inhibitory effect on glutathione utilization through the γ-GT cycle. In pubertal rats, opioid receptor antagonist naloxone stimulated the γ-GT activity, indicating endogenous opioid-mediated suppression of glutathione utilization. This is significant with respect to onset of

Table 1. Hypothalamic γ-glutamyl transpeptidase activity in 40-day-old rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>44.14 ± 0.64</td>
<td>43.21 ± 0.8</td>
</tr>
<tr>
<td>Morphine sulphate</td>
<td>42.98 ± 1.2</td>
<td>34.62 ± 1.2*</td>
</tr>
<tr>
<td>Naloxone</td>
<td>52.10 ± 4.3*</td>
<td>46.13 ± 2.4</td>
</tr>
</tbody>
</table>

*P < 0.05.
Values are in μmol p-nitroaniline released/g wt tissue/h.
Values represent mean ± SE of 6 rats in each group.

Table 2. Hypothalamic γ-glutamyl transpeptidase activity in 60-day-old rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>52.96 ± 1.91</td>
<td>51.40 ± 1.0</td>
</tr>
<tr>
<td>Morphine sulphate</td>
<td>44.47 ± 0.5**</td>
<td>46.10 ± 2.5*</td>
</tr>
<tr>
<td>Naloxone</td>
<td>49.93 ± 2.2</td>
<td>55.80 ± 1.3</td>
</tr>
</tbody>
</table>

*P < 0.05.
**P < 0.01; significantly different from control value.
Values are in μmol p-nitroaniline released/g wt tissue/h.
Values represent mean ± SE of 6 rats in each group.
puberty as glutathione level in hypothalamus is reported to reach a peak at puberty. Suppression of γ-GT transpeptidase activity by morphine in both the age groups suggests that this may be one of the mechanism(s) involved in suppression of gonadotropin secretion.


ACKNOWLEDGEMENTS. We thank University Grants Commission for a research grant to EV. PS was a recipient of JRF and SRF from UGC.

Received 9 February 1995; revised accepted 11 September 1995

Inhibition of biogas production by isobutyric and isovaleric acids and their precursors

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Isobutyric and isovaleric acids inhibit the production of biogas in the anaerobic fermentation of cowdung slurry at a concentration of 1000 ppm. Amino acids leucine and valine, whose metabolic products are isovaleric and isobutyric acids, respectively, when added to the fermenting media did inhibit the biogas production, but not to the extent free acids do, as some of the amino acids could have been utilized for protein synthesis.

Methane gas production under anaerobic fermentation of agricultural wastes and animal wastes is a complex multistep system involving different microorganisms, which first degrade cellulose and turn the product into methane with the help of *Methanobacterium* present in the medium1–3. Many investigators have reported about the importance of volatile acids in the production of methane gas from cattle wastes. Varel et al.4 observed that, under conditions of optimum methane production from cattle wastes, total volatile acid is the first to accumulate in large amounts by increasing the total volatile solids in the feed. The additive effect of addition of water hyacinth5–7 and poultry waste8 to cowdung enhanced the volatile fatty acid content. This was further confirmed using a two-phase fermenting system9. Hobson and Shaw10 reported that volatile fatty acids acetate and butyrate were not inhibitory to methane production by *Methanobacterium formicicum*, but propionate was under certain conditions. Kalie and Menon11 reported that methanogenesis was inhibited by branched-chain fatty acids like isobutyric and isovaleric acids below ambient temperature. However, Kalie et al.12 reported that normal mixed cellulosic culture produces straight-chain fatty acids, which could be due to the presence of isovaleric-degrading bacteria present in marine sediments and sewage.

**Figure 1.** Effect of addition of isobutyric or isovaleric acid to the fermenting medium. Isobutyric acid (IBA) or isovaleric acid (IVA) was added on the 15th and 20th days of fermenting to a final concentration of 1000 ppm and the total amounts of volatile acids and biogas produced were measured. a, Control; b, IBA or IVA added; O—O IVA, total volatile acids; O—O IVA, biogas produced; x—x IBA, total volatile acids; x—-x IBA, biogas produced.