Morphine induced inhibition of the activities of accessory reproductive ducts in male rats

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SUMMARY

Adult male albino rats were treated with 0.5 mg and 0.75 mg morphine/100 g body weight intraperitoneally for 30 days. All the animals were autopsied on 31st day. Epididymis and vas deferens were dissected out, weighed and processed for histological and biochemical studies. Morphine has caused a reduction in the weight of epididymis and vas deferens in both the doses of drug treated groups. The total cholesterol content is increased while protein, DNA and RNA contents and epididymal sperm counts are decreased. The acid phosphatase content is decreased 10.12 ± 0.11 in caput, 9.26 ± 0.30 in cauda of epididymis and in vas deferens 8.14 ± 0.15 in 0.5 mg treated groups and in 0.75 mg treated rats shows 9.52 ± 0.27 in caput, 9.14 ± 0.18 in cauda of epididymis and in vas deferens 7.84 ± 0.11 is decreased, whereas alkaline phosphatase is increased. The surface epithelial cell height of these ducts is reduced and secretory activity is inhibited with the disruption of epithelial cell projections. The gravimetric and histometric changes of epididymis and vas deferens may be due to non-availability of androgens in morphine treated rats.

Key words: Morphine; Epididymis; Vas deferens; Rat

INTRODUCTION

Morphine, a narcotic analgesic opiate has been discovered by Sertuner in 1806. It binds to opioid receptors in Central Nervous System (CNS) and exerts its chief pharmacological actions on the central nervous system (Russell et al., 1989). Morphine administered to female rats during parturition inhibits the uterine contractibility, disturbs the normal maternal behavior in lactating rats (Russell and Spears, 1984). Like other CNS influencing drugs morphine is also known to inhibit the release of follicular stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary, acting through hypothalamus blocking the neural stimulus to the gonadotrophin releasing hormone (Blake, 1974; Blake, 1978; Anderson et al., 1982). According to Jaffe and Martin (1985), opioids act on the hypothalamus and inhibit the release of gonadotrophin releasing hormone (GnRH) and corticotrophin releasing factor (CRF), thus decreasing the circulating concentrations of LH, FSH, adrenocorticotrophic hormone (ACTH) and β-endorphin.

Epididymis is an important site at which the spermatozoa undergo progressive morphological and physiological changes. It provides a favourable milieu for acquiring the motility, fertilizing ability, storage and survival of spermatozoa (Kasturi et al., 1995). The vas deferens in addition to its role in
transport of spermatozoa is also involved in maturation and survival of spermatozoa (Orgebin-Crist, 1969; Orgebin-Crist and Danzo, 1975). Therefore, the epididymis and vas deferens instead of remaining mere passage ducts have an important role in male reproduction and fertility. Any drug influencing the CNS activities can also modify the function of gonads and in turn accessory organs. As the development and function of epididymis and vas deferens depends directly on gonadal activities, it is of interest to study the effect of morphine on the accessory reproductive organs.

MATERIALS AND METHODS

Healthy male albino rats of Wistar strain from inbred colony, weighing 180 - 200 g, of 80 - 90 days old were maintained at room temperature of 20 ± 28°C with lighting schedule of 12 h light and 12 h darkness. They were maintained in individual cages and divided in groups each containing six animals and fed with balanced diet as described by CFTRI (Central Food and Technological Research Institute) Mysore, Karnataka, India and water ad libitum.

The animals were divided into following groups

- Group-I: Received 0.2 ml saline/100 g body weight i.p. for 30 days and served as control.
- Group-II: Received 0.5 mg morphine/100 g body weight i.p. for 30 days in 0.2 ml saline.
- Group-III: Received 0.75 mg morphine/100g body weight i.p. for 30 days in 0.2 ml saline.

All the animals were sacrificed by cervical dislocation after 24 h of the last injection. The epididymis and vas deferens were dissected out immediately and separated from adherent tissue, weighed up to the nearest mg on electronic balance. Organs from one side of each animal were fixed in Bouin's fluid for histological studies. They were embedded in paraffin, sectioned at 5 µ, stained with Ehrlich hematoxylin and Eosin. The micrometric measurements like diameter of epididymis, its epithelial cell height and lumen diameter of vas deferens were made from randomly chosen 20 sections appearing round at cross sections from each group using ocular and stage micrometers. The sperm count from cauda epididymis was done by using haemocytometer (Kempinas and Lamano-Carvalho, 1987). Organs from other side were used for biochemical estimations.

The total cholesterol (Peters and Vanslyke, 1946), protein (Lowry et al., 1951), nucleic acids (Glick, 1985) and phosphatases (Toussaky and Shorr, 1953) of epididymis and vas deferens were estimated and “Student’s t-test” was employed for statistical analysis.

RESULTS

Changes in the epididymis

Gravimetric changes (Table 1)

The weight of caput and cauda epididymis is decreased significantly \((P < 0.05)\) with 0.5 mg of morphine treatment and highly significantly \((P < 0.001)\) with 0.75 mg of morphine treatment when

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight of epididymis (mg/100 g body weight)</th>
<th>Diameter (µm)</th>
<th>Epithelial cell height (µm)</th>
<th>Caudal sperm count (million/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caput</td>
<td>Cauda</td>
<td>Caput</td>
<td>Cauda</td>
<td>Caput</td>
</tr>
<tr>
<td>Control</td>
<td>395.84 ± 2.05</td>
<td>289.68 ± 2.12</td>
<td>2,645.24 ± 25.5</td>
<td>2324.45 ± 22.26</td>
</tr>
<tr>
<td>0.5 mg</td>
<td>380.27 ± 3.01</td>
<td>277.52 ± 2.36</td>
<td>2,486.35 ± 22.54</td>
<td>2238.81 ± 25.32</td>
</tr>
<tr>
<td>0.75 mg</td>
<td>352.40 ± 6.10</td>
<td>263.45 ± 3.20</td>
<td>2,291.73 ± 29.80</td>
<td>2,214.27 ± 18.09</td>
</tr>
</tbody>
</table>

Dose: 0.5 mg and 0.75 mg/100 g body weight. Duration: 30 days. Minimum 6 animals were maintained in each group. Each datum represents the mean ± S.E. \(^* P < 0.05, ^{**} P < 0.01\).
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Biochemical changes (Table 3)

Protein
There is significant \((P < 0.005)\) decrease in the protein content of caput and cauda epididymis due to 0.5 mg morphine treatment, but it is highly significant \((P < 0.001)\) due to 0.75 mg morphine treatment.

Cholesterol
The total Cholesterol content of caput and cauda epididymis is increased significantly \((P < 0.05)\) with 0.5 mg morphine treatment and highly significantly \((P < 0.01)\) with 0.75 mg morphine treatment when compared to control group.

Nucleic acids
The DNA and RNA content of caput and cauda epididymis is reduced almost significantly \((P < 0.005\) with 0.05) with 0.5 mg morphine treatment and highly significantly \((P < 0.001)\) with 0.75 mg morphine administration.

Phosphatases
The acid phosphatase is decreased significantly \((P < 0.05)\) in caput and non-significantly in cauda epididymis due to 0.5 mg morphine administration. But highly significant reduction \((P < 0.01)\) in caput and significant \((P < 0.005)\) reduction in cauda epididymis is observed with 0.75 mg morphine treatment. There is a significant increase \((P < 0.05)\) in alkaline phosphatase activity of caput and non-significant increase in cauda epididymis with 0.5 mg morphine treatment. Highly significant \((P < 0.01)\) increase in both caput and cauda epididymis is observed after the administration of 0.75 mg morphine.

Histometric changes (Table 1)
The epithelial cells of saline treated control group are tall, healthy, columnar and filled with secretory material. But those of morphine treated cells are smaller, disrupted and are with less secretory material. The diameter of caput epididymis is reduced almost significantly \((P < 0.05)\) with 0.75 mg morphine treatment.

Sperm morphology and number
The cauda epididymal sperms of normal rat shows sickle shaped head and straight tailpiece. But in morphine treated rats the sperms are abnormal as their head region reduced and the tail is wrinkled or coiled. A significant reduction \((P < 0.05)\) in the sperm count of cauda epididymis with 0.5 mg morphine and highly significant \((P < 0.01)\) reduction with 0.75 mg morphine is observed.

Changes in vas deferens

Gravimetric changes (Table 2)
A non-significant decrease with 0.5 mg morphine and significant decrease \((P < 0.01)\) with 0.75 mg morphine is observed in the weight of vas deferens.

Biochemical changes (Table 3)
Protein
There is non-significant decrease in the protein content of vas deferens due to 0.5 mg morphine treatment is observed but it is almost significant \((P < 0.05)\) due to 0.75 mg of morphine treatment.

Table 2. Effect of morphine on gravimetric and histometric changes of vas deferens

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight of Vas Deferens (mg/100 g body weight)</th>
<th>Diameter (µm)</th>
<th>Epithelial cell height (µm)</th>
<th>Diameter of Lumen (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>83.19 ± 1.8</td>
<td>278.00 ± 22.13</td>
<td>28.00 ± 1.28</td>
<td>225.32 ± 2.61</td>
</tr>
<tr>
<td>0.5 mg</td>
<td>76.85 ± 1.99</td>
<td>251.62 ± 26.00</td>
<td>27.16 ± 1.24</td>
<td>236.20 ± 4.25</td>
</tr>
<tr>
<td>0.75 mg</td>
<td>52.31 ± 1.60**</td>
<td>200.28 ± 15.10**</td>
<td>21.38 ± 1.05**</td>
<td>243.21 ± 3.55**</td>
</tr>
</tbody>
</table>

Dose: 0.5 mg and 0.75 mg/100 g body weight. Duration: 30 days. Minimum 6 animals were maintained in each group. Each datum represents the mean ± S.E. *\(P < 0.05\), **\(P < 0.01\).
Though the total cholesterol content of vas deferens is increased with both the doses, it is significant \( (P < 0.01) \) only with 0.75 mg morphine treatment.

Nucleic acids
The DNA and RNA content of vas deferens is reduced in both the treated groups, but it is significant \( (P < 0.05) \) only with 0.75 mg morphine treatment.

Phosphatases
Acid phosphatase activity of vas deferens is significantly \( (P < 0.01) \) reduced but the alkaline phosphatase activity is significantly increased \( (P < 0.01) \) with 0.75 mg morphine treatment. Similar changes are observed with 0.5 mg morphine administration.

Fig. 1. Cross section of the cauda epididymis of normal adult rat showing tall healthy and secretary epithelial cells. The surface villi are projecting towards the lumen. The nuclei of the cells give pseudostratified appearance and spermatozoa are spermatozoa are clearly seen in the lumen. H & E Staining (400×). AC, Apical Cell; BC, Basal Cell; HC, Halo Cell; L, Lumen; N, Nucleus; PC, Principal Cell; SP, Spermatozoa; V, Villi.

Histometric changes (Table 2)
Morphine administration has caused the reduction in the diameter of vas deferens and its epithelial cell eight, thereby increasing the lumen diameter.

Table 3. Biochemical changes in epididymis and vas deferens

<table>
<thead>
<tr>
<th>Contents</th>
<th>Groups</th>
<th>Epididymis</th>
<th>Vas deferens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Caput</td>
<td>Cauda</td>
</tr>
<tr>
<td>Protein (mg/100 mg)</td>
<td>Control</td>
<td>16.46 ± 0.22</td>
<td>15.80 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>0.5 mg</td>
<td>15.78 ± 0.16</td>
<td>13.82 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>0.75 mg</td>
<td>15.23 ± 0.09</td>
<td>12.56 ± 0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>146.45 ± 2.21</td>
<td>100.05 ± 2.12</td>
</tr>
<tr>
<td>Cholesterol (µg/100 mg)</td>
<td>Control</td>
<td>158.89 ± 3.13</td>
<td>124.56 ± 2.64</td>
</tr>
<tr>
<td></td>
<td>0.5 mg</td>
<td>178.58 ± 5.11</td>
<td>149.11 ± 20.20</td>
</tr>
<tr>
<td></td>
<td>0.75 mg</td>
<td>186.10 ± 2.91</td>
<td>162.30 ± 3.21</td>
</tr>
<tr>
<td>DNA (µg/100 mg)</td>
<td>Control</td>
<td>173.61 ± 4.00</td>
<td>150.00 ± 3.11</td>
</tr>
<tr>
<td></td>
<td>0.5 mg</td>
<td>165.00 ± 2.21</td>
<td>146.22 ± 3.20</td>
</tr>
<tr>
<td></td>
<td>0.75 mg</td>
<td>824.10 ± 4.72</td>
<td>735.02 ± 2.31</td>
</tr>
<tr>
<td>RNA (µg/100 mg)</td>
<td>Control</td>
<td>835.01 ± 9.50</td>
<td>758.60 ± 4.23</td>
</tr>
<tr>
<td></td>
<td>0.5 mg</td>
<td>824.10 ± 4.72</td>
<td>735.02 ± 2.31</td>
</tr>
<tr>
<td></td>
<td>0.75 mg</td>
<td>9.52 ± 0.27</td>
<td>9.14 ± 0.18</td>
</tr>
<tr>
<td>Acid Phosphatase (µg/100 g)</td>
<td>Control</td>
<td>5.75 ± 0.10</td>
<td>5.33 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>0.5 mg</td>
<td>6.26 ± 0.12</td>
<td>5.67 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>0.75 mg</td>
<td>6.64 ± 0.09</td>
<td>6.21 ± 0.16</td>
</tr>
</tbody>
</table>

Dose: 0.5 mg and 0.75 mg/100 g body weight. Duration: 30 days. Minimum 6 animals were maintained in each group. Each datum represents the mean ± S.E. *\( P < 0.05 \), **\( P < 0.01 \).
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These changes are significant only with 0.75 mg morphine administration.

**DISCUSSION**

The male accessory reproductive organs play an important role in the sperm maturation, motility and formation of semen (Orgebin-Crist, 1969; Hamilton, 1975; Martin and Sloan 1977). Spermatozoas formed in the seminiferous tubules are transported from the testis into epididymis and remain in the duct system for varying periods of time before being ejaculated (Gaddum and Glover, 1965). During this period they acquire motility and fertilizing capacity in the epididymis (Gaddum and Glover, 1965).

Opioids by acting through the hypothalamus inhibit the release of GnRH and CRF, thus decreasing the circulating concentrations of LH, FSH and ACTH (Reisine and Pasternak, 1996). The epididymis and the accessory glands of reproduction depend on testicular androgens (Price and William Ashman, 1961). Testosterone being an important factor for the maintenance of accessory sex organs (Ojeda and Urbanski, 1994). In turn the synthesis and release of androgens depends on the availability of pituitary gonadotrophins like FSH and LH/ICHS (Connel and Eik-Nes, 1868; Johnson and Ewing, 1971; Hanson et al., 1973).

Morphine being a µ-opioid receptor against inhibits the release of pituitary gonadotrophin (Barraclough and Sawyer, 1955; Bruni et al., 1977), which is essential for the androgen synthesis. Therefore, reduction in the weight of epididymis and vas deferens is observed in the present study. As the androgen regulate the synthesis of DNA, RNA and protein (Brooks, 1981; Duggan and North, 1983; Martin, 1984). The decrease in the DNA, RNA and protein contents after morphine treatment supports the reduced androgen productivity by the testis. Significant increase in the cholesterol content of these ducts indicates the hampered conversion of androgen from cholesterol, which is dependent on the availability of pituitary gonadotrophins.

Decrease in the acid phosphatase activity and increase in the alkaline phosphatase activity after morphine treatment may be due to decline in the endogenous androgen production (Kasturi et al., 1995). It is also one of the factors for the suppression of spermatogenesis, which is evidenced by the lowered sperm count in the cauda epididymis after morphine treatment.

**REFERENCES**


