Responses of parathyroid gland, C cells, and plasma calcium and inorganic phosphate levels in rat to sub-lethal heroin administration

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Abstract: In order to record the effects of heroin on plasma calcium (Ca) and inorganic phosphate (Pi) levels as well as parathyroid gland and C cells, two sub-lethal doses (0.50 LD₅₀ and 0.75 LD₅₀) of the drug were administered intramuscularly in Rattus norvegicus for 30 days. Plasma Ca level of control rats ranged between 9.53±0.32 - 9.88±0.22 mg 100 ml⁻¹ while plasma Pi concentration fluctuated between 4.55±0.18 - 4.71±0.24 mg 100 ml⁻¹. Sub-lethal heroin administration induced progressive increase in plasma Ca level during the first seven days (p<0.001), thereafter the level declined on day 15 and 30. However, plasma Pi level of the heroin-treated rats registered increase with the peak value (p<0.001) on day 30. The treatment elicited degenerative changes in parathyroid gland as evident by cytoplasmic vacuolation, presence of more pyknotic nuclei and occurrence of patchy areas among the chief cells. Degenerative changes were also noticed in crista of mitochondria, Golgi complex and endoplasmic reticulum. There was decrease in chromatin material in the nucleus and loss of hormone granules in the cytoplasm. Oxyphil cells of the heroin-treated rat depicted dilation of endoplasmic reticulum and damaged cristae. Sub-lethal heroin administration in the rat for 30 days induced dilation in endoplasmic reticulum and loss of secretory granules in C cells.

Key words: Heroin, Plasma calcium, Plasma inorganic phosphate, Parathyroid gland, Oxyphil cells, C cells, Rattus norvegicus

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Introduction

Heroin abuse is the burning problem of the society world over and associated with multiple poor health manifestations, reduced quality of life and encourage criminal behaviour (Sawynok, 1986; Neri-Semer and Modesti, 1991; Sporer, 1999; Cami and Farre, 2003; Brown et al., 2006; Barai et al., 2009). The drug (diacetylmorphine) is initially metabolized to 6-acetylmorphine and subsequently to morphine in human body (Sawynok, 1986; Goldberger et al., 1994; Jenkins et al., 1994). Chronic abuse of heroin has diverse effects on various body systems due to widespread distribution of specific receptors in many tissues and organs (Martin, 1984; Sawynok, 1986; Hashemi et al., 2006). Nephrototoxic and hepatotoxic effects of the drug have been documented in mammals (Weller et al., 1984; Gomez-Lechon et al., 1987; Kringsholm and Christoffersen, 1989, Barai et al., 2004a). Hematological as well as biochemical profiles of serum to exogenous drug administration have also been recorded (Hussain and Kumar, 1988; El-Daly, 1994; Barai et al., 2004b). Furthermore, there are few reports suggesting that the chronic heroin administration/addiction modulates hypothalamo-pituitary-gonadal (HPG) as well as hypothalamo-pituitary-adrenal (HPA) axes in mammals (Pechnick, 1993; Spanagel, 1999; Samyai et al., 2001; Laorden et al., 2002; George et al., 2005; Blesener et al., 2005; Brown et al., 2006). Though there are a few records on the altered serum calcitonin level and bone metabolism in heroin addicts (Tagliaro et al., 1984; Spagnolli et al., 1987, 1988; Pedrazzoni et al., 1993; Kim et al., 2006), the observations are highly contradictory (Tagliaro et al., 1992). An attempt has, therefore, been made to record the changes occurring in plasma Ca and Pi levels as well as parathyroid gland and calcitonin-producing C cells of rat, Rattus norvegicus, in response to sub-lethal heroin administration.

Materials and Methods

Healthy male rat, R. norvegicus weighing 150-200 g were procured from the Brihanmumbai Municipal Corporation, Mumbai. They were acclimatized under the ambient laboratory conditions (temperature 28±2°C; photoperiod 14L:10 D) for 10 days, fed ad libitum on rat feed (Lipton, Bangalore) and clean water was provided for drinking. 60 male rats were randomly selected and divided into three equal groups - two experimental and one control. Heroin was dissolved initially in small quantity of alcohol and diluted with physiological saline to prepare two test doses - 0.50 LD₅₀ (10.9 mg kg⁻¹) and 0.75 LD₅₀ (16.4 mg kg⁻¹). The drug was administered through intramuscular route to the experimental rats while the control rats received equal volume (0.2 ml kg⁻¹ body weight) of the physiological saline. Animals from both the groups were killed on day 1, 7, 15 and 30 of the treatment. Blood samples were
examined under the light microscope. For electron microscopy, samples were cut from the selected area with glass knife and mounted on a specimen holder. The sections were also stained with toluidine blue and examined under the light microscope. For electron microscopic observations, the tissues were fixed in 3% glutaraldehyde maintained at 4°C. They were washed thoroughly with 0.1N cacodylate buffer to remove traces of glutaraldehyde. Ultrathin sections (600-800Å) were cut from the selected area with glass knife and mounted on 400 mesh copper grids. The tissues were double stained with 10% alcoholic uranyl acetate for 20 min and with Reynold’s lead citrate for 10 minutes. Sections were scanned under Jeol-100 electron microscope.

Table - 1: Effects of heroin administration on plasma calcium and inorganic phosphate levels (mg 100 ml⁻¹) of rat, Rattus norvegicus.

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration (days)</th>
<th>Plasma calcium</th>
<th>Plasma inorganic phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Control</td>
<td>9.88±0.22</td>
<td>9.64±0.16</td>
<td>9.69±0.57</td>
</tr>
<tr>
<td>Heroin (0.50 LD₅₀)</td>
<td>10.82±0.31⁷ (+9)</td>
<td>12.16±0.22⁷ (+26)</td>
<td>9.02±0.12⁷ (-7)</td>
</tr>
<tr>
<td>Heroin (0.75 LD₅₀)</td>
<td>10.93±0.28⁷ (+11)</td>
<td>12.71±0.28⁷ (+32)</td>
<td>9.24±0.35⁷ (-5)</td>
</tr>
<tr>
<td>Control</td>
<td>4.62±0.21</td>
<td>4.68±0.32</td>
<td>4.55±0.18</td>
</tr>
<tr>
<td>Heroin (0.50 LD₅₀)</td>
<td>5.31±0.28⁷ (+15)</td>
<td>5.68±0.28⁷ (+21)</td>
<td>6.25±0.21⁷ (+37)</td>
</tr>
<tr>
<td>Heroin (0.75 LD₅₀)</td>
<td>5.24±0.36⁷ (+13)</td>
<td>5.83±0.14⁷ (+25)</td>
<td>6.35±0.35⁷ (+40)</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 5 specimens. Values in parentheses indicate % increase (+) or decrease (-) over control. Significance: *p <0.05, **p <0.01; ***p <0.001

Results and Discussion

Variations in plasma calcium (Ca) and inorganic phosphate (Pi) levels of Rattus norvegicus in response to sub-lethal heroin administration have been summarized in Table 1. Plasma Ca level of control rats ranged between 9.53±0.32 to 9.88±0.22 mg 100 ml⁻¹ while Pi concentration fluctuated between 4.55±0.18 and 4.71±0.24 mg 100 ml⁻¹. Sub-lethal heroin administration elicited significant increase in plasma Ca level during the first seven days (p<0.001), thereafter the level registered a steady decline approaching normocalcemia on day 15 while by day 30, frank hypocalcemia was observed in both the experimental groups. However, plasma Pi level of the heroin-treated rats registered a progressive increase (p <0.05) by 24 hr and attained the peak (p <0.001) on day 30.

Parathyroid glands of control rat, consisted mainly of chief cells arranged in elongated and branching cords, separated by connective tissue stroma, capillaries and sinusoids (Fig. 1). Ultrastructurally, they were bound by unit membranes and...
Fig. 3: Chief cells of control rat depicting nucleus (N), nucleolus (nu), mitochondria (M), lipid droplet (L), dense body (db) and rough endoplasmic reticulum x 3,000

Fig. 4: Chief cell of control rat showing prominent nucleus (N), rough endoplasmic reticulum (RER), Golgi complex (G), mitochondria (M) and multi-vesicular body (MVb) x 15,000

Fig. 5: Chief cell of rat treated with heroin (0.75 LD₅₀) for 30 days exhibiting part of nucleus (N), rough endoplasmic reticulum (RER), lipid droplet (L) and disintegrated mitochondria (M) x 15,000

Fig. 6: Oxyphil cell of control rat depicting nucleus (N), dense body (db), mitochondria (M) and Golgi complex (G) x 8,000

Fig. 7: Oxyphil cell of rat treated with heroin (0.75 LD₅₀) for 30 days showing mitochondria with damaged cristae (M), dilated endoplasmic reticulum (ER) and lipid body (L) x 10,000

Fig. 8: C cell of control rat exhibiting prominent nucleus (N), mitochondria (M), multi-vesicular body (MVb) and large number of secretory granules (Sg) x 6,000
showed clear desmosomes and terminal bars joining the plasma membranes. The nucleus was large, spherical or oval structure containing many small granules concentrated more towards the periphery. The cytoplasm contained prominent rough endoplasmic reticulum and Golgi apparatus was composed of straight or curved stacks of membranes with small vesicles and granules. Mitochondria were distributed throughout the cytoplasm. Only a few electron dense secretory granules could be seen in cytoplasm. Besides these, lipid, glycogen and lysosomal bodies were also noticed in the chief cells (Fig. 3, 4). Sub-lethal heroin administration for 30 days induced degenerative changes in the parathyroid gland as evident by cytoplasmic vacuolization, presence of more pyknotic nuclei and occurrence of patchy areas among the chief cells (Fig. 2). Degenerative changes were also noticed in cristae of mitochondria, Golgi complex and endoplasmic reticulum. There was decrease in chromatin material in nucleus and loss of hormone granules in cytoplasm of the chief cells (Fig. 5).

A few oxyphil cells, polygonal in shape and larger than the chief cells, were also encountered in the parathyroid gland. Their nuclei were smaller, irregular and denser than those of the chief cells. The abundant cytoplasmic area of the oxyphil cells was filled with numerous large mitochondria. The endoplasmic reticulum, Golgi apparatus and secretory granules were poorly developed in these cells (Fig. 6). Oxyphil cells of the heroin-treated rats depicted dilatation of endoplasmic reticulum and damaged cristae of mitochondria on day 30 (Fig. 7).

Calcitonin-producing C cells of the rat were unevenly distributed in thyroid follicular cells. They were larger in size with more electronlucent cytoplasm as compared to those of thyroid follicular cells. Ultrastructurally, they possessed conspicuous endoplasmic reticulum, prominent Golgi apparatus, numerous mitochondria (both circular and elongated types) and large number of electron dense secretory granules in their cytoplasm (Fig. 8). In a few cells, desmosomes and terminal bars were also encountered. Sub-lethal heroin administration in the rat for 30 days induced dilatation in endoplasmic reticulum and loss of secretory granules (Fig. 9). It is pertinent to remark that the degenerative changes in these glands were more pronounced in higher dose (0.75 $LD_{50}$) of heroin as compared to those given 0.50 $LD_{50}$ dose.

Parathyroid glands made their first phylogenetic appearance only in tetrapods (Clark et al., 1986; Pandey, 1991, 1992), probably to protect against the development of hypocalcaemia and to maintain skeletal integrity in terrestrial animals (Wendalaar Bonga and Pang, 1991; Pandey, 1992). Parathyroid hormone (PTH) is a predominant hypercalcemic and hypophosphatemic factor which controls the plasma Ca and Pi metabolism of mammals in concert with calcitonin (CT) and 1,25-dihydroxyvitamin D$_3$ (active metabolite of vitamin D$_3$). These hormones exert their control in an integrated manner through three main processes: (i) the balance between the rate of deposition and mobilization of Ca and Pi in the bone (reservoir), (ii) the urinary excretion of Ca and Pi and (iii) the absorption of Ca and Pi from the gastro-intestinal tract (Tayler, 1984; Pang and Schreibman, 1989; Wendalaar Bonga and Pang, 1991; Aurbach et al., 1992; Dacke et al., 1996; Dacke, 2000). There exist limited observations on the effects of opioid drugs on calcium-regulating hormones suggesting that the circulating levels of calcitonin may be higher in heroin addicts (Tagliaro et al., 1984, 1985; Spagnoli et al., 1987, 1988), however, Tagliaro et al. (1992) observed increase only in "calcitonin-like" material, but not immunoreactive calcitonin, in such subjects. The opioids may play a role in regulation of calcitonin secretion in rat (Gozariu et al., 1985). In the present study, sub-lethal heroin administration in rat for 30 days elicited degenerative changes in chief and oxyphil cells of the parathyroid gland as well as calcitonin-producing C cells. Conversely, Padrazzoni et al. (1993) recorded increased blood ionized and urinary calcium in heroin addicts. Though oxyphil cells made their first pyletic appearance in parathyroid gland of mammals, its function remains obscure except an increase in number with advancing age (Roth and Schiller, 1976; Setoguti, 1977; Clark et al., 1986). The observed hypocalcaemia and hyperphosphataemia in the rat due to prolonged sub-lethal heroin administration appears to be due to degenerative changes in the chief cells which are the source of parathyroid hormone (PTH) in mammals.

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References


