On the Degranulation of Mesenteric Mast Cells caused by Morphine and Meperidine Hydrochloride in White Rats

Kyu Sik Lee and Soo Yun Pak

Department of Anatomy
Yonsei University College of Medicine, Seoul, Korea

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ABSTRACT

Histological studies were carried out on the degranulation of mesenteric mast cells of white rats caused by injections of morphine hydrochloride and meperidine hydrochloride intravenously, intraperitoneally, and by local injection of the rat's mesentery and the following conclusions were obtained.

1. In the groups of intravenous, intraperitoneal, and local injections of morphine hydrochloride, a fairly significant degradation of the mesenteric mast cell was observed, which was probably associated with the concomitant liberation of tissue histamine derived from its source.

2. In the groups of intravenous and intraperitoneal injections of meperidine hydrochloride, the significant degradation of the mesenteric mast cell was recognized. However, the local injections displayed no cytological change of the cell and no increased permeability of dermal capillaries was observed at the injecting site.

3. The degranulation of the mesenteric mast cell followed by an administration of meperidine hydrochloride was effectively inhibited after an adrenalectomy.

INTRODUCTION

The tissue mast cells were so named by Ehrlich (1879) and many investigators have tried to make a distinction between the activities of the cells as a whole and the physiological activities and relation of those granules.

Metachromatic granules of tissue mast cells were readily diffused from the cells by means of various chemical and physical inducers, and biological agents. The physiological implications relative to distribution of mast cells are important because mast cells are thought to store the components for a readily available supply of heparin (Holmgren and Wilander, 1937), hyaluronic acid (Asboe-Hansen, 1952), histamine (Riley and West, 1953), serotonin (Benditt et al. 1955), and other polysaccharides and enzymes.

Fawcett (1954a, 1954b, 1955), Parratt and West (1957a) and Klaus and Winkelmann (1959) demonstrated that the release of mast cell granules by experimental means is accompanied by the release of histamine. It has repeatedly been shown that injection of histamine liberators, such as compound 48/80, reserpine and polymyxin B caused the degranulation of mast cells associated with the release of histamine in the connective tissue.

It has been shown also that the opium alkaloids and the morphine derivative, apomorphine, have to be added to the class of histamine liberators. Goodman and Gillman (1941), Feldberg and Paton (1950, 1951), Nasmyth and Stewart (1950), Evans et al. (1952), and Parratt and West (1956) demonstrated that in experimental animals, either
opium alkaloids or chemical histamine liberators injected intravenously, caused a fall in arterial blood pressure and increased plasma histamine levels simultaneously. Lee (1965) described that the administration of morphine and meperidine induced vasodilatation, which was associated with a fall of blood pressure, due to histamine action.

Based upon above mentioned researches, it is well known that various histamine liberators induce degranulation and disruption of tissue mast cells and some narcotics release histamine.

This study was made to determine the effects of morphine which is classified as a natural narcotic and meperidine as a synthetic narcotic to induce degranulation of mesenteric mast cells and to compare the effects of both narcotics upon mesenteric mast cells and the permeability of dermal capillary of the white rat.

MATERIALS AND METHODS

Experimental animals used in this study were 72 healthy mature white rats consisting of 52 males and 20 females weighing about 200 grams. Numbers of experimental animals allocated for each group, dosages of natural and synthetic narcotics given to each group, and dosages of meperidine hydrochloride given to the group of adrenalectomized rats are listed in Table 1 and 2.

For degranulation of mast cells due to intravenous and intraperitoneal administrations of meperidine hydrochloride which may have an indirect effect through certain action on the adrenal glands, a group of adrenalectomized rats was added in this experimental series.

For an adrenalectomy the rat was lightly anesthetized with ether, the back skin, just below the 12th rib, was shaved, and prepared for a dorsal approach to the retroperitoneal space. A transverse incision was made just below the 12th rib and the erector spinae muscle was vertically split, the adrenal glands were exposed and completely excised one by one. When adrenalectomized rats were sacrificed, a search was made for any remaining adrenal tissue or adrenal tissue grown in the abdominal cavity. Adrenalectomized rats were available for use in this experiment 2 weeks after the removal of the glands. In the post-operative period, 1 per cent saline solution instead of tap water was supplied ad libitum to the rats in order to compensate for the loss of sodium. For intravenous injection tail veins were used.

To study the direct effect of narcotics on mesenteric mast cells to cause degranulation of mast cells, the local injection of narcotics into the mesentery was carried out by insertion of 26 gauge needle into the fat tissue of the mesentery attached to the small intestine or into the perivascular fat of the mesenteric visceral vessels after an incision was made in the anterior median area of the abdominal wall under light ether anesthesia. Mast cells near the locally injected site were examined for degranulation of metachromatic granules of mast cells.

The rats injected intravenously or locally were sacrificed after 2 hours and those given drugs intraperitoneally were slaughtered after 4 hours. To induce a morphological change of mesenteric mast cells or degranulation of the cells by means of an administration of histamine liberator the authors thought that, from the preliminary study, the durations of 2 hours after local and intravenous injections and 4 hours after intraperitoneal injection respectively, were suitable.

By occipital blows the rats were sacrificed and absolute methanol was, infused into the peritoneal cavity through a small incision of the anterior median abdominal wall and then fixed for 20 minutes in situ in order to reduce the direct mechanical injury to the mesenteric mast cells. A few pieces of the mesentery obtained gently were stained with Pugh solution for 3 minutes, a technique which was used by Leblanc and Rosenberg (1957) for metachromatic granule of mast cells. The tissue was then prepared for the permanent slides through ordinary histological procedures.

In the groups of the intravenous and intraperito-
Table 1. Dosages of narcotics and vehicles given to each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Intravenous injection</th>
<th>Intraperitoneal injection</th>
<th>Local injection</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Nos. of rats</td>
<td>Dosages</td>
<td>Nos. of rats</td>
</tr>
<tr>
<td>Morphine HCl group</td>
<td>9</td>
<td>1 ml (12 mg./kg.) of morphine HCl in normal saline solution</td>
<td>4</td>
</tr>
<tr>
<td>Meperidine HCl group</td>
<td>5</td>
<td>1 ml (2.4 mg./kg.) of meperidine HCl in normal saline solution</td>
<td>5</td>
</tr>
<tr>
<td>Control group</td>
<td>5</td>
<td>1 ml of normal saline solution</td>
<td>5</td>
</tr>
</tbody>
</table>

Morphine hydrochloride (Evans Medical Supply Ltd. Liverpool and London)
Meperidine hydrochloride (Winthrop Laboratories, New York)

Table 2. Dosages of narcotics and vehicles given to the adrenalectomized group

<table>
<thead>
<tr>
<th>Group</th>
<th>Intravenous injection</th>
<th>Intraperitoneal injection</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Nos. of rats</td>
<td>Dosages</td>
</tr>
<tr>
<td>Meperidine HCl group of an adrenalectomized rat</td>
<td>5</td>
<td>1 ml (2.5 mg./kg.) of meperidine HCl in normal saline solution</td>
</tr>
<tr>
<td>Control group of an adrenalectomized rat</td>
<td>4</td>
<td>1 ml of normal saline solution</td>
</tr>
</tbody>
</table>

neal injections, the degrees of degranulation of mast cells were divided into 4 grades by the criteria of An (1964) as follows: 1. the normal type of mast cell which displays a round form mostly (Fig. 1). The mast cell showing one or two extracellular metachromatic granules in the vicinity of it, was described as the normal type, 2. the grade I type or slight degranulation of a mast cell showing a few to several metachromatic granules in the vicinity of it (Fig. 2), 3. the grade II type or moderate degranulation of a mast cell whose contour is clear (Fig. 3), and 4. the grade III type, severe degranulation or disruption of a mast cell in which the clear contour of the cell is hard to indentify due to severe degranulation or a disruption of the mast cell (Fig. 4).

For the potency of morphine hydrochloride and meperidine hydrochloride as histamine liberators the method used by Lee et al. (1960), which is a modification of the method devised by Miles and Miles (1952), was applied in this study.

RESULTS

1. The experimental groups with intravenous injections.

a. The group given 12 mg./kg. of morphine hydrochloride intravenously:

In the rats of this group there was a significant increase of slightly degranulated mesenteric mast cells (Grade I type of mast cell) present in 14.0±0.17 per cent of the cells, compared with an incidence of 2.2±0.08 per cent of slightly degranulated ones occurring in the control group. This result may indicate that morphine hydrochloride injection has a degranulating effect on the mast cell of the rat’s mesentery.

b. The group given 2.4 mg./kg. of meperidine hydrochloride intravenously:

There was a significant increase of slightly degranulated mast cells, 22.8±0.22 per cent compared with the control result of 2.2±0.08 per cent. Also this result may prove that meperidine hydrochloride injection causes a degranulation of mesenteric mast cells as well as morphine hydrochloride.

c. The control group given normal saline solution intravenously:

In this group the majority of the mesenteric mast cells, 97.8±0.08 per cent displayed a normal
type. The slight degree of its degranulation, which showed a 2.2±0.08 per cent incidence of the grade I type of mast cells, is probably considered due to the effect of stress caused from pain of the injection without anesthesia.

2. The experimental group for intraperitoneal injection

a. The group given 50 mg./kg. of morphine hydrochloride intraperitoneally:

In this group there was a slight increase of grade I type of a mast cell with an incidence of 6.7±0.14 per cent compared with 3.2±0.12 per cent in the control group. Comparing the intraperitoneal administration with the result of the experimental group given morphine hydrochloride intravenously, the frequency of degranulated mast cells was less than noted following the intravenous injection probably due to dilution of morphine hydrochloride in Tyrode solution.

b. The group given 12.5 mg./kg. of meperidine hydrochloride intraperitoneally:

Following administration of meperidine hydrochloride, there was a significant increase of grade I type mast cells, 18.9±0.22 per cent of the total mast cells, compared with 3.2±0.12 per cent of the control group. In this group the degranulating effect of meperidine hydrochloride administration upon the mast cell was also clearly proved.

c. The control group given Tyrode solution intraperitoneally:

In the control group the majority of the mesenteric mast cells were of normal type except for 3.2±0.12 per cent of the grade I type.

3. The experimental group for the local injection

a. The group given 0.1 mg. of morphine hydrochloride locally into the mesentery:

Several mast cells near the injecting site of the mesentery showed relatively evident morphological changes of slight degranulation and deformity of the cell contour compared with the normal one, while the mesenteric mast cell which is located far from the injecting site displayed no morphological changes at all. This finding suggests strongly that morphine hydrochloride affects the mast cell directly and causes degranulation of it.

b. The group given 0.5 mg of meperidine hydrochloride locally into the mesentery:

At the local site of the injection almost no cytological changes of mesenteric mast cells were observed in the rats of this group. This finding indicates strongly that the local injection of meperidine HCl does not affect the degranulation of the cell directly in such a dose.

c. The control group given normal saline solution locally into the mesentery:

The rats given normal saline solution locally displayed no degranulation of the cell in the neighbouring area of the injecting site.

4. The experimental group having an adrenalectomy prior to meperidine hydrochloride injection

a. The group given 2.5 mg./kg. of meperidine hydrochloride intravenously:

Normal or intact mesenteric mast cells of the adrenalectomized rats in this group occurred in an incidence of 92.9±0.12 per cent compared with the result of the control adrenalectomized rats in which the incidence of normal or intact mesenteric mast cells was about 92.2±0.17 per cent. By this comparison, results showed there was no difference. Also an inhibition of the mast cell degranulation by means of an adrenalectomy was evidently found by comparison of the result of this group which occurred in an incidence of 6.6±0.12 per cent of the grade I type and that of the group given meperidine hydrochloride intravenously without an adrenalectomy which displayed an incidence of 22.8±0.22 per cent of the grade I type of degranulation.

b. The control group given normal saline solution intravenously:

In this group the slightly degranulated mast cell of the grade I type which occurred in an incidence of 7.6±0.17 per cent was higher than that of the group given normal saline solution intravenously without an adrenalectomy which had an incidence
of 2.2±0.08 per cent of the grade I type of the cell. This finding is considered to result from the increased susceptibility to stresses of the adrenalectomized rat when the injection was performed without any anesthesia.

c. The group given 12.5 mg./kg. of meperidine hydrochloride intraperitoneally and d. the control group given Tyrode solution intravenously:

In the former group the incidence of the slightly degranulated mast cell was about 6.7±0.13 per cent compared with 2.6±0.11 per cent in the later control group. There was no remarkable difference in the incidence between these results.

5. The experimental groups for study of the permeability of dermal capillaries by the administration of morphine and meperidine hydrochloride intradermally

At the injecting sites of histamine and morphine hydrochloride a remarkable dermal blueing was seen while no dermal reaction at the injection sites of normal saline solution and meperidine hydrochloride was observed at all.

DISCUSSION

Riley (1953a) reported that the origin of tissue mast cells and their relationship to blood vessels in the rat have been observed in intact tissue spreads from the subcutis, mesentery and omentum.

He added that there are two types of mast cells observed in all three sites: Type-I cells, found chiefly in the adventitia or vessels with muscle coats, often lying there in rows, which stain orthochromatically and so densely as to obscure the nucleus and Type-II cells, larger and filled with metachromatic granules, which are found mainly around capillaries and free in the tissue spaces. It is considered that Type-I orthochromatic perivascular cells are the early forms and that Type-II metachromatic cells are derived from them.

The occurrence of extracellular granules in the neighborhood of mast cells has often been considered as an artifact of specimen preparation or a degenerative phenomenon. However, Nakajima (1928), Asboe-Hansen (1950), Kelsall and Crab (1954) and Fawcett (1954a) have interpreted this occurrence as an evidence of physiological secretory activity. We have had experience with artificial damage (degranulation of metachromatic granules and disruption) of mesenteric mast cells in the case of roughly handled flat preparations of the rat as well as Mota et al. (1953) who described an artificial bursting of mesenteric mast cells during dissection.

Riley and West (1953) suggested that tissue mast cells are rich in histamine. Blaschkö (1956) and Schild (1956) indicated that most of the cellular contents of histamine, as well as of biogenic amines, is stored by being bound to intracellular cytoplasmic granules.

Table 3. Results of degranulation of mesenteric mast cells of rats injected with morphine and meperidine hydrochloride intraperitoneally and intravenously

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mast cells observed</th>
<th>Normal type(%)</th>
<th>Grade I type(%)</th>
<th>Grade II type(%)</th>
<th>Grade III type(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous injection</td>
<td></td>
<td></td>
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<tr>
<td>Morphine HCI group</td>
<td>41,164</td>
<td>85.5±0.17</td>
<td>14.0±0.17</td>
<td>0.4±0.03</td>
<td>0</td>
</tr>
<tr>
<td>Meperidine HCI group</td>
<td>36,104</td>
<td>74.5±0.22</td>
<td>22.8±0.22</td>
<td>2.3±0.07</td>
<td>0.3±0.03</td>
</tr>
<tr>
<td>Normal saline solution group</td>
<td>32,261</td>
<td>97.8±0.08</td>
<td>2.2±0.08</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adrenal-ectomized group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meperidine HCI group</td>
<td>39,631</td>
<td>92.9±0.12</td>
<td>6.6±0.12</td>
<td>0.3±0.03</td>
<td>0.04±0.1</td>
</tr>
<tr>
<td>Normal saline sol. group</td>
<td>23,315</td>
<td>92.2±0.17</td>
<td>7.7±0.17</td>
<td>0.09±0.02</td>
<td>0</td>
</tr>
<tr>
<td>Intraperitoneal injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine HCI group</td>
<td>28,021</td>
<td>90.5±0.17</td>
<td>6.7±0.14</td>
<td>0.8±0.05</td>
<td>1.9±0.08</td>
</tr>
<tr>
<td>Meperidine HCI group</td>
<td>30,030</td>
<td>77.4±0.24</td>
<td>18.9±0.22</td>
<td>2.7±0.09</td>
<td>1.0±0.05</td>
</tr>
<tr>
<td>Normal saline solution group</td>
<td>21,069</td>
<td>95.7±0.12</td>
<td>3.2±0.12</td>
<td>0.09±0.02</td>
<td>0</td>
</tr>
<tr>
<td>Adrenal-ectomized group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meperidine HCI group</td>
<td>33,596</td>
<td>92.8±0.13</td>
<td>6.7±0.13</td>
<td>0.3±0.03</td>
<td>0.1±0.01</td>
</tr>
<tr>
<td>Tyrode sol. group</td>
<td>18,021</td>
<td>97.3±0.21</td>
<td>2.6±0.11</td>
<td>0.09±0.02</td>
<td>0</td>
</tr>
</tbody>
</table>

M(%)±standard error(%)
Kelsall and Crabb (1959) stated the release of mast cell granules by experimental means is accompanied by the release of histamine. Histamine liberation associated with degranulation of mast cells has occurred when the injections of histamine liberators, such as compound 48/80 by Hunt and Hunt (1956), Fawcett (1954 b), Mota et al. (1953) and Riley (1956), stilbamidine by Mota et al. (1953), Riley (1953 b), Riley and West (1953, 1955), and ovomucin by Benditt et al. (1955) respectively, burst or explode the mast cells of rats.

Feldberg and Paton (1951), studying release of histamine from skin and muscle by administrations of opium alkaloids and other histamine liberators, suggested the release of histamine from the perfused tissues by histamine liberators occurs explosively.

Bhattacharya and Lewis (1956) postulated that the peritoneal mast cells of the rat show much more degranulation than do the subcutaneous mast cells after intraperitoneal injection of morphine. Parratt and West (1957 b) showed that intraperitoneal injection of morphine has but little damaging effect upon tissue mast cells and releases correspondingly little histamine in the rat.

In this study the authors have observed that intraperitoneal and intravenous injections of morphine hydrochloride caused obvious morphological changes of degranulation in many mesenteric mast cells of the rat and local injection of it into the mesentery displayed an evident degranulation of the cells which were located in the neighbourhood of the injecting site. Also the authors, studying the permeability of dermal capillaries with morphine hydrochloride injection, found that the dermal site given morphine hydrochloride intradermally showed an increased permeability of dermal capillaries probably due to local release of histamine from its source or reservoir which was scattered in the vicinity of the injecting site. By the above mentioned results of morphine hydrochloride injections in various sites it is deduced that the degranulation of the mast cell occurs at least from the direct action of morphine hydrochloride on the mast cell associated with possible release of histamine from its source. However, histamine released from the mast cell by the administration of morphine was not chemically proved.

In the experimental animals given a synthetic narcotic of meperidine hydrochloride the authors have found that intravenous and intraperitoneal injections of it caused a distinct degranulation of the mesenteric mast cell as well. However, the local injection of it into the mesentery showed no degranulation of the mast cells scattered in the vicinity of the injecting site and no sign of an increased permeability of the dermal capillary at the dermally injected site. Consequently the authors deduced that meperidine, a synthetic narcotic, affected indirectly the mast cell and caused its degranulation in comparison with the direct acting mechanism of morphine, a natural narcotic.

Räsänen (1961) reported that the administration of ACTH in intact rats caused almost complete degranulation of the mucosal mast cells and its degranulation effect was inhibited by an adrenalectomy. Oh et al. (1964) presented that the whole body irradiation by X-ray induced the degranulation of mesenteric mast cells of the rat and it’s degranulating effect upon mesenteric mast cells was markedly inhibited after the removal of the adrenal gland.

The authors, studying the inhibiting effect of the degranulation of the mast cell after an adrenalectomy, have found that the degranulating effect upon mesenteric mast cells by administration of meperidine hydrochloride was generally inhibited by the removal of the adrenal gland. By this result of meperidine hydrochloride injection it is easily deduced that the degranulating effect of meperidine hydrochloride upon mesenteric mast cells was indirectly carried out by some function of adrenal glands, as yet not clearly defined.

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Fig. 1. Normal type of rat's mesenteric mast cell. Stained with Pugh solution. 400×

Fig. 2. Grade I type of slight degranulation of rat's mesenteric mast cell. Stained with Pugh solution. 400×

Fig. 3. Grade II type of moderate degranulation of rat's mesenteric mast cell. Stained with Pugh solution. 400×

Fig. 4. Grade III type of marked degranulation or disruption of rat's mesenteric mast cell. Stained with Pugh solution. 400×

Fig. 5. Skin of the rat. The positive dermal reaction or dermal blueing at the injecting sites of histamine (H) and morphine HCl (M) are observed. While no dermal reaction at the injecting sites of normal saline solution is seen at all. Lee's modified method.

Fig. 6. Skin of the rat. No dermal reactions or blueing at the injecting sites of meperidine HCl (D) and normal saline solution except the site of histamine (H) are observed. Lee's modified method.