Yonsei Medical Journal
Volume 12, 1971

On the Degranulation of Rat's Mesenteric Mast Cells Caused by Morphine and Meperidine in Vitro

Hyun Sam Shin, Ho Suck Kang and Soo Yun Park

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

ABSTRACT

Histological studies were carried out on the degranulation of mesenteric mast cells of albino rats in which excised pieces of rat mesentery were incubated in media containing morphine and meperidine hydrochloride. The following conclusions were obtained.

1. The experimental dose of 0.04 mg./ml. of morphine hydrochloride in Tyrode solution for the incubated mesenteric pieces brought about the degranulation of mast cells.

2. The experimental dose of 0.04 mg./ml. of meperidine hydrochloride in Tyrode solution for the incubation of the mesenteric pieces did not effect the cytological changes of the mast cells.

3. By the addition of metabolic inhibitor such as iodoacetic acid to the incubating medium the degranulation of the mast cells was remarkably inhibited for the group in which the incubation was carried out for 20 minutes. However, the inhibition of the degranulation of the mast cells due to the metabolic inhibitor was abolished after 30 minutes of incubation.

Consequently the authors have demonstrated the effect of morphine hydrochloride in its ability to induce a degranulation of mesenteric mast cells in vitro.

INTRODUCTION

It is common knowledge that tissue mast cells contain heparin, histamine, hyaluronic acid, serotonin and other components. In cases of extreme need the cells will secrete these cytoplasmic contents. Under certain pathological conditions and by the use of experimental means as, for example, with an injection of histamine liberator mast cells will release cytoplasmic metachromatic granules or disrupt readily.

Junqeira and Beiguelman (1955) studied the action of compound 48/80, in vitro, at low concentrations, promoting a vigorous extrusion of rat's mesenteric mast cell granules. They observed that temperatures below 25 degrees C., acid pH of the incubating media, and several metabolic inhibitors prevented granule extrusion after the addition of compound 48/80. This consequently suggested that metabolic phenomena occurring in mast cells are of vast importance considering their response to the action of compound 48/80.

Lee (1968) and Park et al. (1970) studied the action of morphine and meperidine hydrochloride by means of intravenous injection. In those studies the degranulation of

mesenteric mast cells of albino rats was consequently induced by the administration of morphine and meperidine hydrochloride solution. However, the mechanism of the degranulation of mast cells due to a direct or indirect effect of these narcotics was not determined.

The present study of albino rats was undertaken to determine the effect of morphine and meperidine hydrochloride and to observe whether or not the metabolic inhibitor effects the action of narcotics on the degranulation and disruption of mesenteric mast cells in vitro.

MATERIALS AND METHODS

Experimental animals used in this study were 70 healthy mature male rats of Sprague-Dawley strain weighing approximately 200 Gm. each. For the purpose of studying the effects of morphine and meperidine hydrochloride on mesenteric mast cells in vitro, the experimental groups were divided as follows.

A. The groups incubated in morphine hydrochloride solution:

a. The experimental group consisting of pieces incubated at 0.01 mg./ml. of morphine hydrochloride in Tyrode solution.

b. The experimental group consisting of pieces incubated at 0.02 mg./ml. of morphine hydrochloride in Tyrode solution.

c. The experimental group consisting of pieces incubated at 0.04 mg./ml. of morphine hydrochloride in Tyrode solution.

d. The control group incubated in Tyrode solution.

B. The groups incubated in meperidine hydrochloride solution:

a. The experimental group consisting of pieces incubated at 0.02 mg./ml. of meperidine hydrochloride in Tyrode solution.

b. The experimental group consisting of pieces incubated at 0.04 mg./ml. of meperidine hydrochlorde in Tyrode solution.

c. The control group incubated in Tyrode solution

C. The groups incubated with morphine hydrochloride solution plus iodoacetic acid:

a. The experimental group consisting of pieces incubated at 0.02 mg./ml. of morphine hydrochloride plus 0.03×10^{-2} M of iodoacetic acid in Tyrode solution. The concentration of iodoacetic acid was that used by Junqeira and Beiguelman (1955) in their study of mast cells.

b. The experimental group consisting of pieces incubated at 0.02 mg./ml. of morphine hydrochloride in Tyrode solution.

c. The control group consisting of pieces incubated at 0.02 mg./ml. of morphine hydro-chloride in Tyrode solution.

d. The control group consisting of pieces incubated at 0.04 mg./ml. of morphine hydro-chloride in Tyrode solution.

In each experimental group mesenteric pieces were carefully excised from rats immediately after being sacrified by an occipital blow. Pieces of the various mesenteries were then immediately incubated in prewarmed Tyrode solution at 37 degrees C containing the above mentioned doses of morphine hydrochloride, meperidine hydrochloride and morphine hydrochloride plus iodoacetic acid for periods of 10, 20, and 30 minutes respectively. The pH of the incubating media was adjusted about 7.4 before the incubation and these were twice corrected at 10 and 20 minute-periods during the incubation. After the incubation the pieces were fixed in absolute methanol for 20 minutes and then washed lightly in distilled water.

Each piece of the mesentery was stained in Pugh's solution which had been used by LeBlanc and Rosenberg(1957). A permanent histological slide was prepared for observation using a light microscope at high power magnification.

For the different degrees of degranulation of mesenteric mast cells 4 grades of cytological changes were adapted by the criteria of An (1964) as follow:

1. The normal type of mast cell displays mostly a round form without any evident dispersion of metachromatic granules from the cell. However, the mast cells showing one or two extracellular metachromatic granules were also counted as normal types (Fig. 1).

2. The grade 1 type showing a slight degranulation of a mast cell with a few to several metachromatic granules in its vicinity (Fig. 2).

3. The grade 2 type showing a moderate degranulation of a mast cell with a clear contour of the cell (Fig. 3).

4. The grade 3 type having a severe degranulation or disruption of the mast cell in which the clear contour of the cell is difficult to identify due to the severe degranulation or disruption (Fig. 4).

In each experimental group about 10,000 to 14,000 mesenteric mast cells were observed and scrutinized into 4 types mentioned already.

RESULTS

A. The groups incubated in morphine hydrochloride solution.

In the groups slightly degranulated mast cells of the mesenteries incubated with 0.04 mg./ml. of morphine hydrochloride for 20 and 30 minutes occurred in relatively greater incidence than those results of the control and other groups. The results of slightly degranulated mast cells incubated with 0.04 mg./ml. of morphine hydrochloride for 20 and 30 minutes were in the incidence of $7.64\pm0.45\%$ and $5.92\pm0.35\%$ respectively. Additionally the result of moderately degranulated mast cells of the mesenteries incubated at 0.04 mg./ml. of morphine hydrochloride in Tyrode solution for 30 minutes was the greatest incidence of $1.53\pm0.18\%$ among those experimental groups.

By these results it is known that the experimental dose of 0.04 mg./ml. of morphine hydrochloride in Tyrode solution for the incubation of the mesenteric piece, in vitro, brings about the degranulation of the mast cells (Table 1).

B. The groups incubated in meperidine hydrochloride solution.

Among the cytological change of these groups slightly degranulated mast cells of the mesenteries incubated at 0.02 mg./ml. and 0.04 mg./ml. of meperidine hydrochloride in Tyrode solution for 30 minutes were in the incidence of $1.19\pm0.12\%$ and $2.87\pm0.49\%$ respectively. Additionally the latter result of $2.87\pm0.49\%$ comparing with $3.85\pm0.24\%$ of the control group incubated for 30 minutes was not significantly different each other.

Consequently it is deduced that this experimental dose of meperidine hydrochloride, which has 0.04 mg./ml. of it in Tyrode solution for the incubation of the mesentery, in vitro, did not bring about the cytological changes of the mesenteric mast cells (Table 2).

C. The groups incubated with morphine hydrochloride solution plus iodoacetic acid.

In the groups with slight degranulation of mast cells of the mesenteries incubated

Table 1. Results of degranulation of mesenteric mast cells in the groups incubated at various doses of morphine HCl in Tyrode solution

Experimental group	Dose of morphine HCl	Normal cell (%)	Grade 1 cell (%)	Grade 2 cell (%)	Grade 3 cell (%)
a. (10 minutes)	0.01mg./ml.	95.85±0.24	4.25±0.24	0	0
(10 minutes)	0.02mg./ml.	98.87±0.09	1.13±0.09	0	0
(10 minutes)	0.04mg./ml.	97.55±0.22	2.45±0.22	0	0
control(10 minutes)		97.96±0.11	1.98±0.11	0.05±0.02	0.01±0.01
b. (20 minutes)	0.01mg./ml.	98.83±0.15	1.11±0.15	0.06±0.04	0
(20 minutes)	0.02mg./ml.	97.75±0.12	2.24±0.12	0.01±0.01	0
(20 minutes)	0.04mg./ml.	92.22±0.45	7.64±0.45	0	0.14±0.06
control (20 minutes)		98.38±0.09	1.55±0.08	0.06±0.02	0
c. (30 minutes)	0.01mg./ml.	97.15±0.18	2.72±0.20	0.07±0.03	0.06±0.03
(30 minutes)	0.02mg./ml.	96.37±0.17	3.12±0.16	0.37±0.06	0.14±0.03
(30 minutes)	0.04mg./ml.	92.37±0.38	5.92±0.35	1.53±0.18	0.17±0.06
control (30 minutes)		96.39±0.15	3.57±0.15	0.02±0.01	0.02±0.01

mean±standard error (%)

Table 2. Results of degranulation of mesenteric mast cells in the groups incubated at various doses of meperidine HCl in Tyrode solution

Experimental group	Dose of meperidine HCl	Normal cell (%)	Grade 1 cell (%)	Grade 2 cell (%)	Grade 3 cell (%)
a. (10 minutes)	0.02mg./ml.	99.46±0.09	0.51±0.09	0.03±0.02	0
control (10 minutes)		98.25±0.17	1.75±0.17	0	0
b. (20 minutes)	0.02mg./ml.	99.43±0.08	0.53±0.08	0.04±0.02	0
control (20 minutes)		98.89±0.11	1.02±0.11	0.08±0.03	0
c. (30 minutes)	0.02mg./ml.	98.77±0.12	1.19±0.12	0.02±0.01	0
(30 minutes)	0.04mg./ml.	97.12±0.50	2.87±0.49	0	0
control (30 minutes)		96.15±0.24	3.85±0.24	0	0

mean ± standard error (%)

Table 3. Results of degranulation of mesenteric mast cells in the groups incubated at various doses of morphine HCl plus iodoacetic acid in Tyrode solution

Experimental groups	Dose of substrates	Normal cell (%)	Grade 1 cell (%)	Grade 2 cell (%)	Grade 3 cell (%)
a. (10 minutes)	I.+M. (0.02mg./ml.)	98.44±0.19	1.53±0.18	0.02±0.02	0
(10 minutes)	I.+M. (0.04mg./ml.)	97.51±1.09	2.48±0.48	0.17±0.13	0
control(10 minutes)	M. (0.02mg./ml.)	98.87±0.09	1.13±0.09	0	0
control(10 minutes)	M. (0.04mg./ml.)	97.55±0.22	2.45±0.22	0	0
b. (20 minutes)	I.+M. (0.02mg./ml.)	98.83±0.18	1.17±0.18	0	0
(20 minutes)	I.+M. (0.04mg./ml.)	96.31±0.55	2.58±0.50	0.59±0.24	0
control(20 minutes)	M. (0.02mg./ml.)	97.75±0.12	2.24±0.12	0.01±0.01	0
control(20 minutes)	M. (0.04mg./ml.)	92.22±0.46	7.64±0.45	0	0
c. (30 minutes)	I.+M. (0.02mg./ml.)	94.15±0.33	5.85±0.33	0	0
(30 minutes)	I.+M. (0.04mg./ml.)	69.47±1.01	29.89±1.00	0.63±0.17	0
control(30 minutes)	M. (0.02mg./ml.)	96.37±0.17	3.12±0.16	0.37±0.06	0.14±0.03
control(30 minutes)	M. (0.04mg./ml.)	92.37±0.38	5.92±0.35	1.53±0.18	0.17±0.06

mean±standard error %, I=iodoacetic acid (0.03×10^{-2} M) and M.=morphne hydrochloride

at 0.04 mg./ml. of morphine hydrochloride plus 0.03×10^{-2} M of iodoacetic acid in Tyrode solution for 20 minutes the incidence was $2.58\pm.0.50\%$ compared to the result of $7.64\pm0.45\%$ which was the incidence of the slightly degranulated mast cells incubated at 0.04 mg./ml. of morphine hydrochloride in Tyrode solution for 20 minutes as the control of the experimental group.

The incidence of the slightly degranulated mast cells incubated with 0.04 mg./ml. of morphine hydrochloride in Tyrode solution plus 0.03×10^{-2} M of iodoacetic acid for 30 minutes was about $29.89\pm1.00\%$ and differed with the result of $5.92\pm0.35\%$ which was the incidence of those cells incubated at 0.04 mg./ml. of morphine hydrochloride in Tyrode solution for 30 minutes as the control of this experimental group.

By the comparison of these results it is suggested that the addition of metabolic inhibitor such as iodoacetic acid to the incubating medium containing morphine hydrochloride inhibited remarkably the degranulation of the mast cells. However, the inhibition of the degranulation of the mast cells due to metabolic inhibitor was fairly abolished after the incubation continued for 30 minutes.

DISCUSSION

The degranulation of metachromatic granules of peritoneal mast cells by the intraperitoneal injection of morphine was studied by Bhattacharya and Lewis (1956) and postulated that the peritoneal mast cells of the albino rat show much more degranulation than do the subcutaneous mast cells after intraperitoneal injecton of morphine. However, Parratt and West (1957) showed that intraperitoneal injection of morphine has but little damaging effect upon tissue mast cells and releases correspondingly little histamine in the rat. Lee and Pak (1969) demonstrated that fairly significant degranulation of rat's mesenteric mast cells occurred after the injection of morphine hydrochloride, which was probably associated with the concomitant liberation of tissue histamine derived from its source.

Several authors have already studied the effect of morphine upon the cytological change of tissue mast cells in vivo, though it is not yet resolved whether or not such an effect of morphine is of a direct acting mechanism to tissue mast cells. In this study the authors have observed that the incubation of the rat's mesentery in morphine containing medium in vitro, caused obvious cytological changes of degranulation in many mesenteric mast cells.

The cytological change due to morphine (a natural narcotic) in vitro, was quite different from the change due to meperidine (a synthetic narcotic) and it was deduced that the latter was almost inert for the dispersion of metachromatic granules of the mesenteric mast cells.

In the groups incubated with morphine hydrochloride in Tyrode solution plus iodoacetic acid (a metabolic inhibitor) for 20 minutes, it was obivously demonstrated that the addition of a metabolic inhibitor into the incubating media reduced the cytological change or degranulation of metachromatic granules of the mast cells compared with the change of the control in which the metabolic inhibitor was omitted in the incubating media.

From these results the authors concluded that the effect of morphine upon the degranulation of mast cells might take place through a certain metabolic process.

In this experimental group incubated for

30 minutes (with morphine plus iodoacetic acid) the effect of iodoacetic acid, which is to suppress that of morphine upon the degranulation of the mast cell, was abolished and reversed slightly the degranulation of the mast cells which were promptly increased more than that of the control. This phenomenon was thought to be due to the probable death of the mast cells because of the prolonged incubation in which the metabolic inhibitor was present in the incubating media.

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= Legend for Figures =

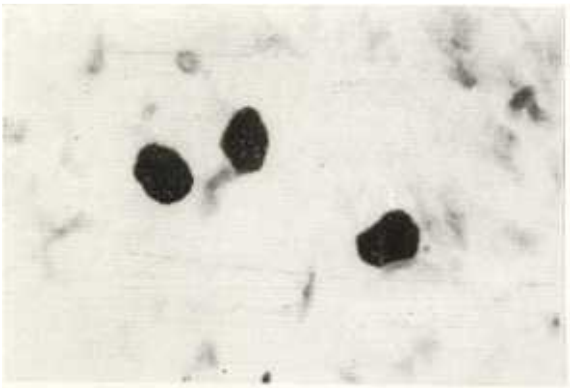

Fig. 1. Normal type of rat's mesenteric mast cell. Stained with Pugh's solution, 400 ×.

Fig. 2. Grade 1 type of slightly degranulated rat's mesenteric mast cell. Stained with Pugh's solution, 400 ×.

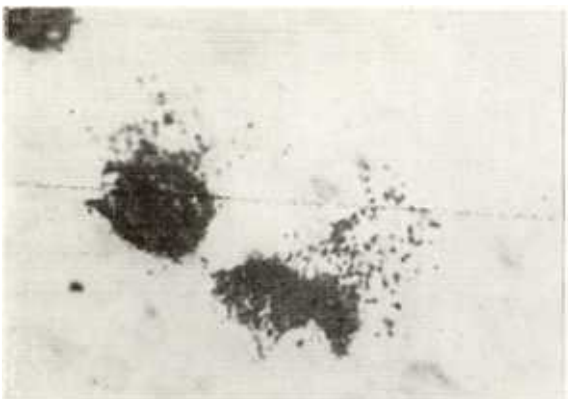

Fig. 3. Grade 2 type of moderate degranulation of rat's mesenteric mast cell. Stained with Pugh's solution, 400 ×.

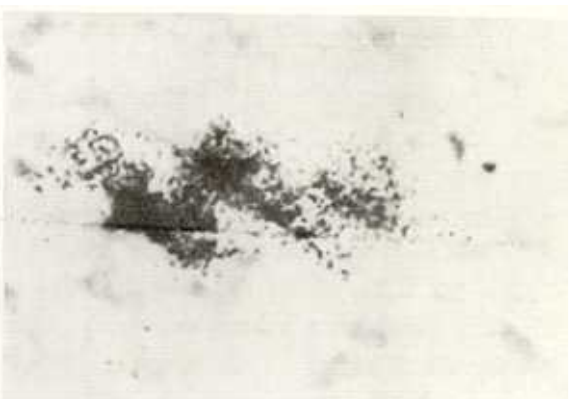

Fig. 4. Grade 3 type of marked degranulation or disruption of rat's mesenteric mast cell. Stained with Pugh's solution, 400 ×.