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# AN IN VITRO STUDY ON THE EFFECT OF HISTAMINE ON THE CONTRACTILE RESPONSIVENESS OF RAT SUBCUTANEOUS FASCIA AND WOUND GRANULATION TISSUE TO MEPYRAMINE

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**Abstract**

Myofibroblasts play an important central role in wound contraction and repair, and are believed to develop from resident fibroblasts in the adjacent uninjured connective tissues. However, the factors involved in their differentiation into more contractile cells are not fully understood. The aim of the present study was to elucidate the role of histamine on the pharmacological responsiveness of both subcutaneous fascia and wound granulation tissues. In the present in vitro study, using superfusion technique, the effects of histamine (1, 10 and 50 μM following 30 and 60 min incubation) on the pharmacological responsiveness of excisional wound granulation tissue were compared with superficial fascia to mepyramine. The results showed that incubation with histamine caused an increase in responsiveness of normal fascia to mepyramine, while it had no effect on excisional wound granulation tissue. In conclusion, it seems that histamine plays an important role in modulating the contractile behaviour of the normal fibroblasts and promotes the development of a more contractile cell resembling the myofibroblasts.

**Keywords:**

Histamine, Mepyramine, Contractility, Subcutaneous fascia, Wound granulation tissue, Wound healing, Rat.

## Introduction

Wound healing is a dynamic, interactive process involving an orchestrated and complex interactions between many factors in the wound space leading to tissue remoulding and restoration of the integrity of the damaged tissues (1). In humans it is always accompanied by discernible scar formation (2). Among the many integrated interactions in the early stages of wound repair is the appearance of a large number of fibroblasts, which are recruited from neighbouring connective tissues (3). It appears that these fibroblasts differentiate into a more active contractile myofibroblasts (4).

Once the tissue integrity is restored, the myofibroblasts disappear, leaving an acellular fibrous collagen scar filling the damaged area (4).

While the histological changes that follow tissue injury have been well known for many years, the factors regulating the modulation of various cellular activities

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still remains unknown. The aim of the present study was therefore to elucidate the role of histamine, a substance known to be released from mast cells that are found in large numbers in the connective tissues, in the modulation of the contractile responsiveness to mepyramine, a potent $H_1$ antagonist found by previous studies to induce contraction (5-8) in both normal subcutaneous fascia and granulation tissue prepared from an excisional wound in rat.

## Materials and Methods

*Materials*

Isolated strips of the loose subcutaneous connective tissues were taken from eight male, Hooded Lister rats (Bradford University strain) with body weight ranging from 250-350g. All rats were housed in polycarbonate, clean sawdust floored, cages (59x38x22 cm) in groups of five under experimental room temperature ranging from 21-25 °C and light/dark cycle of 12h. The sawdust and water were changed twice a week. They had free access to water and standard food pellets (CRM-P-Special Diet Services, Witham, Essex, UK). Mepyramine hydrochloride and other reagents employed in this study were purchased from Sigma (UK).

*Preparation of the strips of the loose connective tissue*

Samples of the superficial fascia were always selected from the lower dorsal site. Following the sacrifice of each animal with an intraperitoneally administered lethal dose of sodium pentobarbital (200mg/kg) and cervical dislocation. A fat free, thin semi-transparent strip of 1x2 cm was mounted under 2g isometric tension in a superfusion set up (9).

*Preparation of strips of excisional wound granulation tissue*

The method used was as those reported by Cross et al. (1995) (7), in which a 15 x 15 mm full thickness wound down to the level of the loose subcutaneous connective tissue was made on the lower dorsal site of each animal. No dressings were applied to the wound surface and each animal was placed in a separate cage and allowed to recover from anaesthesia (20-40% halothane/$O_2$ mixture) before being returned to the holding room, and had free access to food and water.

At the end of the seventh day, the animals were sacrificed with sodium pentobarbital (200mg/kg, IP) and cervical dislocation. The granulation wound tissue (2 x 0.5 cm) was removed and suspended under 2g isometric tension in the path of the superfusate solution.

*Investigation of the effect of incubation of histamine with both superfiscial fascia and granulation tissues*

In order to assess the influence of histamine on the responsiveness of both subcutaneous fascia and wound granulation tissues (n=8 for each set), 128 µM of mepyramine was used (the concentration that was found in the preliminary control experiments to produce maximal contractile responses in these tissues). Both tissue preparations were incubated with histamine (1, 10 and 50 µM) for 30 and 60 min, followed by test responses to same concentration of mepyramine and results were recorded. The superfusate Krebs' Henseleit solution was pumped at a rate of 3 mL/min.

In this experiment the superfusion technique was modified, so that the incubation period was performed in the same superfusion arrangement, by closure of the draining valve and oxygenated Krebs' Henseleit-containing histamine at the relevant concentrations was gently added from the top of the tissue bath and the superfusion pump was switched off during the incubation period. At the end of the incubation period, the valve was gently opened and the tissues were allowed to equilibrate to pre-incubation

tension before recording the test responses. Control vehicle effects were also performed, in which the same tissues were incubated with oxygenated (5% $CO_2/O_2$ mixture) Krebs' Henseleit alone (n=8 for all experiments) for the corresponding periods.

*Statistical Evaluation*

The data, presented as mean ± SEM, were analysed using ANOVA, followed by Dunnet's test, with $P<0.05$ was considered as the level of significance.

## Results

The initial mean control contractile responses to 128 µM mepyramine in the subcutaneous fascia and granulation tissues were 76 ± 12 and 192 ± 15mg, respectively.

Incubation with histamine did not alter the baseline tension nor caused any contractile response in either of the tissues. Incubation with Krebs' Henseleit alone also did not produce significant changes in the responsiveness to mepyramine in either of the tissues employed. The responses in superficial fascia were found to increase following incubation with histamine in a dose-dependent employed following both 30 and 60 min incubation periods (Tables 1 and 2).

Table 1: Effects of 30-min incubation with histamine (1, 10 and 50 µM) on the contractile responses (mg) of rat connective tissue superficial fascia to 128 µM mepyramine

| | | Histamine ( M) after 30-min incubation | | |
|---|---|---|---|---|
| | Control | (1) | (10) | (50) |
| Contractile response | 76 ± 12 | 90 ± 7 | 99 ± 8 | 113 ± 9 |
| % increase in response | 0 | 18 ± 11 * | 30 ± 6 ***, $^{+}$ | 49 ± 9 *** |

All figures are expressed as mean ± SEM and as percentage change relative to control before incubation (*P<0.05 and ***P<0.001, ANOVA followed by Dunnet's test, n=8) prior to incubation with histamine. $^{+}$P<0.05 between 30- and 60-min incubation.

Table 2: Effects of 60-min incubation with histamine (1, 10 and 50 µM) on the contractile responses (mg) of rat connective tissue superficial fascia to 128 µM mepyramine

| | | Histamine ( M) after 60-min incubation | | |
|---|---|---|---|---|
| | Control | (1) | (10) | (50) |
| Mepyramine | 76 ± 12 | 91 ± 8 | 110 ± 7 | 119 ±10 |
| % increase in response | 0 | 19 ± 7 * | 45 ± 5 *** | 57 ± 11 *** |

All figures are expressed as mean ± SEM and percentage increase of response relative to control before incubation (*P<0.05 and ***P<0.001, ANOVA followed by Dunnet's test, n=8) prior to incubation with histamine.

The threshold concentration of histamine that produced an increase in the response to mepyramine, after incubation for 30 min, was observed at 1μM (Table 1). This concentration produced an increase of 18 ± 11% relative to control values before incubation ($P<0.05$). This percentage increase in the response was found to be dose and duration of incubation dependent. After an incubation period of 60 min with histamine, the increase in the responsiveness, at corresponding doses, to mepyramine was significantly greater as compared to 30 min incubation period. Incubation for 30 and 60 min with 10μM histamine caused 30±6 and 45 ± 5% increase relative to control, respectively ($P<0.001$). However, the maximum increase in fascia contraction to mepyramine, following 60-min incubation with 50 μM of histamine, was still significantly lower than those obtained from the wound granulation tissues. In contrast, the responses of the wound granulation tissues were not found to be influenced by incubation with histamine at all of the doses and durations of incubation periods. The responses did not change even after 90 min period of incubation producing a mean ± SEM response of 205 ± 18 mg.

## Discussion

In this study, it was demonstrated that incubation of histamine with wound granulation tissues did not induce a significant change in response to mepyramine. This could be that the myofibroblasts in wound granulation tissue are already in a fully activated and mature state. On the other hand, the fibroblasts in the fascia were brought into a more contractile state following incubation with histamine, a substance known to be present in abundance in its neighboring mast cells (10). This interesting observation suggests an important role for histamine in the activation process of the fibroblast, which is in agreement with the report that mast cells have a role in the reparative processes (11).

Modification of the superfusion set up, as a mean for mimicking in vivo presence of excessive histamine in wound environment has not, to our knowledge, been reported previously. Although histamine, even after a 60 min incubation, did not produce any direct stimulatory effect on the superficial fascia, but was found to induce changes in its responsiveness towards mepyramine, suggesting that histamine was a potent modulator upon the resident fibroblasts. These effects were observed at low concentration of 1 μM and after what may be considered to be a short incubation period of 30 min.

The findings of this study may have physiological significance, since it is well known that mast cells, the storage site for histamine (12), are in close association with fibroblasts and are one of the predominant cells in these tissues. Furthermore, histamine is released, at a high concentration, from mast cells within seconds, following injury (13). All these findings support the notion that similar contributing action of histamine under normal physiological conditions may prevail. However, in order to assess this hypothesis, further in vivo studies are clearly needed.

Explaining the effects of histamine is complicated because histamine has been reported to have metabolic effects on synovial fibroblasts inducing the secretion of hyaluronic acid and proliferation of fibroblast via $H_1$ and $H_2$ receptors in a dose-dependent manner (14). It has been reported that histamine induced the production of matrix metalloprotienase-1 by human synovial fibroblasts and this action is believed to be mediated via $H_1$ receptors (15).

Furthermore, a number of in vivo and in vitro studies demonstrated the interactions between mast cells and fibroblasts in the repairative processes in both inflammatory conditions and wound repair. Some of these studies were related to tissue repair and showed that an external stimulus induces inflammatory and injury responses which are accompanied with mast cell degranulation and the release of histamine and heparin. In a study conducted by Fozard and colleagues in 1996 (16), it was shown that adenosine can be an inducer of mast cell degranulation. While Nagata et al. (14), found that histamine acts via histamine receptors on the fibroblasts and increases hyaluronic acid and DNA synthesis. Alternatively, histamine was shown to enhance fibroblast migration and proliferation in an in vitro cell culture model of wound (11). Inase et al. (17), found that the released heparin had an inhibitory effect on the release of histamine from mast cells. Earlier investigations (18) suggested that rat foetal tissues have a high histamine forming capacity as compared to adults and attributed this to the rapid growth in the foetus. In addition, foetal tissues are known to be the only tissues that heal without leaving scars (18). Clearly, further studies are needed to assess the role of histamine in the development of scarless tissues both in foetal and adulthood wound healing studies.

Although the maximum dose and duration of exposure to histamine employed in this study, did not increase the responsiveness of wound granulation tissues towards mepyramine to similar magnitude as those of fascia tissues, our findings, showed the first to demonstrate, that mast cells contribute to evolvement of the fibroblast into a more contractile cells, which in turn carry out their vital role in the mystifying phenomenon of wound closure. Elucidation of the underlying mechanism(s) that govern the observed findings and clarification of the intracellular effects associated with exposure to histamine may provide an inroad towards better understanding the nature of this complicated phenomena and deserves further investigations.

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