

## ORIGINAL PAPER

# Differential corticosterone responses to stress in the lung in two strains of Flinders rats

H. Amano<sup>\*1</sup>, H. Akiyama<sup>†1</sup> and J. Bienenstock<sup>‡</sup>

<sup>\*</sup>Department of Dermatology, Gunma University Graduate School of Medicine, Maebashi, Japan, <sup>†</sup>Division of Novel Foods and Immunochemistry, National Institute of Health Sciences, Tokyo, Japan and <sup>‡</sup>Departments of Medicine, Pathology and Molecular Medicine, McMaster University and Brain-Body Institute, St Joseph's Healthcare, Hamilton, ON, Canada

## Clinical and Experimental Allergy

### Summary

**Background** Acute stress affects a variety of organs and cellular systems. These include the hypothalamic–pituitary–adrenal (HPA) axis, corticotropin-releasing factor (CRF), mast cells and nerves. Flinders-sensitive (FSL) rat strains have hypercholinergic responses and are more sensitive than Flinders-resistant rats (FRL) to anaphylaxis.

**Objective** To investigate the effects of acute water avoidance stress (1 h) on FSL and FRL tracheal epithelial tissue.

**Methods** We measured short circuit current ( $I_{sc}$ ) as a measure of tracheal response, and the effect of substance P (SP) on tracheal epithelium in Ussing chambers. Electron microscopy was performed to assess mast cell activation.

**Results** Both strains showed increased  $I_{sc}$  responses to stress, inhibited by prior injection of the CRF receptor 1 and 2 antagonist,  $\alpha$ -helical CRF-(9–41). No increases in conductance were seen. Stress responses were accompanied by electron microscopic morphologic evidence for mast cell degranulation, which was not completely inhibited by  $\alpha$ -helical CRF-(9–41) pre-treatment. Stress primed the epithelium for an enhanced response to SP in FSL, but this again was not inhibited by  $\alpha$ -helical CRF-(9–41). FRL had 2.5 times the corticosterone response of FSL.

**Conclusion** Acute stress affects the tracheal epithelium, not accompanied by changes in ion permeability, but associated with mast cell degranulation. Because blunted HPA axis responses are associated with vulnerability to inflammation, this may partially explain the findings. These stress effects on the lung have a genetic basis associated with relative corticosterone responses, are complex and only in part mediated by CRF.

**Keywords** corticosterone, hypothalamic–pituitary–adrenal (HPA) axis, lung epithelium, short circuit current ( $I_{sc}$ ), stress

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### Correspondence:

Hiroo Amano, Department of Dermatology, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan.  
E-mail: hamano@showa.gunma-u.ac.jp

### Introduction

Stress is a subject that has received increasing attention since the pioneering work of Selye [1]. Much work has been carried out in relation to the body's general adaptation to acute and chronic stress. Most work until recently have focussed on the effects of stress on the hypothalamic–pituitary–adrenal (HPA) axis [2], inflammation and immunity [3] and various psychologic and social parameters [4]. Recently, there has been a considerable expansion of the scientific literature on intestinal responses to

stress [5–7]. However, the effects of stress on various physiologic and other parameters in the lung have hitherto largely been unexplored and the subject remains controversial. One recent study in mildly asthmatic students has shown that examination stress caused changes in lung physiology that correlated with an increase in eosinophil-derived products in the sputum [8].

Wright et al. [9] have suggested in a population study of a large birth cohort that there is an association between maternal stress and wheeze in early childhood. We have compared stress responses in the two Flinders rat strains: Flinders-sensitive (FSL) and Flinders-resistant (FRL) [10]. The former strain is more sensitive to cholinergic

<sup>1</sup>These authors contributed equally to this study.

(muscarinic) agonists than the latter and is more sensitive to systemic anaphylactic effects such as hypothermia despite no differences in IgE levels [11, 12]. Our study explored the effects of acute stress on FSL and FRL tracheal tissue as reflected by changes in epithelial responses in Ussing chambers. We have shown increases in tracheal short circuit current ( $I_{sc}$ ) responses to acute water avoidance stress, a model that has been used in the examination of stress effects in the intestine [13]. This model appears to have effects on intestinal responses similar to those seen with other models of stress, namely restraint stress with or without cold [14]. The FSL, in accord with its responses in anaphylaxis, had a greater tracheal secretory response to stress than the FRL and also a greater response after stress to the neuropeptide, substance P (SP). In contrast, the FSL had a reduced HPA axis response to stress in terms of corticosterone levels, which may in part account for these findings.

Others have shown in two distinct rat strains, the Lewis and Fischer, that the Lewis vulnerability to experimental arthritis was dependent on its comparatively blunted corticosterone response [15]. We conclude that the Flinders rat strains may be appropriate for further studies of stress effects in the lung, and particularly relevant to study the role of stress in asthma.

## Methods

### Animals

Male rats of the FSL or FRL line were selected from breeding colonies maintained in the animal quarters at McMaster university. They were housed and bred under specific pathogen-free (SPF) conditions and maintained on a normal 12:12 h light-dark cycle. Rats were provided with food and water *ad libitum*. They weighed between 300 and 400 g at sacrifice. All experiments were approved by the Animal Care Committee at McMaster University.

### The stress model

Acute water avoidance stress (WAS) was used as the standard stressor model [5, 16] and all experiments were performed between 10:00 and 13:00 hours to minimize the effects of circadian rhythm. In addition, rats were handled daily by the same investigator for 1 week before the study to minimize the effects of stress occasioned by human handling. WAS consisted of placing the rat for 1 h on a glass platform (height from floor, 8 cm; diameter, 6 cm) located in the middle of a plastic container (56 × 50 cm) filled with water up to 3 cm below the top level of the platform. Sham rats were placed on the same platform above a waterless container for 1 h.

### Drugs

Corticotropin-releasing factor (CRF) and  $\alpha$ -helical CRF-(9–41) ( $\alpha$ -helical CRF), a CRF antagonist (Peninsula Lab, Belmont, CA, USA), were dissolved in phosphate-buffered saline (PBS) according to the manufacturer's instructions, aliquoted and kept frozen at  $-70^{\circ}\text{C}$  until used. The neutral endopeptidase inhibitor, phosphoramidon, was obtained from Sigma Chemical Co. (St Louis, MO, USA). The reagent was dissolved in PBS. SP (Peninsula Lab) was dissolved in PBS, aliquoted and stored at  $0^{\circ}\text{C}$  before use. All drugs were used fresh. Immediately before the experiments, the drugs were diluted in PBS to the appropriate concentrations.

### Experimental design

A pilot study showed that colonic epithelial abnormalities were maximal at 1 h after initiation of WAS. CRF was injected intraperitoneally (i.p.) at  $50\text{ }\mu\text{g/kg}$  1 h before mounting tissues in the chambers. Others had determined previously that this dose was maximal in intestinal  $I_{sc}$  responses [5]. The dose of  $\alpha$ -helical CRF ( $250\text{ }\mu\text{g/kg}$ ) has been shown to block intestinal responses to WAS effectively [5].  $\alpha$ -helical CRF-(9–41) ( $250\text{ }\mu\text{g/kg}$ ) was injected i.p. 30 min before WAS (90 min before mounting).

### Ussing chamber experiments

The rats were euthanized at the end of each stress session.

After sacrifice, the trachea was isolated from the surrounding tissues, removed and immersed in  $37^{\circ}\text{C}$  oxygenated Krebs buffer. Krebs buffer contained (in mM) 115.0 NaCl, 8.0 KCl, 1.25  $\text{CaCl}_2$ , 1.2  $\text{MgCl}_2$ , 2.0  $\text{KH}_2\text{PO}_4$  and 25.0  $\text{NaHCO}_3$ , pH 7.33–7.37. The tissue was opened along the anterior margin, and then immediately mounted in Ussing chambers (surface area,  $15\text{ mm} \times 4\text{ mm}$ ,  $0.6\text{ cm}^2$ ) [17]. The tissue was bathed in 10 mL of oxygenated Krebs buffer containing 10 mM glucose. The serosal buffer included 10 mM glucose as an energy source, osmotically balanced by 10 mM mannitol in the luminal buffer. Tissues were short circuited at 0 V with an automated voltage clamp (W-P Instruments, Narco Scientific, Mississauga, ON, Canada), and the ( $I_{sc}$ ,  $\mu\text{A/cm}^2$ ) was continuously monitored. Initially, baseline values for  $I_{sc}$ , as an indicator of ion secretion, and  $G$ , as an indicator of ion permeability, were measured 15 min after mounting the tissues, and then every 5 min for 40 min [18].

Tissue conductance ( $G$ ) was calculated according to Ohm's law and expressed as  $\text{mS/cm}^2$ , and reflects the relative epithelial integrity in terms of ion transport.

### Tracheal responses to substance P in vitro

In all experiments, phosphoramidon (final concentration:  $10^{-5}\text{ M}$ ) was added to the chamber buffer 10 min before

adding SP [19]. SP was added to the luminal or the serosal buffer of the Ussing chambers 50 min after mounting the tissues in amounts of 10  $\mu$ L to a final concentration of  $10^{-8}$  M. This concentration was chosen because it has been used before by many authors examining SP effects on tracheal  $I_{sc}$  and smooth muscle responses. After adding SP to the luminal buffer, immediate, sharp increases in  $I_{sc}$  were observed. The  $I_{sc}$  was calculated as the difference between the baseline  $I_{sc}$  value and the maximum value within 10 s of addition of SP.

### Electron microscopy

Electron microscopy (EM) was performed following after the method of Akiyama et al. [18]. The tracheal tissue samples were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 h at room temperature, washed, left overnight at 4 °C in 0.2 M sodium cacodylate buffer, pH 7.4, and then washed three times for 5 min each in the same buffer. They were post-fixed in 2% osmium tetroxide for 1 h, finally dehydrated in a graded ethanol series and embedded in Spurr's resin. The tissue samples were cut lengthwise to obtain longitudinal sections of all layers. Ultrathin sections (70 nm) were placed on 200 mesh copper/palladium grids and stained with saturated uranyl acetate (alcoholic) and lead citrate (Reynolds). The sections were observed and photographed using a transmission electron microscope (JEOL 1200 EX, Tokyo, Japan). EM photomicrographs were taken at a magnification of  $\times 3000$ . At least 20 mast cells from each rat were analysed by two investigators who were blind to the experimental conditions. Interobserver error did not vary by more than 3%. Activation of mast cells was defined by the presence of granules with altered or absent electron-dense content. The loss of electron-dense content was often associated with large empty intragranular spaces, or complete dissolution of electron-dense content. We regarded all these characteristics as evidence of mast cell activation.

### Measurement of serum corticosterone

Serum corticosterone levels were measured following the method of Wong et al. [20] with some modification using a high-performance liquid chromatography system [18].

Animals were killed at the end of each stress session or immediately upon removal from cages. After decapitation, the blood was collected, allowed to clot at room temperature for 1 h and centrifuged at  $500 \times g$  for 15 min. Serum was collected and stored at  $-20$  °C before analysis.

### Statistical analysis

The data were expressed as mean  $\pm$  SEM. A *P*-value of less than 0.05 was considered to be significant. Differences between the values were tested using the Mann–Whitney

*U*-test for two groups or the Kruskal–Wallis test to compare more than two groups.

## Results

### Corticosterone levels

An initial pilot experiment was conducted in FRL rats, which consisted of four controls, four shams and four stressed animals. The sham ( $545.1 \pm 146.1$  ng/mL) differed significantly from the control ( $98.3 \pm 17.4$  ng/mL) ( $*P < 0.05$ ). The stress group ( $1126.2 \pm 174.0$  ng/mL) differed significantly from both the sham ( $*P < 0.05$ ) and the control ( $**P < 0.01$ ). From this, it can be seen that the sham group exhibited stress behaviour and therefore we omitted the sham group in all subsequent experiments, and compared all results with the control baseline (Fig. 1) as have others in similar studies [21, 22]. In FRL, the stress response in serum corticosterone levels reached a level 2.5 times than seen in FSL. Nevertheless, both were significantly increased relative to controls: FSL ( $**P < 0.01$ ) and FRL ( $**P < 0.01$ ).

CRF injection led to an increase in the value over controls in both groups: FSL ( $**P < 0.01$ ), FRL ( $*P < 0.05$ ). As predicted, pretreatment with  $\alpha$ -helical CRF before stress reduced levels seen in both the FSL group ( $^{\dagger}P < 0.05$ ) and the FRL group ( $^{\dagger\dagger}P < 0.01$ ), but only reduced the levels to that of controls in the FSL. In the FRL, the reduced levels still differed from controls.  $\alpha$ -helical CRF alone had no effect on constitutive corticosterone levels ( $51.5 \pm 21.9$  ng/mL).

### Effects of stress, corticotropin-releasing factor and $\alpha$ -helical corticotropin-releasing factor on tracheal short-circuit current responses

The effects found in FSL and FRL animals can be seen in Fig. 2. The baseline control values in FRL were markedly increased relative to FSL ( $*P < 0.05$ ). Therefore, because the levels achieved after WAS were equivalent, the increase was greater in FSL than in FRL. In FSL, it can be seen that stress significantly increased the short circuit current relative to the baseline values seen in controls ( $**P < 0.01$ ). Surprisingly, the injection of a high dose of CRF did not increase the  $I_{sc}$  above baseline.  $\alpha$ -helical CRF before stress reduced the values significantly ( $^{\dagger}P < 0.05$ ) to stress. The effects of  $\alpha$ -helical CRF by itself were insignificant.

In FRL, stress caused a significant increase above baseline control ( $*P < 0.05$ ). In a manner similar to the FSL group, CRF injection did not elevate short circuit current above baseline.  $\alpha$ -helical CRF injection before stress again reduced the stress values significantly ( $^{\dagger}P < 0.05$ ) to stress. The effect of  $\alpha$ -helical CRF by itself was not significant.

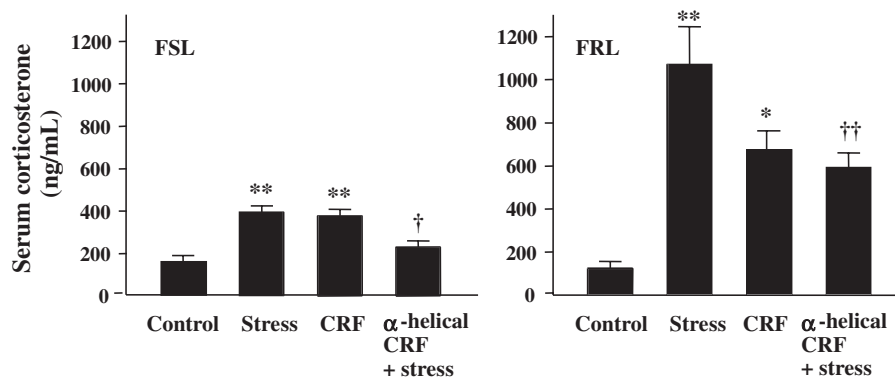


Fig. 1. Effects of stress and corticotropin-releasing factor (CRF) on serum corticosterone levels in Flinders-sensitive (FSL) and Flinders-resistant (FRL) rats.  $n = 5-7$ . \*\* $P < 0.01$ ; \* $P < 0.05$  vs. control; †† $P < 0.01$  vs. stress; † $P < 0.05$  vs. stress.

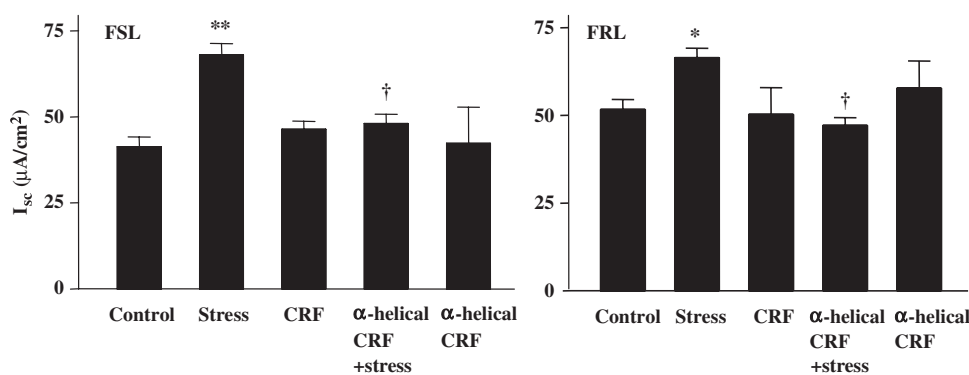


Fig. 2. Effect of water avoidance stress on tracheal baseline short-circuit current ( $I_{sc}$ ) in Flinders-sensitive (FSL) and Flinders-resistant (FRL) rats. Rats were subjected to water avoidance stress or sham stress for 1 h or injected intraperitoneally with corticotropin-releasing factor (CRF) (50  $\mu g/kg$ ) or  $\alpha$ -helical CRF-(9-41) (250  $\mu g/kg$ ) 30 min before stress or CRF protocol. Bars represent mean  $\pm$  SEM;  $n = 6-9$  rats/group.  $\alpha$ -helical CRF-(9-41);  $n = 3$ . \*\* $P < 0.01$  vs. control; \* $P < 0.05$  vs. control; † $P < 0.05$  vs. stress.

No changes in conductance were seen with any of these treatments in either of the groups.

#### Effects of stress and corticotropin-releasing factor on short-circuit current responses to substance P

Previous experiments have suggested that CRF may increase the effect of SP on the contraction of bronchial smooth muscle [23]. We therefore examined the effects of stress, CRF and the CRF receptor 1 and 2 inhibitor [24],  $\alpha$ -helical CRF, on the  $I_{sc}$  responses to a standard dose of SP.

Initial experiments (not shown) indicated an optimal  $I_{sc}$  response at a concentration of SP of  $10^{-8}$  M delivered on the luminal side. There appeared to be no difference in response to tissue bathed in buffer containing phosphoramidon ( $10^{-5}$  M). All reported experiments were conducted with phosphoramidon in the bathing medium.

It can be seen in Fig. 3 that there was a significant difference in control baseline responsiveness of tracheal epithelium to SP in the FRL group compared with the FSL group (†† $P < 0.01$ ). Stress caused a significant (\*\* $P < 0.01$ ) increase in response to a standard dose of

SP only in the FSL group, there being no significant difference between controls ( $176.3 \pm 37.3$ ) and stress ( $233.5 \pm 56.5$ ) in FRL, although a tendency towards this was seen. It should be noted that in the FRL, the baseline control response and the level reached after SP were both considerably elevated relative to FSL. The result was that the increase in  $I_{sc}$  in FSL was greater than in FRL after stress despite overall weaker responses. Prior injection with CRF did significantly enhance the effect of SP only in FSL (\*\* $P < 0.01$ ).

Prior treatment with  $\alpha$ -helical CRF before stress reduced the response to SP but this did not reach statistical significance in either group.  $\alpha$ -helical CRF injection alone similarly caused no significant change from baseline control.

#### Effect of stress on mast cell morphology

As can be seen in Tables 1 and 2, there was extensive EM evidence of activation of mast cells following stress in both FSL (Fig. 4) and FRL. Figure 4 shows representative findings from control (a) and stress (b) rats. Both strains

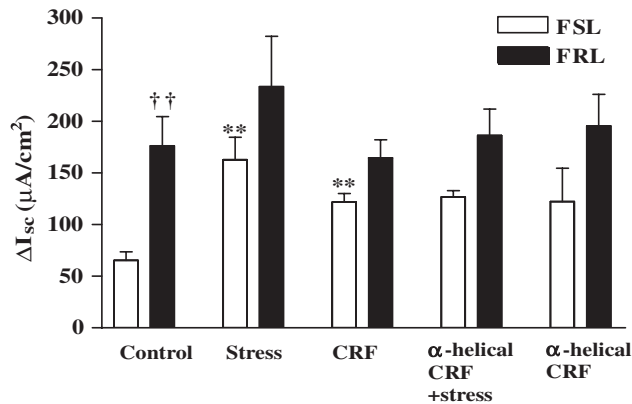


Fig. 3. Effect of stress, corticotropin-releasing factor (CRF) or  $\alpha$ -helical CRF tracheal responses to substance P (SP). Rats were subjected to water avoidance stress for 1 h or injected intraperitoneally (i.p.) with CRF (50  $\mu$ g/kg). One hour after the initiation of stress, 90 min after i.p. injection of  $\alpha$ -helical CRF or 60 min after CRF injection alone, tracheas were mounted in Ussing chambers. SP was added to the luminal side of the chamber 50 min after mounting.  $n = 4$ –5 Flinders-sensitive (FSL);  $n = 4$ –6 Flinders-resistant (FRL) \*\* $P < 0.01$  vs. respective control; †† $P < 0.01$  vs. FSL control.

Table 1. Effects of water avoidance stress on mast cells in FSL and FRL rats under different conditions

| Strain | Condition         | Mast cell activation granular change |                                |                                 |
|--------|-------------------|--------------------------------------|--------------------------------|---------------------------------|
|        |                   | Total granules*                      | Total low density <sup>†</sup> | % Total activation <sup>‡</sup> |
| FSL    | Control           | 2070                                 | 511                            | 24.9 $\pm$ 2.0                  |
|        | Stress            | 3878                                 | 2167                           | 56.5 $\pm$ 4.8**                |
|        | $\alpha$ -helical | 3071                                 | 1099                           | 34.0 $\pm$ 4.7 <sup>†</sup>     |
|        | CRF+stress        |                                      |                                |                                 |
| FRL    | Control           | 2498                                 | 567                            | 22.4 $\pm$ 1.3                  |
|        | Stress            | 3069                                 | 1414                           | 47.0 $\pm$ 3.1**                |
|        | $\alpha$ -helical | 2522                                 | 846                            | 32.7 $\pm$ 1.3 <sup>†, **</sup> |
|        | CRF+stress        |                                      |                                |                                 |

At least 20 mast cells from each rat were evaluated ( $n = 4$  in each group).

\*Total granules.

<sup>†</sup>Loss of granule electron density.

<sup>‡</sup>Indicates the percentage of changed granules (mean  $\pm$  SEM).

\*\* $P < 0.01$  vs. control; <sup>†</sup> $P < 0.05$  vs. stress.

FSL, Flinders-sensitive; FRL, Flinders-resistant; CRF, corticotropin-releasing factor.

showed strong evidence of mast cell degranulation/activation, relative to that seen in controls (FSL and FRL, \*\* $P < 0.01$ ) (Fig. 4b). The effects of  $\alpha$ -helical CRF on mast cell activation caused by stress showed a significant reduction in activation in both FSL and FRL ( $^{\dagger}P < 0.05$ ). In neither of the strains did the levels return to control values and this achieved significance in FRL (\*\* $P < 0.01$ ).

Table 2. Effects of water avoidance stress on mast cells in FSL and FRL rats under different conditions

| Strain | Condition                    | Mast cell activation granular change |                          |                                 |
|--------|------------------------------|--------------------------------------|--------------------------|---------------------------------|
|        |                              | Granules*                            | Low density <sup>†</sup> | % Total activation <sup>‡</sup> |
| FSL    | Control                      | 204                                  | 52                       | 25.5                            |
|        |                              | 368                                  | 104                      | 28.3                            |
|        |                              | 592                                  | 113                      | 19.1                            |
|        |                              | 906                                  | 242                      | 26.7                            |
|        | Stress                       | 1429                                 | 760                      | 53.2                            |
|        |                              | 812                                  | 573                      | 70.6                            |
|        |                              | 922                                  | 457                      | 49.6                            |
|        |                              | 715                                  | 377                      | 52.7                            |
|        | $\alpha$ -helical CRF+stress | 409                                  | 125                      | 30.6                            |
|        |                              | 1423                                 | 568                      | 39.9                            |
|        |                              | 614                                  | 137                      | 22.3                            |
|        |                              | 625                                  | 269                      | 43.0                            |
| FRL    | Control                      | 256                                  | 53                       | 20.7                            |
|        |                              | 955                                  | 223                      | 23.4                            |
|        |                              | 618                                  | 158                      | 25.6                            |
|        |                              | 669                                  | 133                      | 19.9                            |
|        | Stress                       | 457                                  | 199                      | 43.5                            |
|        |                              | 1581                                 | 694                      | 43.9                            |
|        |                              | 493                                  | 219                      | 44.4                            |
|        |                              | 538                                  | 302                      | 56.1                            |
|        | $\alpha$ -helical CRF+stress | 681                                  | 222                      | 32.6                            |
|        |                              | 408                                  | 140                      | 34.3                            |
|        |                              | 1203                                 | 417                      | 34.7                            |
|        |                              | 230                                  | 67                       | 29.1                            |

At least 20 mast cells from each rat were evaluated ( $n = 4$  in each group).

\*Total granules.

<sup>†</sup>Loss of granule electron density.

<sup>‡</sup>Indicates the percentage of changed granules (mean  $\pm$  SEM).

FSL, Flinders-sensitive; FRL, Flinders-resistant; CRF, corticotropin-releasing factor.

## Discussion

To date, very limited efforts appear to have been focussed on stress effects in the lung [25]. Therefore, we initiated a series of studies to look at the effects of acute stress on the physiology of the trachea in Wistar rats [18]. We report here our first studies on rat tracheal  $I_{sc}$  responses in the FSL and FRL rat strains. These selectively bred rats have significant differences in allergic responses [11], the FSL being more sensitive than the FRL to anaphylaxis and more responsive to cholinergic (muscarinic) agonists [10]. Others have shown that smooth muscle responses of the tracheo-bronchial tree in FSL were greater than in FRL when tissue was exposed to cholinergic challenge [12].

Recent evidence suggests that stress may play a role as a risk determinant in asthma [9] and serves to enhance eosinophil-associated lung inflammation [8]. Acoustic stress enhanced both airway reactivity and inflammation in a murine model of allergic asthma [26]. The HPA axis in several rat strains have significant differences, and HPA

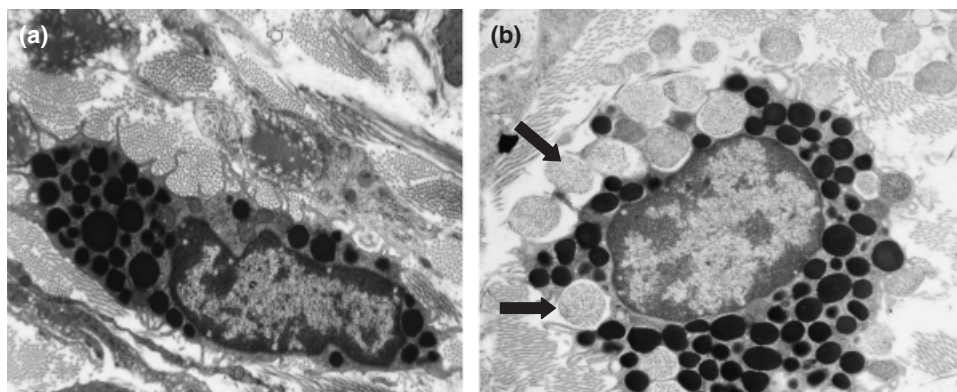


Fig. 4. Representative electron micrographs of mast cells in Flinders-sensitive (FSL) rat tracheas from control (a) and stress (b) rats. Similar findings were seen in Flinders-resistant (FRL) experiments. Mast cells from stress groups were more activated than those from the control groups, as indicated by loss of granule density (arrows point to such granules in b). Magnification  $\times 3000$ .

hypo-responsiveness of the Lewis rat and hyperresponsiveness in the Fischer lines underlie the reason why Lewis are susceptible and the Fischer resistant to induction of experimental arthritis [2, 27]. In our hands, FRL had far greater corticosterone responses to stress than FSL. We believe that this underlies the relative increased susceptibility to anaphylaxis by the FSL [11] as well as some of the physiologic differences. Atopic patients with atopic dermatitis or asthma have similarly blunted cortisol responses and the authors have suggested that this may contribute to the disease states [28, 29]. The potential significance of such differences in HPA responses to stress in many chronic disease states has been reviewed by Shanks and Lightman [30].

In terms of  $I_{sc}$  responses to stress, there were significant increases in the tracheal responses of both FSL and FRL. The responses in both strains were inhibited by prior  $\alpha$ -helical CRF in accord with results in the intestine [5, 21]. Only in FRL, however, did they clearly return to baseline, suggesting that at least in FSL other factors such as the known hypercholinergic response were probably involved in the production of stress effects. Indeed, CRF injection by itself had no effect on  $I_{sc}$  responses. We reported previously that Wistar rats have strong HPA axis responses to psychological stress, and CRF in that strain caused increases in  $I_{sc}$  [18]. The fact that the Flinders strain has either a hypercholinergic or a resistant response leads us to conclude that the exaggerated cholinergic pathway is responsible for the differing responses to stress and corticosterone synthesis that we have found. Indeed, CRF clearly has effects on vagal activity [31].

However, stress may have complex effects in addition to those induced by CRF because for example, in CRF transgenic knock-out mice, behavioural changes dependent on the CRF 1 receptor were still apparent [4], and it was suggested that a CRF-like molecule such as urocortin may have been involved. We assume that CRF enhances and/or triggers stress responses via neuronal especially

cholinergic pathways [31]. In the cholinergic-resistant strain, this pathway is relatively deficient and this likely explains why exogenously administered CRF did not alter  $I_{sc}$  in our experiment.

However, increased changes in conductance, which reflects the relative permeability of the epithelium to ions, were not seen in our experiments, whereas they were clearly seen in the intestine [5, 14]. This may reflect a general organ difference between the trachea and the intestine, as also described by ourselves and others [17, 32].

Our EM experiments support the suggestion that stress effects may be due to factors in addition to CRF because in animals treated with  $\alpha$ -helical CRF before stress, mast cell activation was significantly reduced but did not reach control values. Either factors other than CRF were involved, or different receptors, affinity or numbers of classical receptors for CRF are to be found in these strains but such examinations were beyond the scope of the present study.

The effects of CRF are complex and CRF has been shown to have both pro-inflammatory [33] and anti-inflammatory effects [34], and CRF is involved in the exaggerated response to inflammation following stress in a model of hapten-induced colitis [35]. CRF causes both mucosal and connective tissue mast cells to degranulate as evidenced in experiments involving rat intestine [36], skin [33] and heart [37]. Thus, the differential effect that we have observed is not due to the relative predominance of mucosal vs. connective tissue types of mast cells in the trachea [38].

Stress also only caused a significantly enhanced response to SP in FSL, the hypercholinergic strain and the one in which baseline responses to SP were much lower than those seen with the FRL. Thus, the FSL seems to have a much higher sensitivity in this model to SP effects as would be predicted with a hypercholinergic strain but administration of  $\alpha$ -helical CRF did not appreciably reduce the effects of stress similar to findings in Wistar rats.

Despite the lack of any effect of CRF injections on tracheal  $I_{sc}$ , CRF did lead to, as already stated, an enhanced response to SP in FSL. This is in keeping with observations in the guinea-pig that prior CRF exposure led to an enhanced response of bronchial smooth muscle to SP [23]. These results again underline the likelihood that the effects of stress on enhancement of SP responses were due to the effects of CRF in a hypercholinergic state.

Steroids prevent mast cell activation and even promote mast cell apoptosis and removal [39]. Tracheal mast cell activation after stress was less in FRL, the strain with the greater corticosterone response. It is tempting to suggest that these observations are correlatable but the conclusion must await further experimentation.

In summary, our experiments have revealed differences in tracheal responses to stress in two related strains of rats. Many but not all these responses appear to be mediated by CRF. Greater  $I_{sc}$  responses were seen in the FSL in which factors in addition to CRF such as the added effect of cholinergic activity are probably included. The effects of SP were also markedly enhanced in FSL for the same reasons. In contrast, FRL had far greater corticosterone responses that correlate with the observations but do not prove cause and effect relationships. Our experiments point to differences in HPA axis responses being involved in the regulation of lung responses to stress. These stress effects on the lung have a genetic basis, are complex and only in part mediated by CRF.

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