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Impairment in connective tissue mast cells degranulation in spontaneously hypertensive rats: stimulus dependent resistance

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1 Microvascular permeability in the mesentery and consequent leakage of protein into the peritoneum of spontaneously hypertensive rats (SHR) and normotensive rats (NTR) was measured *in vivo* by the extravasation of Evans blue dye.

2 In sensitized NTR, challenge with antigen produced extensive increases in dye extravasation in the mesentery and in peritoneal lavage fluid within 10 min.

3 In sensitized SHR there was no increase in the permeability of the mesentery and a very weak increase in dye extravasation in the peritoneal cavity following challenge.

4 The glucocorticoid antagonist RU38486 did not change the permeability response induced by antigen in sensitized NTR and SHR.

5 However, compound 48/80 was equally effective in either NTR or SHR in causing increased vasopermeability.

6 Mesenteric mast cells in the NTR were degranulated after immunological challenge, whereas those in the SHR were resistant, as measured histologically.

7 Similarly, challenge *ex vivo* of mesentery from sensitized NTR induced contraction of guinea-pig ileum in co-incubation experiments, whereas SHR mesentery was unresponsive.

8 Plasma levels of antigen-specific IgE and IgG2a in sensitized NTR and SHR were identical.

9 Immune serum from SHR was unable to induce a passive cutaneous anaphylaxis (PCA) reaction in the skin of NTR and SHR did not develop a PCA reaction upon passive sensitization with NTR immune serum.

10 We conclude that the mast cells of SHR are resistant to degranulation following immunological challenge, although the relevant antibodies are present.

Keywords: Mast cells; anaphylaxis; vascular permeability; anaphylactic antibodies; spontaneously hypertensive rats

Introduction

In rats sensitized to antigen, challenge with the same antigen results in a rapid and striking degranulation of mast cells, and subsequent increase in local microvascular permeability. The latter effect is a consequence of the mast cell degranulation and is readily assessed by the Evans blue extravasation method, where the dye injected intravenously binds to plasma albumin and thus acts as a marker for increased vascular permeability (Wilhelm *et al*., 1958). We have previously observed that following intraperitoneal administration of antigen to sensitized rats there is a marked increase in dye extravasation in several visceral tissues and within the peritoneal cavity, and that this increased vascular permeability is associated with release of platelet-activating factor (PAF) and histamine (Jancar *et al*., 1991). We were therefore surprised to observe a group of sensitized animals in which dye extravasation in the peritoneal cavity was absent following immunological challenge. Further inquiry revealed this group to have been drawn, inadvertently, from a different stock of rats, the spontaneously hypertensive Wistar-Kyoto strain (SHR).

We therefore set out to study the reasons for the apparent failure of the SHR to respond to the immunological challenge, especially as work with peritoneal mast cells from SHR had shown that these cells *in vitro* released more histamine than mast cells from the related normotensive strain (Amon *et al*., 1990; Masini *et al*., 1991).

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Methods

Animals

Male Wistar normotensive rats (NTR), Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR), 5 to 6 months old with mean weights 375–400 g, from our own facilities were used (Central Bioterium of Biomedical Sciences Institute, University of São Paulo, Brazil).

Sensitization and challenge

Rats were sensitized with an intraperitoneal injection of ovalbumin (10 mg per rat) adsorbed to 10 μg of aluminum hydroxide ('Alumen', Aldrox, Wyeth). Fourteen days after the sensitization, the animals were challenged with an intraperitoneal (i.p.) injection of ovalbumin (1 mg) in 0.5 ml of isotonic saline. Increases in vascular permeability were measured 10 min later. Control groups consisted of sensitized animals which received only the dye intravenously (i.v.) to assess the basal levels of dye extravasation and unsensitized animals which received ovalbumin (1 mg) i.p.

Evaluation of vascular permeability

Increased vascular permeability was assessed by the Evans blue dye extravasation method as modified by Sirois *et al*. (1988). Briefly, Evans blue (20 mg kg^{-1}) was injected i.v. immediately before the stimulus, i.e. antigen (1 mg, i.p.) in sensitized rats or compound 48/80 (2 mg kg^{-1}, i.p.) in unsensitized rats. Ten minutes later the animals were anaesthetized with ether and exsanguinated. The peritoneal cavity was washed with 5 ml of saline containing heparin (5 u ml^{-1}) and the lavage fluid was recovered with a Pasteur pipette. The mesentery was then excised and weighed. A sample of mesentery was incubated with formamide (4 ml g^{-1} tissue) for 24 h. The concentration of Evans blue extracted into the supernatant solution was measured spectrophotometrically in an ELISA reader, at 620 nm. Dye extravasation in the mesentery was expressed as μg dye g^{-1} wet weight of tissue. Dye in lavage fluid was expressed as μg dye ml^{-1} of lavage volume recovered (estimate 4 ± 0.5 ml).

Sensitized rats of either strain were given RU38486 for 3 days (10 mg kg^{-1}, i.p.) and then challenged with antigen 24 h after the last injection of RU38486 (Moura & Moraes, 1994). Dye extravasation was then measured as described above.

Measurement of ovalbumin specific antibodies by ELISA

The animals were anaesthetized with ether, blood withdrawn by cardiac puncture, allowed to clot in an ice bath and the serum collected. To determine the IgE concentration, 96-well microplates were coated overnight at 4°C with 1 μg per well of a mouse monoclonal antibody to rat IgE Fc, diluted in borate buffer (0.2 M, pH 8.6). The plates were washed with PBS containing 0.05% of Tween 20 (PBS-T), and incubated for 3 h at 37°C with borate buffer containing gelatin (3%) and Tween 20 (1%). After washing with PBS-T an aliquot of 100 μl of the individual serum samples, diluted in PBS-T containing 0.5% of gelatin (PBS-T-gel), was added to each well and incubated for 1 h at 37°C. After washing, 1 μg of biotin-conjugated ovalbumin diluted in PBS-T-gel was added to each well and incubated for 1 h at 37°C. The plates were again washed with PBS-T, and 100 μl of horseradish peroxidase-conjugated streptavidin (1 : 2000 in PBS-T gel) was added to each well, and the plates were again incubated for 1 h at 37°C.

For IgG2a determination, 96-well microplates were coated overnight at 4°C with 1 μg per well of ovalbumin. The plates were then washed with PBS-T and a volume of 100 μl of serial dilutions of the sera was applied and incubated for 2 h at 37°C. After washing, a peroxidase labelled mouse monoclonal antibody to rat IgG2a Fc, diluted in PBS-T (1 : 1000) was added and incubated for 1 h at 37°C.

Peroxidase activity in both assays was measured by addition of 100 μl per well of *o*-phenylene-diamine (OPD) (0.01% in citrate buffer 0.1 M pH 5.5), in the presence of 10 μl of H_2O_2. After 15 min the reaction was stopped with citric acid 0.2 M (50 μl per well) and quantified spectrophotometrically at 490 nm wavelength.

Evaluation of mast cell degranulation

To examine mast cell degranulation after immunological challenge, rats (SHR or NTR) sensitized or unsensitized (control) received the antigen (1 mg) i.p. and ten minutes later the animals were anaesthetized and exsanguinated. The abdomen was opened and the mesentery was dissected away from the small intestine. Fragments of mesentery were fixed and stained for 15 min in a solution containing 50% ethanol, 10% formaldehyde, 5% acetic acid and 0.2% toluidine blue. Mesentery fragments were then mounted on a glass slide, care being taken not to fold or stretch the tissue. Mast cell degranulation was assessed by counting the % of cells with extruded granules (12 microscopic fields counted; $250 \times$ magnification). In rats, degranulated cells can be visualized because the granules are still stained by the dye when they have been discharged from the cell and are extracellular (Mota, 1964).

Measurement of anaphylactic reaction in vitro

Untreated guinea-pigs were killed by cervical dislocation and pieces of terminal ileum (2 cm) were removed and suspended under 2 g tension in organ baths containing oxygenated (95% O_2, 5% CO_2) and warmed (37°C) Krebs solution of the following composition (in mM): NaCl 115.0, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 2.5, $NaHCO_3$ 25.0 and glucose 11.0. This solution also contained a mixture of antagonists in the final concentrations shown (in mM): dibenamine 100, methysergide 57, hyoscine, 52 and propranolol 880. The tissue was left to stabilize for 45 min with changes of Krebs solution every 15 min. Isometric contractions to histamine (3–300 ng ml^{-1}) were recorded and a dose-effect curve constructed. A piece of mesentery (about 650 mg) from sensitized animals of either strain was then added to the Krebs solution in the organ bath and, if necessary, a new baseline established. After a minimum of 15 min, ovalbumin was added to the organ bath to give a final concentration of 125 μg ml^{-1} and any resulting contraction recorded. The piece of mesentery was then removed and the ileum allowed to relax with several changes of Krebs solution. A further dose-effect curve for histamine was then generated and the response to ovalbumin expressed as ng equivalents of histamine released g^{-1} of mesentery added, by reference to the mean values of the dose-effect curves.

Passive cutaneous anaphylaxis (PCA)

PCA reactions were produced by the technique of Mota & Wong (1969). Sera from sensitized SHR or NTR were serially diluted and injected intradermally (100 μl/site) in the shaved dorsal skin of unsensitized SHR or NTR. After 24 h, the animals were injected i.v. with 1 ml of a solution containing 500 μg of ovalbumin and 2.5 mg of Evans blue in saline. Thirty minutes later the rats were killed, the skin removed and the diameter of the dye stain measured on the inner surface of the skin. The PCA titres represent the highest dilution of the serum which gave a dye stain of more than 5 mm in diameter.

Statistical analysis

Results from the vascular permeability responses were analysed with a two-way analysis of variance, followed by Tukey's multiple comparisons. The *in vitro* experiments were analysed by Student's *t* test. Values of $P < 0.05$ were taken as showing a significant difference between means.

Materials

The following compounds were purchased from Sigma Chemical Co. (St. Louis, U.S.A.): ovalbumin (three times crystallized), compound 48/80, formamide, *o*-phenylene diamine (OPD), dibenamine, hyoscine, propranolol, Evans blue and toluidine blue. Aluminum hydroxide (Aldrox) was obtained from Wyeth (Whitehall Ltd., Brazil). Methysergide

was obtained from Sandoz S.A. (Brazil), and heparin from Roche Produtos Químicos e Farmacêuticos S.A. (Rio de Janeiro, Brazil). Mouse monoclonal antibody (IgG1 isotype, clone MARE-1) to rat IgE (Fc specific), and peroxidase labelled mouse monoclonal antibody (IgG1 isotype, clone MARG-2a) to rat IgG2a (Fc specific) were purchased from Serotec Ltd. (Kidlington, Oxford, U.K.). Horseradish peroxidase-conjugated streptavidin was purchased from Amersham International (Amersham, U.K.), and hydrogen peroxide from Merck S.A. (Rio de Janeiro, Brazil).

RU38486 (11-β-17-β-dihydroxy-6-methyl-17 α-(1-propynyl) androsta-1,4,6-trien-3-one) was received as a gift from Dr D. Philibert from the Centre de Recherches Roussel UCLAF (France).

Results

Increased vascular permeability induced by immunological challenge

The mean resting systolic blood pressure, measured by tail cuff, in SHR before immunization was 198.2 ± 7.9 and in NTR was 112.5 ± 4.6 mmHg.

Animals from both groups were sensitized with ovalbumin/alumen. After 14 days, the animals received an i.p. injection of the antigen, together with an i.v. injection of Evans blue, and 10 min later the vascular permeability was evaluated by measuring the concentration of extravasated dye. The results obtained confirmed the earlier observation of a greatly diminished vascular permeability response to immunological challenge in SHR. In these experiments another strain of normotensive rats, the Wistar-Kyoto (WKY), more closely related to the SHR strain was also used.

As summarized in Figure 1, the amounts of dye in peritoneal lavage or in the extravascular space within the mesentery in the SHRs was only 10–15% of that observed in NTR or WKY after challenge with ovalbumin.

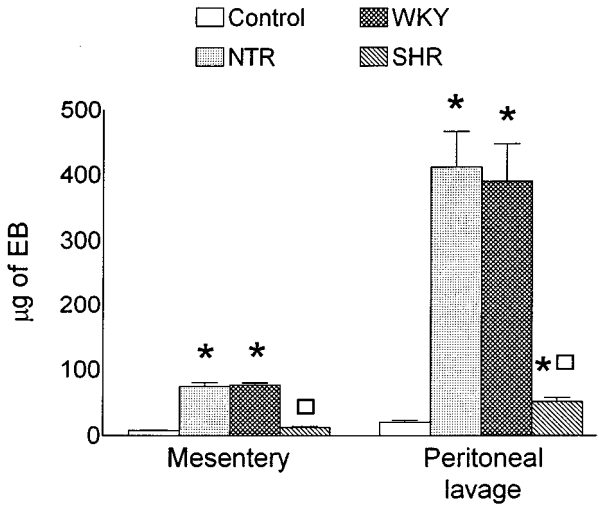

Figure 1 Increased vascular permeability induced by immunological challenge. Results are shown for four groups of animals–one control, unsensitized and three sensitized groups, normotensive Wistar (NTR), normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). Both the normotensive groups exhibited increased mesenteric permeability and extensive dye leakage into the peritoneal cavity on challenge. Responses of the SHR group were markedly decreased, relative to the other two groups. The values shown represent the means $\pm$ s.e.mean of 20 to 22 animals per group. *$P<0.05$ compared to the control group; □$P<0.05$ compared to the NTR and to the Wistar-Kyoto (WKY) group.

The levels of dye found extravascularly 10 min after being injected i.v. in unsensitized rats (basal levels) were similar in SHR, NTR and WKY animals. Injection of ovalbumin i.p. in unsensitized rats of the SHR strain (control groups, $n=12$) induced low levels of dye extravasation (17.8 ± 1.4 μg and 8.0 ± 0.5 μg g^{-1} in peritoneal lavage and mesentery, respectively), not different from the values in unsensitized NTR rats (23.9 ± 4.6 μg and 7.6 ± 1.1 μg g^{-1}). These values were also not different from the basal values in the unsensitized rats. Since control values were similar between the strains, we have combined these control values to give a single mean value as shown in Figure 1.

In a limited series of experiments, the effect of a glucocorticoid antagonist RU38486 (Philibert, 1984) on dye extravasation after immunological challenge was assessed in both strains. The results (Table 1) disclosed no changes in the permeability response of any of the tissues measured.

Increased vascular permeability induced by compound 48/80

Compound 48/80 is a non-immunological mast cell degranulator which does not require immunoglobulins attached to the mast cell membrane, and that has selectivity for connective tissue mast cells (Hunt & Hunt, 1960; Pearce *et al*., 1982). We assessed the degranulation of mesenteric mast cells on treatment with this compound when given directly into the peritoneal cavity, by measuring microvascular permeability. In these experiments, no difference in the dye leakage either in mesentery or in lavage fluid was seen between strains (Figure 2).

Production of anaphylactic antibodies against ovalbumin

Another possible reason for the difference between the responses of the SHR and NTR to immunological challenge was that antibody production was impaired in SHR. We therefore measured the amounts of circulating antibodies specific for ovalbumin of the two types relevant to mast cell responses in rats, IgE and IgG2a. As shown in Figure 3, no differences were detected in the amounts of ovalbumin-specific IgE and IgG2a, in the serum of SHR and NTR 14 days after sensitization.

Histological examination of mesenteric mast cells and ex vivo *studies*

We then counted the number of mast cells present in the mesentery and the percentage of mast cell with extruded

Table 1 Effect of the glucocorticoid antagonist RU38486 on vascular permeability after immunological challenge

		Vascular permeability (μg dye g^{-1} tissue)	
	Treatment	*Mesentery*	*Peritoneal lavage*
NTR	Control	67.3 ± 4.5	340.7 ± 38.3
	RU38486	76.3 ± 6.0	250.2 ± 34.2
SHR	Control	9.6 ± 0.5	53.0 ± 7.4
	RU38486	10.8 ± 0.5	39.4 ± 10.4

Results are expressed as μg of Evans blue dye g^{-1} of wet tissue (mesentery) or total dye in peritoneal washings (lavage) and represent the mean $\pm$ s.e.mean of 4–5 rats per group. Control values were obtained without pretreatment with RU38486 (see Methods for details). The antagonist did not affect permeability responses in either strain.

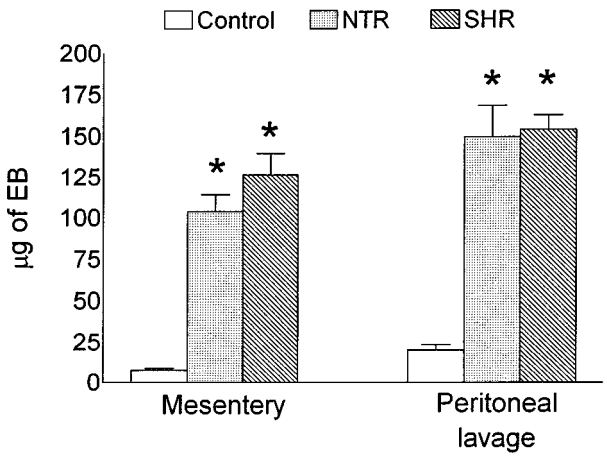

Figure 2 Increased mesenteric vascular permeability induced by compound 48/80. Compound 48/80 (2 mg kg^{-1}) was injected i.p. and dye extravasation in the mesentery and peritoneal lavage fluid was measured as described in the Methods. Control results here show dye extravasation in rats of either strain injected i.p. with saline; they were not different and have been combined to give a single value. Following compound 48/80, large increases in dye extravasation were recorded in the mesentery and in peritoneal lavage; these results also did not differ between strains. The values shown are expressed as μg dye g^{-1} of tissue for mesentery or total μg dye per rat for peritoneal lavage and represent the mean $\pm$ s.e.mean of 10 animals per group. $^*P<0.05$ compared to the control group.

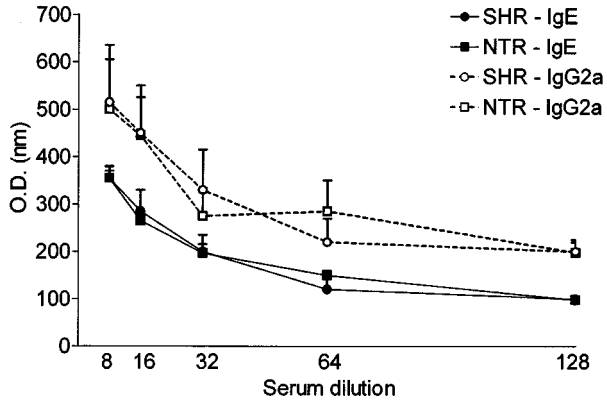

Figure 3 Quantitation of anti-ovalbumin antibodies in the serum of SHR and NTR. The animals were immunized as described in the Methods and blood samples taken 14 days later. The concentration of specific antibodies of IgE (solid symbols) and of IgG2a (open symbols) types in the serum was determined by ELISA and is expressed as O.D. at 490 nm. The values shown (means $\pm$ s.e.mean of 5 animals per group) have been corrected for the low values obtained in unsensitized animals of either strain.

granules in whole-mount preparation of mesentery following challenge in SHR and NTR. The histological preparation of the mesentery has been described in Methods. There were no differences in the numbers of mast cells in the mesentery between strains before challenge (Table 2), although when the sensitized animals were challenged with the antigen, extensive degranulation was noted in NTR (about 80%) in contrast to less than 4% degranulated mast cells in SHRs (Table 2).

The next set of experiments was designed to investigate whether the impaired capacity of the SHR mast cells would persist if they were isolated and stimulated *ex vivo*. Pieces of mesentery taken from sensitized rats were co-incubated in an organ bath with a preparation of isolated ileum from normal

Table 2 Mesenteric mast cells in NTR and SHR

	NTR	*SHR*
Mast cell number before challenge (cells/field)	34.3 $\pm$ 2.7	35.8 $\pm$ 2.2
% degranulated after challenge in sensitized rats	81.8 $\pm$ 8.9	3.9 $\pm$ 4.3*
% degranulated after challenge in unsensitized rats	2.8 $\pm$ 2.4	3.7 $\pm$ 2.4

The values shown are the means $\pm$ s.e.mean of 5–7 rats in each group. Although there were the same number of mast cells in the mesentery of either strain, there was essentially no degranulation of mast cells in the mesentery of sensitized SHR after challenge, in contrast to the extensive degranulation seen in the NTR. $^*P<0.05$ different from the corresponding value for NTR.

guinea-pigs. Upon addition of the antigen to the organ bath, the guinea-pig ileum contracted only when co-incubated with the mesentery from sensitized NTR (Figure 4). This difference was quantified in terms of contractions of the ileum to histamine. The peak height of responses to immunological challenge of NTR mesentery was equivalent to 34.5 $\pm$ 6.6 ng histamine (mean $\pm$ s.e.mean; $n=5$), whereas very small contractions (equivalent to 6.0 $\pm$ 2.4 ng histamine; $n=7$) were observed with challenge of SHR-derived tissue. No attempt was made to assess the extent or duration of the long lasting contraction of the ileum to mesenteric challenge (see Figure 4).

Passive cutaneous anaphylaxis (PCA) in NTR and SHR

Since the mesenteric mast cells in the SHR appeared to be abnormal only to immunological challenge, the reactivity of other similar connective tissue mast cells was assessed by the PCA reaction in a crossover design using immune sera from either strain tested in each strain. As shown in Figure 5, the PCA reaction in NTR with NTR immune serum gave a high titre, expressed as the dilution of the serum giving a minimum reaction (see Methods). Furthermore immune sera from NTR tested in SHR skin gave a very low titre confirming the resistance of SHR skin mast cells to immunological challenge. However, immune sera from SHR which contain high levels of IgE and IgG2a antibodies (see Figure 3) was not able to induce a PCA in the NTR skin.

Discussion

Our present experiments have supported and extended our preliminary findings that whereas injection of antigen into sensitized rats induces an increase in vascular permeability in the mesentery and peritoneal cavity of NTR, equivalent immunological challenge in the SHR did not change the vascular permeability. This finding together with the normal response of the SHR to compound 48/80 showed that the defect in the SHR was confined to immunological stimulation. In our analysis of this defect these results led us to focus on the components of the anaphylactic response itself.

Clearly, the first requirement for active anaphylaxis to take place is the production of anaphylactic antibodies that bind to the mast cell membrane. In rats, IgG2a and IgE are considered anaphylactic antibodies (Bazin & Pauwels, 1982) and our data show clearly that ovalbumin-specific IgE and IgG2a were produced in equal amounts by SHR and NTR. There is one report that the SHR produce lower levels of antibodies against sheep red blood cells (Takeichi *et al.*, 1981). However,

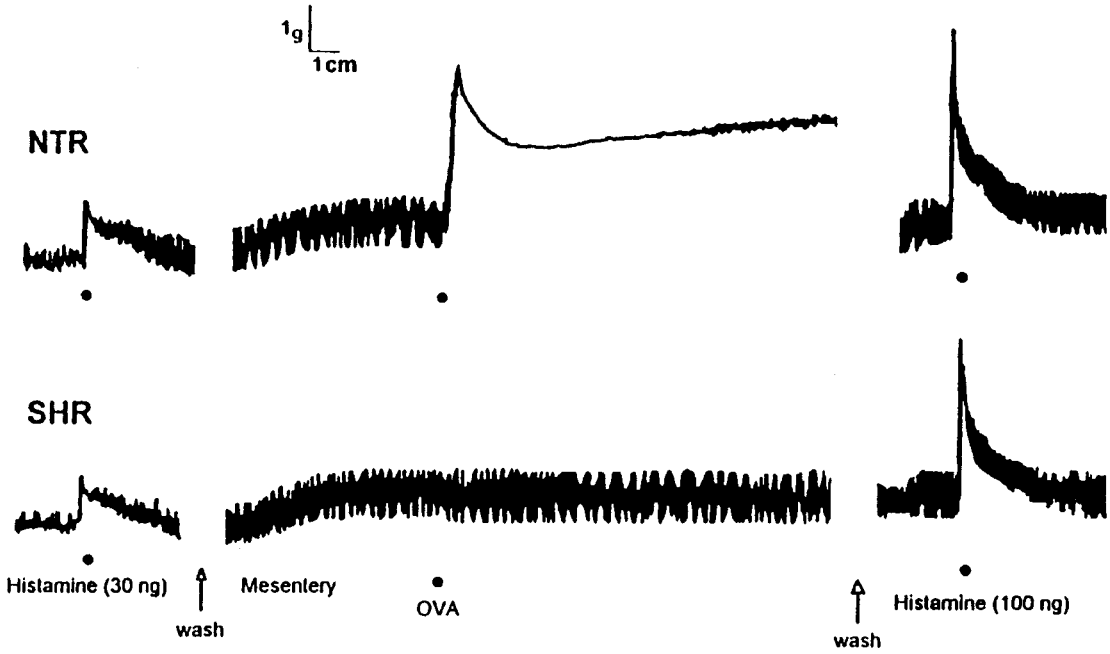

Figure 4 Release of myotropic bioactivity following challenge of sensitized rat mesentery *ex vivo*. Contractions of guinea-pig isolated ileum to standard doses of histamine are shown as the first and last responses. The middle response of the upper trace shows the lack of response when a sample of mesenteric tissue from a sensitized rat was added to the organ bath containing the ileum. However, after addition of ovalbumin (OVA) to the organ bath containing mesenteric sample from a sensitized NTR, a marked and long lasting contraction was observed. The lower trace shows that there was essentially no contraction after addition of OVA in presence of mesentery taken from a sensitized SHR. Addition of mesenteric samples from unsensitized rats of either strain released no bioactivity with or without challenge with ovalbumin (responses not shown).

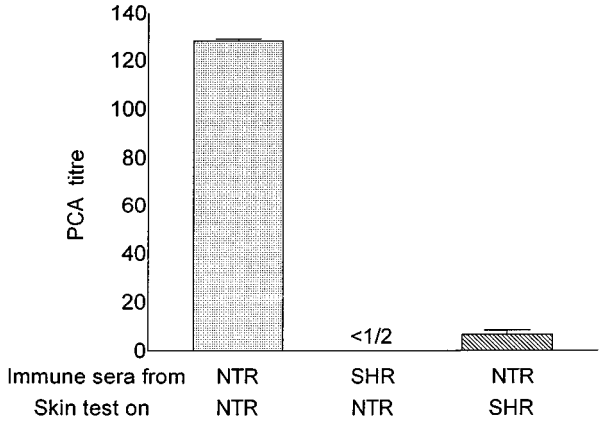

Figure 5 Passive cutaneous anaphylaxis reaction in SHR or NTR. Serum taken from NTR or SHR was serially diluted and injected in the skin of unsensitized SHR or NTR. After 24 h the antigen (ovalbumin) was injected i.v. together with Evans blue dye and the animals were killed 30 min later. Immune serum from NTR induce high PCA titre when injected in NTR skin but very low titre when injected in SHR skin. Immune serum from SHR did not induce a PCA reaction in the NTR skin. Results are expressed as the reciprocal of the highest dilution of the immune serum that gave a lesion of more than 5 mm of diameter. Data represent the median of 6 to 8 animals per group $\pm$ s.d.

immunization with particulate antigens, as used by Takeichi *et al.* leads preferentially to production of IgM antibodies, whereas soluble antigen in alumen as used in our experiments induces preferentially the production of IgE antibodies. Our results would thus rule out defects in the sensitization process as an explanation for the observed difference in immunological response. Although several other anomalies in the immune function of the SHR have been described including lymphocyte defects (Pascual *et al.*, 1992; 1993; Offosu-Appiah & Ruggiero, 1992; Purcell & Gattone, 1992; Purcell *et al.*, 1993) and enhanced macrophage suppressor activity (Pascual *et al.*, 1993), no estimate of the production of anaphylactic antibodies in the SHR has previously been made. From our results it is clear that the ability to produce anaphylactic antibodies is not defective in the SHR.

However, these antibodies produced by the SHR could still be inactive in triggering the mast cell Fc receptor even though their reaction with the antibodies in the ELISA was normal. It is certainly possible that the recognition sites for the mast cell receptor and for the antibodies used in ELISA are quite different and capable of being changed independently of each other. Since we have no evidence for such a change, the simplest logical assumption is that the antibodies in the SHR were indeed effective at the mast cell receptor as well.

If then the antibodies fixing to the mast cell are normal, the mast cells themselves in the SHR may be abnormal. Here we were able to show that although SHR and NTR had equal numbers of mast cells in the mesentery, the mast cells of SHR did not degranulate in response to antigen challenge *in vitro*. This finding was unexpected both in so far as there is no obvious reason for rats with hypertension also to have abnormal mast cell function and because where differences have been reported in mast cells from SHRs (Masini *et al.*, 1991), those differences have been shown as increases in function, not decreases. There are two possible explanations for this discrepancy. The first one is that the experiments described by these authors were *in vitro* with mast cells from peritoneal washings and our experiments were performed *in vivo* and *ex vivo* with mast cells *in situ*. The second and perhaps more likely reason, is that the results obtained by Masini *et al.* (1991) were obtained in mast cells stimulated with non-

immunological stimuli such as compound 48/80 or calcium ionophore. In our experiments SHR mesenteric mast cells were able to respond normally to non-immunological stimuli. The histamine content in peritoneal fluid after challenge was not measured, so we cannot comment on the possibility that mesenteric mast cells from SHR may release more histamine when activated by a non-immunological stimulus, as found for mast cells free within the peritoneal cavity by Masini *et al.* (1991).

The results of the co-incubation experiments showed that the difference between SHR and NTR was still present when anaphylaxis was induced *ex vivo*, indicating that the mast cells from the SHR were still unable to respond, even when removed from the animal. The resistance *in vivo* and *ex vivo* to immunological degranulation combined with a normal susceptibility to compound 48/80 strongly suggests a defect in the mesenteric mast cells of the SHR, a defect only exhibited towards immunological stimulation. This could be explained at the signal transduction level, since non-immune stimulation of mast cells involves G protein (Mousli *et al*., 1989; 1991; Aridor *et al*., 1990) but does not require external calcium (Bronner *et al*., 1987), whereas in immune stimulation the participation of G proteins is not clear (Beaven & Metzger, 1993) and extracellular calcium is essential for degranulation (Foreman & Mongar, 1972).

Assuming normal antibodies, these findings suggest a defect in the mast cell receptor and our PCA experiments were designed to expose such a difference between NTR and SHR. The first crossover experiment with immune serum from NTR tested in SHR skin appeared to confirm the SHR mast cell as the defective component. Since both mesenteric and skin mast cells are connective tissue mast cells, our original findings would be explained by a failure of the SHR mast cell either in binding antibody or perhaps in the subsequent signalling pathways leading to degranulation.

However, the failure of the SHR immune serum to provide a PCA in NTR skin pointed to a defect in the SHR immune serum. This defect could not be due to insufficient antibodies as the ELISA had already shown that the antibodies in SHR and NTR were quantitatively equal (see Figure 3). Apart from the possible qualitative discrepancy discussed above, another possible explanation of the 'defective SHR serum' is the presence of some factor inhibitory to immunological activation.

One candidate for the inhibitory factor would be an endogenous glucocorticoid. The SHR strain have a well documented abnormality in the hypothalamic-pituitary-adrenal system which is probably responsible for the development of hypertension (Aoki, 1963; Aoki *et al*., 1963; Hashimoto *et al*., 1989). In man, as in laboratory animals essential hypertension is associated with higher glucocorticoid levels (Whitworth, 1987). In more recent studies, impairment of leukocyte adhesion in SHR was mediated by high levels of glucocorticoids, since adrenalectomy and the use of a glucocorticoid antagonist prevented the defect (Suzuki *et al*., 1994; 1995). Furthermore, exogenous glucocorticoid inhibited almost completely dye extravasation induced by PCA in normotensive rats (Miura *et al*., 1992) and in rabbits, local dexamethasone inhibited IgE-induced PCA reactions generated 72 h later (Hellewell & Williams, 1989). Pretreatment with glucocorticoids inhibited degranulation of connective tissue mast cells induced by immunological stimulation but not that induced by compound 48/80 or substance P (Heiman & Newton, 1995). This selective effect of glucocorticoids on responses to immunological stimuli could be related to the inhibition of Fcε receptors expression by dexamethasone (Crabtree *et al*., 1979; Yodoi *et al*., 1981). However, our finding that the glucocorticoid antagonist RU38486 did not modify the response to immunological challenge in the SHR would not support the possibility that glucocorticoids are the inhibitory component of SHR serum.

Our results have shown that the SHR are abnormal in aspects apart from blood pressure like the abnormal response to immunological challenge shown here.

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