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Endogenous nitric oxide does not modulate mesenteric mast cell degranulation in rats

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Abstract

The inhibitory effects of endogenous nitric oxide could explain the decreased mesenteric mast cell degranulation after anaphylaxis in genetically hypertensive rats (SHR). SHR and normotensive rats (NT) were sensitized to ovalbumin and challenged 14 days later. Degranulation of mast cells was assessed in duodenum, mesentery and skin by increased microvascular permeability using extravasation of Evans blue dye (20 mg/kg, i.v.), and in the mesentery also by light microscopy after staining with toluidine blue. Pretreatment with an inhibitor of nitric oxide synthesis, L-NAME (30 mg/kg, i.v.) did not change dye extravasation after immunological challenge or after compound 48/80 in mesentery of either SHR or NT. PCA was also defective in SHR. Pretreatment with L-NAME did not affect either the defective PCA in SHR or the normal PCA reaction in NT. Our results show that inhibition by endogenous nitric oxide is not the cause of the defective mast cell degranulation in the SHR nor did it modulate degranulation of mesenteric or skin mast cells in NT.

Keywords: Mast cells; Nitric oxide; Anaphylaxis; Vascular permeability; Spontaneously hypertensive rat

1. Introduction

In an earlier paper, we reported that mast cells in the mesentery of sensitized rats of the genetically hypertensive strain (SHR) were resistant to degranulation following immunological challenge *in vivo*, relative to mast cells in normotensive sensitized animals, although normal levels of antigen-specific antibodies were present in serum [1]. One endogenous inhibitor of mast cell degranulation appears to be nitric oxide, highly effective *in vitro* [2–5] and *in vivo*, particularly in the rat mesenteric microcirculation [6,7]. It has been postulated that SHR produce, constitutively, higher levels of nitric oxide than the normotensive strain as a compensatory mechanism for their higher blood pressure [8,9]. Such an increased production of nitric oxide could explain the resistance to degranulation of SHR mast cells that we had observed. However, Masini *et al.* [10] showed that peritoneal mast cells from SHR generated less nitric oxide than mast cells from normotensive animals and released more histamine on degranulation.

There are three major differences between the conditions of mast cell degranulation in the two sets of experiments [1,10]. We used immunological challenge as the stimulus for degranulation and assessed degranulation *in vivo* in mast cells, within tissue [1], whereas Masini *et al.* [10] used nonimmunological stimuli (compound 48/80 or calcium ionophore A23187) and assessed degranulation *in vitro*, using free mast cells from peritoneal washings. One possible resolution of the discrepancy between the findings of Masini *et al.* [10] and ours is to postulate that the cells in the mesentery adjacent to the mast cells generate nitric oxide and that, on challenge, the mesenteric mast cells could be exposed to nitric oxide derived from the neighboring cells, which would more than compensate for any lack of nitric oxide production by the mast cells themselves. This proposition would preserve the general

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Abbreviations: SHR, spontaneously hypertensive rats; NT, normotensive rats; L-NAME, *N*ω–nitro-L-arginine methyl ester; WKY, Wistar–Kyoto rats; PCA, passive cutaneous anaphylaxis.

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principle of nitric oxide as an inhibitor of mast cell degranulation and attribute the difference in findings to the different cellular environments involved. Thus, the lack of mast cell degranulation in the SHR could be postulated as being due to an overproduction, relative to the normotensive strain, of nitric oxide generated by adjacent cells as a result of the immunological challenge. For instance, vascular smooth muscle cells from SHR produce higher levels of nitric oxide than those from normotensive rats (NT) [11]. A test of this proposition would be that inhibition of endogenous nitric oxide synthesis should increase degranulation in the SHR, measured under our original conditions.

We have therefore assessed the contribution of endogenous nitric oxide to our model of *in vivo* degranulation of mesenteric mast cells following antigen challenge in sensitized animals of the SHR and normotensive strains. Because the mast cells in the rat mesentery are predominantly of the connective tissue type, we also studied responses in rat duodenum where the other type of mast cell, mucosal mast cells, are a major component [7,12]. Degranulation of mast cells in skin through the PCA reaction was essentially absent in the SHR [1] and the contribution of endogenous nitric oxide to this defect was also assessed. As an index of mast cell degranulation *in vivo* we have used increases in microvascular permeability, measured by extravasation of Evans blue dye, given intravenously (i.v.). We have also measured degranulation by histological examination.

Our results did not support our initial proposition as inhibition of endogenous nitric oxide synthesis had no effect on mesenteric mast cell degranulation in the SHR. However and more surprisingly, we could not demonstrate that endogenous nitric oxide modulated the degranulation of mesenteric mast cells after challenge even in the control, normotensive strain. This suggests that the widely observed inhibitory effects of nitric oxide on mast cell degranulation *in vitro* may not be demonstrable *in vivo*.

2. Materials and methods

2.1. Animals

Male Wistar NT, SHR and the closely related normotensive Wistar–Kyoto strain (WKY), 5–6 months old (body weight 280–350 g), from our own facilities were used (Central Bioterium of Biomedical Sciences Institute, University of São Paulo). The systolic blood pressure, measured by the tail cuff method, was 112.5 (±4.6) mmHg in the NT (mean ± SEM; N = 15), 117.2 (±3.7) mmHg in the WKY (N = 6), and higher, 171.8 (±7.9) mmHg, in the SHR (N = 14) ($P < 0.05$). Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation and approved by the Biomedical Sciences Institute/USP–Ethical Committee for Animal Research (CEEA).

2.2. Stimuli for mast cell degranulation

2.2.1. Immunological challenge

Rats were sensitized with an intraperitoneal (i.p.) injection of ovalbumin (10 μg/rat) in aluminium hydroxide ("Alumen," Aldrox®—Wyeth; 10 mg/rat). Fourteen days after the sensitization, the animals were challenged with an i.p. dose of ovalbumin (1 mg/rat) in 0.5 mL of isotonic saline. Increases in vascular permeability were measured 10 min later.

2.2.2. Compound 48/80

In another set of sensitized animals, degranulation of mast cells was induced by i.p. injection of compound 48/80 (2 mg/kg in isotonic saline), immediately after i.v. injection of dye. Increases in vascular permeability were measured 10 min later.

For either stimulus, the basal levels of dye extravasation were assessed in control groups consisting of unsensitized animals given only the dye i.v. or the dye with ovalbumin (1 mg/rat, i.p.). The dye extravasation in these two groups was not different and values were combined to provide the value shown as Control in the figures.

2.3. Measurement of vascular permeability

Increased vascular permeability was assessed by the Evans blue dye method modified by Sirois *et al.* [13]. Briefly, Evans blue (20 mg/kg) was injected i.v. immediately before the stimulus. Ten minutes later the animals were anesthetized with ether and exsanguinated. The mesentery and duodenum were then excised and a weighed sample incubated with formamide (4 mL/g tissue) for 24 hr. The tissues were then removed from the formamide and the solution centrifuged for 10 min at 1700 *g*. The concentration of Evans blue in the supernatant solution was measured spectrophotometrically in an ELISA reader, at 620 nm. Values of dye extravasation in the tissues were expressed as μg dye/g wet weight.

2.4. Histological assessment of mesenteric mast cell degranulation

Sensitized or unsensitized (control) rats (SHR or NT) were given antigen (ovalbumin, 1 mg/rat, i.p.) and 10 min later the animals were anaesthetized and exsanguinated. The mesentery was removed and placed in an aqueous staining solution containing 50% ethanol, 10% formaldehyde, 5% acetic acid (v/v) and 2% toluidine blue (w/v). After 15 min, the mesentery was blotted dry on filter paper and then divided into 3–4 approximately equal pieces. These were mounted on a glass slide, care being taken not to fold or stretch the tissue. Mast cell degranulation was expressed as the proportion (as %) of mast cells with extruded granules relative to the total mast cells present in 12 microscopic fields (250× magnification), per piece of

mesentery. With approximately 35 mast cells per microscopic field, the total numbers of mast cells counted per mesentery was 900–1000. This total was not different between strains [1]. 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 [14].

2.5. PCA reaction

The degranulation of skin mast cells induced by immunological challenge was assessed by the PCA reaction [15]. Sera from sensitized NT were pooled (5 rats/pool), serially diluted and injected intradermally (100 μL per site) in the shaved dorsal skin of unsensitized NT or SHR animals. After 24 hr, the rats were given an i.v. injection (1 mL) of isotonic saline containing the antigen (500 μg) and Evans blue (25 mg). Thirty minutes later, the rats were anaesthetized with ether, exsanguinated and the dorsal skin removed. The diameter of the stain of extravasated dye on the inner surface of the skin was measured. The PCA titres represent the highest dilution of the serum which gave a dye stain of more than 5 mm in diameter.

2.6. Inhibition of endogenous nitric oxide synthesis

Animals were treated with an inhibitor of nitric oxide synthesis, L-NAME, dissolved in isotonic saline and given i.v. 30 min before the challenge.

2.7. Statistical analysis

Results from the vascular permeability responses are shown as means (±SEM) and were analyzed with a two-way analysis of variance, followed by Tukey's multiple comparisons. Values of $P < 0.05$ were taken as showing a significant difference between means.

2.8. Materials

The following compounds were purchased from Sigma Chemical Co—ovalbumin (Grade III), Evans blue, toluidine blue, formamide and L-NAME. Aluminium hydroxide (Aldrox®) was obtained from Wyeth. Heparin was obtained from Roche Produtos Químicos e Farmacêuticos S.A.

3. Results

3.1. Permeability responses of peritoneal tissues

Microvascular permeability in the duodenum and mesentery of sensitized rats challenged *in vivo* with antigen was measured by extravasation of Evans blue dye. As shown in Fig. 1, dye extravasation in the duodenum of the SHR was markedly increased after antigen challenge. This response in the SHR was not different from the responses observed in the duodenum of the WKY strain or from that in the NT strain. As we have previously shown [1], although the mesentery in the NT and the normotensive WYK strain exhibited a clearly increased dye extravasation after challenge, the response of the mesentery in the SHR was so low as to be indistinguishable from that of the control group. This latter group comprised sensitized animals challenged with saline and unsensitized animals challenged with antigen. As the responses were not different between these two control sub-groups, the values have been combined into a single control value, shown in Fig. 1.

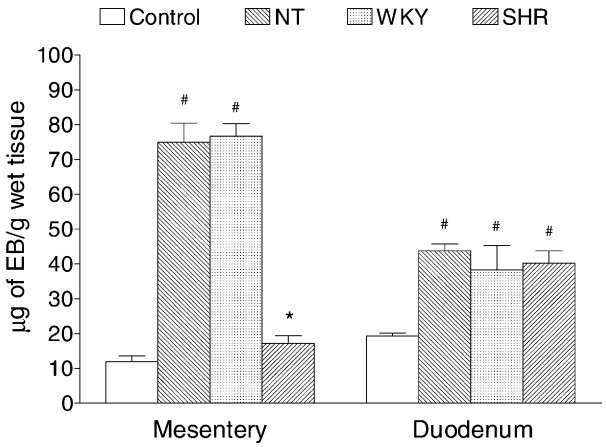

Fig. 1. Strain-dependent dye extravasation following immunological challenge in sensitized rats. In either tissue from any strain, values of dye extravasation in nonsensitized rats challenged with antigen or in rats challenged with saline were low and have been combined to give the control value shown (Control). In the WKY and NT, dye extravasation was significantly increased in both tissues sampled after antigen challenge ($N = 6$ for WKY and 30 for NT; $P < 0.05$), relative to the control value. In the SHR, only the duodenum showed increased dye extravasation after challenge ($N = 30$; $P < 0.05$) to values not different from those in the NT and WKY strains. *Significantly different from corresponding values in NT or WKY. #Significantly different from corresponding values in control rats. Data represent the means (±SEM) of extravasation of Evans blue/g of wet tissue.

The contribution of endogenous nitric oxide to these permeability responses was assessed by pretreatment of animals with 30 mg/kg of L-NAME given i.v. 30 min before challenge. The systemic arterial blood pressure in NT and SHR animals pretreated with L-NAME was measured just before challenge and increased from 115 ± 5 mmHg to 140 ± 8 mmHg for the NT and from 175 ± 4 mmHg to 225 ± 14 mmHg for the SHR strains ($P < 0.05$; $N = 4$–5 rats in each group).

Following treatment with L-NAME (30 mg/kg), the increased microvascular permeability induced by antigen challenge in the duodenum of either strain was diminished (Fig. 2). In the SHR, dye in the duodenum was reduced to control, that is, unstimulated levels and in the NT strain, dye extravasation after L-NAME was decreased to midway

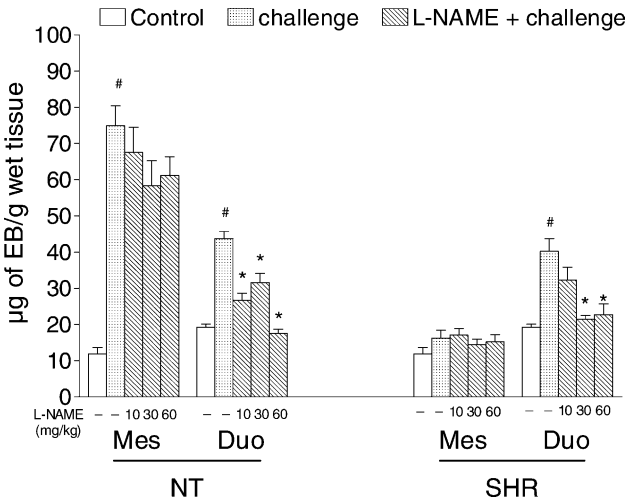

Fig. 2. Tissue-dependent effects of nitric oxide synthase inhibition on dye extravasation in sensitized rats. In both strains, the postchallenge dye values in the mesentery were unchanged following treatment *in vivo* with L-NAME (10, 30, 60 mg/kg). However, in both strains, dye extravasation in duodenum was decreased. Control values for dye extravasation in either tissue were not affected by inhibition of endogenous nitric oxide synthesis. *Significantly different from value without L-NAME pretreatment; $P < 0.05$ (N = 12 for SHR and N = 20 for NT, for the dose of 30 mg/kg and N = 6 to 7 for the other doses). #Significantly different from corresponding values in control rats. Data represent the means (±SEM) of extravasation of Evans blue/g of wet tissue.

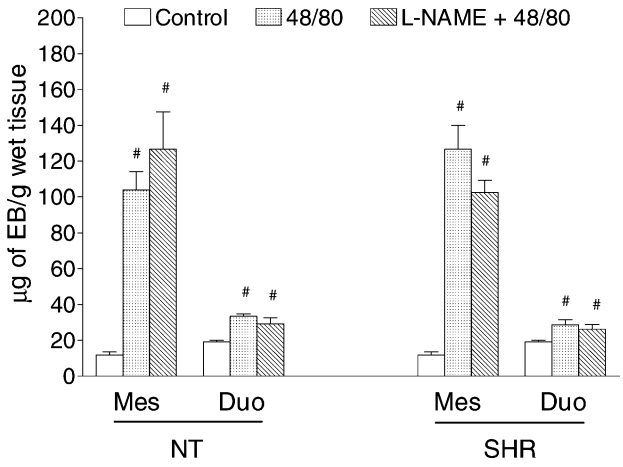

Fig. 3. Lack of effect of nitric oxide synthase inhibition on dye extravasation after degranulation of mast cells by compound 48/80. With this stimulus, the dye values in the mesentery and duodenum in both strains increased significantly and comparably. Pretreatment with L-NAME (30 mg/kg) did not affect degranulation as assayed by dye extravasation, in either tissue from either strain. #Significantly different from corresponding values in control rats. All values shown are means (±SEM) of results from 5 rats per group, and represent the extravasation of Evans blue/g of wet tissue.

between the unstimulated values and those in animals not receiving L-NAME. However, mesenteric dye extravasation values were not affected in either strain by pretreatment with L-NAME (Fig. 2). In animals from either strain, pretreatment with L-NAME did not alter the low basal, that is, without challenge, level of dye extravasation in any of the peritoneal samples taken (Table 1).

Because Masini *et al*. [10] had used compound 48/80 as a degranulating stimulus in their *ex vivo* study of mast cells, we gave compound 48/80 i.p. to sensitized animals, with or without pretreatment with L-NAME. With this stimulus, although microvascular permeability was increased in both mesentery and duodenum, there were no strain-dependent differences in rats without L-NAME pretreatment (Fig. 3). Furthermore, pretreatment with L-NAME did not alter the responses to compound 48/80 in either strain.

3.2. Histological assessment of mesenteric mast cells

Degranulation of mast cells was assessed histologically after immunological challenge *in vivo* and the results confirmed the defective response in the SHR (Table 2). Pretreatment with L-NAME did not affect the low proportion of degranulated cells in the mesentery of the SHR. Further, the degranulation in the mesentery of the NT animals was similarly unaffected by L-NAME pretreatment.

3.3. Responses of skin mast cells

In earlier work we had shown that the PCA reaction, generated by serum from sensitized NT, a response dependent on immunological stimulation of skin mast cells, was essentially absent in SHR animals, though easily demonstrable in the NT strain [1]. We therefore assessed the effect of pretreatment with L-NAME on the PCA reaction in

Table 1
Lack of effect of pretreatment with L-NAME on basal microvascular permeability in NT or SHR

	Evans blue dye extravasation (as µg/g of tissue)			
	NT		SHR	
	Mesentery	Duodenum	Mesentery	Duodenum
Control (treated with saline)	14.5 ± 0.7	19.3 ± 1.2	10.6 ± 1.0	19.6 ± 0.8
Treated with L-NAME	11.1 ± 2.0	23.8 ± 2.6	9.0 ± 1.0	24.9 ± 2.2

This table shows the basal microvascular permeability values in peritoneal tissues from sensitized rats, assessed 10 min after the administration of Evans blue dye. The control groups received an i.v. injection of isotonic saline and the treated groups, L-NAME (30 mg/kg, i.v.), 30 min before Evans blue dye. There were no changes in dye extravasation in either tissue from either strain, following treatment with L-NAME. Values shown are the mean (±SEM) results from 5 animals in each group.

Table 2
Histological assessment of degranulation in mesenteric mast cells after immunological challenge in NT and SHR; effect of L-NAME pretreatment

	Extent of degranulation (as % of mast cells counted)			
	No treatment		Pretreatment with L-NAME	
	NT	SHR	NT	SHR
Unchallenged	2.8 ± 2.3 (N = 4)	3.7 ± 2.4 (N = 4)	2.2 ± 0.7 (N = 5)	5.2 ± 2.0 (N = 5)
Challenged	57.1 ± 1.8* (N = 6)	4.4 ± 2.2 (N = 5)	59.7 ± 11.4* (N = 4)	2.4 ± 1.8 (N = 7)

This table illustrates the degranulation of mesenteric mast cells following antigen challenge *in vivo*, assessed histologically. Although mast cell degranulation, as measured by staining with toluidine blue (see Section 2), in mesentery from the NT was markedly increased after challenge, that in SHR mesentery was not changed. Pretreatment with L-NAME (30 mg/kg), 30 min before challenge *in vivo*, did not change this differential degranulation. The total numbers of mast cells in mesentery was not different between strains (about 35 per microscopic field; see Ref. [1]).

* Significantly different from unchallenged value; $P < 0.05$. The values shown represent the proportion of degranulated mast cells present over 12 high power fields. Mean (±SEM) results are shown, from N animals.

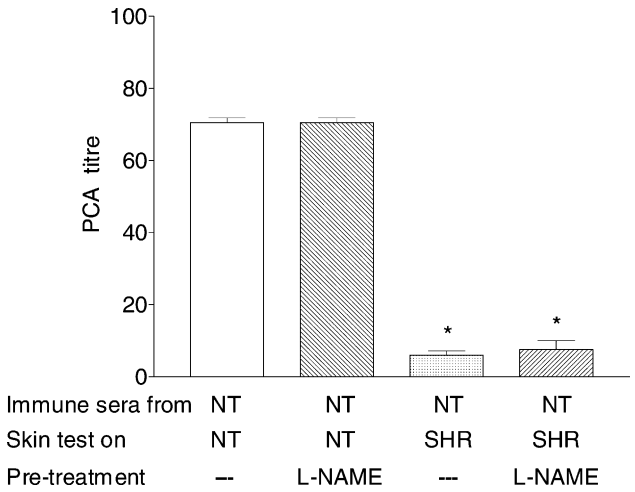

Fig. 4. Resistance of the PCA reaction to inhibition of nitric oxide synthesis. The PCA reaction in unsensitized rats to immune serum from NT was essentially absent in the SHR recipients, relative to the marked reaction in NT recipients. Neither level of PCA was modified by pretreatment of recipient animals with L-NAME (30 mg/kg). *Significantly different from corresponding values in NT. Values shown are means (±SEM) from 5–6 recipient rats per group, using immune serum pooled from 5 sensitized rats.

animals from either the NT or SHR strains. As shown in Fig. 4, the intensity of the reaction was assessed by the highest dilution of immune serum from (donor) sensitized NT that was able to produce a given magnitude of reaction in the skin of recipient, unsensitized animals (see Section 2). The low level of reaction in SHR rats was not altered by inhibition of endogenous nitric oxide synthesis nor was the high level of PCA in the NT recipients.

4. Discussion

We undertook these experiments to test our hypothesis that excessive production of endogenous nitric oxide was the reason for the decreased mast cell degranulation induced by immunological challenge in the SHR. This hypothesis was based on two premises, the deficient degranulation of mast cells in the SHR [1] and the widely supported concept of nitric oxide as an endogenous inhibitor of mast cell degranulation [2,6,7]. The role of endogenous nitric oxide in our model was deduced from the effects of pretreatment with L-NAME, an inhibitor of nitric oxide synthase [16]. The treatment regimen we chose increased arterial blood pressure in both normotensive and SHR strains, an indication of effective inhibition of endogenous nitric oxide synthesis.

However, the results of our experiments did not support our hypothesis in that pretreatment with L-NAME did not increase the degranulation of mast cells in the mesentery of the SHR, in response to immunological challenge. From these results we would conclude that an excess of endogenous nitric oxide does not contribute to the depressed mast cell degranulation in the mesentery of the SHR. Moreover, similar levels of NO synthase activity (both calcium-dependent and calcium-independent) were found in homogenates of duodenum from SHR and NT. These levels were not changed by immunological challenge (data not shown).

Unexpectedly, and more importantly, in the NT strain also, there was no evidence for modulation of mesenteric mast cell degranulation by endogenous nitric oxide. Thus, dye extravasation in the mesentery following antigen challenge was unaffected by pretreatment with L-NAME. There was also no evidence for an endogenous nitric oxide 'tone' controlling mast cell function as basal levels of dye extravasation were not altered by pretreatment with L-NAME, in either the mesentery or duodenum. Another, mast cell-dependent, immunological reaction—PCA—which was strongly expressed in the NT strain, was equally unaffected by systemic treatment with L-NAME. Since both the skin and the mesenteric mast cells are of the connective tissue type, it is difficult to resist the conclusion that in this type of mast cell, endogenous nitric oxide is not involved in degranulation after immunological challenge in the rat. More surprisingly, even when degranulation of mesenteric mast cells was induced, *in vivo*, by compound

48/80—a commonly used, nonimmunological stimulus, there was still no modulation of the degranulation after treatment with L-NAME.

In all these studies we used increased microvascular permeability leading to dye extravasation, as an indicator of mast cell degranulation because the stimuli we used (immunological challenge, compound 48/80) are well established as inducers of mast cell degranulation. Furthermore, there was no increase in microvascular permeability in nonsensitized animals challenged with antigen or in sensitized animals challenged with saline. These functional assays of mast cell degranulation were supported by histological assessment of mesenteric mast cells challenged with antigen in either strain and after L-NAME pretreatment. Essentially, there were very few degranulated mesenteric mast cells in the SHR and extensive degranulation in the NT. This difference was not altered by pretreatment with L-NAME. The absence of a microvascular permeability response in the SHR after immunological challenge did not reflect a defect in the microvasculature itself as exogenous histamine induced equal amounts of dye extravasation in the mesentery or the duodenum of both strains (unpublished experiments).

We sought to extend our analysis to mucosal-type mast cells which are found in abundance in the duodenum and here two differences were readily apparent. In the SHR, permeability increase in the duodenum in response to immunological challenge was equal to that in NT and, for both NT and SHR strains, there was clear inhibition of dye extravasation after L-NAME. These two findings would suggest that mucosal mast cells in the SHR were not defective in their response to immunological challenge and, further, that degranulation of this type of mast cell was sensitive to nitric oxide. Differences in responses to compound 48/80 and other strongly basic degranulating agents between different types of mast cell are long established [7,12,17]. In a more recent analysis of phenotypic differences in mast cell behaviour *in vitro* [5], variability in the inhibitory effects of nitric oxide on degranulation were reported between peritoneal mast cells, an immature, bone marrow-derived mast cell line and a basophil cell line, in short term cultures. Our results would add to this diversity of response between types of mast cell. Some part of this diversity could lie in the cellular source of nitric oxide in any particular tissue since many of the cells normally adjacent to mast cells—endothelium, epithelium, and smooth muscle—can also produce nitric oxide. There is already evidence for a nonmast cell source of nitric oxide in preparations of peritoneal mast cells [3,18] and in the experiments of Koranteng *et al*. [5], *unfractionated* mouse peritoneal cells were used as a source of nitric oxide. A further functional difference between isolated, free mast cells and those *in situ* could lie in the interaction with components of the extracellular matrix (fibronectin, vitronectin) which can upregulate cytokine output from mast cells [19].

However, in our experiments, when endogenous nitric oxide did appear to modulate degranulation, in the duodenum, its effect was to potentiate degranulation *in vivo*, that is, inhibition of nitric oxide synthesis decreased dye extravasation. This finding is in contrast to that reported from assays using mast cells isolated from rat peritoneal washings [3–5,10,20], where nitric oxide decreased degranulation. Indeed, a protective role has been attributed to nitric oxide in the intestinal microvasculature through its downregulation of release of mediators such as histamine and platelet activating factor from mast cells [7]. One possible explanation for this discrepancy is that our experiments involved mast cells *in situ* whereas the work quoted used mast cells washed from the peritoneum and subjected to a series of purification procedures. As we suggest later, such manipulations may activate alternative, nitric oxide-dependent, mechanisms not functional *in situ*. Further, these peritoneal mast cells are more likely to be of the connective tissue type whereas there will be many more mucosal-type mast cells in the duodenum.

What is clear from our results is that the inhibition of endogenous nitric oxide synthase had no effect on the responses, *in vivo*, of mast cells in the mesentery and skin of either the SHR or the NT strain. This latter group of results—in normal rats—is in sharp contrast to many papers describing strong interactions between nitric oxide and mast cells in the microvasculature of the mesentery [6,7,21,22]. A free radical scavenging role has been attributed to endogenous nitric oxide in order to explain the effects of inhibition of nitric oxide synthase [20]. Our results may not have relevance to the permeability changes measured in the intravital models of microvascular function, such as the hamster cheek pouch and rat mesentery preparations, in which leukocyte adhesion and migration are frequently observed. In these models, measurements are made over a longer time, at least 30 min poststimulus, and after an equilibration period, typically 30–45 min, after setting up the tissue. Within this time, mechanisms involving nitric oxide synthesis and action, different from those operating immediately (5–10 min poststimulus) may be activated. Because of this temporal difference in the models, we do not consider that leukocytes participated in the increased microvascular permeability we measured and further that our results may not necessarily relate to the control of leukocyte function in the microvasculature.

There are other reports of divergences between nitric oxide synthase inhibition, mast cell degranulation and microvascular changes. For instance, in a model with minimum surgical intervention, the rolling of polymorphonuclear leukocytes in mesenteric venules was unchanged by treatment with L-NAME [23]. Also, mast cells may not be the only target for L-NAME, as in rats genetically deficient in mast cells, L-NAME still enhanced leukocyte rolling and adhesion in rat mesenteric venules [24]. Another possible source of discrepancy is the different mode of administration of L-NAME. In many papers,

L-NAME was given locally, by superfusion of the mesenteric tissue, thus avoiding systemic effects with the potential for gross changes in blood flow. However in other work and particularly in a recent report [25], L-NAME was given i.v. and induced degranulation of mast cells and increased leukocyte rolling and adhesion. The general proposition that nitric oxide is a modulator of mast cell degranulation has been further questioned recently, by an extensive report (which appeared while this paper was in preparation) on histamine release from rat peritoneal mast cells [26]. Using a range of stimuli and a range of nitric oxide donors and inhibitors, the authors concluded that nitric oxide did not play a significant role in histamine release from mast cells. It will be necessary to study other models in which mast cell behaviour can be measured with minimum intervention, in order to make a full analysis of the contribution of nitric oxide to mast cell function in the rat, but there is now reason to question the role of nitric oxide as an endogenous regulator of mesenteric mast cell degranulation *in vivo*.

The lack of effect of L-NAME pretreatment on degranulation in skin mast cells in the NT strain was also unexpected as nitric oxide is known to modulate microvascular permeability in the skin following endogenous (released from sensory nerves) or exogenous, substance P [27,28]. This neuropeptide will induce mast cell degranulation, along with its direct effects on microvascular permeability. It would seem from our results that the nitric oxide–substance P interactions reported probably did not involve mast cells.

The causes of the failure of PCA reactions in the SHR are still not elucidated but such a failure in humans could have interesting clinical consequences. Immunological reactions in skin (skin tests) are frequently used to test for allergy to common antigens such as pollens or pet hair. Both hypertension and allergy are increasingly prevalent in the general population and it would be interesting to search for correlations between the intensity or incidence of such skin reactions and systemic blood pressure.

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