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Mast cells promote lung vascular remodelling in pulmonary hypertension

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ABSTRACT: Left heart disease (LHD) frequently causes lung vascular remodelling and pulmonary hypertension (PH). Yet pharmacological treatment for PH in LHD is lacking and its pathophysiological basis remains obscure. We aimed to identify candidate mechanisms of PH in LHD and to test their relevance and therapeutic potential.

In rats, LHD was induced by supracoronary aortic banding. Whole genome microarray analyses were performed, candidate genes were confirmed by RT-PCR and Western blots and functional relevance was tested *in vivo* by genetic and pharmacological strategies.

In lungs of LHD rats, mast cell activation was the most prominently upregulated gene ontology cluster. Mast cell gene upregulation was confirmed at RNA and protein levels and remodelled vessels showed perivascular mast cell accumulations. In LHD rats treated with the mast cell stabiliser ketotifen, or in mast cell deficient *Ws*/*Ws* rats, PH and vascular remodelling were largely attenuated. Both strategies also reduced PH and vascular remodelling in monocrotaline-induced pulmonary arterial hypertension, suggesting that the role of mast cells extends to non-cardiogenic PH.

In PH of different aetiologies, mast cells accumulate around pulmonary blood vessels and contribute to vascular remodelling and PH. Mast cells and mast cell-derived mediators may present promising targets for the treatment of PH.

KEYWORDS: Heart failure, mast cells, pulmonary hypertension, vascular remodelling

Two thirds of patients with chronic severe left-sided heart disease develop a pulmonary arterial pressure (P_{pa}) of $\geqslant$25 mmHg at rest [1]. According to the 2009 World Health Organization clinical classification, these patients fall into the second and presumably largest category of pulmonary hypertension (PH), *i.e.* PH owing to left heart disease (LHD) [2]. In chronic LHD, PH is not only caused by a congestive increase in lung vascular pressures, but is frequently aggravated by a concomitant rise in pulmonary vascular resistance (PVR), which further increases right ventricular afterload, limits right ventricular output and ultimately promotes right ventricular failure [3]. The clinical relevance of this scenario is underlined by the fact that PH, reduced right ventricular ejection fraction or increased PVR each constitute independent predictors of mortality in chronic LHD [4]. However, in contrast to other forms of PH, efficient and/or approved treatment strategies for PH owing to LHD are still lacking.

The pathology of PH owing to LHD is characterised by endothelial dysfunction, increased vessel tone and vascular remodelling as a result of chronically elevated pressures in the pulmonary circulation [5]. In a model of supracoronary aortic banding in rats that replicates these characteristics [6], we aimed to elucidate underlying cellular and molecular mechanisms as a rational basis for the design of new treatment strategies. Using genome-wide expression analyses we identified potential target genes involved in vascular regulation, and inflammatory or developmental pathways. Notably, the strongest differential regulation was an upregulation of genes associated with mast cell activation. Based on this finding we hypothesised a putative role for mast cells in vascular remodelling and PH owing to LHD and potentially in other, non-cardiogenic forms of PH as well. We tested this hypothesis both by pharmacological intervention and in a genetic model of mast cell deficient rats. Our findings identify mast cells as critical

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promoters of lung vascular remodelling in PH of different aetiologies and suggest mast cell-targeted strategies as promising interventions for the treatment of PH.

METHODS

A detailed material and methods section is provided in the online supplementary material. In brief, PH owing to LHD was induced by supracoronary aortic banding in Sprague–Dawley rats, mast cell deficient *Ws/Ws* rats or corresponding BN/*f*Mai wildtypes, as described previously [6]. Sham-operated rats served as controls. Non-cardiogenic PH was induced by intraperitoneal injection of monocrotaline (MCT) (60 $mg \cdot kg^{-1}$ in Sprague–Dawley and 40 $mg \cdot kg^{-1}$ in *Ws/Ws* or BN/*f*Mai rats). Ketotifen (1 $mg \cdot kg^{-1}$ $bodyweight \cdot day^{-1}$) was given with the drinking water. Cardiopulmonary characterisation was performed 9 weeks after aortic banding or sham operation, and 3 weeks after MCT injection, as described previously [6]. Using echocardiographic transthoracic two-dimensional and M-mode imaging, right and left ventricular end-diastolic areas were determined and tricuspid annular plane systolic excursion (TAPSE) was measured for evaluation of right ventricular systolic longitudinal function.

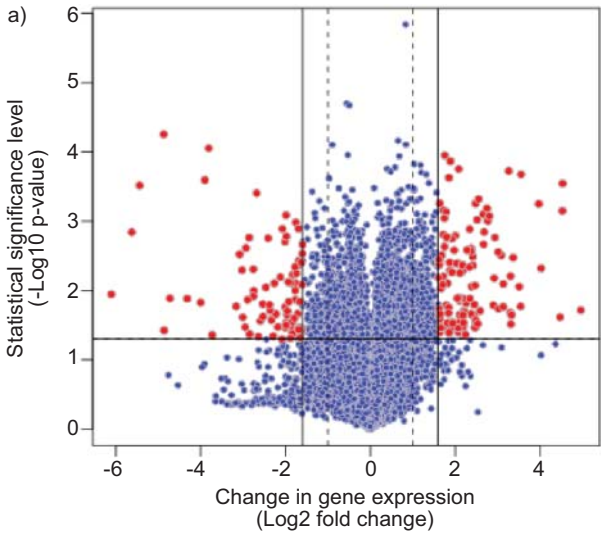
Whole rat genome microarray analyses of lungs from banded and control rats and subsequent bioinformatic analyses were performed as outlined in the supplementary material. Differentially expressed genes were visualised by heat maps and analysed for enrichments of gene ontology (GO) classes. For selected genes, differential expression was confirmed at the transcriptional and translational level by RT-PCR and Western blot analysis. Vascular remodelling, and mast cell accumulation and degranulation were quantified by blinded analysis of histological sections as described in the supplementary material. In brief, medial wall thickness was quantified in small (20–50 μm diameter), medium sized (50–100 μm) and larger (>100 μm) pulmonary arterioles, and the fraction of non-muscularised, partially muscularised or fully muscularised vessels and the vascular lumen area were determined in small arterioles. Density of mast cells was measured in toluidine blue-stained lung sections and the number of perivascular mast cells surrounding each vessel was determined for different vessel calibres. An index of granulation was calculated as ratio of granulated over degranulated mast cells. All data are presented as mean±SEM and statistical significance was assumed at $p<0.05$.

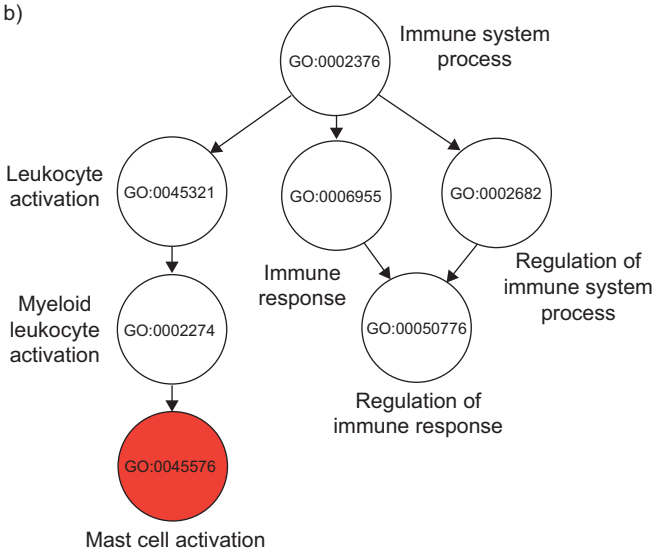
RESULTS

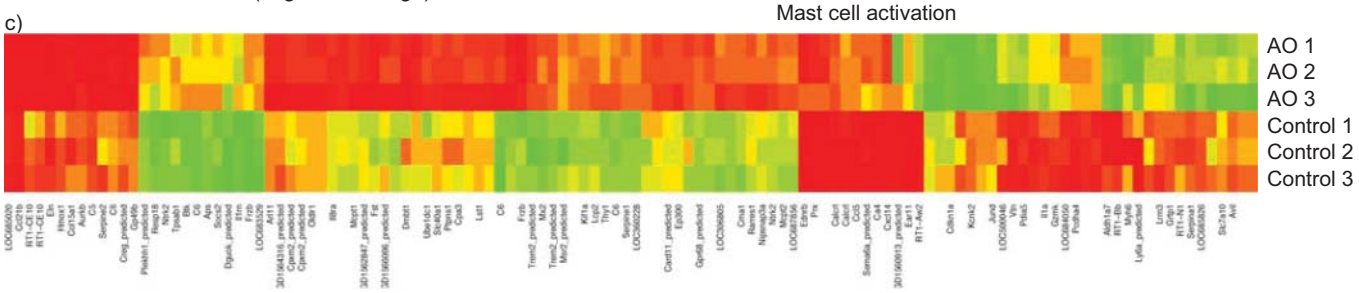
Whole rat genome microarray analyses were performed in lung homogenates of three rats with established PH following supracoronary aortic banding and in three sham-operated controls. The complete microarray data set is accessible at the Gene Expression Omnibus (GEO) repository of the National Center for Biotechnology Information. [7]. Out of a total of 28,000 analysed genes, differential expression defined as ⩾4-fold change with $p<0.05$ was evident for 120 genes. Of these, 76 were upregulated and 44 downregulated in aortic banding compared with control lungs, as illustrated by a volcano blot (fig. 1a). Differentially regulated genes, presented as a heat map in figure 1c, encode proteins involved in inflammatory and developmental pathways, complement and coagulation cascades, vascular regulation or extracellular matrix composition (table 1). However, the gene GO class with the most pronounced differential regulation was the GO class ''mast cell activation'' in which 13 out of 20 genes were significantly upregulated in aortic banding compared with control lungs (fig. 1b). The notion of an increased expression of mast cell genes in PH owing to LHD was confirmed for selected candidate genes. RT-PCR (fig. 1d and e) and Western blot analyses (fig. 1f and g) demonstrate upregulation of mast cell chymase and mast cell peptidase 2 in aortic banding lungs at both the genomic and proteomic level.

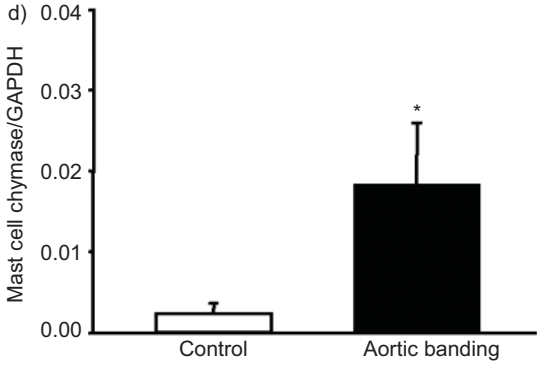
In order to test for a putative functional role of mast cells in lung vascular remodelling and PH in LHD, we applied both a pharmacological approach by treatment of aortic banding rats with the mast cell stabiliser ketotifen and a genetic strategy by use of *Ws/Ws* rats which are deficient in mast cells [8]. Ketotifen treatment from post-operative day-1 significantly attenuated the development of PH and right ventricular hypertrophy over 9 weeks of aortic banding, as demonstrated by a reduced P_{pa} and right ventricular weight in ketotifen-treated compared with untreated banded rats (fig. 2a–c). In sham-operated control rats without banding, ketotifen treatment had no detectable effect on pulmonary haemodynamics. The attenuation of PH by ketotifen was predominantly attributable to a normalisation of PVR, while left atrial pressure and aortic flow did not differ significantly between ketotifen-treated and -untreated rats (online supplementary material table 2), indicating that ketotifen did not mitigate underlying congestive heart failure. Consistent with our previous findings [6], arterial pressure and systemic vascular resistance did not differ between banded and control rats and were similarly unaffected by ketotifen treatment (online supplementary material table 2). Echocardiographic analyses 9 weeks after aortic banding revealed an increased right ventricular end-diastolic area compared with controls consistent with the development of cor pulmonale (fig. 2d–i). Ketotifen treatment largely normalised right ventricular end-diastolic dimensions to control values. Left ventricular end-diastolic area showed a trend to increase in rats with aortic banding independent of ketotifen treatment, yet without reaching significance. TAPSE was reduced in aortic banding rats compared with controls, indicating right ventricular dysfunction, but was restored in part by ketotifen treatment (fig. 2g–i).

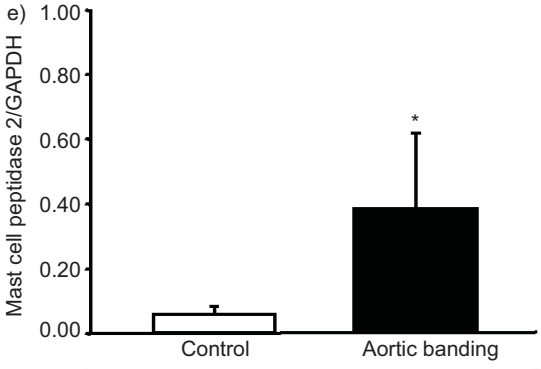
Considerable lung vascular remodelling was evident in banded rats as medial wall thickening in all analysed vessel categories, *i.e.* in small- and medium-sized pulmonary arterioles of 20–50 μm and 50–100 μm diameter, respectively, as well as in larger arterioles of >100 μm diameter (fig. 3a–f). While the majority of small pulmonary arterioles were non-muscularised or partially muscularised in control rats, a marked increase in fully muscularised arterioles was evident in banded rats and was associated with a concomitant decrease in vascular lumen area (fig. 3g and h). Ketotifen treatment significantly attenuated the increase in medial wall thickness in all analysed vessel categories, reduced microvascular muscularisation, increased vascular lumen area and thus, largely prevented lung microvascular remodelling in banded rats (fig. 3a–h). Significant mast cell accumulation and degranulation in lungs of banded compared with control rats was evident as increased mast cell density, reduced granularity and perivascular mast cell accumulation in all analysed vessel

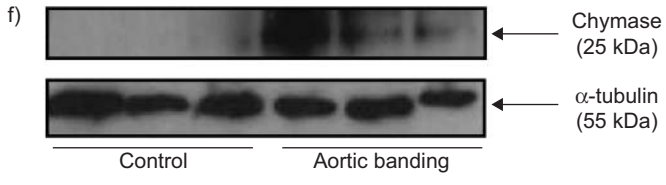

a)
Statistical significance level (-Log10 p-value)
Change in gene expression (Log2 fold change)

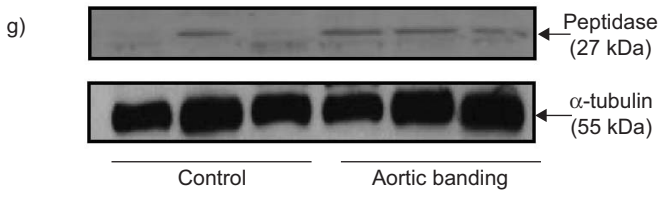

b)
GO:0002376
Immune system process
Leukocyte activation
GO:0045321
GO:0006955
GO:0002682
Immune response
Regulation of immune system process
Myeloid leukocyte activation
GO:0002274
GO:00050776
Regulation of immune response
GO:0045576
Mast cell activation

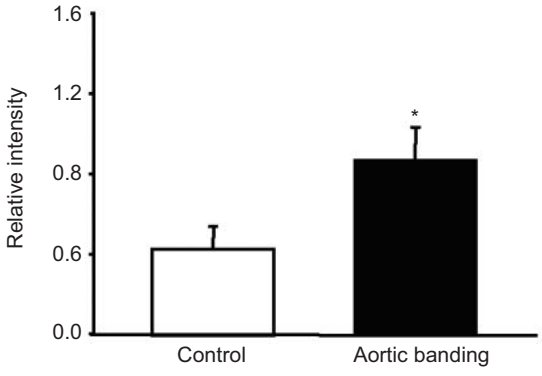

c)
AO 1
AO 2
AO 3
Control 1
Control 2
Control 3


d)
Mast cell chymase/GAPDH
Control
Aortic banding


e)
Mast cell peptidase 2/GAPDH
Control
Aortic banding


f)
Chymase (25 kDa)
α-tubulin (55 kDa)
Control
Aortic banding


g)
Peptidase (27 kDa)
α-tubulin (55 kDa)
Control
Aortic banding


Relative intensity
Control
Aortic banding

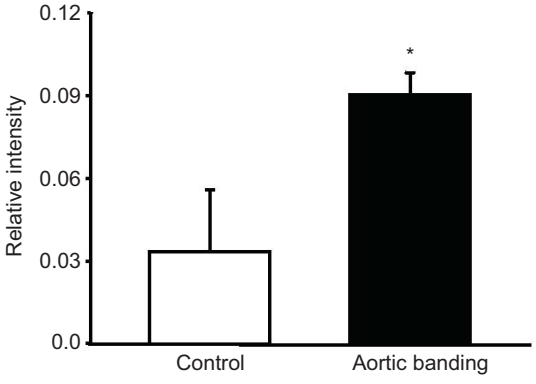

Relative intensity
Control
Aortic banding

FIGURE 1. Lung mast cell accumulation in rats with supracoronary aortic banding. a) Volcano plot from whole rat genome microarray analyses (28,000 genes in total) shows differential gene expression in lung homogenates of rats with aortic banding compared with controls (n=3 each). Red spots depict 120 genes that are significantly ($p<0.05$; -Log10 >1.3) and more than four-fold upregulated (Log2 >2) or downregulated (Log2 <-2) in banded compared with control rats. Conversely, blue dots depict the residual 27,880 genes which were not significantly and/or less than four-fold upregulated or downregulated. b) Dendrogram of the gene ontology (GO) cluster ''immune system process'' with respective GO terms and accession numbers. Within the entire microarray, differential regulation was most pronounced in the GO class ''mast cell activation''. c) Heat map depicts expression pattern of the 120 differentially regulated genes from three lungs of rats with aortic banding (AO) and three control lungs. The colour code (red: upregulation; green: downregulation) shows individual gene expression relative to mean gene expression of the whole array. Annotation according to National Center for Biotechnology Information gene symbols. Bar graphs show upregulation of d) mast cell chymase and e) mast cell peptidase 2 in aortic banding compared with control rats. Data from real time RT-PCR of lung homogenates were quantified in comparison to standard curves produced under the same cycling conditions and normalised to glyceraldehyde-3-phosphate dehyrogenase (GAPDH) expression from the same experiment. Bar graphs and representative Western blots from lung homogenate show increased protein expression of f) mast cell chymase and g) mast cell peptidase 2 in lungs from aortic banding rats compared with controls (α-tubulin serves as loading control). Data from n=3 each. *: $p<0.05$ *versus* control.

calibres (fig. 3i–k). Mast cells did not co-localise with the occasional occurrence of vascular thrombosis or microvascular haemorrhage in the banding model, suggesting that these pathologies did not act as the primary trigger for mast cell recruitment. In line with its proposed mode of action as a mast cell stabiliser, ketotifen treatment prevented mast cell degranulation and increased granularity even above control levels. In addition, ketotifen attenuated the increase in lung mast cell density and prevented in particular the perivascular accumulation of mast cells around small pulmonary microvessels of 20–50 μm diameter (fig. 3i–k).

In a second, non-pharmacological approach we addressed the role of mast cells by subjecting mast cell deficient *Ws/Ws* and corresponding BN/*f*Mai wildtype rats to 9 weeks of aortic banding. Compared with wildtypes, the increase in P_{pa} and PVR following aortic banding was significantly attenuated in *Ws/Ws* rats (fig. 4a–c). Likewise, right ventricular hypertrophy was reduced (fig. 4a–c) and vascular remodelling, *i.e.* the increase in medial wall thickness and muscularisation and the loss of vessel lumen, was markedly attenuated in banded *Ws/Ws* compared with wildtype rats (fig. 4d–h). Sham-operated *Ws/Ws* and wildtype rats did not differ in any of the assessed parameters. In line with the results obtained in Sprague–Dawley rats, aortic banding resulted in pulmonary mast cell accumulation and degranulation in wildtype BN/*f*Mai rats. In agreement with their reported phenotype, no mast cells were detectable in lung sections from *Ws/Ws* rats (fig. 4i–k).

To test whether the role of mast cells also expands to non-cardiogenic forms of PH, we analysed the effects of ketotifen in MCT-induced pulmonary arterial hypertension. Within 3 weeks of MCT injection, Sprague–Dawley rats developed PH and lung vascular remodelling as demonstrated by increased P_{pa}, PVR and right ventricular hypertrophy (fig. 5a–c) and an increase in mean pulmonary arteriolar wall thickness (fig. 5d–f), that was

TABLE 1 Selected differentially regulated genes in pulmonary hypertension owing to left heart disease

Category	Gene	↑/↓	Fold change#	p-value
Inflammation/immune response	IL-1α	↓	0.17	0.009
	IL-1RA	↑	4.43	0.025
	IL-8Rα	↑	8.65	0.003
	oxLDL receptor 1	↑	5.65	0.025
	Mast cell protease 1	↑	10.31	0.030
	Mast cell peptidase 2	↑	4.53	0.003
	Tryptase	↑	4.12	0.013
	Chymase 1	↑	6.81	0.001
Complement system	Complement factor 1	↑	5.71	<0.001
	Complement component 6	↑	31.77	0.020
Coagulation system	PAI-1	↑	6.47	<0.001
Vascular regulation	ETB receptor	↓	0.23	0.049
	Haem oxygenase 1	↑	6.98	0.016
	Endothelial lipase	↓	0.17	0.019
Extracellular matrix composition	Vitronectin	↓	0.05	0.007
	Procollagen type XV	↑	4.42	0.040
	Elastin	↑	4.58	0.030
Developmental pathways	Semaphorin 6A	↓	0.12	0.014

Selected genes out of a total of 120 genes with differential expression in lungs of rats with supracoronary aortic banding compared with sham-operated controls. Differential expression was defined as ⩾4-fold change with a $p<0.05$. Data from n=3 animals each. ↑/↓: upregulation and downregulation in banded compared with control rats IL: interleukin; IL-1RA: IL-1 receptor antagonist; IL-8Rα: IL-8 receptor α; oxLDL: oxidised low-density lipoprotein; PAI-1: plasminogen activator inhibitor-1 (serpinE1); ETB: endothelin-1 type B receptor. #: fold change is expressed as ratio of gene expression in banded/control rats. Mast cell specific genes are shown in bold.

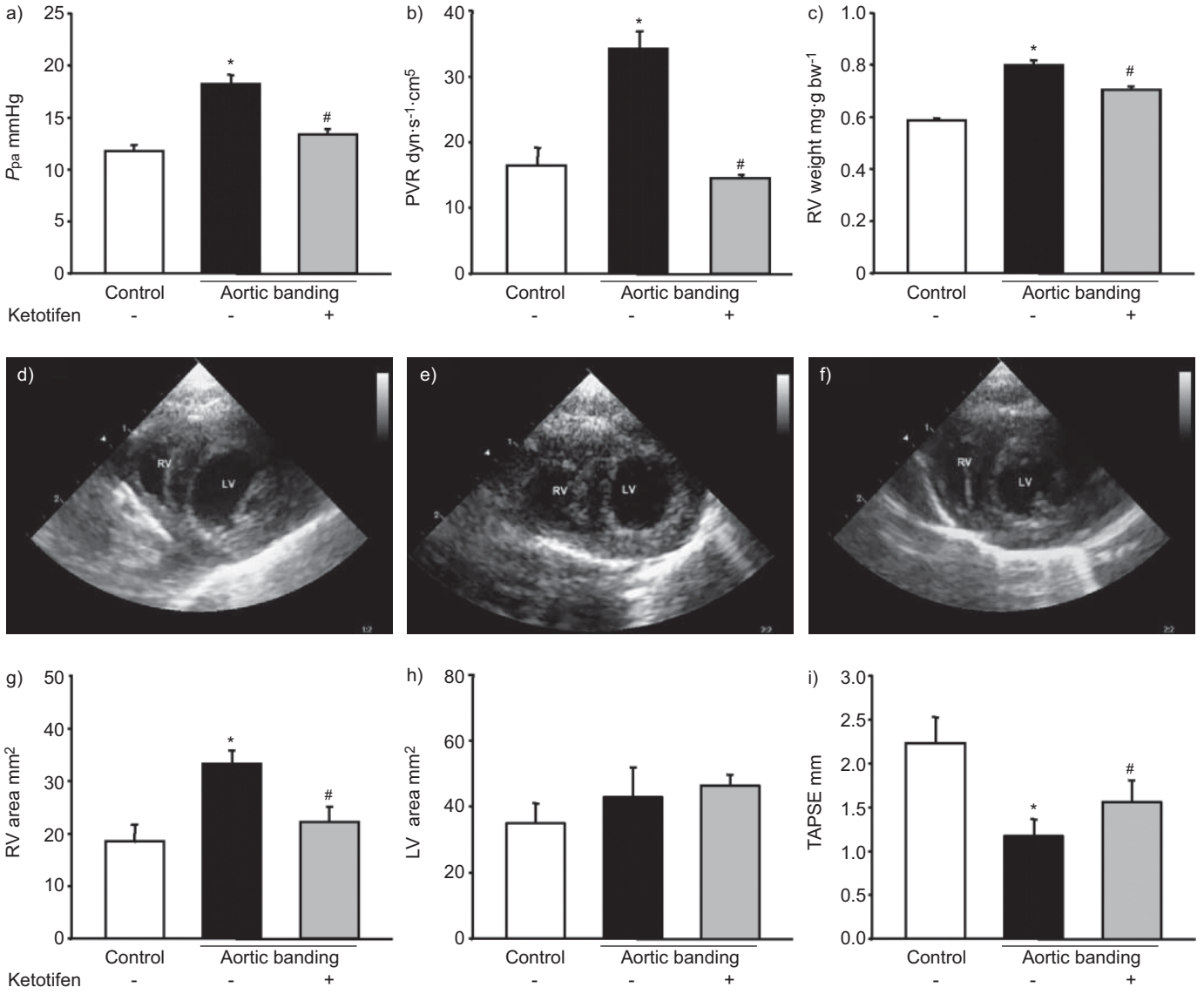


FIGURE 2. Effect of the mast cell stabiliser ketotifen on haemodynamics and right ventricular dysfunction in pulmonary hypertension owing to left heart disease. a) Pulmonary arterial pressure (P_{pa}), b) pulmonary vascular resistance (PVR) and c) right ventricular (RV) weight relative to bodyweight (bw) in controls, banded rats and ketotifen-treated banded rats. Data from n=6 each. d–f) Representative echocardiographic two-dimensional-images show left ventricle (LV) and right ventricle (RV) in end-diastole in d) control, e) banded and f) ketotifen-treated banded rats. Note marked dilation of the RV in banded rats, which is attenuated by ketotifen. Replicated in n=10 each. g) RV and h) LV end-diastolic area and i) tricuspid annular plane systolic excursion (TAPSE) in control, banded and ketotifen-treated banded rats. Data from n=10 each; *: $p<0.05$ *versus* control; #: $p<0.05$ *versus* aortic banding.

associated with an accumulation and degranulation of mast cells (fig. 5g–i). Analogous to our findings in banded rats, ketotifen treatment mitigated these effects and attenuated the MCT-induced increase in right ventricular end-diastolic area as assessed by echocardiography (fig. 5d–f). Left ventricular end-diastolic area was not altered by either MCT or ketotifen treatment. To substantiate the notion that mast cells may be equally relevant in pulmonary arterial hypertension, we next

FIGURE 3. Effect of the mast cell stabiliser ketotifen on lung vascular remodelling and pulmonary mast cells in pulmonary hypertension owing to left heart disease. Representative images of haematoxylin–eosin-stained lung sections from a) control, b) banded and c) ketotifen-treated banded rats. Scale bars=9~50 μm. Medial thickening in lung vessels from banded rats is largely abrogated by ketotifen. Replicated in n=5 each. Medial wall thickness for pulmonary arterioles sized d) 25–50 μm, e) 50–100 μm and f) >100 μm in diameter in lungs from control, banded and ketotifen-treated banded rats. The degree of g) muscularisation and h) internal lumen area of pulmonary arterioles of 20–50 μm diameter in lungs from control, banded and ketotifen-treated banded rats. Degree of muscularisation is expressed as proportion of non-, partially or fully muscularised pulmonary arterioles in percentage of total pulmonary arteriolar cross-section. Data from n=5 each. i) Mast cell density in lung histological sections, j) index of granulation and k) number of perivascular mast cells surrounding pulmonary vessels sized 20–50 μm, 50–150 μm and >150 μm in diameter in lungs from control, banded and ketotifen-treated banded rats. Data from n=8 each. *: $p<0.05$ *versus* control; #: $p<0.05$ *versus* aortic banding.

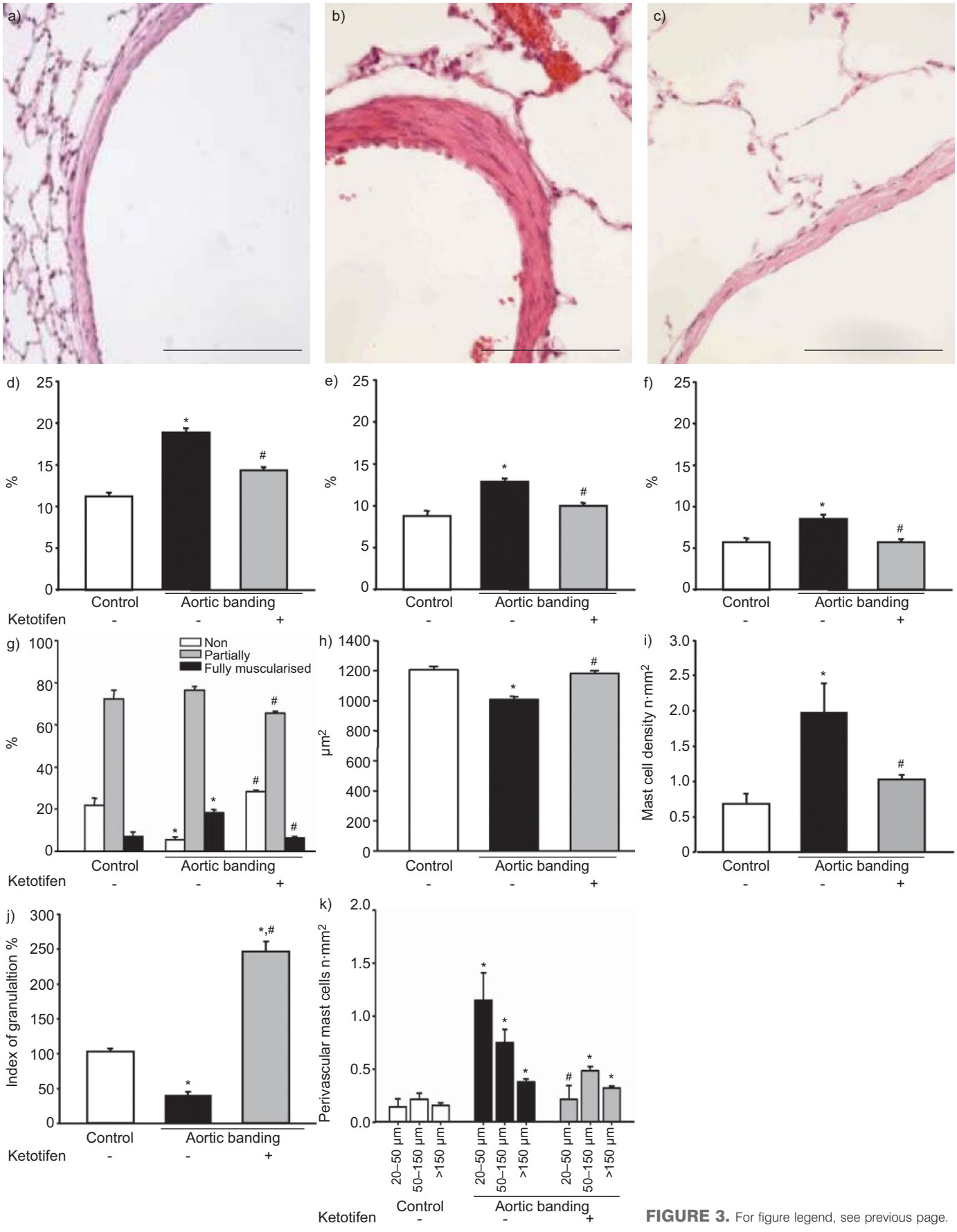


FIGURE 3. For figure legend, see previous page.

compared the effects of MCT in mast cell-deficient *Ws/Ws* and wildtype BN/*f*Mai rats. As BN/*f*Mai rats showed the characteristic features of the MCT model, *i.e.* an increased P_{pa}, PVR and right ventricular hypertrophy, echocardiographic signs of cor pulmonale, right ventricular dysfunction and extensive vascular remodelling, these effects were markedly attenuated or even abrogated in mast cell deficient *Ws/Ws* rats (fig. 6a–f). These findings consolidate the view that the role of mast cells in lung vascular remodelling and PH is not restricted to LHD, but extend to other categories of PH, and as such may constitute a unifying disease mechanism independent of the individual aetiology.

DISCUSSION

Here, we identify mast cells as catalysts of lung vascular remodelling in PH. Gene array analyses identified ''mast cell activation'' as the most prominently upregulated GO class in PH subsequent to aortic banding, and perivascular mast cell accumulation was substantiated on the genomic, proteomic and histological level. A functional role of mast cells in PH owing to LHD was demonstrated by a dual pharmacological and genetic strategy in that both the mast cell stabiliser, ketotifen, or the use of mast cell deficient *Ws/Ws* rats attenuated: 1) PH; 2) the increase in PVR; 3) lung vascular remodelling; 4) right ventricular hypertrophy; and 5) end-diastolic right ventricular dilation in aortic banding rats. The protective effects of ketotifen or mast cell deficiency were likewise evident in a model of MCT-induced pulmonary arterial hypertension, suggesting that the role of mast cells expands to other forms of PH. Mast cells may thus present a novel and promising target in the treatment of PH.

Whereas mast cells have traditionally been recognised as critical in allergic and nonallergic immune responses, a growing body of evidence implicates these cells in cardiovascular disease. Mast cells stimulate the proliferation of endothelial [9] and smooth muscle cells [10], which present hallmarks of PH. In the present study, the upregulation of mast cell genes and the accumulation of mast cells in heart failure lungs led us to speculate on a pathophysiological role for mast cells in vascular remodelling and PH. Notably, the notion that mast cells accumulate in lungs in LHD is not new. EHRLICH [11], who first identified this cell type in 1878, noted that mast cells were abundant in ''brown induration of the lung'', *i.e.* in haemosiderosis following mitral stenosis. Since then, several studies have confirmed the accumulation of mast cells in lungs of patients with PH secondary to congenital cardiac septal defects or mitral stenosis, as well as in patients with idiopathic PH [12, 13]. These clinical findings were paralleled by similar observations in experimental models of PH [14, 15]. Although these studies cohesively proposed that the accumulation of mast cells in the lung may have potential functional relevance for the pathophysiology of PH and lung vascular remodelling, this hypothesis has never been systematically tested as yet.

Several lines of argument led us to propose that mast cells may play a critical role in PH. Mast cells release biogenic amines including serotonin, which plays a key role in pulmonary arterial vasoconstriction and smooth muscle cell proliferation [16], or histamine, which is a vasoconstrictor in pulmonary veins. Mast cells activate the renin-angiotensin system which has been implicated in the pathogenesis of PH [17] by the release of renin and mast cell chymase which converts angiotensin I to angiotensin II [18]. Mast cell chymase also processes pro-matrix metalloprotease 9 (pro-MMP-9) to active MMP-9 which is a biomarker for scleroderma-associated PH [19]. Furthermore mast cells produce collagen-cleaving MMP-13 [20] as well as platelet-derived growth factor and transforming growth factor (TGF)-β, which both stimulate smooth muscle cell proliferation [21]. Mast cells secrete activin A [10], a member of the TGF-β superfamily that is elevated in PH and again promotes smooth muscle cell proliferation. Last but not least, mast cells release interleukin-6 which plays a critical role in animal models of PH [22] and is elevated in serum of patients with idiopathic PH [23]. Taken together, these data not only consolidate the hypothesis that mast cells may contribute to PH, but provide a potential mechanistic basis for this notion.

Remarkably, the postulated role for mast cells may also be at the bottom of or at least contribute to the effectiveness of kinase inhibitors in the treatment of PH. Mast cells express high levels of the tyrosine kinase stem cell factor receptor c-kit which regulates their maturation, differentiation and activation [24]. Importantly, multikinase and tyrosine kinase inhibitors such as sorafenib and imatinib, which have recently emerged as powerful new treatment strategies for PH [25, 26], are potent inhibitors of c-kit [27] and, as such, may exert their protective effects at least in part by inhibition of mast cell maturation and function.

Based on these concurrent data, we postulated a functional role for mast cells in lung vascular remodelling and PH. In the first set of experiments we tested this hypothesis by treating rats after aortic banding with the mast cell stabiliser ketotifen. Notably, ketotifen is clinically approved as oral therapy for asthma in children. Ketotifen treatment effectively attenuated the development of PH, lung vascular remodelling and right ventricular hypertrophy over 9 weeks after banding. Notably, ketotifen not only blocked mast cell degranulation but equally mitigated mast cell accumulation in the lung parenchyma and around pulmonary blood vessels, suggesting that mast cell activation and accumulation may mutually promote each other in PH. However, some caution as to the specificity of this pharmacological intervention is warranted, since ketotifen is not only a mast cell stabiliser, but also a histamine H1-receptor

FIGURE 4. Vascular remodelling and pulmonary hypertension owing to left heart disease in mast cell deficient *Ws/Ws* rats. a) Pulmonary arterial pressure (P_{pa}), b) pulmonary vascular resistance (PVR) and c) right ventricular (RV) weight relative to bodyweight (bw) in control wildtype (WT) rats, banded WT rats and banded *Ws/Ws* rats. Data from n=6 each. Medial wall thickness for pulmonary arterioles sized d) 25–50 μm, e) 50–100 μm; and f) >100 μm in diameter in lungs from control WT, banded WT and banded *Ws/Ws* rats. g) The degree of muscularisation and h) internal lumen area of pulmonary arterioles of 20–50 μm diameter in lungs from control WT, banded WT and banded *Ws/Ws* rats. Data from n=5 each. i) Mast cell density in lung histological sections from control WT, banded WT and banded *Ws/Ws* rats, j) index of granulation and k) number of perivascular mast cells surrounding pulmonary vessels sized 20–50 μm, 50–150 μm and >150 μm in diameter in lungs from control WT and banded WT rats. Data from n=8 each, *: $p<0.05$ *versus* control WT; #: $p<0.05$ *versus* aortic banding WT.

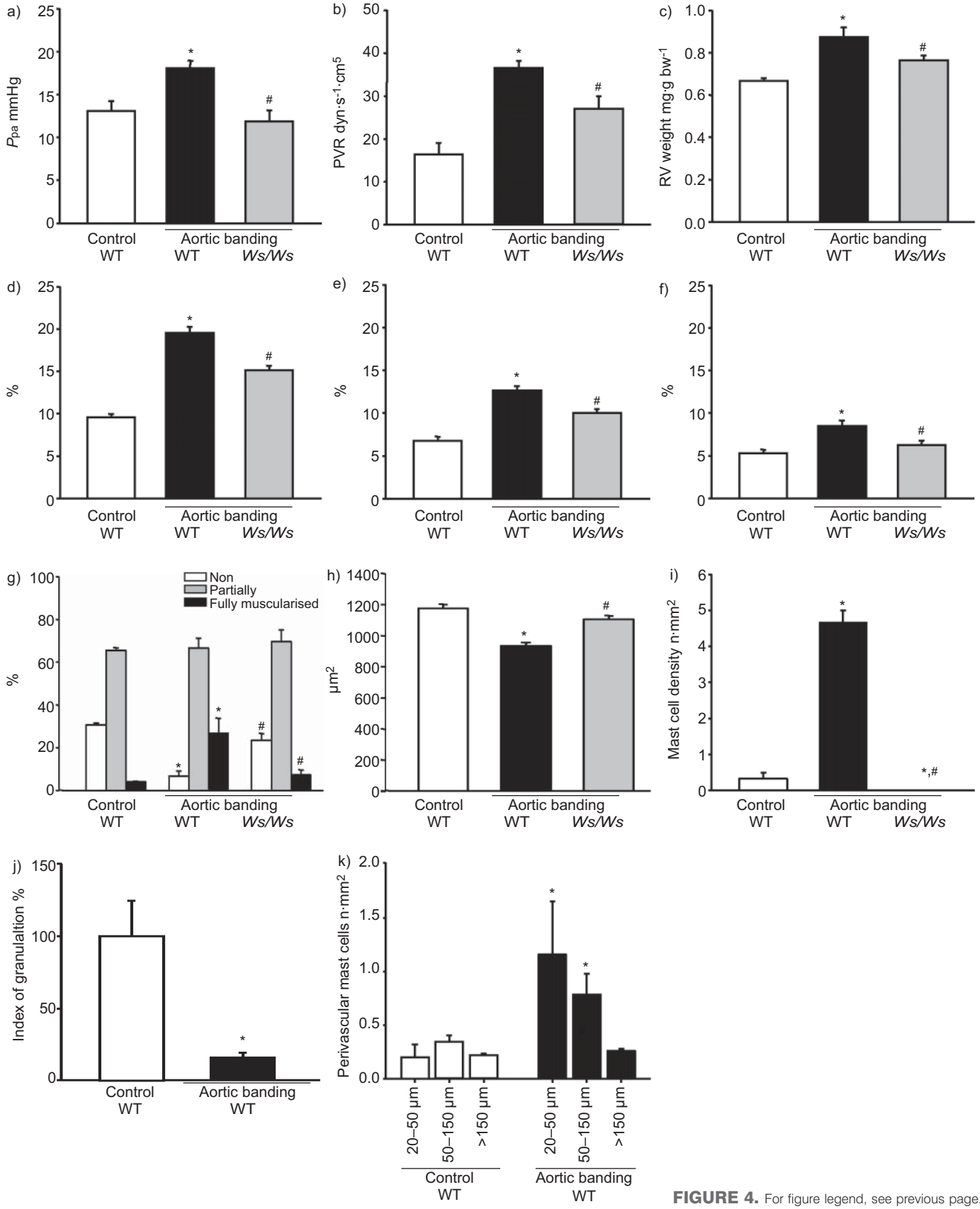


FIGURE 4. For figure legend, see previous page.

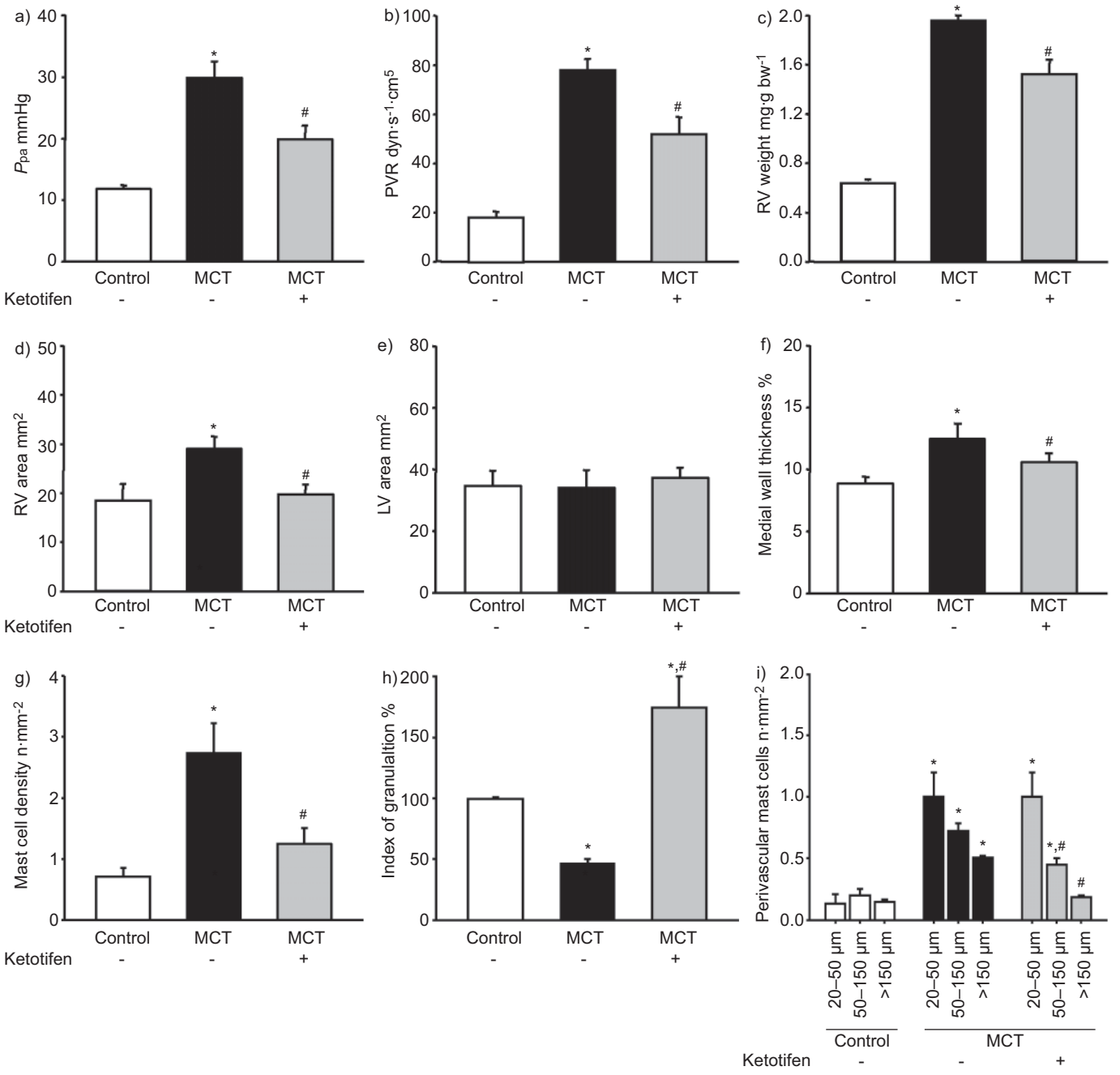


FIGURE 5. Effect of the mast cell stabiliser ketotifen in monocrotaline (MCT)-induced pulmonary hypertension. a) Pulmonary arterial pressure (P_{pa}), b) pulmonary vascular resistance (PVR) and c) right ventricular (RV) weight relative to bodyweight (bw) in control rats, MCT-treated rats and ketotifen-treated MCT rats. Data from n=6 each. d) RV and e) left ventricular (LV) end-diastolic area and medial wall thickness of pulmonary arteries in control, MCT and ketotifen-treated MCT rats. Data from n=10 each. g) Mast cell density in lung histological sections, h) index of granulation and i) number of perivascular mast cells surrounding pulmonary vessels sized 20–50 μm, 50–150 μm and >150 μm in diameter in lungs from control, MCT and ketotifen-treated MCT rats. Data from n=8 each, *: $p<0.05$ *versus* control; #: $p<0.05$ *versus* MCT.

antagonist, and has been proposed to have additional off-target effects which may include reduced phosphodiesterase and increased methyltransferase activity, as well as calcium antagonistic properties [28, 29]. Therefore, we applied a second, genetic strategy to substantiate a potential role of mast cells in PH.

Ws/Ws rats possess two mutant alleles at the white spotting (*Ws*) locus which is identical with the *c-kit* proto-oncogene. As a consequence, suckling *Ws/Ws* rats are deficient in mast cells and severely anaemic due to a defect of mast cell and erythrocyte precursors. The magnitude of anaemia is abated in adult *Ws/Ws* rats, while that of mast cell deficiency is enhanced, making this mutant a useful model for the study of mast cell functions [8]. In the present study, haematocrit did not differ between *Ws/Ws* and corresponding BN/*f*Mai wild-type rats at the time of investigations (online supplementary material table 3), while c-kit positive cells were completely

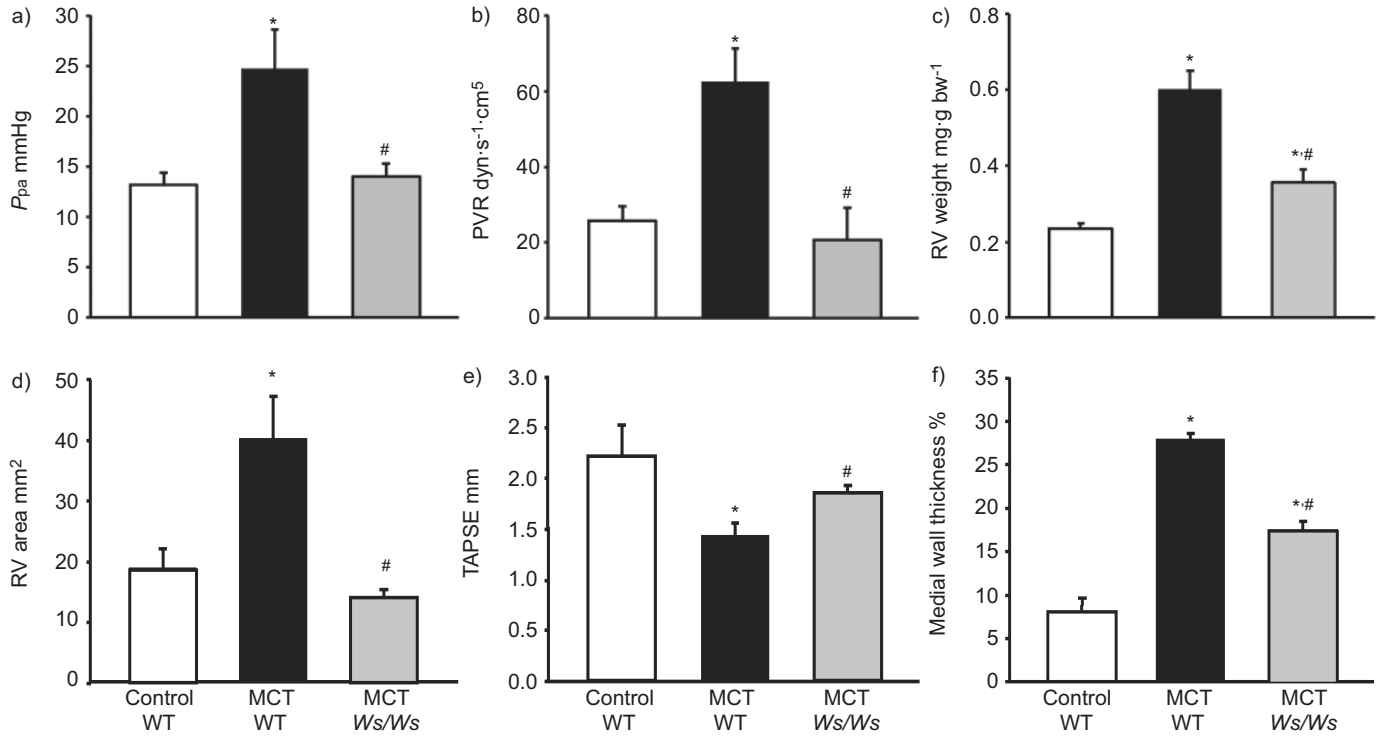


FIGURE 6. Vascular remodelling and pulmonary hypertension in monocrotaline (MCT)-treated mast cell-deficient *Ws/Ws* rats. a). Pulmonary arterial pressure (P_{pa}), b) pulmonary vascular resistance (PVR) and c) right ventricular (RV) weight relative to bodyweight (bw) in control wildtype (WT) rats, MCT-treated WT rats and MCT-treated *Ws/Ws* rats. Data from n=6 each. d) RV end-diastolic area, e) tricuspid annular plane systolic excursion (TAPSE) and f) medial wall thickness of pulmonary arteries in control WT, MCT WT and MCT *Ws/Ws* rats. Data from n=10 each. *: $p<0.05$ *versus* control WT; #: $p<0.05$ *versus* MCT WT.

absent in histological lung sections from *Ws/Ws* rats (online supplementary material fig. 1). In banded rats, the effects of the *c-kit* mutation paralleled those of ketotifen, in that PH, lung vascular and right ventricular remodelling were mitigated in *Ws/Ws* rats. The notion that c-kit positive mast cells accumulate in the lung in PH is in line with previous findings in a bovine model of hypoxia-induced PH demonstrating the mobilisation of c-kit+ cells from the bone marrow into the circulation, and their accumulation in the remodelled pulmonary arterial vessel walls [30]. The fact that the receptor tyrosine kinase c-kit is essential for the maturation, differentiation and activation of mast cells may furthermore provide an alternative mechanistic explanation for the emerging therapeutic potency of multikinase and tyrosine kinase inhibitors in the treatment of PH [25, 26]. However, expression and function of c-kit are not exclusive to mast cells, but apply to haematopoietic precursor cells and stem cells, germ cells, melanocytes, Cajal cells and a considerable number of neoplasms [31]. Thus, while the attenuation of vascular remodelling and PH in *Ws/Ws* rats alone cannot be unequivocally attributed to their mast cell deficiency, we believe that the concurrent results obtained by both a pharmacological mast cell stabiliser and in the genetic model of a c-kit mutant rat provide substantial evidence for a functional role of mast cells in lung vascular remodelling.

Since pulmonary accumulation of mast cells is a hallmark not only of LHD but similarly of other forms of PH, we used the MCT model to test whether this concept also applies to non-cardiogenic PH. Analogous to their effects in banded rats, ketotifen treatment or genetic mast cell deficiency attenuated MCT-induced pulmonary vascular, haemodynamic and right ventricular changes, suggesting that mast cells may also play a critical role in pulmonary arterial hypertension. Notably, prevention of mast cell degranulation by disodium cromoglycate was recently shown to attenuate the development of hypoxic PH in rats [32], while mast cell deficiency did not prevent PH and vascular remodelling in W/W^v mice, which similar to *Ws/Ws* rats have two mutant alleles at the *c-kit* locus [33]. While this apparent difference between species may be attributable to the relative paucity of mast cells in the mouse [33], it raises the question to which degree data from animal models reflect the clinical situation in humans. The abundance of mast cells in lungs of patients with PH [12, 13], and the fact that humoral mediators secreted from human mast cells stimulate proliferation of human smooth muscle cells support the notion that mast cells also play a fundamental role in PH in humans [21]. However, this view will ultimately have to be put to the test in appropriate clinical trials.

Our mechanistic insights into the emerging role of mast cells in nonallergic diseases are still scarce. Future studies both *in vivo* and *in vitro* are essential to advance our understanding of the how, when and where mast cells contribute to vascular remodelling and PH. In view of the abundance of potential candidate pathways outlined previously, a detailed dissection of intra- and intercellular signalling events pertinent to the role of mast cells in PH was beyond the scope of this study. Importantly, the present recognition of the mast cell as a critical promoter of lung vascular remodelling may give rise to

a new spectrum of therapeutic strategies, ranging from clinically approved mast cell stabilisers to inhibitors of specific constituents of the mast cell releasate, or interventions targeted to prevent mobilisation of bone marrow-derived mast cells and mast cell precursors or to block mast cell adhesion and recruitment to the lung.

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STATEMENT OF INTEREST

Statements of interest for I. Saarikko and R.T. Schermuly can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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