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Substance P induces adverse myocardial remodelling via a mechanism involving cardiac mast cells

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Aims Substance P and neurokinin A (NKA) are sensory nerve neuropeptides encoded by the *TAC1* gene. Substance P is a mast cell secretagogue and mast cells are known to play a role in adverse myocardial remodelling. Therefore, we wondered whether substance P and/or NKA modulates myocardial remodelling via a mast cell-mediated mechanism.

Methods and results Volume overload was induced by aortocaval fistula in $TAC1^{-/-}$ mice and their respective wild types. Left ventricular internal diameter of wild-type (WT) fistulas increased by 31.9%; this was prevented in $TAC1^{-/-}$ mice (4.2%). Matrix metalloproteinase (MMP) activity was significantly increased in WT fistula mice and was prevented in $TAC1^{-/-}$ mice. Myocardial collagen volume fraction was decreased in WT fistula mice; this collagen degradation was not observed in the $TAC1^{-/-}$ group. There were no significant differences between any groups in tumour necrosis factor (TNF)-α or cell death. Cardiac mast cells were isolated from rat hearts and stimulated with substance P or NKA. We found that these cells degranulated only to substance P, via the neurokinin-1 receptor. To determine the effect of substance P on mast cells *in vivo*, volume overload was created in Sprague-Dawley rats treated with the NK-1 receptor antagonist L732138 (5 mg/kg/day) for a period of 3 days. L732138 prevented: (i) increases in cardiac mast cell density; (ii) increased myocardial TNF-α; and (iii) collagen degradation.

Conclusions Our studies suggest that substance P may be important in mediating adverse myocardial remodelling secondary to volume overload by activating cardiac mast cells, leading to increased TNF-α and MMP activation with subsequent degradation of the extracellular matrix.

Keywords Sensory nerves • Substance P • Mast cell • TAC1 • Heart

1. Introduction

The sensory nerve neuropeptides, substance P and neurokinin A (NKA), are both encoded by alternative splicing of the *TAC1* gene[1,2] and have long been known to have negative inotropic and chronotropic effects on the heart.[3,4] Recently, there has been renewed interest in the role of sensory nerve neuropeptides in the heart, with D'Souza *et al.*[5] demonstrating that substance P is associated with dilated cardiomyopathy in a mouse model of parasitic myocarditis. They found increased myocardial levels of substance P in the hearts of infected wild type (WT) mice, and hearts from mice deficient in substance P were protected from hypertrophy, cell death, and inflammatory cell infiltration. Robinson *et al.*[6] subsequently reported similar findings in a mouse model of viral-induced myocarditis.

Mast cells play a prominent role in initiating adverse myocardial remodelling,[7–10] however, the underlying stimulus responsible for their activation is poorly understood. Until now, no one has investigated the possibility that interactions between substance P and/or NKA and cardiac mast cells mediate myocardial remodelling. A significant number of mast cells in the heart lie in close proximity to nerves,[11,12] thus, we hypothesized that sensory nerve neuropeptides may be important activators of mast cells, thereby initiating adverse myocardial remodelling. In this study, we used isolated cardiac mast cells and a model of volume overload induced myocardial remodelling and found

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that: (i) substance P, but not NKA, induces cardiac mast cell activation via the neurokinin (NK)-1 receptor; (ii) both NK-1 receptor antagonism and deletion of the *TAC1* gene prevented adverse remodelling of the left ventricle (LV); and (iii) increased myocardial tumour necrosis factor (TNF)-α and matrix metalloproteinase (MMP) activation were prevented by NK-1 receptor blockade and deletion of *TAC1*, respectively.

2. Methods

2.1 Animals

All of the animal studies conformed to the principles of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and all protocols were approved by our Institution's Animal Care and Use Committee. Sprague-Dawley rats were obtained from Harlan Laboratories. $TAC1^{-/-}$ mice congenic in the C57BL/6J background were acquired from Jackson Laboratories (Bar Harbor, MA, USA) and a breeding colony was established with genotyping performed according to standard procedures. All animals were housed under standard environmental conditions and maintained on commercial rat or mouse chow and tap water *ad libitum*. Rats and mice were anaesthetized with inhaled isoflurane (3% for rats, 2% for mice) for survival surgeries. Rat terminal surgeries were performed after intra-peritoneal (IP) injection of a combination of ketamine (20 mg) and xylazine (10 mg). Mice terminal surgeries were performed after IP injection of avertin (250 mg/kg). Proper analgesia was evaluated by palpebral reflex, toe pinch reflex, and corneal reflex. At the experimental endpoint euthanasia was accomplished by removal of the heart.

2.2 Long-term *in vivo* studies

All experiments were performed using 8-week-old male mice of C57BL/6J background. Volume overload was induced by creation of an aortocaval fistula using a previously described procedure for rats[7,8,13,14] using a $27\frac{1}{2}$-gauge needle inserted into the abdominal aorta and advanced through the medial wall into the vena cava. The mice were divided into four groups: (i) WT sham ($n = 8$); (ii) WT fistula ($n = 7$); (iii) $TAC1^{-/-}$ sham ($n = 7$); and (iv) $TAC1^{-/-}$ fistula ($n = 7$). Twenty-eight days post-fistula was chosen as the experimental endpoint based on pilot studies in WT mice, which showed extensive remodelling at this time-point. At the experimental endpoint, the fistula was visually confirmed by identification of turbulent blood flow in the vena cava to ensure that it had remained patent, and the mice were euthanized by removal of the heart. The right ventricle (RV) and LV including septum were separated and weighed. The LV was then sectioned into apical and mid-ventricular sections. The apical section was snap-frozen for biochemical analysis and the mid-ventricular section was fixed in Carnoy's fixative for histological analysis. The lungs were removed and their plural surfaces blotted dry, and weighed.

2.3 Echocardiography studies

Echocardiography was performed using a Vevo 660 small animal echocardiographic system (Visual Sonics). Mice were anaesthetized by continual inhalation of 1.5% isoflurane. Measurements of LV posterior wall thickness and internal chamber diameter were made using two-dimensional M-Mode taken at mid-papillary level. LV function was assessed by fractional shortening (FS), calculated as follows:

$$FS = \frac{LVIDd - LVIDs}{LVIDd} \times 100,$$

where LVIDd and LVIDs represent left ventricular internal diameter in diastole and systole, respectively.

2.4 Short-term *in vivo* studies

Our previous studies in rats have shown that 3 days post-fistula is a key time-point when mast cell activity is at its peak.[7] The role of the NK-1 receptor in mediating mast cell-mediated effects was examined using the previously described aortocaval fistula model of volume overload.[7–9,13] All experiments were performed using 8-week-old male Sprague-Dawley rats randomly divided into three groups: (i) sham-operated ($n = 14$); (ii) fistula ($n = 12$); and (iii) fistula + the NK-1 receptor antagonist (L 732 138, 5 mg/kg/day, S.Q., $n = 11$) beginning 1 day prior to surgery. At 3 days post-surgery, the fistula was visually confirmed by identification of turbulent blood flow in the vena cava to ensure that it had remained patent, and the rats were euthanized and the LV and septum were separated from the RV and weighed. The lungs were removed and their plural surface blotted dry and weighed. A transverse section of the LV was then fixed in Carnoy's fixative and the apical section was snap frozen in liquid nitrogen and stored at −80°C for subsequent analysis.

2.5 Myocardial TNF-α levels

TNF-α levels were determined from myocardial samples using a commercially available ELISA kit (BD Biosciences). Protein was extracted from myocardial tissue by homogenization followed by sonication. Each sample was then incubated with triton-X before being separated into cytosolic/extracellular and membrane fractions by centrifugation. TNF-α was measured in the cytosolic/extracellular fraction with each sample run in duplicate.

2.6 Matrix metalloproteinase activity

MMP activity was measured from the cytosolic/extracellular myocardial protein extract using a colorimetric MMP activity assay kit (AnaSpec). This was a non-specific assay which measures activity of MMP-1, -2, -3, -7, -8, -9, -12, -13, and -14. All samples were run in duplicate.

2.7 Mast cell density and collagen volume fraction

Five micrometre thick coronal sections were stained with the mast cell-specific stain, toluidine blue. Mast cell density was determined by dividing the total number of mast cells per LV cross-section by the tissue area of the corresponding section. Collagen volume fraction was determined as previously described[15–18] with 5 μm thick paraffin-embedded sections stained with picrosirius red (0.1% Sirius Red F3BA in picric acid) following incubation in phosphomolybdic acid (0.2%). Twenty random images per LV section were acquired and analysed with Image J software (NIH). Perivascular areas were excluded from the collagen analysis.

2.8 TUNEL assay

Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) (Roche Diagnostics) was applied to tissue sections and the amount of apoptotic nuclei per tissue section was quantified. Slides were also co-stained with DAPI (Sigma) to verify nuclei presence. Counts of TUNEL positive cells normalized to LV area were used to quantify changes in cell death between groups.

2.9 Cardiac inflammatory cell isolation procedure (*in vitro* studies)

Cardiac inflammatory cells were isolated from rat hearts as previously described.[19] Briefly, a thoracotomy exposed the intact pericardial sac, which was then filled with Hanks balanced salt solution (HBSS, 7.4 pH; Sigma Aldrich) using a teflon catheter sleeve attached to a sterile 10 cc syringe. The buffer was then aspirated into a new sterile 10 cc syringe. This was repeated several times. This procedure results in the collection of predominantly mast cells, T cells, and monocyte/macrophages.[17] 2×10^5 of this mixed population of cells per well were incubated with substance P (100 μM) for 20 h in Dulbeccos Modified Eagle media containing

10% FBS, penicillin, streptomycin, amphotceterin B, and gentamicin. At completion of the incubation period, the media was assayed for TNF-α using a commercially available TNF-α ELISA kit (BD Biosciences). Specific cardiac mast cell responses were examined by incubating 4×10^3 mast cells per treatment tube in HyClone buffer (Thermo Scientific) containing substance P (0, 3×10^{-6}, 1×10^{-5}, 3×10^{-5}, 1×10^{-4}, and 3×10^{-4} μM) or NKA (0, 3×10^{-6}, 1×10^{-5}, 3×10^{-5}, and 1×10^{-4} μM) at 37°C for 20 min. The post-treatment supernatants and pellets were separated for subsequent analysis of histamine as a marker of mast cell degranulation using a commercial ELISA kit (Neogen, Lexington, KY, USA). Per cent histamine release was determined by dividing the histamine value from the supernatant by total histamine (supernatant plus pellet). To determine the contribution of NK-1 and -2 receptors to the activation of cardiac mast cells, additional groups of isolated cells were pre-incubated for 20 min with the NK-1 or -2 receptor antagonists, L 732 138 (20 μM) and GR 159897 (10 μM), respectively, before treatment with substance P (100 μM).

2.10 Statistical analysis

All grouped data were expressed as mean ± SD or SEM as appropriate. Grouped data comparisons were made by one-way ANOVA, using SPSS 11.5 software (SPSS, Inc., Chicago, IL, USA). When a significant *F*-test ($P < 0.05$) was obtained, intergroup comparisons were analysed using the Fisher protected least-significant difference *post hoc* testing. Statistical significance was taken to be $P < 0.05$.

3. Results

3.1 Long-term myocardial remodelling in TAC1$^{-/-}$ mice

The results for body, LV, RV, and lung weight are displayed in *Table 1*. Volume overload in the WT led to a significant increase in all parameters measured when compared with the WT sham animals.

Table 1 Biometric parameters

Groups	*n*	BW (g)	LV weight (mg)	RV weight (mg)	Lung weight (mg)
WT sham	8	26 ± 1	90.9 ± 10.1	23.8 ± 2.4	138.3 ± 21.2
WT fistula	7	29 ± 1*	111.9 ± 24*	33.6 ± 8.9*	160.4 ± 19.9*
TAC1$^{-/-}$ sham	7	24 ± 1*,**	78.4 ± 3.5**	20.4 ± 1.3**	131.9 ± 10.7**
TAC1$^{-/-}$ fistula	7	25 ± 3**	80.9 ± 8.2**	22.9 ± 3.1**	130.1 ± 9.3**

Long-term studies in mice at 28 days post-fistula and their respective shams.
All values are mean ± SD. BW, body weight; LV, left ventricle; RV, right ventricle.
*$P < 0.05$ vs. WT sham.
**$P < 0.05$ vs. WT fistula.

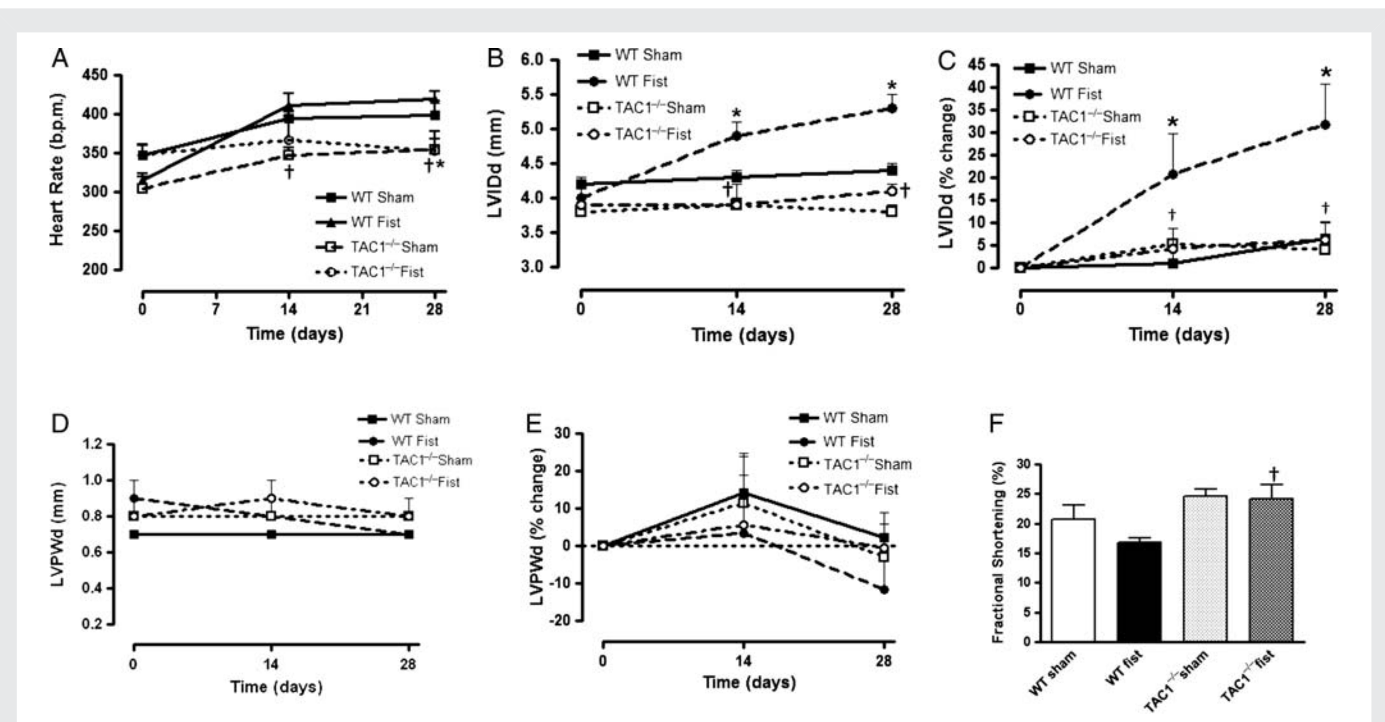

Figure 1 Echocardiographic parameters in WT sham ($n = 8$), WT Fistula ($n = 7$), TAC1$^{-/-}$ Sham ($n = 7$), and TAC1$^{-/-}$ Fistula ($n = 7$) at 0, 14 and 28 days post fistula. (*A*) Echocardiographic-derived heart rate, (*B*) left ventricular internal diameter in diastole (LVIDd), (*C*) Per cent change in left ventricular internal diameter in diastole, (*D*) Left ventricle posterior wall thickness in diastole (LVPWd), (*E*) Per cent change of left ventricle posterior wall thickness in diastole, and (*F*) Fractional shortening at 28 days post fistula. All values are mean ± SEM. *$P < 0.05$ vs. WT sham and †$P < 0.05$ vs. WT fist.

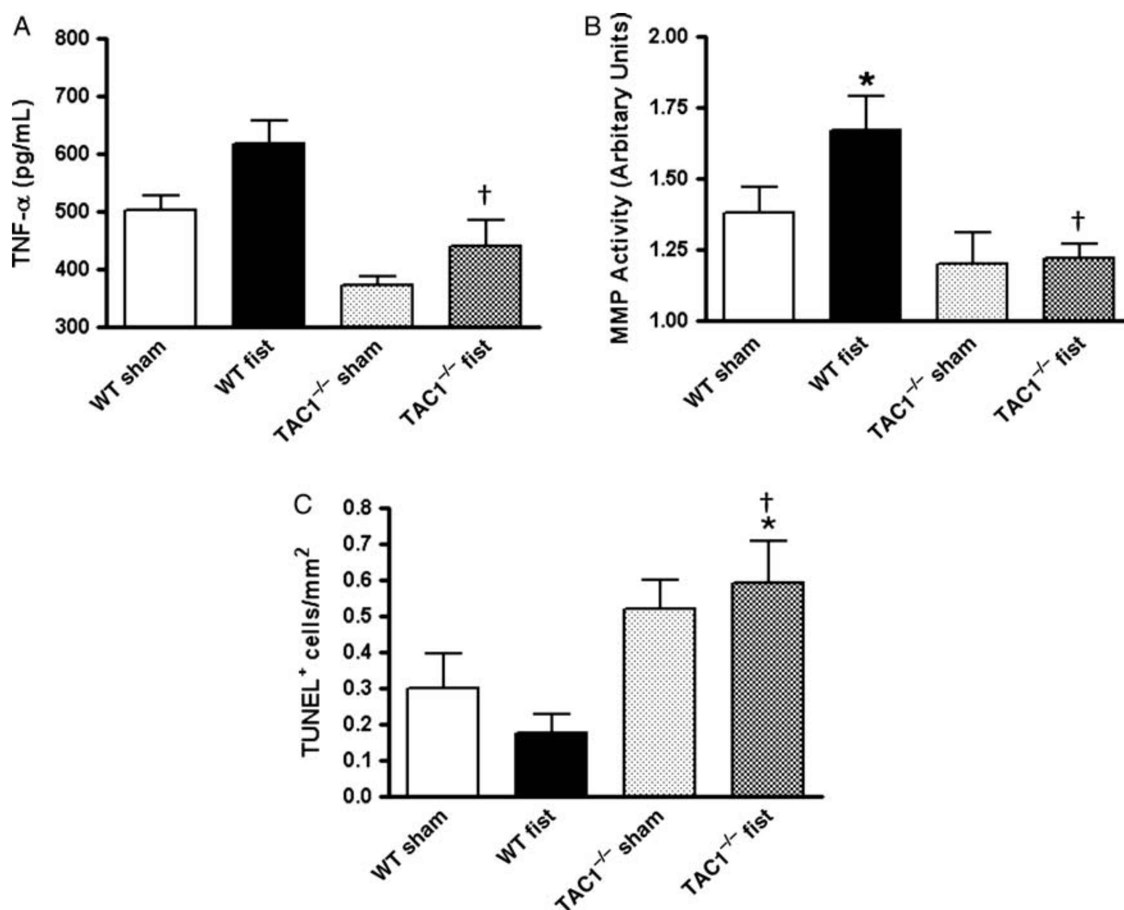

Figure 2 (*A*) Myocardial levels of TNF-α, (*B*) myocardial matrix metalloproteinase (MMP) activity, and (*C*) number of $TUNEL^+$ nuclei per square millimetre of left ventricular section in WT sham ($n = 8$), WT fistula ($n = 7$), $TAC1^{-/-}$ sham ($n = 7$), and $TAC1^{-/-}$ fistula ($n = 7$). All values are mean ± SEM. *$P < 0.05$ vs. WT sham and †$P < 0.05$ vs. WT fist.

This is indicative of a progression towards heart failure in the WT fistula mice. The $TAC1^{-/-}$ mice were slightly smaller than their WT counterparts. In contrast to the WT, none of the parameters in the $TAC1^{-/-}$ mice with fistula was significantly different from those in the $TAC1^{-/-}$ sham mice; moreover, all parameters were significantly different from the WT fistula animals.

3.2 Echocardiography

Heart rate was not significantly different between WT sham and WT fistula mice at any time-point measured (*Figure 1A*). Heart rates of the $TAC1^{-/-}$ groups did not differ significantly from each other, however, both $TAC1^{-/-}$ sham and fistula mice had significantly lower heart rates than WT sham and WT fistula mice at 14 and 28 days post-fistula. LV chamber diameter and wall thickness are expressed both as absolute values and as per cent change from baseline. Following fistula, WT hearts showed a continual increase in LV chamber size reaching 5.3 ± 0.2 mm, which represents a 31.9% increase above baseline at 28 days post-fistula (*Figure 1B* and *C*). In contrast, WT sham hearts increased in chamber dimension above baseline by only 6.5% (reaching 4.4 ± 0.1 mm), likely indicative of normal growth. $TAC1^{-/-}$ sham hearts as well as $TAC1^{-/-}$ fistula hearts increased by 4.1% (reaching 3.8 ± 0.1 mm) and 6.3% (reaching 4.1 ± 0.1 mm), respectively. Thus, there were no significant differences between these two groups. While there was no significant difference in the size or per cent change for LV posterior wall thickness, there was a definite trend towards wall thinning in the WT fistula group (*Figure 1D* and *E*). This trend was not apparent in the $TAC1^{-/-}$ fistula group. FS was determined as a measure of cardiac function (*Figure 1F*). While there was no statistically significant difference between WT sham and WT fistula groups at 28 days post-fistula, there was a definite trend towards a decrease in FS in the WT fistula group. This trend was not present in the $TAC1^{-/-}$ fistula group.

3.3 Myocardial TNF-α levels

There were no significant differences in TNF-α in the WT groups (*Figure 2A*). Myocardial levels of TNF-α in the $TAC1^{-/-}$ fistula were significantly lower than the WT fistula. Overall, $TAC1^{-/-}$ mice had lower levels of TNF-α.

3.4 Myocardial MMP activity

MMP activity (arbitrary units) was significantly increased in the WT fistula group when compared with WT sham group (*Figure 2B*). The $TAC1^{-/-}$ fistula group was not significantly different from the $TAC1^{-/-}$ sham.

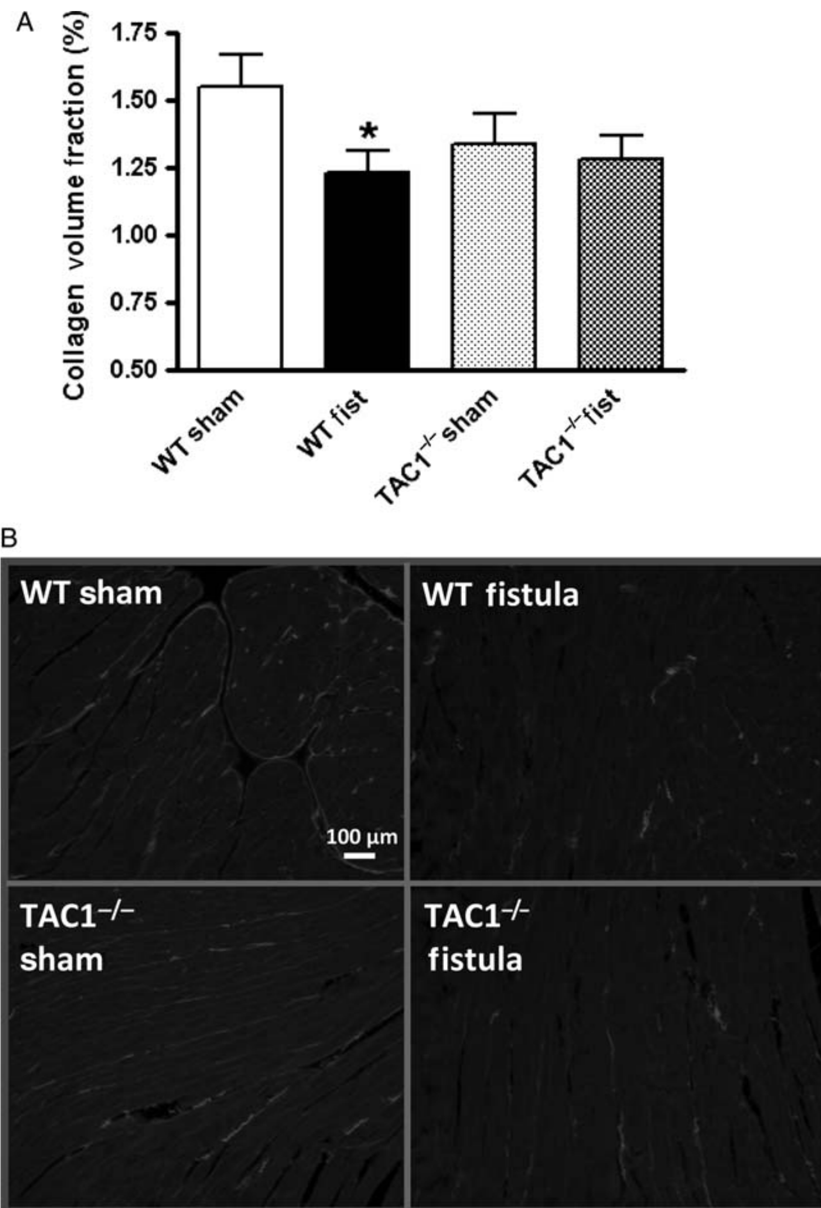

Figure 3 (*A*) Graphical representation of collagen volume fraction and (*B*) representative images of picrosirius red stained sections taken at 20× magnification from WT sham ($n = 8$), WT fistula ($n = 7$), *TAC1*$^{-/-}$ sham ($n = 7$), and *TAC1*$^{-/-}$ fistula ($n = 7$) after 28 days post-surgery. All values are mean ± SEM.*$P < 0.05$ vs. WT sham.

3.5 TUNEL assay

TUNEL staining was used to identify dying cells in the myocardium. There were no significant differences between both fistula groups and their respective controls (*Figure 2C*). Both the *TAC1*$^{-/-}$ sham and fistula groups had more TUNEL^{+} cells/mm^2 than the WT fistula group.

3.6 Collagen volume fraction

Collagen volume fraction was significantly decreased at 28 days post-fistula in WT fistula mice when compared with the WT sham (*Figure 3A* and *B*). This decrease in collagen volume fraction did not occur in the *TAC1*$^{-/-}$ fistula animals compared with the *TAC1*$^{-/-}$ shams.

3.7 Degranulation of cardiac mast cells

The ability of substance P and NKA to induce cardiac mast cell degranulation was examined using isolated cardiac mast cells. The concentration–response curves for histamine release are displayed in *Figure 4A*. Substance P elicited a strong concentration-dependent secretagogue effect with the maximum per cent of histamine released being 64 ± 4% (EC50 −log 4.4). In contrast, cardiac mast cells released essentially no histamine in response to NKA. Pre-treatment with the selective NK-1 receptor antagonist, L 732 138 prior to stimulation with substance P prevented the release of histamine (*Figure 4B*). Pre-treatment with the selective NK-2 receptor antagonist, GR 159 897 had no effect. Neither L 732 138 or GR 159 897 had any effect on histamine release when administered alone (data not shown).

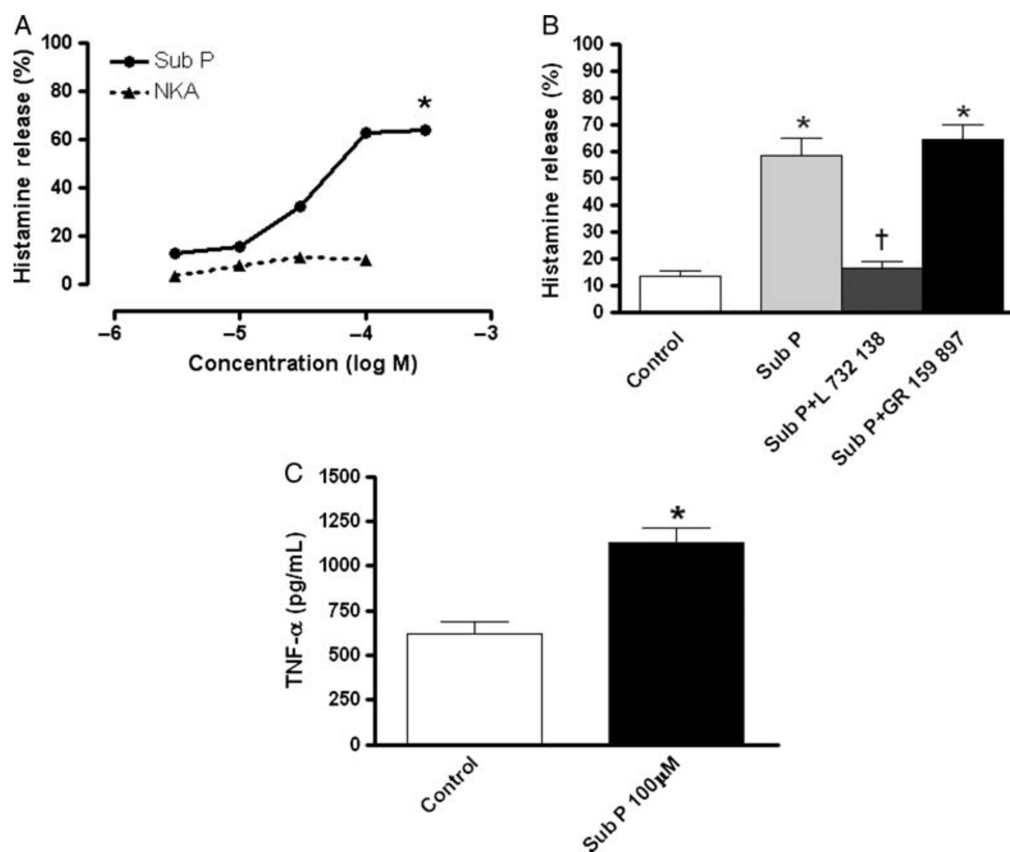

Figure 4 (*A*) Concentration–response curves for histamine release in response to substance P ($n = 5$) and NKA ($n = 4$) (*$P < 0.05$ vs. NKA), (*B*) Histamine release from isolated cardiac mast cells that were untreated (control), substance P-treated (100 μM), pre-treated with L 732 198 (20 μM) prior to stimulation with substance P, or pre-treated with GR 159 897 (10 μM) prior to stimulation with substance P (*$P < 0.05$ vs. cont; †$P < 0.05$ vs. substance P), and (*C*) TNF-α release from a mixed population of isolated cardiac inflammatory cells in response to substance P (100 μM). All values are mean ± SEM (*$P < 0.05$ vs. control).

3.8 Substance P-induced release of TNF-α from isolated cardiac inflammatory cells

A mixed population of isolated cardiac inflammatory cells containing T cells, mast cells, and macrophages was stimulated with substance P (100 μM) to investigate the ability of substance P to induce TNF-α release. Substance P was found to significantly increase TNF-α production (*Figure 4C*).

3.9 Effect of NK-1 receptor antagonism on short-term myocardial remodelling, cardiac mast cell density and myocardial TNF-α in rats

The importance of the NK-1 receptor in the initial phase of volume overload-induced adverse myocardial remodelling, when mast cell density is at its greatest, was investigated using the fistula model. At 3 days post-fistula there were no changes in body, RV or lung weight (*Table 2*). However, LV weight was increased in the untreated and treated fistula groups. No biometric parameters were affected by treatment with L 732 138 in any group. In untreated fistula rats at 3 days post-fistula, there was a characteristic decrease in collagen volume fraction when compared with sham-operated controls, indicative of collagen degradation (*Figure 5A* and *B*). This degradation of collagen was prevented by the selective NK-1 receptor antagonist, L 732 138. Cardiac mast cell density was increased in untreated fistula rats compared with shams (*Figure 5C*). Treatment with L 732 138 prevented this increase in mast cell density. Creation of a fistula caused a significant increase in myocardial TNF-α levels at 3 days post-fistula (*Figure 5D*). NK-1 receptor blockade prevented the increased levels of TNF-α in the fistula.

4. Discussion

In addition to the central nervous system, substance P and NKA are often co-localized in peripheral sensory nerves[1] with these nerves being associated with numerous areas of the heart, including the ventricle, atria, valves, and connective linings.[20] In this study, we used the fistula model of volume overload to assess the effect of deletion of the *TAC1* gene, which encodes for substance P and NKA, on myocardial remodelling. Although a fistula is not a common clinical condition, the pattern of remodelling that occurs in this model mimics that of the human myocardium in response to volume overload.[21] That is, a hypertrophic remodelling that is insufficient to normalize diastolic wall stress and ultimately can no longer meet the demands of the body for blood supply, and as a result edematous increases in lung and body weights become apparent.[8,9,13,22] The first finding of the

Table 2 Biometric parameters

Groups	n	BW (g)	LV weight (mg)	RV weight (mg)	Lung weight (mg)
Sham	14	262 ± 13	595.9 ± 38.4	163.9 ± 11.0	1421.8 ± 198.3
Fistula	12	289 ± 41	687.2 ± 81.0*	182.2 ± 38.4	1446.1 ± 164.9
Fistula + L732138	11	269 ± 17	665.6 ± 78.8*	185.1 ± 26.8	1322.0 ± 78.2

Short-term studies were done in rats at 3 days post-fistula and their respective shams.
All values are mean ± SD. BW, body weight; LV, left ventricle; RV, right ventricle. $P < 0.05$ vs sham.

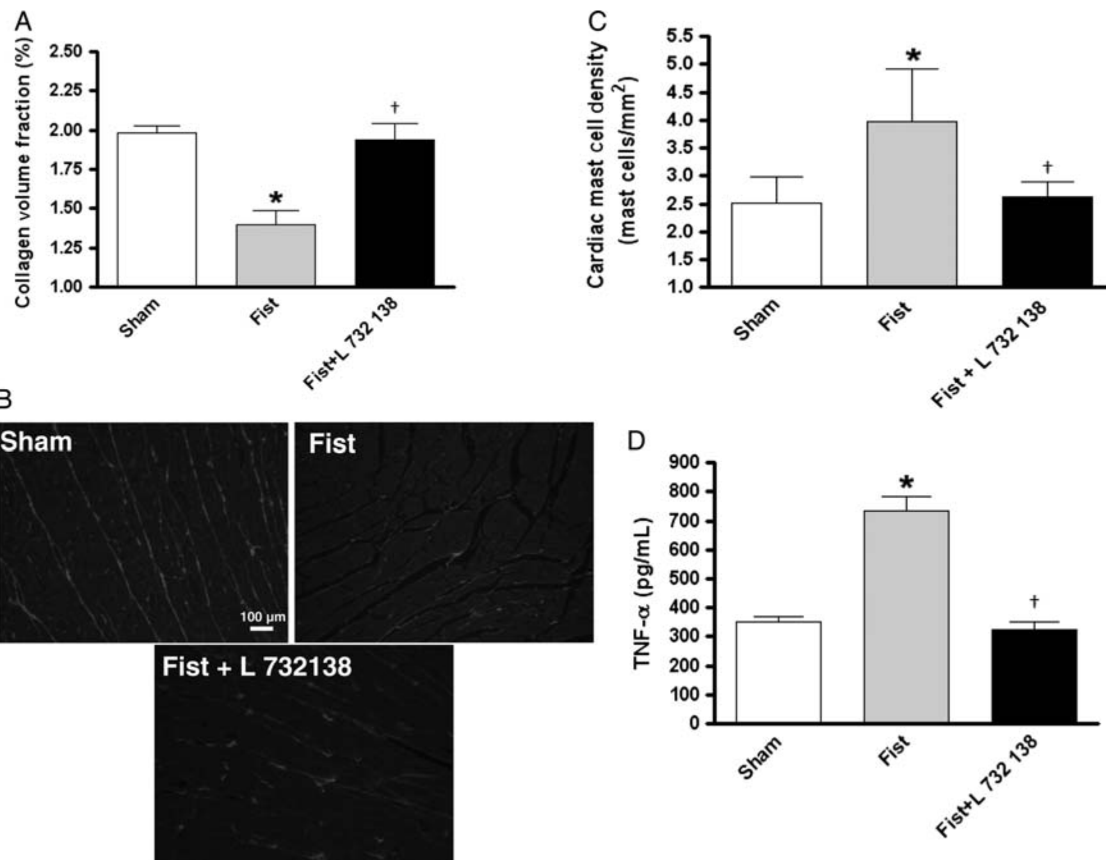

Figure 5 (*A*) Graphic representation of LV collagen volume fraction ($n = 5$). Values are mean ± SEM, (*B*) representative images from sham, untreated fistula, and fistula treated with the NK-1 receptor antagonist, L 732 138, (*C*) changes in cardiac mast cell density for sham, untreated fistula, and fistula treated with the NK-1 receptor antagonist, L 732 138 ($n = 5$). Values are mean ± SD, and (*D*) myocardial TNF-α levels following treatment with the selective NK-1 receptor antagonist, L 732 138 (5 mg/kg/day) ($n = 5$). Values are mean ± SEM. $^{*}P < 0.05$ vs. sham; $^{\dagger}P < 0.05$ vs. untreated fist.

current study is that in contrast to WT animals, $TAC1^{-/-}$ mice did not develop adverse structural remodelling following induction of volume overload. Echocardiographic analysis revealed that $TAC1^{-/-}$ hearts did not progressively dilate as is characteristic of volume overload. Further, WT fistula hearts showed a decreasing FS over the final 14 days of the 28 day study period. Although this decrease did not reach significance, this together with the increase in body, LV, RV, and lung weights, strongly suggests that these hearts were beginning to fail. This trend towards a decreased FS was absent in the $TAC1^{-/-}$ mice and they did not have significant increases in body, LV, RV, or lung weights. D'Souza *et al.*[5] had previously used mice that did not express substance P to investigate remodelling of the heart in response to Taenia crassiceps infection. While they did not identify the specific gene deleted in these mice, it was more than likely *TAC1* since this is the only gene that encodes substance P. They found that unlike the WT, their knockout mice did not develop ventricular hypertrophy following infection. Similarly, Robinson *et al.*[6] also observed that myocardial hypertrophy was prevented following infection with encephalomyocarditis virus in mice not expressing substance P. In these myocarditis studies, the authors

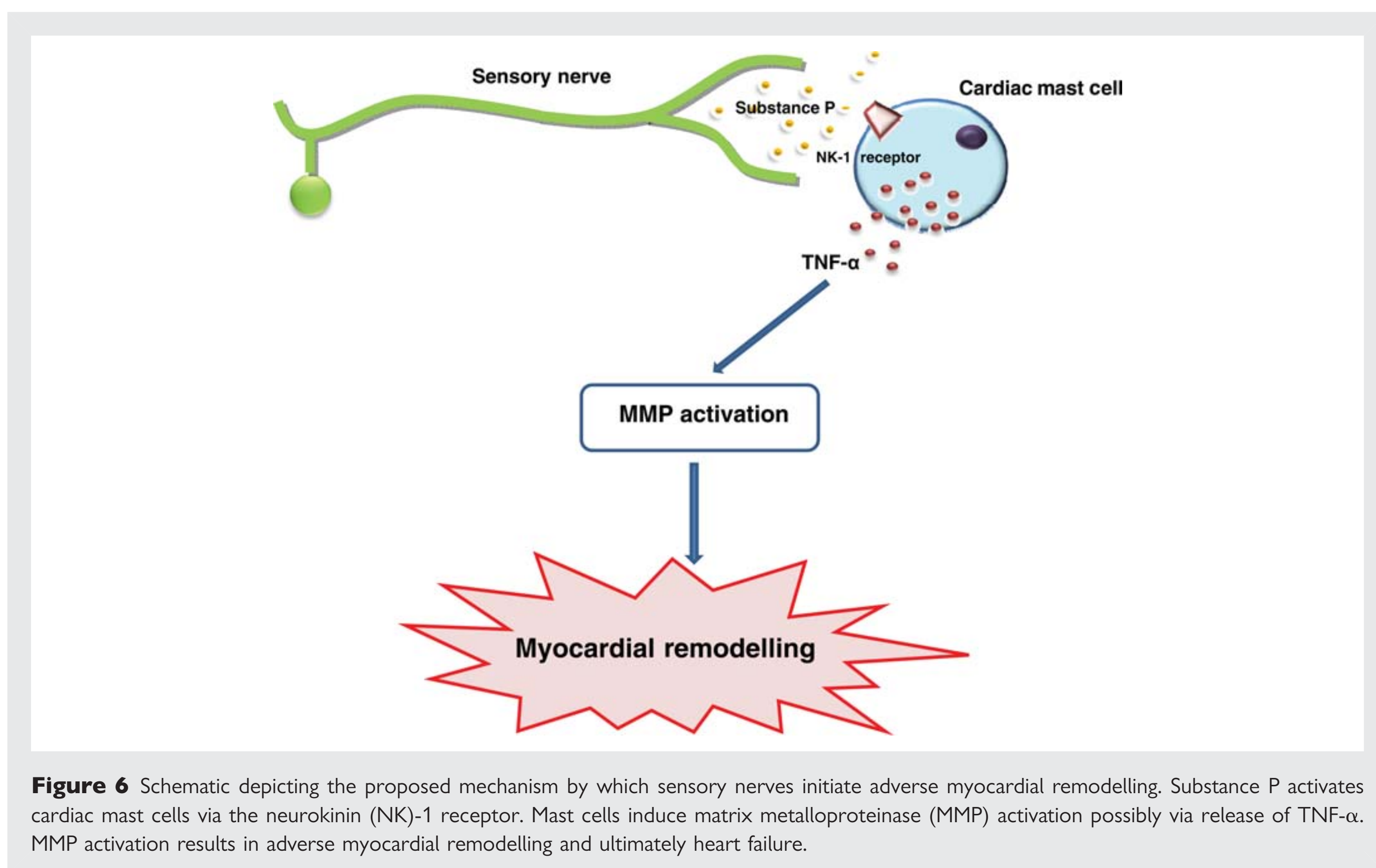

Figure 6 Schematic depicting the proposed mechanism by which sensory nerves initiate adverse myocardial remodelling. Substance P activates cardiac mast cells via the neurokinin (NK)-1 receptor. Mast cells induce matrix metalloproteinase (MMP) activation possibly via release of TNF-α. MMP activation results in adverse myocardial remodelling and ultimately heart failure.

attributed a decrease in cell death as a possible mechanism of protection in the knockout animals; however, we found cell death not to be important in our model. This may reflect differences between myocarditis-induced remodelling and remodelling induced by increased volume overload. Instead, we found that deletion of the *TAC1* gene prevented the increased activity of MMPs that was present in the WT fistula. Many MMPs are active during the remodelling process and MMPs are critical to initiating collagen degradation and inducing subsequent LV dilatation.[23] In keeping with this lack of MMP activation, hearts from $TAC1^{-/-}$ fistula animals did not undergo collagen degradation. This prevention of MMP activity by deletion of *TAC1* is consistent with observations in human lung fibroblasts where substance P has been shown to increase MMP-1 (collagenase) and collagen degradation,[24] and human gingival fibroblasts where substance P increased the quantity of numerous MMPs.[25] Volume overload also induces myocardial TNF-α production,[26] which is critical in driving adverse myocardial remodelling, including the degradation of collagen.[26,27] However, TNF-α was not significantly elevated at 28 days post-fistula in either group. However, this does not rule out the possibility that TNF-α was modulated by *TAC1* at an earlier time-point as was the case with the 3 day fistula animals.

Cardiac mast cells are known to be important in driving adverse myocardial remodelling.[7–10] Mast cells are often spatially located close to nerves[11,12] and a wide range of mast cells are known to respond to substance P.[19,28–31] With this in mind, we sought to determine whether substance P and NKA could activate isolated cardiac mast cells. Herein, we demonstrate that substance P elicited a strong concentration-dependent secretagogue effect on isolated cardiac mast cells, mediated via the NK-1 receptor. Conversely, NKA elicited virtually no response. NKA binds to NK-2 receptors, and the very small histamine release observed with NKA, coupled with the finding that the NK-2 receptor antagonist did not prevent cardiac mast cell degranulation in response to substance P, suggests that NK-2 receptors may not be present on cardiac mast cells. In fact, a study of rat hearts has previously revealed a lack of expression of the NK-2 receptor in this organ.[32] Having identified substance P and not NKA as the more likely mediator of mast cell activation and hence myocardial remodelling, we wanted to test the effects of substance P on TNF-α release. Since we also know that all inflammatory cells in the heart produce TNF-α following volume overload,[33] and substance P is known to stimulate production of TNF-α by numerous cell types including mast cells and lymphocytes,[34–36] we stimulated with substance P a mixed population of inflammatory cells (lymphocytes, mast cells, and macrophages)[17] isolated from rat hearts. As expected, TNF-α release was increased following stimulation. While we cannot rule out non-specific effects due to the high concentration of substance P required to induce an effect, TNF-α release was not due to changes in cell viability (data not shown).

Having determined that substance P activation of cardiac mast cells occurred via the NK-1 receptor, we sought to determine the importance of this receptor to myocardial remodelling *in vivo*. Peak mast cell activity occurs at ~3 days post-fistula, with concomitant collagen degradation.[7] It is this early mast cell activation that initiates the long-term remodelling of the heart.[8] Accordingly, we treated rats with the NK-1 receptor antagonist, L 732 138 for 3 days post-fistula and found that blockade of this receptor prevented mast cell density from increasing. Consequently, collagen degradation did not occur. NK-1 receptor blockade also prevented fistula-induced increases in

myocardial TNF-α, consistent with our *in vitro* findings that substance P induces TNF-α production.

In summary, we demonstrate for the first time that sensory nerve neuropeptides mediate adverse myocardial remodelling via a mechanism involving cardiac mast cells, TNF-α, and MMPs (*Figure 6*). While we cannot say definitively that it is substance P and not NKA that is the critical neuropeptide, evidence presented in this study would suggest that this is the case. Clearly these results need to be tested in other models of cardiac disease, such as pressure overload, since there are clear differences in the remodelling processes. While both volume and pressure overload induce a similar degree of hypertrophy in response to similarly elevated wall stresses, both differ in patterns of gene regulation, calcium handling, and extracellular matrix response,[37] indicating that different loads may require specific pharmacological interventions. However, if these findings should subsequently be found to be relevant to human cardiac disease, then these findings are particularly exciting and relevant given that antagonists of the NK-1 receptor are already in use at the clinical trial level, undergoing testing for the prevention of post-operative nausea and vomiting,[38] depression,[39,40] diabetic neuropathy,[41] chemotherapy-induced nausea,[42,43] and migraines,[44] and thus may represent a treatment strategy for the prevention of adverse cardiac remodelling in the foreseeable future.

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Conflict of interest: none declared.

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