Induction of the calcineurin variant CnAβ1 after myocardial infarction reduces post-infarction ventricular remodelling by promoting infarct vascularization

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Aims
Ventricular remodelling following myocardial infarction progressively leads to loss of contractile capacity and heart failure. Although calcineurin promotes maladaptive cardiac hypertrophy, we recently showed that the calcineurin splicing variant, CnAβ1, has beneficial effects on the infarcted heart. However, whether this variant limits necrosis or improves remodelling is still unknown, precluding translation to the clinical arena. Here, we explored the effects and therapeutic potential of CnAβ1 overexpression post-infarction.

Methods and results
Double transgenic mice with inducible cardiomyocyte-specific overexpression of CnAβ1 underwent left coronary artery ligation followed by reperfusion. Echocardiographic analysis showed depressed cardiac function in all infarcted mice 3 days post-infarction. Induction of CnAβ1 overexpression 1 week after infarction improved function and reduced ventricular dilatation. CnAβ1-overexpressing mice showed shorter, thicker scars, and reduced infarct expansion, accompanied by reduced myocardial remodelling. CnAβ1 induced vascular endothelial growth factor (VEGF) expression in cardiomyocytes, which resulted in increased infarct vascularization. This paracrine angiogenic effect of CnAβ1 was mediated by activation of the Akt/mammalian target of rapamycin pathway and VEGF.

Conclusions
Our results indicate that CnAβ1 exerts beneficial effects on the infarcted heart by promoting infarct vascularization and preventing infarct expansion. These findings emphasize the translational potential of CnAβ1 for gene-based therapies.

Keywords
CnAβ1 • Calcineurin • Myocardial infarction • Cardiac remodelling • Akt

1. Introduction
Myocardial infarction (MI) remains a major cause of mortality and hospitalization worldwide. Reperfusion of the blocked artery through percutaneous coronary intervention or thrombolytic therapy reduces infarct size and increases survival.1 However, even if reperfusion therapies have allowed a dramatic reduction in immediate mortality, infarction survivors are at high risk of suffering from heart failure, arrhythmia, and sudden death. Shortly after reperfusion, millions of cardiomyocytes die due to oxidative stress and are substituted by scar tissue. The mechanical stress experienced by the ventricle progressively causes scar thinning and expansion, leading to dilatation of the left ventricle (LV) and other structural changes collectively known as remodelling.2,3 The surviving cardiomyocytes undergo hypertrophy to compensate the loss of contractile capacity, but these changes progressively lead to a decline in cardiac function and eventually to the development of heart failure.

Far from being a passive tissue, post-infarction scar tissue is populated by different cell types that contribute to maintaining its integrity, regulate
extracellular matrix (ECM) turnover, and prevent dilatation. A few days after infarction a granulation tissue is formed, integrated by leucocytes, new blood vessels, fibroblasts, and myofibroblasts that secrete different ECM components, mainly collagen. Inflammatory cells progressively disappear from the area and the scar matures into an ECM-rich fibrotic tissue. The persistence of myofibroblasts in the infarct region contributes to tissue stability. Progressive loss of these cells causes thinning of the infarcted area and left ventricular dilatation. Whereas a strong ECM in the scar region prevents structural remodelling, collagen accumulation in the remote myocardium has the opposite effect. Interstitial fibrosis increases passive stiffness, causes electrical remodelling, and enhances arrhythmogenicity, further contributing to cardiac remodelling and dysfunction. Therefore, ideal therapies will aim at promoting scar maturation while reducing interstitial fibrosis in the remote myocardium.

The molecular mechanisms involved in post-infarction remodelling are not entirely understood. Calcineurin generally plays a detrimental role in the heart, and both protective and deleterious effects have been described for this protein in response to ischaemia/reperfusion. It is both sufficient and necessary to induce maladaptive cardiac hypertrophy, and activation of the transcription factor NFAT (nuclear factor of activated T cells) by calcineurin promotes myocyte hypertrophy and cardiac fibrosis. Recently described that, in contrast to other calcineurin A isoforms, the naturally occurring splicing variant, CnA1, has a beneficial effect on the heart. CnA1 has a unique C-terminal domain, not present in any other known protein, that confers its distinct properties. In a chronic MI model with permanent occlusion of the left coronary artery, cardiac-specific overexpression of CnA1 has a protective effect, improving cardiac function and reducing long-term scar size.

The purpose of the present work was to investigate the potential benefit of CnA1 overexpression in response to ischaemia/reperfusion injury, to determine whether CnA1 is cardioprotective or whether its beneficial action results from post-infarction effects (i.e. improving post-infarction remodelling), and to explore the potential of CnA1 for gene-based therapies.

2. Methods

2.1 Transgenic mice
Reverse Tet transactivator (rtTA)-CnA1 mice express the rtTA in a cardiomyocyte-specific manner, which, in turn, induces CnA1 overexpression from a second transgene in a doxycycline (Dox)-inducible fashion (Figure 1). rtTA mice also express the rtTA in a cardiomyocyte-specific manner, but lack the CnA1 transgene and therefore do not overexpress CnA1. The rtTA-CnA1 mouse line was generated by crossing the original TetO-CnA1 transgenic line, carrying the Tet operator and the CnA1 cDNA (Figure 1) with the rtTA mouse line. Both rtTA-CnA1 and rtTA mice were generated in a CBA/Black10 genetic background and inbred in this background for at least eight generations. Male littersmates between 3 and 5 months of age were used for experiments. Dox was administered to mice with diet (0.3%) starting either 3 weeks before infarction or 1 week after infarction, and maintained until mice were sacrificed.

2.2 Surgeries and echocardiographic analysis
Myocardial infarction was induced in rtTA-CnA1 (n = 28) and rtTA mice (n = 21) by ligation of the left coronary artery for 30 min followed by reperfusion of the artery. Surgeries were performed under mechanical ventilation with 3–3.5% sevoflurane. Mice received analgesic treatment with buprenorphine (0.3 mg/kg, s.c.) after surgery. The mortality rate in the first 24 h post-infarction was 38%; no mortality was found afterwards. Cardiac function, chamber dilatation, and wall thickness were analysed by transthoracic echocardiography 3 and 28 days after infarction, as well as in uninfarcted mice, using a Vevo 2100 system and a 45-MHz probe (Visualsonics, Toronto, Canada). Measurements were taken by a blinded operator with mice placed on a heating pad under light anaesthesia with sevoflurane adjusted to obtain a target heart rate of 500 ± 50 bpm. Two-dimensional (2D) and M-mode echocardiography images were recorded in a long and short view at the level of the papillary muscles. LV end-systolic and end-diastolic volumes as well as LV ejection fraction were measured from 2D parasternal long axis using the area-length method. Animals were sacrificed by gradually filling the chamber with carbon dioxide.

For the analysis of infarct size using echocardiography, regional left ventricular function was evaluated in the parasternal long-axis view. The LV wall was subdivided into six segments (basal, mid, and apical in the anterior and posterior walls). Each segment was scored by an independent blinded evaluator based on its motion and systolic thickening, according to the guidelines of the American Society of Echocardiography. We recently described that, in contrast to other calcineurin A isoforms, the naturally occurring splicing variant, CnA1, has a beneficial effect on the heart. CnA1 has a unique C-terminal domain, not present in any other known protein, that confers its distinct properties. In a chronic MI model with permanent occlusion of the left coronary artery, cardiac-specific overexpression of CnA1 has a protective effect, improving cardiac function and reducing long-term scar size.

The purpose of the present work was to investigate the potential benefit of CnA1 overexpression in response to ischaemia/reperfusion injury, to determine whether CnA1 is cardioprotective or whether its beneficial action results from post-infarction effects (i.e. improving post-infarction remodelling), and to explore the potential of CnA1 for gene-based therapies.

2.3 Western blot
Western blot was carried out using the following primary antibodies as previously described: anti-phospho-Akt-Ser473, anti-Akt, anti-phospho-mTOR-Ser 2448, anti-mammalian target of rapamycin (mTOR) (Cell Signaling), anti-hypoxia-inducible factor 1α (HIF1α) and anti-Periostin (Novus Biochemicals), anti-vascular endothelial growth factor (VEGF), anti-Lox and anti-CD31 (Abcam), and anti-fibronectin (Sigma). Anti-CnA1 has been previously described. Brightness and contrast were linearly adjusted using Photoshop CS5.

2.4 Histology and immunohistochemistry
Samples were fixed in parafomaldehyde (4% in PBS) for 48 h, washed in PBS, dehydrated, and included in paraffin. Five-micron thick sections were stained following Masson’s trichrome protocol. Images were quantified using ImageJ (NIH, USA). Scar length was determined in Masson’s trichrome-stained sections using the midline method, which best correlates with functional measurements. In this method, the infarct length is measured as the length of the midline of the infarcted wall, in which >50% of the wall thickness is composed of scar tissue. Scar length represents the percentage of infarct length with respect to the length of the whole LV circumference. Medium/large blood vessels in the infarct region were visually scored in Masson trichrome-stained sections. These vessels would be >20 μm in diameter and typically surrounded by a smooth muscle layer that makes them easily identifiable. In parallel, vessels were stained by immunohistochemistry using anti-a-smooth muscle actin (SMA). In both experiments, the number of vessels was determined in the infarct region only and divided by the infarct area. Vessels were quantified in three non-consecutive sections for each mouse. Capillaries were stained using biotin-conjugated Isolectin B4 (Sigma) and scored in two separate 40× microscope fields. Apoptosis in cardiomyocytes (in the whole section) and fibroblasts (in the infarct region) was analysed using Tunel and immunostaining with anti-tropinin I (cardiomyocytes) or anti-periostin (fibroblasts).
Cardio-specific inducible transgenic mice used in this study. (A) rtTA-CnAβ1 mice overexpress the rtTA specifically in cardiomyocytes under the control of the Xenopus MLC2v promoter. Upon Dox administration in the diet, rtTA activates overexpression of CnAβ1 from a second transgene. (B) Schematic showing the different experimental groups and Dox administration regime. (C) CnAβ1 mRNA expression was analysed in the remote myocardium 28 days post-infarction by qRT-PCR. Results are expressed as mean fold induction ± SE over the values of uninjured hearts (indicated by a dashed line). *P < 0.05 Dox-treated vs. no Dox. (D) rtTA mice were used as negative controls to test for the effect of rtTA overexpression and Dox administration themselves. These mice overexpress rtTA in cardiomyocytes but not CnAβ1, since they lack the second transgene present in rtTA-CnAβ1 mice. (E) Schematic showing the rtTA mouse groups and Dox administration regime. (F) Analysis of CnAβ1 mRNA expression in the remote myocardium 28 days post-infarction by qRT-PCR. Results are expressed as mean fold induction ± SE over the values of uninjured hearts (dashed line). n = 6–15 per group. (G) qRT-PCR analysis of CnAβ1 and CnAβ2 mRNA expression in uninjured hearts and in the remote and infarct regions of the heart 28 days post-infarction. *P < 0.05 compared with no infarction, one-way ANOVA plus Bonferroni post-test.
2.5 RNA isolation and quantitative reverse-transcribed polymerase chain reaction

After sacrificing the animals, mice were perfused with PBS, hearts were excised, and samples from the infarct region, border zone, and remote myocardium were separated and snap-frozen in liquid nitrogen. Total RNA was isolated using the RNeasy kit from Qiagen, with DNase digestion on the column. cDNA was synthesized from 100 ng of total RNA using random hexamers and a High Capacity cDNA Reverse Transcription kit (Applied Biosystems) in a 10-µL reaction. Quantitative reverse-transcribed polymerase chain reaction (qRT-PCR) was carried out in an AB9700 thermocycler (Applied Biosystems) using Taqman chemistry or SYBR green (Applied Biosystems). The following Taqman probes were used: Acta1 (Mm00808218_g1), Nppb/brain natriuretic peptide (BNP) (Mm01255770_g1), Col1a1 (Mm00801666_g1), Lox (Mm00495386_m1), Thy1 (Mm00493681_m1), and Acta2 (Mm01204962_g1). Gene expression was normalized to 18S rRNA levels quantified simultaneously using a VIC-labelled probe and Acta2 (Mm01204962_gh). Gene expression was normalized to 18S, Lox, Thy1, Nppb, Col1a1, and CD31 expression were quantified using SYBR green and the following primers and conditions: CnA forward: 5′-AGCAA GTGTGCAATACATCATT-3′, CnA reverse: 5′-AGGCAAGGGAGGAGGGTTAGGT; 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C, 30 s at 60°C. CnA2 primers were previously described. qRT-PCR data were analysed using the LinReg software in order to estimate the efficiency rates and the Ct values.

2.6 Angiotubes

Neonatal cardiomyocytes were isolated from rtTA-CnA1 and rtTA mice as described previously. Neonatal mice were sacrificed by cervical dislocation. A total of 750 000 cells/well were seeded in 12-well plates and grown in the presence of 10% foetal calf serum for 2 days. Cells were then washed and cultured in serum-free medium for 2 days in the presence of 2 μg/mL of Dox, 0.1 mM rapamycin, or DMSO (1 : 1000) as indicated in the figures. Conditioned medium was cleared by centrifugation and used for angiobase assays as follows. Matrigel (Becton Dickinson) diluted 1 : 3 in DMEM was added to 96-well plates (100 µL/well) and allowed to polymerize for 60 min. Human umbilical cord vascular endothelial cells (HUVECs; Promocell) were seeded at a density of 30 000 cells/well in the presence of the different conditioned mediums and allowed to form tubes for 6 h. Where indicated, neutralizing anti-VEGF antibody (R&D Systems) was added to the culture (2 ng/µL). Anti-troponin I (Abcam) was used as a negative control antibody. Pictures were taken with a Nikon eclipse TI microscope, and the number of tube network nodes was quantified for each well. Experiments were performed in triplicate and all experiments were repeated at least three times.

2.7 Statistics

Data are presented as mean ± SE. In echocardiographic data, the same mice were analysed 3 and 28 days post-infarction, with non-infarcted animals representing a different group of mice. To test for statistical significance, data were analysed by two-way ANOVA followed by Bonferroni post-test for multiple comparisons. In addition, a two-way ANOVA with repeated measures followed by Bonferroni post-test was applied to compare mice at 3 vs. 28 days post-infarction. Significant differences in qRT-PCR and histological quantifications were analysed by one-way ANOVA followed by Dunnett’s post-test to compare Dox-untreated mice. Angiotubes were analysed by Student’s t-test or two-way ANOVA, followed by Bonferroni’s post-test depending on the number of variables. Data were analysed with GraphPad Prism.
3. Results

3.1 Cnα1 improves cardiac function and remodelling after ischaemia/reperfusion

To determine the potential benefit of Cnα1 overexpression in the context of MI with reperfusion, we developed double transgenic mice in which Cnα1 expression is induced three- to four-fold specifically in cardiomyocytes upon administration of Dox in the diet (rtTA-Cnα1 mice, Figure 1). As negative controls, to test the effect of Dox itself, we used mice that overexpress the same rtTA transactivator but lack the Cnα1 transgene (rtTA mice). As a first approach, we administered Dox starting 3 weeks before surgery and maintained it throughout the experiment. We induced MI by occluding the left coronary artery for 30 min followed by reperfusion, and analysed the mice 3 and 28 days later by echocardiography. rtTA-Cnα1 and rtTA mice showed functional decline and chamber dilatation 3 days post-infarction regardless of Dox treatment (Figure 2A–D and Table 1), suggesting that initial infarct size and myocardial loss were analogous among all groups. At Day 28 post-infarction, we detected a significant improvement in cardiac function (left ventricular ejection fraction, LVEF) in mice overexpressing Cnα1 from 3 weeks before surgery (Dox pre-MI; Figure 2A, grey bars), while animals not receiving Dox showed no improvement (No Dox; Figure 2A, white bars). Improved function was accompanied by reduced ventricular dilatation in Dox-treated rtTA-Cnα1 mice (Figure 2C and Table 1). In contrast, Dox administered to rtTA mice had no effect on cardiac function or chamber dilatation (Figure 2B and D). Of note, neither Cnα1 nor Cnα2 (the Cnα1-splicing isomorph that carries the full Cnα autoinhibitory domain) mRNA expression showed much variation in response to MI itself (Figure 1G).

3.2 Cnα1 induces recovery rather than protection

The results obtained at 3 days post-infarction suggested that Cnα1 offered no protection against reperfusion injury, whereas the improvement observed at 28 days suggested a positive effect post-infarction. To further discriminate between the protective and recovery effects of Cnα1 overexpression, we treated rtTA-Cnα1 mice with Dox starting 1 week post-infarction, once the scar has developed (Dox post-MI, black bars). Interestingly, a significant enhancement of cardiac function was observed in these mice (Figure 2A), which was accompanied by reduced ventricular dilatation (Figure 2C). Importantly, in all the study,

### Table 1 Cnα1 reduces remodelling and improves cardiac function after myocardial infarction and reperfusion

<table>
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<td>0.65 ± 0.03</td>
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<td>0.53 ± 0.03</td>
<td>0.67 ± 0.07</td>
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<tr>
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<tr>
<td>LVDDV (μL)</td>
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<td>IVSd (mm)</td>
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Ligation of the left coronary artery (30’ ischaemia followed by reperfusion) was performed in rtTA and rtTA-Cnα1 male transgenic mice, and echocardiography analysis was carried out 3 and 28 days later. Mean values ± SE are shown.

- EF: ejection fraction; LVEVF: left ventricular end-diastolic volume; LVDDV: left ventricular end-diastolic volume; LVSPDd: left ventricular posterior wall in diastole; IVSd: interventricular septum in diastole; HW/BW: heart weight/body weight ratio; n: number of mice analysed.
- *p < 0.05 treated vs. No Dox.
- †p < 0.05 Dox-treated vs. No Dox, 2-way ANOVA plus Bonferroni post-test.
- ‡p < 0.05 28 days post-MI vs. 3 days post-MI repeated-measures two-way ANOVA plus Bonferroni post-test.
Figure 3 CnAβ1 reduces infarct expansion. Scar length (A) and thickness (B) were analysed 28 days post-infarction using histological methods. (C–F) mRNA expression of collagen Ia1 (C), lysyl oxidase (D), Thy1 (E), and α-smooth muscle actin (F) were analysed by qRT-PCR in the infarct region only. Results are expressed as mean fold induction ± SE over the values of uninjured hearts (dashed line). n = 6–15 per group. *P < 0.05 Dox-treated vs. no Dox, one-way ANOVA followed by Dunnett’s post-test. (G) Western blot analysis of lysyl oxidase (Lox), fibronectin (Fn), periostin (Postn), and CD31 in the infarct region of rtTA-CnAβ1 and rtTA mice 28 days post-infarction.
the same mice were sequentially analysed at 3 and 28 days. The functional decline observed at 3 days post-infarction and the subsequent improvement observed at 28 days in Dox-treated rtTA-CnAβ1 mice (see Supplementary material online, Figure S1 and Table 1) demonstrate that CnAβ1 induces functional recovery, rather than protection from infarction.

### 3.3 CnAβ1 prevents infarct expansion and improves remote myocardium remodelling

We next analysed the impact that CnAβ1 overexpression has on infarct expansion. We found that overexpression of CnAβ1 before infarction resulted in reduced scar length 28 days post-infarction, compared with mice untreated with Dox (Figure 3A and Supplementary material online, Figure S2). Importantly, a similar effect was achieved when CnAβ1 was induced after infarction. In Dox-treated rtTA-CnAβ1 mice, scar length at 28 days post-infarction (20.47 ± 3.85% in mice treated pre-MI and 14.23 ± 4.16% in mice treated post-MI) remained similar to that observed at 7 days in untreated mice (21.52 ± 2.90%).

Echocardiographic analysis showed that CnAβ1 overexpression results in a reduction in the number of LV segments with dysfunctional motility 28 days after infarction (see Supplementary material online, Table S1). This was particularly evident in the mid-anterior segment, confirming the reduced infarct size observed by histological methods. In addition, CnAβ1-overexpressing mice showed thicker scars than control mice (Figure 3B), suggesting that CnAβ1 prevents infarct expansion. Induction of CnAβ1 also resulted in higher expression of collagen Iα1 in the scar region, together with increased expression of lysyl oxidase, which crosslinks collagen and elastin molecules into mature fibres, the fibroblast proliferation marker Thy1/CD90, and α-smooth muscle actin (Figure 3C–F). We also detected increased expression of perioestin, fibronectin, and lysyl oxidase proteins in the infarct region of CnAβ1-overexpressing mice (Figure 3G). rtTA mice showed no changes in scar length, thickness, or expression of fibrosis markers upon Dox administration (Figure 3A–G and Supplementary material online, Table S1).

To investigate whether reduced infarct expansion was accompanied by improved fibroblast survival, we quantified the percentage of apoptotic fibroblasts by Tunel staining. As shown in Supplementary material online, Figure S3A, only a very low degree of fibroblast apoptosis was detected in the infarct region 28 days post-infarction, and no significant difference was observed among the groups.

To determine whether changes in infarct expansion were paralleled by improved remodelling of the remote myocardium, we analysed the cardiomyocyte area and the expression of heart failure markers in this region. CnAβ1 overexpression either before or after infarction significantly reduced the cross-sectional area of cardiomyocytes and the wall thickness in the remote myocardium (Figure 4A and Table 1). This was accompanied by a significant reduction of α-skeletal actin, BNP, and collagen Iα1 in the remote myocardium of these mice (Figure 4B–D). A trend towards reduced cardiomyocyte apoptosis was also detected in CnAβ1-overexpressing mice, although the percentage of apoptotic cardiomyocytes 28 days post-infarction was low (see Supplementary material online, Figure S3B). In contrast, Dox administered to rtTA mice had no effect on cardiomyocyte size or

**Figure 4** Reduced myocardial remodelling in CnAβ1-overexpressing mice. (A) Cardiomyocyte cross-sectional area was analysed 28 days post-infarction by histological methods. (B–D) The expression of α-skeletal actin (B), BNP (C), and collagen Iα1 (D) mRNA was analysed by qRT-PCR in the remote myocardium. Results are expressed as mean fold induction ± SE over the values of uninjured hearts (dashed line). n = 6–15 per group. *P < 0.05 Dox-treated vs. no Dox, one-way ANOVA followed by Dunnett’s post-test.
expression, heart failure, or fibrosis markers or apoptosis (Figure 4A–D and Supplementary material online, Figure S3B).

3.4 Improved vascularization in the infarct region of CnAβ1-overexpressing mice

The effect of CnAβ1 on infarcted hearts is reminiscent of cardiomyocyte activation of the HIF1α, which drives the expression of angiogenic factors and promotes infarct vascularization.23 To determine whether more active, thicker scars were supported by enhanced vascularization in CnAβ1-overexpressing mice, we quantified the amount of blood vessels in the scar region. We observed that induction of CnAβ1 overexpression either pre- or post-infarction increased the number of blood vessels in the infarcted region (Figures 5 and 6A and B). This was confirmed by an up-regulation of CD31 and α-SMA mRNA in the same region (Figures 3F and G and 6C). A mild induction in CD31 mRNA expression and in the number of capillaries were also detected in the remote area upon CnAβ1 induction (Figure 6E and F).

3.5 CnAβ1 promotes vascularization by activating the Akt signalling pathway

We have previously shown that CnAβ1 activates the Akt pathway through its unique C-terminal domain.15 Akt is known to induce angiogenesis in the heart by promoting expression of VEGF.24 We therefore investigated the role of this signalling pathway in the angiogenic response elicited by CnAβ1. We observed that Dox stimulation of rtTA-CnAβ1 mice induced expression of VEGF and the HIF1α in the remote myocardium (Figure 7A). This was accompanied by increased CD31 expression and activation of the Akt/mTOR pathway. No changes were observed in rtTA mice upon Dox treatment.

To test whether the paracrine angiogenic effect of CnAβ1 was reproduced in culture, we used the assay for HUVEC angiobtube formation on matrigel.25 As shown in Figure 7B, conditioned medium from Dox-treated rtTA-CnAβ1 cardiomyocytes stimulated angiobtube formation by HUVEC, whereas that of untreated cells or rtTA cardiomyocytes had no effect. This effect was blocked by a neutralizing anti-VEGF antibody (Figure 7C), suggesting that VEGF mediates the paracrine effect of CnAβ1-overexpressing cardiomyocytes on endothelial cells. Interestingly, treatment of rtTA-CnAβ1 cardiomyocytes with the inhibitor of the Akt/mTOR pathway rapamycin blocked the induction of angiobtube formation by the conditioned medium (Figure 7D). Rapamycin also prevented the induction of VEGF secretion by Dox-treated rtTA-CnAβ1 cardiomyocytes (Figure 7E). These results suggest that CnAβ1 activates Akt/mTOR in cardiomyocytes to induce VEGF secretion and paracrine activation of angiogenesis.

4. Discussion

We show here that, in contrast to other calcineurin isoforms, CnAβ1 has beneficial effects on the heart after reperfusion injury. CnAβ1 reduces ventricular dilatation and improves function even when overexpressed 1 week after infarction. By activating the Akt/mTOR pathway, CnAβ1 promotes secretion of angiogenic mediators by cardiomyocytes and enhances vascularization of the infarct region, thus precluding infarct expansion and improving heart remodelling.

Our results suggest that the beneficial effects of CnAβ1 overexpression involve the prevention of heart remodelling rather than cardioprotection. The significant ventricular dilatation and decline in cardiac function observed here at Day 3 post-infarction in all experimental groups strongly suggest that CnAβ1 exerts its beneficial effects by improving myocardial remodelling after infarction, rather than by protecting from reperfusion injury. This is further supported by the fact that induction of CnAβ1 as late as 1 week post-infarction improves cardiac function and reduces ventricular dilatation. The scar tissue in CnAβ1-overexpressing mice is thicker and richer in collagen, matrix crosslinking enzymes and activated fibroblasts, which result in reduced infarct expansion.

We previously showed that CnAβ1 overexpression leads to reduced scar formation after permanent occlusion of the left coronary artery.15 In the chronic infarction model, scars from CnAβ1-overexpressing mice showed reduced fibroblast number and collagen expression. In contrast, Dox-treated rtTA-CnAβ1 mice show thicker scars with increased collagen expression after ischaemia reperfusion. This apparent discrepancy is likely due to the different response to permanent ischaemia and to
reperfusion injury. Chronic MI eventually results in the death of most cells in the infarct region, leading to loss of ECM production and infarct expansion. In that context, the reduced infarct size observed in the presence of CnAβ1 is likely due to reduced myocardial damage, which is consistent with the improved function already observed 7 days post-infarction. In contrast, reperfusion injury reduces cardiomyocyte death and promotes a stronger angiogenic response in the infarcted myocardium. Our results suggest that CnAβ1 promotes VEGF expression from cardiomyocytes to improve vascularization of the infarcted region, while stimulating fibroblast proliferation and ECM production in this area. In addition, although we only detected a very low degree of fibroblast apoptosis 28 days post-infarction, we cannot exclude the possibility that better vascularization improves fibroblast survival at earlier time points. The increase in fibroblasts and myofibroblasts in the scar tissue will contribute to increased ECM turnover and to the maintenance of the scar structure, thus preventing remodelling. Distinct effects on heart remodelling in response to permanent ischaemia and reperfusion injury have been described for other factors like growth differentiation factor 15.

In addition, the remote myocardium of CnAβ1-overexpressing mice shows reduced cardiomyocyte hypertrophy, together with lower expression of collagen and heart failure markers. This reduction in
myocardial remodelling is likely the result of the limited infarct expansion. Importantly, all these effects were observed even when CnAβ1 overexpression was induced late after infarction, indicating that CnAβ1’s main target is not only the reduction of cardiomyocyte death or inflammation, but also the prevention of infarct expansion and chamber dilatation.

Molecular therapies capable of improving ventricular function after infarction are scarce. Overexpression of sonic hedgehog starting 5 days post-infarction attenuates remodelling and improves function.28 Inhibition of Wnt signalling with Frizzled antagonist peptides reduces infarct expansion and prevents ventricular dilatation.29 This effect was also achieved when the peptide was administered 2 weeks after infarction, suggesting that Wnt signalling has a detrimental effect on heart remodelling rather than on early infarct healing. In both cases, the improvement in cardiac remodelling was accompanied by enhanced infarct vascularization. In this same regard, overexpression of the angiogenesis regulator HIF1α also improves vascularization and function, and reduces infarct size in transgenic mice.23 However, chronic overexpression of HIF1α has detrimental effects on the heart.10 Similarly, short-term Akt activation in cardiomyocytes induces VEGF and angiogenesis, but sustained activation induces maladaptive hypertrophy and fibrosis.24 We show here that CnAβ1 induces angiogenesis by promoting VEGF expression in an Akt/mTOR-dependent manner. In contrast to sustained Akt activation, CnAβ1 overexpression in cardiomyocytes improves infarct vascularization, cardiac remodelling, and function without inducing the detrimental side effects observed after sustained Akt or HIF1α overexpression.

Taken together, our results emphasize the therapeutic potential of CnAβ1 and suggest that it may be an excellent candidate for gene therapies aimed at reducing ventricular remodelling and improving cardiac function post-infarction. We demonstrate that these beneficial effects can be achieved by inducing CnAβ1 expression late after infarction. Our work constitutes a first step for the translation of these findings to the clinical setting.

Supplementary material
Supplementary material is available at Cardiovascular Research online.

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