Overexpression of Cardiomyocyte Alpha$_{1A}$-Adrenergic Receptors Attenuates Post-Infarct Remodeling by Inducing Angiogenesis Through Heterocellular Signaling

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ABSTRACT

Objective— Stimulation of cardiac α_{1A}-adrenergic receptors (α_{1A}-ARs) has been proposed for treatment of heart failure, since it increases myocardial contractility. We investigated a different mechanism, induction of angiogenesis.

Approach and Results—Four weeks after permanent coronary artery occlusion (CAO), transgenic (TG) rats with cardiomyocyte-specific α_{1A}-AR overexpression had less remodeling than their non-transgenic littermates (NTLs), with 29% less fibrosis and 15% smaller cardiomyocyte cell size in non-ischemic zone, 16% reduced left ventricular heart weight, 15% reduced lung weight to tibial length and smaller scar size (18±2% vs. 28±3%) (all $p<0.05$). Coronary blood flow, measured with microspheres, increased in the infarct zone in TG compared to NTLs (1.4±0.2 vs. 0.5±0.08ml/min/g) ($p<0.05$), which is consistent with angiogenesis, as reflected by a 21% increase in capillary density in the zone adjacent to the infarct, compared to NTLs. VEGF-A mRNA and protein was increased in isolated TG cardiomyocytes. In addition, treatment of NTL cardiomyocytes with the selective α_{1A}-agonist, A61603, increased VEGF-A expression, which was blocked by the α_1-antagonist, prazosin. Conditioned medium from cultured TG cardiomyocytes enhanced human umbilical vein endothelial cell (HUVEC) tubule formation, which was blocked by an anti-VEGF-A antibody. Moreover, improved cardiac function, blood flow and increased capillary density after chronic CAO in TG rats were blocked by either a MEK or a VEGF-A inhibitor.

Conclusions—Cardiomyocyte-specific overexpression of the α_{1A}-AR in rats resulted in enhanced MEK-dependent cardiomyocyte VEGF-A expression, which stimulates angiogenesis and improves myocardial blood flow *via* a paracrine mechanism involving heterocellular cardiomyocyte/endothelial cell signalling, protecting against remodeling and heart failure following chronic CAO.

ABBREVIATIONS

α_{1A}-AR	α_{1A}-adrenergic receptor
NTL	Non-transgenic littermates
TG	Transgenic
CAO	Coronary artery occlusion
MI	myocardial infarction
HUVEC	Human umbilical vein endothelial cell

INTRODUCTION

Cardiac-specific α_{1A}-adrenergic receptor (α_{1A}-AR) stimulation has been proposed as a therapeutic strategy for heart failure[1-5], as it increases myocardial contractility[6, 7], and blockade of the α_1-AR exacerbates heart failure[2, 3]. We investigated a different mechanism, whereby,cardiomyocyte-specific overexpression of the α_{1A}-AR induces angiogenesis, which could be therapeutically beneficial for heart failure, particularly that due to chronic myocardial infarction (MI). This is because, in the presence of permanent coronary artery occlusion (CAO), even cardioprotective interventions are destined to fail as they do not increase blood flow distal to the CAO, even to that observed in hibernating myocardium[8, 9]. This is required to limit ischemic damage to the myocardium. In fact, despite the hundreds, if not thousands, of studies identifying molecular pathways protecting the heart, there has been little clinical translation of these findings, and even approaches to enhance myocardial regeneration have met with uneven success[10]. In the permanent absence of blood flow to the ischemic heart, and in the absence of preformed collateral channels, almost any intervention is destined to fail. Although several TG models have shown cardioprotection in the setting of ischemia/reperfusion[11-13], the key is that in reperfusion models blood flow is restored after a relatively short duration of ischemia, generally from 15 min to an hour which is not the case in permanent CAO.

For these reasons, we investigated here if α_{1A}-AR overexpression also limits cardiac remodeling after chronic myocardial infarction resulting from permanent CAO. Specifically, we examined the effects of permanent CAO on the development of remodeling and heart failure in a rat model with 40-fold cardiomyocyte overexpression of the α_{1A}-AR. Our hypothesis was that if we observed protection from remodelling after 4 weeks of permanent CAO in the TG rats, then

there must have been some sustained blood flow to the ischemic myocardium (See Figure 1). Indeed, we did observe that the transgenic (TG) α_{1A}-AR rat heart was protected from remodeling after permanent CAO, and since, unlike the dog and hamster[14], the rat has few preformed collateral vessels, we also examined the extent to which protection against remodeling is due to neo-angiogenesis. Accordingly, we measured coronary blood flow to the ischemic zone and quantified the viable myocardium within this zone, as well as the development of newly formed coronary vessels. Since overexpression of the α_{1A}-AR was restricted to cardiomyocytes, we then determined the mechanism underlying neo-angiogenesis in the TG hearts. Given that microarray studies, verified by qPCR, identified vascular endothelial growth factor (VEGF-A) as the sole angiogenesis gene upregulated in TG myocytes, we focused on this factor and also examined MEK/ERK signalling, which has been found to be activated with α_{1A}-AR stimulation[15-17] and to induce VEGF-A mediated angiogenesis[18].

RESULTS

Attenuated cardiac remodeling in transgenic rats after chronic MI

At 4 weeks post MI, compared to NTLs, TG rats showed significantly less fibrotic tissue deposition in the areas adjacent (25% less) and remote (33% less) to the ischemic zone (Fig.2A). Compared to baseline, the amount of cellular hypertrophy post MI was significantly less in the TG group in both the adjacent (573±14mm^2) and remote zones (485±34mm^2) *vs*. those seen in the NTLs (652±24mm^2 in adjacent zone and 582±18mm^2 in remote zone, $p<0.05$, Fig.2B). Consistent with these findings, the ratio of left ventricular (LV) weight to tibial length was reduced by 16% in TG rats compared to NTLs (Fig.2C, $p<0.05$), the ratio of lung weight to tibial length was also lower in TGs (44±1.1mg/mm) than in NTLs (52±2.2mg/mm, $p<0.05$), which supports decreased post MI remodeling in the TG hearts. Scar size, measured by fibrotic tissue quantification within the ischemic zone and expressed as the percentage of the whole myocardial area, was markedly less in TG rats (19±1.1%) compared to NTLs (25±2.0%, $p<0.05$, Fig.2E). This reduction in infarct size was accompanied by increased viable tissue within the ischemic zone in TGs (12±1.0% vs. 7.9±1.2% in NTLs, $p<0.05$, Fig.2D, E). LV ejection fraction fell to 38% in NTL, but was significantly preserved at 63% in TG compared to baseline(Table 1). The low LV ejection fraction in NTLs 4 wks after CAO (39%) is at a level found in other studies of rats with heart failure; the latter evident in this study by the increase in LV weight/tibial length and lung weight/tibial length in NTLs. Survival, by chi square analysis(?), was significantly enhanced in TG vs NTL rats (Fig. 2F)?.

Increased angiogenesis in transgenic rats with upregulation of VEGF-A

Capillary density was increased by 21% in the zone adjacent to the infarct in TGs compared to NTLs at 4- 6 weeks after MI, $p<0.05$, (Fig. 3C). This was consistent with the finding of more viable tissue within the ischemic zone in TGs (Fig 2E), secondary to collateral blood flow through angiogenesis.

Myocardial blood flow was studied with microspheres injected at 20 min, 1 day, 1wk, 2wk and 4wk post-MI. Over the 4wk period of permanent CAO, both groups showed a gradual recovery of blood flow within the ischemic zone. However, the rate of recovery was significantly faster and greater in TG rats compared to their NTLs (Fig.3A). At 4wk post-MI, the blood flow in the central ischemic, adjacent and remote zones was consistently higher in TG group compared to NTL rats ($p<0.05$, Fig. 3B).

Microarray analysis revealed 6 upregulated angiogenesis-related genes in cardiomyocytes of TG rats. Amongst the six genes validated by qPCR , only VEGF-A mRNA was significantly increased compared to NTLs (Fig. 3D). In addition, we found a 3-fold increase in VEGF-A protein levels in TG mouse cardiomyocytes compared with NTLs ($p<0.05$) (Fig 4A).

Activation of the α_{1A}-AR in cardiomyocytes induced angiogenesis via a paracrine mechanism

In isolated mouse cardiomyocytes, VEGF-A expression was found to be upregulated at both the protein and mRNA levels (Fig. 4A, B). VEGF-A levels also increased significantly after stimulation of NTL cardiomyocytes with α_{1A}-AR agonist, A61603 (25nM) (Fig. 4A, B). A61603 mediated upregulation of VEGF-A mRNA was abolished by pre-treatment of cardiomyocytes with the α_{1A}-AR antagonist, prazosin (1μM) (Fig. 4B), indicating that the upregulation of VEGF-A is mediated by the α_{1A}-AR.

To evaluate the underlying cellular mechanism of the α_{1A}-AR-mediated angiogenic effect, we employed a Matrigel culture system using HUVECs. Tubule formation was increased after treating HUVECs with conditioned medium collected either from cultured TG cardiomyocytes, or from A61603-treated NTL cardiomyocytes, but was completely abolished with pre- treatment with an anti-VEGF-A antibody (Fig. 4C) but not by pre-treatment with control IgG (data not shown). This suggests that the growth factor, VEGF-A, is required for endothelial cell growth and organization into tubules, after its release from cardiomyocytes upon activation or with overexpression of the α_{1A}-AR. Thus, the α_{1A}-AR appears to program an angiogenic response

within the myocardium that enhances endothelial cell growth and organization through a paracrine mechanism involving heterocellular signalling.

Diminished myocardial remodeling in TG rats was abolished by inhibition of the MEK-VEGF-A pathway

U0126, a MEK inhibitor, or SU5416, a VEGF receptor inhibitor, was administered throughout the 4wk post MI period in both NTL and TG rats. Table 1 compares the effects of the MEK and VEGF receptor inhibitors in NTL and TG rats with that observed in NTL and TG rats given only vehicle. At 4wk post MI, the preserved cardiac function and attenuated remodeling observed in vehicle-treated TG rats, as compared to their vehicle-treated NTLs, were abolished after treatment with either inhibitor. Thus, with inhibitor treatment, cardiac function, reflected by ejection fraction, LV +dP/dt, and LV wall stress were now similar in the TG animals and their NTLs (Table 2). Also, scar size was not significantly different between U0126- or SU5416-treated NTL and TG hearts at 4 wk post MI (Fig. 5A). Compared to vehicle treated TG hearts post MI, TG hearts treated with U0126 or SU5416 did not show an increase in myocardial blood flow, suggesting that treatment with either inhibitor caused regression of collateral vessels (Fig. 5B). Capillary density within the adjacent zone was no longer increased in TG rats treated with either inhibitor but was similar among the inhibitor-treated NTL and TG groups (Fig. 5C). In A61603 treated NTL cardiomyocytes, pre-treatment with U0126 markedly reduced VEGF-A mRNA and protein levels (Fig. 5D).

DISCUSSION

In the present investigation, we demonstrated that overexpression of α_{1A}-AR in cardiomyocytes protected the heart from the adverse effects of remodeling and heart failure that occurs after permanent CAO. In the rat model of heart failure induced by permanent CAO, others have reported that LV ejection fraction falls to levels of 40-45% and heart failure develops[19-21]. These values are similar to those we observed with CAO of NTLs in the present study, where LV ejection fraction fell to 38%, 4 wks after permanent CAO. Moreover, LV function was better preserved and remodeling after CAO, in terms of fibrosis, myocyte hypertrophy, LV weight/tibial length and lung weight/tibial length and scar size, was less in the TG rats. Whereas α_{1A}-AR stimulation has been proposed previously for the treatment of heart failure on the basis of its increased inotropic properties, and because α_1-AR blockade exerts an adverse effect in heart failure[1-3], the results of the current investigation provide an additional novel mechanism that confers α_{1A}-AR mediated protection against the remodeling and the

development of heart failure that occurs after permanent CAO, i.e., α_{1A} receptor-stimulated angiogenesis.

With complete CAO modulators of apoptosis or preconditioning are without effect given the lack of any blood flow to maintain tissue viability, we concluded that the chronic protection observed I the TG rats must be due to the induction of neo-angiogenesis, which provides collateral blood flow to the central ischemic and adjacent zones in the face of permanent CAO. This restored blood flow to the ischemic myocardium, resulting in less cell death with more viable tissue within the ischemic zone, and within the central infarct area. It is important to appreciate that additional salvage of myocardium can come later in the process, due to the positive effects of remodeling, resulting in reduced LV wall stress and myocardial oxygen demand.

To our knowledge, this is the first report of the α_{1A}-AR being linked to angiogenesis. Although norepinephrine was found to induce cardiomyocyte VEGF-A expression and angiogenesis[22], the adrenergic receptor involved was not determined. There is evidence that the β-AR can stimulate angiogenesis[24], however, our studies indicate that, in the heart, the α_{1A}-AR/MEK/VEGF-A pathway appears to be the mediator of angiogenesis via heterocellular myocyte/endothelial cell signaling. Elucidation of this pathway was greatly aided by the availability of the TG rat model, which is more amenable to detailed physiological investigations than the mouse.

Involvement of the α_{1A}-AR/MEK/VEGF-A pathway in neo-angiogenesis is evident from the following observations. Firstly, we demonstrated that the TG rats with α_{1A}-AR overexpression had preserved cardiac function with higher ejection fraction and lower LV wall stress than NTLs (Table 1). TG rats had similar LV systolic pressure with smaller LV chambers and significantly greater wall thickness at systole and their LV systolic wall stress was markedly lower than in the NTLs (Table 1). Reduced wall stress, *per se*, could have contributed to the cardioprotection, since it reduces myocardial oxygen requirements. However, as noted above, this by itself, in the absence of some preservation of blood supply, is not sufficient to protect the heart subjected to 4 weeks of permanent CAO. Secondly, using histological staining, scar size, measured as the percentage of the total myocardium, was markedly smaller in TG hearts and was accompanied by more viable tissue and capillaries within the scar and less fibrosis in ischemic and adjacent zones, suggesting that the mechanism of protection involved neo-angiogenesis. The mechanisms mediating adjacent and remote remodeling are complex and likely different. The adjacent zone, which also suffers from some myocardial ischemia is influenced positively by

angiogenesis and improved myocardial perfusion. This is not the case for the remote zone, which never experiences myocardial ischemia. The improvement in the remote zone in the TG rats is due more to the positive effects of decreased remodeling and reduced LV wall stress and myocardial oxygen demand, which protects against further myocyte necrosis and secondary myocyte hypertrophy and fibrosis.

Proposed differential regulation of α-ARs in endothelial cells and cardiomyocytes remains a controversial issue[23, 24]. Some studies have implied that alpha receptors on endothelial cells may induce vasodilation[23, 24], where others have suggested vasoconstriction[25, 26]. Studies have found different results regarding which subtypes of alpha receptors are predominant[23, 24], and whether there are differences between regulation of RV vs. LV, or normal vs. heart failure[27].We found that overexpression of the α_{1A}-AR specifically in cardiomyocytes induced angiogenesis both *in vivo* and *in vitro* and that the improved myocardial blood flow led to preserved function of the heart following chronic CAO. Previous studies on α_{1A}-AR-induced angiogenesis have been controversial; it was shown that inhibition of endothelial α_1-ARs promoted neo-angiogenesis *in vitro* and in a hindlimb ischemia model[28], while in another study involving systemic, chronic administration of an α_1-AR antibody significantly reduced vascular area with decreased blood flow[29]. To our knowledge, there is no extant evidence showing that overexpression of any alpha receptor subtype in the heart can induce angiogenesis, and there are relatively few myocyte specific TG models for any gene that have been shown to protect against permanent CAO by inducing angiogenesis, unless that model also affected angiogenesis directly[30], or through a pathway that induced angiogenesis, e.g., elaboration of an associated growth factor[31].

We next determined the angiogenic factor that mediates the increase in blood flow and in capillary density in the α_{1A}-AR TG rat model with permanent CAO. Microarray analysis revealed enhanced expression of 6 angiogenic growth factor genes in TG vs NTL cardiomyocytes. However, only enhanced VEGF-A expression in TG cardiomyocytes could be verified by qPCR (Fig. 3C). VEGF-A promotes endothelial proliferation and migration resulting in tubule formation[32-34], and as a proangiogenic factor, is known to be activated by hypoxia and has an important role in angiogenesis and in reducing hypoxic cellular damage[33, 35].

We postulated that the mechanism by which angiogenesis was induced in the current myocyte-specific TG model was through a crosstalk between the cardiomyocytes and endothelial cells, also known as heterocellular signalling[36-39]. Whereas heterocellular signalling has been described between several cell types[36-40], the current investigation focused on crosstalk between cardiomyocytes overexpressing the α_{1A}-AR and endothelial cells to induce angiogenesis. This paracrine mechanism appears to involve transmission of a signal from one

cell type to an adjacent cell via the elaboration by the former of a secreted factor - in this case VEGF-A secretion from cardiomyocytes acting on endothelial cells and/or vascular smooth muscle cells, to induce angiogenesis as a result of their proliferation and migration[30, 36, 38, 41, 42]. To further confirm the link between α_{1A}-AR stimulation and VEGF-A secretion, we examined the effects of α_{1A}-AR agonist stimulation, using A61603[43], directly on cultured NTL mouse cardiomyocytes. We elected to use cardiomyocytes isolated from mice instead of rats for the mechanistic studies because endogenous α_{1A}-AR expression in mice is several fold less than rats, and is more akin to that in humans[3]. Treatment with A61603 enhanced VEGF-A expression at both the mRNA and protein levels; effects blocked by the non-specific α_1-AR antagonist, prazosin (Fig 4B). In addition, conditioned medium from cultured TG myocytes or from A61603 treated NTL myocytes induced vascular tubule formation by HUVECs (which do no express α_{1A}-ARs) significantly more than conditioned medium obtained from cultured untreated NTL cardiomyocytes. These findings support our concept of heterocellular signaling between the α_{1A}-AR expressing cardiomyocytes and endothelial cells. In keeping with this notion, enhanced myocardial VEGF-A production, observed in response to hypertrophic or ischemic signals, has been shown to activate endothelial cells and to induce neovascularization[41, 42].

The MEK/ERK pathway has been found to be upregulated in hearts with cardiomyocyte-specific α_{1A}-AR overexpression[15, 16], and is also activated with *in vitro* receptor stimulation of cardiomyocytes expressing native levels of the α_{1A}-AR[44]. In the current study, inhibition of the MEK pathway with U0126 abolished the attenuated cardiac remodeling observed after MI in TG rats, by not only increasing LV wall stress and decreasing LV contractility, but also by decreasing capillary density. In agreement with our findings, previous studies have suggested that MEK/ERK signaling is essential for VEGF-regulated endothelial cell proliferation[18, 45, 46]. The MEK/ERK pathway has also been shown to mediate preconditioning in the CAO/reperfusion model[11]. However, as noted above, the molecular mechanism is not effective in the absence of flow.

In summary, the results of this investigation suggest a novel therapeutic target for improved clinical outcomes by limiting post MI loss of myocardium and, hence, remodeling and heart failure as a result of α_{1A}-AR -MEK/VEGF-A-mediated angiogenesis; an effect that also protects the function of marginally ischemic myocardium adjacent to the infarct. Protection against remodelling (less fibrosis and myocyte hypertrophy) also protects the non-ischemic zone after chronic CAO, a mechanism, unlike the adjacent zone, not dependent upon increased blood flow. Another novel finding was that overexpression of cardiomyocyte specific α_{1A}-AR was

able to induce angiogenesis, a mechanism that might be available for other myocyte proteins, and might promote a new line of research in the angiogenesis field. Although α_{1A}-AR stimulation has been proposed as a potential treatment for heart failure based on its ability to augment inotropy[1], this is the first time that the α_{1A}-AR has been linked to the enhanced expression of a potent proangiogenic factor, VEGF-A; a factor that also induces neo-angiogenesis in response to ischemia. This mechanism improving perfusion to ischemic myocardium changes the paradigm explaining α_{1A}-AR's salutary action in heart failure, from simply increasing inotropy, which also increases myocardial oxygen consumption, to increasing inotropy while improving perfusion, which protects myocardial oxygen consumption.

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SIGNIFICANCE

This investigation demonstrates at several levels a novel mechanism for α_{1A}-AR mediated protection from remodelling and heart failure following permanent CAO, i.e., angiogenesis. First, stimulation of the α_{1A}-AR in cardiomyocytes has not been demonstrated previously to induce angiogenesis. Moreover, the fact that this occurs through heterocellular signalling from myocytes to coronary vessels will open new areas of investigation into the mechanisms of myo-vascular coupling, and potentially will lead to new therapeutic modalities. This is not only significant at the basic science level, but also from a clinical perspective, since cardiomyocyte α_{1A}-AR stimulation has been proposed for the treatment of heart failure based on its action to increase myocardial contractility. Given the current findings, there is perhaps a more important reason to support α_{1A}-AR stimulation for treatment of heart failure, particularly when it is of myocardial ischemic etiology, i.e., improvement of myocardial blood flow through angiogenesis.

Table 1

Cardiac Function at Baseline and at 4 Weeks after MI

	Baseline	4wk MI
Heart Rate (beat/min)		
NTL	387±6.3	354±13
TG	358±11	329±7.6
Mean Arterial Pressure (mmHg)		
NTL	112±14	113±12
TG	108±8.9	111±6.2
LV Systolic Pressure (mmHg)		
NTL	143±3.9	143±3.9
TG	135±5.0	142±9.1
LV Ejection Fraction (%)		
NTL	71±0.5	38±1.1
TG	87±1.2*	63±1.8*
LV End-diastolic Diameter (mm)		
NTL	6.9±0.3	9.6±0.3
TG	6.7±0.3	7.8±0.1*
LV Systolic Wall Stress		
NTL	36±6.3	185±21
TG	16±3.5*	94±9.3*

n=4-5 at baseline, n=8-9 at 4wk MI, *p<0.05 vs. NTL

Table 2

Cardiac Function at 4 weeks after Myocardial Infarction Treated with Vehicle, U0126 or SU5416

	NTL	TG
Heart Rate (beat/min)		
Vehicle	397±12	333±13*
U0126	409±24	350±11*
SU5416	425±31	397±18
Mean Arterial Pressure (mmHg)		
Vehicle	89±5.5	100±3.1
U0126	72±3.3	80±2.2*
SU5416	71±4.2	74±2.7
LV Systolic Pressure (mmHg)		
Vehicle	102±5.6	112±3.2
U0126	87±3.7	94±3.0
SU5416	91±5.2	88±3.3
LV Ejection Fraction (%)		
Vehicle	39±2.3	55±1.1*
U0126	37±0.9	40±1.8
SU5416	33±2.1	27±1.0
LV End-diastolic Diameter (mm)		
Vehicle	10±0.4	8.6±0.4*
U0126	11±0.2	11±0.5
SU5416	9.7±0.3	9.5±0.6
LV Systolic Wall Stress (dynes/cm^2)		
Vehicle	193±22	137±11*
U0126	218±9.3	221±12
SU5416	229±4.2	236±7.6

n=4, *p<0.05 vs. NTL

Fig. 1 (A) Mechanism for reduced remodeling after chronic myocardial infarction in α_{1A}-AR TG rats. Angiogenesis induced through α_{1A}-AR -MEK/VEGF-A pathway, provides blood flow to ischemic zone, despite permanent coronary artery occlusion, to allow cardiomyocyte survival, reduce remodeling and preserve cardiac function, which was presented with preserved (B) LV ejection fraction and (C) wall stress. Results are expressed as the mean ± SEM. n=4-5/group; $p<0.05$ vs. NTLs.

Fig. 2 Responses to 4 wk of permanent CAO in TG rats (open bars) and their NTLs (closed bars). (A) Fibrosis, quantified using picro-sirius red staining, was decreased in TG. (B) Myocyte size, determined using WGA fluorescent staining, was decreased in the zones bordering and remote to the ischemic area. (C) Left ventricular weight (LV) to tibial length (TL) ratio was decreased in TG rats. (D) Trichrome staining of LV rings (above) and transverse sections (below) of both NTL and TG hearts after chronic myocardial infarction demonstrates reduced scar size and fibrosis in the TG heart, as well as evidence of viable myocardium within the scar. (E) Scar size, presented as the percentage of scar size to LV surface area, and the amount of viable myocardium within the ischemic zone, were decreased in TG vs. NTL hearts. (F) Survival rate of 4 week MI, using Chi square analysis. Survival was significantly greater in the TG. Results are expressed as the mean ± SEM. n=6-8/group; *$p<0.05$ vs. NTL.

Fig. 3 (A) Coronary blood flow in the ischemic zone at 20min, 1 day, 1 week, 2 weeks and 4 weeks after permanent CAO, showing that recovery of flow was significantly faster and of greater magnitude in TG vs. NTL rat hearts at the times indicated (n=4-6, $p<0.05$) . (B) Capillary density was increased in TG hearts at 4 wk post CAO in the ischemic and adjacent zones. (C) Upregulation of angiogenic genes expressed in TG and NTL cardiomyocytes was determined by microarray analysis. The mRNA expression was further verified using qPCR. The significant increase in VEGF-A expression in TG hearts was verified. Results are expressed as the mean ± SEM. n=4-8; *$p<0.05$ vs. NTL. VEGF-A, vascular endothelial growth factor-A; Hbegf, Heparin-

binding EGF-like growth factor; cTGF, connective tissue growth factor; FGF2, Fibroblast growth factor-2; Angpt-2, angiopoietin 2; FGF6, fibroblast growth factor 6.

Fig. 4 (A) Protein expression of VEGF-A was increased in cardiomyocytes isolated from TG vs. NTL mouse hearts, and increased to similar levels with A61603 treatment of NTL cardiomyocytes. (B) mRNA levels of VEGF-A are expressed as relative to the internal control, HPRT (hypoxanthine-guanine phosphoribosyltransferase). Compared to untreated NTL mouse cardiomyocytes, VEGF-A mRNA was increased in both untreated TG and 25nM A61603 treated cardiomyocytes in NTL. 1μM Prazosin blocked these increases in both NTL and TG VEGF-A mRNA levels. (C) HUVECs were cultured with conditioned medium collected from NTL, TG, A61603 treated NTL or anti-VEGF-A antibody treated NTL cardiomyocytes. Tubule formation, presented as fold change from unconditioned medium, was increased in HUVECs cultured with conditioned medium from TG or A61603-treated NTL cardiomyocytes, compared to medium from vehicle or 2μg/ml anti-VEGF-A antibody treated NTL cardiomyocytes. (D) Representative pictures of tubule formation in HUVECs treated with conditioned medium collected from vehicle or A61603 or VEGF antibody (Ab) treated NTL mouse myocytes. Results are expressed as the mean ± SEM. n=3-6; *$p<0.05$ vs. NTL.

Fig. 5 Responses of NTL and TG rats to 4 wk of CAO and treatment with vehicle (10% DMSO), the MEK inhibitor, U0126 (400μg/kg/day), or the VEGF receptor inhibitor, SU5416 (25mg/kg/day). (A) Scar size was significantly smaller in the vehicle-treated TG vs. NTL hearts; a difference that was no longer observed after inhibitor treatment. (B) Coronary blood flow in the ischemic zone after 4 wk of CAO was significantly higher in TG vs. NTL hearts, and the increase in the TG hearts was abolished with either U0126 or SU5416 treatment for 4 wk. (C) Capillary density was increased in the adjacent zone of TG compared with NTL hearts after 4 wk of CAO, and this increase was abolished with either U0126 or SU5416 treatment for 4 wk. (D) In NTL cardiomyocytes, A61603 increased VEGF-A mRNA and protein levels, and these increases were prevented when the α_{1A}-AR agonist was combined with U0126. Results are expressed as the mean ± SEM. n=4-6; *$p<0.05$ vs. NTL + vehicle; $^{\dagger}p<0.05$ vs. TG + Vehicle; $^{\ddagger}p<0.05$ vs. A61603 treated NTL cardiomyocytes.