

Activin A Levels Are Associated With Abnormal Glucose Regulation in Patients With Myocardial Infarction

Potential Counteracting Effects of Activin A on Inflammation

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OBJECTIVE—On the basis of the role of activin A in inflammation, atherogenesis, and glucose homeostasis, we investigated whether activin A could be related to glucometabolic abnormalities in patients with acute myocardial infarction (MI).

RESEARCH DESIGN AND METHODS—Activin A measurement and oral glucose tolerance tests (OGTTs) were performed in patients ($n = 115$) with acute MI, without previously known diabetes, and repeated after 3 months. Release of activin A and potential anti-inflammatory effects of activin A were measured in human endothelial cells. Activin A effects on insulin secretion and inflammation were tested in human pancreatic islet cells.

RESULTS—1) In patients with acute MI, serum levels of activin A were significantly higher in those with abnormal glucose regulation (AGR) compared with those with normal glucose regulation. Activin A levels were associated with the presence of AGR 3 months later (adjusted odds ratio 5.1 [95% CI 1.73–15.17], $P = 0.003$). 2) In endothelial cells, glucose enhanced the release of activin A, whereas activin A attenuated the release of interleukin (IL)-8 and enhanced the mRNA levels of the antioxidant metallothionein. 3) In islet cells, activin A attenuated the suppressive effect of inflammatory cytokines on insulin release, counteracted the ability of these inflammatory cytokines to induce mRNA expression of IL-8, and induced the expression of transforming growth factor- β .

CONCLUSIONS—We found a significant association between activin A and newly detected AGR in patients with acute MI. Our in vitro findings suggest that this association represents a counteracting mechanism to protect against inflammation, hyperglycemia, and oxidative stress. *Diabetes* 60:1544–1551, 2011

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Type 2 diabetes is a chronic disease with rapidly increasing prevalence, and patients with type 2 diabetes have an increased risk of developing coronary artery disease (CAD) (1,2). The coexistence of type 2 diabetes and CAD is considerable, and type 2 diabetes has been rated as an equivalent of CAD. In contrast, many patients with established CAD have type 2 diabetes or its pre-states (3). Thus, a high prevalence of impaired glucose tolerance (IGT) and unknown type 2 diabetes has been reported in patients with CAD with no previous diagnosis of diabetes (4–6). Abnormal glucose regulation (AGR) (impaired fasting glucose [IFG], IGT, or type 2 diabetes) is approximately twice as common among patients with myocardial infarction (MI) as in population-based controls (7), and the presence of AGR is a strong risk factor for new cardiovascular events after acute MI (3,8,9).

The association between cardiovascular disease and hyperglycemia may be explained by an accumulation of cardiovascular risk factors associated with the metabolic syndrome in patients with AGR (10). It may, however, also relate to mechanisms triggered by hyperglycemia and insulin resistance leading to cardiovascular damage before the onset of overt diabetes (7,11). Moreover, experimental and clinical data have illuminated a role of inflammation in atherogenesis, and it has been suggested that atherosclerosis, type 2 diabetes, and the metabolic syndrome are multifactorial diseases characterized by chronic inflammation (12). However, the reasons for the association between AGR and atherosclerotic disorders are not fully understood.

Activin A, a member of the transforming growth factor (TGF)- β superfamily (13), has been recognized as a multifunctional cytokine with roles in regulation of wound repair, cell differentiation, and inflammation, and growing evidence implicates a role for activin A in inflammatory disorders potentially mediating both inflammatory and anti-inflammatory effects (14). Activin A also has been suggested to be important for glucose homeostasis, at least partly through direct stimulatory effects on pancreatic β -cells (15). However, reports on activin A levels in patients with type 2 diabetes are few and include a small number of patients (16,17). Furthermore, there are only a few reports on activin A levels during acute coronary syndrome (ACS) and no reports on patients with ST-elevation MI (STEMI) exclusively. Moreover, no data exist on the association between activin A and AGR in patients with CAD.

On the basis of the role of activin A in inflammation, atherogenesis, and glucose homeostasis, we investigated whether activin A could be related to glucose abnormalities associated with STEMI, potentially representing a counteracting mechanism in response to AGR. This hypothesis was investigated by different approaches, including studies in a well-characterized population with STEMI and experimental studies in endothelial cells and pancreatic β -cells.

RESEARCH DESIGN AND METHODS

Study population. A total of 115 patients with a primary percutaneous coronary intervention (PCI)-treated STEMI, originally included in a prospective observational cohort study on the prevalence of AGR classified by an oral glucose tolerance test (OGTT) (5), were investigated for a possible association between circulating levels of activin A and AGR. In brief, patients with a primary PCI-treated STEMI were included if they were stable, without chest pain or nausea, and aged <85 years. Patients with previously known type 2 diabetes, persistent hyperglycemia (admission plasma glucose >11 mmol/L and a fasting capillary glucose level >8 mmol/L), or renal failure (creatinine >200 μ mol/L) were excluded. Only 17 patients had increased creatinine levels (>90 μ mol/L). Seven patients had comorbidity that could potentially influence activin A levels: cancer ($n = 1$), polymyalgia rheumatica ($n = 2$), vasculitis ($n = 1$), rheumatoid arthritis ($n = 1$), and arthritis urica ($n = 2$).

The patients in this substudy were randomly selected from the total study population, and the baseline parameters (Table 1) did not differ from those of the total population. All patients met at a 3-month follow-up, which included a clinical examination, additional fasting blood sampling, and a repeated OGTT. Activin A was measured in blood samples collected the first morning after a primary PCI-treated STEMI and at 3 months. For activin A analyses, venous blood was drawn into pyrogen-free blood collection tubes without any anticoagulant, and serum was allowed to clot before centrifugation (2,500g for 10 min). All samples were stored at -80°C and thawed only once. In addition, blood samples for activin A analyses were collected in 45 of the patients before and 2 h after a standardized OGTT at the 3-month visit. For comparison, activin A levels also were measured in 72 patients with stable CAD (61 [53, 69] years of age, 61 male [85%]). The diagnosis of CAD was confirmed in these patients by coronary angiography showing at least 1-vessel disease.

TABLE 1
Baseline characteristics of 115 patients with an acute PCI-treated STEMI

	Patients
Age (years)	59 (52, 68)
Male, n (%)	97 (84)
Previous disorder:	
MI, n (%)	5 (4.3)
Angina pectoris, n (%)	3 (2.6)
Hypertension (treated), n (%)	23 (20.0)
Hyperlipidemia (treated), n (%)	9 (7.8)
Status at baseline:	
Current smoker, n (%)	49 (42.6)
cTnTmax ($\mu\text{g/L}$)	5.0 (2.5, 9.7)
BMI (kg/m^2)	26 (24.3, 28.6)
Waist circumference (cm)	99 (94, 106)
Stent in culprit lesion, n (%)	109 (94.8)
Gp IIb/IIIa inhibitor-treated, n (%)	39 (33.9)
Single-coronary vessel disease, n (%)	73 (63.5)
Double-coronary vessel disease, n (%)	34 (29.6)
Triple-coronary vessel disease, n (%)	8 (7.0)
Time from symptoms to balloon (min)	239 (155, 370)
Medication at admission:	
Aspirin	10 (8.7)
β -Blockers	11 (9.6)
Lipid-lowering agents	7 (6.1)
ACE inhibitors	3 (2.6)
Angiotensin II receptor blockers	11 (9.6)

Data are presented as median values (25th, 75th percentiles) or proportions (%). cTnTmax, cTnT maximum; Gp, glycoprotein.

The regional ethics committee approved the study. All patients provided written and oral informed consent.

Definition of STEMI. STEMI was defined as the typical increase and decrease of troponin T with at least one value above the 99th percentile of the upper reference limit in patients with symptoms of ischemia and new ST-elevation at the J-point in two contiguous leads with the cutoff points of 0.2 mV in men or 0.15 mV in women in leads V2–V3 or 0.1 mV in other leads or new left bundle branch block (18).

OGTT. A standardized 75 g OGTT (plasma glucose measurements at 0 and 120 min) was performed after an overnight fast (19). The patients were classified glucometabolically according to the World Health Organization guidelines (20) into one of the following categories (glucose in millimoles/liter): 1) normal glucose tolerance, i.e., OGTT <6.1 (baseline) and <7.8 (2 h); 2) IFG, i.e., OGTT ≥ 6.1 <7.0 (baseline) and <7.8 (2 h); 3) IGT, i.e., OGTT <7.0 (baseline) and ≥ 7.8 <11.1 (2 h); 4) type 2 diabetes, i.e., OGTT ≥ 7.0 (baseline) or OGTT ≥ 11.1 (2 h). Patients with AGR were defined as the sum of patients with IFG, IGT, and type 2 diabetes (7).

Routine laboratory methods. Routine analyses, including plasma glucose and HbA_{1c}, were analyzed by use of conventional methods. Serum levels of cardiac-specific troponin T (cTnT) were measured by electrochemiluminescence technology for quantitative measurement (third-generation cTnT, Elecsys 2010; Roche Diagnostics GmbH, Mannheim, Germany). The lower detection limit of the assay is 0.01 $\mu\text{g/L}$, with a recommended diagnostic threshold of 0.03 $\mu\text{g/L}$. Insulin was measured by a competitive radioimmunoassay (Linco Research, Inc., St. Charles, MO). C-peptide was determined by Immulite 2000 (Diagnostic Product Corporation, Los Angeles, CA). Proinsulin and C-reactive protein (CRP) were measured by enzyme immunoassays (EIAs) from DRG Instruments (Munich, Germany).

EIA. Serum levels of activin A were measured by a precoated EIA (R&D Systems, Minneapolis, MI), whereas activin A in human umbilical vein endothelial cell (HUVEC) supernatants was measured by EIA using matched antibodies (R&D Systems) after activation with 3M Urea provided by the manufacturer (St. Paul, MN). Interleukin (IL)-8 was measured by EIA from R&D Systems. The inter- and intra-assay coefficients of variation were <10% for all EIAs.

Endothelial cell culture. HUVECs were obtained from umbilical cord veins by digestion with 0.1% collagenase A (Boehringer Mannheim, Mannheim, Germany) and cultured as described previously (21). HUVECs were stimulated with different concentrations of recombinant human activin A (R&D Systems), recombinant human IL-1 β (5 ng/mL, R&D Systems), or a combination thereof. In a separate set of experiment, HUVECs were cultured in different concentrations of glucose, insulin (Sigma-Aldrich, St. Louis, MO), or a combination thereof in a glucose-free Dulbecco's modified Eagle's medium containing glucogenic and ketogenic amino acids (Life Technologies, Invitrogen, Carlsbad, CA). At different time points, cell pellets and cell-free supernatants were collected and stored at -80°C . In all experiments, the vehicle of the stimulus was added as control.

Human islet isolation and culture. Following obtained consent from the organ donor registry or relatives, pancreata from deceased donors were transported from the donor hospital to the laboratory for islet isolation in Uppsala, Sweden. Islets were isolated according to the automated method, refined by the Nordic Network for Islet Transplantation (22). Within 2–5 days from isolation, aliquots of purified islet preparations were stimulated with activin A (100 ng/mL; R&D Systems), a mixture of inflammatory cytokines (IL-1 β [10 ng/mL], interferon- γ [IFN- γ , 50 ng/mL], and tumor necrosis factor- α [TNF- α , 10 ng/mL]; all from R&D Systems), or a combination thereof for 24 h in CMRL-1066 media (ICN Biomedicals, Costa Mesa, CA) with 10% ABO-identical serum. Cell pellets and supernatants were harvested and stored at -80°C until further analyses.

Glucose-stimulated insulin secretion in human islet cells. For static insulin secretion in response to a glucose challenger, 20 islets were handpicked, transferred into 24-transwell trays (Costar, Cambridge, MA), and preincubated in 1.67 mmol/L glucose at 37°C (5% CO₂) for 30 min. Thereafter, the islets were incubated for 1 h in 1.67 mmol/L glucose (basal), before additional incubation for 1 h in 20.0 mmol/L glucose (stimulated). Secreted insulin in the media was measured by EIA (Mercodia AB, Uppsala, Sweden). The capacity for insulin release was expressed as insulin stimulation index, calculated as the ratio of stimulated to basal insulin secretion.

Real-time quantitative RT-PCR. Total RNA was extracted by MagNa Pure LC RNA isolation kit III (Roche Diagnostics GmbH) and stored at -80°C . Primers for metallothionein, IL-1 β , IL-8/CXCL8, monocyte chemoattractant protein-1/CCL2, and TGF- β were designed using the Primer Express software, version 2.0 (Applied Biosystems, Foster City, CA). Primer sequences could be provided by request. Quantification of mRNA was performed using the ABI Prism 7500 (Applied Biosystems). Gene expression of the housekeeping gene β -actin and glyceraldehyde-3-phosphate dehydrogenase (Applied Biosystems) was used for normalization.

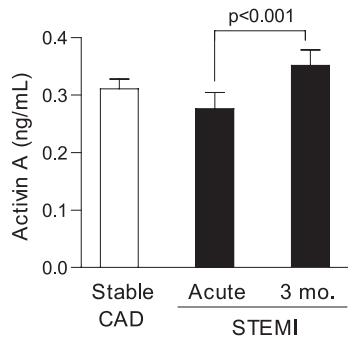


FIG. 1. Circulating activin A in patients with STEMI. Serum levels of activin A measured in patients with STEMI ($n = 115$) the first morning after primary PCI and in a stable phase after 3 months. Serum levels of activin A from patients ($n = 72$) with stable CAD are shown for comparison. Data are mean \pm SEM.

Statistics. Continuous variables are presented as median values with 25th to 75th percentiles or mean \pm SEM as appropriate. Categorical variables are presented as proportions if not otherwise stated. Nonparametric statistics were used throughout. Differences among groups were analyzed by Mann-Whitney test for continuous variables. The Mantel-Haenszel method was used to highlight potential effect modification by the Breslow-Day test of

heterogeneity and to quantify potential confounders (23). Continuous variables were categorized into quartiles, and the variables were dichotomized into high and low levels. A χ^2 for trend across the quartiles of a variable identified the cutoff point used. A logistic regression model, including a backward elimination procedure, was performed to adjust for potential confounders. Potential confounders (i.e., gender, age, current smoking, treated hypertension, treated hyperlipidemia, previous MI, multivessel disease, prior medication, time from symptoms to balloon, cTnT maximum, CRP, creatinine, BMI, cholesterol, and triglycerides) that were associated with activin A levels or AGR with a $P < 0.2$ were included in the model. $P < 0.05$ was considered statistically significant.

RESULTS

Study population. Baseline characteristics of the study population are shown in Table 1. The patients were relatively young, very few had a previous diagnosis of CAD, and a majority of the patients had single-vessel disease. The prevalence of AGR classified by an OGTT in-hospital and 3 months later was 44 and 23%, respectively.

Association between circulating activin A and clinical and glucometabolic variables. Serum levels of activin A measured acutely, within a median time of 16.5 h of a primary PCI-treated STEMI ($n = 115$), were 0.23 (0.17, 0.29) ng/mL, which increased after 3 months, comparable to activin A levels in a group of patients with stable CAD ($n = 72$) (Fig. 1).

TABLE 2

Clinical and biochemical variables in patients with acute STEMI related to circulating activin A

Variables	Activin A (<0.23 ng/mL)	Activin A (\geq 0.23 ng/mL)	P value
Measured in-hospital			
Age (years)	54 (50, 63)	62 (56, 73)	0.6
Male, n (%)	49 (86.0)	48 (82.8)	0.0001
Hypertension (treated), n (%)	7 (12.3)	16 (27.6)	0.04
Statin treatment, n (%)	7 (12.3)	0 (0)	0.06
Aspirin treatment, n (%)	3 (30)	7 (70)	0.2
ACE inhibitor/ARB treatment, n (%)	4 (29)	10 (72)	0.08
Current smoker, n (%)	26 (45.6)	23 (39.7)	0.5
BMI (kg/m^2)	26 (24.3, 28.7)	26 (24.3, 27.8)	0.96
Comorbidity, n (%)*	1 (1.8)	6 (10.3)	0.06
cTnTmax ($\mu\text{g}/\text{L}$)	4.7 (2.1, 8.2)	6.9 (3.3, 10.8)	0.10
Cholesterol (mmol/L)	5.2 (4.4, 6.0)	5.2 (4.7, 5.8)	0.7
Triglycerides (mmol/L)	1.3 (1.0, 1.7)	1.2 (0.9, 1.8)	0.5
CRP (mg/L)	10.7 (4.4, 27.3)	17.7 (7.8, 47.6)	0.047
Creatinine ($\mu\text{mol}/\text{L}^{-1}$)	70 (60, 82)	77 (66, 90)	0.06
Admission glucose (mmol/L)	6.5 (5.7, 7.7)	6.9 (6.4, 8.1)	0.035
Fasting glucose (mmol/L)	5.1 (4.8, 5.5)	5.5 (5.0, 6.3)	0.009
Two-hour glucose (mmol/L)	6.4 (5.6, 7.8)	7.6 (6.5, 8.9)	0.004
HbA _{1c} (%)	5.4 (5.2, 5.5)	5.6 (5.4, 5.8)	0.0003
Insulin (pM)	69 (50, 86)	75 (51, 110)	0.3
C-peptide (pM)	788 (622, 1,029)	1,034 (746, 1,212)	0.01
Proinsulin (pM)	7.3 (5.0, 9.3)	9.5 (5.8, 14.1)	0.051
IL-8 (pg/mL)	14.9 (13.7, 17.1)	15.3 (12.6, 18.3)	0.84
Measured at 3 months			
Fasting glucose (mmol/L)	4.9 (4.6, 5.4)	5.2 (4.9, 5.6)	0.007
Two-hour glucose (mmol/L)	5.4 (4.4, 6.6)	5.7 (4.8, 7.8)	0.16
HbA _{1c} (%)	5.5 (5.3, 5.7)	5.6 (5.4, 5.9)	0.007
Insulin (pM)	68 (46, 86)	72 (53, 98)	0.3
C-peptide (pM)	781 (603, 992)	853 (685, 1,192)	0.052
Proinsulin (pM)	6.3 (4.6, 8.1)	6.3 (4.8, 10.2)	0.5
CRP (mg/L)	2.02 (0.79, 2.96)	3.27 (1.58, 5.88)	0.02
BMI (kg/m^2)	26.1 (24.4, 28.7)	26.2 (23.8, 28.4)	0.6
Current smoker, n (%)	14 (24.6)	7 (12.1)	0.08
Cholesterol (mmol/L)	4.2 (4.0, 4.6)	4.0 (3.6, 4.6)	0.06
Triglycerides (mmol/L)	1.2 (0.9, 1.5)	1.2 (0.9, 1.5)	0.7

Data are presented as median values (25th, 75th percentiles) or proportions. Clinical and biochemical variables in patients with acute STEMI related to high or low activin A concentrations measured within 24 h after an acute PCI (median value as cutoff point). ARB, angiotensin II receptor; cTnTmax, cTnT maximum. *One patient with cancer and six patients with inflammatory diseases.

Patients with high levels (i.e., above median) of activin A at baseline were older, were more likely to have hypertension, had higher CRP and creatinine levels, and were more unlikely to use statins (Table 2).

Moreover, patients with high activin A levels had significantly more glucometabolic abnormalities (Table 2). Thus, patients with high activin A levels at baseline had higher glucose levels at admission, higher levels of glucose during the OGTT, and higher levels of HbA_{1c} and C-peptide in-hospital. After 3 months, these patients had higher fasting glucose levels and higher HbA_{1c} compared with those with low activin A levels (Table 2). In line with this, serum levels of activin A measured in-hospital were significantly higher in patients with abnormal compared with normal glucose regulation, both when classified by an OGTT in-hospital and after 3 months (Table 3). In addition, activin A levels measured after 3 months remained higher, although only borderline statistically significant, in the patients classified into AGR on the basis of the OGTT performed at the same time point (Table 3).

High levels of activin A at baseline are associated with AGR after 3 months. Activin A measured in-hospital was strongly associated with the presence of AGR classified by an OGTT at 3 months (Table 3). Thus, trend analysis for the presence of AGR across quartiles of activin A demonstrated a significant trend for the odds ratio (OR) of having AGR at 3 months with increasing activin A concentrations ($P = 0.012$), identifying a threshold of activin A at the median value (≥ 0.23 ng/mL, Fig. 2). In univariate analyses, high levels of activin A (≥ 0.23 ng/mL) measured in-hospital were associated with AGR classified 3 months after the acute STEMI with an OR of 5.9 (95% CI 2.04–17.10; $P = 0.004$). Activin A remained associated with AGR after adjustment for potential confounders, including creatinine levels and use of prior medication (see RESEARCH DESIGN AND METHODS), with an OR of 5.1 (95% CI 1.73–15.17, $P = 0.003$).

Patients with comorbidity ($n = 7$) (i.e., autoimmune diseases or cancer, Table 2) had significantly higher activin A levels at baseline (data not shown), but notably, the

TABLE 3
Activin A levels related to glucometabolic state in patients with STEMI

	NGR		AGR		<i>P</i> value
	(in-hospital) (<i>N</i> = 64)		(in-hospital) (<i>N</i> = 51)		
Activin A (ng/mL) (in-hospital)	0.212 (0.169, 0.261)		0.251 (0.182, 0.339)		0.023
	NGR		AGR		<i>P</i> value
	(at 3 months) (<i>N</i> = 89)		(at 3 months) (<i>N</i> = 26)		
Activin A (ng/mL) (in-hospital)	0.213 (0.168, 0.273)		0.261 (0.239, 0.334)		0.009
Activin A (ng/mL) (at 3 months)	0.290 (0.237, 0.367)		0.366 (0.286, 0.464)		0.052

Data are presented as median values (25th, 75th percentiles). Activin A measured in-hospital and at 3 months (in patients with STEMI) according to glucometabolic classification by an OGTT in-hospital and at 3 months. NGR, normal glucose regulation.

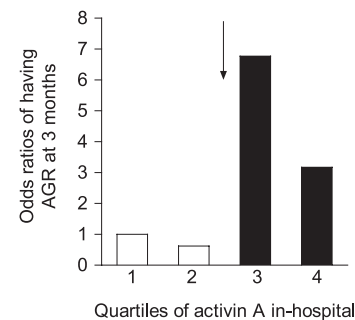


FIG. 2. Association between activin A and AGR. Activin A was measured in-hospital in patients with acute STEMI, classified 3 months later by an OGTT into AGR and normal glucose regulation. ORs of having AGR by quartiles of activin A are shown (P for trend; $P = 0.012$). The median value (arrow) was used as the cutoff for dichotomizing activin A into high and low levels.

significant association between activin A at baseline and the presence of AGR at 3 months was still present when patients with comorbidity were excluded ($n = 108$, OR 8.1 [95% CI 2.5–25.9], $P < 0.0001$).

High activin A levels are associated with high levels of glucose parameters during OGTT. In 45 patients, activin A levels were measured during OGTT at the 3-month follow-up. When the patients were classified according to activin A levels measured 2 h after oral glucose loading (above or below the 75th percentile, 0.302 ng/mL), those with high activin A levels had significantly higher levels of glucose, insulin, and C-peptide at the same time point than the other patients (Fig. 3).

Glucose induces activin A release in endothelial cells. The endothelium is a crucial target in both atherosclerosis and diabetes and an important cellular source of activin A. Therefore, to further elucidate the association between AGR and activin A, we examined the ability of glucose and insulin to promote activin A release in endothelial cells. When HUVECs were exposed to glucose, there was a dose-dependent increase in activin A release (Fig. 4A). The combined effect of insulin was more complex. Although insulin showed an enhancing effect on the glucose-stimulated activin A release at a concentration of 5 nmol/L, comparable to the insulin concentration seen during OGTT in those with high activin A levels, a biphasic pattern was seen at higher and potentially supraphysiologic insulin concentration (25 nmol/L), with enhancing effects at low

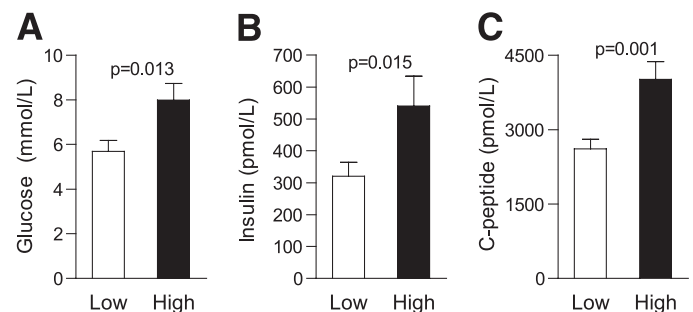


FIG. 3. Association between activin A levels and glucometabolic parameters during an OGTT. An OGTT was performed in stable patients ($n = 46$) 3 months after an acute STEMI. Glucose (A), insulin (B), and C-peptide (C) levels, measured 2 hours after glucose loading, in patients with high (≥ 75 th percentile, 0.302 ng/mL) and low (< 75 th percentile) activin A measured at the same time point. Data are mean \pm SEM.

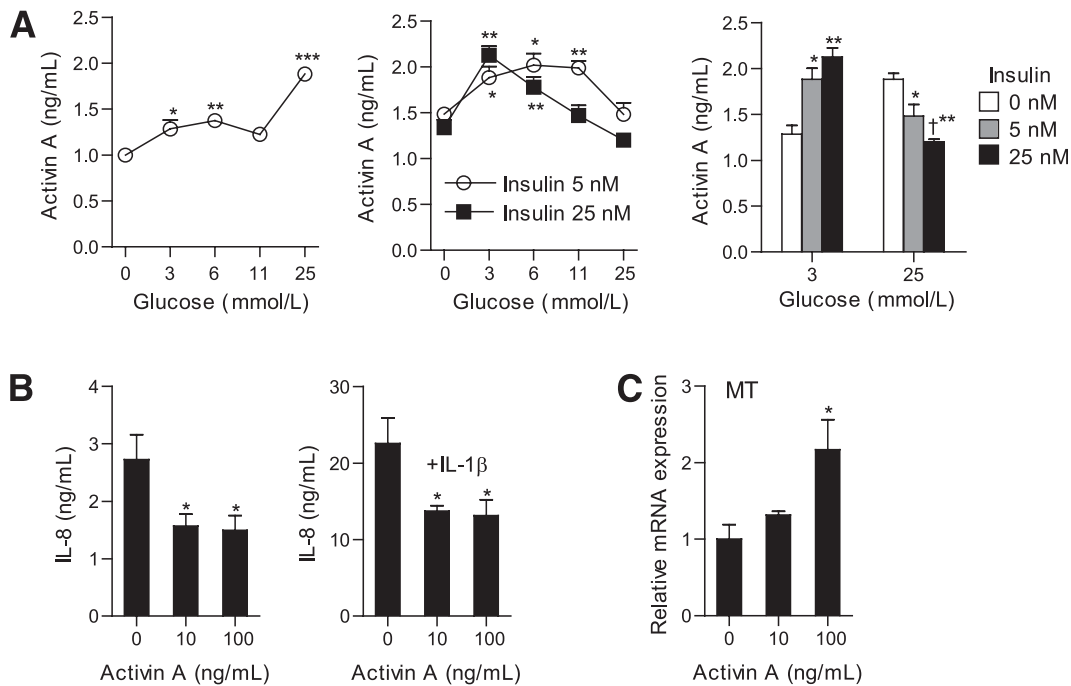


FIG. 4. Interaction between activin A and endothelial cells. **A:** The effect of various concentrations of glucose, insulin, or a combination thereof on activin A levels in HUVEC supernatants after culturing for 48 h as assessed by EIA measurements. Data are mean \pm SEM ($n = 4$). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. no glucose (two left panels). * $P < 0.05$ and ** $P < 0.01$ vs. no insulin. † $P < 0.05$ vs. 5 nmol/L insulin (right panel). The effect of different concentrations of activin A on the spontaneous and IL-1 β -stimulated (5 ng/mL) release of IL-8 (**B**) and the unstimulated expression of metallothionein (**C**) after culturing for 24 and 4 h, respectively. IL-8 was measured by EIA, and metallothionein was assessed by RT-PCR in relation to the control gene β -actin. Data are mean \pm SEM ($n = 5$). * $P < 0.05$ vs. unstimulated (or IL-1 β -stimulated) cells. MT, metallothionein.

glucose concentrations and suppressive effects at high glucose concentrations.

Anti-inflammatory and antioxidative effects of activin A in endothelial cells. On the basis of our previous findings with anti-inflammatory effects of activin A in peripheral blood mononuclear cells from patients with CAD (24), we next investigated whether activin A could have similar effects in endothelial cells, potentially mediating protective responses during AGR. Indeed, activin A attenuated the release of IL-8, a prototypical endothelial-derived chemokine, in both unstimulated and IL-1 β -stimulated cells after culturing for 24 h (Fig. 4B). In contrast, activin A enhanced the mRNA levels of metallothionein (Fig. 4C), an antioxidant enzyme that has been found to be protective in relation to oxidative stress-induced damage in diabetes (25).

Our in vitro findings may suggest an interaction between activin A and IL-8, but we found no significant correlations in patients with STEMI, either acutely or at the 3-month follow-up, between serum levels of IL-8 and activin A (data not shown). However, a positive correlation between IL-8 and activin A measured 2 h after glucose loading at 3 months was observed (0.32, $P = 0.03$).

Effects of activin A in human pancreatic islet cells. To further elucidate the effects of increased activin A levels in relation to AGR in patients with PCI-treated STEMI, we next examined the ability of activin A to modulate insulin secretion and inflammation in human islet cells. Inflammation, and in particular IL-1 β , has been implicated in the pathogenesis of β -cell dysfunction in relation to type 2 diabetes (26). Therefore, the cells were also costimulated with a combination of the inflammatory cytokines IL-1 β , TNF- α , and IFN- γ . Although activin A had no significant effect on the capacity for insulin release (i.e., insulin

stimulation index) on its own, it totally abolished the suppressive effect of the inflammatory cytokines on the insulin stimulation index (Fig. 5A). Although activin A had no effect on its own, it attenuated the enhancing effect of the inflammatory cytokines on mRNA expression of IL-8 and IL-1 β , but not of monocyte chemoattractant protein-1, in pancreatic islet cells (Fig. 5B). Finally, although activin A markedly increased mRNA levels of TGF- β on its own, it did not counteract the suppressive effect of the inflammatory “cocktail” on this anti-inflammatory cytokine (Fig. 5B).

DISCUSSION

The high prevalence of AGR in patients with CAD and no previous diagnosis of type 2 diabetes is an emerging problem in clinical medicine. The current study showed that serum levels of activin A are associated with AGR in patients with acute STEMI without known type 2 diabetes. The association between high activin A levels and high glucose levels was also seen during OGTT. Our in vitro studies suggest that activin A may exert anti-inflammatory and antioxidative effects in endothelium, as well as insulin-stimulating and anti-inflammatory effects in human islet cells. It is tempting to hypothesize that the increased activin A levels in relation to AGR could represent a counteracting mechanism to protect against hyperglycemia and hyperinsulinemia and their consequences.

There are a few previous reports on activin A levels during ACS showing increased, similar, or, as in the current study, lower levels of activin A in patients with ACS compared with stable CAD (24,27). The current study, however, is the first report on an association between serum activin A levels and AGR in patients with STEMI

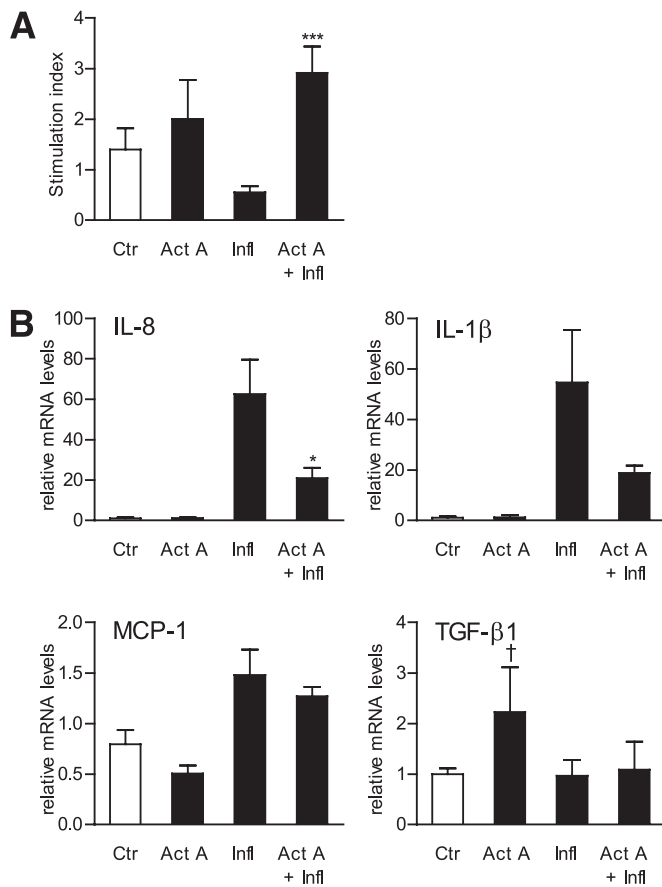


FIG. 5. Effects of activin A in human pancreatic islet cells. The effect of activin A (100 ng/mL), a mixture of inflammatory cytokines (IL-1 β [10 ng/mL], IFN- γ [50 ng/mL], and TNF- α [10 ng/mL]), or a combination thereof on the capacity for glucose-stimulated insulin release, expressed as insulin stimulation index (culture time 1 h after determination of basal insulin secretion, **A**), and on mRNA levels of IL-8, IL-1 β , monocyte chemoattractant protein-1, and TGF- β (culture time 24 h, **B**) in human pancreatic islet cells. mRNA was assessed by RT-PCR in relation to the control gene β -actin. Data are mean \pm SEM ($n = 4-6$). * $P < 0.05$ and *** $P < 0.001$ vs. inflammatory cytokines without activin A. † $P < 0.05$ vs. control cells (vehicle). Act A, activin A; Ctrl, control; Infl, inflammatory cytokines; MCP, monocyte chemoattractant protein.

without known type 2 diabetes. High activin A levels, measured the 1st day after a PCI-treated STEMI, were associated with a fivefold increase in the odds of being classified with AGR 3 months later. This association was also present when adjusting for covariates. Moreover, patients with high activin A levels during STEMI had significantly elevated levels of HbA_{1c} and C-peptide in-hospital, suggesting that the association between activin A and glucometabolic disturbances also may have been present before the acute MI.

The reason for the association between activin A levels and AGR is at present not clear, but our findings may suggest that glucose and insulin directly could induce activin A release. During OGTT, performed at the 3-month follow-up visit, there was a significant association between high activin A responses and high levels of glucose, insulin, and C-peptide. In addition, in endothelial cells, being an important cellular source of activin A (28), glucose induced a significant increase in activin A release. At an insulin concentration comparable to that seen during OGTT, insulin further enhanced activin A levels. Insulin has been shown to induce activin A production in macrophages,

potentially contributing to the anti-inflammatory effects of insulin during systemic inflammation (29). Our findings suggest that the association between activin A levels and AGR could reflect a direct stimulatory effect of glucose and insulin on activin A release, at least partly involving effects on endothelial cells.

Activin A has been shown to possess anti-inflammatory properties under certain circumstances. Activin A is rapidly induced during systemic inflammation (30) and has been found to antagonize IL-6-mediated effects within hepatocytes (31), and we have previously shown anti-inflammatory effects in preactivated peripheral blood mononuclear cells from patients with CAD (24). The endothelium is an important target for vascular complication in atherosclerosis and type 2 diabetes (32), and the current study showed that activin A attenuated the release of the proatherogenic chemokine IL-8 from endothelial cells and enhanced the expression of the antioxidative enzyme metallothionein. Metallothionein may protect heart and kidney against diabetes-induced pathophysiology (33) and has been related to protection against cardiac endoplasmic reticulum stress in diabetes (34). Although our findings will have to be confirmed in the in vivo situation, our data suggest that activin A has the potential to attenuate vascular inflammation and oxidative stress in disorders, such as atherosclerosis and type 2 diabetes. The current study found no correlation between activin A and IL-8 in serum. However, an interaction between activin A and IL-8 within the endothelial microenvironment may not necessarily be reflected in a correlation in serum levels, which reflect the contribution from many organ systems. However, our finding of a positive correlation between IL-8 and activin A during OGTT suggests some interaction between activin A and IL-8 during hyperglycemia also in vivo.

Several studies have demonstrated a role for TGF- β family cytokines in regulating pancreatic β -cell function (35). Activin A has been shown to be expressed in human pancreatic islets cells (36) and was found to act as an autocrine factor enhancing insulin secretion in the presence of glucose in the same cell type (15). Our results confirmed the proposed enhancing effect of activin A on insulin release and added new information to the possible protecting effects of activin A on pancreatic islet cells. Inflammation, in particular enhanced IL-1 activity, seems to be important in the pathogenesis of type 2 diabetes at least partly by inhibiting the function of pancreatic β -cells (26). The current study showed that activin A 1) abolished the suppressive effect of IL-1 β , in combination with TNF- α and IFN- γ , on the insulin stimulation index; 2) attenuated the inflammatory response of these inflammatory cytokines; and 3) induced the expression of the anti-inflammatory cytokine TGF- β in human pancreatic islet cells. TGF- β and activin A share common signal transduction pathways involving increased phosphorylation of Smads (35,37), and TGF- β has been shown to protect pancreatic β -cell function in mice models (38). If similar effects may be seen of activin A or if the anti-inflammatory effects of activin A are mediated through TGF- β activation would need to be clarified. However, the inability of activin A to counteract the suppressive effects of the inflammatory cytokines on TGF- β levels suggests that the counteracting effects of activin A on IL-8 and IL-1 β expression in cytokine-exposed pancreatic islet cells do not involve TGF- β .

Activin A is released during various inflammatory condition, potentially as a counteracting mechanism (30). It is possible that similar mechanisms could be operating as

a response to the glucometabolic disturbances during acute MI. Thus, activin A may be released as a consequence of hyperglycemia and low-grade hyperinsulinemia as a counteracting response to protect various compartments, such as endothelium and pancreatic β -cells against inflammation and oxidative stress, as well as other harmful consequences of these glucometabolic disturbances. The current study has certain limitations, such as a relatively low number of patients. Because we lack data on activin A clearance, we cannot exclude that the increased serum activin A levels, at least in part, are due to reduced clearance and not increased production of activin A. One might also argue that the concentrations of activin A used in the in vitro experiments (10 and 100 ng/mL) are not relevant to the in vivo situation (serum levels \sim 0.3 ng/mL). However, activin A concentrations in supernatants from endothelial cells that were exposed to glucose and insulin were 2–2.5 ng/mL, and it is not inconceivable that activin A concentrations may be even higher in endothelial cells within an inflammatory microenvironment, such as in patients with atherosclerotic disorders.

The current study demonstrated an association between circulating activin A and AGR in patients with acute STEMI without previously known diabetes. Our findings suggest that this association may reflect a direct interaction between insulin and glucose and activin A, potentially representing a counteracting mechanism to protect against inflammation and oxidative stress in these patients. Further studies are needed to clarify whether activin A could represent a therapeutic target in these overlapping disorders (i.e., atherosclerosis and type 2 diabetes).

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G.Ø.A. conceived, designed, and initiated the study (together with P.A.); recruited patients; performed statistical analyses; interpreted data; and drafted and revised the article (together with P.A.). T.U. conceived and designed the study; performed EIA, endothelial cell culture experiments, and statistical analyses; interpreted data; and revised the article. E.C.K. conceived and designed the study, recruited patients, performed OGTT and statistical analyses, interpreted data, and revised the article. H.S. conceived and designed the study, performed islet cell culture experiments and mRNA analyses, and revised the article. A.Y. conceived and designed the study, performed mRNA analyses, and revised the article. A.S. conceived and designed the study, performed islet cell culture experiments and mRNA analyses, and revised the article. C.S. conceived and designed the study; performed endothelial cell experiments, EIAs, and mRNA analyses; and revised the article. T.L. conceived and designed the study, performed endothelial cell experiments, and revised the article. K.O. conceived and designed the study, performed endothelial cell experiments, and revised the article. B.H. conceived and designed the study, interpreted data, and revised the article. I.S. conceived and designed the study, interpreted data, and revised the article. P.A. conceived, designed, and initiated the study (together with G.Ø.A.); interpreted data; and drafted and revised the article (together with G.Ø.A.).

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