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REFERENCES

1. Goff DC, Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2014;63:2935-59.
2. Conroy RM, Pyörälä K, Fitzgerald AP, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J* 2003;24:987-1003.
3. Nordestgaard BG, Chapman MJ, Ray K, et al. Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur Heart J* 2010;31:2844-53.
4. Willeit P, Kiechl S, Kronenberg F, et al. Discrimination and net reclassification of cardiovascular risk with lipoprotein(a): prospective 15-year outcomes in the Bruneck Study. *J Am Coll Cardiol* 2014;64:851-60.
5. Emerging Risk Factors Collaboration, Di Angelantonio E, Gao P, et al. Lipid-related markers and cardiovascular disease prediction. *JAMA* 2012;307:2499-506.

GRK2 Regulates α_2 -Adrenergic Receptor-Dependent Catecholamine Release in Human Adrenal Chromaffin Cells



Alterations in catecholamine (CA) synthesis and catabolism predispose to or aggravate the course of cardiovascular disorders. In heart failure (HF), excessive norepinephrine, resulting from persistent sympathetic efferent fiber activation, contributes to CA spillover, which is a major prognostic and therapeutic index (1).

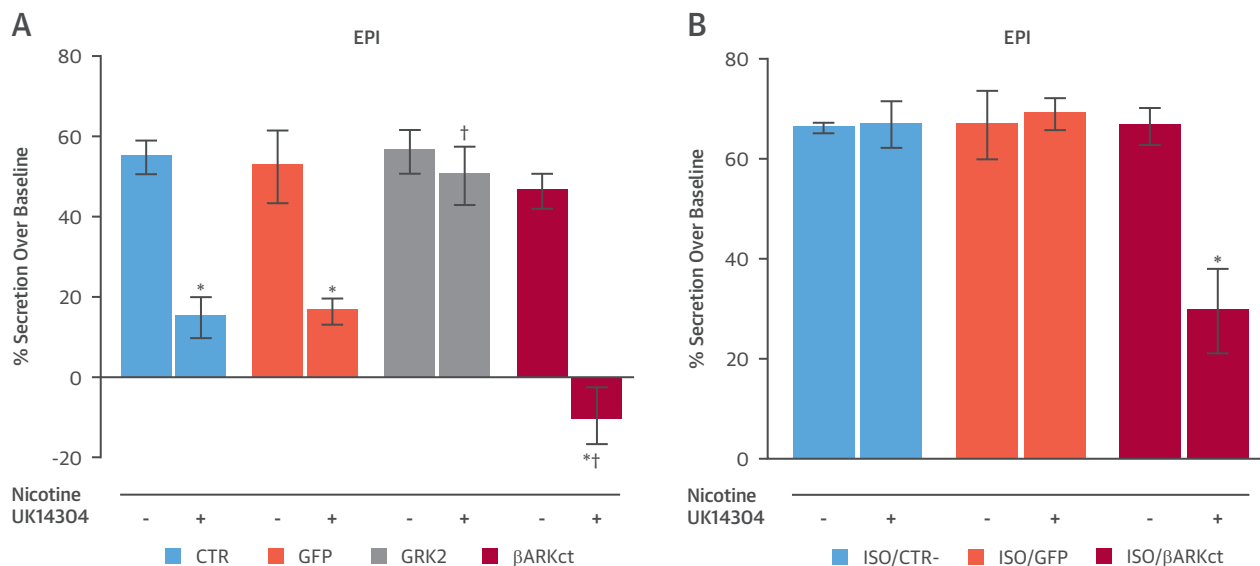
Exaggerated epinephrine (EPI) from the chromaffin cells of the adrenal glands (2) is also involved in HF progression. EPI secretion is controlled by β -adrenergic receptors (β ARs), which facilitate its release, and α_2 -adrenergic receptors (α_2 ARs), which

provide negative feedback, preventing unremitted EPI (and norepinephrine) adrenal discharge (1,2). In chromaffin cells of HF rodents, G protein-coupled receptor kinase 2 (GRK2) expression is up-regulated, impairing α_2 AR-gated EPI secretion via desensitization of these receptors (2). It is unknown whether a similar mechanism is present in humans. Importantly, in ex vivo cultured human pheochromocytoma explants, gallein, a GRK2 inhibitor, prevents uncontrolled CA secretion (3). However, it remains to be determined whether physiologically relevant CA concentrations can up-regulate GRK2 expression in normal human adrenal glands, preventing sympathoinhibitory α_2 AR effects on EPI (and norepinephrine) release.

To this aim, we used human chromaffin cells isolated from adrenal glands (n = 13) from deceased kidney transplant donors. The local ethics committee of the University “Federico II” reviewed and approved this protocol. All experimental methods were described previously (2). Statistical significance was determined using the Student t test or analysis of variance followed by Bonferroni post hoc correction for multiple comparisons. All data were analyzed using GraphPad Prism version 7 (GraphPad Software, La Jolla, California).

We first increased GRK2 expression and activity via adenovirus (Ad), whereas in separate experiments we expressed β ARKct, an inhibitor peptide of GRK2 activation via $G_{\beta\gamma}$ sequestration. Noninfected cells, or Ad-green fluorescent protein (GFP) cells, were used as controls. We stimulated human chromaffin cells with nicotine to induce a marked EPI secretion; this effect was equipotent in all experimental groups (Figure 1A). Interestingly, pre-treating cells with the selective α_2 AR agonist UK14304 resulted in an abated nicotine-induced EPI release in control (CTR) and AdGFP-infected cells (Figure 1A). However, the α_2 AR's sympathoinhibitory effect was markedly blunted in AdGRK2-infected cells, whereas the presence of β ARKct potentiated it.

Stimulation with CA up-regulates GRK2 in the heart (2). Similar direct evidence in human chromaffin cells is lacking. Therefore, we pre-treated in vitro human chromaffin cells with isoproterenol (1 μ mol/l) for 12 h and found a significant rise in GRK2 protein levels (GRK2 levels fold over CTR, CTR 1.00 \pm 0.04 vs. isoproterenol 1.88 \pm 0.42; p < 0.05 vs. CTR). High GRK2 levels abolished the α_2 AR-mediated negative feedback on EPI release, in both CTR and GFP-expressing cells (Figure 1B). Importantly, β ARKct expression rescued α_2 AR-mediated inhibition on EPI secretion from these human cells (Figure 1B).

FIGURE 1 In Vitro Modulation of Adrenal G Protein-Coupled Receptor Kinase 2 Activity Regulates Human Chromaffin Cell α_2 AR Function and Thus Catecholamine Secretion

Epinephrine (EPI) secretion, measured by enzyme-linked immunosorbent assay, from chromaffin cells isolated from human adrenal glands. **(A)** Cells were infected with adenovirus-G protein-coupled receptor kinase 2 (AdGRK2) or adenovirus- β -adrenergic receptor Kct (Ad β ARKct). Noninfected cells (CTR) or adenovirus-green fluorescent protein (AdGFP)-infected cells were used as controls. Cells were stimulated with nicotine (20 μ mol/l) for 30 min, following pre-treatment with vehicle or with the α_2 -adrenergic receptor (α_2 AR) agonist UK14304 (10 μ mol/l) for 30 min (* p < 0.05 vs. CTR + nicotine; $\dagger p$ < 0.05 vs. CTR + nicotine + UK14304; n = 6 per group). **(B)** Cells were infected with Ad β ARKct. CTR or AdGFP-infected cells were used as controls. Twenty-four h post-infection, the cells were pre-treated with isoproterenol (ISO) (1 μ mol/l) for 12 h. Following ISO stimulation, the media were replaced, and the cells were stimulated with nicotine (20 μ mol/l) for 30 min. After ISO, and prior nicotine stimulation, a group of cells was pre-treated with the α_2 AR agonist UK14304 (10 μ mol/l) (* p < 0.05 vs. ISO/CTR + nicotine; n = 6 per group).

Our study is the first to show that β AR-dependent GRK2 up-regulation accounts for α_2 AR desensitization in human chromaffin cells. GRK2 elevation abolishes the feedback inhibition operated by α_2 AR activation on further CA release from adrenal glands.

Our present findings offer a major advancement in our understanding of the pathophysiology of diseases characterized by elevated sympathetic nervous system activity. At the same time, our data suggest that GRK2 blockade could be useful in advanced human HF as well as in other disorders aggravated by the hyperactivity of the central and/or peripheral sympathetic nervous system, namely, hyperthyroidism, hypertension, and pheochromocytoma, all conditions in which excessive circulating CA levels may be pathogenic as well.

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REFERENCES

1. Florea VG, Cohn JN. The autonomic nervous system and heart failure. *Circ Res* 2014;114:1815-26.
2. Lymperopoulos A, Rengo G, Funakoshi H, et al. Adrenal GRK2 upregulation mediates sympathetic overdrive in heart failure. *Nat Med* 2007;13:315-23.
3. Kamal FA, Mickelsen DM, Wegman KM, et al. Simultaneous adrenal and cardiac α -protein-coupled receptor-G β γ inhibition halts heart failure progression. *J Am Coll Cardiol* 2014;63:2549-57.

Characteristic Left Ventricular Regional Wall Motion Abnormality Can Help Prevent Unnecessary Ischemic Workup



I have read the paper by Dr. Doshi and colleagues (1) with great interest, and I completely agree that a better job can be done in identifying ischemic etiology in new-onset heart failure. While deciding who should undergo an ischemic workup, the observation of a characteristic regional wall motion pattern in dilated cardiomyopathy may be helpful (2). In >40 patients with dilatative cardiomyopathy, I observed inferior (2Ch [2chamber]) and inferolateral (3Ch) akinesia with preserved contractility in the proximal third of the inferolateral wall and in the proximal third of the anterolateral wall (4Ch), together with various degrees of hypocontractility in the remainder of the left ventricle. None of these patients had obstructive coronary artery disease on coronary angiography. I believe that the presence of this simple 2-dimensional echocardiography parameter can help prevent unnecessary ischemic workup in patients with new-onset heart failure who do not have baseline coronary artery disease.

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REFERENCES

1. Doshi D, Ben-Yehuda O, Bonafede M, et al. Underutilization of coronary artery disease testing among patients hospitalized with new-onset heart failure. *J Am Coll Cardiol* 2016;68:450-8.
2. Drinkovic N. Characteristic LV regional wall motion abnormality: sign of cardiotoxicity? *J Am Coll Cardiol* 2015;65:758-9.

REPLY: Characteristic Left Ventricular Regional Wall Motion Abnormality Can Help Prevent Unnecessary Ischemic Workup



We thank Dr. Drinkovic for his comments on our study. Attempts to further explore noninvasive methodologies to sufficiently differentiate between nonischemic and ischemic etiologies of dilated cardiomyopathy are of clear interest (1–4) because they have the potential to obviate the need for ischemic testing for selected patients. However, identification of ischemic etiologies for congestive heart failure with reduced ejection fraction can lead to treatment modifications (both related to secondary preventive medical therapies and revascularization). Furthermore, because revascularization of ischemic cardiomyopathy has been associated with a survival advantage over medical therapy alone (5), any such methodology would be required to have sufficient negative predictive value so patients are not left with a potentially reversible cause for heart failure unaddressed or undiagnosed.

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