ADENOSINE RECEPTOR SUBTYPES AND THE HEART FAILURE PHENOTYPE: TRANSLATING LESSONS FROM MICE TO MAN

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ABSTRACT

Adenosine plays an important role in the pathophysiology of heart failure and in myocardial protection during ischemia and reperfusion. The action of adenosine in the heart is mediated by four G-protein-coupled receptors: A_1-AR and A_3-AR, which act via $G\alpha_1$, and A_{2A}-AR and A_{2B}-AR, which act via $G\alpha_s$. Understanding of cellular signaling pathways triggered by adenosine has been complicated by the availability of only partially specific adenosine agonists/antagonists. Adenosine signaling appears to be at times redundant in receptor function, and cellular signaling pathways for adenosine are multiple, parallel, and interrelated. Data obtained about the specific role of individual adenosine receptors, through the genetic modulation of receptors in murine hearts have provided important information about the role of adenosine receptors in the heart. Here we review existing data and present new results that clarify the function of individual adenosine receptors in the heart and their role in the development of left ventricular dysfunction, and about the downstream signaling systems that are modified by adenosine receptor activation.

In 1929, Drury and Szent-Gyorgyi published their landmark article in the *Journal of Physiology* in which they demonstrated that simple extracts from heart muscle, brain, kidney, and spleen had a "definite and transient effect upon the mammalian heart," and reported their finding that the substance responsible for this effect was adenosine (1). They recognized that adenosine would "slow the rate of beating, impair conduction from auricle to ventricle, and arrest experimentally produced auricular fibrillation." Dr. Robert M. Berne found in 1963 that adenosine was a critical signaling molecule in the heart, and hypoth-

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esized that adenosine, released by cardiac myocytes, exerts its effects on the vasomotor tone of the coronary arteries and thus couples metabolic demands of the myocardium to coronary blood flow (2).

More recently, interest in the role of adenosine in the heart has been due to a series of studies demonstrating that myocardial adenosine levels increase in response to metabolic stress and cell damage, such as inflammation, hypoxia, ischemia, and trauma (3, 4), and that this protects the heart during ischemia and reperfusion (5). Furthermore, adenosine mediates the effects of pre-ischemic conditioning—the phenomenon whereby repetitive and brief episodes of ischemia "pre-condition" the heart so that the subsequent total occlusion of a coronary artery results in a myocardial infarction that is small in relation to that seen in animals that have not had such pre-conditioning (6). Nonetheless, the exact mechanisms responsible for the salutary effects of adenosine remain undefined (7, 8).

Adenosine is a ubiquitous purine nucleoside generated by the dephosphorylation of the 5^1 adenosine monophosphate (AMP) and by the hydrolysis of S-adenosylhomocysteine (9). Adenosine action in the heart is mediated by four G-protein-coupled receptors: A_1-AR and A_3-AR, which act via $G\alpha_{i,}$ and A_{2A}-AR and A_{2B}-AR which act via $G\alpha$ (9). When a receptor is stimulated, the α subunit releases guanosine diphosphate (GDP), allowing guanosine triphosphate (GTP) to bind in its place, and the G-protein complex dissociates into two activated components: an α subunit and a $\beta\gamma$ subunit. $G\alpha_i$ and $G\alpha_s$ have been classically associated with inhibition and stimulation (respectively) of adenyl cyclase, and activation of the $G\alpha_s$ pathway is important in the modulation of calcium handling (Figure 1) (9). $G\beta\gamma$ mediates adenosine action in the cell nucleus by modifying the expression of genes involved in cell growth and remodeling (10, 11).

Although the role of adenosine in mediating the effects of ischemic pre-conditioning and protection from reperfusion injury is well known, we hypothesized that adenosine and adenosine receptor subtypes could play a critical role in the development of left ventricular (LV) dysfunction, hypertrophy, and failure. This hypothesis was due in large part to the fact that the family of cardiac adenosine receptors activated a cascade of downstream signaling molecules, many of which were implicated in cardiac hypertrophy, apoptosis, or the regulation of intracellular calcium fluxes (Figure 1). These critical signaling molecules, which act downstream of the adenosine receptors, include Akt, protein kinase C (PKC), the nuclear regulatory proteins MEF2, CERB, HDAC, and c-Fos, the mitochondrial K_{ATP} channel, and the protein kinase A

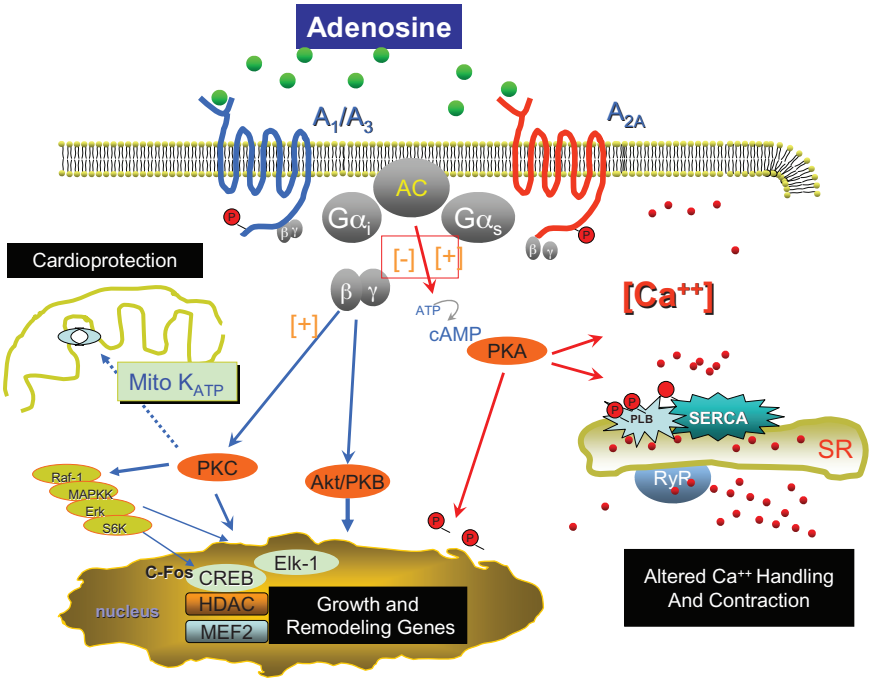

FIG. 1. Signaling pathway for adenosine receptors.

(PKA) signaling system that regulates the function of the calcium regulatory proteins phospholamban and Ca^{2+} ATP-ase.

Our speculation that adenosine and adenosine receptors might behave quite differently in the failing heart than in the presence of cardiac ischemia was also supported by our studies in mice with heart failure secondary to over-expression of the pro–inflammatory cytokine TNFα or to trans-aortic banding. While hearts exposed to cardiac ischemia demonstrate marked increases in tissue levels of adenosine, adenosine levels were decreased by 70% in hearts with LV dysfunction secondary to over–expression of TNFα. In addition, in the mice with heart failure, A_1-AR levels were increased by 4-fold, and a 50% reduction in the levels of A_{2A}-AR was observed (12).

Early attempts to tease apart the regulatory properties of the adenosine receptors have been limited by the highly homologous nature of the three cardiac adenosine receptors and by the fact that adenosine receptors are ubiquitous and can be found in the vasculature as well as in the central nervous system and the kidney. Therefore, we and others have utilized transgenic models and gene-transfer technology to per-

turb the expression of selected adenosine receptor subtypes in the myocardium.

GENETIC MANIPULATION OF CARDIOVASCULAR A_1-ADENOSINE RECEPTORS

Matherne and colleagues created one of the first transgenic murine models with altered adenosine signaling (13). They designed a construct containing a rat A_1-adenosine receptor, human growth hormone polyadenylylation signal, and mutated α-Major histocompatibility complex (MHC) promoter. Over-expression of up to 100-fold of this functional A_1-adenosine receptor in C57/black mice was associated with bradycardia, delayed conduction through the sinoatrial (SA)and atrioventricular(AV) nodes, atrial arrhythmias, and moderate ventricular hypertrophy (13–16). Altered response to β-adrenoceptor signaling (13) was associated with augmented A_1-AR signaling. Furthermore, A_1-AR over-expression mediated enhanced ischemic tolerance during ischemia-reperfusion injury (14) and hypertrophic remodeling of the myocardium (13, 16). A limitation of these studies was that the transcript was expressed during late fetal development as well as during the early post-natal period. Furthermore, the studies were performed in C57/black mice, a strain that may obviate the ability to discern the development of heart failure.

To mitigate some of the limitations of constitutive over-expression of a G-protein-coupled receptor transgene, we created transgenic mice in a FVB background (12), using a construct of human A_1-AR cDNA cloned into a cardiac-specific vector composed of a modified mouse a-myosin heavy-chain promoter fused with nucleotide binding sites for tetracycline transactivating factor (tTA). This experimental design allowed on/off switching of A_1-AR over-expression. In the presence of doxycycline in the diet of pregnant or nursing mouse mothers or in the food of young mice that had been weaned, the transgene was turned-off, whereas in the absence of doxycycline the transgene was turned-on. Constitutive A_1-AR overexpression induced a dilated cardiomyopathy and death at 6 to 12 weeks (Figure 2). Mice in which A_1-AR over-expression was induced in adulthood achieved a normal phenotype after birth and a had more robust survival than did mice with constitutive over-expression of the transgene; however, these mice still demonstrated a decreased survival and LV dysfunction later in life as compared with non-transgenic littermate controls.

Both constitutive and controlled overexpression of the A_1-AR was associated with ventricular hypertrophy, an increase in the size and

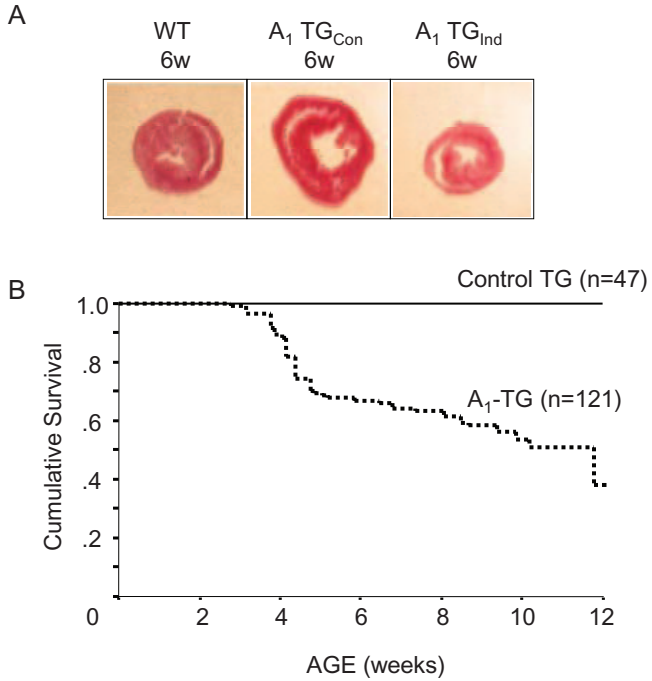

FIG. 2. Constitutive induction (Con) and adult induction (Ind) of A_1-AR expression in transgenic mice. For adult A_1-AR induction doxycycline, (DOX) was removed from transgenic mice at 3 weeks of age (*Panel A*): HE staining of 6-Week-old mouse myocardium cross-section. (*Panel B*): Kaplan-Meier survival curves for mice constitutively expressing A_1-AR (A_1-transgenic construct [TG_{Con}]) and for mice with A_1-AR induced at 3 weeks of age by removal of DOX (A_1-TG_{Ind}).

length of individual myocytes, a lower heart rate, increased ventricular fibrosis, diminished calcium cycling, and recapitulation of the heart-failure "genotype," including increased production of atrial natriuretic peptide (ANP), and decreased sarco(endo) plasmic reticulum calcium-ATPase (SERCA) and phospholamban expression. Activity of the cardoprotective kinase. Akt was also decreased (17). The change in ventricular function could not be attributed to a decrease in heart rate, because diminished ventricular function was also seen when hearts from transgenic mice were isolated, perfused, and paced at a defined heart rate.

A_1-AR-induced cardiomyopathy was also associated with a marked decrease in the expression of caveolin-3: levels of both caveolin-3 mRNA and caveolin-3 protein were decreased (Figure 3) (18). Caveolin-3 anchors cardiac receptors and calcium-signaling proteins in the caveolae of the cardiac T-tubule system, and is also crucial in A_1-AR

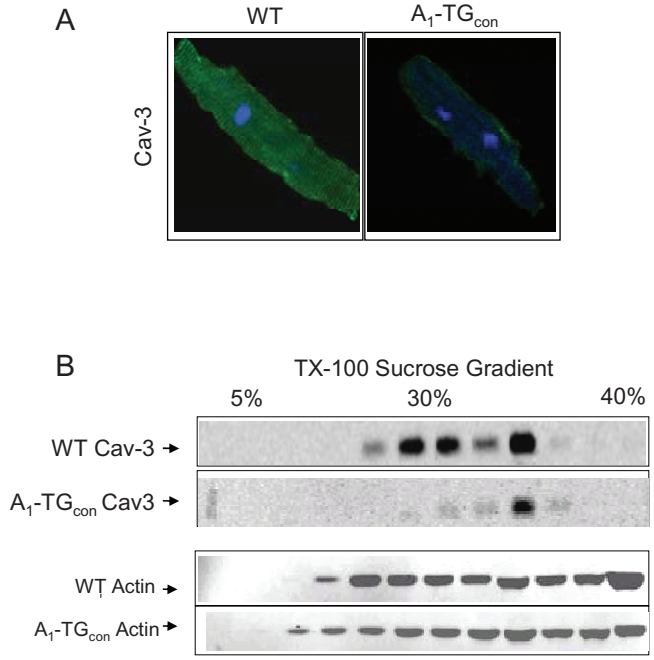

FIG. 3. (*Panel A*): Immunofluorescence staining of caveolin-3 isolated myocytes. Confocal images represent at least 3 mice/genotype group and a minimum of 20 myocytes/mice examined. (*Panel B*): Caveolin-3 (Cav3) protein expression profile in sucrose gradient. Ventricular extracts from 10-week-old WT and A_1-TG_{con} mice were separated in a 5%-30%–40% step-sucrose gradient and probed with indicated antibodies.

internalization, recycling, and signal transduction. The nuclear regulatory protein myogenin is both necessary and sufficient for expression of the caveolin-3 gene, while the protein ID2 can limit the ability of myogenin to interact with its binding sites in the promoter region of the caveolin-3 gene. Over-expression of the A_1-AR had no effect on cardiac levels of myogenin or ID2. Therefore, the mechanism whereby over-expression of A_1-AR decreases the expression of caveolin-3 remains to be defined.

GENETIC MANIPULATION OF CARDIOVASCULAR A_{2A}-ADENOSINE RECEPTOR

To better understand the role of the A_{2A}-AR, we generated transgenic mouse lines with high and low expression of the A_{2A}-AR by putting the cDNA for the human receptor under control of the cardiac-

specific promoter, with the on/off switch for gene expression that we used in generating mice with A_1-AR over-expression. In the absence of doxycycline, there was expression of the A_{2A}-AR at levels four-times those seen in the normal non-transgenic mice in the line with high levels of A_{2A}-AR expression and approximately a two-fold greater expression than in the mouse line with low levels of A_{2A}-AR expression. Constitutive overexpression of the A_{2A}-AR in young mice was associated with increased cardiac contractility, higher heart rates, and a small increase in LV mass (Figure 4A). Increased SERCA2 expression and Ca^{2+} uptake by the sarcoplasmic reticulum were found in association with augmented signaling through the A_{2A}-AR, and correlated with physiologic changes (19). The role of A_{2A}-AR-mediated signaling in this mouse model during ischemia-reperfusion injury suggested salutary benefits of the A_{2A}-AR on cardiac function and cardiac hemodynamics, but the effects of long-term, controlled over-expression of the A_{2A}-AR remain to be determined.

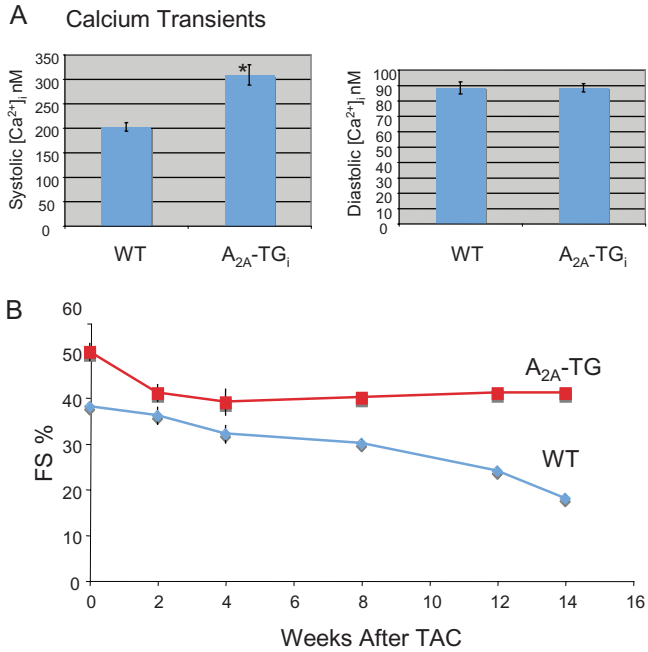

FIG. 4. (*Panel A*): Calcium transient response in WT and A_{2A}-TG mice. Adult myocytes from WT and A_{2A}-TG mice were used to measure systolic and diastolic $[Ca^{2+}]_{i*}$ $*P < 0.01$. (*Panel B*): Echocardiography of A_{2A}-TG and WT mice. Wild-type ($n = 17$) and A_{2A}-TG ($n = 10$) mice were evaluated at 0, 2, 4, 8, and 14 weeks after TAC—transaortic constriction Graph shows percent fractional shortening (% FS).

Recently, we have shown that adult over-expression of the cardic A_{2A}-AR modifies the heart's response to pressure overload. Transthoracic banding in wild-type (WT) mice leads to decreased cardiac function, an increase in end-systolic and end-diastolic dimensions, a greater ratio of heart weight to body weight, and marked fibrosis as compared with these measures in sham-operated controls. These changes were markedly attenuated by over expression of the A_{2A}-AR ($P < 0.001$ for each measure) (Figure 4B).

Interestingly, over-expression of A_{2A}-AR is not cardioprotective in all experimental situations. When WT mice were injected intra-perioneally with adriamycin (5 mg/kg/wk for 4 weeks), all of the mice survived. However, when mice over-expressing the A_{2A}-AR were given this same treatment, all of the mice died by 5 weeks (Figure 5). Telemetry showed progressive prolonation of the QT interval, bradyarrhythmias, heart block, and sudden death in the A_{2A}-AR over-expressing mice as compared with WT mice. The duration of the action potential also increased dramatically in the A_{2A}-AR-expressing myocyte of adriamycin-treated mice (20).

Perhaps the most important finding was that over-expression of the A_{2A}-AR could rescue the heart-failure phenotype in mice with constitutive over-expression of the A_1-AR. Concurrent over-expression of A_{2A}-AR and A_1-AR resulted in mice with a normal or near-normal cardiac phenotype in terms of ventricular function, survival, and Ca^{2+} handling (19). In addition, mice concurrently over-expressing the A_1-AR and A_{2A}-AR had levels of brain natriuretic peptide (BNP),

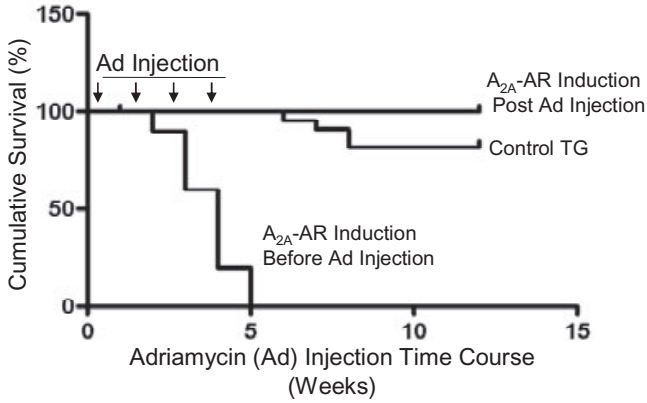

FIG. 5. Timing of A_{2A}-AR expression affected survival after adriamycin treatment. Eight-week-old control and A_{2A}-TG mice were injected with adriamycin (Ad) and monitored over 15 weeks. Expression of the A_{2A}-AR transgene was induced either before (starting at 3 weeks of age) or after cessation of adriamycin administration.

phosphlamban, and Ca^{2+}-ATPase that mirrored those in non-transgenic controls, and their expression of caveolin-3 was normal. Importantly, low levels of over-expression of the A_{2A}-AR were unable to overcome LV dysfunction in A_1-AR over-expressing mice. These results suggested that the normal homeostatic function of the heart requires balanced activation of the A_1-AR and A_{2A}-AR—a stoichoimetric relationship that occurs under physiologic conditions because of similarities in the inherent affinity of the A_1-AR and A_{2A}-AR for both endogenous and exogenous adenosine. Our results also suggest that pharmacologic manipulation of a single adenosine receptor subtype or a genetic mutation that alters the function of a single adenosine receptor subtype might have adverse affects on the heart.

ADENOSINE RECEPTOR "KNOCKOUT" STUDIES

Studies in which adenosine receptors have been genetically kocked-out have not significantly improved our understanding of their role in heart failure, owing largely to the fact that in most of these models, adenosine receptors have been deleted from all organs. For example, mice in which the A_1-AR has been genetically deleted appear to have a normal cardiac phenotype in the absence of stress (21, 22). The effect of the A_1-AR deletion on coronary blood flow, vascular sensitivity (23–25), and ischemic preconditioning (26, 27) remain to be defined (26–28).

Studies done with A_{2A}-AR-knockout transgenic mice have highlighted the role of the A_{2A}-AR in blood pressure control, local vasodilatation, and coronary vasoregulation, as well as in regulating the inflammatory response following myocardial ischemia-reperfusion injury and in regulating angiogenesis. Deletion of the A_{2A}-AR was associated with a significant increase in blood pressure and associated bradycardia (29, 30), and with reduced aortic relaxation and endothelial function (31–33). Although A_{2A}-AR-independent arterial dilation in A_{2A}-AR-knockout hearts is probably mediated by the A_{2B}-AR, the receptor was found to be up-regulated in the coronary arteries of A_{2A}-AR knockout mice (34).

Deletion of A_{2A}-AR-mediated signaling augments inflammation in a variety of cells and model systems (35–37), through effects including inhibition of the bone-marrow-derived cell response to injury, specifically by T-lymphocytes (38). Studies of A_{2A}-AR knockout have also implicated a role for the A_{2A}-AR in angiogenesis (39–41). However, the effects of A_{2A}-AR ablation on the development of cardiac hypertrophy and failiure are unknown.

GENETIC MANIPULATION OF CARDIOVASCULAR A_3-AR TRANSGENIC A_3-AR OVER-EXPRESSION.

Far less is known about the role of the A_3-AR in development of the heart failure phenotype than is known about the role of the A_{2A}-AR. Enhanced expression of the A_3-AR during early embryogenesis in some strains of mice is lethal (42). In other strains with high levels of receptor expression, a phenotype with prominent bradycardia and arrhythmogenesis emerges (42, 43), while experimental approaches inducing lower levels of A_3-AR overexpression have observed its effects only on the cardiac response to myocardial ischemia (42–44). The basis of cardiomyopathy triggered by A_3-AR overexpression is thought to be secondary to enhanced $G\alpha_i$ signaling (42).

Mice with an A_3-AR-knockout transgene (45) were found to exhibit modulation of inflammatory processes (45), elevations in cAMP in cardiac and vascular tissue, and an exaggerated systemic hypotensive response to exogenous adenosine (although the animals baseline blood pressure was unaltered) (46). Talukder and colleagues provide evidence that A_3-AR agonism modestly counters vasodilatation mediated by A_{2A}-ARs (47), and a group of studies demonstrated a cardioprotective effect of the A_3-AR (48, 49). Thus, for example, deletion of the A_3-AR reduced infarct size (48) without significantly modifying the intracellular energy state (49). However, both the genetic background of the mouse model and the design of ischemic preconditioning studies may be important in understanding the role of the A_3-AR (7, 50, 51). The only information regarding the role of the A_3-AR in heart failure comes from studies in which this receptor has been knocked out. Lu and colleagues found that genetic ablation of the A_3-AR blunted pathologic cardiac remodeling, including hypertrophic growth and fibrotic change (52).

TRANSLATING MOUSE BIOLOGY TO HUMANS: ADENOSINE IN HEART FAILURE

Rolofylline KW-3902, Merck Research Laboratories, West Point, PA was the first receptor-subtype-specific adenosine antagonist to be evalutated for the treatment of decompensated heart failure. This specific A_1-AR antagonist, with 890-fold binding selectivity for the A_1-AR over the A_{2A}-AR, and no effect on the A_3-AR, exerts its effect by inhibiting sodium reabsorption in the proximal tubule of the kidney and by blocking adenosine-mediated constriction of the afferent glomerular arterioles. This results in diuresis and natriuresis. Thus, rolofylline was developed because of its presumed effect on

renal function, independently of whatever effects it might have on cardiac muscle.

In early phase I clinical trials diuresis was seen at 3 hours, after a dose of 30 mg of rolofylline, and the effect persisted for up to 8 hours after active drug administration (88). In a phase II trial, 146 patients with decompensated class II-III congestive heart failure (CHF) and an estimated creatinine clearance of 20 to 80 mL/min were randomized to receive placebo or from 1 to 4 doses of rolofylline infused for up to 2 hours daily for up to 3 days. The drug increased urine output, enhanced diuresis, and reduced the need for concomitant intravenous furosemide. One seizure was reported in a patient receiving the highest dose of rolofylline (53). With these encouraging results in preliminary studies, 2,033 patients hospitalized for CHF were randomized in a 2:1 ratio to receive either rolofylline 30 mg/day or placebo, administered as a 4-hour daily infusion that was repeated for 3 days. The primary end-point of the PROTECT trial was a three-category ordered outcome of treatment success, unchanged, patient condition, or treatment failure (54). Secondary end-points were time to death or rehospitalization for cardiovascular or renal causes, and persistent renal impairment.

No significant difference was found among rolofylline-and placebo-treated patients with respect to the primary end-point or the secondary endpoints. A higher rate of persistent renal impairment was noted in the rolofylline group. In addition, more patients in the rolofylline group experienced seizures (11 [0.8%] vs. 0 in the placebo group) as well as strokes (16[1.2%] vs. 3 [0.5%]). Thus, despite promising preliminary findings, the PROTECT trial did not yield a positive results in heart failure, owing in large part to the increased incidence of neurologic events in patients receiving rolofylline. Although the mechanisms responsible for these adverse effects were not identified by the study, one could only conclude that the use of receptor-specific antagonists (or agonists) is not a rational approach to treating heart failure in light of the basic science data showing an adverse effect of receptor subtype-specific interventions.

To further evaluate this hypothesis, we sought to identify whether mutations in individual adenosine-receptor subtypes could predict outcomes in patients with heart failure. We sequenced the genes encoding the human A_1-, A_{2A}-, and A_3-AR receptors in a cohort of patients with normal cardiac function and in a matched group of patients with idiopathic dilated cardiomyopathy or ischemic heart failure (55). These studies identified a group of genetic variants that occurred in a relevant percentage of the general population. Several of these variants

were of particular interest. These consisted of three variants in the 3′-untranslated region of the A_1-AR gene (nt 1689 C/T; nt 2206 Tdel, nt 2683 del 36) that predicted a change in the three-dimensional structure of the mRNA tail and thus a change in stability of the mRNA for the receptor; a non-informative single nucleiotide polymorphism (SNP) in the coding region of the A_1-AR gene (nt 717 T/G); and an informative SNP in the coding region of the A_{2A} adenosine gene (nt 1509 A/C). The four genetic variants in the A_1-AR gene were associated with either an increase or a decrease in infarct size in patients with heart failure secondary to ischemic heart disease and myocardial infarction. In addition, the non-informative SNP at nt 717(T/G) in the coding region of the A_1-AR gene was associated with an increase in infarct size as well as with an increase in the combined end-point of death or cardiovascular hospitalization in the same population (Feldman, AM, unpublished data). Thus, the finding that alterations in the genetic structure—and presumably function—of a sub-type-specific AR gene could change the outcome of disease in a population of patients with heart failure supports the findings in mice with controlled or constitutive over-expression of the A_1-AR.

CONCLUSIONS AND FUTURE DIRECTIONS

Since 1963, when adenosine was initially identified as an important signaling molecule in the heart by Robert M. Berne, its role in the heart has been extensively studied; however, the vast majority of these studies have focused on the role of adenosine in hearts with ischemic injury. Our recent studies demonstrate that adenosine receptors can play an important role in the development of heart failure. In particular, we have found that normal cardiac homeostasis requires a stoichiometric balance between signaling through the A_1/A_3 $G\alpha_i$ protein-coupled receptors and the A_{2A} $G\alpha_s$ protein-coupled receptor. The need for "balanced" signaling provides a teleologic explanation for how nature could develop a signaling system in which a single ligand binds to multiple receptor subtypes—some of which activate down-stream signaling pathways having diametrically opposite cellular effects Additional studies will be required to identify the role of novel down-stream signaling proteins and pathways that transform the signals for activation of a specific-receptor subtype into physiologic effects, as these downstream signaling pathways may provide more appropriate targets for future therapeutic interventions for altering the heart failure phenotype and modifying disease progression.

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DISCUSSION

Hasday, Baltimore: It's been 25 years since I've done anything with adenosine receptors. One of the few things that I remember about them is that they have different β_{max} values and different affinities for adenosine, and I wonder how the concentration of adenosine may affect the balance between the two signaling pathways.

Feldman, Philadelphia: We've looked at that, and actually the affinity of adenosine for the A_1 and the A_{2A} receptor is somewhat similar. Nonetheless, what was interesting is that we have three different lines of the A_{2A} adenosine receptor. Two of the lines that had very low levels of expression would not rescue the mouse cardiac phenotype with A_1 receptor over-expression, but the higher expression line would dido this. So we were actually able to tinker with those lines and find the one that actually gave the correct stoichiometric relationship.

Blantz, San Diego: I have two sorts of unrelated questions: In your over-expressed models, did you ever look at the feedback regulatory loop in terms of adenosine generation? Does endonucleotidase activity change or do tissue adenosine levels change as a result of an over-expressed model, because that might spill into the other receptor and have secondary effects that would be confounding?

Feldman, Philadelphia: That's a great question. We actually did look at adenosine levels and they were not different in the two models.

Blantz, San Diego: One second, smaller question. In terms of the human studies, or I guess the ones you described at the end, the kidney has adenosine receptors that act in somewhat opposite directions. Is that part of the complexity?

Feldman, Philadelphia: The interesting part of that story is that there were two large trials done over the past 3 or 4 years, and both looked at an A_1 adenosine receptor antagonist and it's role in treating patients with congestive heart failure. The hypothesis was, as you alluded to, that you could antagonize the renal A_1 adenosine receptors and in so doing improve diuresis. In fact, those molecules did what we wanted them to do in terms of increasing diuresis. But in the large morbidity-mortality trials, there was actually a signal toward an increase in mortality with an A_1 adenosine receptor antagonist, which we think happens because it changes the critical balance in the myocardium between A_1- and A_{2A}-mediated receptor signaling. In addition, those patients receiving active drug showed a substantially higher incidence of seizures, because they were also experienced perturbed adenosine receptor balance in the central nervous system. As a result of these studies, further study of both drugs was discontinued.

Shannon, Philadelphia: Elegant stuff as always. Does the A_{2A} receptor undergo down-regulation or desensitization like other G-coupled protein receptors, or do we know the answer to that?

Feldman, Philadelphia: That's a great question. It turns out that if you look at models of failing hearts—and we've looked at three models—you see very interesting changes in receptor levels. We've looked at a banding model; we've looked at a TNFα overexpression model; and we've looked at a model of cardiac-specific overexpression of calsequestrin (CSQ). In all cases, when heart failure occurs, the levels of adenosine actually go down. On the basis of data from models of cardiac ischemia, you would expect that they would go up, but they don't. In these same models, the decrease in adenosine levels is accompanied by up-regulation of the A_1 adenosine receptor but down-regulation of the A_{2A} adenosine receptor. Thus, this system is far more complex than we might imagine.

Abboud, Iowa City: Is the expression of A_{2A} and A_1 in a protective function related in any way to cellular effects on potassium channels versus calcium channels?

Feldman, Philadelphia: That is an excellent question, but at this point in time we don't know the answer to it. We actually embarked on this project on the basis of work by fellow who was working in my laboratory at the time, Dr. Daniel Wagner. He found that adenosine was the most potent inhibitor of TNF2. Recognizing that TNFα could adversely influence the cardiac phenotype, we thought that adenosine molecules would be excellent molecules with which to modulate the expression of the pro–inflammatory cytokines that were over-expressed in congestive heart failure. Then we were faced with the confounding problems that I discussed today, with over-expression models demonstrating heart failure. We need to get back to that underlying hypothesis eventually, but as yet we haven't.

Oates, Nashville: This is certainly beautiful work in selecting out the different adenosine receptors. I'm curious about some of the effects other than contractility as we go forward in thinking about therapeutic interventions focused on a specific receptor subtype.

Feldman, Philadelphia: I think we have to understand what these receptors are doing to a greater degree before we start putting molecules into humans, and I think that we often make assumptions about what the biology of the receptor is without really knowing what it's going to do in a clinical situation.