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Genetic Modulation of Adrenergic Activity in the Heart and Vasculature: Implications for Perioperative Medicine

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The adrenergic system provides the primary control for cardiac, vascular, pulmonal, and metabolic functions. Seven of the nine adrenergic receptor subtypes display mutations that affect their function. Results from transgenic mouse models and from association studies in human populations allow to link protein dysfunctions to cardiovascular diseases or to risk for disease development. The disease contribution by single nucleotide polymorphisms may be small. Series of single nucleotide polymorphisms along a chromosome are combined in haplotypes and inherited together. Individual single nucleotide polymorphisms in a haplotype can influence each other and lead to new, unpredictable phenotypes. Haplotypes vary widely among different ethnic groups. In this review, we discuss the genetic organization of single nucleotide polymorphisms and haplotypes in the adrenergic system and their implications for the heart and vasculature with special reference to perioperative medicine. With the advent of powerful genomic technologies, genotyping may become standard in patient evaluation and will help to individualize therapeutic approaches.

THE widely distributed adrenergic receptors (ARs) play important roles in regulating cardiac, vascular, pulmonary, and metabolic functions. Beneficial effects of antiadrenergic treatment regimens in perioperative medicine have been confirmed in observational studies, meta-analyses,[1–3] and randomized controlled clinical trials.[4–9] Although moderate changes in sympathetic nervous system activity are required to optimize cardiac performance, undue liberation of excitotoxic catecholamines, particularly during emergence from anesthesia and the painful postoperative period, facilitates the occurrence of cardiovascular complications. The "fight-or-flight" response can become a potentially hazardous life-threatening maladaptation. "Blunting" adrenergic activity in response to the perioperative stress is regarded as a pivotal task in anesthetic practice and perioperative medicine.[10] However, the (too) simple concept of "sympatholysis" as an effective treatment modality needs refinement in light of the many new experimental and clinical findings, as it erroneously equates annihilation of any type of adrenergic activity with cardioprotection. The perception of the significance of the sympathetic nervous system in the delicate equilibrium of health and disease has continuously changed over the last decades.

In 1960, increased adrenergic drive in patients with heart failure was regarded as a life-supportive adaptation because of the observed reduced norepinephrine levels in failing myocardium and the detrimental short-term effects of high doses of antiadrenergic agents.[6] In the late 1970s, new findings enforced the development of a "counterintuitive" antiadrenergic therapeutic strategy.[11] First, chronic β-adrenergic antagonism increased survival in idiopathic dilated cardiomyopathies. Second, β-AR down-regulation was recognized as a direct result of the excessive adrenergic drive. Third, coronary sinus blood exhibited increased norepinephrine release despite decreased norepinephrine stores. After almost 20 yr of dominance of this "counterintuitive" therapeutic approach, new experimental and clinical findings cast doubts on this dogma.[12] Moxonidine, a centrally active imidazoline/α2-agonist, which decreases norepinephrine spillover and reverses remodeling in the myocardium, increased mortality by >50% in the Moxonidine Congestive Heart Failure Trial (MOXCON).[13] Moreover, in the β-Blocker Evaluation of Survival Trial (BEST), bucindolol increased mortality disproportionately in African-American New York Heart Association class IV patients, although the overall benefit of bucindolol still prevailed in the whole bucindolol cohort when compared with placebo.[14] Excessive β2-AR antagonism decreasing presynaptic norepinephrine release in conjunction with unopposed α1-blockade were accused of these adverse effects. Obviously, removal of adrenergic activity with the inability to recruit compensation when required to maintain adequate cardiac function is detri-

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mental. Collectively, these changing therapeutic approaches reflect the complex biologic effects of adrenergic activity and seriously challenge the (too simple) dogma of "sympatholytic equals beneficial."

The usefulness of β-AR antagonists in perioperative medicine relies on a limited number of studies with relatively small sample sizes.[15] However, there is a substantial base of large clinical studies strongly supporting their use in acute coronary syndromes, myocardial infarction, congestive heart failure, and arrhythmias. Such support does not exist for the α2-AR agonists. In chronic heart failure, down-regulation of β1-AR concomitant with up-regulation of the inhibitory Gαi signaling protein,[16,17] together with changes in the intracellular Ca^{2+} handling,[18] leads to progressive contractile dysfunction. Contractile impairment is also found as an acute response during the perioperative period as the result of a variety of mechanisms such as myocardial ischemia-reperfusion injury, hibernation, and stunning, as well as myocardial β-AR desensitization.[19] Markedly increased catecholamine levels found during cardiac surgery results in agonist-induced desensitization. Acute administration of a β-AR blocker in an open chest dog model of coronary artery bypass graft surgery was shown to attenuate β-AR desensitization. On the other hand, neither chronic oral nor intraoperative intravenous administration of β-AR blockers attenuated β-AR desensitization in patients undergoing coronary artery bypass graft surgery.[19]

After briefly discussing the essentials of adrenergic signaling in the myocardium and vasculature, the present review will provide new insights into the role of the adrenergic system in the cardiovascular system, as gained from gene-targeted animal models and studies on genetic polymorphisms. Therapeutic potential from transgenic approaches and implications of adrenergic genomics for perioperative medicine will be briefly outlined.

Adrenergic Signaling and Function

Acute and long-term regulation of myocardial function, including heart rate, ventricular systolic and diastolic function, and metabolism, is primarily governed by the β1-AR and β2-AR signaling pathways. A functional role for the α-AR is less well established, although positive and negative inotropic effects have been attributed to specific α-AR subtypes, particularly in cardiomyopathies and heart failure.[17,20–25] The human AR family consists of nine subtypes originating from different genes: α1A, α1B, α1D, α2A, α2B, α2C, β1, β2, and β3. These receptors comprise seven trans-membrane α-helices and couple to signal transferring guanine nucleotide-binding G-proteins (G-protein coupled receptors, GPCRs).[17,20,26–30] The ARs belong to a GPCRs super-

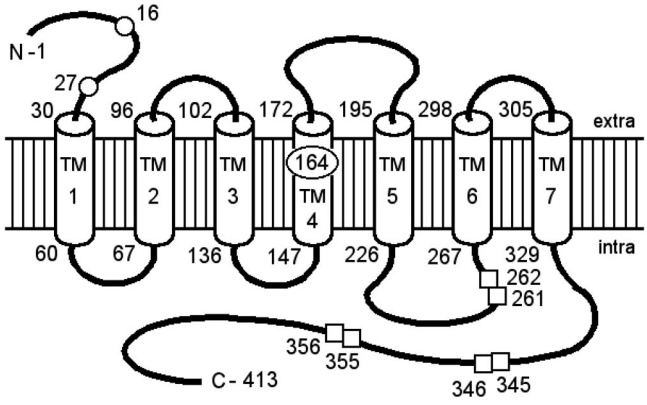

Fig. 1. Predicted structure of the human β2-adrenergic receptor based on protein sequence homology with the bovine photoreceptor rhodopsin, displaying approximate location of the polymorphisms at amino acid positions 16, 27, and 164 (*circles*) as well as the serine phosphorylation sites (*squares*) for receptor desensitization by protein kinase-A (261,262,345,346) and by G-protein-coupled receptor kinase (355,356).[17,31–33] The seven transmembrane α-helices (*cylinders* labeled *TM1* through *TM7*) contain around 30 amino acid residues each (number of first and last residue given for each helix). The agonists are thought to bind to the extracellular domains of TM3-TM4-TM5-TM6, whereas the Gα subunit binds to the first intracellular loop (between TM1 and TM2) and the carboxyterminal domain. N-1 = amino-terminal residue; C-413 = carboxy-terminal residue; extra = extracellular side; intra = intracellular side.

family with over 1500 members.[17,31–33] The three-dimensional structure has not yet been resolved for any of the GPCRs except for the bovine photoreceptor rhodopsin. Figure 1 shows a scheme of the predicted structure of the β2-AR based on its amino acid sequence homology with that of rhodopsin. The GPCRs couple at the inner side of the cell membrane to the heterotrimeric G-protein complex (Gα, Gβ, and Gγ), of which Gα binds guanosine triphosphate. On receptor activation, the Gα subunit releases guanosine diphosphate in exchange for guanosine triphosphate, which causes dissociation from the complex, leaving the βγ-subunits as undissociable heterodimer. Both the Gα as well as the Gβγ subunits can inhibit or activate a variety of intracellular effectors including ion channels, phospholipases, adenylyl cyclase (AC) isoforms, phosphoinositide-3 kinase, mitogen activated protein kinase (MAPK), and others. The downstream signaling pathways involve sequential protein phosphorylation cascades ultimately affecting targets in the cytoplasm or operating *via* transcriptional factors affecting the gene expression profile (fig. 2 and table 1). A second group of enzymes, the protein phosphatases, are responsible for dephosphorylation and termination of the signaling. Many signaling components contain multiple phosphorylation sites and phosphorylation may either stimulate or inhibit signaling depending on their specific localization on the protein. All intracellular signaling pathways are interconnected and form a robust network with considerable redundancy.[17]

A decrease in the stimulating agent (circulating hor-

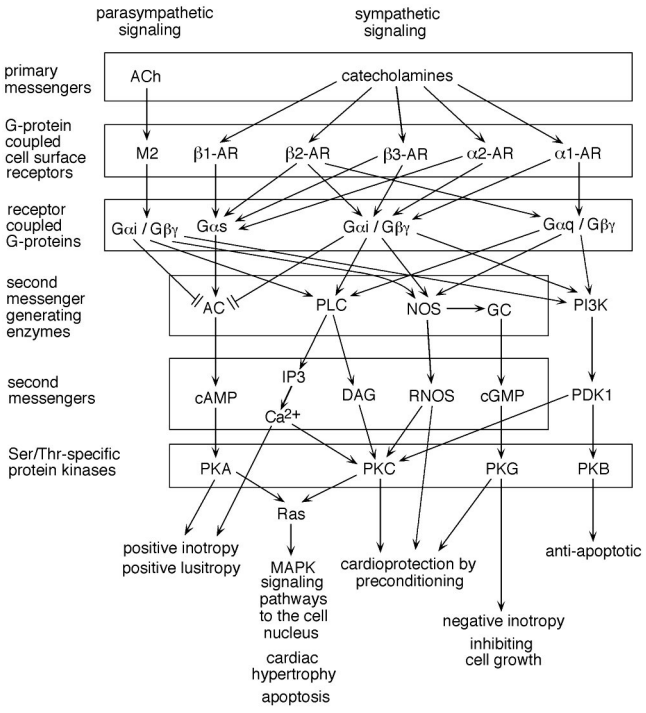

Fig. 2. Simplified scheme of sympathetic and parasympathetic signaling cascades of GPCRs down to the level of cellular responses (modified from[17]). Note the intimate crosstalk between the various signaling pathways. Lines with blunted ends (=) indicate inhibition. Information taken from[21,22,25–27,29,30,56,58]. AC = adenylyl cyclase; ACh = acetylcholine; AR = adrenergic receptor; cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; DAG = diacylglycerol; GC = guanylyl cyclase; Gαs, Gαi, Gαq, Gβγ = G-protein signaling components; IP3 = inositol trisphosphate; M2 = muscarinic acetylcholine receptor; MAPK = mitogen activated protein kinase; NOS = nitric oxide synthase; PDK1 = phosphoinositide-dependent kinase-1; PI3K = phosphatidylinositol 3-kinase; PKA, PKB, PKC, PKG = target-specific serine/threonine protein kinases; PLC = phospholipase-C; Ras, small monomeric GTPase; RNOS = reactive nitric oxide species. From Reference 17, used with permission from Oxford University Press.

mones or exogenously administered drugs) does not terminate receptor signaling at a clearly defined time point. Rapid signal termination is rather induced by desensitization of the agonist-activated receptor through specific phosphorylation (homologous desensitization) by a GPCR kinase. Alternatively, desensitization may also be achieved by phosphorylation *via* a specific serine/threonine-kinase in the absence of agonist occupancy. This heterologous desensitization is regulated by the signaling of another receptor *via* an intermediary second messenger that stimulates a particular serine/threonine-kinase like protein kinase-A (PKA) or protein kinase-C.[17,34] Desensitization may or may not be followed by internalization of the GPCRs, which subsequently is either degraded or recycled back to a functional membrane receptor.

The accepted paradigm for signaling of GPCRs holds that a receptor functions as a single unit (monomer), independently capable of coupling to a particular G-protein complex. However, recent reports on homodimerization and heterodimerization between GPCR subtypes suggest an additional receptor complexity that could account for previously unexplained pharmacological diversity.[17,35,36] For instance, β2-ARs form homodimers that seem to represent the agonist-induced active receptor species. Heterodimerization between β1-AR and β2-AR prevents agonist-induced internalization of β2-AR and its ability to activate the MAPK signaling cascades. Another interesting point concerns the finding that α1D-ARs accumulate intracellularly and become only surface membrane targeted in smooth muscle cells when α1B-ARs are coexpressed, forming heterodimers with the α1D species.[37] Furthermore, functional interaction between β-AR and angiotensin-II type-1 receptor signaling described by *in vitro* and *in vivo* experiments reveals that β-AR blockers and type-1 receptor blockers each may gain dual control over these two signaling pathways.[38] Because one of the receptors in the complex has to be free of antagonist to permit efficient coupling and signaling of the ligand-activated receptor, it may have a role in stabilizing the interaction of the activated receptor with its cognate G-protein. Thus each antagonist blocks not only its own receptor but also the signaling of the reciprocal receptor by a transinhibition mechanism. These few examples demonstrate the functional significance of GPCR dimerization, but this may just be the tip of the iceberg with much more to be uncovered by future research.[39,40]. In particular, it may be possible that dimerized GPCRs could represent the functional unit.

Different types of Gα-proteins stimulate (Gαs) or inhibit (Gαi) the AC, which generates the second messenger cyclic adenosine monophosphate (cAMP) (fig. 2).[17,20,29] cAMP activates PKA, which is responsible for an increase in intracellular Ca^{2+}, positive inotropy, and accelerated lusitropy (accelerated relaxation). The enhanced lusitropy is tightly coupled to positive inotropy to accommodate a faster heart rate, thereby increasing the efficiency of the pump. β1-ARs exclusively couple to Gαs, whereas β2-ARs may signal *via* Gαs, Gαi, and the Gβγ dimer. Diverse signaling of β2-AR has been demonstrated both for rodent and human hearts.[41] The signaling of the Gαi complex is able to follow several different routes: i) Gαi may activate phospholipase-C, resulting in generation of the two messengers inositol trisphosphate 3 and diacylglycerol. Diacylglycerol activates protein kinase-C, and inositol trisphosphate 3 releases Ca^{2+} from the sarcoplasmic reticulum. ii) Gαi from the β3-AR may stimulate the endogenous myocardial nitric oxide synthase producing nitric oxide, which stimulates the guanylyl cyclase producing cyclic guanosine monophosphate, which in turn activates the protein kinase-G. This signaling pathway results in negative inotropy and cytoprotection. iii) The Gβγ dimer from the β2-AR may initiate another cytoprotective signaling cascade involv-

Table 1. Main Adrenergic Receptor Subtype Signaling Pathways and Functions in Nervous, Cardiac and Vascular Tissues

Adrenergic receptor subtype	Main signaling pathways	Effects on nervous and cardiac tissue	Effects on vascular tissue
beta 1	Gs-AC-cAMP-PKA	Positive effects on heart rate, inotropy, lusitropy, metabolism, growth, myocyte toxicity	–
beta 2	Gs-AC-cAMP-PKA, Gi-inhibiting AC, Gi-PLC-DAG-PKC, and Gβγ-PI3K-PDK1-PKB	Positive effects on inotropy, lusitropy, metabolism, growth, myocyte survival	Relaxation of vascular smooth muscle cells
		Presynaptic stimulation of NA release	–
beta 3	Gi-inhibiting AC, Gi-PLC-DAG-PKC, and Gi-NOS-NO-GC-cGMP-PKG	Negative inotropy, and myocyte survival	–
alpha 1A	Gq-PLC-IP3-Ca^{2+}	Positive effects on heart rate, inotropy and growth in myocytes	Vasoconstriction
alpha 1B	Gq-PLC-IP3-Ca^{2+}		
alpha 1D	Gq-PLC-IP3-Ca^{2+}	Contraction of smooth muscle cells	Vasoconstriction
alpha 2A	Gi-inhibiting AC	Presynaptically reduces sympathetic outflow	Lowers blood pressure
alpha 2B	Gi-inhibiting AC and also Gs-stimulating AC	Postsynaptically counteracts the effects of alpha 2A	Vasoconstriction in the periphery
alpha 2C	Gi-inhibiting AC	Presynaptically reduces sympathetic outflow Postsynaptically lowers cAMP	Lowers blood pressure Participation in vasoconstriction after exposure to cold temperatures

AC = adenylyl cyclase; cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; DAG = diacyglycerol; Gs, Gi, Gq, Gβγ = G-protein signaling components; GC = guanylyl cyclase; IP3 = inositol trisphosphate; NO = nitric oxide; NOS = NO synthase; PDK1 = PI3K-dependent kinase; PI3K = phosphatidylinositol 3-kinase; PKA, PKB, PKC, PKG = target-specific serine/threonine protein kinases; PLC = phospholipase-C.

ing phosphoinositide-3 kinase, phosphoinositide-3 kinase-dependent kinase-1, and protein kinase-B (also called Akt). This leads to increased expression of the antiapoptotic protein Bcl-2 and inhibition of proapoptotic factors such as caspase-9 and Fas-ligand (death factor) expression. The α1-AR subtypes all signal *via* the Gαq- phospholipase-C-IP3-Ca^{2+} pathway increasing cytoplasmic Ca^{2+}, whereas the α2-AR subtypes inhibit AC by the Gαi component, thus decreasing intracellular Ca^{2+}.

In general, the type-2 ARs (α2 and β2) are found at the prejunctional site in the central and peripheral sympathetic nervous system, where activation of α2-AR inhibits, and activation of β2-AR enhances norepinephrine release (fig. 3 and table 1). Presynaptically localized α2A-AR and α2C-AR subtypes are important in decreasing sympathotonus in the central nervous system as well as in decreasing the norepinephrine release in cardiac sympathetic nerve terminals.[21,24,42]

In most effector cells such as cardiomyocytes, endothelial or smooth muscle cells, the type-2 ARs are also present postsynaptically together with α1, β1, and β3-ARs. Acute changes in myocardial function are, however, almost exclusively governed by the β-AR signaling pathways. A functionally relevant contribution of α1-ARs appears unlikely in humans under normal conditions. However, in heart failure, β-ARs are desensitized and down-regulated (β1>β2), whereas the total amount of α1-AR subtypes remains constant or may even be upregulated. Under these conditions, the α1-ARs-mediated inotropy may prove important.[20,23,42,43] Although all three types of α1-AR are expressed in the heart, the α1A is the dominant subtype. Evidence from both *in vitro* and *in vivo* studies shows that the α1A-AR is the main subtype (approximately 90%) responsible for the sympathetic regulation of vascular tone and blood pressure.[44,45] Some α1B-AR and α1D-AR can be found in different parts of the vascular system, but little is known about their specific function.

No direct α2-AR-mediated effects are discernible on

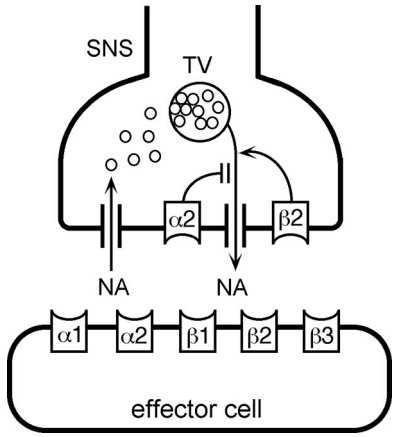

Fig. 3. Schematic representation of adrenergic synaptic transmission in the central and peripheral nervous system and at the site of target tissues. The effector cells may comprise cardiomyocytes, smooth muscle cells, or endothelial cells. SNS = sympathetic nervous system; TV = transmitter vesicle; NE = norepinephrine. Line with blunted end (=) indicates inhibition. From Reference 17, used with permission from Oxford University Press.

the myocardium. Also, the negative inotropy of β3-AR signaling mentioned above appears negligible under normal conditions.[16,46] However, in the failing heart where β3-ARs are up-regulated with concomitant down-regulation of β1-ARs, this negative inotropic effect may precipitate the deterioration of cardiac function.

The heart rate is directly linked to β-AR signaling *via* the Gαs component raising the cAMP levels. Specific pacemaker channels reside in the sinoatrial node controlling heart rate and rhythm.[47–49] These voltage-gated cation channels open during hyperpolarization after the action potential and allow Na^+ to enter the cell. This results in slow depolarization of the membrane potential until it reaches threshold level and elicits a new action potential. Stimulation of the pacemaker channels by sympathetic activity does not depend on phosphorylation but is induced by binding of cAMP to the channel protein. In contrast, stimulation of muscarinic acetylcholine receptors reduces cAMP levels *via* Gαi-mediated inhibition of AC, thus decreasing the heart rate (fig. 2).

The presynaptic inhibitory α2-ARs are the main site of action for the nonsubtype-specific α2-AR agonists clonidine and dexmedetomidine in the treatment of hypertension and postoperative pain, although some transitory peripheral vasoconstriction may be observed as a result of the postsynaptic α2B-AR.[50–52] Also, β-AR blockers used as antihypertensive drugs may reduce sympathetic drive by dampening the signaling activity of presynaptic β2-ARs, whereas their cardioprotective effects are predominantly mediated at the peripheral postsynaptic receptor level by decreasing β1- and β2-AR signaling in cardiomyocytes.

Lessons from Transgenic Animal Models

A Note of Caution

Given the complexity and intimate cross-connections of the adrenergic signaling network (fig. 2), it would not have been possible to delineate some well-defined basic mechanisms of the different AR subtypes without the advent of transgenic modification and gene knockout technology. However, it is unwise to directly translate insights from transgenic mice into human health and disease states. The following points of caution seem appropriate to consider before reporting some new findings: i) compensatory changes such as up-regulation or down-regulation of another component of a signaling pathway could offset the loss of a functional receptor, ii) altered expression of a receptor could cause different phenotypes in young and old mice, male and female, different genetic backgrounds or different environment, iii) in potentially lethal deletions, surviving animals may represent a phenotype that facilitated survival but that is not directly related to the deleted gene, iv) there may be differences between endogenous and overexpressed proteins with respect to their localization, interaction with other proteins, and specificity of signaling, and v) there may be a different temporal activation pattern of an overexpressed signaling component. The latter point may be illustrated by overexpression of the catalytic subunit of PKA that constitutes a constitutively active component whereas overexpression of AC is under the control of Gαs and thus dependent on β-AR activation.

Most of the knowledge regarding the β-AR subtype physiology *in vivo* has been derived from the use of subtype-selective agonists and antagonists. In contrast, no strictly subtype-specific agents are available for the characterization of the different α-ARs. Also, tissue-specific overlapping of the α-AR subtypes bias experimental and clinical observations.

The β-adrenergic Receptor

Myocyte-specific transgenic overexpression of β1-ARs, β2-ARs, and β3-ARs as well as knockout approaches for β1, β2, β3, and double knockout of β1/β2 have been applied.[16,53–55] Surprisingly, mice lacking either β1 or β2, or the double knockout of β1/2 do not exhibit cardiovascular limitations under basal conditions but show significant impairment in chronotropic range and vascular reactivity. β1 knockout mice achieve normal exercise capacity with normal metabolic response, whereas β2 knockout mice have increased exercise capacity with a concomitant shift from glycolytic to fatty acid metabolism and development of hypertension, probably resulting from unopposed α1-mediated peripheral vasoconstriction. β1/2 double knockout mice have normal exercise capacity but an impaired energy production, indicating that β1 and β2 serve both separate and common metabolic functions during exercise. Only ablation of both β-ARs reveals this stress-induced metabolic abnormality. The concomitant down-regulation of the cardiac muscarinic cholinergic receptor is thought to counterbalance the lack of adrenergic stimulation in the double knockout mice.

Cardiac-specific overexpression more than normal of either β1-AR (5–15-fold) or its receptor-coupled Gαs protein (threefold to fivefold) induces hypertrophy and apoptosis, resulting in congestive heart failure with premature death.[53–58] Propranolol, an unspecific β-AR antagonist, abolished the hypertrophic response and the development of dilated chambers, thereby improving survival. Overexpression of β2-ARs exhibits a biphasic gene-dose response: i) overexpression up to 60-fold induces enhanced ventricular contractility without hypertrophy or functional decompensation, ii) with overexpression greater than 100-fold, contractility was enhanced in young mice, but functional deterioration occurred over a 1-yr period, iii) overexpression in the range of 350-fold results in aggressive fibrotic dilated cardiomyopathy leading to death in mice older than 40 weeks, and iv) with overexpression of approximately 1000-fold, the mice died before the age of 5 weeks. In

conclusion, both β1-AR and β2-AR signaling enhance myocardial function, but the toxic effects of β2 become apparent only at much higher expression levels. The detrimental effects at high levels of β2-AR overexpression may be attributable either to a preponderance of Gαs signaling, as in the case of low β1-AR overexpression, or to an unduly high basal heart rate in these mice. Characteristically, physiologic and low level β2-AR overexpression signaling is accompanied by only a modest increase of cAMP.

Overexpression of human β3-AR does not affect cardiac morphology but is associated with an increase in basal heart rate and an acceleration of basal contractility *ex vivo*.[46] Stimulation with β3-AR agonists induces a significant negative inotropic response in these transgenic mice, as one would expect from the major β3-signaling pathway *via* Gαi. These findings agree with the observation that stimulation of endogenous β3-AR produces a decrease of contractility in human, dog, and guinea pig hearts.

Signaling Components Downstream of the β-adrenergic Receptors

Cardiac-specific overexpression of Gαs with a 38-fold increase of the mRNA and a threefold to fivefold increase of Gαs protein led to a continuously enhanced signaling, resulting in an augmented heart rate and contractility in young mice.[59] Over the lifespan, however, a dilated cardiomyopathy with fibrosis and degeneration atrophy developed. As expected, chronic β-AR blockade prevents the development of cardiomyopathy. Surprisingly, overexpression of the next downstream element in the β1-AR signaling cascade after Gαs, namely AC, is reported to augment adrenergically stimulated cAMP levels and to improve cardiac function in the absence of fibrosis and heart failure even in older animals.[57,60] Consequently, overexpression of AC was reported to rescue function in a heart failure model.[61] On the other hand, overexpression of the catalytic subunit of PKA, the next downstream signaling component, finds its way back into the line of canonical β1-signaling with all characteristic cardiotoxic symptoms.[62]

β-AR regulation of inotropy and heart rate largely depends on Ca^{2+} as a central messenger in the various signaling pathways (signaling for contraction, metabolism, and gene expression).[17] Thus, it is not surprising that overexpression of the sarcolemmal L-type Ca^{2+}-channel (more Ca^{2+} entering the cell) or of a "superinhibitory variant" of phospholamban (increased cytoplasmic Ca^{2+} because of inhibition of the cardiac Ca^{2+}-pump of the sarcoplasmic reticulum, SERCA2a), produces the symptoms of hypertrophy, fibrosis, apoptosis, and heart failure, as does overexpression of β1-AR.[55,63] Overexpression of wild-type phospholamban specifically entails depressed contractility (increased Ca^{2+} influx) followed by heart failure and premature mortality, but ablation of phospholamban concomitant with overexpression of β1-AR rescues functional, morphological, and molecular characteristics of heart failure in transgenic mice.[64] On the other hand, homozygous ablation of the genes for NCX1 (sarcolemmal Na^{+}-Ca^{2+}-exchanger), SERCA2a, or the sarcoplasmic reticulum Ca^{2+} release channel RYR2 (called ryanodine receptor) causes embryonic lethality. Taken together, these findings imply a primary role for Ca^{2+} signaling in myocyte toxicity whether affected directly by altered proteins involved in the intracellular Ca^{2+} handling or affected indirectly by the β1-AR system.

Results on transgenic expression of Gαi, mainly linked to β2-AR signaling, are complex and difficult to interpret.[55,65–67] In human congestive heart failure, Gαi is up-regulated. The resulting reduced activity of AC is thought to be responsible for the impaired systolic function in these hearts. Intuitively, the enhanced Gαi signaling may be regarded as compensatory response to β-AR overstimulation in patients with congestive heart failure. Whether the up-regulation of Gαi under these conditions is maladaptive or beneficial remains elusive. Chronic overexpression of Gαi in mice proves, however, to have detrimental effects by impairing excitability (wide QRS complex in the electrocardiogram) and rapidly leading to dilated cardiomyopathy. On the other hand, Gαi signaling prevents pacing-mediated enhanced contractility in mice overexpressing the Gαi-coupled receptor Ro1. Ro1 is a modified κ-opioid receptor that couples to Gαi and retains normal signaling in response to an exogenous administered drug, spiradoline.[68] Short-term overexpression of Gαi in isolated hearts and myocytes attenuates, as expected, positive inotropic effects resulting from β-AR stimulation with isoproterenol. To test the potential cardioprotective effect of Gαi in response to strong chronic β2-AR signaling, mice with targeted deletion of the Gαi2 (heterozygous and homozygous) were crossbred with mice highly expressing β2-ARs. β2-AR overexpressing mice developed severe hypertrophy and died of heart failure if not crossbred with Gαi2. When missing Gαi2, the deleterious effects are significantly precipitated. The results from intercrossed species indicate that the isoform Gαi2 adopts an essentially protective part in the signaling of β2-AR by preventing hypertrophy and increasing lifespan. However, the isoform Gαi3 is compensatorily up-regulated in the β2-AR overexpressing mice and seems to be responsible for the disturbed excitability. In conclusion, overexpression of Gαi may hold no promise for future therapeutic use.

The α-adrenergic System

The many reports on overexpression and knockout of α-AR subtypes, albeit with sometimes controversial outcomes, allow to characterize their principal physiologic functions.[22,23,54–57] The α1A-ARs and α1B-ARs are func-

tionally important for both the myocardium and the vasculature, whereas the α1D-ARs seem to play a role in the resistance and conduit arteries. Knockout of either α1A-ARs or α1B-ARs or double knockout of α1A/B-ARs all result in hypotension and reduced pressor response to phenylephrine but little effect on contractility of the normal myocardium. However, α1-AR-mediated inotropy may serve as backup system when β1-AR signaling is down-regulated during chronic heart failure. The double knockout of α1A/B-ARs causes a retardation of cardiac growth.

Overexpression of α1-AR (100-fold) has hardly any hypertrophic effect on the heart. Mice and humans seem to have rather low levels of α1-ARs in the myocardium, which are much higher in the rat. Therefore, species differences must always be taken into account. On the other hand, overexpression of a constitutively active mutant of α1B-AR (threefold and 26-fold) as well as overexpression of the wild-type α1B-AR (20–40-fold), both induce hypertrophy with up-regulation of the characteristic protein markers, which may lead to heart failure with increasing age. Overexpression of the α1B-AR is further known to interfere with β-adrenergic signaling by activation of protein kinase-C (fig. 2) and to induce heterologous desensitization of β-ARs.[69] In other words, molecular crosstalk may uncouple the β-AR system.

Overexpression of either the α1A-AR or the α1B-AR induces only moderate changes in the normal heart. It may be hypothesized that both receptor subtypes could act together in a concerted way to induce pronounced hypertrophic signaling, as both activate primarily Gαq. However, Gαq also serves as a downstream signaling element of receptors stimulated by angiotensin-II and endothelin, both strong mediators of cardiac hypertrophy. It is thus not surprising that overexpression of Gαq (mimicking α1-AR hyperactivation, as α1-ARs preferentially couple to Gαq) induces similar cardiotoxicity, such as heart failure and premature death, as observed with overexpression of β-AR signaling elements. Gαq signaling involves the phospholipase-C and the phosphoinositide-3 kinase pathways, both of which are ramified and complex, as in the case of β2-AR signaling (fig. 2). Overexpression of Gαq induces the cardiomyopathic changes by activating all three major MAPK pathways, extracellular signal regulated kinase, c-Jun amino-terminal kinase, and p38 MAPK. This sequence of events is comparable to what has been described under experimental pressure overload induced by aortic constriction.

Mice with ablation of any of the three α2-AR subtypes develop normally.[21,23,24,52] In the case of α2B-AR knockout, however, the animals are not born at the expected Mendelian ratios, indicating that this receptor may play a role in embryonic development. Because of the widespread and varied distribution over the central and the peripheral nervous system and the lack of sufficiently subtype-specific agents, it is difficult to assign distinct functions to the three α2-AR subtypes. Only the knockout approach allowed unraveling subtype-specific properties, which however extend over diverse physiologic areas such as regulation of sympathetic tone, neurotransmitter release, blood pressure, intraocular pressure, analgesia, sedation, and some aspects of behavior. For instance, the vascular α2-AR subtypes are differentially distributed between vascular beds. Several functions are even overlapping, especially among the α2A-ARs and α2C-ARs. Both α2A-ARs and α2C-ARs fulfill a feedback function by operating as presynaptic receptors in sympathetic neurons, complementing each other to integrate central nervous system function and behavior (fig. 2). Shortly, the α2A-AR activity predominantly monitors the antinociceptive, sedative, and hypotensive actions. Stimulation of α2B-AR elicits transitory vasoconstriction. Finally, α2C-ARs modulate dopaminergic neurotransmission, various behavioral responses, induce hypothermia, and, in mice, contribute to spinal antinociception of the imidazoline and α2-agonist monoxidine.[70] In the periphery the α2C-ARs represent a silent intracellular receptor population that on moderate cooling translocate from the Golgi complex to the cell membrane of smooth muscle cells, causing vasoconstriction *via* activation of the monomeric small guanosine triphosphatase signaling component RhoA and Rho-dependent kinase.[17,71]

The biphasic vascular response upon nonspecific α2-AR stimulation can now be attributed to an initial constriction mediated by the postsynaptic peripheral α2B-AR lasting as long as the agonist is present, followed by nitric oxide release and centrally mediated hypotension depending on α2A-ARs possibly adjoined by α2C-ARs.[52] Mice lacking either α2A-AR or α2C-AR seem to acquire a risk for hypertension and coronary events. Double knockout of α2A-ARs and α2C-ARs produces mice with severe insufficient feedback inhibition of neuronal catecholamine release developing cardiac hypertrophy, fibrosis, and ultimately heart failure.

Therapeutic Potential from Transgenic Approaches

Enhanced sympathetic drive induces myocardial remodeling that results in hypertrophy and progression to heart failure. Chronically increased angiotensin-II and endothelin levels, besides the catecholamines, represent equally potent stimuli for detrimental cardiac remodeling. All three stimuli use the major signaling cascades involving Gαs and Gαq.[30] The cardiotoxic outcome from overstimulation of these signaling pathways was experimentally documented including the transgenic approaches. Perioperatively, increased long-term basal sympathetic activity along with repetitive short-term peaks stimulate the same signaling pathways. It is therefore of interest whether the damaging adrenergic over-

stimulation can be attenuated by transient transfection *in vivo* at least over a limited period of time. A number of long-term and short-term studies have attempted to prove the validity of a therapeutic genetic approach in principle.[54–58,62]

As counterbalancing β-adrenergic overstimulation by overexpression of Gαi was not successful, efforts focused on preventing overstimulation of Gαq, one of the central pivotal signaling elements. Moreover, β1-AR overstimulation can efficiently be controlled by systemic application of specific antagonists like atenolol or metoprolol. Transgenic targeting of a therapeutic molecule to the heart by myocyte-specific promoters, however, prevents extracardiac side effects. The protective effects of β2 signaling can be transgenically applied to rescue the cardiotoxicity of Gαq. Mice with different expression levels of β2-AR (30-fold, 140-fold, and 1000-fold above background) were crossed with strains overexpressing Gαq fivefold. The low expression (30-fold) of β2-AR completely prevents development of the characteristic pathologic Gαq phenotype. Medium expression level (140-fold) does not further improve ventricular function, and with the high gene-dose (1000-fold) the animals die from cardiomegaly within 5 weeks.

Another approach is based on the finding that in chronic heart failure β1-ARs are down-regulated concomitant with up-regulation of the β-AR kinases (GRKs), giving rise to a vicious circle because GRKs initiate homologous desensitization of the receptors. A 194 amino acid inhibitory peptide (194GRK) from the carboxyl terminus of GRK2 was shown to bind to Gβγ.[57] This prevents recruitment of the endogenous GRK and thereby stops homologous desensitization of β-ARs. As expected, overexpression of the aforementioned inhibitor 194GRK alone produces an increase in β-ARs with enhanced ventricular contractility both at baseline and in response to isoproterenol. Crossing animals overexpressing 194GRK with several genetic mouse models of cardiomyopathy leads in all cases to functional improvement and prevention of chamber dilation. Both approaches, overexpression of β2-AR or of 194GRK, have been adapted for adenoviral-mediated *in vivo* transfection of normal rabbit hearts.[54,57] Enhancement of global left ventricular contractility is manifest 3 and 6 days after intracoronary application indicating that sufficient gene transfer has occurred *via* this route. Three weeks after coronary artery ligation with ensuing myocardial infarction, rabbit hearts appear to recapitulate the biochemical characteristics of human heart failure, including a decrease in β-AR density and signaling accompanied by an increased expression of GRKs. Delivery of the 194GRK transgene at the time of coronary ligation prevents the biochemical abnormalities as well as the hemodynamic failing. Gene delivery 3 weeks after arterial ligation, *i.e.*, when post-myocardial infarction heart failure is already developing, is still able to reverse heart failure despite the loss of functional myocardium. Taken together, these findings indicate that adrenergic modulation by transient genetics serving as "molecular ventricular assistance" may become feasible as a novel therapeutic strategy in perioperative medicine.

Further approaches to rescue failing heart conditions have been reported. First, heart-specific expression of a 54 amino acid inhibitory peptide (54Gαq) derived from the carboxyl-terminus of Gαq itself. 54Gαq inhibits the interaction of Gαq with the agonist-occupied receptors and is able to attenuate the pressure-induced cardiac hypertrophy after aortic constriction in transgenic mice by blocking Gαq signaling.[56] Second, AC activity as rate limiting step in β-AR signaling represents another potential candidate for maintaining cardiac function without untoward adrenergic consequences.[72] In particular, the two major cardiac isoforms AC5 and AC6 expressing mice have been bred separately into Gαq transgenic animals. Both AC isoforms are able to rescue the Gαq-associated cardiomyopathy by improving contractile function and increasing survival. Last, mice lacking the dopamine β-hydroxylase that converts dopamine into norepinephrine have neither norepinephrine nor epinephrine in their circulation (gene-targeted sympathectomy).[56] This is associated with enhanced β-AR responsiveness and a reduction in GRK2 that desensitizes β-ARs. The pressure-overload-induced cardiomyopathy elicited by aortic constriction is significantly attenuated in dopamine β-hydroxylase knockout mice. In addition, the activation of all three MAPK signaling pathways (extracellular signal regulated kinase, c-Jun amino-terminal kinase, and p38 MAPK) induced by pressure overload in control mice is completely absent in the dopamine β-hydroxylase knockout animals. These data demonstrate the absolute requirement of endogenous epinephrine and norepinephrine for *in vivo* induction of cardiac remodeling and heart failure. Whether any of these latter transgenic approaches may attain clinical significance remains to be determined in future randomized controlled trials.

Collectively, the adrenergic signaling network seems to follow a tightrope walk; anything too much may harm the cardiovascular system, and anything too little may not be enough to meet the hemodynamic demands.

Human Genetic Polymorphisms: Clinical Correlates of Modified Adrenergic Responses

Genetic heterogeneity in populations forms the basis of interindividual variations, influences cardiovascular phenotypes, and significantly affects the natural history of many cardiovascular disease states. In contrast to rare mutations, which may be the single basis of an inherited disease, a polymorphism has by definition an allele frequency of >1% in a population. Most polymorphisms in the human genome occur at single nucleotide sites and have been designated "single nucleotide polymor-

Table 2. Nonsynonymous Polymorphisms of Adrenergic Receptors (Adapted From Ref. 33 and References Therein)

Receptor	Amino acid position	Alleles coding for amino acids: Major	Minor	Caucasians (%)	African Americans (%)	*In vitro* functional effects from studies on cell expression systems	Pathophysiological effects from human studies
beta 1	49, near N-term	Ser	Gly	15	13	Gly49 enhances agonist-promoted downregulation	Gly49 allele is associated with improved survival
	389, in Gs-coupling domain	Arg	Gly	27	42	Arg389 strongly stimulates AC; Gly389 impairs Gs-coupling	Arg389 risk for heart failure only in combination with alpha2C Del322–325
beta 2	16, near N-term	Gly	Arg	39	50	Gly16 enhances agonist-promoted downregulation	Gly16 segregates with hypertension
	27, near N-term	Gln	Glu	43	27	Glu27 resistant to agonist-promoted downregulation	Glu27 segregates with hypertension
	164, in the fourth transmembrane domain	Thr	Ile	2–5	2–5	Ile164 impairs coupling to the stimulatory Gs-protein	Heterozygous Ile164 entails rapid progression of heart failure with significant decrease of survival
beta 3	64, end of first transmembrane domain	Trp	Arg	10	?	Arg64 possibly displays lower G-protein coupling	Arg64 may be involved in obesity and type-2 diabetes, relevance for the heart not well established
alpha 1A	492, near C-term	Cys	Arg	46	70	Similar pharmacological and functional characteristics	Similar prevalence of each polymorphic allele in various diseases
alpha 2A	251, in the third intracellular loop	Asn	Lys	0.4	5	Lys251, a rare polymorphism enhanced Gi-coupling-function	Centrally mediated hypotension
alpha 2B	301–303, Glu-Glu-Glu	No deletion	Deletion	31	12	Only partially phosphorylatable, resistant to desensitization	Increased vasoconstriction, risk for coronary events if homozygous
alpha 2C	322–325, Gly-Ala-Gly-Pro	No deletion	Deletion	4	38	Control of basal NE-release, impaired Gi-coupling	Risk for idiopathic and ischemic cardiomyopathies (homozygous)

AC = adenylyl cyclase; Ala = alanine; Arg = arginine; Asn = asparagine; C-term = carboxyl-terminal residue; Cys = cysteine; Gln = glutamine; Glu = glutamic acid; Gly = glycine; Gs, Gi = G-protein signaling components; Ile = isoleucine; Lys = lysine; N-term = amino-terminal residue; NE = norepinephrine; Pro = proline; Ser = serine; Thr = threonine; Trp = tryptophan.

phisms" (SNPs).[73] By mid-2004 the human genome will include a catalog of around seven million of the total of approximately 10 million SNPs. Other mutations involving insertion/deletion polymorphisms consist of changes in larger segments of DNA but are less common. Within coding regions of genes, SNPs may encode for different amino acids (nonsynonymous polymorphisms) or, because of redundancy of the genetic code, may have no effect on the encoded amino acids (synonymous polymorphisms). Nonsynonymous polymorphisms may have no effect, may have effects that are clinically silent but can be delineated by physiologic testing, may represent a risk factor because of their altered physiologic function, can act to modify diseases, or can alter the response to therapeutic drug administration. Single nucleotide polymorphisms not directly connected with functional alterations may serve as markers for identification of chromosomal disease loci. Currently, seven of the nine adrenergic receptor subtypes have been found to display polymorphisms of nonsynonymous point mutations as well as insertion/deletion mutations (table 2). However, their clinical relevance is not known in all cases.[33]

The functional effects of relevant polymorphisms as derived from *in vitro* and *in vivo* experimental approaches require examination and verification in the clinical setting. Acute effects may be tested by administration of specific adrenergic agonists or antagonists to healthy volunteers or to patients and measuring pulmonal function (bronchial dilatation), vasodilatation, or cardiac function (heart rate and contractility). Insight into chronic consequences of polymorphisms on a particular type of tissue may be obtained by assessing their association frequencies with certain pathophysiological states such as essential hypertension or cardiac hypertrophy.

β1-AR Polymorphisms

β1-AR polymorphisms at codons 49 (serine or glycine in the extracellular amino-terminal domain) and 389 (ar-

Table 3. Combined Polymorphisms in the Major Haplotypes of the Human β2-adrenergic Receptor and Their Ethnic Frequencies (Adapted from References 33 and 81)

	Amino acid position with nonsynonymous polymorphism				Frequency in different ethnic groups (%)			
Haplotype	Cys19Arg in the upstream regulatory peptide (BUP)	Gly16Arg	Gln27Glu	Thr164Ile	Caucasian	African-American	Asian	Hispanic-Latino
1	Cysteine	Arginine	Glutamine	Threonine	0.7	25.0	12.5	10.0
4	Cysteine	Arginine	Glutamine	Threonine	33.0	29.7	45.0	40.0
2	Arginine	Glycine	Glutamate	Threonine	48.3	6.3	10.0	26.7
6	Cysteine	Glycine	Glutamine	Threonine	13.2	31.3	30.0	13.3

Haplotype numbers correspond to those used in (33) (Table 2 therein). Haplotypes 1 and 4 vary in the promoter region further upstream of the regulatory peptide whose functional consequences are presently not known. Isoleucine (Ile) at position 164 is found only in one of the eight rare haplotypes in heterozygotes precipitating heart failure and mortality (see Table 2 and text section on β2-AR polymorphisms, third paragraph).

ginine or glycine in the carboxyl-terminal cytoplasmic tail of the receptor, which is involved in G-protein binding) have been previously described.[33] One study found an improved prognosis in heart failure associated with glycine49 as compared with serine49. In contrast, patients homozygous for arginine389, which is somewhat more frequent in Caucasians than African-Americans and exhibits enhanced Gαs-coupling, develop higher baseline heart rate and diastolic blood pressure, and have an increased risk of hypertension. The substitution of arginine at position 389 instead of glycine has recently been shown by transgenic overexpression in mice to represent a gain-of-function variant of the β1-AR.[74] Overexpression of β1-arginine389 in young animals (in comparison to overexpression of the β1-glycine389 variant) resulted in enhanced AC activity, which in turn stimulated cAMP-dependent PKA activity, producing an increased basal and agonist-induced cardiac contractility and heart rate. With advancing age, however, the arginine389 mice developed cardiac depression with a reduction of AC activity, of Gαs expression, of phospholamban phosphorylation, and of the sarcoplasmic reticulum Ca^{2+}-pump SERCA2a. Agonist-induced contractility and heart rate reserve were also depressed. Thus the human β1-arginine389 variant also predisposes in the mouse model to heart failure by instigating hyperactive signaling programs.

β2-AR Polymorphisms

The most intricate array of polymorphisms has been described for the β2-AR gene so far. The intronless gene displays nine different point mutations within the coding region, of which four are nonsynonymous resulting in amino acid changes (glycine16arginine, glutamine27glutamate, valine34methionine, and threonine164isoleucine).[33,75] The allele frequency at codon 34 is less than 1%. The other three mutations (at the codons 16, 27, and 164) are functionally relevant, the first two affecting agonist-induced down-regulation of the receptor and the third altering its coupling properties to the stimulatory Gαs-protein (table 2). These findings stem from experiments with heterologous expression of receptor constructs as well as from primary cell cultures endogenously expressing these variants and reflect the molecular properties of the individual polymorphisms. The approximate location of the pathophysiologically relevant polymorphisms at positions 16, 27, and 164, are given in figure 1.

Subsequent clinical studies assessed the association of these β2-AR polymorphisms with diseases such as asthma or hypertension on the basis of the role of the β2-AR in modulating bronchial and vascular smooth muscle tone.[33,76] The presumption in these studies was that patients with the glycine16 polymorphism were prone to bronchoconstriction or vasoconstriction as a result of the enhanced down-regulation of the receptors by endogenous catecholamines (as compared with those having the arginine16 receptor) entailing a reduced potential for agonist-induced relaxation of the smooth muscle cells. In contrast to the expected adverse effects of glycine16, the glutamate27 polymorphism, based on the *in vitro* experimental data, is supposed rather to induce smooth muscle relaxation because its resistance to down-regulation (as compared with the glutamine27 species) may account for the higher responsiveness to catecholamines. However, differences in frequency of these two common polymorphisms were not observed between asthmatics and nonasthmatics, although they were found to act as significant disease modifiers.[76] The glutamate27 allele enhances vasodilation to variable degrees, probably depending on zygosity and its combination with either glycine16 or arginine16.[77] Studies on the glycine16arginine polymorphism are even more equivocal because either the glycine16 or the arginine16 allele, or none of them, was found to be associated with hypertension.[33,78] An explanation for the combination of glycine16 with glutamate27 remaining clinically silent could be relate to the balancing of the adverse (glycine16) and the beneficial (glutamate27) effects. Both polymorphisms display moderate differences in allele frequency between Caucasians and African-Americans (table 3).

Conversely, the rare isoleucine164 (2–5%) polymorphism displays a reduced formation of the agonist-receptor-Gαs complex with more consistent functional consequences.[33,79] A decreased AC activity followed by impaired exercise capacity and significant decrease in survival was found in isoleucine164 receptor transgenic mice, which corresponds to the depressed agonist-promoted function. As the β2-AR accounts for 20–30% of total cardiac β-AR, it was expected that the heart function may be affected as well. Normal volunteers with the isoleucine164 allele showed a decreased responsiveness of heart rate and contractility to infusion of the β2-AR agonist terbutaline.[80] In long-term observations, a worsened outcome of congestive heart failure was indeed clearly associated with the heterozygous isoleucine164 polymorphism.[33] The 1-yr survival for this polymorphism was 42% as compared with 76% for patients with the usual threonine164 β2-AR. In a 3-yr follow-up, individuals with the isoleucine164 allele showed a rapid progression toward either death or transplantation. Homozygosity for isoleucine164 was never found. These findings uphold the notion that a normally functioning β2-AR plays a life-supporting (antiapoptotic) compensatory role in patients with chronic heart failure.

A mechanistic explanation for the equivocal results in the clinical setting, at least in part, may derive from the strong linkage disequilibrium between the codons for the positions 16 and 27.[33,76,81,82] Eight more polymorphisms found in the promoter region immediately 5'-upstream of the start codon (nucleotide triplet ATG) add to the complexity. This region contains several putative cis-acting regulatory elements including a short stretch with an open reading frame coding for a regulatory peptide of 19 amino acids (β2-AR upstream peptide or BUP). *In vitro* and *in vivo* studies indicate that the BUP impedes translation of β2-AR mRNA and thus regulates cellular expression of the receptor. One polymorphism concerns the last 3'-codon, changing the carboxyl-terminal cysteine (homozygous in 39%) to arginine (homozygous in 13%), with the remainder being heterozygous. Thus, the allele frequency is 0.63 for cysteine and 0.37 for arginine in a Caucasian population without acute or chronic disease. β2-AR expression in a cell culture system was almost half as much with the arginine-containing BUP compared with the cysteine-BUP.

Linkage analysis of 13 synonymous and nonsynonymous polymorphisms (eight in the promoter region including the BUP, and five in the protein coding region) in the sequence stretching from nucleotide minus-1100 into the coding sequence down to nucleotide 700 of the β2-AR gene yielded only 12 haplotype combinations of 8192 possibilities.[33,76,82] The coding region contains four more SNPs further downstream whose hyplotypic organization is not known. The specific set of SNPs (or alleles) observed on a single chromosome or part of a chromosome is called a haplotype. New haplotypes are formed by additional mutations, or by recombination when the parental chromosomes exchange corresponding DNA segments, resulting in a chromosome that is a mosaic of the two parental haplotypes.[83] The coinheritance of SNP alleles on these haplotypes over many thousands of generations in a population is known as linkage disequilibrium. The likelihood of separation of two SNP alleles by recombination events increases with the distance between them; thus its linkage equilibrium decreases. Nearby SNPs usually have a high linkage disequilibrium. Such established haplotypes are strongly conserved in the human genome because recombinations preferentially happen in hot spots outside the genes.[84]

Of the 12 β2-AR haplotypes, only four are common (table 3); the remaining eight are uncommon (<1% in Caucasians and <5% in other ethnicities). The four common haplotypes display marked frequency differences between ethnic groups. The two most common Caucasian homozygous haplotypes (2/2) and (4/4) were studied in a transient expression system to ascertain the functional relevance of these haplotypes. β2-AR mRNA and protein expression were indeed lower for the type-2 (with arginine-BUP, glycine16, and glutamate27) than for the type-4 (with cysteine-BUP, arginine16, and glutamine27) haplotype. This was paralleled by a three times larger increase of expiratory volume in response to the β2-AR agonist albuterol in asthmatic patients homozygous with the 2/2 as compared with those having the 4/4 haplotype.[82] With hindsight this could be explained as follows: i) the haplotype-2 retains a high receptor level ready to respond to albuterol because glutamate27 resists agonist-induced desensitization, thus glutamate27 apparently overcomes the adverse effects of arginine-BUP with its lower rate of receptor synthesis as well as the potentially enhanced agonist-promoted down-regulation by glycine16, ii) although the haplotype-4 with cysteine-BUP displays a higher rate of receptor synthesis, the normal desensitization rate by the endogenous catecholamine levels results in a low receptor reserve ready for an acute agonist response.

The results observed in the airways correspond to an enhanced desensitization of vascular β2-AR in healthy subjects homozygous for both arginine16 and glutamine27 (haplotype-4) when challenged by isoproterenol in comparison with subjects homozygous for both glycine16 and glutamate27 (haplotype-2) displaying resistance to agonist-mediated down-regulation (enhanced vasodilation due to increased agonist responsiveness).[77] In another study, the systemic effect of salbutamol on diastolic blood pressure indicated an agonist-induced down-regulation of the β2-AR in Caucasian asthmatic subjects homozygous for arginine16/glutamine27 (haplotype-4) and a resistance to down-regulation with homozygosity of glycine16/glutamate27 (haplotype-2).[85] A similar resistance to down-regulation also occurred with

homozygous glycine16/glutamine27 that corresponds to the common haplotype-6 (table 3). No rational explanation can be offered for this latter finding, as in the absence of glutamate27 the enhanced down-regulation ascribed to glycine16 would be expected. A slower desensitization was also observed in genotyped healthy subjects homozygous for glutamate27 when challenged by terbutaline and assessed for cardiac function (heart rate and contractility), although with ongoing agonist treatment over several days, glutamate27/glutamate27 reached as low a responsiveness as did the glycine16/glycine16 homozygosity.[86] Taken together, these findings seem to indicate that *in vivo* the homozygous arginine16/glutamine27 (haplotype-4) is responsible for fast agonist-mediated down-regulation, whereas homozygous glutamate27 is required to confer resistance to desensitization. This holds for acute testing of smooth muscle (in the airways and in the vasculature) and of the myocardium.

However, heterozygous asthmatics with the 4/6 haplotype combination (table 3) displayed an even larger response than did those with the 2/2 homozygotes to the challenge with albuterol.[82] In this case no intelligible explanation can be offered based on the effects of the individual allelic SNPs. The increased rate of receptor synthesis by cysteine-BUP was of no benefit in the 4/4 homozygotes and the glycine16 in the haplotype-6 would even enhance the normal agonist-promoted down-regulation and diminish the receptor reserve, yet the 4/6 combination exhibits the highest response to albuterol. Thus, additional effects hidden in the two different haplotypes and possibly deriving from their interplay, must contribute to the observed functional phenotype. Nevertheless, distinct functional phenotypes could be attributed to each of the five most common haplotype pairs (together accounting for almost 88%) in Caucasians: 2/2, 4/4, 2/4, 2/6, and 4/6. The homozygous pair 6/6 has not been found so far. Preliminary evidence suggests that the coding region polymorphisms are also in linkage disequilibrium with the promoter polymorphisms and hence some of the unexplainable physiologic effects could possibly be related to altered gene expression patterns.[81]

Among the various, somewhat conflicting, reports on linkage of β2-AR polymorphisms with essential hypertension, one of the most reliable studies involving sib-pairs from 55 pedigrees and approximately 2500 individuals from 589 families found a consistently increased risk in subjects with either glycine16 or glutamate27 alleles.[87] The odds ratio for the occurrence of hypertension was 1.80 for the glutamate27 allele. There was a linear increase of blood pressure levels with increasing number of glutamate27 alleles (glycine16/glycine16 < glycine16/glutamate27 < glutamate27/glutamate27). With regard to position 16, the glycine16 allele was more frequent than arginine16 among hypertensive individuals, indicating that this position is also significantly associated with the diagnosis of hypertension. Glycine16, as opposed to arginine16, exhibited greater down-regulation in cellular transfection assays in response to isoproterenol, resulting in a reduced agonist sensitivity.[75] A blunted agonist response could likely produce an increase in blood pressure. Conversely, glutamate27 was resistant to down-regulation in cell transfection experiments, suggesting an increased sensitivity to catecholamine agonists for cells carrying this allele.[33] Nevertheless, this latter effect was only evident in the presence of the arginine16 allele, and cells transfected with the glycine16/glutamate27 combination displayed an increased down-regulation. Consequently, the haplotypic combination of arginine16/glutamate27 should be associated with a low blood pressure as a result of its high agonist sensitivity. However, the arginine16/glutamate27 combination is found in none of the 12 naturally occurring haplotypes.[82] On the other hand, the glycine16/glutamate27 combination segregates with high blood pressure because of its down-regulation and subsequent agonist insensitivity. At the same time, glycine16/glutamate27 represents the most common haplotype in Caucasians (table 3). Furthermore, individuals homozygous with glutamate27 are almost exclusively homozygous also for glycine16.[88] In accordance, a higher glycine16 allele frequency (in the possible combination either with glutamine27 or glutamate27) was also reported for hypertensive African-Caribbean men and women and an Austrian male population as well.[89,90]

In conclusion, both amino-terminal polymorphic positions 16 and 27 contribute to the long-term development of essential hypertension. Given the multigenic nature of the hypertensive status, it may be no surprise that the β2-AR polymorphisms account for only approximately 2% of the total variation of blood pressure among a Caucasian population.[87] The effect size does not, however, preclude the possibility that the β2-AR polymorphisms may have a larger impact in population subsets. As observed with the acute testing, some haplotype combinations may also generate unpredictable outcomes during their long-term influence on the cardiovascular system. The hypothesis that the agonist insensitivity resulting from enhanced down-regulation of the β2-AR with the glycine16/glutamate27 haplotype being causally linked to hypertension rests on *in vitro* transfection experiments.[33,87] It is at odds, however, with its high agonist responsiveness and resistance to down-regulation found in acute testing on airways, vasculature, and heart functions.[77,82,85,86] The chronic development of hypertension may be laden with adaptational and unforeseeable environmental interactions, which may not allow to distinguish between the primary phenomenon and secondary processes. This calls for caution with interpretational attempts when comparing the results from acute functional testing of defined haplotypes

and their association frequencies with chronic disease states.

β3-AR Polymorphisms

In one nonsynonymous polymorphism (tryptophan64-arginine) of the β3-AR, arginine present in approximately 10% of Caucasians replaces the Trp most proximal to the first intracellular loop at the end of the first transmembrane domain (table 2).[91] Interestingly, Japanese and Alaskan Eskimos have higher arginine64 allele frequencies.[92] Arginine64 seems to cause a lower G-protein coupling with a decreased agonist-stimulation of lipolysis. This is discussed within the context of adipose tissue and diabetes but has not yet been linked to cardiovascular effects. An interesting point of note is that arginine64 is found in all β3-AR genes cloned from various species except for humans.[33] The pharmacological properties between rodent and human β3-AR are quite different, suggesting that this receptor serves somewhat different functions in the two species.

α-AR Polymorphisms

Only one nonsynonymous polymorphism cysteine492-arginine has been described for the α1A-AR subtype, which is fairly common among Caucasians (46%) and African-Americans (70%), but without apparent pathophysiological consequences (table 2).[33,93] Two exonic synonymous polymorphisms not changing the amino acid sequence have been reported for the α1B-AR gene.[94] A rare polymorphism concerns the α2A-AR (Asn251Lys), in which the Lys251 genotype induces enhanced coupling function with Gαi, leading to centrally-mediated hypotension.[95]

In the α2B-AR a deletion polymorphism (Del301–303) was found to be a risk factor for experiencing acute coronary events.[96] More recently, the homozygous deletion was shown in Caucasian men to be associated with coronary microvascular constrictions and sequelae such as myocardial infarction and sudden cardiac death.[97] This deletion variant lacks three glutamate residues within the third intracellular loop essential for phosphorylation by GRK.[17,98] The deletion entails a reduced degree of phosphorylation in that region that results in a complete loss of agonist-promoted desensitization and a concomitant sustained activity. This deletion polymorphism represents a "loss of structure gain of function" variant. The molecular properties and physiologic consequences tie in with the clinical observation of increased coronary events in patients carrying this deletion polymorphism.[52] On the other hand, the fact that the postsynaptic peripheral α2B-AR induces only transitory vasoconstriction is compatible with its deletion polymorphism not being associated with essential hypertension *per se*.[96]

A recent study evaluated the significance of another deletion polymorphism, in the α2C-AR gene (Del322–325) coupled with the β1-arginine389 polymorphism in patients with congestive heart failure. The four-amino acid deletion in the third intracellular receptor loop impairs its binding to the AC inhibiting Gαi-protein, thus reducing the inhibition of norepinephrine release.[99] As a result of its presynaptical localization in cardiac nerve terminals, this leads to chronically increased norepinephrine liberation. In addition, several intracellular signaling pathways are impaired, such as inositol phosphate release *via* phospholipase-C and MAPK cascades. On the other hand, the β1-arginine389 variant exhibits enhanced coupling to AC *via* interaction with Gαs, thus also increasing catecholamine levels.[33] Hence, both polymorphisms markedly potentiate catecholamine effects. The α2C-Del322–325 is as frequent as 38% among African-Americans compared with only 4% in Caucasians and, when homozygous, proved a strong risk factor for heart failure. The risk becomes even greater among persons with simultaneous homozygosity for α2C-Del322–325 and β1-arginine389. However, the β1-arginine389 genotype alone was not associated with increased risk for heart failure, whereas the frequency of the α2C-Del322–325 was greater among heart failure patients, at least in Caucasians. These observations imply that the simultaneous presence of two mechanisms potentiating the norepinephrine effects substantially increases the risk for heart failure. They further provide evidence that α2C-AR stimulation is clearly associated with cardioprotection. The importance of catecholamine-induced feedback inhibition *via* two α2-AR subtypes (α2A-AR and α2C-AR) was experimentally confirmed in transgenic animal models.[21,24]

Polymorphisms of Receptor Downstream Signaling Components and More

Polymorphisms may occur at any level of the multistep signaling cascades, including the Ca^{2+} handling proteins and other components interwoven with the adrenergic system (fig. 2).[17] A few cases may be paradigmatically mentioned here. Signaling affecting polymorphisms have been identified in the stimulatory Gαs1 and Gβ3 subunits of the receptor-coupled G-protein complex.[100–103] Both these signaling components may couple to several different adrenergic receptors (fig. 2), and their polymorphic deviations cause enhanced signaling, which originally was associated with hypertension. The gene (GNAS1) for Gαs1 displays a common synonymous polymorphism at the nucleotide level T393C involving a change of the codon 131 from ATT to ATC (approximately 50% each) leaving the amino acid isoleucine unchanged. TT and TC allele combinations segregate with the risk for development of hypertension, whereas the CC homozygosity protects against hypertension. Carriers of the T allele show a poor response to β-AR agonists because of uncontrolled constitutively active signaling, whereas homozygous CC carriers retain the receptor controlled signaling and are strongly sensi-

tive to agonist stimulation.[102] As the amino acid isoleucine is unchanged by this nucleotide polymorphism, an association of the T allele with a haplotype leading to a dysfunctional Gαs1 protein is suggested.

Another silent nucleotide polymorphism at nucleotide position C825T in exon-10 of the gene (GNB3) for the Gβ3 subunit also affects signaling *via* the Gβγ dimer.[101] The T825 allele is associated with a splice variant yielding a shorter protein missing 41 amino acids normally encoded by exon-9.[104] This protein variant is constitutively active and found to be associated with patients with essential hypertension. It remains unresolved how the remote nucleotide exchange in exon-10 could affect splicing of exon-9 located over 1000 base pairs upstream. Additional polymorphisms have been detected in the promoter and in intron-9, which are in almost complete linkage disequilibrium defining a complex "C-haplotype" and "T-haplotype." Approximately 10% of Caucasians carry the TT, 45% the TC, and 45% the CC genotype.[105] African-Americans and other ancient ethnicities such as Australian aborigines have an extremely high T allele frequency and only an approximate 10% CC homozygosity. The T allele originally associated with hypertension induces vasoconstriction in the skin microcirculation and the coronary arteries on agonist stimulation. On the other hand, drugs such as clonidine and endothelin receptor antagonists evoke increased vasodilation in T allele carriers. Interestingly, both these nucleotide polymorphisms residing in the Gαs1 and in the Gβ3 subunits have been shown to constitute independent risks for orthostatic hypotension.[102] Orthostatic dysregulation of blood pressure resulting from inadequate baroreflex sensitivity and sympathetic nervous stimulation was found to be associated with an increased incidence of future cardiovascular events.

Most recently two polymorphisms of phospholamban have been recognized that may drastically affect β-adrenergic signaling.[63] First, an arginine9 to cysteine9 mutation greatly increases the binding affinity of phospholamban to PKA, causing an undissociable complex at the sarcoplasmic reticulum Ca^{2+}-pump SERCA2a.[106] Consequently, the pump is chronically inhibited and no more amenable to β-adrenergic regulation by phosphorylation of intact phospholamban, ultimately leading to congestive heart failure. In a second human phospholamban polymorphism, the Leu39 codon is mutated to a stop codon producing a truncated phospholamban protein.[107] This shortened protein fragment is unstable or misrouted to membranes other than the sarcoplasmic reticulum. In this case, the SERCA2a is unimpededly active and in the absence of functional phospholamban, resistant to adrenergic regulation. This state was also found to lead to lethal dilated cardiomyopathy.

The known insertion/deletion polymorphism of the angiotensin-converting enzyme gene misses a fragment of 287 bases in the deletion-allele.[108] Although II and ID (I stands for insertion, D for deletion) combinations exhibit no obvious phenotype, homozygous DD is associated with increased angiotensin-converting enzyme activity and higher levels of circulating angiotensin-II. High angiotensin-II induces myocardial remodeling, leading to depressed contractile function with a poor prognosis. Accordingly, patients with the DD genotype also exhibit decreased transplant survival. Interestingly, this genotype-dependent risk is not observed in patients treated with β-AR blockers, implying an interdependence of the sympathetic and renin-angiotensin signaling pathways. Such an interdependence between these two systems is further substantiated by clinical studies indicating that β-AR blockers may exert their favorable effects in chronic heart failure on cardiac morbidity and mortality at least in part by renin inhibition.[109] In addition, as mentioned previously, recent experimental evidence shows that a direct interaction between β-ARs and type-1 receptors has significant consequences on the overall response to drugs that antagonize these receptors.[38]

Defects in drug-metabolizing and drug-transport pathways are certainly also important contributors to variability in the action of commonly used agents such as β-AR antagonists.[108,110] For instance, a significant delay in the metabolic inactivation of metoprolol and carvedilol (two of the most commonly used β-blockers) occurs in patients homozygous for loss-of-function alleles in the gene encoding the specific drug-metabolizing enzyme CYP2D6 of the cytochrome-P450 family.

These few examples demonstrate the wide scope of interrelations and interdependencies of the adrenergic signaling network.

Some Afterthoughts of Caution

Current research focuses on testing new drugs or well-known drugs with new applications in ever-larger population cohorts. However, this entails the danger that, as a result of genetic variability, the outcome of new treatment regimens with a high beneficial potential may fail to reach the required significance. Selective population studies comprising genotyped individuals may yield more reliable information. This is particularly true for the genetic variability of the adrenergic signaling system acting as first and fast "fire-wire" control over the cardiovascular system. Evidence-based medicine should not simply rely on large numbers but should take into account biologic complexity, thus attaining a more personalized approach in medical treatment and preventive health care. Nevertheless, some caution seems appropriate when routinely genotyping patients before treatment.

First, several classes of genetic variation require differentiation.[76] One is composed of mutations that alter signaling function and are the direct cause of a disease. These diseases are typically rare (<1%) and the mutation is not found in unaffected individuals. As such mutations

may spontaneously arise in genes containing mutational hot spots, diagnostic genotype screening for prophylaxis only makes sense in afflicted families with a hereditary disease history. A second class comprises variations that alter adrenergic receptor functions or protein expression but do not appear to be the direct cause of a disease. In this case, the variation can be common in apparently healthy carriers. These polymorphisms may act as disease modifiers with pathophysiological consequences becoming apparent when signaling function is critical for compensation in the diseased state or for the response to therapy. Finally, in complex diseases that are likely multigenetic, polymorphisms may also directly contribute to the disease or to its development, but only in context with multiple other genes or other mutations. From a public health perspective, genes with common mutations that are less highly penetrant but much more prevalent have a greater effect on the population than genes that are highly penetrant but uncommon (the latter often are typical for monogenetic diseases with Mendelian inheritance).

Second, the identification of gene variants connected with complex cardiovascular disorders is often complicated by the presence of multiple risk factors deriving from gene-gene and gene-environment interactions such as geographic patterns of gene variations, ethnic differences, age, sex, coexisting other diseases, diet, and medications. Two general strategies to identify complex trait loci are in use.[111,112] One is the candidate gene approach, which is motivated by what is biologically known about the trait and may be called the "hypothesis-testing" approach. The other one is the "hypothesis-generating" approach encompassing a genome-wide search for previously unknown disease-related loci. Both approaches can be evaluated by two methods: "linkage analysis" and "association studies" in human populations. Linkage analysis is used to identify the chromosomal localization of disease-related gene variants. This approach was successfully used to map hundreds of genes for rare monogenetic disorders. However, in complex diseases a multitude of genes with either rare or common alleles produces an apparently chaotic pattern of heterogeneity within and between patient families. The consequence of this, together with the potentially weak influence of many loci, presents a formidable task for the statistics in detecting individually contributing genes. Furthermore, hundreds of genes may reside within the broad chromosomal regions identified by scanning for markers, which may act alone or in concert with each other. This adds to the difficulty of determining their contribution to the traits to which they segregate.

Association studies examine the frequency of DNA variants in groups of unrelated individuals with disease and unaffected controls. An increased statistical power and no need for family-based sample collections are the advantages of this approach over linkage analysis. One weakness of the association approach is that it has no power to detect and map trait loci unless the marker of interest is connected in disequilibrium to a functional variant or the marker SNP allele itself represents the actual functional variant. A recent meta-analysis identified underpowered studies to constitute a main source for inconsistent results in subsequent tests, and proposed more stringent statistical criteria to exclude false-positive findings.[113]

Third, several types of markers may serve to perform gene association studies. The discovery of restriction fragment length polymorphisms represented the first highly polymorphic DNA markers and led to the first human restriction fragment length polymorphisms map in 1987 with approximately 400 markers at a density of one in every 10 million bases.[114] Inclusion of over 5000 simple sequence length polymorphisms derived from alleles containing different numbers of short DNA repeat units brought the map in 1994 to an average marker density of one per 600,000 bases. Such a region could still comprise several hundreds of genes (the average gene consists of approximately 300 coding bases plus additional noncoding intron sequences).

Today, the catalog of around 7 million SNPs allows scanning the human genome with a resolution one thousand times higher. As the association of nearby markers varies dramatically across the genome, it is not efficient to use SNPs selected at random or evenly spaced throughout the genome sequence. Instead, the patterns of association must be empirically determined for efficient selection of so-called "tag SNPs."[73] These studies revealed that only a limited number of all possible haplotypes containing several individual SNPs are preserved in the human genome. Exactly this has been shown for the haplotypes of the β2-AR gene. For common SNPs, which tend to be older than rare SNPs, the patterns of linkage disequilibrium (haplotypes) largely reflects historical recombinations and demographic events. The reduced number of haplotypes that have survived in the genome suggests that only these are compatible with life. The occurrence of few haplotypes in a chromosomal region allows reduction of indiscriminate searching for individual SNPs considerably. Whereas a SNP represents a single nucleotide variant, a haplotype covers a considerably longer sequence of nucleotides (averaging about 25,000 bases) with any additional variants in this region that tend to be inherited together. A few carefully chosen tag SNPs will provide enough information to predict the colocalization of the remainder common SNPs in that region. These observations are the conceptual and empirical foundation for developing the "haplotype map of the human genome" (International HapMap Project) including the tag SNPs selected for most efficient and comprehensive search approaches.[73] Genome-wide scanning by haplotype mapping will prob-

ably be the key to future association studies yielding greatly increased sensitivity and specificity of predicting how allotypic variation may affect specific clinical outcomes.

Finally, we emphasize that identification of a positive association between a specific genotype and clinical outcome does not necessarily imply causality. Experimental approaches *in vitro* and *in vivo* are required to establish the causal relationship between the pathophysiological effects of a putative relevant SNP and the phenotypic trait. In the past, experimental testing and association studies were often done to characterize single specific SNPs. However, as can be learned from the ensemble of multiple SNPs in the β2-AR gene, distinct physiologic effects of individual SNPs may become modified or even obscured by the interplay between the nearby SNPs. This presents an additional reason as to why functional phenotypes should rather be associated to commonly occurring haplotypes. With the advent of the new more powerful technologies, a time is envisaged when genotyping will be included in the standard evaluation of patients and will help to personalize the therapeutic approaches.

Implications of Adrenergic Genomics for Perioperative Medicine

Recent studies of human gene mutations have provided new insights into the role of the adrenergic system in the heart and vasculature. Pharmacogenetic testing has so far focused on pharmacokinetic mechanisms involving the large families of drug metabolizing enzymes.[115] However, pharmacodynamic mechanisms appear now to account for most of the cardiovascular variations in response to drug therapy. The deficiency in understanding the factors responsible for the interindividual variation constitutes a main cause of treatment failure in more than 50% of treated hypertensive patients. Although this line of research is still in the early stages, its impact on perioperative patient management will be significant. Genotyping for chromosomal haplotype regions associated with cardiovascular traits may become feasible at affordable costs and will help to individualize drug therapy with the goal of maximizing efficacy and limiting toxicity. Patients with coronary artery disease or risk factors of coronary artery disease and specific adrenergic genetic polymorphisms may be particularly sensitive to catecholamine toxicity and prone to ischemia and cardiac complications. The new technical abilities may allow moving from treatment based on average effects observed in large patient groups to treatments based on individual patient characteristics. Identification of patients with critical genetic polymorphisms linked to adverse outcome during the process of perioperative risk assessment, may directly improve patient management by specific pharmacological interventions and decrease perioperative mortality.[116] In the era of genomic perioperative medicine, crisis-driven interventions may be at least partly replaced by predictive medicine with individually tailored interventions.

Conclusions and Perspectives

Hyperadrenergic activity and maladaptive alterations in the autonomic nervous system are hallmarks of the perioperative stress response. Activation of the sympathetic nervous system, particularly the β-ARs, increases heart rate and oxygen consumption and plays a pivotal role in the development of perioperative myocardial damage. Although significant protection from maladaptive adrenergic activity can be achieved by selective inhibition or activation of specific β/α-adrenergic receptor subtypes,[16] the current pharmacological armamentarium is still limited with respect to receptor-subtype selectivity. Results from gene-targeted animal models suggest that modulation of adrenergic receptor subtypes exerts beneficial or detrimental effects on the myocardium. Thus, pan-adrenergic inhibition of the sympathetic nervous system may not represent the optimal cardioprotective treatment modality in perioperative medicine. Irreversible removal of adrenergic support with the inability to maintain adequate cardiac function may be detrimental. In contrast, fine-tuning of the complex adrenergic signaling may provide maximal cardioprotection, as suggested by novel findings from transgenic animal models and genetic polymorphisms in humans.

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