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PERSPECTIVE

β-Adrenergic Receptors, Transgenic Mice, and Pharmacological Model Systems

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Several important issues are raised in the current article by Engelhardt et al. (2001) (see pp. 712–717). These include: the utility of transgenic mouse models to investigate β-adrenergic receptor (β-AR) signaling and pharmacology; the relative sensitivity of biological preparations to investigate pharmacological characteristic such as inverse agonism and intrinsic sympathomimetic activity (ISA); the presence or absence of constitutive or spontaneous activity of β-ARs; and lastly, the connection between model system pharmacology and therapeutic response.

During the past few years, there has been an explosion in the use of transgenic technology to investigate the role of G-protein-coupled receptors and components of their downstream signaling pathways, particularly in the cardiac context. Beginning with the now classic paper of Milano et al. (1994), describing overexpression of the human β_2-AR in mouse heart, considerable insight has been gained into the biochemical basis of β-AR signaling, the role of the β-AR in modulating cardiac function, and, on a subtype-specific basis, the relative virtues or hazards of overexpressing β-ARs in the heart (Milano et al., 1994; Drazner et al., 1997; Engelhardt et al., 1999; Bisognano et al., 2000; Liggett et al., 2000; Shah et al., 2000; Freeman et al., 2001). The maturity of this field is exemplified by its movement beyond descriptive studies on signaling pathways or phenotypes to exploitation of transgenic technology as useful physiological and pharmacological model systems. One such concept is spontaneous activity of β-ARs, as first described in transgenic mice overexpressing the human β_2-AR (Bond et al., 1995). Another more applied use of transgenic mouse models is the screening of new or existing therapeutic agents to affect the transgene-induced phenotype.

The concept of spontaneous activity of β-ARs is both a biochemically interesting and therapeutically relevant pharmacological principle. Figure 1 illustrates, in simplistic fashion, the concept of the inactive receptor (R), with high affinity for inverse agonists, and the activated receptor (R*), with high affinity for agonists. The R* conformation of the β-AR, which has been investigated almost exclusively in overexpression systems, couples spontaneously (e.g., in the absence of agonist) to adenylyl cyclase activation. Further investigation, however, indicates that the simple binary model may have serious theoretical inconsistencies, requiring, for example, a distinction from ligand-induced R* (**L**R*) in the case of L-type Ca^{2+} channel activation (Zhou et al., 1999a) and consideration of the influence of G-protein heterotrimer dissociation kinetics (Krumins and Barber, 1997). Just as distinctions between spontaneously activated states (above) can be made, it is also possible that further differences in activated states produced by mutagenesis (constitutively activated mutants) (Samama et al., 1993; Lattion et al., 1999) or by naturally occurring polymorphisms that alter coupling efficiency [e.g., the Ile164Thr allele of the human β_2-AR or the Arg389Gly allele of the human β_1-AR (Liggett, 2000)] may also be made. Thus, defining the presence or absence of spontaneous activity of a receptor may depend ultimately on the basis of receptor activation (agonist independent, agonist-dependent, constitutively activated mutants, or natural polymorphism).

Although spontaneous activity for the β_2-AR subtype is well documented in a variety of model systems (Samama et al., 1993; Chidiac et al., 1994, 1996; Milano et al., 1994; Bond et al., 1995), there is controversy regarding the presence or absence of spontaneous activity for the β_1-AR. However, it is possible that the current lack of concordance for the β_1-AR may be based solely on the method used to determine the presence of spontaneous activity.

In an article published recently in *Molecular Pharmacology*, Zhou et al. (2000) investigated β_1-AR spontaneous activity using the highly novel system of adenovirus-mediated gene transfer of β-AR subtypes into isolated ventricular cardiomyocytes obtained from the hearts of β_1/β_2-AR double

ABBREVIATIONS: AR, adrenergic receptor; ISA, intrinsic sympathomimetic activity; GNMB, guanine nucleotide modulatable binding.

knockout mice. Their conclusion that overexpressed β_1-ARs lacked spontaneous activity was based on the observation that increased abundance (multiplicities of infection) of β_1-ARs failed to increased basal cAMP accumulation and did not result in increased contractile amplitude of cardiomyocytes. This finding was in sharp contrast to what was observed in the same system with overexpressed β_2-ARs.

In the current article by Engelhardt et al. (2001), evidence of spontaneous activity for the β_1-AR was obtained by using a transgenic mouse overexpressing the human β_1-AR in a cardiac-selective context. In marked distinction to the article by Zhou et al. (2000), Engelhardt et al. (2001) used a different functional response: assessment of heart rate in isolated atrial preparations. An increased heart rate in β_1-AR TG mice was observed even after reserpinization, virtually eliminating the possibility of a contribution by endogenous catecholamines; this finding supports the conclusion that β_1-ARs can exhibit spontaneous activity. The disparity of results between these two articles cannot be completely explained by model systems or endpoints, however, because Engelhardt et al., also demonstrated β_1-AR spontaneous activity in COS7 cells in which increasing degrees of overexpression correlated with increasing basal cAMP accumulation. Clearly, further investigation into these interesting findings, neither without apparent flaw, is warranted.

The existence of spontaneous activity is a property readily exploited to investigate the presence of either inverse agonism or ISA and considerable and relatively consistent information is available for a variety of β-AR agonists and antagonists with β_2-AR overexpression systems (Chidiac et al., 1994, 1996; Bond et al., 1995; Yoshikawa et al., 1996; Gurdal et al., 1997; Zhou et al., 1999a,b; Zhou et al., 2000). Whether overexpression represents an advantage over previously described forskolin-mediated signal amplification systems (Jasper et al., 1990) remains to be determined.

The observation of spontaneous activity for the β_1-AR in transgenic mice has now been used to further examine the presence of inverse agonism and ISA for several well described β-blocking agents. As expected, Engelhardt et al. demonstrated inverse agonism for the β_1-AR selective antagonists, CGP20712A, bisoprolol, and metoprolol, and for the nonselective antagonist, propranolol. Perhaps one of the most intriguing findings is that the β-blocking agent carvedilol, which in clinical use has a markedly antiadrenergic profile, seems to have (very) modest ISA or at least a distinct absence of inverse agonism, as assessed by an increase in right atrial beat frequency. What should readers make of these results?

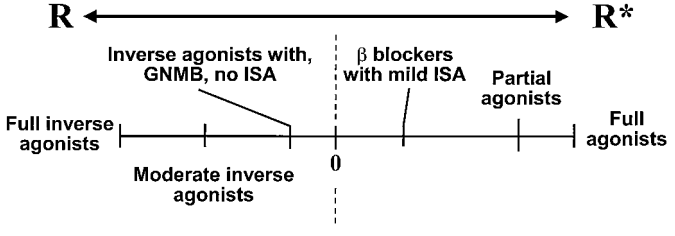

Fig. 1. States of activation of β-adrenergic receptors and the continuum of β-adrenergic antagonist/agonist activity. β-Adrenergic receptors are simplistically described as a binary model. In the resting state (R), receptors are uncoupled from the signaling pathway(s) and have a high affinity for inverse agonists (antagonists). Conversely, receptors activated state (R*), demonstrate a high affinity for agonists and an increased association with the stimulatory G-protein, Gs. (GNMB, guanine-nucleotide modulatable binding; ISA, intrinsic sympathomimetic activity)

Carvedilol and bucindolol are both third-generation β-blocking agents with vasodilator properties, which in isolated human cardiac membranes exhibit the property of guanine nucleotide modulatable binding (GNMB; Yoshikawa et al., 1996). This phenomenon is ordinarily thought to be an agonist-like property, such that the presence of Mg^{2+}, a ligand binds to the high affinity "agonist binding" states of receptors that are precoupled to GTP. In radioligand–nonradioligand competition experiments, this binding state is then uncoupled by high concentrations of nonhydrolyzable guanine nucleotides, such as 5′-guanylylimidophosphate. Yet, in human receptor systems, neither carvedilol nor bucindolol have yielded substantive evidence of agonist activity (Bristow, 2000a; Maack et al., 2000; Sederberg et al., 2000). In rat cardiac preparations, however, bucindolol has detectable ISA, whereas carvedilol does not. This apparent difference between rat and human cardiac preparations with regard to bucindolol and ISA is probably caused by differences in the degree of amplification of signal transduction, which is greater in rats than humans, as deduced from the EC_{50} value of isoproterenol—right ventricular contraction dose-response curves performed in nonfailing preparations under similar conditions [rat isoproterenol $EC_{50} \sim 20$ nM (Sederberg et al., 2000), human $EC_{50} \sim 300$ nM (M. Bristow, et al., unpublished observations)]. An extension of this observation is that when signal transduction is amplified even further by β_1-receptor overexpression, compounds such as carvedilol (+ GNMB but undetectable ISA in other model systems or in human heart preparations) may then have detectable ISA. This series of observations would place GNMB, weak ISA, and strong ISA on a continuum, as shown in Fig. 1. Also, according to this paradigm, compounds with low levels of inverse agonism reside on the continuum just beyond or perhaps overlapping with GNMB. In overexpressed human β_2 receptors, bucindolol has a low inverse agonist profile (Yoshikawa et al., 1996), which correlates with its minimal reduction in Holter-monitored lowest 24-h heart rate (Lowes et al., 1994), a clinical measure of β-AR inverse agonism. For bucindolol or carvedilol, low respective amounts of inverse agonism or ISA identified in overexpressed human β_2- (Yoshikawa et al., 1996) or β_1-receptor (Engelhardt et al., 2001) systems probably contribute to the good tolerability of these agents in advanced heart failure (Beta-Blocker Evaluation of Survival Trial Investigators, 2001; Packer et al., 2001). This finding is in comparison with nonselective β-blockers with higher inverse agonist profiles, such as propranolol (Beta-Blocker Evaluation of Survival Trial Investigators, 2001; Yoshikawa et al., 1996).

What do small amounts of ISA detectable only in highly amplified systems mean in terms of clinical efficacy? Probably nothing; as mentioned earlier, neither carvedilol nor bucindolol seem to have ISA in human myocardium in vivo (Beta-Blocker Evaluation of Survival Trial Investigators, 2001) or in amplified in vitro systems (Bristow, 2000a; Sederberg et al., 2000). Moreover, if ISA is associated with a detrimental response in chronic heart failure (Xamoterol in Severe Heart Failure, 1990), there is no evidence of this for carvedilol, because this agent's left ventricular functional and clinical responses are at least as efficacious as 2nd-generation compounds (Yoshikawa et al., 1996; Bristow,

2000b; Packer et al., 2001). On the other hand, bucindolol has produced clinical results in advanced, class IV chronic heart failure (Beta-Blocker Evaluation of Survival Trial Investigators, 2001) that may be inferior to carvedilol, whereas in class III heart failure, the bucindolol results are roughly comparable with those of second-generation β-blocking agents (CIBIS Investigators and Committees, 1994; CIBIS Investigators and Committees II, 1999; MERIT, 1999). However, bucindolol differs from carvedilol in several potentially important pharmacological respects. These include the ability of bucindolol to produce β-AR down-regulation in primary cultures of cardiac myocytes via a mechanism independent of agonist-induced down-regulation (versus "pseudo–down-regulation" produced by carvedilol) (Asano et al., 2001), powerful sympatholytic properties for bucindolol but not carvedilol (Lowes et al., 2000), and potent α-blockade for carvedilol compared with bucindolol (Yoshikawa et al., 1996). Of these potential differentiating features between carvedilol and bucindolol, the sympatholytic effects of bucindolol seem to be the most likely explanation for this compound's disappointing clinical results in advanced heart failure subpopulations (Beta-Blocker Evaluation of Survival Trial Investigators, 2001; Bristow et al., 2001).

In summary, genetically engineered/amplified β-receptor signal transduction systems may have utility in detecting useful or harmful properties of therapeutic agents which can modify signal transduction. Potentially useful properties identifiable by such systems include low inverse agonist properties, which may correlate clinically with improved tolerability (Lowes et al., 1994) and ISA, higher degrees of which may preclude clinical efficacy of β-blocking agents (Xamoterol in Severe Heart Failure, 1990). However, as illustrated for carvedilol, the data derived from these screens need to be interpreted in the context of effects produced in intact and isolated human cardiac systems. In this particular case, the amplified screening system used by Engelhardt et al. (2001) was sufficiently powerful to have detected a minute amount of ISA that seems to have no negative clinical consequences.

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