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How carvedilol does not activate β_2 -adrenoceptors

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The introduction of β -adrenergic receptor blockers, “ β -blockers”, as a treatment for heart failure decades ago was a major advance in cardiovascular medicine^{1–4}. Moreover, it has been clear for a number of years that some β -blockers with “intrinsic sympathomimetic activity” (ISA) due to weak partial agonist activity mediated through G protein stimulation did not share this salutary effect^{5,6}. Yet, a recent paper by Benkel et al.⁷, “How carvedilol activates β_2 -adrenoceptors”, using engineered cells that lack either G proteins or arrestins, proposes that carvedilol, a widely used and effective β -blocker for heart failure, signals through β_2 AR-mediated G protein activation. Here, we explain why we believe the conclusions put forward by Benkel et al. are incorrect and certainly cannot be extended to clinical implications and should not guide decisions in the clinic.

Contrary to the claims of Benkel et al.⁷, it has previously been established that in systolic heart failure (currently known as Heart Failure with reduced Ejection Fraction, HFrEF), β -blockers without ISA have beneficial effects whereas those with ISA do not^{5,6}. None of the three FDA-approved β -blockers for the treatment of heart failure (carvedilol, metoprolol and bisoprolol) and nebivolol (approved in Europe) are classified as having ISA⁸. This is in concert with clinical trial evidence that drugs such as milrinone and ibopamine, which enhance cyclic AMP (as is the case for β -blockers with ISA), have deleterious effects in systolic heart failure^{9,10}. Benkel et al. cite one post-myocardial infarction trial¹¹ of a β -blocker with ISA. Yet when considering the 25 post myocardial infarction β -blocker trials carried out in the 1970’s and 1980’s, those with ISA showed substantially less beneficial, or even negative effects on patient outcomes¹².

It has been established that cells such as those used in most of the studies in Benkel et al., which have been transfected with robust expression vectors consisting of cDNAs encoding GPCRs (in this case the β_2 adrenoceptor, β_2 AR), express receptors at levels that are

generally at least 10- to 100-fold higher than the levels of endogenous expression¹³. Benkel et al. do not directly report the level of receptor expression in their cells. It can be readily demonstrated that in untransfected HEK-293 cells, which express low (physiologic) levels of endogenous β_2 ARs, carvedilol does not stimulate an elevation in the levels of cyclic AMP¹⁴, and this is true even in some systems with high level overexpression of β_2 ARs¹⁵. Not until receptor levels are dramatically raised by overexpression, and at high concentrations of carvedilol, is very low-level stimulation observed. Indeed, the authors themselves point out “...that the majority of assays to disentangle G-protein versus Arrestin biased signaling were performed in over-expression systems. Hence extrapolation of our data to primary cells or the in vivo situation must be performed with caution...”. The concentrations of carvedilol used in Benkel’s overexpressing cells, 10 micromolar for many studies and above one micromolar in most, are well above the plasma levels the drug ever achieves clinically. For example, maximal plasma levels of carvedilol after a typical 50 mg dose in humans are well below 100 nM, of which 95 to 98% is protein bound, meaning the effective therapeutic concentration is, in fact, an order of magnitude lower^{16,17}.

Our review of the literature finds at least 9 papers in which more physiological cardiovascular systems were used to test whether carvedilol possesses ISA^{18–26}. These studies used a wide array of in vivo and ex vivo cardiac preparations ranging from in vivo pithed rats^{24,25} to isolated myocytes^{21,24,26} to isolated atrial and ventricular myocardial preparations^{18–20,22,23}, from multiple species including guinea pig²⁶, mouse²¹, rat^{18,24,25}, and human failing and non-failing myocardium^{18–20,22,23}. In no case was any stimulatory effect seen with carvedilol at the level of cyclic AMP^{18,19,24,26}, isolated muscle force generation^{19,20,22,23}, contractility²¹ or beating rate^{24,25}. None of these studies were cited in Benkel et al.⁷, and all failed to show that carvedilol

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demonstrates ISA in cardiac preparations with endogenous levels of adrenergic receptor expression. These results are in conflict with the findings reported by Benkel et al. in the set of experiments in which isolated neonatal mouse cardiac myocytes are utilized (Fig. 3 in Benkel et al.). Additionally, in our view, several of the papers that are referenced in support of carvedilol possessing ISA are cited misleadingly. For example, Benkel et al. cite reference 24 as stating that carvedilol binding is sensitive to GTP²⁷. While it is true that the Maack et al. paper claimed that carvedilol has ISA, in fact, their data showed that carvedilol has no ISA in 6 out of 7 experiments performed²⁷. Other such papers cited either explicitly state that “carvedilol has no intrinsic sympathomimetic activity”²⁸, make no statements about ISA²⁹, or use non physiologic overexpression systems^{30–32}.

Furthermore, the finding that the full agonist isoproterenol and the β -blocker carvedilol through the β_2 AR have quite similar (70%) effect on enhancing heart cell beating rate is inconsistent with fundamental pharmacological principles for the action of a full agonist and a competitive antagonist on receptor activation (Benkel et al., Fig. 3f: + CGP-20712A, 3rd column -22 bpm, vs. Fig. 3h: + CGP-20712A, 2nd column -15 bpm). Moreover, the data in Fig. 3 with myocardial cells that indicate the chronotropic effects of isoproterenol and carvedilol are being driven primarily by the β_2 AR is inconsistent with previous studies with β_1 and β_2 AR knockout mice, both in neonatal cardiac myocytes³³ and in the intact heart^{34,35} (none cited). These previous studies clearly indicate that such responses to β -agonists are driven either primarily or exclusively by the β_1 AR. Patients who begin taking carvedilol do not develop a tachycardia, and indeed, lowering heart rate in heart failure can be beneficial³⁶.

Several laboratories have documented that carvedilol is a weak β -arrestin biased ligand that can stimulate ERK activity in a β -arrestin and/or GRK dependent fashion although most demonstrations have been in the same type of overexpression, high drug concentration systems used here^{14,21,37–40}. This understanding of the actions of carvedilol still casts it as a very useful tool for biophysical studies probing the molecular mechanisms of biased signaling. This is evidenced by several studies which demonstrate that the drug stabilizes unique partially active conformations of the β_2 AR^{41,42} with characteristics in common with β -arrestin biased conformations of other GPCRs such as the angiotensin receptor featuring repositioning of transmembrane helix 7 and the short non membrane embedded helix 8⁴³. Nonetheless, in Fig. 1 Benkel et al. report that they failed to observe any alteration in carvedilol-stimulated ERK activation in comparing the response between one particular β -arrestin 1/2 double CRISPR knockout HEK-293 cell line and its cognate parental HEK-293 cell line. The authors have published this particular finding before⁴⁴. However, it has been demonstrated by others that independent derivation of such CRISPR β -arrestin knockout lines in two other laboratories leads to cells with very different properties than the ones utilized here³⁷ (not cited in this context). For example, in the two distinct parental HEK-293 cells used for derivation of the other two CRISPR knockout lines, transfection with β -arrestin1/2 siRNA led to near elimination of carvedilol-stimulated ERK activation. Moreover, in CRISPR β -arrestin knockout lines derived from these parental cells, no carvedilol stimulation of ERK activity could be demonstrated but could be restored by reintroduction of β -arrestin 1/2 by transfection³⁷. These results could not be replicated with the parental and knockout HEK-293 cells used by Benkel et al. These striking differences in β -arrestin signaling amongst the three independently derived CRISPR β -arrestin knockout lines and the parental HEK-293 clones from which they were derived are presumably due to differential “rewiring of the cells” during their derivation and underscore a shortcoming in the Benkel et al. study. Highly engineered systems call for orthogonal approaches to validate the findings, for example using more than one of the published clonal β -arrestin knockout lines and use of siRNA to knock down β -arrestin expression. In fact, in contrast with these variable results obtained with

the different CRISPR lines, siRNA mediated reductions in β -arrestin levels in all three parental HEK-293 lines led to consistent reductions in ERK activation not only through the β_2 AR and β_1 AR, but also through the AVP and FSH receptors as well³⁷.

In summary, over 30 years of clinical studies support the fact that β -blockers without ISA have beneficial effects, and those with ISA have no benefit or increased mortality in systolic heart failure. The proposal of Benkel et al. that the mechanism responsible for the beneficial actions of carvedilol is ISA is not consistent with the overwhelming evidence that rests securely on decades of basic science studies and numerous clinical trials, and should not guide clinical practice.

References

1. Bristow, M. R. beta-adrenergic receptor blockade in chronic heart failure. *Circulation* **101**, 558–569 (2000).
2. Packer, M. Beta-adrenergic blockade in chronic heart failure: principles, progress, and practice. *Prog. Cardiovasc. Dis.* **41**, 39–52 (1998).
3. Packer, M. et al. Effect of carvedilol on survival in severe chronic heart failure. *N. Engl. J. Med.* **344**, 1651–1658 (2001).
4. Ruffolo, R. R. & Feuerstein, G. Z. Carvedilol case history: the discovery and development of the first beta-blocker for the treatment of congestive heart failure. *Expert Opin. Drug Discov.* **1**, 85–89 (2006).
5. The Xamoterol in Severe Heart Failure Study Group. Xamoterol in severe heart failure. *Lancet* **336**, 1–6 (1990).
6. Witchitz, S. et al. Treatment of heart failure with celiprolol, a cardioselective beta blocker with beta-2 agonist vasodilatory properties. The CELICARD Group. *Am. J. Cardiol.* **85**, 1467–1471 (2000).
7. Benkel, T. et al. How carvedilol activates beta(2)-adrenoceptors. *Nat. Commun.* **13**, 7109 (2022).
8. Podrid P. J. *Major Side Effects of Beta Blockers*. <https://www.uptodate.com/contents/major-side-effects-of-beta-blockers> (2022).
9. Packer, M. et al. Effect of oral milrinone on mortality in severe chronic heart failure. The PROMISE study research group. *N. Engl. J. Med.* **325**, 1468–1475 (1991).
10. Hampton, J. R. et al. Randomised study of effect of ibopamine on survival in patients with advanced severe heart failure. Second Prospective Randomised study of Ibopamine on Mortality and Efficacy (PRIME II) investigators. *Lancet* **349**, 971–977 (1997).
11. Boissel, J. P., Leizorovicz, A., Picolet, H. & Peyrieux, J. C. Secondary prevention after high-risk acute myocardial infarction with low-dose acebutolol. *Am. J. Cardiol.* **66**, 251–260 (1990).
12. Yusuf, S., Peto, R., Lewis, J., Collins, R. & Sleight, P. Beta blockade during and after myocardial infarction: an overview of the randomized trials. *Prog. Cardiovasc. Dis.* **27**, 335–371 (1985).
13. Shenoy, S. K. et al. beta-arrestin-dependent, G protein-independent ERK1/2 activation by the beta2 adrenergic receptor. *J. Biol. Chem.* **281**, 1261–1273 (2006).
14. Pani, B. et al. Unique positive cooperativity between the beta-arrestin-biased beta-blocker carvedilol and a small molecule positive allosteric modulator of the beta2-adrenergic receptor. *Mol. Pharm.* **100**, 513–525 (2021).
15. Galandrin, S. & Bouvier, M. Distinct signaling profiles of beta1 and beta2 adrenergic receptor ligands toward adenylyl cyclase and mitogen-activated protein kinase reveals the pluridimensionality of efficacy. *Mol. Pharm.* **70**, 1575–1584 (2006).
16. Morgan, T. Clinical pharmacokinetics and pharmacodynamics of carvedilol. *Clin. Pharmacokinet.* **26**, 335–346 (1994).
17. Packer, M. et al. Pharmacokinetic profile of controlled-release carvedilol in patients with left ventricular dysfunction associated with chronic heart failure or after myocardial infarction. *Am. J. Cardiol.* **98**, 39L–45L (2006).

18. Andreka, P. et al. Bucindolol displays intrinsic sympathomimetic activity in human myocardium. *Circulation* **105**, 2429–2434 (2002).
19. Bristow, M. R. et al. Receptor pharmacology of carvedilol in the human heart. *J. Cardiovasc. Pharm.* **19**, S68–S80 (1992).
20. Brixius, K., Bundkirchen, A., Böck, B., Mehlhorn, U. & Schwinger, R. H. Nebivolol, bucindolol, metoprolol and carvedilol are devoid of intrinsic sympathomimetic activity in human myocardium. *Br. J. Pharm.* **133**, 1330–1338 (2001).
21. Carr, R. 3rd et al. beta-arrestin-biased signaling through the beta2-adrenergic receptor promotes cardiomyocyte contraction. *Proc. Natl Acad. Sci. USA* **113**, E4107–E4116 (2016).
22. Liggett, S. B. et al. A polymorphism within a conserved beta(1)-adrenergic receptor motif alters cardiac function and beta-blocker response in human heart failure. *Proc. Natl Acad. Sci. USA* **103**, 11288–11293 (2006).
23. Maack, C. et al. Characterization of beta(1)-selectivity, adrenoceptor-G(s)-protein interaction and inverse agonism of nebivolol in human myocardium. *Br. J. Pharm.* **132**, 1817–1826 (2001).
24. Willette, R. N. et al. In vitro and in vivo characterization of intrinsic sympathomimetic activity in normal and heart failure rats. *J. Pharm. Exp. Ther.* **289**, 48–53 (1999).
25. Willette, R. N., Mitchell, M. P., Ohlstein, E. H., Lukas, M. A. & Ruffolo, R. R. Jr. Evaluation of intrinsic sympathomimetic activity of bucindolol and carvedilol in rat heart. *Pharmacology* **56**, 30–36 (1998).
26. Yao, A. et al. Characteristic effects of alpha1-beta1,2-adrenergic blocking agent, carvedilol, on [Ca²⁺]_i in ventricular myocytes compared with those of timolol and atenolol. *Circ. J.* **67**, 83–90 (2003).
27. Maack, C. et al. Different intrinsic activities of bucindolol, carvedilol and metoprolol in human failing myocardium. *Br. J. Pharm.* **130**, 1131–1139 (2000).
28. Böhm, M. et al. Beta-adrenergic signal transduction following carvedilol treatment in hypertensive cardiac hypertrophy. *Cardiovasc. Res.* **40**, 146–155 (1998).
29. Moser, M. & Frishman, W. Results of therapy with carvedilol, a beta-blocker vasodilator with antioxidant properties, in hypertensive patients. *Am. J. Hypertens.* **11**, 15s–22s (1998).
30. Baker, J. G., Hall, I. P. & Hill, S. J. Agonist and inverse agonist actions of beta-blockers at the human beta 2-adrenoceptor provide evidence for agonist-directed signaling. *Mol. Pharm.* **64**, 1357–1369 (2003).
31. Baker, J. G., Hall, I. P. & Hill, S. J. Agonist actions of “beta-blockers” provide evidence for two agonist activation sites or conformations of the human beta1-adrenoceptor. *Mol. Pharm.* **63**, 1312–1321 (2003).
32. Shen, A. et al. β-blockers augment L-type Ca(2+) channel activity by targeting spatially restricted β(2)AR signaling in neurons. *Elife* **8**, e49464 (2019).
33. Devic, E., Xiang, Y., Gould, D. & Kobilka, B. Beta-adrenergic receptor subtype-specific signaling in cardiac myocytes from beta(1) and beta(2) adrenoceptor knockout mice. *Mol. Pharm.* **60**, 577–583 (2001).
34. Yoo, B. et al. Beta1-adrenergic receptors stimulate cardiac contractility and CaMKII activation in vivo and enhance cardiac dysfunction following myocardial infarction. *Am. J. Physiol. Heart Circ. Physiol.* **297**, H1377–H1386 (2009).
35. Rohrer, D. K. et al. Targeted disruption of the mouse beta1-adrenergic receptor gene: developmental and cardiovascular effects. *Proc. Natl Acad. Sci. USA* **93**, 7375–7380 (1996).
36. Swedberg, K. et al. Ivabradine and outcomes in chronic heart failure (SHIFT): a randomised placebo-controlled study. *Lancet* **376**, 875–885 (2010).
37. Luttrell, L. M. et al. Manifold roles of beta-arrestins in GPCR signaling elucidated with siRNA and CRISPR/Cas9. *Sci. Signal* **11**, eaat7650 (2018).
38. Nobles, K. N. et al. Distinct phosphorylation sites on the beta(2)-adrenergic receptor establish a barcode that encodes differential functions of beta-arrestin. *Sci. Signal* **4**, ra51 (2011).
39. Wang, J. et al. beta-Arrestin-biased allosteric modulator potentiates carvedilol-stimulated beta adrenergic receptor cardioprotection. *Mol. Pharm.* **100**, 568–579 (2021).
40. Wisler, J. W. et al. A unique mechanism of beta-blocker action: carvedilol stimulates beta-arrestin signaling. *Proc. Natl Acad. Sci. USA* **104**, 16657–16662 (2007).
41. Liu, J. J., Horst, R., Katritch, V., Stevens, R. C. & Wuthrich, K. Biased signaling pathways in beta2-adrenergic receptor characterized by 19F-NMR. *Science* **335**, 1106–1110 (2012).
42. Khsai, A. W. et al. Multiple ligand-specific conformations of the beta2-adrenergic receptor. *Nat. Chem. Biol.* **7**, 692–700 (2011).
43. Wingler, L. M. et al. Angiotensin analogs with divergent bias stabilize distinct receptor conformations. *Cell* **176**, 468–478 e411 (2019).
44. Grundmann, M. et al. Lack of beta-arrestin signaling in the absence of active G proteins. *Nat. Commun.* **9**, 341 (2018).

Author contributions

All authors jointly wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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