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COMMENTARY

Do low-affinity states of β-adrenoceptors have roles in physiology and medicine?

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The pharmacology once ascribed to the 'β_4-adrenoceptor' is now believed to be that of a low-affinity state of the β_1-adrenoceptor. The β_2-adrenoceptor may also have a low-affinity state or site, while the β_3-adrenoceptor – the original low-affinity β-adrenoceptor – can display more than one pharmacology. In this issue, Mallem *et al*. show that CGP-12177 relaxes thoracic aorta rings from normal rats by stimulating vascular smooth muscle low-affinity β_1-adrenoceptors, apparently linked in part to G_i protein. By contrast, in rings from hypertensive rats, CGP-12177 acts mainly *via* endothelial β_3-adrenoceptors. This work raises the possibility that low-affinity states of β-adrenoceptors have physiological roles, and suggests that they might be drug targets.
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Abbreviations: CGP-12177, (±)-4-(3-*t*-butylamino-2-hydroxypropoxy)benzimidazol-2-one; CGP-20712, (±)-l-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazoyl)phenoxy]-2-propanol

If only receptor pharmacology were as simple as the textbooks suggest... but then it would be a lot less interesting, as Mallem *et al*. (2004) demonstrate in this issue. Consider β-adrenoceptors. For many years classical pharmacology suggested that there were just two subtypes: β_1 and β_2. Then, the discovery of agonists with a novel tissue selectivity backed up earlier evidence for a third subtype, 'an atypical β-adrenoceptor', that is resistant to blockade by most β_1- and β_2-adrenoceptor antagonists. Once β_3-adrenoceptor had been cloned, everything seemed to fit into place: atypical β-adrenoceptors were β_3-adrenoceptors (see Arch, 2002).

But this cosy story did not survive for long. It was undermined in part by studies using 'nonconventional partial agonists'. These are potent antagonists of β_1- or β_2-adrenoceptors which, at higher concentrations, stimulate β_3-adrenoceptors, including cloned β_3-adrenoceptors. One such compound is CGP-12177 ((±)-4-(3-*t*-butylamino-2-hydroxypropoxy)benzimidazol-2-one), which stimulates β_3-adrenoceptors at concentrations roughly 1000-fold higher than those that antagonise β_1-and β_2-adrenoceptors. CGP-12177 was used in many studies to demonstrate a role for β_3-adrenoceptors in tissues such as human white adipocytes. It gradually became clear, however, that the pharmacology of CGP-12177 in some tissues (initially cardiac tissues, but subsequently human white adipocytes) is not like that of cloned β_3-adrenoceptors or of CGP-12177 in tissues where the β_3-adrenoceptor clearly predominates: CGP-12177 was too potent; it was antagonised too well by the β_1-adenoceptor antagonist CGP-20712 ((±)-l-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazoyl)phenoxy]-2-propanol) and too weakly by propranolol. The existence of a fourth type of β-adrenoceptor pharmacology was finally confirmed when it was shown that (−)-CGP-12177 behaves as an atypical β-adrenoceptor agonist in atria and brown adipocytes from β_3-adrenoceptor knockout mice (Kaumann *et al*., 1998; Cohen *et al*., 2000).

This new pharmacology was ascribed to a 'β_4-adrenoceptor'. Nobody was able to discover a novel gene that coded for such a molecule, however. Eventually, it was realised that cloned β_1-adrenoceptors could respond to CGP-12177 with a pharmacology that resembled the 'β_4-adrenoceptor' pharmacology found in atria and human adipocytes. Moreover, studies in knockout mice showed that this pharmacology was dependent upon the presence of β_1-adrenoceptors (Konkar *et al*., 2000; Kaumann *et al*., 2001). The 'β_4-adrenoceptor' is in fact a low-affinity state of the β_1-adrenoceptor – perhaps a state more like the 'classical' β_3-adrenoceptor; one that binds standard β_1- and β_2-adrenceptor antagonists poorly.

If there is more than one affinity state of the β_1-adrenoceptor, why should there not be others? Another β-adrenoceptor pharmacology has been described for rat isolated mesenteric artery (Kozlowska *et al*., 2003). Moreover, the β_2-adrenoceptor seems to have more than one binding site or state (Seifert *et al*., 1999; Heubach *et al*., 2004), which perhaps explains some peculiarities of responses to β-adrenoceptor agonists in skeletal muscle (Liu *et al*., 1996). Even the β_3-adrenoceptor – once seen as claiming a novel pharmacology all to itself – can display multiple pharmacologies: binding, adenylyl cyclase activation in membranes and cyclic accumulation in cells that express human cloned β_3-adrenoceptors give totally different orders of potency for ligands (Arch, 2002).

So, what is the relevance of low-affinity states of β-adrenoceptors to physiology and medicine? Mallem *et al*. (2004) have made a small step towards answering this question by studying the roles of the β_3-adrenoceptor and the low-affinity state of the β_1-adrenoceptor in thoracic aortic rings from spontaneously hypertensive and control (Wistar Kyoto)

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rats. Their goal was to understand why there is a decrease in vascular relaxation in response to β-adrenoceptor stimulation in hypertension.

In control rats, Mallem *et al*. found an endothelium and nitric oxide-*independent* relaxation in response to CGP-12177, which was reduced by high concentrations of the β_1-adrenoceptor antagonist CGP-20712, but not by two β_3-adrenoceptor antagonists. In other words, the response appeared to be mediated by low-affinity β_1-adrenoceptors ('β_4'-adrenoceptors) in vascular smooth muscle. By contrast, in thoracic aortic rings from hypertensive rats, CGP-12177 produced mainly an endothelium and nitric oxide-*dependent* relaxation that was strongly reduced by the β_3-adrenoceptor antagonists, but not by the β_1- and β_2-adrenoceptor antagonist nadolol. In other words, this appeared to be a β_3-adrenoceptor-mediated response, and they were able to link it to increased β_3-adrenoceptor expression in endothelium in vessels from hypertensive rats.

The endothelium-independent response in tissue from the control rats was reduced by adenylyl cyclase inhibitors, but was amplified in preparations from pertussis toxin-pretreated rats. Pertussis toxin also partially restored the weak endothelium-independent relaxation in tissue from hypertensive rats. The authors link these findings to a report that G_i protein expression is upregulated in a rat model of heart failure and raise the possibility that the low-affinity state of the β_1-adrenoceptor is linked to G_i. Coupling to G_i (as well as G_s) is also a feature of the β_3-adrenoceptor and the low-affinity state of the β_2-adrenoceptor (Heubach *et al*., 2004), so it may be common to all low-affinity β-adrenoceptors.

The work of Mallem *et al*. may be relevant to our understanding of vasodilating (third-generation) β-blockers. First, it raises the possibility that vasodilating β-blockers that have β_3-adrenoceptor agonist activity might be more effective vasodilators in hypertensive subjects. Mallem *et al*. were, however, unable to demonstrate any difference between aortic rings from control and hypertensive rats in the response to the β_3-adrenoceptor agonist SR-58611 A, a paradox that they ascribe to decreased NO bioavailability in the hypertensive rings.

Secondly, their work suggests that some third-generation β-blockers might exert their vasodilatory activity through the low-affinity state of the β_1-adrenoceptor. But do therapeutic concentrations of β-blockers ever reach the concentrations required to activate this – or any other – low-affinity state? Does the potency plus efficacy of β-blockers at the low-affinity state relative to potency at the high-affinity state correlate with vasodilator relative to β-blocker efficacy in the clinic? And are the relative contributions of the low- and high-affinity states to the overall pharmacology of one β_1-adrenoceptor ligand constant, or do they vary in response, for example, to the tissue G protein profile?

The pharmacology of β-adrenoceptors is, indeed, complex, and we have much more to learn about their role in physiology, disease and pharmacotherapy.

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