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COMMENTARY

Atypical pharmacologies at β-adrenoceptors

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β-Adrenoceptors are one of the most widely studied groups of G-protein-coupled receptors but continue to provide surprises and insights that have general relevance to pharmacology. Atypical pharmacologies have been described for ligands formerly (and still) described as antagonists acting at β_1-, β_2- and β_3-adrenoceptors that involve ligand-directed signalling and possibly allosteric interactions at the receptors. In the article by Ngala *et al.*, in this issue of the *Br J Pharmacol*, another example of atypical interactions with β-adrenoceptors is described, this time for agonists. Some of the responses to BRL37344 and clenbuterol can be explained in terms of actions at β_2-adrenoceptors, whereas others such as the increased glucose uptake and palmitate oxidation observed with pM concentrations of BRL37344 may involve interactions with other (possibly allosteric) sites. Atypical pharmacologies of ligands acting at β-adrenoceptors have already indicated new ways in which ligands can interact with G-protein-coupled receptors and these mechanisms are likely to have important consequences for future drug development.
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Abbreviation: GPCR, G-protein coupled receptor

It is widely accepted that the largest group of 'druggable' proteins are cell surface receptors. Of these, the G-protein-coupled receptors (GPCRs) comprise the largest single grouping and have been widely exploited for the treatment of a wide range of diseases. With few exceptions, ligands have been developed that mimic or block the actions of the endogenous hormone or neurotransmitter acting at what has become known as the orthosteric site (Christopoulos and Kenakin, 2002). Although this paradigm will continue to deliver new drugs acting at the orthosteric site of recently deorphanized or poorly understood GPCRs, it is now becoming clear that ligands can produce changes in functional properties of GPCRs by interacting with topographically distinct (allosteric) sites or with distinct conformations of receptors (Christopoulos and Kenakin, 2002; Galandrin and Bouvier, 2006; Kenakin, 2007; Galandrin *et al.*, 2008). Novel binding sites can also be produced by interaction of GPCRs with receptor activity-modifying proteins or by receptor oligomerization.

One of the most widely studied groups of GPCRs are the β-adrenoceptors of which there are three members, all activated by the endogenous catecholamines, noradrenaline and adrenaline. A wide variety of drugs have been developed on the basis of the orthosteric site identified by the catecholamines and used to treat a wide variety of diseases including hypertension, cardiac arrhythmias, cardiac failure and asthma. There have also been attempts (so far unsuccessful) to develop anti-obesity drugs that target β_3-adrenoceptors. The activities of the pharmaceutical companies to synthesize compounds with activity at the β-adrenoceptors has led to a wide variety of ligands that selectively activate or block the individual subtypes. These selective compounds have proved extremely useful in identifying sites or receptor conformations with characteristics suggesting that they differ from the classical orthosteric sites (Galandrin and Bouvier, 2006; Sato *et al.*, 2007; Galandrin *et al.*, 2008). An early atypical pharmacology was identified at the β_1-adrenoceptor when it was shown that there was a class of drugs with high antagonist affinity that possessed agonist actions at much higher concentrations than those required to fully occupy the receptors (Kaumann and Molenaar, 2008). These non-conventional partial agonists included various pindolol derivatives, carazolol and most notably CGP12177A (Kaumann and Molenaar, 2008). Although these findings have been interpreted in various ways including the suggestion that the agonist effects were mediated by a novel (β_4) adrenoceptor, it is now generally accepted that the effects are only seen in the presence of β_1-adrenoceptors and probably represent a state or conformation of this receptor (Kaumann and Molenaar, 2008). It is also possible that CGP12177A and other non-conventional partial agonists may interact with both an allosteric and an orthosteric site on the receptor with different affinity. More recently, it has been recognized that many β-adrenoceptor ligands formerly classified as antagonists can interact with

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β_1-, β_2- or β_3-adrenoceptors to produce ligand-directed signalling and that many of these compounds are particularly powerful activators of mitogen-activated protein kinase signalling (Galandrin and Bouvier, 2006; Sato *et al*., 2007; Galandrin *et al*., 2008). These properties have been easiest to identify with ligands that were originally classified as antagonists when their agonist properties in other signalling paradigms are relatively easily seen.

In the study reported in this issue, Ngala *et al*. (2008) show what may be another example of atypical pharmacology at β-adrenoceptors displayed by agonists. It has been known for some time that the β_3-adrenoceptor agonist BRL37344 produces increases in glucose uptake in soleus and extensor digitorum longus muscles of mice *in vitro* at concentrations significantly below those that activate β_3-adrenoceptors and far below those that activate β_1- or β_2-adrenoceptors (Liu *et al*., 1996). The effect is not blocked to any marked degree by any of the subtype-selective β-adrenoceptor antagonists (Liu *et al*., 1996), and similar concentrations of BRL37344 also stimulated palmitate and pyruvate oxidation in soleus muscle (Board *et al*., 2000). Higher concentrations of BRL37344 acting via β_2-adrenoceptors inhibited rather than stimulated glucose uptake in soleus muscle (Liu *et al*., 1996). However, studies in L6 rat skeletal muscle cells show consistently that higher concentrations of BRL37344, zinterol and isoprenaline increase glucose uptake by activating β_2-adrenoceptors (Nevzorova *et al*., 2002; Nevzorova *et al*., 2006). The current study by Ngala *et al*. (2008) extends these studies and compares the responses to BRL37344 and two recognized β_2-adrenoceptor agonists salbutamol and clenbuterol in mouse soleus muscle and also in C2C12 mouse skeletal muscle cells. Low (pM) concentrations of BRL37344 produced increases (30–60%) of glucose uptake that were unaffected by the β_3-adrenoceptor antagonist SR59230A or by appropriate concentrations of the β_2-adrenoceptor antagonist ICI118551 or the β_1-adrenoceptor antagonist CGP20712A. The effects were blocked by the β-adrenoceptor antagonist atenolol (1 μM), although, given the lack of effect of CGP20712A, it is hard to see how this effect could be related to blockade of β_1-adrenoceptors. Clenbuterol and salbutamol were also capable of stimulating glucose uptake at low concentrations but, in contrast to the responses elicited by BRL37344, they were blocked by ICI118551 and therefore are likely to be mediated by β_2-adrenoceptors. High (nM) concentrations of BRL37344 increased and clenbuterol or salbutamol decreased glucose uptake, with responses to all three agonists being blocked by ICI118551, suggesting involvement of β_2-adrenoceptors. Interestingly, the inhibitory effect of clenbuterol was reversed to a stimulatory effect in the presence of ICI118551. It is possible that clenbuterol but not BRL37344 directs signalling to G_i possibly by inducing greater phosphorylation of the β_2-adrenoceptor and that this is prevented by ICI118551.

Ngala *et al*. also examined the effects of BRL37344 and clenbuterol on pyruvate and palmitate oxidation in soleus muscle. They found that the effects of BRL37344 on pyruvate oxidation were similar to those on glucose uptake and that the effect of nM agonist concentrations was blocked by ICI118551, suggesting a β_2-adrenoceptor-mediated mechanism. Palmitate prevented the effects of BRL37344 at both low and high concentrations on glucose uptake but did not reveal an inhibitory effect at the high concentration. This suggested both that BRL37344 preferentially stimulates fat oxidation rather than carbohydrate metabolism and that the inhibitory effect of 100 nM clenbuterol is unlikely to result from fat oxidation.

The studies conducted so far indicate the presence of a response with atypical pharmacology, produced by low concentrations of a number of β-adrenoceptor agonists, and responses with β_2-adrenoceptor pharmacology, produced by higher concentrations. The responses to pM concentrations of BRL37344 are mediated by an undefined site, whereas those to clenbuterol are blocked by ICI118551. It is possible that the response to BRL37344 may be explained by an interaction with an allosteric site with distinct pharmacology from that utilized by clenbuterol. Responses to nM concentrations of both agonists appear to be mediated by β_2-adrenoceptors, although clenbuterol inhibits glucose uptake and does not affect palmitate oxidation and BRL37344 increases both parameters. Similar responses were produced in C2C12 mouse skeletal muscle cells that, as judged by reverse transcription-PCR, express predominantly β_2-adrenoceptors, suggesting that this subtype may be responsible for all of the effects observed. It is unlikely (although still possible) that BRL37344 produces the effect at low concentrations by interacting with another receptor, although it has been shown to have only low affinity for muscarinic and α_1-adrenoceptors. It would be of interest to determine if the response to low concentrations of BRL37344 is still present in soleus muscles from β_1-, β_2-, or β_3-adrenoceptor knockout mice (preliminary work suggests that the effect is still present in β_3-adrenoceptor knockouts; see Ngala *et al*. (2008) for details). Experiments conducted in cells transfected with the particular receptor subtypes may also help in determining the mechanisms involved and could provide the basis for the examination of whether BRL37344 and other ligands (including antagonists) can interact with particular receptor conformations, using techniques such as bioluminescence resonance energy transfer (Galandrin *et al*., 2008). What is clear is that we can expect to hear a lot more about atypical pharmacologies of ligands acting at β-adrenoceptors that have already changed the way we think about the interaction between ligands and GPCRs and is likely to have important consequences for future drug development.

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