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COMMENTARY

On the mechanism of the persistent action of salmeterol: what is the current position?

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The mechanism of the long duration of action of salmeterol at β_2-adrenoceptors has long been a matter of debate, and is still unresolved. Szczuka and colleagues have both summarized the position to date and suggested a new mechanistic contender, receptor rebinding. Despite this, they still do not come to any clear conclusion. Much of the literature data that they have drawn upon appears contradictory, and mathematical models are inevitably flawed by the questionable validity of key values applied to them. Although the issue will undoubtedly eventually be resolved, it will probably require investigators to apply carefully designed studies on simple experimental systems such as isolated membranes or cultured cells. Only then should studies be extended to more complex systems such as isolated preparations of airways smooth muscle, where tissue bulk inevitably presents a complicating factor, particularly where relatively lipophilic compounds are concerned.
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Abbreviations: LABA, long-acting β_2-adrenoceptor agonist

The mechanism of persistence of the long-acting β_2-adrenoceptor agonist (LABA), salmeterol, clearly evident both *in vitro* and *in vivo*, has been a matter of considerable controversy for nearly two decades, and despite various studies having been performed to shed light on the issue, there is still no general agreement. The present report from Szczuka and colleagues (2009) not only provides a useful summary, but also adds a new mechanistic possibility. However, it ultimately fails to provide any strong indication as to which of the various proposed mechanisms is likely to be responsible.

The primary mechanistic contenders that they consider are the plasmalemma diffusion microkinetic (microkinetic) model of Anderson *et al.* (1994) and the exosite model, originally proposed by Jack (1991), as well as their own suggestion, β_2-adrenoceptor rebinding. This additional mechanism, although presenting an interesting addition to the debate, does not appear to stand close scrutiny as a mechanistic contender to explain salmeterol's duration of action. Such receptor rebinding can only contribute where there are available free β_2-adrenoceptors with which dissociated agonist molecules can interact. It is therefore difficult to see how such a mechanism could explain the agonist reassertion witnessed with salmeterol following treatment with (and subsequent removal of) high concentrations of β_2-adrenoceptor blocking drugs, a situation where effectively all available 'free receptors' are eliminated. Furthermore, as admitted by the authors, it could only provide any realistic extension of drug retention if associated with another mechanism acting to delay diffusion out of the local environment. This is a key consideration; otherwise, all agonists would be subject to such a phenomenon, and those with higher efficacy (i.e. lower occupancy, therefore, more free receptors) would be most affected. There is little or no evidence for an association between receptor efficacy and duration of action, and it should be remembered that salmeterol is a relatively low efficacy agonist. In short, although there is little doubt that receptor rebinding would be expected to contribute to a delay in the diffusion of agonists away from their active environment, it seems highly unlikely to be a critical determining factor in the case of salmeterol.

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One difficulty in determining which, if either of the microkinetic and exosite mechanism is the primary determinant of salmeterol's duration of action, is that microkinetics will undoubtedly have some influence on the persistence of action of any compound with a degree of lipid solubility. The real issue is whether microkinetics alone is sufficient to explain salmeterol's duration of action, and if not, whether there is any validity to the exosite hypothesis. Although this may at first seem a somewhat academic issue, it is of considerable importance to understand the contribution of the various mechanisms influencing duration of action, if improved compounds of this type are to be developed.

The case for microkinetics has been fuelled by the enthusiasm of various groups to compare salmeterol with the other prototypical LABA, formoterol (Anderson *et al.*, 1994; Bergendal *et al.*, 1996; Teschemacher and Lemoine, 1999; Austin *et al.*, 2003), for which this mechanism may very well play the primary role in the determination of duration of action. These groups have argued in essence that any difference in the *in vitro* duration profiles of these two compounds is quantitative rather than qualitative, and simply reflects salmeterol's higher log *P* value. It is undoubtedly true that in the absence of other contributory factors, lipophilic drugs are inherently slower in both onset and offset than hydrophilic drugs, but the question here is whether salmeterol's lipid solubility alone is sufficient to explain its persistence of action.

The results of the mathematical model proposed here by Szczuka and colleagues, as well as that of Austin *et al.* (2003), have broadly favoured lipophilicity as at least the primary duration determining factor for LABAs in general. However, a mathematical model can only be as good as the data applied to it. Reliance on estimations of key factors, such as durations of action and dissociation constants, which not only resist accurate determination, but may also be drawn from the work of others over which the authors have had no control, detract from the value of their conclusions. Indeed, Szczuka and colleagues have used values for the release of salmeterol from the plasmalemma membrane (k_{rel}) taken from two different studies (Rhodes *et al.*, 1992; Austin *et al.*, 2003), which differ by a factor of six, and the conclusions drawn critically depend on which of the two values they apply.

Another problem encountered is in the variety of experimental techniques and compounds used. Austin *et al.* (2003), for example, performed a comprehensive analysis on results obtained with an extensive range of compounds, from which they concluded that duration of action is a function of lipid solubility, along with basicity of the secondary amine, and they felt that their data provided no support for the exosite hypothesis. Although this may have been the case for the compounds under study, these compounds were not salmeterol, and the durations of action determined ($\leq$3 h) were considerably shorter than that of salmeterol, and thus too limited to permit any firm conclusions to be drawn.

Exosite binding is still very much a hypothesis, but there are certain features of salmeterol's profile of action (see Coleman *et al.*, 1996) that appear to be hard to explain by other mechanisms: (i) the repeated ability of responses to salmeterol to fully reassert, following treatment with high concentrations of β-adrenoceptor blocking drugs and subsequent washout, despite the addition of no further agonist; (ii) the relatively short duration of action of responses to salmeterol that are mediated by mechanisms other than β_2-adrenoceptors, including β_1- and β_3-adrenoceptors and other undefined 'non-β_2-adrenoceptor' mediated mechanisms; (iii) the results of receptor mutation studies in which an amino acid sequence within the fourth transmembrane domain of the β_2-adrenoceptor, when replaced with the corresponding sequence from the β_1-adrenoceptor, resulted in a less persistent duration of action of salmeterol. Furthermore, when the wild-type sequence from the β_2-adrenoceptor replaced its counterpart in the β_1-adrenoceptor, it resulted in a prolonged duration of action of salmeterol mediated via this β_1-adrenoceptor mutant.

At the risk of further complicating the issue, it is probably worth mentioning the introduction of a novel 'ultra-LABA', indacaterol, that uniquely combines a duration of action reportedly longer than that of salmeterol, with a rapid onset of action (Battram *et al.*, 2006). This compound appears to be of relatively low lipid solubility (consistent with its rapid onset of action) and lacks the arylalkoxyalkyl chain key to salmeterol's prolonged duration of action, and central to the exosite hypothesis. The impressive duration of action of indacaterol would therefore appear unlikely to result from either microkinetics or exosite binding, suggesting that a further mechanism is at play, which may or may not have relevance to salmeterol. Interestingly, the authors make no comment on this issue.

One of the confounding factors in most of the studies performed to date is the use of tissue as an experimental model. Tissue bulk provides a huge potential lipid sink that will undoubtedly act as a reservoir for any lipophilic compound, having a marked influence on apparent offset of action, whether or not it is the prime determinant. Perhaps the most clearly 'anti-exosite' data presented to date are contained in the report by Teschemacher and Lemoine (1999), who used lung membranes rather than intact tissue for their studies, and limited the amount of drug to which the membranes were exposed. Using this model, they reported that salmeterol could behave as a fast onset, fast offset β_2-adrenoceptor agonist, demonstrating little or no persistence of action. Although their findings seem to be at odds with those of others who have used isolated membranes or cells rather than tissue in their studies, this approach probably indicates the way forward, that is, simplicity of experimental system. There is little doubt that this debate will eventually be resolved, but it will take very carefully designed experiments with clearly quantifiable functional read-outs conducted in simple systems, whether cells or isolated membranes, to limit the potentially confusing influence of tissue bulk, to achieve it. Only when such studies have been completed, and some clarity of mechanism has emerged, should the studies be extended to more complex systems, initially isolated preparations of airways smooth muscle *in vitro*, and ultimately airways function *in vivo*.

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