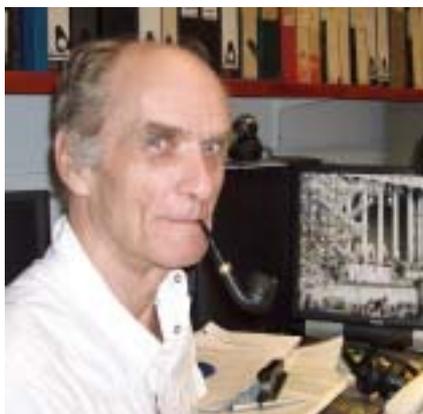


# Stephenson, affinity and efficacy in 2005



Professor David Colquhoun

The death last year of Robert Stephenson (Kelly & Stephenson, 2004) provides a good excuse to take a look at the current status of his ideas, and to recall the Pharmacology department in Edinburgh in the 1960s.

I joined that department in 1960, to do my PhD under Walter Perry and W E Brocklehurst. I had a large lab (and a technician), opposite Henry Adam's office (Vickerman, 2004), in the old and beautiful medical school building in Teviot Place. That was before the rape of George Square, and the reincarnation of the pharmacology department in the style of concrete brutalism. Now, of course, the department has vanished altogether in another bit of brutalism, but this time perpetrated by academic administrators rather than architects.

Perry had been my external examiner in Leeds, and his charm made me think that Edinburgh would be a good place to go. He thought it would be a great idea to measure the binding of radiolabelled immunoglobulins to lung tissue during passive sensitisation, so I spent four happy years teaching myself immunoelectrophoresis and ligand-binding methods (before the latter existed). The less said about the project the better. Despite discovering the perfect control (the very similar g1 and g2 globulins of the guinea pig, only one of which sensitised), any specific binding that there may have been was drowned in the large non-specific binding.

At about the same time, Humphrey Rang in Oxford was measuring the binding of radiolabelled atropine to smooth muscle, with much more success. Paton & Rang (1965) was the first radioligand binding paper of the modern age, and a great deal better than many of its successors.

I didn't really see much of Walter Perry during my PhD: he would appear from time to time in the lab, but mainly when he wanted a cigarette between committee meetings, and a bit of genial conversation. We didn't talk science much, and the fact that his radiation badge was the only one that got fogged turned out to be a result of a radium needle that had been sitting in a drawer in the departmental office, irradiating generations of professors and secretaries.

The highlights of my time there were, in many ways, the departmental coffee room and the staff club in Chambers Street, where I met Peter Higgs (later famous for his elusive boson). In the coffee room I met, in particular, 'Steve' Stephenson, Dick Barlow and Bernard Ginsborg, and I learnt as much in conversation with them as I did in the rest of my PhD. At that time, there was a big debate about the relative merits of Stephenson's (1956) ideas about efficacy (published only four years before I arrived) and the Dutch group of Ariens and van Rossum who characterised partial agonists in a simpler way in terms of 'intrinsic activity' (just the observed relative maximum response).

Stephenson had seen that an agonist, unlike an antagonist, could not be characterised by an equilibrium constant alone. An agonist must first bind (with a certain affinity), but then it must do something to produce a response.

The discovery of partial agonists meant that it could not be supposed that every agonist-receptor complex was equal. Some must produce more response than others, and the amount of response produced by the complex

was measured, he proposed, by a constant called 'efficacy' that was small for partial agonists, indefinitely large for full agonists and zero for competitive antagonists.

These ideas arose from the need to explain the phenomena of partial agonism and 'spare' receptors. Stephenson realised that they were also essential for rational drug design. The structural features that control the ability to bind (affinity) might be quite different from those that control the ability to activate the receptor once bound (efficacy). In order to understand how changes in the structure of an agonist change its effectiveness, it was essential to understand whether it was affinity or efficacy that was altered. This idea is as important now as it was in 1956. The fact that these quantities have proved so hard to measure may be one reason why our ability to predict the effects of changing the structure of the agonist is scarcely better now than it was in 1956.

The Dutch approach was purely descriptive, and could not cope, for example, with the quite common case in which there are more receptors than are needed to produce the maximum response.

It was pretty obvious, even in 1960, that Stephenson's ideas were much more sensible, as the Dutch school came eventually to agree (van Rossum, 1966). Nevertheless, there were heated arguments about what exactly the new ideas meant (in the course of which I realised that if I had been a bit older and more confident I should have asked to do my PhD with Bernard Ginsborg, whose intellect I came to admire enormously). It has to be said that Steve was not easily drawn beyond his original paper, and it was left to Barlow and Ginsborg to try to exploit the ideas (Barlow *et al.*, 1967; Barlow *et al.*, 1969; Stephenson & Ginsborg, 1969).

The strength (and, as it turned out, the fatal weakness) in Stephenson's famous paper was its attempt to come to conclusions without

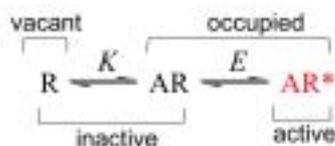
considering mechanisms. After all, Heinz Schild had done this with spectacular success for antagonists, so why not for agonists?

The formalism of Stephenson's theory was simple. The response ( $R$ ) was supposed to be some unspecified function of the receptor occupancy ( $p$ ) times a constant that he called *efficacy* ( $e$ ), so  $R = f(ep)$ . And occupancy was written as a simple Hill-Langmuir expression,  $p = c/(c + K)$ , where  $c$  is the free agonist concentration and  $K$  is an equilibrium constant (the theory was restricted to systems at equilibrium).

There were several assumptions (apart from the now rather implausible idea that two constants are enough to describe the action of an agonist). It was assumed explicitly that the function,  $f$ , though unknown, was the same for different agonists. It was also assumed implicitly that the two free parameters,  $e$  (measuring efficacy) and  $K$  (measure of affinity) were independent. The latter was the fatal mistake.

These deceptively simple equations were repeated from textbook to textbook, and in generations of undergraduate handouts (including mine). The discussion of what they meant often had that slightly bemused air that one sees also in the thermodynamics section of biochemistry textbooks, as though it had been copied from the previous textbook in the hope that the reader would not spot that the author did not quite understand it.

The whole question was clarified by another paper that was published a year after Stephenson's. Bernard Katz, at University College London, was also seeking to explain partial agonism, in particular the action of decamethonium, which appeared to be a partial agonist at the nicotinic receptors of the neuromuscular junction. Katz's approach was entirely different from Stephenson's. Rather than trying to be general, he wrote down a simple explicit reaction scheme, the now famous del Castillo-Katz (1957) mechanism (del Castillo & Katz, 1957). This can be written as follows:



My final year in Leeds had been something of a do-it-yourself job. I stumbled across some of Katz's papers in the library, though without realising at once their enormous importance. That was explained to me over coffee by Bernard Ginsborg, who had previously worked in Katz's Biophysics department at UCL (eg Burke & Ginsborg, 1956; Fatt & Ginsborg, 1958).

Clearly the first step in Katz's mechanism and its equilibrium constant,  $K$ , measures the ability of the agonist to bind in the first place. It therefore represents 'affinity', in the sense that Stephenson intended. The second step, an isomerisation between inactive and active receptors, both with agonist bound, and its equilibrium constant,  $E$ , equally clearly measures efficacy in Stephenson's sense.

But a glance at Katz's mechanism shows that there are two different species with agonist bound to them, AR and AR\*. Clearly the fraction of receptors that are occupied depends on  $E$  as well as on  $K$ . This means that a ligand binding experiment will not measure  $K$  but will measure  $K/(1 + E)$ . Some of the complications that result from that fact were dealt with in 1973 (Colquhoun, 1973), but it was sometime later before I realised that the theory on which I had been brought up was simply wrong.

Sometime in the mid-1980s I asked Stephenson whether the word 'occupancy', as used in his 1956 paper, was intended to be the quantity that would be measured in a ligand-binding experiment. When he replied "yes" it was clear that the whole basis for the quantitative formulation in Stephenson's paper was simply wrong.

The binding of an agonist, as measured in a binding experiment, can never depend only on the initial binding constant, because binding of an agonist elicits a response, and if binding affects activation then activation must affect binding. Stephenson's equation  $p = c/(c + K)$

asserts that occupancy depends only on affinity, whereas in fact it must depend also on efficacy. This is a very general conclusion, of which the Castillo-Katz mechanism provides the simplest concrete example.

Once this is realised, it follows that the various methods (Stephenson, 1956, Furchgott, 1966) that have been proposed for measurement of Stephenson's affinity and efficacy, simply do not work (Colquhoun, 1987). I should have seen this much sooner than I did, because while in Edinburgh I had bought the 1958 Edsall & Wyman's marvellous textbook, *Biophysical Chemistry* (from Donald Ferrier's bookshop, opposite the medical school, in 1962). The answer is implicit in their section on 'linked functions', and, less accessibly, in Wyman & Allen, 1951.

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*"drinking coffee with people cleverer than oneself is not a waste of time, but one of the best ways of expanding horizons. The 'sandwich at the desk syndrome' is not just unsociable: it may seriously harm your career"*

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The heart of the confusion that blights so many textbooks lies in the fact that the word affinity has two different meanings, and these meanings are often confused. Both uses are legitimate, but they are different.

One meaning of 'affinity' refers to what you measure when agonist binding is measured in a ligand binding experiment (it is appropriate to refer to this as macroscopic binding, and it depends on more than one equilibrium constant).

The other meaning of 'affinity' refers to the equilibrium constant for a single binding step, such as the first step in the del Castillo-Katz mechanism (microscopic affinity). It is the latter which represents the spirit of Stephenson's idea, but the former was what he got.

Stephenson's idea was magnificent. It is as important today as it was in 1956. Nowadays, the same question is put in a slightly different way. It amounts to identifying a reaction mechanism for the receptor, and estimating the rate constants for each step in the scheme. The only sort of receptors where we are anywhere near to being able to achieve this is for a handful of ion channels (Colquhoun, 1998). The vocabulary has changed a bit, but the ideas are the same. We are a long way from being able to measure these quantities for any G protein-coupled receptor. But, as he so presciently said, drug design cannot become a rational process until that (or some modern-day equivalent) becomes possible.

Two lessons can be drawn from this story. First, always follow Katz's example, and postulate an explicit mechanism, even if it is oversimplified, to test the results of intuitive thinking.

Second, drinking coffee with people cleverer than oneself is not a waste of time, but one of the best ways of expanding horizons. The 'sandwich at the desk syndrome' is not just unsociable: it may seriously harm your career.

**David Colquhoun**  
**Dept of Pharmacology**  
**University College London**

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