Kinetics of agonist conductance changes during hyperpolarization at frog endplates

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Commentary by

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I was surprised to learn that my little 1974 paper on voltage jump relaxations of agonist-induced conductance changes at frog endplates is to be reprinted in the BJP Anniversary edition, since it is a paper that I, and the world, forgot. I think that it elicited only 3 reprint requests, far fewer than any of my other productions, however trivial. Nevertheless I harbour some affection for this sickly and stunted first-born, partly because it *was* my first paper, partly because it triggered (or at least symbolised) a series of professional and scientific accidents, and partly because the idea that it embodies still has interest.

The study arose from my PhD thesis work, which began as a sort of freelance investigation of two interesting, but apparently unrelated, discoveries by others. The first was the well known observation that synaptic transmission is often more vulnerable to local anaesthetic agents than is nerve conduction, a difference that was thought to reflect the greater sensitivity of conduction in fine nerve terminals (Straughan, 1961; Donald Straughan became Chairman of the Department of Pharmacology at the School of Pharmacy, a position subsequently held by former colleagues at Bart's Medical School, David Brown and Norman Bowery). The second was the observation by my Professor that general anesthetics could block a muscle's response to acetylcholine but not its nerve-evoked twitch (Quilliam, 1955), which my fellow student Haydon Cash extended to the endplate potential produced either by nerve or agonist.

I was extremely fortunate to be shown how to record intracellularly from frog muscle by John Nicholls during one of his Christmas visits to the Department. Armed with this new technique I taught myself how to record extracellularly from nerve terminals and how to apply drugs iontophoretically. It soon became evident that procaine's effect was mainly a strange postsynaptic one, and that barbiturates could block rapid responses to acetylcholine much more effectively than they block depolarisations produced by the natural neurotransmitter. When I presented the latter result, at my first Society meeting, (Adams, Cash and Quilliam 1970) Jimmy Mitchell got up and insisted that the abstract be changed so that "pharmacologists in Wagga Wagga" would not conclude that Dale was wrong about the transmitter being acetylcholine!

To investigate these strange phenomena further I decided to try the voltage clamp technique used at the neuromuscular junction by the Takeuchis and by Kordas (1969). It immediately became apparent that all sorts of problems to do with agonist and drug action at synapses could be investigated in this way, and this became the topic of my thesis. I quickly found that the conductance change produced by carbachol and other agonists increased exponentially with membrane hyperpolarisation (Adams 1976a). Humphrey Rang was also doing similar experiments (Hammes *et al.*, 1971). Indeed Humphrey seemed to be on many of the same tracks (see also his review in *Nature*; 1971) but was deflected by career pressures and sailing. The explanation was made clear in an astonishing Royal Society lecture that Katz gave in 1969 (see Katz and Miledi, 1970). This lecture was ostensibly about transmitter release, but at the end Katz rather diffidently dropped what I felt then, and still feel, was the most amazing scientific bombshell - the discovery of "membrane noise" and its interpretation in terms of random opening and closing of single ion channels. Combined with Kordas' finding that the decay rate of endplate currents was prolonged by hyperpolarisation, the conclusion from Katz' analysis was inescapable - hyperpolarisation prolonged the open time of the

endplate channel, so shifting the equilibrium to favour channel opening. Similar inferences were drawn by Magleby and Stevens (1972) and Anderson and Stevens (1973).

My parallel analysis of barbiturate and procaine actions lead me to the idea that these drugs worked by entering and plugging open endplate channels (Adams 1974; 1975; see Lester 1990). Once again Humphrey was on the right trail, for he suggested that I look at Alan Steinbach's abstruse but important papers about local anesthetic action at the endplate. Steinbach's brother Joe Henry was later to popularise my idea in a single channel study done with Erwin Neher (Neher & Steinbach 1978). Equally important to me was a brief discussion with Don Mason (Reader in the Department), who suggested, most presciently, that the block of the receptor that seemed to be triggered by activation might be of the pore itself. In the first version of my full paper "Drug Blockade of Open End-Plate Channels" (Adams, 1976b) I discussed the general implications of open channel block for pharmacology. Unfortunately the reviewers insisted on excising this, the most important section of the paper, as being too speculative. I have invariably found that journal referees, while fair and efficient, are totally unsympathetic to ideas, which are after all the most important part of science. Some premature ideas wither by neglect, but most are aborted by the coat hangers of convention. As a result of analysing the behavior of channel blockers, I became interested in chemical kinetics. Manfred Eigen had recently been awarded the Nobel Prize for discovering relaxation methods, and I was particularly intrigued by his "voltage jump" technique. I reasoned that a voltage clamp step could be used to perturb the agonist-receptor reaction, and reveal the underlying kinetics and molecular mechanism. This lead to the experiments described in this 1974 *British Journal* article.

The experiment was quite tricky. Katz had shown that the time constants involved were about 1 msec, much shorter than the membrane time constant (which is particularly slow in muscle because of the capacity of the T-system). Although the synapse is quite short, and rapidly clamped, the capacity transients are very large and prolonged. I overcame this problem in 2 ways. Firstly, I cooled the preparation using a thermoelectric module. Because it was winter I could cool the preparation down further (to 2°C) by the simple expedient of opening the nearby window and wearing gloves. Secondly, I measured the local endplate current using a focal extracellular electrode. By these means I could measure the very quick relaxation of the carbachol-induced current. Of course I was very proud of these results - I thought of them as the first ever relaxation measurements on a biological membrane. In retrospect I had of course reinvented the wheel, since this was exactly the approach used by Hodgkin and Huxley to analyse the action potential. However, viewing voltage clamp as a relaxation experiment did offer some advantages.

The relaxation times I saw were very similar to those found using noise analysis by Katz and Miledi. I made the erroneous assumption that in my experiments quite high local concentrations of agonist were achieved, sufficient to saturate the receptors. I then drew the reasonable, but incorrect, conclusion that the agonists tested did not open more than a small fraction of the endplate channels. The attempt to estimate the efficacy of agonists using relaxation and noise techniques was to be a theme of the next 3 years (Sakmann and Adams, 1978). However the matter was only settled by the use of single channel analysis (Colquhoun and Sakmann, 1985).

Eigen was my hero, so I wrote to him asking if I could continue the relaxation experiments in his group. He wrote back saying that he had moved from chemical kinetics to the problem of the origin of life (Eigen, 1971), but that he was passing on my enquiry to a young researcher who had just joined his institute. This was Bert Sakmann, of whom I had never heard. I sent Sakmann a preprint of my *Brit. J.* paper, and he responded with a preprint of a paper in *Proceedings of the National Academy of Science* in which was reported almost exactly the same experiment (Neher & Sakman 1975), and an invitation to work in Göttingen! In Germany I used the relaxation method to further analyse the procaine effect (Adams, 1977), and persuaded Bert to abandon single channel recording to use noise and relaxation methods to analyse agonist action. My main claim to fame in science is that I impeded the patch clamp revolution by a year!

Later in Texas I joined forces with my former Bart's colleague David Brown to analyse the other main class of agonist actions (nowadays called metabotropic as opposed to ionotropic) using voltage jumps (Brown and Adams 1980). This decisively influenced both of our subsequent careers. My own recent work uses imaging techniques to understand neural circuits in brain slices and behaving zebrafish. However the most amazing

effect of the 1974 paper has only just emerged. When, in a hammock slung between two coconut palms, I read Eigen's beautiful little book "Steps Towards Life" (1994) during a sabbatical at my beach house in Eleuthera, I suddenly realised that Eigen's version of Darwinian evolution could also explain how the brain works. It turns out that synapses and genes follow the same rules, and that mental adaptations evolve in exactly the same way that physical adaptations do. I will spend the rest of my life exploring the ramifications of this extraordinary idea.

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