Antagonist discrimination between ganglionic and ileal muscarinic receptors

D.A. Brown, A. Forward & S. Marsh

Commentary by

M.P. Caulfield

In 1979, Donald Straughan moved from the Wellcome Chair of Pharmacology at the School of Pharmacy in London to take over the Pharmacology Division at Glaxo in Greenford. One of the projects at Glaxo was to investigate the possibility that there were subtypes of the muscarinic receptor, with special focus on neuronal receptors, and to define any potential therapeutic utility of subtype-selective agonists or antagonists; some of the possible targets included Alzheimer's disease, Parkinson's disease and pain. I joined this project at the beginning of 1980, under the impression that all muscarinic receptors were identical. Straughan, however, had a different view. He recalled a novel compound, 4-diphenylacetoxy N-methyl piperidine methiodide (4-DAMP) which had been made and tested by R.B. Barlow in Edinburgh, when he and Straughan were both members of the Pharmacology Department. Barlow had found that 4-DAMP was a more potent muscarinic antagonist at receptors mediating contraction of ileum, than at the receptors mediating slowing of atrial beating (Barlow *et al.*, 1976), suggesting that gut muscarinic receptors differed from those in heart.

Also, during a visit in 1979 to the British Pharmacological Society meeting at Leeds, Straughan heard a communication from Nigel Birdsall's group, describing curious neuronal muscarinic receptor binding characteristics of the Boehringer Ingelheim anti-ulcer drug pirenzepine. This compound has been spotted by Rudi Hammer of Boehringer as having peculiar selectivity *in vivo*, and he had initiated a collaboration with Birdsall and Ed Hulme to study this in radioligand binding experiments. Birdsall and Hulme had a rare understanding of radioligand binding, and two further advantages: they could do very accurate binding determinations, enabling them to detect deviation of a binding curve from that predicted by mass action (using radiolabelled ligands which they had developed), and they also had a non-linear iterative curve fitting program (a very rare thing in those days) which could model non-mass action behaviour by multiple ligand binding sites with more than one ligand affinity. The upshot was that their binding work strongly indicated the presence of multiple muscarinic binding sites in different brain regions, which could be discriminated by differing pirenzepine affinities (Hammer *et al.*, 1980).

Given this, Straughan was keen to see whether the neuronal muscarinic receptor subtypes indicated by the Birdsall group's binding studies could be confirmed in functional experiments, particularly on receptors modulating neuronal excitability (the wish to do functional tests was intended to balance the prevailing enthusiasm for binding experiments, with the concern that binding sites do not necessarily represent functional receptors). We had both been at the School of Pharmacy in London when David Brown's group were doing a study on extracellularly-recorded muscarinic depolarizations in sympathetic ganglia (Brown et al., 1980a), and it seemed logical to ask him to take a look at these possibly selective compounds. David Brown was very supportive of our ideas, and extremely interested in the concept of neuronal subtypes of muscarinic receptors, but his priority at the time had moved on from recording extracellular depolarizations, to voltage-clamping and measuring neurotransmitter effects on something called an "M-current". Nevertheless, because of his interest in the science, he agreed to collaborate with us, and dig out the old extracellular recording set-ups and do some experiments. I think he regretted this somewhat when I asked that he compare neuronal responses with contractile responses in rat ileum, as this involved Steve Marsh invading the third

year teaching lab to set up arrays of organ baths, isotonic levers and (horror of horrors) smoked drums. He has only recently recovered from this techno-embarrassment.

The results, of course, were fascinating. The Schild-type experiments reported in Brown *et al.* (1980b) showed that pirenzepine had higher affinity for ganglionic muscarinic receptors than ileal receptors, (about 10-fold) while 4-DAMP did not discriminate. The pirenzepine data were consistent with the discrimination reported by the Birdsall group for the high and low-affinity binding sites in brain. This, coupled with Barlow's ileal versus atrial selectivity of 4-DAMP (again, about 10-fold) provided good evidence that there were three distinct functional muscarinic receptor subtypes, and confirmed pirenzepine and 4-DAMP as useful discriminatory tools. Others made a contribution by calling these receptors M_1, M_2 and M_3. The immediate impact of this demonstration of heterogeneity was to galvanize research into the role and location of muscarinic receptor subtypes, aided by the use of these selective compounds in both functional and binding studies. Further compounds with new profiles of selectivity came from modifications of the pirenzepine molecule by the Boehringer chemists (e.g. AF-DX 116), and other chemical approaches (e.g. methoctramine from Melchiorre's group, and hexahydrosiladifenidol from the Lambrecht group). Also, a number of pharmaceutical companies busied themselves setting up ganglion recordings, and producing muscarinic agonists in the hope of finding one with M_1 receptor selectivity, which Straughan and I had suggested might be useful in treatment of the memory disorder in Alzheimer's disease (Caulfield & Straughan, 1983).

The high level of interest in muscarinic receptor subtypes stimulated by the work of Barlow, Birdsall and Brown was rewarded in 1986-7, when the existence of distinct receptors was confirmed at the molecular level by the efforts of molecular biologists in the groups of Numa/Haga, Peralta and Bonner/Brann/Buckley, with (as predicted) three different genes, the first (m1) expressed predominantly in hippocampus and cerebral cortex, and also in sympathetic ganglia, m2 expressed in heart, and m3 expressed in smooth muscle. Knowledge of receptor sequences also resulted in the cloning of two further members of the muscarinic receptor family, and although pharmacologists had no way of knowing about M_4 and m5, it was salutory that the molecular biology confirmed what the pharmacologists had concluded some six years previously about M_1, M_2 and M_3 receptors. It has also been gratifying to see the merging of receptor pharmacology, receptor molecular biology, and that new-fangled M-current! (see Brown *et al.*, 1995). It was also nice that one of our Glaxo project members (Sebastian Lazareno) caught up with the molecular biologists by defining a unique pharmacology for the M_4 receptor (Lazareno *et al.*, 1990), which Brown and I subsequently demonstrated in a functional assay (coupling to inhibit voltage-gated Ca^{2+} currents: Caulfield & Brown, 1991). The knowledge of the pharmacology described by Lazareno has been invaluable in assigning further functions to this novel receptor subtype.

All of this work led to definition of muscarinic receptor subtypes which not only differed pharmacologically (i.e. at the ligand recognition sites), but also differed in terms of their effector coupling. The reason behind this became clear during the same period as the subtype work, as the other thing happening in parallel was the finding that many of the receptors we had been studying (including muscarinic receptors) produced their effects by coupling via G-proteins. Thus, the different effects of the muscarinic receptor subtypes (including those on ion channels) could often be attributed to coupling to different G-proteins, and the group of David Brown has also been at the forefront of progress here (reviewed by Caulfield, 1993; Brown *et al.*, 1995). This complex coupling (and the lack of understanding of it) probably contributes to the difficulty encountered in trying to develop subtype-selective agonists. For the future, we hope that increasing knowledge of receptor structure, and of how agonists change receptor structures to bring about coupling to G-proteins, will allow us to understand how agonist occupancy of receptors brings about responses. Additionally, increasing knowledge of how G-protein subunits couple to effectors (including ion channels) will allow us to describe these interacting mechanisms in a quantitative (that is, pharmacological) manner. And this is why good old-fashioned quantitative pharmacology, of the sort seen in Brown *et al.* (1980b) is still indispensible, even in the modern world of molecular biologists and patch clamps.

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