The pharmacological actions of polymethylene bistrimethylammonium salts

W.D.M. Paton & E.J. Zaimis

Commentary by

D. Colquhoun

This paper was an important milestone in the development of ideas about muscle and neuronal nicotinic receptors. It is about the pharmacological actions of the series of compounds that consist of two quaternary ammonium groups joined by a polymethylene chain of variable length. Quaternary ammonium compounds were probably the first synthetic compounds to be investigated with a view to revealing a relationship between structure and activity (Crum Brown & Fraser, 1868, 1869), and they have featured in many studies since then. Although the paper by Paton & Zaimis (1949) is ostensibly a purely pharmacological investigation, it has actually contributed as much to physiology as to pharmacology. The paper describes in some detail the actions of compounds with 2 to 13 (and 18) methylene groups between the quaternary nitrogens. A whole range of actions is investigated: neuromuscular block and its (considerable) species-dependence, antagonism of neuromuscular block, direct actions on skeletal muscle (frog isolated *rectus abdominis*), ganglion blocking activity, anticholinesterase activity, oral activity, and surface activity effects. The main emphasis, though, was on neuromuscular block by the 10-methylene compound, C10, or decamethonium; the ganglion block effects were investigated in more detail by Paton & Zaimis (1951).

There are really two separate areas in which this paper was important. First, it gave the first detailed description of the action of a compound that produced paralysis of voluntary muscle by a depolarising action at the end-plate, as opposed to the non-depolarising effects of previous blockers like tubocurarine. And secondly, it provided a clear and quantitative distinction between the acetylcholine receptors that are responsible for transmission in skeletal muscle, and in autonomic ganglia. These two contributions are best dealt with separately.

Depolarising neuromuscular block

Decamethonium, and the compounds near it in the series, were shown to block nerve-evoked twitches of skeletal muscle, for example the cat *tibialis*. The block (in the cat) was found to be faster in onset and recovery than that produced by tubocurarine (one property that led to its clinical use for a while). More interestingly, they found that decamethonium differed radically from tubocurarine in many ways. It produced, initially, muscle fasciculation, and often a transient increase in twitch strength, and it did not affect the ability of either nerve or muscle fibres to conduct action potentials. It also, like acetylcholine itself, caused a contracture of the frog *rectus abdominis* muscle, and the paralysis caused by decamethonium was not reversed by anticholinesterase agents. In fact they say "We do not wish, however, to underestimate the resemblance of some of our tracings to those resulting from injections of potent anticholinesterases", though they later conclude (correctly), that (*a*) the weak cholinesterase inhibition produced by decamethonium is insufficient to explain its effects, and they also speculate (again correctly) that the paralysis produced by anticholinesterases may, in any case, not be caused only by inhibition of cholinesterase (Aracava *et al.*, 1987). They conclude that decamethonium is not "an inactive competitor with acetylcholine but that it is itself active in some respect at the neuromuscular junction". In fact they were already aware that decamethonium could depolarise the end-plate, and say "the relationship of this depolarisation to the neuromuscular block remains, however, to be elucidated". The only statement with which one would disagree today is that "it is difficult to account for these facts on the supposition that tubocurarine and decamethonium work at the same point".

Shortly afterwards, Burns and Paton (1951) took the analysis of the mechanism of the block much further. From a very impressive piece of work, done by external electrical recording from the cat *gracilis* muscle, they concluded that the depolarisation produced by decamethonium is centred round the end-plate region and spreads only slightly beyond it, and that "the inexcitability of the muscle membrane around the point at which the end-plate potential is set up is therefore a principal cause of the neuromuscular block produced by decamethonium". They found that "removal of the end-plate depolarisation, by passing an anodal current at the end-plate region, restores neuromuscular transmission in a muscle blocked by decamethonium", and that "all the principal features of block by decamethonium can be reproduced with acetylcholine". They conclude that "the characteristic features of block by decamethonium are simply those of any persistent cathode" – in more modern jargon, they concluded that persistent depolarisation itself, whether produced electrically or by means of an agonist like decamethonium or acetylcholine, would render the perijunctional region inexcitable, and so block transmission. This impeccable description needs no alteration today. All that is missing is an allusion to the inactivation of perijunctional sodium channels by persistent depolarisation.

This was not quite the end of the story, because subsequently it was suggested (Thesleff, 1955) that decamethonium and related agents might work by desensitizing acetylcholine receptors, rather than by depolarisation *per se*. This led to a prolonged, and sometimes vituperative battle in which Zaimis, in particular, defended the original proposition with considerable vigour. Still later, it became apparent that decamethonium and similar compounds are also powerful channel blockers (Adams & Sakmann, 1978). There is no doubt that desensitization and channel block occur, but equally there is little doubt that Paton & Zaimis (and Burns & Paton) had got it essentially right. Under the conditions of their experiments, and under clinical conditions, the only important difference between acetylcholine and decamethonium is that the latter is around for longer, and the consequent prolonged depolarisation renders the perijunctional muscle membrane inexcitable, so action potentials cannot be initiated by transmitter in the normal way.

Muscle and neuronal nicotinic acetylcholine receptors

By the time this paper was written it was already well-established that synaptic transmission in both sympathetic ganglia and at the neuromuscular junction were chemically mediated, and that the transmitter was the same at both sites, namely acetylcholine. It must be remembered that, at this time, the term 'receptor' was not used nearly as much as it is now. It is clear, nevertheless, that this was more a matter of caution than of substance. For example, A. V. Hill's (1909) derivation of the Langmuir equation in the context of nicotinic responses of the frog *rectus*, clearly implied that even then the action of acetylcholine was supposed to be on discrete binding sites on some 'receptive substance' (as Langley had termed it). In fact the term 'nicotinic receptor' is never used in the paper of Paton & Zaimis. At the outset it is mentioned that the compounds being tested have actions that are "commonly called nicotine-like". And in the Discussion it is said that "it is difficult to avoid the conclusion that the extreme sensitivity of the series to chain length implies some rather specific 'fit' between the extended molecule and its *effector site*" ..."...corresponds to the spacing of some regularly recurring receptor groups" "A recurrent acidic residue on a polypeptide chain would provide an array of the type required": it is clear from all this that, although the term nicotinic receptor is never used, the authors were assuming that the compounds they used did act on a polypeptide receptor.

It was certainly appreciated already, when this paper was written, that the actions of acetylcholine were by no means the same on muscle and on sympathetic ganglia, but Paton and Zaimis provided the most quantitative description of these differences to be published. The fact that the quaternary compounds being tested show a sharp peak in potency for neuromuscular block with decamethonium, but a similarly sharp peak for ganglion block with hexamethonium, provided a dramatic picture of the difference between what we would now call muscle-type and neuronal-type nicotinic receptors, and their graph showing the relationship between activity and chain length is still frequently reproduced. They also appreciated that the mechanisms of block were quite different at these two sites. Hexamethonium did *not* produce block by depolarisation. For that reason it was often in

those times (and sometimes still is) referred to as being 'competitive'. It was not until much later that it was realised that hexamethonium does not compete with acetylcholine to any noticeable extent, but blocks ion channels, and is so long-lived because it can get trapped inside the channels (Gurney & Rang, 1984), though this suggestion was first made in the 1950s (Blackman 1959).

Still less could Paton & Zaimis have foreseen the multiple types of subunits for muscle, and especially neuronal, nicotinic receptors that have now been cloned. This new work has shown that the story started by their paper (among others) is still far from complete. The subunit composition of neuronal nicotinic receptors is still unclear, as is the function of the many nicotinic receptors found in the brain (e.g. Sivillotti & Colquhoun, 1995).

The paper of Paton & Zaimis is still a pleasure to read, and not only because of the quality of the experiments, and the accuracy of its speculations. It is written in plain English, totally free of unnecessary jargon, exaggeration or sanctimonious euphemisms (animals are killed, not 'sacrificed'). The style is that of an age when plain facts, and plain words were more important than grantsmanship, and that makes a nice change.

References

ADAMS, P.R. & SAKMANN, B. (1978). Decamethonium both opens and blocks end-plate channels. *Proceedings of the National Academy of Sciences, USA*, **75**, 2994-2998.

ARACAVA, Y., DESHPANDE, S.S., RICKETT, D.L., BROSSI, A., SCHONENBERGER, B. & ALBUQUERQUE, E.X. (1987). The molecular basis of anticholinesterase actions on nicotinic and glutamatergic synapses. *Annals. New York Academy of Sciences*, **505**, 225-226.

BLACKMAN, J.G. (1959). Mechanism of Ganglion Blockers, Ph.D. thesis, University of New Zealand.

BURNS, B.D. & PATON, W.D.M. (1951). Depolarization of the motor end-plate by decamethonium and acetylcholine. *Journal of Physiology (London)*, **115**, 41-73.

GURNEY, A.M. & RANG, H.P. (1984). The channel-blocking action of methonium compounds on rat submandibular ganglion cells. *British Journal of Pharmacology*, **82**, 623-642.

HILL, A.V. (1909). The mode of action of nicotine and curari determined by the form of the contraction curve and the method of temperature coefficients. *Journal of Physiology (London)*, **39**, 361-373.

PATON, W.D.M. & ZAIMIS, E.J. (1949). The pharmacological actions of polymethylene bistrimethylammonium salts. *British Journal of Pharmacology*, **4**, 381-400.

PATON, W.D.M. & ZAIMIS, E.J. (1951). Paralysis of autonomic ganglia by methonium salts. *British Journal of Pharmacology*, **6**, 155-168.

SIVILOTTI, L.G. & COLQUHOUN, D. (1995). Acetylcholine receptors: too many channels, too few functions. *Science*, **269**, 1681-1682.

THESLEFF, S. (1955). The mode of neuromuscular block caused by acetylcholine, nicotine, decamethonium and succinylcholine. *Acta Physiologica Scandinavica*, **34**, 218-231.