# The channel-blocking action of methonium compounds on rat submandibular ganglion cells

A.M. Gurney & H.P. Rang

## *Commentary by*

H.P. Rang

In two classic papers in the *British Journal of Pharmacology*, Paton & Zaimis (1949, 1951) described the effects of the methonium series of compounds on neuromuscular and ganglionic transmission. One of their most striking findings was the bell-shaped relationship between the length of the interquaternary polymethylene chain and the ganglion blocking effect of the compound, the maximum activity being observed with hexamethonium (C6), with a drop-off in activity at shorter or longer chain lengths. In the early 1960s I had the very good fortune to work in Oxford as a graduate student under Bill Paton's supervision. This was shortly after the publication of his Rate Theory of drug action (Paton, 1959), which attributed the balance between agonist and antagonist activity essentially to the dissociation rate constant of the drug-receptor complex. It was generally believed, on the basis of their chemical resemblance to acetylcholine, that the methonium compounds must act by binding at the receptor site, and Bill felt that the explanation for the chain length relationship must somehow be related to the on-off kinetics of the interaction of these compounds with the receptor. It was, however, difficult to formulate the hypothesis convincingly, much less to test it experimentally. I became involved in other projects, and thought no more about the problem until about 15 years later, when a spate of papers appeared which showed that many compounds, including local anaesthetics (Adams, 1976), atropine (Feltz *et al* 1977) and tubocurarine (Colquhoun *et al* 1979) could inhibit cholinergic transmission by 'open channel block', that is, by blocking the ion channel without binding directly to the acetylcholine receptor site. Operationally, the main characteristics which distinguished channel block from receptor block were its strong dependence on the membrane potential, and 'use-dependence' – the tendency for repeated opening of the channels by an agonist to increase the amount of block.

When Philippe Ascher joined me for a year's sabbatical leave, we decided to set up a preparation of the rat submandibular ganglion for voltage clamp measurements, and these experiments (Ascher *et al* 1979) quickly revealed that both the methonium compounds and tubocurarine owed their ganglion blocking activity mainly or entirely to open channel block, in contrast to other ganglion blocking agents, such as trimetaphan, which showed quite different characteristics, and probably acted at the receptor site. This reinterpretation of the mechanism of action of the much-studied methonium compounds succeeded in overturning the accepted dogma, but we were still left with a paradox: decamethonium and hexamethonium appeared to associate with the open channel in a very similar way - the rate constants for their association with the open channel were not much different - yet decamethonium was a much weaker blocking agent (in line with the earlier work of Paton & Zaimis) under steady-state conditions. We seemed to have upset a number of apple-carts, but were no nearer to an explanation of Paton & Zaimis' bell-shaped curve.

Soon after I moved to University College London, Alison Gurney joined my laboratory as a graduate student. Initially, we set out to use the ganglion preparation to investigate the postulated presynaptic blocking action of drugs such as tubocurarine; this proved difficult and progress was slow, so we decided to take another look at the methonium problem, which was by now annoying me just as it had Paton. We were fortunately able to obtain, through the generosity of Edward Gill, an earlier Oxford colleague, samples of the whole series of methonium compounds from C4 to C10,

and so were able to study the series systematically. All turned out to be open channel blockers, as we had suspected. With compounds shorter than C6, the association rate constant dropped off sharply, explaining their low potency. The longer chain compounds all had very similar association rate constants, so it seemed that the drop-off in potency must be due to an increase in the dissociation rate constant as the chain-length increased - a counter-intuitive hypothesis, but one which we could at least test. Comparing C6 and C10 at equiactive concentrations, we indeed found that the use-dependent component of the block equilibrated much more slowly with C6 than with C10, consistent with a lower rate of dissociation from the channel. To measure the dissociation rate more directly meant inducing the use-dependent channel block, then allowing a fixed recovery period before testing with a pulse of acetylcholine to assess how much block remained. These were fiddly experiments, but with C6 they seemed to show that no recovery whatever was occurring, even if we waited several minutes before applying the test pulse. We simply could not understand what was going on, as theory led us to expect recovery in a few seconds. Then we tested C10, and found that it did indeed dissociate in about 5 seconds. Mercifully, as Alison was by this time growing nervous about her thesis, the penny dropped at this point. When C6 blocks the ion channel, the channel can still close when the acetylcholine molecule dissociates, and in doing so traps the C6 molecule until such time as the channel reopens in response to a pulse of acetylcholine. C10 is too big to allow the channel to close, so it escapes readily even if no acetylcholine is applied. This effect with C6, which we called the 'trapping phenomenon' finally provided an explanation of why the potency dropped off as the chain-length increased beyond a certain point; compounds shorter than C9 are small enough to become trapped, whereas beyond this size, they are large enough to prevent channel closure and thus elude the trap, which makes them very much less effective as blocking agents under physiological conditions.

Unknown to us at the time, Lingle had developed the same idea to account for the actions of methonium compounds on the response of lobster muscle to acetylcholine, and his findings, published shortly before ours (Lingle, 1983) were very similar. We also realised that Armstrong (1971) had much earlier come to a similar conclusion about the interaction of alkylammonium compounds of varying chain-lengths with potassium channel gating in the squid axon. The trapping phenomenon, in various forms, has subsequently been invoked to account for a number of effects with both voltage-gated and ligand-gated ion channels, but so far as I know, Lingle's paper and ours were the first to develop the concept to explain the effects of synaptic blocking drugs. In 1984 very little was known about the molecular mechanism of channel gating, and we imagined a kind of trapdoor mechanism. However, the recent X-ray studies of Unwin (1993) on the Torpedo acetylcholine receptor suggest that gating is associated with constriction and dilatation of the channel pore, as the pore-lining transmembrane helices alter their conformation. To accommodate the trapping phenomenon, we have to postulate that the smaller molecules, such as C6, somehow get stuck when the pore constricts, whereas larger molecules, such as C10, are able to prevent the pore from constricting without getting stuck - a trick worthy of Houdini. Whether this is chemically feasible remains an open question.

Subsequently, neither Alison nor I worked any more on this problem, but we felt that we had cleared the air, and confirmed yet again the old pharmacological truth that simple molecules often work in complex ways.

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