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Modes of action of gallamine at the neuromuscular junction

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Gallamine is normally classified as a competitive neuromuscular blocking agent (e.g. Rang & Ritter, 1969). We have studied gallamine by means of voltage-jump measurements. Figure 1a shows the normal relaxation of end-plate current induced by acetylcholine (3μM), following step changes of membrane potential from −70 mV to −130 mV, and back. Figure 1b shows a similar experiment but carried out in the presence of gallamine (5 μM). On hyperpolarization there is initially a rapid *decrease* in current, followed by opening of channels that is slower than the normal rate shown in Figure 1a. When the internal potential is reduced to −70 mV again there is, paradoxically, a rapid *increase* in current at first, followed by the usual decrease which is again slower than normal. Similar observations have been made at end-plates of both mouse (Figure 1) and frog.

The effects shown in Figure 1 strongly resemble those found with procaine at the neuromuscular junction by Adams (1977). This suggests that gallamine can produce a potential-dependent block of open ion channels, as tubocurarine appears to do (Colquhoun, Dreyer & Sheridan, 1978), though much faster. These observations are consistent with those made recently by Katz & Miledi (1978). The relative importance of competitive block and ion-channel block by gallamine is being investigated.

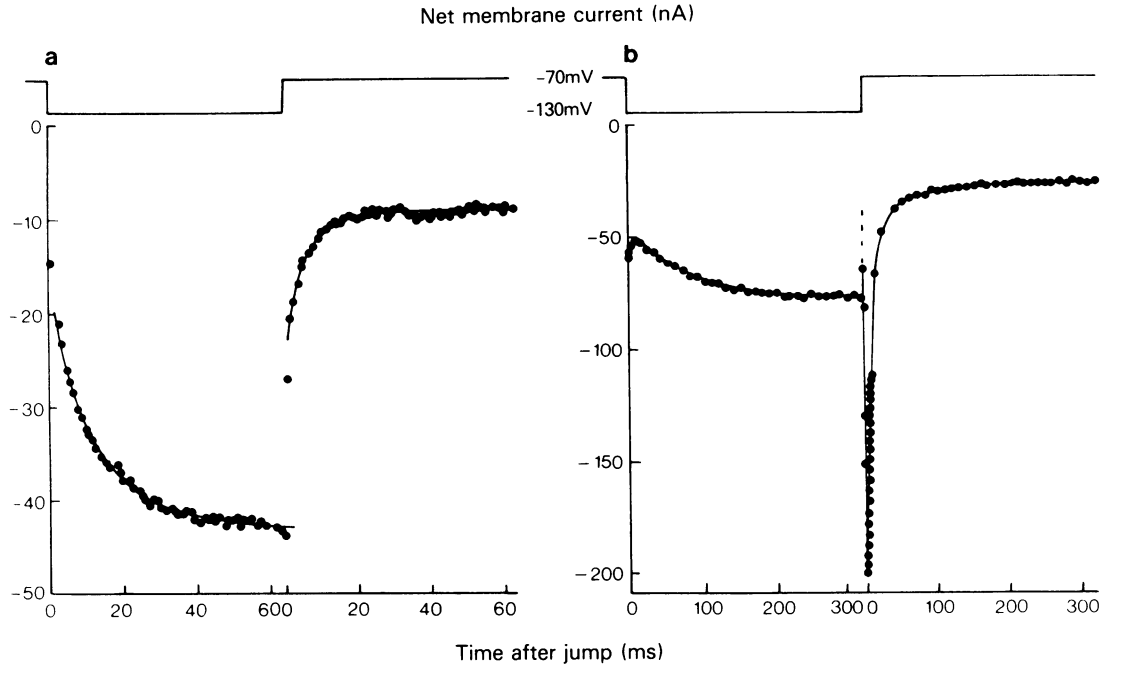

Figure 1 Mouse omohyoid muscle, at 8°C. Cholinesterase inhibited with methane sulphonyl fluoride. Membrane potential clamped as shown schematically above (a) and (b). Inward (negative) current is plotted against the time after the voltage jump. (a) Net currents induced by acetylcholine (3μM). (b) Net currents induced by acetylcholine (10μM) in the presence of gallamine (5μM). The dashed line is extrapolated to the expected instantaneous current immediately following repolarization. Note slower time scale in (b).

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Two modes of action of ganglionic blocking drugs

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Non-depolarizing ganglion blocking drugs are usually thought to block acetylcholine receptors competitively, though Blackman (1959) suggested that hexamethonium acts by blocking ionic channels rather than by competition.

We have used a two microelectrode voltage clamp technique on rat submandibular ganglion cells (Ascher, Large & Rang, 1978) to investigate the actions of blocking drugs in more detail.

With the membrane potential held constant application of carbachol (by perfusion or microiontophoresis) causes an inward current whose magnitude increases markedly with hyperpolarization. Some antagonists (trimetaphan, 2.5μM; surugatoxin, 0.2 μM) reduce the carbachol-induced current equally at all membrane potentials (i.e. the block is not voltage-dependent). Others (tubocurarine, 5 μM; hexamethonium 2 μM; decamethonium, 50 μM) reduce the current proportionately more as the membrane is hyperpolarized (voltage-dependent block). Defining Λ as the ratio of current in the absence and in the presence of antagonist, voltage dependence can be expressed as $(\Lambda_{-80}-1)/(\Lambda_{-50}-1)$ where the subscript represents membrane potential. Values obtained were: trimetaphan 1.18, surugatoxin 0.86, tubocurarine 3.13, hexamethonium 3.17, decamethonium 2.87. Marked voltage dependence suggests, but does not prove, a mechanism other than competitive block.

With competitive block $\Lambda-1$ should decrease as the agonist concentration is increased. We tested this by measuring $(\Lambda_{large}-1)/(\Lambda_{small}-1)$ with alternate large and small responses to iontophoretically applied carbachol, differing 3–4 fold in amplitude. With trimetaphan this ratio is slightly less than one; with voltage-dependent antagonists this ratio ranges from 1.3 to 1.7, which is not consistent with competitive block.

This pattern of block increasing with agonist concentration and with membrane potential favours a channel block mechanism such as that described for procaine and other agents at the neuromuscular junction (Adams, 1977; Feltz, Large & Trautmann, 1977, Neher & Steinbach, 1978) and for various acetylcholine antagonists on Aplysia neurones (Ascher, Marty & Neild, 1978).

Kinetic studies were carried out with voltage jumps, in which the change of current with time was followed after a step in membrane potential from −50mV to −80mV. With carbachol alone the current increases to a new level within 10 ms (the limit of resolution of our voltage clamp) and no relaxation is detectable. With hexamethonium or tubocurarine present, the current increases rapidly and then decreases exponentially with a time constant of 1–2 s. This relaxation becomes faster if the antagonist concentration is increased, as expected for a slowly-dissociating channel blocker. With decamethonium the slow relaxation is in the opposite direction and becomes slower if the decamethonium concentration is increased, as predicted for a rapidly dissociating channel blocker (Adams, 1977, Ascher, Large & Rang, 1978).

It is concluded that tubocurarine, hexamethonium and decamethonium act mainly or entirely by blocking ionic channels, whereas trimetaphan and surugatoxin probably block receptors.

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