Br. J. Pharmac. (1984), **81**, 215–224

The response of cat spinal motoneurones to the intracellular application of agents with local anaesthetic action

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1 QX-222 (the trimethyl analogue of lignocaine), methylxylocholine, lignocaine and pentobarbitone were iontophoresed intracellularly into cat lumbosacral motoneurones. Iontophoresis and recording was either from a triple-barrelled microelectrode unit or from two separately advanced microelectrodes.

2 QX-222 and methylxylocholine caused a very slow reversible block of the current-evoked and antidromic action potentials (AP) with no significant change of membrane potential (E_M). Lignocaine had a minimal blocking effect on the AP.

3 No change, or only a small decrease, of membrane slope conductance (G_M) was seen when the APs had been totally abolished.

4 QX-222 and methylxylocholine reduced the massive G_M increase evoked by the passage of large depolarizing currents and converted the post-current hyperpolarization (time constant 120–150 ms) into a depolarization of similar time course. It is suggested that the quaternary local anaesthetics can reduce the fast and slow voltage-dependent potassium conductances.

5 Both agents totally blocked AP generation without decreasing the magnitude of the Ia e.p.s.p.

6 It is suggested that intracellularly iontophoresed QX-222 (on account of its low lipid solubility) could be used as a pharmacological tool to block specifically the active Na and K channels in only the cell impaled by the microelectrodes.

Introduction

The action of the classical local anaesthetic agents such as procaine and lignocaine has been well characterized for nerve fibres (see reviews by Ritchie, 1975; Strichartz, 1976) and for other tissues (see review by Seeman, 1972). Experiments have shown that the charged (cationic) form of the amine local anaesthetics, when present intracellularly, plays an important role in inhibiting the generation of the action potential (Narahashi, Frazier & Moore, 1972).

The action potential (AP) mechanism of cat spinal motoneurones has been shown to be sensitive to tetrodotoxin (Blankenship, 1968; Zieglgänsberger & Puil, 1972) which indicates that there is a close similarity between 'active' electrogenesis in motoneurones and in peripheral nerve fibres. Experiments in which motoneurones have been exposed to the action of classical local anaesthetic agents by microiontophoresis (Curtis & Phillis, 1960; Engberg, Flatman, Kadzielawa & Lambert, 1975) provide further evidence of this similarity. The large size of the motoneurone allows relatively easy and stable penetration by one or more microelectrodes, and agents under investigation can be ejected intracellularly by microiontophoresis (Constanti, Krnjević & Nistri, 1980). Thus the intracellular action of the permanently charged, cationic analogues of lignocaine can be identified in the knowledge that little of the iontophoresed agent will escape to the extracellular environment as these agents are very lipid insoluble (Strichartz, 1973; Hille, 1977).

In this article we investigate the action of agents with local anaesthetic action (including pentobarbitone) applied intracellularly by 'push-pull' iontophoresis (Eccles, Eccles & Ito, 1964) from triple-barrelled microelectrode assemblies. To overcome the problems of recording membrane potential at the same time as injecting the large currents required to plot current/voltage curves, we have also used inde-

pendent impalement of the motoneurone with two microelectrodes (Engberg, Källström & Marshall, 1972; Engberg & Marshall, 1979).

Preliminary accounts of this work have appeared previously (Flatman, Lambert & Engberg, 1978; 1980; 1981; Flatman & Lambert, 1979).

Methods

Experiments were performed on 20 cats (2–5 kg, either sex) anaesthetized with pentobarbitone (35 mg kg^{-1} intraperitoneally at first and then 5–10 mg intravenously as required), paralysed with gallamine triethiodide and artificially respired with intermittent positive pressure ventilation. Spinal transection was performed at the lower thoracic region. Full details of the basic experimental procedure and maintenance of animal welfare have been given previously (Engberg *et al.*, 1979).

Left hind limb nerves were dissected free and laid upon bipolar silver electrodes (within a pool of mineral oil) for nerve stimulation. E.p.s.ps were evoked in a motoneurone by stimulating muscle nerves at known stimulus strengths just above threshold (T) for the group Ia muscle spindle afferents (as judged by monitoring the afferent volley at the entry zone of the dorsal root at the cord dorsum). The L_7 and S_1 ventral spinal roots were sectioned and laid upon bipolar silver electrodes to allow antidromic stimulation of the penetrated motoneurone.

Arrangements for simultaneous potential recording and microiontophoresis used in this study

(i) Triple-barrelled microelectrodes The electrodes were prepared as described previously (Flatman *et al.*, 1978). The tips were broken back to an overall diameter of 2–2.5 μm. Two barrels were filled with KCl: one (3 M) for recording and one (0.3 M) for current balance during iontophoresis. The third barrel contained one of the following drugs at a pH of 7: 0.3 M N-(2,6-dimethylphenylcarbamoylmethyl) trimethylammonium chloride (QX-222 Cl) or 0.05 M in 0.3 M lignocaine hydrochloride or 0.05 M in 0.3 M KCl; 0.3 M methylxylocholine chloride. The concentrations were chosen so as to give a reasonable compromise between passive drug leakage and transport number during iontophoresis. The currents used to iontophorese drugs from the third barrel were counterbalanced by equal currents of opposite polarity through the second KCl barrel. In some early experiments this was done manually; later it was automatically controlled by electronic connections between the current generators – the actual current from one generator was converted to a command signal for the other. With some electrodes drug leakage, as indicated by an increasing block of the action potential (AP), had to be restrained by retaining currents (similarly balanced). The electrode assemblies were screened with graphite to within 5 mm of the tips (for electrical connections and functions of the screen, see Engberg, Flatman & Lambert, 1975, 1979).

(ii) Independent impalement by two separately advanced microelectrodes The micromanipulators used have been described previously (Engberg *et al.*, 1972). One electrode contained 2.5 M KCl in 2% agar (tip diameter, 1.5–2 μm, resistance, 2–6 MΩ). The second contained one of the following solutions: (i) 3 M KCl; (ii) QX-222 (in concentrations ranging from 0.05 M to 0.5 M) in 3 M or 5 M KCl; (iii) 0.3 M methylxylocholine in 3 M KCl (tip diameter, 1–2 μm, resistances, 3–8 MΩ). Both electrodes were screened with graphite, which extended to within 4–5 mm of the tip (see above).

A motoneurone was first impaled with the agar-containing electrode and an AP evoked by a 6 ms current pulse. The second electrode was then advanced carefully while observing the extracellularly recorded AP until penetration was achieved. Occasionally penetration was not achieved after a series of 'runs' at the cell, possibly because there is a tendency for the fine electrode tips to bend in the cord. The second electrode was then removed from the spinal cord, its co-ordinates changed slightly and the electrode tracked again.

Current pulses or linear current ramps (of either polarity, to a maximum of 250 nA and a maximal duration of 1.2 s) were applied through either electrode, usually the second (drug-containing) electrode. A train of 5–8 pairs of Ia e.p.s.ps and small negative current pulses (of −3 to −10 nA, to measure the interelectrode 'coupling' and membrane slope conductance) was applied during the course of the current ramp or pulse (see Figure 3). A minimum of 120 ms was allowed between each of the individual e.p.s.ps in the train.

Each pair of e.p.s.p. and conductance (G_M) measuring pulse was sampled via a 'clamp' amplifier, to allow a high d.c. gain with zero suppression, and recorded photographically in the 'raster' mode of a MEDELEC MS6 oscilloscope system (see Figure 3d). The output of the 'clamp' amplifier was also electronically averaged with either a DIDAC 800 or DATALAB DL4000B. High gain and low gain d.c. recordings were also stored on tape with a Brüel and Kjaer tape-recorder (Type 7003, Modification 5185) for subsequent detailed analysis.

Throughout all experiements the membrane potential (E_M) was recorded on an Elema Siemens Mingograph and on a slow, flat bed recorder. Recordings of fast transient responses were photo-

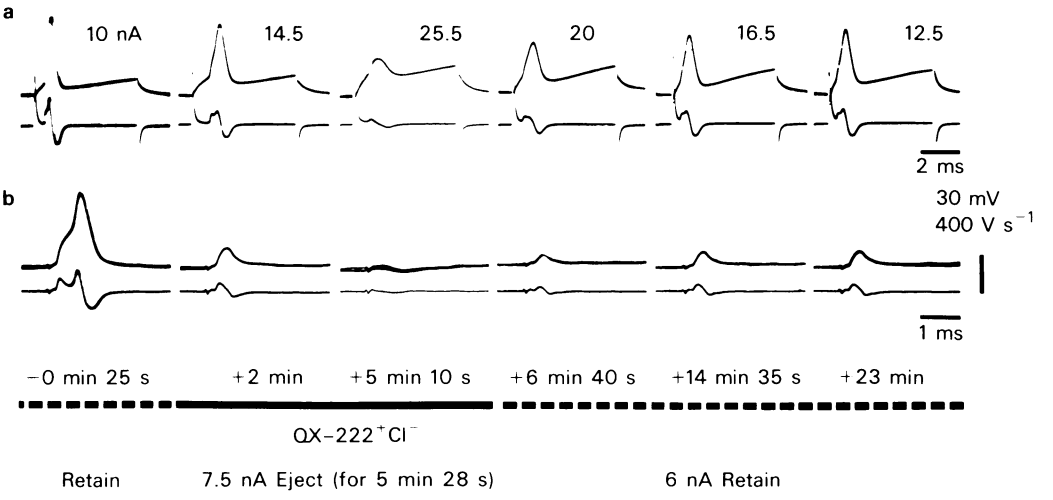

Figure 1 The action of intracellularly iontophoresed QX-222 on the current-evoked (a), and antidromic (b) action potentials (APs) (unidentified motoneurone). Resting membrane potential (E_M), −78 mV. The consecutive AP records have been mounted at levels which correspond to the actual E_M at which they were taken. Further, the AP records are accompanied by a trace below showing the rate of rise and fall of the APs (dV/dt). The neurone was stimulated with current pulses (6 ms duration) of sufficient intensity to give a maximal rate of rise of the AP. The current intensity (nA) to evoke an AP in 50% of trials (rheobase) is noted above each AP. The records were taken before, during and after the balanced ejection of QX-222 (electrode contained 0.3 M QX-222) at the times shown beneath the records.

graphed from Tektronix oscilloscope screens with a Nihon-Kohden camera. The potentials were also displayed on the screen of a Gould OS 4000 digital storage oscilloscope and the response recorded on Polaroid film.

Results

Effects on the action potential

Intracellular iontophoresis of QX-222 (the trimethyl derivative of lignocaine) produced a rapid block of spike generation in spinal motoneurones. In Figure 1 an iontophoretic current of 7.5 nA caused a block of the soma-dendritic (SD) component of the antidromically evoked AP (Figure 1b) and then total block of the AP within 3 min. Confirmation of block could also be gained from the records of current evoked spikes (Figure 1a). During the passage of the iontophoretic current the rate of rise (dV/dt) and the magnitude of the SD component decreased rapidly and eventually the spike failed to fire even in response to a large intracellular current injection. At the same time the injected current threshold (rheobase) increased as did the level of depolarization needed to reach the firing threshold of the initial segment (IS) spike. Following cessation of the ejection of QX-222, spike generation slowly recovered although full restitution was not achieved, even after 21 min. An earlier short-duration ejection of 25 nA QX-222 (for 1 min) into the same cell produced a less marked and nearly completely reversible AP inhibition. However, more than 20 min elapsed before the soma-dendritic (SD) component of the antidromic spike reappeared. The recovery of the components returned in reverse order to their disappearance. The increase of the magnitude of the IS component of the antidromic AP with time paralleled the recovery of the rate of rise of the SD component of the current evoked spike.

We were unable to restore a maximally blocked AP by hyperpolarizing the motoneurone with current.

There was a clear increase in the duration of the current evoked spike (reflected in the dV/dt traces) during and after the action of QX-222. The longlasting action, poor reversibility, and marked potency of QX-222 (and methylxylocholine) in blocking the AP was a consistent finding in our experiments.

There was a slight change in E_M following the action of QX-222 in Figure 1, probably due to inexact counterbalance of the iontophoretic current. In later experiments with automatically balanced currents, QX-222 caused total block of the AP without significant E_M change (E_M before QX-222 or methylxylocholine (mean ± s.e.mean): −64.8 ± 1.5 mV; E_M when complete block developed: −65.0 ± 1.6 mV, 31 cells).

Lignocaine, the parent compound of QX-222, was injected for comparison on a few occasions. It produced little block of spike generation. During an injection of +50 nA lignocaine, the SD component of the antidromic AP was still fired and there was only a slight decrease of the SD component of the current evoked AP. Even when large (> 200 nA), long (3 min) iontophoretic lignocaine currents were used, there was only a 10% depression of the dV/dt. These massive injections were more difficult to control and tended to produce a membrane depolarization. The local anaesthetic actions of lignocaine were rapidly reversible.

Intracellularly iontophoresed pentobarbitone was as ineffective as lignocaine in producing AP blockade. At low iontophoretic currents no response was seen and during the passage of −100 nA there was a decrease in the voltage threshold for spike generation, a decrease in the IS–SD delay in generation of the antidromic AP, and an increase in the rate of development of the SD components of the antidromically and current evoked APs. Following the current evoked spike a small after-depolarization appeared which was followed by a repolarization to a level more negative than that recorded during the control pulse.

Effects on membrane conductance

The current-voltage relationships of the motoneurones were assessed in two ways: firstly, by observing the E_M change in response to short duration (< 20 ms) current pulses, of both polarities, injected through the recording electrode, and secondly, by observing E_M changes in response to intracellularly injected current 'ramps'. For the latter technique current was passed through one electrode while E_M was recorded through a second one (see Methods).

In the experiments in which the motoneurone was penetrated by a triple-barrelled microelectrode and the slope G_M assessed by means of small current pulse injection, intracellular iontophoresis of quaternary local anaesthetics caused either a small decrease in G_M (11% ± 5%, SD, 8 cells) or no measurable change (9 cells). Figure 2 shows an example of the results from a motoneurone in which there was no detectable alteration of resting slope G_M at a time when the current evoked AP was abolished. Our later experiments with the double-penetration technique confirmed these findings.

The passage of a +250 nA (depolarizing) ramp through an electrode containing 0.5 M QX-222 in 5 M KCl did not produce the initial sustained burst of repetitive firing normally seen when current is passed from a drug-free electrode (Figure 3, cf. Figure 1 in Flatman *et al.*, 1981). It was, however, still possible to evoke an AP in response to a short duration depolarizing pulse. The membrane rapidly depolarized during the first part of the ramp, but when a potential of about −40 mV was reached, the potential trajectory became less steep (Figure 3a). During this latter phase, examination of conductance-measuring pulses showed that the slope conductance of the

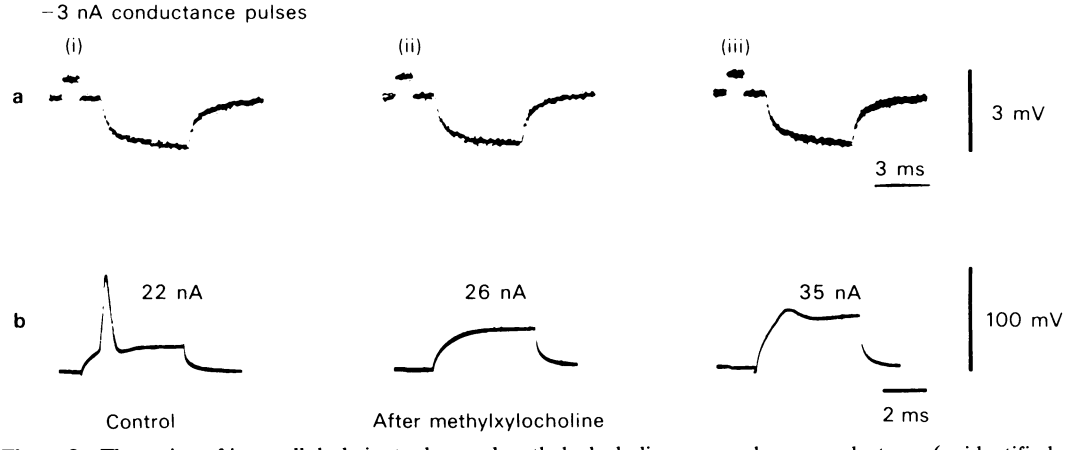

Figure 2 The action of intracellularly iontophoresed methylxylocholine on membrane conductance (unidentified motoneurone). Resting membrane potential, −65 mV. The iontophoretic electrode contained 0.3 M methylxylocholine which was ejected with 30 nA for 3 min. (a) Potential changes produced by the intracellular injection of −3 nA pulses through the recording electrode; 30 samples were averaged just before (i) and approximately 1.5 min after the drug ejection (ii). Records (i) and (ii) are superimposed in (iii). (b) Responses to intracellular stimulation (current strength given above records). The first record was taken before, the second 0.5 min and the third 3 min after the drug ejection. Despite the strong depression of the action potential there is no change in resting membrane conductance.

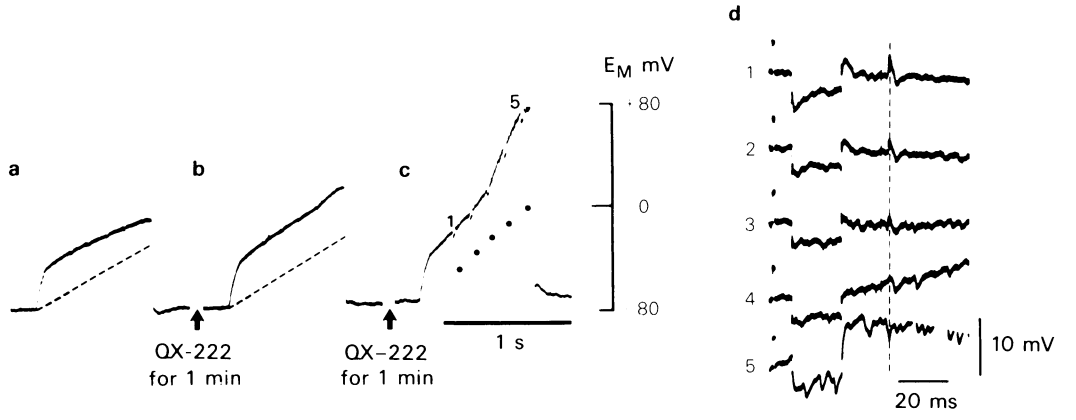

Figure 3 The action of intracellularly iontophoresed QX-222 on a deep peroneal motoneurone impaled by two independent microelectrodes. Membrane potential (E_M) before current injection, −76 mV. Records (a), (b), and (c) were all evoked by passing linearly rising current ramps (peak +250 nA) through an electrode containing 0.5 M QX-222 in 5 M KCl, while recording E_M through a second, drug-free electrode. The potential developed across the interelectrode 'coupling' resistance during current passage is shown as the broken lines in (a) and (b), and by the filled symbols (●) in (c) (see below). To calculate the 'true' E_M at each point, the value of the 'coupling' potential should be subtracted from the measured E_M. Trace (a) was recorded before and (b) after, a 1 min current of + 50 nA through the QX-222 electrode and (c) after another min with the same current. (d) 'Rastered' recording of G_M pulses (−10 nA) taken during the final ramp (c). Values of the 'coupling' resistance at consecutive points throughout the ramp (1–5) were obtained from the fast potential jumps at the start and end of each −10 nA current pulse in the train. They were measured in enlargements of the original records and used to calculate the potentials plotted as the filled symbols (●) in (c). The vertical broken line in (d) marks the peak of a Ia e.p.s.p. evoked during the ramp (stimulus strength: 1.5 × threshold).

membrane had risen to very high values. Indeed the extra depolarization developed during the ramp after an E_M of −40 mV had been reached could simply be accounted for by the potential developed over the interelectrode 'coupling' resistance.

In Figure 3 the potential generated over the 'coupling' resistance alone is indicated by either the broken lines or by the individual symbols (●). The 'coupling' resistance is calculated from the very fast part of the potential transients recorded by the potential recording electrode when the conductance measuring pulses are turned on and off (Figure 3d). The 'coupling' resistance is therefore calculated at intervals throughout the current injections, and values of E_M can be corrected accordingly. This is important as the 'coupling' resistance can change inconsistently throughout the course of a current injection. The coupling resistance was typically between 120–160 kΩ, but varied from 80 kΩ to over 300 kΩ on rare occasions. When the 'coupling' resistance was measured with both electrode tips in the extracellular environment just outside the motoneurone and with the tips separated by the same distance as within the cell, the coupling resistance ranged from 10 kΩ to 25 kΩ.

At the end of the current ramp before prolonged QX-222 iontophoresis, the E_M recovered to, and overshot, resting E_M; the E_M returned from this hyperpolarized level with a time constant of about 160 ms (Figure 3a). Then a steady current of + 50 nA was passed for 1 min through the electrode containing QX-222. This injection did not alter the resting E_M (−76 mV), but abolished the current evoked spike (not illustrated). However, during the current ramp in Figure 3b the E_M trajectory paralleled the 'coupling' potential line suggesting that a high G_M state still developed (cf. Figure 4). The E_M now returned directly to resting E_M after the ramp. A further 1 min application of + 50 nA through the QX-222-containing electrode was given (before Figure 3c), which did not alter resting E_M but prevented the full development of the high G_M state. E_M now reached positive levels (even when allowance was made for the potential developed over the 'coupling' resistance). The E_M record developed a characteristic appearance with the tail of the curve turning-up towards the end. Following the current ramp in Figure 3c, E_M recovered rapidly to about −65 mV and thereafter to resting E_M with a time constant similar to that for the recovery of the previous after-hyperpolarization. Very similar curves were seen when methylxylocholine was used instead of QX-222.

The results of another experiment are shown in Figure 4. In this figure we have superimposed the E_M responses to + 250 nA ramps, before and after QX-

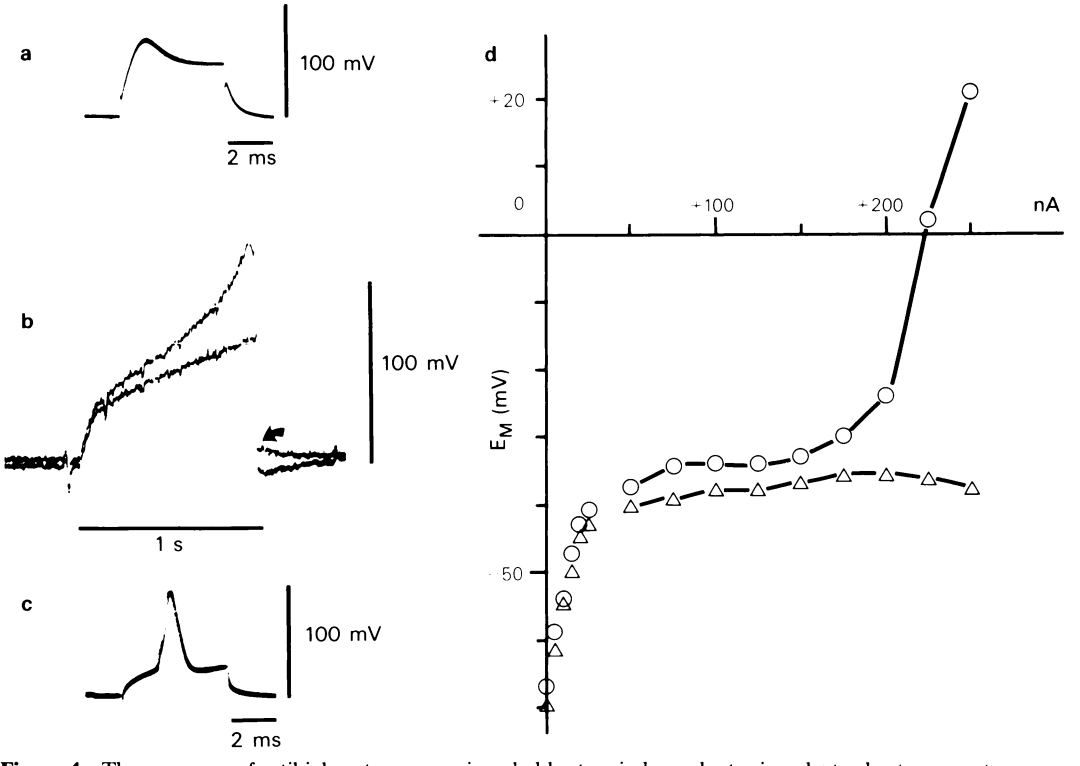

Figure 4 The response of a tibial motoneurone impaled by two independent microelectrodes to current ramps under the influence of QX-222. Resting membrane potential (E_M), −70 mV. (c) Shows the current-evoked (+ 18 nA) action potential before and (a) the response to a + 100 nA current pulse after, the passage of + 50 nA for 2.5 min through an intracellular QX-222 containing electrode (0.3 M in 3 M KCl). Note the coupling 'jump' at the beginning and end of the current pulse. (b) Shows the superimposed E_M responses to current ramps before (lower trace), and after (upper trace), the iontophoresis of QX-222 (recorded from a digital storage oscilloscope screen). The maximum current of both ramps was + 250 nA. The ramp current was delivered through the QX-222 electrode and E_M measured by the other electrode (containing only KCl-agar). After QX-222, the E_M returned at the end of the ramp, to a level more depolarized than resting E_M (as marked by the arrow head). Before QX-222 there was, instead, an after-hyperpolarization. (d) Current-voltage curves plotted on the basis of the E_M records (b), before (△) and after (○) the action of QX-222; plotted potentials have been corrected for 'coupling' (calculated as in Figure 3).

222 had abolished the AP (Figure 4b). A small depolarization (3 mV) had occurred between the times when the control and test ramps were passed. The appearance of both trajectories is typical (see Figure 3). When allowance is made for the 'coupling' resistance, the current-voltage curves during the current ramps appear as in Figure 4d. Note that at low current intensities the two current-voltage curves run a parallel course, that is, drug application has not affected membrane slope conductance. At an E_M around −40 mV the two curves diverge, the control curve (△) shows the development of a very high membrane conductance (the voltage parallels the current axis, i.e. a virtual short circuit). The curve obtained after QX-222 treatment initially displays a high conductance (between + 25 and + 150 nA), but then the conductance decreases and the cell depolarizes to positive levels.

In these experiments with double penetration of 18 motoneurones, no significant alteration of the resting E_M was seen after the passage of sufficient QX-222 or methylxylocholine into the cell to abolish totally all spike activity (E_M before application (mean ± s.e.mean): −67.6 ± 1.4 mV; E_M after total spike block: −66.8 ± 1.3 mV). In the ten cells in which slope G_M was assessed by averaging the potential response to small current pulses before and after complete AP blockade had been produced, no change of G_M was seen in six cells, a significant decrease (approximately 25%) was seen in three cells and an increase (4%) in one cell.

Effects on the Ia e.p.s.p.

At an early stage of this investigation we noted that at

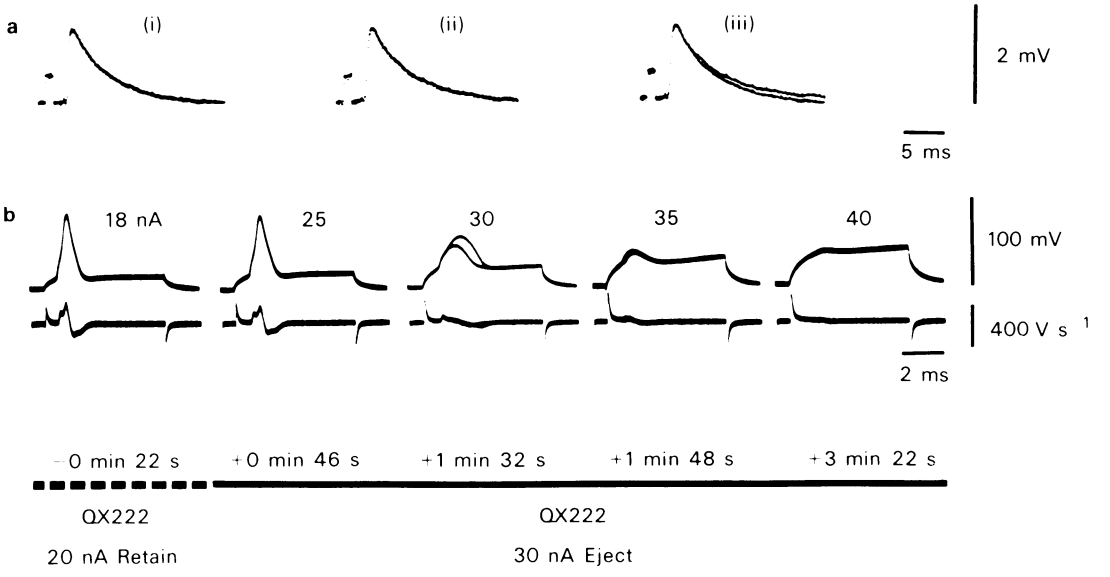

Figure 5 The action of current balanced intracellular iontophoresis of QX-222 from a triple barrelled electrode on Ia e.p.s.ps of a gastrocnemius-soleus motoneurone. Resting membrane potential, −60 mV. (a) Shows fifty Ia e.p.s.ps (1.2 × T) averaged (i) during the passage of a −20 nA retaining current through the QX-222 containing electrode barrel (0.05 M in 3 M KCl), and (ii) 2.25 min after the reversal of the current to a +30 nA ejecting current. (iii) Shows the superimposition of traces (i) and (ii). (b) Responses to intracellular stimulation during the same period (records mounted as in Figure 1, but with stimulus strength indicated). No recovery of the action potential was seen up to 20 min after the iontophoretic ejection of QX-222 was stopped. Despite this marked depression the e.p.s.p. was not decreased.

times when the motoneuronal AP was totally blocked by QX-222 or methylxylocholine, the evoked potentials (Ia e.p.s.p. and i.p.s.p.) were little affected. We therefore investigated more rigorously Ia e.p.s.ps averaged before and during the action of an intracellular dose of QX-222 sufficient to block totally the AP. The results of such experiments are illustrated in Figure 5. QX-222 caused neither a reduction of the peak amplitude of the Ia e.p.s.p. nor an alteration in resting E_M at a time when the antidromic AP was completely abolished and only a minute residual local response could be evoked by current injection. The decay phase of the Ia e.p.s.p. was slightly prolonged. Overall, no change in Ia e.p.s.p. magnitude was seen in five cells, about 5% decrease in three cells and a 10% increase in one cell.

The results of the experiments using triple-barrelled electrodes were replicated in later experiments in which the motoneurones were penetrated by two independent microelectrodes, and the Ia e.p.s.p. averaged before and after total AP blockade. There was no discernible alteration of the Ia e.p.s.p. in six cells and an increase (about 10%) in three cells. It may be of relevance that these were the three cells in which the slope G_M was decreased significantly by intracellular QX-222.

Discussion

Effects on the action potential

Like other quaternary analogues of lignocaine (e.g. QX-314), QX-222, when applied intracellularly to nerve fibres, is very potent in blocking the development of the AP (Narahashi *et al.*, 1972; Strichartz, 1973; Hille, 1977; Cahalan, Shapiro & Almers, 1980). We show here that the AP generating mechanism of cat spinal motoneurones is similarly sensitive to intracellularly applied QX-222. Results similar to ours have been obtained with QX-314 on cat motoneurones by Schwindt & Crill (1980 a,b), on guinea-pig hippocampal pyramidal neurones with QX-314 by Connors & Prince (1982) and with QX-222 by Puil & Carlen (1982 and personal communication). Little change of resting E_M was seen during the action of QX-222 (and methylxylocholine), with applications which were sufficient to block totally both the antidromically and current evoked APs. Intracellular applications with large currents occasionally caused cell depolarization, but it is difficult to determine whether this was due to the action of the drug itself (a lytic action, Engberg, Flatman & Kadzielawa, 1976) or was a result of the large currents passed. The AP generating mechanism was progres-

sively blocked during the action of the quaternary agents. The following list is in order of decreasing sensitivity of active electrogenesis to quaternary agents: (i) The ability of the membrane to generate repetitive firing during maintained depolarization; indeed the ability of a motoneurone to develop repetitive firing has been used as an index of the 'healthiness' of the ionic channels (Schwindt, 1973; Schwindt & Crill, 1980 a, b). (ii) The development of the SD component of the antidromically evoked AP. (iii) The magnitude of the antidromically evoked IS spike and the current evoked AP; they decreased in parallel; concurrently with this decrease, the duration of the spike increased. (iv) The IS component of the current evoked AP; this decreased gradually, leaving only a slow local response which finally disappeared. The total block of all the elements of AP generation in the motoneurone, even in response to massive stimulating currents, suggests that the quaternary local anaesthetic agents diffuse extensively in the cell.

The order in which the elements of the motoneuronal AP are inhibited can possibly be correlated with the morphology of the cell and the assumed distribution of active, electrogenic, areas of membrane. In the majority of motoneurones, the tip of the iontophoretic electrode is probably located in the soma or within a large dendrite. Thus the ejected cation will first affect the spike generating mechanism of the soma and will probably progressively inhibit active regions of the membrane in the dendrites and the initial segment of the axon as the drug diffuses along them. The SD component of the antidromically evoked AP was blocked earlier than this component of the current-evoked AP. This is explained by the inability of the limited current stimulation by the IS component of the antidromically evoked AP to excite effectively the partially blocked sodium channels in the soma-dendritic membrane. However, somatically-applied stimulating current not only evoked an IS spike but itself depolarized the soma membrane, aiding AP invasion at the soma-dendritic membrane. The greater resistance of the IS spike is probably due to the distribution of the drug within the cell, but also to the density and extent of active sites in the IS as compared to dendrites (see analyses by Dodge & Cooley, 1973; Traub & Llinás, 1977).

The block of the spike produced by intracellularly applied quaternary analogues of local anaesthetic agents was poorly reversible. This is probably a consequence of the poor lipid solubility of these cationic agents which hinders their passage through the cell membrane. If this is the main route for elimination, it would take a very long time for the cell to recover.

The spike generating mechanism of cat spinal motoneurones is sensitive to the action of tetrodotoxin (Blankenship, 1968; Zieglgänsberger & Puil, 1972), thus it is likely that 'fast' Na channels take part in the electrogenesis. In frog nerve fibres, these channels are blocked by QX-222 (Strichartz, 1973). The AP block seen in our experiments thus gives further support to the notion that Na channels participate in motoneuronal AP production.

The means by which we administer quaternary agents intracellularly and the method of assessing membrane properties would be expected to give maximal block of 'classical' voltage-dependent Na channels. The large, positive currents used to eject QX-222 from the intracellular electrode would also carry this quaternary cation into the Na channels which have been opened by the current-evoked depolarization and block them. In addition the depolarization inactivates the Na channels and thereby reduces the Na conductance. Further, this inactivation is in itself affected by both the quaternary and the tertiary form of local anaesthetics, i.e. they produce a use-dependent, or voltage-dependent, block as discussed by Courtney, Kendig & Cohen (1978) and Cahalan *et al.* (1980).

Lignocaine was surprisingly ineffective in blocking the AP. This is probably related to the fact that the lignocaine cation, rapidly dissociates on reaching the intracellular environment. The uncharged form diffuses rapidly away from the intracellular medium.

Effects on membrane conductance

We were able to assess the effect of the quaternary agents on various G_M channels from the results of experiments with current ramp injections (see Figures 3 and 4).

Despite the use of the twin-electrode technique, part of the potential change recorded with one electrode during the passage of current through the second was due to the potential developed across the 'interelectrode coupling resistance'. The magnitude of this 'coupling' resistance (80–300 kΩ), and its inconsistent variation during current passage, was of some concern to us (see Flatman *et al.*, 1981). Careful evaluation of our results led us to believe that the high values for 'coupling' resistance calculated in our experiments are a true reflection of the behaviour of electrodes placed intracellularly (see Eisenberg & Engel, 1970). It is possible that the higher values seen were related in some way to the presence of QX-222 in one of the electrodes. The 'coupling' resistance values measured extracellularly (10–25 kΩ) were only a fraction of those measured intracellularly.

We are unable to explain the small decrease of slope G_M (measured in response to small hyperpolarizing current pulses) that occasionally occurs without alteration of resting E_M after the injection of quaternary agents. A decrease of G_M has also been

noted by Nistri, Krnjević & Lamour (1978) following the iontophoresis of procaine into cat spinal motoneurones and by Puil & Carlen (personal communication) following QX-222 iontophoresis into hippocampal neurones. It is possible that a reduction of the resting permeability to both Na and K by intracellular QX-222 could produce a G_M reduction in the absence of changes in E_M. A further explanation may be that QX-222 can block the channels subserving inwardly directed anomalous rectification (see Barrett, Barrett & Crill, 1980; Schwindt & Crill, 1980 a, b; Halliwell & Adams, 1982). A similar inhibition of anomalous rectification during depolarization was suggested by the rectilinear trajectory at the very early part of our current-voltage (i/v) curves (see Figure 4 and Figure 1 in Flatman *et al.*, 1978).

When untreated motoneurones were depolarized to an E_M between -40 and -45 mV, G_M rose to such a degree that the membrane was virtually short-circuited (see Figures 3 and 4). This G_M increase was probably due to an opening of the voltage-sensitive, delayed rectification channels (I_{Kf}, Barrett *et al.*, 1980) and/or to the opening of the slow potassium channels (I_{Ks}) which mediate the development of the spike after-hyperpolarization.

The above interpretations suggest that both Na and K channels may be sensitive to the action of intracellularly applied quaternary analogues, Na channels being more sensitive to block than K channels.

It is possible that the after-depolarization seen following large current-evoked depolarizations (see Figures 3 and 4) represents the decay of a residual ionic current, possibly an inwardly directed calcium current. However, it is difficult to exclude the possibility that it was related to a redistribution of ions that occurred during the current passage.

Effects on the Ia e.p.s.p.

Intracellularly applied QX-222 or QX-314 does not block the channels which are opened by acetylcholine in myoballs of neonatal rat thigh muscles (Horn, Brodwick & Dickey, 1980). Thus it was of interest to see whether synaptic transmission to the motoneurone could be affected by quaternary local anaesthetics.

Ia synapses on the motoneurone have been demonstrated to occur throughout the dendritic tree, but primarily on the proximal half (Brown & Fyffe, 1981). It is probable that QX-222 had diffused to the sites of many of these synapses during experiments in which the AP was totally abolished. Thus, the total lack of inhibition of the Ia e.p.s.p. by the intracellularly applied quaternary agents should not be due to failure of the drug to reach the cytoplasmic side of the synapses.

The technical assistance of Dorthe Ledet Jensen, Marianne Stürup-Johansen, Susanne Andersen, Aage Jensen, Finn Marquard and Robert Langley is gratefully acknowledged. Karen Damgaard Ottesen is thanked for her assistance in preparing the manuscript. Astra is thanked for the gift of QX-222. Part of this study was supported by the Danish Medical Research Council.

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(*Received August 29, 1983.*)