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FAILURE OF CHRONIC DENERVATION TO ALTER THE SENSITIVITY OF THE RAT DIAPHRAGM TO LOCAL ANAESTHETICS OR QUINIDINE

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1 The actions of procaine, nupercaine and quinidine on the membrane resting and action potentials of the normal and chronically denervated rat diaphragm were studied *in vitro.*

2 The degree of suppression of the rate of rise of the action potential by any of the drugs was the same in denervated as in normal muscle.

3 The degree of suppression of the rate of rise of the action potential by procaine in denervated muscle was about the same in the presence and absence of tetrodotoxin.

4 Three possible interpretations of the lack of effect of denervation on the response to these drugs are discussed:

(a) In the sodium channel, the structural alteration that confers resistance to tetrodotoxin is quite limited and does not affect groups which interact with local anaesthetics.

(b) Local anaesthetics are non-selective in their binding so that alteration in membrane structure around the channel has little influence on the efficiency of the local anaesthetic.

(c) Denervation may affect the outer surface of the membrane, where acetylcholine and tetrodotoxin act, more than the intracellular surface, where local anaesthetics appear to act.

5 The slope of the log concentration-effect line for quinidine was steeper than that for procaine or nupercaine.

Introduction

The skeletal muscle fibre membrane undergoes extensive changes after chronic denervation. The spread of acetylcholine receptors from the region of the motor endplate gradually over the entire muscle fibre membrane has been known for a long time (Ginetzinsky & Shamarina, 1942; Axelsson & Thesleff, 1959; Miledi, 1960). There also develops a partial resistance to the action of tetrodotoxin (Redfern, Lundh & Thesleff, 1970). The resistant sodium channels spread outward from the motor endplate to cover the entire fibre surface, in parallel with the spread of acetylcholine receptors (Harris & Thesleff, 1971; Redfern & Thesleff, 1971b). Other changes indicative of alterations in membrane structure occur: the rate of rise of the action potential diminishes (Albuquerque & Thesleff, 1968; Redfern & Thesleff, 1971a, b; Albuquerque & Warnick, 1972); input resistance, membrane resistance, membrane capacitance, space constant, and time constant all increase (Albuquerque & McIsaac, 1970; Redfern & Thesleff, 1971a). These various changes in membrane properties, especially the development of partial resistance to tetrodotoxin, evoked the question whether there might also be changes in sensitivity to other substances which interfere with the movement of sodium through the skeletal muscle fibre membrane. Consequently, a study was made of the effects of procaine, nupercaine and quinidine on the rate of rise of the action potentials of muscle fibres from normal and chronically denervated rat diaphragm.

Methods

Male Sprague-Dawley rats were used. The left half of the diaphragm was denervated by section of the left phrenic nerve under halothane anaesthesia. An incision was made in the 7th to 9th left intercostal space, and the phrenic nerve was cut close to the diaphragm. Care was taken to avoid damaging the diaphragm. The incision was then closed and sutured. Positive pressure ventilation was used during the short period that the chest was open.

Six to twenty-four days after denervation, the

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rat was anaesthetized with chloroform and exsanguinated. The diaphragm was rapidly removed and placed in a dish of Liley's solution (Liley, 1956) bubbled with 95% O_2 and 5% CO_2 at room temperature. A strip (approximately 1 cm wide) was transferred to a small perspex chamber perfused with continuously flowing Liley's solution at 30°C. The strip was pinned to a silicone resin mat on the bottom of the chamber. A double electrode technique similar to that of Redfern & Thesleff (1971a) was employed. A recording glass microelectrode, filled with 2 M KCl, was first inserted into a fibre for purposes of recording the membrane resting and action potentials. This electrode was connected through a Medistor Model A35 negative capacity electrometer to a storage oscilloscope. The signal was differentiated with a Philbrick operational amplifier, and the derivative was displayed on the second beam of the oscilloscope.

After the recording electrode was inserted, a stimulating microelectrode, filled with 1 M potassium citrate was inserted into the same muscle fibre close to the recording electrode (within approximately 50-100 μm). A hyperpolarizing current was then applied such that the membrane potential at the recording electrode was −95 mv. After 30 s of hyperpolarization, a rectangular depolarizing pulse of 5 ms duration was applied. The ensuing action potential and its derivative, V, were recorded. The resting membrane potential before hyperpolarization, the height of the action potential, the maximum rate of rise (V_{max}), and the membrane potential at V_{max} were recorded.

After ten action potentials were recorded, each from a different fibre, the bathing solution was changed to one containing a drug. One hour was allowed for penetration and equilibration. At the end of this interval, ten more action potentials were recorded. The average V_{max} of each set of ten measurements was determined. Relative V was then calculated as the ratio of the average V_{max} in the presence of drug to that without drug.

To test whether tetrodotoxin (TTX)-resistant sodium channels differed from normal ones in their sensitivity to local anaesthetics, the relative V after procaine was determined in denervated muscle in the absence and presence of TTX. The effect of procaine, expressed as relative V, was first determined in the normal way on a strip of denervated muscle, and the muscle allowed to recover by washing for 1 h with drug-free solution. TTX (2.5×10^{-7} M) was then applied. This concentration diminishes V by approximately 50%, and further increasing the TTX concentration has little further effect (Colquhoun, Rang & Ritchie, unpublished observations). The effect of procaine was then tested again as before, but in the presence of TTX, and the relative decrease in V compared with that obtained in the absence of TTX.

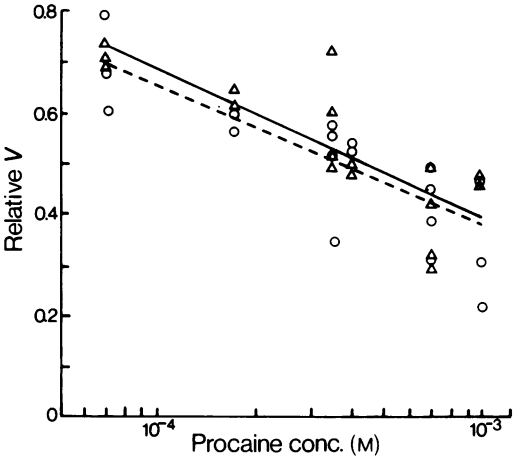

Fig. 1 The relationship of the suppression of the maximum rate of rise of the action potential to the concentration of procaine in the normal (△) and denervated (○, dotted line) rat diaphragm. Relative V is the ratio V_{max} after procaine to V_{max} of the control. The data are plotted semi-logarithmically. The lines are calculated regression lines.

The strips of innervated and denervated muscle that were compared in any one experiment were taken from symmetrical locations in the diaphragm.

Results

Procaine hydrochloride had no effect on the resting membrane potential. It consistently decreased V_{max}. At low concentrations, its effects on overshoot were slight and varied from fibre to fibre; at high concentrations, overshoot was consistently diminished, but not in proportion to the reduction in V_{max}. Procaine usually increased the threshold to stimulation, but this effect often varied greatly among a group of fibres tested in the same preparation. The effects of procaine were fully reversed when the muscle was washed in drug-free solution for 30-60 minutes.

With either normal or denervated muscle, a plot of relative V against the log of the concentration of procaine was essentially rectilinear over more than a ten-fold range of concentration (Figure 1). The regression coefficient of the line representing the denervated muscle was −0.286 and that representing the normal muscle was −0.300. The

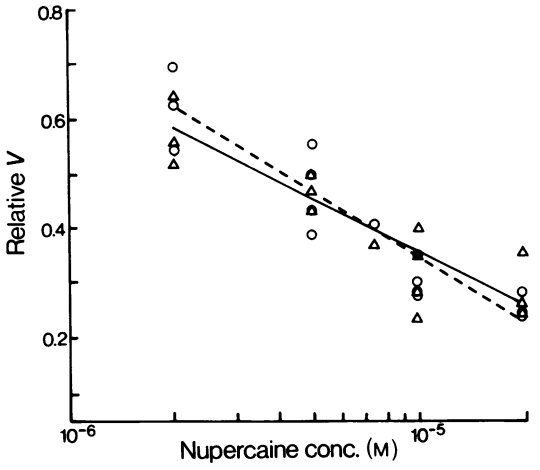

Fig. 2 The relationship of the suppression of the maximum rate of rise of the action potential to the concentration of nupercaine in the normal (△) and denervated (○, dotted line) rat diaphragm. Relative *V* is the ratio of V_{max} after nupercaine to V_{max} of the control. The data are plotted semi-logarithmically. The lines are calculated regression lines.

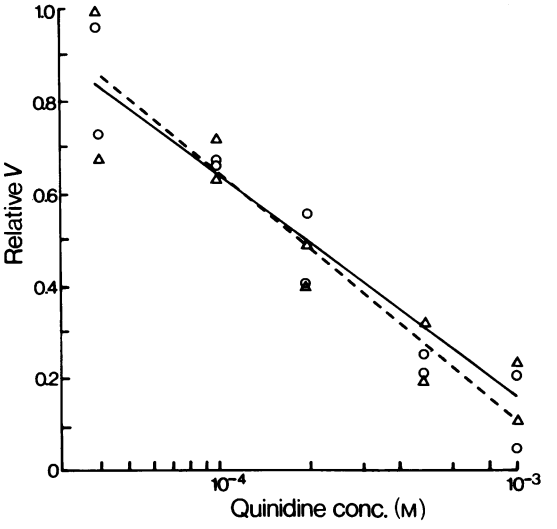

Fig. 3 The relationship of the suppression of the maximum rate of rise of the action potential to the concentration of quinidine in the normal (△) and denervated (○, dotted line) rat diaphragm. Relative *V* is the ratio of V_{max} after quinidine to V_{max} of the control. The data are plotted semi-logarithmically. The lines are calculated regression lines.

50% blocking concentration (EC_{50}) in the denervated muscle did not differ significantly from that in the normal muscle (see Table 1).

The effects of nupercaine were essentially the same as those of procaine, except that they were achieved with approximately 1/100 of the concentration. The regression coefficient of the log concentration-effect line was −0.333 for denervated muscle and −0.393 for normal muscle (Figure 2). The EC_{50} from denervated and normal muscle also did not differ significantly (Table 1).

The effects of quinidine were like those of the local anaesthetics, but they differed in some respects. In concentrations between 10^{-5} M and 10^{-4} M, there occurred sporadic spontaneous discharges and twitches, so that microelectrodes were sometimes dislodged. The drug also appeared to cause a very slight depolarization. The average of the resting membrane potentials of 10 fibres was lower than the average in the corresponding controls in 29 preparations, higher in five and the same in one. The deviations from control ranged from −9.6 to +2.4 mV and averaged −1.8 ± 0.41 mV; by a paired *t* test the probability that this differed from zero by chance was less than 0.005.

The effects on V_{max} and threshold were like those of the local anaesthetics, and the EC_{50} in denervated and control muscle did not differ significantly (Table 1). However, the slope of the log concentration-effect curve (Fig. 3) was larger than that of procaine or nupercaine, being −0.535 for denervated and −0.503 for normal muscle.

Table 1 EC_{50} values of procaine, nupercaine and quinidine in normal and denervated rat diaphragm

	Normal muscle		*Denervated muscle*	
Drug	EC_{50}	*95% confidence limits*	EC_{50}	*95% confidence limits*
Procaine	3.98×10^{-4} M	$(2.85\text{-}5.55) \times 10^{-4}$ M	3.54×10^{-4} M	$(2.46\text{-}5.08) \times 10^{-4}$ M
Nupercaine	3.52×10^{-6} M	$(2.88\text{-}4.30) \times 10^{-6}$ M	3.96×10^{-6} M	$(2.81\text{-}5.57) \times 10^{-6}$ M
Quinidine	1.84×10^{-4} M	$(1.01\text{-}3.35) \times 10^{-4}$ M	1.79×10^{-4} M	$(1.54\text{-}2.07) \times 10^{-4}$ M

Table 2 Relative V in procaine-treated denervated rat diaphragm before and after tetrodotoxin (TTX)

Concentration of procaine	No TTX	TTX 2.5×10^{-7} M
4×10^{-4} M	0.598	0.540
	0.529	0.462
	0.541	0.561
7×10^{-4} M	0.481	0.400

In the experiments with tetrodotoxin-treated denervated muscle the presence of TTX did not appreciably affect the sensitivity to procaine. The data are shown in Table 2. In these experiments TTX itself decreased V_{max} by an average of 52.5%.

Discussion

The results presented in this paper suggest that denervation of mammalian skeletal muscle makes no difference to the susceptibility of the membrane to local anaesthetics, in contrast to the decreased sensitivity to TTX that follows denervation. The use of the maximum rate of rise of the action potential as an index of the blocking effect of drugs on the sodium channels is not altogether satisfactory, since there will undoubtedly be a non-linear relationship between the fraction of channels blocked and fraction by which V_{max} is decreased. This has been shown, for example, by Schwarz, Ulbricht & Wagner (1973) on the node of Ranvier of frog myelinated nerve fibres where a 50% block of the maximum rate of rise of the action potential requires a considerably higher concentration of TTX (10.9 nM) than is needed to decrease by 50% the maximum depolarization-induced increase in sodium conductance, G_{Na} (3.6 nM).

Denervation affects many membrane properties, including the resistance and capacitance of the resting membrane (see Albuquerque & McIsaac, 1970), so the relationship between V_{max} and G_{Na} is likely to be different in denervated and normal muscle, and it is possible that this could obscure a real effect of denervation on the blocking action of local anaesthetics. With TTX, though, the effect of denervation is dramatic and obvious, the fibres continuing to conduct action potentials in the presence of TTX at concentrations which block a normal muscle completely. It therefore seems safe to say that the effect of denervation on sensitivity to local anaesthetics is very much less than for TTX, and is probably absent altogether.

The fact that denervation does not alter the sensitivity of the skeletal muscle membrane to the local anaesthetics or quinidine may tentatively be explained in more than one way. The first possibility is that the structural change around the sodium channel may be minor, perhaps involving only the chemical group that confers selectivity for tetrodotoxin but not for other substances. This possibility is supported by the fact that less resistance develops to saxitoxin than to tetrodotoxin (Harris & Thesleff, 1971), even though the two toxins appear to act at the same site.

A second explanation may be that the local anaesthetics are so non-selective in their interaction with chemical groups along or near the sodium channels that even rather extensive alterations in membrane structure would have only a minor effect on the affinity of local anaesthetics for membrane structures in the critical region. The wide variety of chemical structures that possess local anaesthetic activity of the procaine type indicates low selectivity. In this connection, the ability of local anaesthetics to increase lateral surface pressure in lipid films and to stabilize them, to modify compositional mosaics of membrane lipids (see Skou, 1961; Shanes, 1963; and Ritchie & Greengard, 1966 for reviews), or to increase the degree of disorder in the membrane (Metcalfe & Burgen, 1968) depends non-specifically on hydrophobic moieties in the molecule rather than discrete chemical groups. Furthermore, the ability of local anaesthetics to suppress calcium uptake into phospholipids is demonstrable with at least three different kinds of phospholipid (Feinstein, 1964). It is of course only speculative that any of these actions of local anaesthetics relate to the actions on the intact cellular membrane. The decreased potassium conductance caused by local anaesthetics (see Shanes, 1958a, b; Taylor, 1959), adds to the accumulated evidence that local anaesthetics have a low degree of selectivity.

A third possibility is that the trophic influence of the nerve on the muscle membrane is greater on the outer than on the inner surface. Tetrodotoxin has been shown to act only on the outside surface of the membrane of the giant axon of the squid (Moore, 1965; Moore & Narahashi, 1967), and it can be tentatively assumed that it acts similarly on the skeletal muscle membrane. Acetylcholine has likewise been shown to act only on the outside of the endplate membrane (del Castillo & Katz, 1955). In contrast, the local anaesthetics probably act on the inside of the membrane (Narahashi, Yamada & Frazier, 1969; Frazier, Narahashi & Yamada, 1970; Narahashi, Frazier & Yamada, 1970; Strobel & Bianchi, 1970; Narahashi &

Frazier, 1971; Catchlove, 1972; Strichartz, 1973). Batrachotoxin also appears to act only on the inner surface of the membrane (Narahashi, Albuquerque & Deguchi, 1971), where binding can be prevented by procaine (Albuquerque, Seyama & Narahashi, 1973). Denervation of skeletal muscle does not affect the sensitivity to batrachotoxin (Albuquerque & Warnick, 1972). Thus denervation appears to affect responsiveness only to those agents which act on the outside of the membrane.

Because of the many similarities between the effects of quinidine and the local anaesthetics on heart and nerve, it is common to think of quinidine as acting by the same mechanism as local anaesthetics. There are, however, no critical studies which prove that quinidine and local anaesthetics act upon the sodium channel in the same way.

The fact that the slope of the log concentration-effect curve for quinidine was steeper than that for procaine or nupercaine could be construed as evidence for a different mechanism of action.

Other effects of quinidine which distinguish this drug from local anaesthetics are the slight depolarizing effect and the promotion of spontaneous discharges in the rat diaphragm. Quinidine has also been reported to increase the contractility of the rat diaphragm (Senges, Rüdel & Kuhn, 1973), an action the local anaesthetics appear to lack.

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