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The effect of temperature on end-plate depolarization of the rat diaphragm produced by suxamethonium and acetylcholine

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End-plate depolarization in the rat isolated diaphragm by suxamethonium and acetylcholine was measured at 37° and 20° using a modification of the method of Fatt (1950). At 20° the response of the motor end-plates to the depolarizing activity of acetylcholine and suxamethonium was enhanced. Since the presence of neostigmine in the bath fluid did not modify the response of the motor end-plates to the depolarizing activity of suxamethonium at 37°, the results suggest the enhanced response of the motor end-plates at 20° is due to a change in the properties of the motor end-plate, and not to a change in the activity of cholinesterases present in the tissues.

IT is well known that muscle temperature affects the activity of skeletal neuromuscular blocking agents. Holmes, Jenden & Taylor (1951), investigating the effects of tubocurarine on the rat isolated diaphragm, showed that the neuromuscular blocking activity of tubocurarine was reduced at low temperatures. Using the rat isolated diaphragm, and nerve muscle preparations in anaesthetized cats, dogs and rabbits, Bigland, Goetzee & others (1958) showed that the neuromuscular blocking activity of tubocurarine was reduced at low temperatures, whereas the neuromuscular blocking activity of suxamethonium and decamethonium was enhanced. The nature of the neuromuscular blockade produced by these drugs was qualitatively unaltered. These results suggested that cooling sensitizes the muscle end-plates to the action of depolarizing drugs. Similar results were obtained by Cannard & Zaimis (1959) in man.

Whittaker (1962a, b), using the rat isolated diaphragm, showed that the neuromuscular blocking activity of suxamethonium was enhanced at low temperatures, and also that at these temperatures the characteristics of neuromuscular blockade by suxamethonium more closely resembled pure depolarization block than at normal temperatures.

In view of these results it was of interest to investigate the ability of acetylcholine and suxamethonium to depolarize the end-plate region of the rat isolated diaphragm preparation at normal temperature (37° ± 1°) and at low temperature (20° ± 1°).

Experimental

METHOD

Segments of the right hemidiaphragm, 1 cm wide, were prepared by making parallel cuts in the tissue equidistant from the insertion of the right phrenic nerve. The preparation was immersed in Krebs solution aerated with oxygen 95%, carbon dioxide 5%, and allowed to equilibrate

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for 45 min before commencing the experiment. End-plate depolarization was measured by the method of Fatt (1950), suitably modified for use with mammalian tissue, using the method illustrated in Fig. 1. For recording purposes, aeration was interrupted and the fluid level lowered to dislodge any gas bubbles adhering to the preparation, thus removing a possible source of error; end-plate depolarization was measured as the fluid electrode was allowed to sweep upwards. Electrical changes were recorded from $Ag/AgCl_2$ electrodes embedded in Krebs agar, and measured directly on a Tetronix 502A oscilloscope after amplification through the direct coupled amplifier of the oscilloscope. Records of end-plate

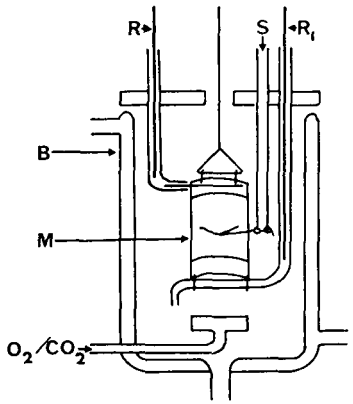

FIG. 1. Method used for measuring end-plate depolarization in the rat isolated diaphragm. B: Jacketed organ bath, 80 ml capacity. M: Segment of rat diaphragm mounted with remnants of rib cage uppermost. R: $Ag/AgCl_2$ electrode in Krebs agar. Contact to nerve-free rib cage remnants made through a pig bristle. R_1: $Ag/AgCl_2$ electrode in Krebs agar, open to Krebs solution. O_2/CO_2: Gas supply; aeration through sintered glass bubbler. S: stimulating electrodes (not used in these experiments).

depolarization were taken 1, 2, 4, 8 and 16 min after the addition of drugs. Bath temperature was maintained at normal (body) temperature (37° ± 1°) or at low (room) temperature (20° ± 1°) by means of a heat exchanger-pump unit. Preparations that exhibited injury potentials of 1 mV, or more were discarded. Drug concentrations refer to acetylcholine bromide and suxamethonium chloride. In experiments in which the end-plate depolarization induced by acetylcholine was measured, neostigmine, 1×10^{-6} g/ml was added to the Krebs solution to inhibit the action of cholinesterases.

The results are presented as end-plate depolarization in mV plotted against log time in min.

Results

At 37°, acetylcholine, $0{\cdot}5 \times 10^{-6}$ g/ml, in the presence of neostigmine, induced a depolarization of the end-plate region which remained constant

for about 4 min, and thereafter slowly declined. The peak depolarization observed was about 3 mV. At 20°, however, the same concentration of acetylcholine depolarized the end-plate region to a greater extent than at 37° and the peak depolarization (about 8 mV) was reached after about 4 min (Fig. 2a). Essentially similar results were observed when end-plate

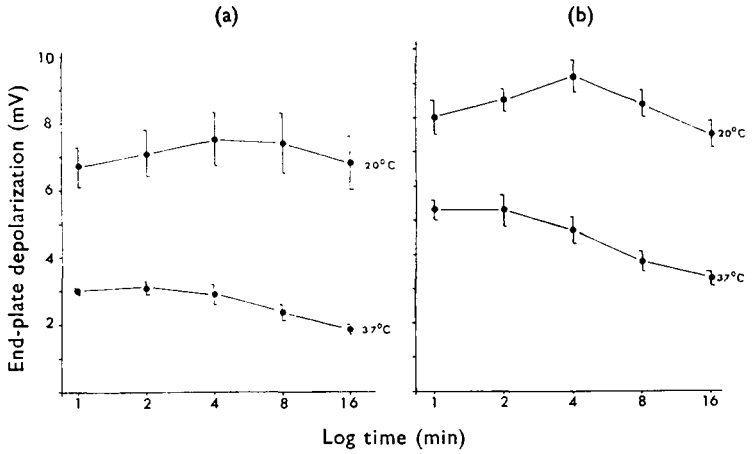

FIG. 2. End-plate depolarization in the isolated rat diaphragm at 20° and 37°. Each point mean ± s.e. of mean (n = 5 or 6). (a) Acetylcholine $0{\cdot}5 \times 10^{-6}$ g/ml in the presence of neostigmine 1×10^{-6}. Note that maximum depolarization at 37° occurs at 2 min with a value of 3·1 ± 0·2 mV. At 20°, maximum depolarization occurs at 4 min, with a value of 7·5 ± 0·8 mV. (b) Suxamethonium, 5×10^{-6} g/ml. Maximum depolarization at 37° occurs at 1–2 min, with a value of 5·3 ± 0·5 mV. At 20°, maximum depolarization occurs at 4 min with a value of 9·2 ± 0·5 mV.

depolarization was measured using suxamethonium, 5×10^{-6} g/ml, as the depolarizing agent (Fig. 2b).

Since suxamethonium is hydrolysed by cholinesterases (Bovet & Bovet-Nitti, 1955), the potentiation of end-plate depolarization by suxamethonium at 20° could be complicated by a reduction in the activity of cholinesterases at the lower temperature, which would result in a higher effective concentration of suxamethonium present in the bath under these conditions.

Fig. 3 illustrates a control experiment in which the end-plate depolarization to suxamethonium at 37° was measured in the presence of 1×10^{-6} g/ml neostigmine, the result being compared to that obtained in the absence of neostigmine. The end-plate depolarization induced by suxamethonium in the presence of neostigmine was insignificantly potentiated, although the decline in depolarization with time was slower, indicating that the hydrolysis of suxamethonium may be a causative factor in the relatively rapid decline of activity of suxamethonium. It is obvious, however, that the reduction of cholinesterase activity at low temperature cannot satisfactorily explain the potentiated end-plate depolarization induced by suxamethonium.

Discussion

The mechanism by which suxamethonium and decamethonium interrupt neuromuscular transmission is complex. In the intact cat (with the exception of certain red muscles, e.g., soleus) neuromuscular blockage is produced by a persistent depolarization of the end-plate region (Burns & Paton, 1951). In most other species, however, the mechanism of action of neuromuscular blockade induced by these drugs has overall intermediate characteristics of block by both depolarization and by competition. In these species, neuromuscular blockade by these drugs is initially of the depolarization type which later changes to a competitive type of action and has been called "dual" by Zaimis (1953). The mechanism of action of these drugs in *in vitro* preparations is confusing. Neuromuscular

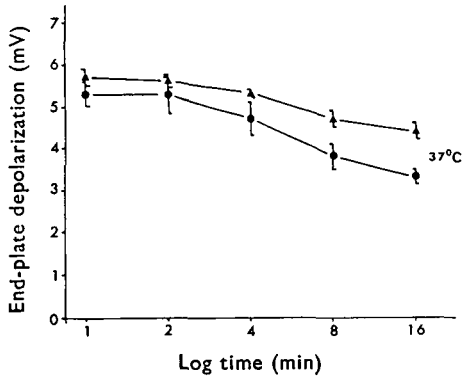

FIG. 3. End-plate depolarization in the isolated rat diaphragm at 37°. Each point mean ± s.e. of mean (n = 4 or 5). Solid circles: Suxamethonium 5×10^{-6} g/ml. Solid triangles: Suxamethonium, 5×10^{-6} g/ml in the presence of neostigmine, 1×10^{-6} g/ml. Note the insignificant potentiation of end-plate depolarization by suxamethonium in the presence of neostigmine at 37° for 2–4 min. There is some potentiation of the end-plate depolarization by suxamethonium in the presence of neostigmine after 4 min.

blockade takes place in two phases. In phase 1, the onset of block is rapid and reaches a maximum in 5–10 min. During this phase, the action of neuromuscular block appears to be by depolarization. After phase 1 neuromuscular transmission is partially restored, and then gives way to phase 2, a period of slowly developing neuromuscular block with the characteristics of block by competition with acetylcholine (Maclagan 1962). This biphasic action does not occur in *in vivo* preparations (Maclagan, 1962). Consideration of these points suggests that caution is necessary when attempts are made to transpose results obtained in one species to a situation in another species, or in attempting to transpose results obtained *in vitro* to a situation *in vivo*.

Suxamethonium is known to be hydrolysed by cholinesterases (Bovet & Bovet-Nitti, 1955). The first hydrolytic product is succinylmonocholine which has been shown to possess a weak neuromuscular blocking activity with features of a competitive type of action (Stovner, 1958). In view of

this, Whittaker (1962, b) suggested that an increase in the neuromuscular blocking activity of suxamethonium at low temperatures might be related to an inhibition of cholinesterases. However, this is unlikely to be the major factor for two reasons. Firstly, from the results here presented, the end-plate depolarizing activity of suxamethonium at 37° is hardly changed by the presence of a concentration of neostigmine known to inhibit more than 99% of total cholinesterases in the isolated homogenized rat diaphragm (Leach, unpublished observation); secondly Bigland & others (1958) have shown that the neuromuscular blocking activity of decamethonium is affected by temperature at least as much as is suxamethonium, even though decamethonium is not hydrolysed by cholinesterases (Zaimis, 1950).

Bigland & others (1958) suggested that their results might be explained by supposing that at low temperatures the end-plates are specifically sensitized to the action of agonist drugs, which would lead to an enhanced action of acetylcholine, suxamethonium and decamethonium at the neuromuscular junction. This suggestion could also be applied to the findings of Letley (1960) who showed that contracture of the isolated denervated rat diaphragm produced by a wide range of drugs including acetylcholine, suxamethonium and decamethonium was potentiated at low temperatures.

Although caution is necessary in attempting to transpose results obtained *in vitro* to a situation *in vivo*, the present results would support the supposition of Bigland & others (1958). Since suxamethonium is capable of depolarizing the end-plate region much more readily at low temperature than at normal temperatures, the obvious implication is that the drug would more readily interrupt neuromuscular transmission at low temperatures.

The present finding that acetylcholine produces larger end-plate depolarization at low temperature suggests that normal neuromuscular transmission may be similarly augmented by cooling. If this is so, it would provide an explanation for the fact that the neuromuscular blocking activity of tubocurarine is reduced at low temperature.

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