Br. J. Pharmac. (1970), **38**, 517–529.

The effect of some foreign anions on suxamethonium blockade of the isolated rat diaphragm preparation

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Summary

1. The pharmacology of suxamethonium blockade of the rat phrenic nerve-diaphragm preparation has been studied in the presence of some foreign anions.

2. Blockade proceeded in two distinct phases in both normal and modified Krebs solution.

3. In normal Krebs solution the characteristics bore no close resemblance to either depolarizing or competitive type blockade.

4. In the presence of foreign anions the characteristics of the blockade more closely resembled those of depolarization.

5. There was an increase in the sensitivity of the motor end-plate region of the muscle to the depolarizing action of acetylcholine, carbachol and suxamethonium in the presence of the anions.

6. Although the anions enhanced the depolarizing activity of suxamethonium, the blocking potency of the drug was unaltered.

7. It is suggested that end-plate depolarization plays little part in suxamethonium blockade of the isolated rat diaphragm and that desensitization is the primary cause of the blockade.

Introduction

The mechanism of action of the " depolarizing " blocking agents at the mammalian neuromuscular junction *in vivo* is dependent upon the species of animal used, and the specific muscle under investigation. In the human, and in fast-twitch (white, phasic) muscles of the cat, blockade is probably due to a persistent depolarization of the motor end-plate region (Burns & Paton, 1951 ; Jewell & Zaimis, 1954 ; Grob, Johns & Harvey, 1956, for example). In slow-twitch (red, tonic) muscles of the cat and in the muscles of all other species examined the blockade commences by motor end-plate depolarization and proceeds by competition with neurally released acetylcholine (Hall & Parkes, 1953 ; Zaimis, 1953 ; Jewell & Zaimis, 1954). This mechanism has been termed " dual " (Zaimis, 1953).

In vitro, the mechanism of action of " depolarizing " blocking agents is much less clear. Among the many interpretations of the characteristics accompanying

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blockade, three are most commonly put forward. Brand (1952), Jenden, Kamijo & Taylor (1954), Jenden (1955), Sabawala & Dillon (1959) and Whittaker (1962a, b) have all suggested that the block commences with motor end-plate depolarization, and proceeds by competition with neurally released acetylcholine. Thesleff (1955, 1958) suggested that the blockade commences with motor end-plate depolarization, but proceeds by a desensitization of motor end-plate receptor sites to the transmitter substance. More recently Freeman (1968a, b) suggested that motor end-plate depolarization is relatively unimportant and that blockade is primarily due to presynaptic activity which results in a reduction of transmitter output.

In amphibian skeletal muscle, foreign anions of a "lyotropic series" (Horowicz, 1964) cause an increase in muscle fibre membrane resistance due to a reduction in chloride conductance. One consequence of this action is that the amplitude of the end-plate potential is increased (Hutter & Padsha, 1959; Oomura & Tomita, 1961).

We felt that if an increase in motor end-plate depolarization were demonstrable in the presence of foreign anions, it should be possible to assess the importance of depolarization in neuromuscular blockade caused by "depolarizing" blocking agents in terms of altered characteristics of the blockade.

This paper describes some observations on the characteristics of suxamethonium blockade of the isolated rat diaphragm preparation in the presence of some lyotropic anions.

Part of this work was communicated to the British Pharmacological Society in July 1966.

Methods

All phrenic nerve-diaphragm preparations were set up in normal or modified Krebs solution at 37° C and vigorously oxygenated with 95% oxygen and 5% carbon dioxide. Each preparation was allowed to equilibrate in the bathing fluid for 45 min before an experiment was started. Preparations were stimulated indirectly using square wave pulses of 0·05 ms or less at a supramaximal voltage (usually 5·0–10·0 V) and at a frequency of 0·2 Hz unless otherwise stated, and the responses were recorded using a semi-isotonic spring loaded lever. In some experiments, isometric recordings of contractile behaviour were made. In these experiments a Kistler pressure transducer (type 701A) and a Southern Instruments U.V. oscillograph (type M1300) were used. Directly stimulated preparations were maintained in curarized Krebs solution (tubocurarine, $1{\cdot}0 \times 10^{-6}$ g/ml), and the stimuli were square wave pulses of 1·0 ms duration and supramaximal strength. The normal Krebs solution had the following composition: Na^+ 135 mM; K^+ 5·9 mM; Ca^{2+} 2·5 mM; Mg^{2+} 1·2 mM; Cl^- 128 mM; HCO_3^{2-} 25 mM; $H_2PO_4^-$ 1·2 mM; SO_4^{2-} 1·2 mM; glucose 11·1 mM. Modified Krebs solutions were prepared using an equivalent quantity of sodium bromide, sodium nitrate or sodium methylsulphate in place of sodium chloride: these solutions were isotonic with and had the same pH as normal Krebs solution.

The retrograde injection of drugs into the isolated diaphragm preparation was carried out as described by Paterson (1965) modified according to Harris & Leach (1965).

The characteristics associated with neuromuscular blockade caused by suxamethonium were investigated using the preparation described by Bülbring (1946).

The potency of suxamethonium as a neuromuscular blocking agent in the isolated rat diaphragm preparation was difficult to determine because successive equal doses of suxamethonium were found to induce an increasing degree of neuromuscular blockade, unless the period of washing between doses was prolonged (1–4 h). The neuromuscular blocking activity of suxamethonium was therefore assessed as follows. Paired diaphragm preparations were individually set up in a twin chamber organ bath and both were initially bathed in normal Krebs solution and maintained under similar experimental conditions. A standard dose of suxamethonium ($5{\cdot}0 \times 10^{-6}$ g/ml or $1{\cdot}0 \times 10^{-5}$ g/ml) was added to each bath during a period of indirect stimulation, and the degree of neuromuscular blockade occurring after a 2 min drug contact time was measured. After the drug contact period, indirect stimulation was stopped and the preparations washed for 8 min using five or six changes of bathing fluid. In this way, responses to a constant dose of suxamethonium were obtained over a period of one hour (six responses). After one hour, the bathing solution in one of the chambers of the organ bath was changed from normal Krebs solution to either " bromide ", " nitrate " or " methylsulphate " Krebs solution, and the experiment continued for a further 1 h. In this way, any change in the slope of the progressively increasing degree of blockade following the use of a modified Krebs solution compared with that developed in the preparation immersed in normal Krebs solution would indicate a change in the potency of suxamethonium.

Motor end-plate depolarization in the isolated diaphragm was measured as described by Harris & Leach (1968).

The drugs used in these experiments were acetylcholine bromide, suxamethonium chloride, tubocurarine chloride, neostigmine methylsulphate, edrophonium bromide, and carbachol chloride. Drugs quoted in this paper refer to the salts, and doses refer to final bath concentrations except in the case of retrograde injection experiments.

Results

Effects of the anions on muscle twitch responses

In the presence of the foreign anions the twitch responses of the preparation to both direct and indirect stimulation were potentiated. The relative " efficacy " of the anions was $Cl^- < Br^- < NO_3^- < CH_3OSO_3^-$; this order of potency is similar to that noted by many other workers (see reviews by Horowicz, 1964 ; Sandow, 1964). Frequently the twitch response was complicated by a " veratrine-like " after contracture (Krayer & Acheson, 1946). Similar activity has been reported by Sandow & Stein (1956) and Lüllman (1961) for isolated rabbit and rat skeletal muscle respectively, and the response has been shown to be due to repetitive firing of the muscle (Lüllman, 1961).

During stimulation at rates above 0·083 Hz, the first response of the preparation was a twitch followed by after contracture. With continuing stimulation, the after contracture decreased in magnitude until only a pure potentiated twitch response was observed (Fig. 1). The initial contractural response was restored following a brief rest period of 1–2 min. After contracture was seen consistently in the presence of methylsulphate, frequently in nitrate but only rarely in bromide.

The potentiated pure twitch response was not due to fibre recruitment, for it was observed in directly stimulated, curarized preparations. Extracellularly recorded

action potentials showed no sign of repetitive firing of the muscle, which precludes the possibility of the response being a brief asynchronous tetanus of the type described by Jones & Laity (1965). The precise cause of the potentiated twitch response was not, however, examined in further detail.

Retrograde intravenous injection

The response of rat diaphragm preparations immersed in normal Krebs solution to retrogradely administered acetylcholine was basically similar to those observed by Paterson (1965), and typical responses are shown in Fig. 2A. A small injection artefact was obtained with the injection of 0·1 ml saline, and the injection of 1·0 μg of acetylcholine caused a response approximately equal to that of the indirect twitch response.

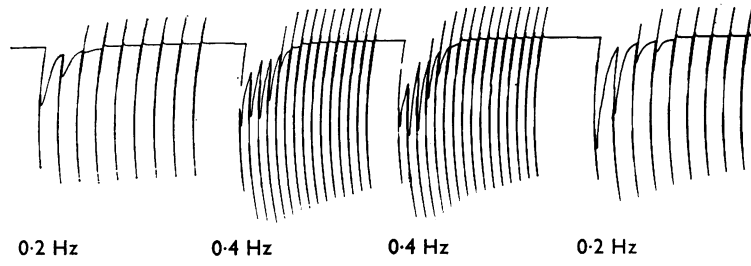

FIG. 1. Semi-isotonic indirect twitch responses recorded from a diaphragm preparation immersed in methylsulphate Krebs solution. Preparation rested for 2 min between each period of stimulation. Stimuli were square wave pulses of 0·1 ms duration, supramaximal voltage. Rate of stimulation: 0·2 and 0·4 Hz. The responses of the muscle to direct stimulation recorded under isometric conditions were qualitatively similar.

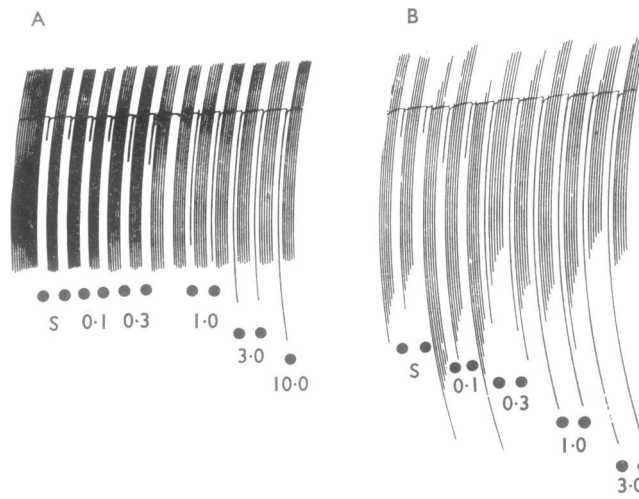

FIG. 2. Responses of diaphragm preparations immersed in normal and modified Krebs solutions to retrograde intravenous injections of acetylcholine. A, Diaphragm preparation in normal Krebs solution; responses to 0·1 ml normal saline (S) and 0·1–10·0 μg acetylcholine in 0·1 ml saline. B, Diaphragm preparation in nitrate Krebs; responses to 0·1 ml normal saline and 0·1–3·0 μg acetylcholine in 0·1 ml saline.

Preparations immersed in the modified Krebs solutions were much more sensitive to injections of acetylcholine (Fig. 2B). Log dose-response curves showed that the degree of potentiation of the twitch response was similar for all of the substituent anions studied.

An increase in sensitivity was also seen in response to injections of carbachol, decamethonium and suxamethonium; injection of these drugs into preparations immersed in normal Krebs solution elicited no twitch-like response but following immersion of the preparation in the modified Krebs solutions, twitch responses were readily obtained (Fig. 3). The preparations exhibited tachyphylaxis to these drugs, however, and no reliable quantitative information concerning these responses could be elicited.

Characteristics of suxamethonium-induced blockade

Normal Krebs solution

The rat diaphragm preparations immersed in normal Krebs solutions were relatively insensitive to the neuromuscular blocking action of suxamethonium, and individual preparations showed a wide variation in sensitivity to suxamethonium. Complete block was rarely achieved with doses less than $5{\cdot}0 \times 10^{-6}$ g/ml, and more commonly $6{\cdot}0$–$8{\cdot}0 \times 10^{-6}$ g/ml was necessary. As described by other workers (Whittaker, 1962a; Gibberd, 1966) suxamethonium induced blockade proceeded in two phases. Phase I consisted of a rapid onset of neuromuscular block, which became maximal after 5–10 min, followed by a partial recovery of neuromuscular transmission. Following this partial recovery, a slowly developing failure of neuromuscular transmission took place (phase II) from which spontaneous recovery was never observed (Fig. 4). Subsequent doses of suxamethonium induced a monophasic block of transmission. Neuromuscular blockade was not preceded by indirect twitch potentiation, and similarly, "sub-blocking" doses of suxamethonium ($0{\cdot}5$–$2{\cdot}0 \times 10^{-6}$ g/ml) were incapable of inducing indirect twitch potentiation (Fig. 5A).

As described by Whittaker (1962a) and Gibberd (1966) suxamethonium and competitive neuromuscular blocking agents such as tubocurarine were mutually

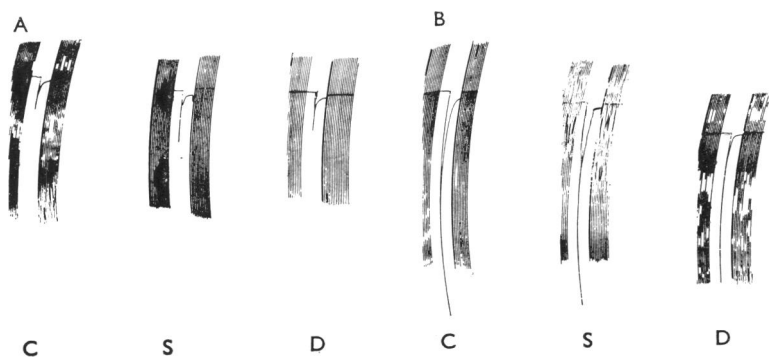

FIG. 3. Responses of diaphragm preparations immersed in normal Krebs solution (A) and nitrate Krebs solution (B) to retrograde intravenous injections of carbachol, 1·0 μg (C), suxamethonium, 1·0 μg (S), and decamethonium, 10·0 μg (D), in 0·1 ml saline.

synergistic. Thus, small doses of suxamethonium ($0{\cdot}5$–$2{\cdot}0 \times 10^{-6}$ g/ml) intensified the blockade caused by tubocurarine (Fig. 5B) and similarly small doses of tubocurarine ($0{\cdot}1$–$0{\cdot}5 \times 10^{-6}$ g/ml) intensified the blockade caused by suxamethonium (Fig. 5C).

Tetanic stimulation of the partially blocked preparations immersed in normal Krebs solution induced a poorly held tetanus of the muscle with no observable post-tetanic facilitation of transmission (Fig. 5D).

Neostigmine and edrophonium had no consistent effect on the blockade.

Modified Krebs solution

Like those described for normal Krebs solution, preparations immersed in the modified Krebs solutions were insensitive to suxamethonium and varied in sensitivity to the drug. In the continuing presence of suxamethonium, the blockade was biphasic and had a similar time sequence to that observed in normal Krebs solution.

In contrast to the observed effects of suxamethonium in normal Krebs solution, however, in the presence of the foreign anions neuromuscular blockade was always preceded by indirect twitch potentiation. The degree of potentiation was dependent upon the dose of suxamethonium. "Sub-blocking" doses of suxamethonium caused only indirect twitch potentiation; increased doses resulted in a decrease of both the extent and duration of this potentiation, until with higher doses ($5{\cdot}0 \times 10^{-6}$

FIG. 4. Biphasic response of an isolated diaphragm preparation immersed in normal Krebs solution in the continuing presence of suxamethonium, $5{\cdot}0 \times 10^{-6}$ g/ml (S). Contact time 75 min.

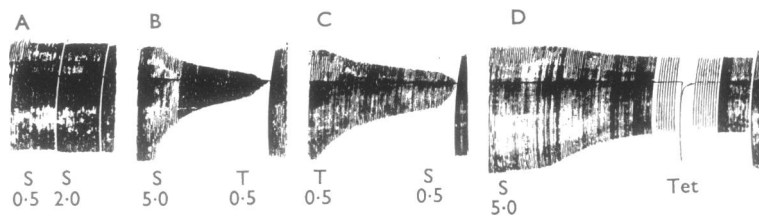

FIG. 5. Characteristics of suxamethonium induced neuromuscular blockade in isolated diaphragm preparations immersed in normal Krebs solution. A, Absence of indirect twitch potentiation preceding neuromuscular blockade. B and C, Mutual synergism between suxamethonium and tubocurarine. D, Poorly held tetanus during partial blockade (drum speed increased during tetanic stimulation). Suxamethonium (S) and tubocurarine (T) expressed as bath concentration $\times 10^{-6}$ g/ml. Tet, Indirect tetanic stimulation at 50 Hz.

g/ml) the potentiation was very short lasting and was rapidly transformed to neuromuscular blockade (Fig. 6A). Immediately following addition to the bath, suxamethonium (and also acetylcholine, carbachol and decamethonium) induced a short lasting " contractural " response in the muscle which appeared to be dose dependent. This latter feature could not be assessed quantitatively, however, because of the very rapid onset of tachyphylaxis.

Furthermore, in the presence of the foreign anions, suxamethonium and tubocurarine were found to be mutually antagonistic. Thus small doses of tubocurarine reversed suxamethonium blockade (Fig. 6B) and prevented indirect twitch potentiation; similarly, suxamethonium reversed tubocurarine blockade, although the reversal was only transient (Fig. 6C).

As with preparations immersed in normal Krebs solution (see Fig. 5D), tetanic stimulation of partially blocked preparations induced a poorly held tetanus of the muscle.

Neostigmine and edrophonium had no consistent effect on the blockade.

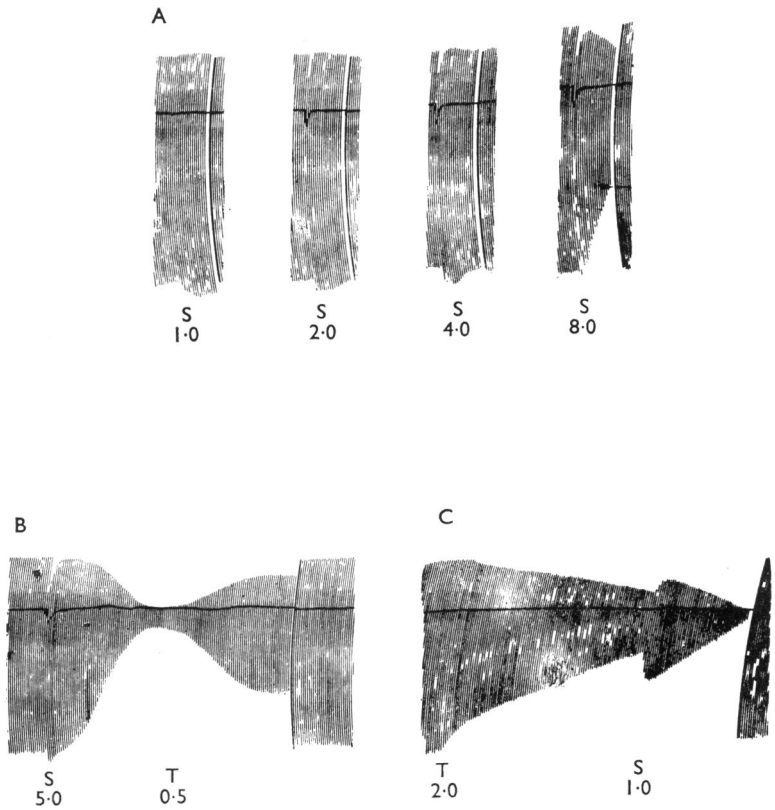

FIG. 6. Characteristics of suxamethonium induced neuromuscular blockade in isolated diaphragm preparations immersed in nitrate Krebs solution. A, Indirect twitch potentiation preceding neuromuscular blockade. B and C, Mutual antagonism between suxamethonium and tubocurarine. Suxamethonium (S) and tubocurarine (T) expressed as bath concentration $\times 10^{-6}$ g/ml.

Potency of suxamethonium

The ability of suxamethonium to cause a blockade of neuromuscular transmission was examined at two dose levels ($5{\cdot}0 \times 10^{-6}$ g/ml and $1{\cdot}0 \times 10^{-5}$ g/ml) as described under **Methods**. The foreign anions had no effect on the potency of suxamethonium as a neuromuscular blocking agent (Fig. 7).

Motor end-plate depolarization

The depolarization of the motor end-plate region caused by acetylcholine, carbachol and suxamethonium was measured in diaphragm preparations immersed in normal or modified Krebs solutions. Peak depolarization was observed after 1 min drug contact time (with the one exception of the depolarization caused by acetylcholine in preparations immersed in normal Krebs solution, in which case peak depolarization was observed after 2 min contact time). Maximum end-plate depolarization in preparations immersed in the modified Krebs solutions was always significantly greater than in normal Krebs solution and peak depolarization in methylsulphate was significantly greater than that obtained in nitrate Krebs solution (Fig. 8). It is not possible to say whether the enhanced response of the end-plates represents either true sensitization of the end-plates (which would result in a shift to the left of the dose/response curve), a potentiation of the maximum response of the preparation (no shift of the dose response curve, but a larger maximum response), or both mechanisms operating together, because this type of analysis requires information in the form of log-dose response curves. Unfortunately, tachyphylaxis develops so rapidly in the rat diaphragm preparation that such information is very difficult to obtain.

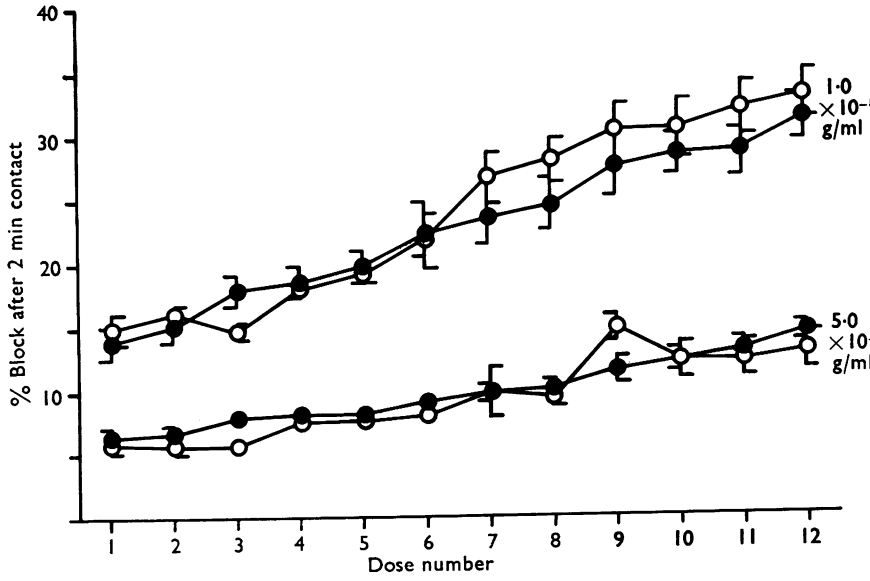

FIG. 7. Responses of paired diaphragm preparations to the repeated administration of suxamethonium ($5{\cdot}0 \times 10^{-6}$ and $1{\cdot}0 \times 10^{-5}$ g/ml bath concentration). ●——●, Preparations immersed in normal Krebs solution for 60 min followed by nitrate Krebs solution for 60 min. ○——○, Preparations immersed in normal Krebs solution for the duration of the experiment. Each point represents mean ± S.E. of four experiments.

End-plate depolarization in the continuing presence of the drug declined very rapidly, so that little difference could be measured between depolarization in normal Krebs solution compared with the modified solutions after 4 min drug contact time (Harris, unpublished observation). The faster rate of end-plate desensitization after a higher initial depolarization is similar to the observations made by Thesleff (1958).

Discussion

The characteristics of neuromuscular blockade induced by suxamethonium in the isolated rat diaphragm preparations immersed in normal Krebs solution reported in this paper are generally similar to those reported by other workers (Brand, 1952 ; Bergh, 1953 ; Stovner, 1958 ; Whittaker, 1962a, b ; Gibberd, 1966, for example). The interpretation of the characteristics is complicated by several factors, all of which are related directly or indirectly to the nature of the isolated nerve-muscle

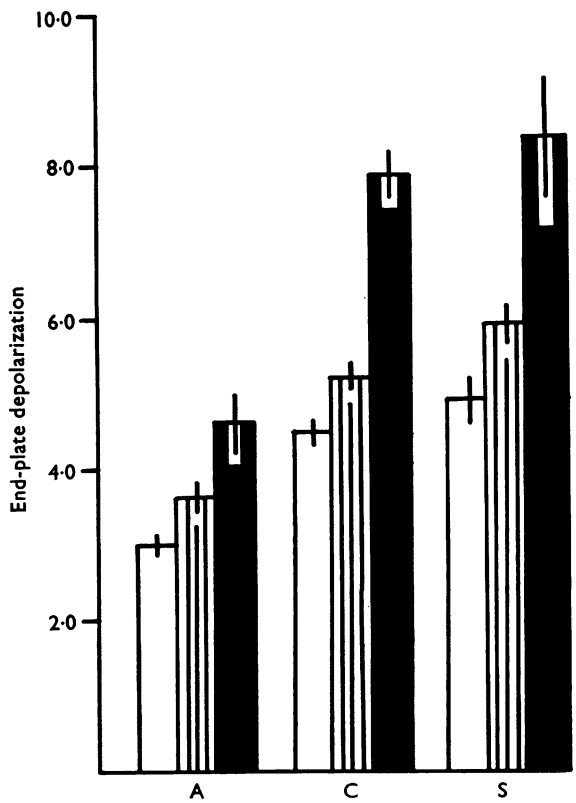

FIG. 8. Mean end-plate depolarization (±S.E., $n=4$–6) caused by acetylcholine ($0{\cdot}5\times10^{-6}$ g/ml, A), carbachol ($5{\cdot}0\times10^{-6}$ g/ml, C) and suxamethonium ($5{\cdot}0\times10^{-6}$ g/ml, S) in isolated rat diaphragm preparations immersed in normal Krebs solution (open columns), nitrate Krebs solution (shaded columns) and methylsulphate Krebs solution (solid columns) after 1 min contact time. The differences between the means were significant at the following levels: Acetylcholine: normal/nitrate, $P=<0{\cdot}05$; normal/methylsulphate, $P=<0{\cdot}01$; nitrate/methylsulphate, $P=<0{\cdot}05$. Carbachol: normal/nitrate, $P=<0{\cdot}05$; normal/methylsulphate, $P=<0{\cdot}01$; nitrate/methylsulphate, $P=<0{\cdot}05$. Suxamethonium: normal/nitrate, $P=<0{\cdot}05$; normal/methylsulphate, $P=<0{\cdot}001$; nitrate/methylsulphate, $P=<0{\cdot}01$.

preparation. Zaimis (1962, 1964), for example, has pointed out that the distinguishing features of neuromuscular blockade in various animal species (Hall & Parkes, 1953 ; Zaimis, 1953) are lost in isolated nerve-muscle preparations of these animals. This loss of distinguishing characteristics is probably related to the changes in ion permeability which occur in isolated tissues (Creese & Northover, 1961 ; Krnjevic & Miledi, 1958), and also to the way in which the drug reaches the receptor sites (Gibberd, 1966).

The most obvious characteristic common to all isolated nerve-muscle preparations is the biphasic response of the muscle to the continuing presence of the blocking agent (Jenden, Kamijo & Taylor, 1954 ; Jenden, 1955 ; Sabawala & Dillon, 1959 ; Maclagan, 1962 ; Taylor, 1962 ; Whittaker, 1962a ; Freeman, 1968a). This response in particular has led to many of the current theories of the mechanism of depolarizing blockade in isolated nerve-muscle preparations.

Whittaker (1962a) observed that tubocurarine intensified suxamethonium induced neuromuscular blockade in the isolated rat diaphragm preparation and suggested that suxamethonium induced blockade primarily by competition with neurally released acetylcholine, with end-plate depolarization as a minor feature. This mechanism has been reclassified as " dual block " (Zaimis, 1953) by Bowman (1964). Essentially similar conclusions were drawn by Jenden, Kamijo & Taylor (1954), Jenden (1955) and Sabawala & Dillon (1959) in their experiments on isolated rabbit, guinea-pig and human nerve-muscle preparations respectively.

In our own experiments we have found little evidence to support the suggestion that suxamethonium blocks either as a straightforward competitive blocking agent of low potency, or by a dual mechanism in the isolated rat diaphragm. Zaimis (1953) in her original use of the term " dual " laid down several criteria all of which she had drawn from observations made on nerve-muscle preparations *in vivo*. Briefly, these criteria were that the " depolarizing " blocking agent should produce early changes in neuromuscular transmission consistent with end-plate depolarization, but that at some later stage the blockade should demonstrate features consistent with competitive blockade. In the isolated rat diaphragm preparation, features of both initial depolarization and later competition are absent. Not only was there an absence of indirect twitch potentiation or " contracture " in the preparation immediately following the addition of suxamethonium, but even the retrograde injection of suxamethonium failed to elicit a " twitch-like " response of the muscle. Furthermore, although tubocurarine was observed to potentiate the blockade in both phase I and phase II, neostigmine, edrophonium and potassium ions were all incapable of consistently producing a reversal of blockade, even in phase II. It is well known that rat muscles are particularly insensitive to " depolarizing " blocking agents (Barlow & Ing, 1948 ; Paton & Zaimis, 1949 ; Jarcho, Eyzaguirre, Talbot & Lilienthal, 1950 ; Bergh, 1953, for example), and this may be the explanation for the absence of excitatory effects of suxamethonium, for it has been noted that the more readily blocked muscles exhibit the most marked evidence of depolarization (Zaimis, 1953) ; but if this is the case, then the argument against the occurrence of dual blockade in the isolated rat diaphragm is merely strengthened.

The situation is complex, however, for both Thesleff (1958) and ourselves (Harris & Leach, 1968) have shown that suxamethonium does induce motor end-plate depolarization. If, therefore, this depolarization is of importance in the onset of neuromuscular blockade, any procedure which increases the sensitivity of the motor

end-plate region to depolarizing blocking agents might be expected to enhance the potency of the drug as a blocking agent. Furthermore, the block would be expected to be accompanied by features generally accepted as being indicative of depolarization blockade. The presence of the foreign anions used in this study does result in an increase in the sensitivity of the motor end-plate region as measured by end-plate depolarization. In the presence of these ions, the isolated rat diaphragm preparation did exhibit many features associated with depolarization blockade when suxamethonium was added to the bath ; for example, we observed indirect twitch potentiation, a twitch-like response following retrograde injection and a mutual antagonism between suxamethonium and tubocurarine. However, suxamethonium was no more potent as a blocking agent in the presence of foreign anions than in normal Krebs solution.

This observation would suggest that end-plate depolarization plays virtually no role in the development of neuromuscular blockade in the isolated rat diaphragm preparation treated with suxamethonium. This, in turn, would imply that motor end-plate desensitization (Thesleff, 1958) or the inhibition of transmitter release (Freeman, 1968a, b) sets in so rapidly that any effect of end-plate depolarization on transmission is masked.

From our own observations it is not possible to decide which of these two mechanisms is operative in the isolated rat diaphragm preparation. Thesleff (1955) and Roberts & Thesleff (1965) have demonstrated that motor end-plate desensitization is an important feature of neuromuscular blockade, and our results are completely consistent with this theory. On the other hand, Freeman & Turner (1968) were unable to make a direct demonstration of a depression of transmitter release by suxamethonium. Moreover, Freeman's (1968a, b) theory is based on the indirect evidence of a suxamethonium induced depression of transmitter release from preparations maintained at 29°–30° C. Her results are therefore complicated by the already existing depression of transmitter substance which is known to occur at low temperatures (Hofmann, Parsons & Feigen, 1966).

Freeman's (1968a) " unifying concept " of suxamethonium blockade *in vitro* is that excitation followed by a stabilization occurs at both pre- and post-synaptic sites. Facilitatory activity may be expected when the excitatory phase is well developed ; block without facilitation when stabilizing actions predominate. Perhaps this theory may be taken one step further to suggest that the pre-synaptic membrane is " stabilized " only at low temperatures, and that at normal temperatures the predominant stabilizing activity is at the post-synaptic membrane and results in motor end-plate desensitization.

REFERENCES

BARLOW, R. B. & ING, H. R. (1948). Curare-like action of polymethylene bisquaternary ammonium salts. *Br. J. Pharmac. Chemother.*, **3**, 298–304.

BERGH, N. P. (1953). Studies on the reaction of the isolated phrenic nerve diaphragm preparations of various species to certain agents causing a neuromuscular block. *Scand. J. clin. lab. Invest.*, **5** (suppl. 7).

BOWMAN, W. C. (1964). In *Evaluation of Drug Activities: Pharmacometrics*, ed. Laurence, D. R. and Bacharach, A. L., vol. 1, pp. 325–352. London: Academic Press.

BRAND, H. (1952). A propos du mode d'action du decamethonium et de l'amyl-trimethyl-ammonium sur la preparation isolée nerf-phrénique-diaphragme du rat. *Experientia*, **8**, 273.

BÜLBRING, E. (1946). Isolated phrenic nerve diaphragm preparations of the rat. *Br. J. Pharmac. Chemother.*, **1**, 38–61.

BURNS, B. D. & PATON, W. D. M. (1951). Depolarisation of the motor end-plate by decamethonium and acetylcholine. *J. Physiol., Lond.*, **115**, 41–73.

CREESE, R. & NORTHOVER, J. (1961). Maintenance of isolated diaphragm with normal sodium content. *J. Physiol., Lond.*, **155**, 343–357.

FREEMAN, S. E. (1968a). Ionic influences on succinylcholine blockade of mammalian neuromuscular junction. *Br. J. Pharmac. Chemother.*, **32**, 546–566.

FREEMAN, S. E. (1968b). Antagonism of succinylcholine blockade of the mammalian neuromuscular junction. *J. Pharmac. exp. Ther.*, **162**, 10–20.

FREEMAN, S. E. & TURNER, R. J. (1968). Succinylcholine blockade of the mammalian neuromuscular junction after acetylcholinesterase inhibition with D.F.P. *Life Sci., Oxford*, **7** (1), 875–881.

GIBBERD, F. B. (1966). Action of decamethonium on rat diaphragm. *Br. J. Pharmac. Chemother.*, **28**, 128–136.

GROB, D., JOHNS, R. J. & HARVEY, A. McG. (1956). Studies in neuromuscular function. *Bull. Johns Hopkins Hosp.*, **99**, 115.

HALL, R. A. & PARKES, M. W. (1953). The effect of drugs upon neuromuscular transmission in the guinea-pig. *J. Physiol., Lond.*, **122**, 274–281.

HARRIS, J. B. & LEACH, G. D. H. (1965). An improved method for obtaining twitch responses to acetylcholine in the rat diaphragm. *J. Pharm. Pharmac.*, **17**, 816–817.

HARRIS, J. B. & LEACH, G. D. H. (1968). The effect of temperature on end-plate depolarisation of the rat diaphragm produced by suxamethonium and acetylcholine. *J. Pharm. Pharmac.*, **20**, 194–198.

HOFMANN, W. W., PARSONS, R. L. & FEIGEN, G. A. (1966). Effects of temperature and drugs on mammalian motor nerve terminals. *Am. J. Physiol.*, **211**, 135–140.

HOROWICZ, P. (1964). The effects of anions on excitable cells. *Pharmac. Rev.*, **16**, 193–221.

HUTTER, O. F. & PADSHA, S. M. (1959). Effect of nitrate and other anions on the membrane resistance of frog skeletal muscle. *J. Physiol., Lond.*, **146**, 117–132.

JARCHO, L. W., EYZAGUIRRE, C., TALBOT, S. A. & LILIENTHAL, J. L. (1950). Neuromuscular excitation: responses of normal and denervated mammalian muscle to bis-trimethyl-ammonium decane (C_{10}) and to *d*-tubocurarine. *Am. J. Physiol.*, **162**, 475–488.

JENDEN, D. J. (1955). The effect of drugs upon neuromuscular transmission in the isolated guinea pig diaphragm. *J. Pharmac. exp. Ther.*, **114**, 398–408.

JENDEN, D. J., KAMIJO, K. & TAYLOR, D. B. (1954). The action of decamethonium on the isolated rabbit lumbrical muscle. *J. Pharmac. exp. Ther.*, **111**, 229–240.

JEWELL, P. H. & ZAIMIS, E. (1954). A differentiation between red and white muscle in the cat based on responses to neuromuscular blocking agents. *J. Physiol., Lond.*, **124**, 417–428.

JONES, J. J. & LAITY, J. L. H. (1965). A note on an unusual effect of gallamine and tubocurarine. *Br. J. Pharmac. Chemother.*, **24**, 360–364.

KRAYER, O. & ACHESON, G. H. (1946). The pharmacology of the veratrine alkaloids. *Physiol. Rev.*, **26**, 383–446.

KRNJEVIC, K. P. & MILEDI, R. (1958). Failure of neuromuscular propagation in rats. *J. Physiol., Lond.*, **140**, 440–461.

LÜLLMAN, H. (1961). Das Verhalten und denervierten Skeletmuskulatur in chloridfreiem Medium (Methylsulfat-Tyrodelösung). *Arch. exp. Path. Pharmak.*, **240**, 351–360.

MACLAGAN, J. (1962). A comparison of the responses of the tenuissimus muscle to neuromuscular blocking drugs *in vivo* and *in vitro*. *Br. J. Pharmac. Chemother.*, **18**, 204–216.

OOMURA, Y. & TOMITA, T. (1961). Some observations concerning the end-plate potential. *Tohuko J. exp. Med.*, **73**, 398–415.

PATERSON, G. (1965). Twitch responses with acetylcholine in the isolated innervated and chronically denervated rat diaphragms and their modification by neuromuscular blocking agents. *J. Pharm. Pharmac.*, **17**, 281–294.

PATON, W. D. M. & ZAIMIS, E. J. (1949). Pharmacological actions of polymethylene bistrimethylammonium salts. *Br. J. Pharmac. Chemother.*, **4**, 381–400.

ROBERTS, D. V. & THESLEFF, S. (1965). Neuromuscular transmission *in vivo* and the actions of decamethonium: a micro-electrode study. *Acta anaesth. scand.*, **9**, 165–172.

SABAWALA, P. B. & DILLON, J. B. (1959). Mode of action of depolarising agents. *Acta anaesth. scand.*, **3**, 83–101.

SANDOW, A. (1964). Potentiation of muscular contraction. *Arch. Phys. Med. Rehabil.*, **45**, 62–81.

SANDOW, A. & STEIN, E. (1956). Effects of nitrate on normal and dystrophic rabbit muscle. *Fedn Proc.*, **15**, 162.

STOVNER, J. (1958). The effect of tetraethyl-ammonium (T.E.A.) on neuromuscular block caused by succinylcholine and succinylmonocholine. *Acta anaesth. scand.*, **2**, 53–67.

TAYLOR, D. B. (1962). Influence of curare on uptake and release of a neuromuscular blocking agent labelled with iodine-131. In *Curare and Curare-like Agents*, pp. 21–27, Ciba Foundation Study Group No. 12. London: Churchill.

THESLEFF, S. (1955). The effects of acetylcholine, decamethonium and succinylcholine on neuromuscular transmission in the rat. *Acta physiol. scand.*, **34**, 386–392.

THESLEFF, S. (1958). A study of the interaction between neuromuscular blocking agents and acetylcholine at the mammalian motor end-plate. *Acta anaesth. scand.*, **2**, 69–79.

WHITTAKER, R. (1962a). The neuromuscular blocking action of suxamethonium on the rat diaphragm. *J. Pharm. Pharmac.*, **14**, 177–181.

WHITTAKER, R. (1962b). The effect of lowered temperature on the neuromuscular blocking action of suxamethonium on the rat diaphragm. *J. Pharm. Pharmac.*, **14**, 803–807.

ZAIMIS, E. J. (1953). Motor end-plate differences as a determining factor in the mode of action of neuromuscular blocking substances. *J. Physiol., Lond.*, **122**, 238–251.

ZAIMIS, E. J. (1962). Experimental hazards and artefact in the study of neuromuscular blocking drugs. In *Curare and Curare-like Agents*, pp. 75–86, Ciba Foundation Study Group No. 12. London: Churchill.

ZAIMIS, E. J. (1964). General physiology and pharmacology of neuromuscular transmission. In *Disorders of Voluntary Muscle*, ed. Walton, J. N., chap. 4. London: Churchill.

(*Received October* 14, 1969)