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THE EFFECT OF (+)-TUBOCURARINE ON NEUROMUSCULAR TRANSMISSION DURING REPETITIVE STIMULATION IN THE RAT, MOUSE, AND FROG

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SUMMARY

1. The effect of tubocurarine on amplitudes of end-plate currents in response to trains of repetitive stimulation (50–150/sec) was investigated in voltage-clamped muscle fibres of the rat, mouse and frog.

2. In rat and mouse muscle, the presence of tubocurarine led to a more rapid decline (rundown) in the amplitudes of successive end-plate currents during trains of impulses. In frog, tubocurarine caused an increase in apparent facilitation of end-plate current amplitudes during the first few impulses of repetitive stimulation; this increase was followed by a more rapid rundown of end-plate current amplitude.

3. These effects of tubocurarine appear not to be an artifact resulting from inadequate control of membrane potential in voltage-clamped fibres.

4. The more rapid rundown during trains of end-plate currents in the presence of tubocurarine showed little variation with membrane potential indicating that voltage-sensitive channel blockade by tubocurarine was not a major factor contributing to the rundown.

5. The effect of tubocurarine on the apparent facilitation and rundown of end-plate current amplitudes was typically decreased by reducing the frequency of stimulation.

6. These results suggest that tubocurarine affects transmitter release at neuromuscular junctions during repetitive stimulation.

INTRODUCTION

Although it is generally accepted that the blockade of neuromuscular transmission caused by tubocurarine arises to a large extent from its binding to receptors in the post-synaptic membrane (e.g. Jenkinson, 1960; Adams, 1975; Colquhoun, Dreyer & Sheridan, 1979) it remains possible that additional actions of tubocurarine may contribute to the blockade that can develop during repetitive nerve stimulation (tetanic fade, see Paton & Zaimis, 1952; Bowman & Webb, 1976). When released acetylcholine is collected and measured during and following repetitive stimulation some investigators report that tubocurarine has no marked effect on release (Strau-

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ghan, 1960; Krnjević & Mitchell, 1961; Chang, Cheng & Chen, 1967; Fletcher & Forrester, 1975) while others suggest that tubocurarine increases (Miledi, Molenaar & Polak, 1978) or decreases (Beani, Bianchi & Ledda, 1964) release. Various effects of tubocurarine on quantal content have been suggested on the basis of electrophysiological studies; it has been concluded that for single stimuli or for the first end-plate potential in a train, quantal content is decreased (Hubbard, Wilson & Miyamoto, 1969; Hubbard & Wilson, 1972), unchanged (Auerbach & Betz, 1971) or increased (Blaber, 1970, 1973) when tubocurarine is present in the bathing solution. During repetitive nerve stimulation, many investigators find that the presence of tubocurarine leads to a more rapid decline (rundown) in the amplitude of successive end-plate potentials or currents (Lilleheil & Naess, 1961; Brooks & Thies, 1962; Elmqvist & Quastel, 1965; Hubbard *et al.* 1969; Blaber, 1970, 1973; Hubbard & Wilson, 1972; Glavinović, 1979); these investigators have interpreted their findings in terms of a presynaptic action of tubocurarine. Auerbach & Betz (1971), however, have reported little presynaptic action.

A factor complicating the interpretation of the rundown of end-plate potentials and currents during repetitive stimulation is the possibility of blockade of open end-plate channels by tubocurarine (Manalis, 1977; Katz & Miledi, 1978; Colquhoun *et al.* 1978, 1979); it has been considered by Colquhoun *et al.* (1979) that end-plate channel blockade might contribute to the effects of tubocurarine during high frequency stimulation.

In the present paper we further examine the effects of tubocurarine on neuromuscular transmission during repetitive stimulation in an effort to resolve some of these questions concerning the action of this drug. Using intact mouse and rat muscle fibres, which have been immobilized by stretch, we confirm that end-plate current amplitudes run down faster in the presence of tubocurarine during repetitive stimulation. In addition, we find that in the frog tubocurarine leads to an increase in the apparent facilitation of end-plate current amplitudes during the first few impulses of repetitive stimulation which is then usually followed by a more rapid rundown of end-plate current amplitudes. These effects of tubocurarine in the rat, mouse and frog are most pronounced at high stimulation rates. They do not appear to be due to possible voltage clamping artifacts or to voltage dependent postsynaptic channel blockade by tubocurarine. We suggest that tubocurarine affects transmitter release at neuromuscular junctions during repetitive stimulation.

METHODS

Mammalian muscles used were mouse omohyoideus and rat diaphragms. For experiments on rat diaphragms a strip of muscle 2–4 mm wide was cut parallel to the long axis of the muscle fibres so that the strip contained the point of entry of the phrenic nerve where the muscle fibres were intact (Clark, Hobbiger & Terrar, 1980); any damaged fibres at the edge of the preparation could easily be recognized and avoided. These narrow strips of diaphragm or intact omohyoideus muscles were mounted in a channel 10 mm wide milled from a Perspex block; a continuous stream of oxygenated solution from a resevoir mounted above the bath, flowed through the channel, bathing the muscle at 10–15 ml. min^{-1}. The composition of this solution could be changed rapidly by a tap connected to the inflow of the bath. The temperature of the preparation, which was monitored by a probe close to the muscle could be varied by means of a heating coil warming the inflowing solution. The majority of experiments were done at 23–24 °C, although a few were at 36 °C. Since contraction was not blocked in these muscles in the absence of drugs or at low concentrations of

(+)-tubocurarine, it was necessary to immobilize the muscle; this was achieved by controlled stretching over a curved Perspex support. End-plate currents uncontaminated by action potentials can be recorded in stretched muscle by the voltage clamp technique (Terrar, 1978).

The solution used for these experiments was that described by Liley (1956) and contained (mM): NaCl, 137; $NaHCO_3$, 24; KCl, 5·0; $CaCl_2$, 2·0; $MgCl_2$, 1·0; NaH_2PO_4, 1·0; glucose, 11.

The frog muscle used was the sartorius from *Rana pipiens*. In these experiments muscle contraction was prevented by pre-treatment with hypertonic glycerol (Gage & Eisenberg, 1967; Howell, 1969); the nerve-muscle preparation was immersed for about 60 min in the standard solution to which 500–900 mM-glycerol had been added. The muscles were then transferred to the standard solution and allowed to recover for 30–60 min before use. The standard solution used in these experiments contained (mM): NaCl, 116; KCl, 2·0; $CaCl_2$, 1·8; Na_2HPO_4, 2·16; NaH_2PO_4, 0·85; glucose, 5; choline, 0·03. Frog experiments were done at 20 ± 0.5 °C by cooling the preparation chamber with a Peltier-effect module.

Motor nerves supplying the muscles were stimulated with trains of stimuli (50–150/sec). The interval between trains was 5–90 sec.

The voltage-clamping apparatus was similar to that described previously (Connor & Stevens, 1971; Magleby & Terrar, 1975). Microelectrodes were filled with 3 M-KCl and had resistances in the range 5–20 MΩ. Voltage and current data were recorded on an FM tape recorder (0–5000 Hz). The data were later photographed from a Tektronix 5103 oscilloscope with a Polaroid camera and enlarged for measurement by hand or digitized and analysed on a PDP-11 computer (sampling rate 50–100 μsec/point).

RESULTS

Effect of tubocurarine on end-plate current amplitudes during repetitive stimulation in mammalian muscle

Fig. 1 shows end-plate currents recorded from mammalian muscle fibres in response to repeated nerve stimulation at 150/sec. Movement of the muscle which would have been expected because neuromuscular transmission was not blocked, was minimized or abolished by stretching the muscles. Under these conditions the effect of tubocurarine could be examined in the absence of other drugs or treatments used to prevent muscle contraction. Trains of end-plate currents recorded from rat diaphragm before the addition of tubocurarine are shown in Fig. 1 A_1; the effects of tubocurarine applied in the solution flowing over the muscle reached a steady level after approximately 1 min exposure to the drug at the flow rate used, and Fig. 1 A_2 shows trains of end-plate currents after 3 min (0·5 μM-tubocurarine). Records after washout of the tubocurarine are shown in Fig. 1 A_3. It can be seen that the presence of tubocurarine in the bathing solution reduces the amplitudes of the end-plate currents as expected from the well known post-synaptic blocking action of this drug (Jenkinson, 1960; Adams, 1975; Colquhoun, Dreyer & Sheridan, 1979). In addition, it can be seen that end-plate currents at the end of the train are proportionately more reduced than the first end-plate current: the train appears to 'rundown' more rapidly in the presence of tubocurarine as found by Glavinović (1979) in cut muscles from rat diaphragm and by others recording end-plate potentials (Lilleheil & Naess, 1961; Hubbard *et al.* 1969; Blaber, 1970, 1973; Hubbard & Wilson, 1972).

These effects of tubocurarine were reversible and were always seen over the range of concentrations studied (0·5–1 μM).

A similar action of tubocurarine of causing a faster rundown of end-plate current amplitudes during repetitive stimulation was also seen in mouse omohyoideus muscle. This is shown in Fig. 1 *B* before (B_1), during (B_2), and after washout of (B_3) of the drug.

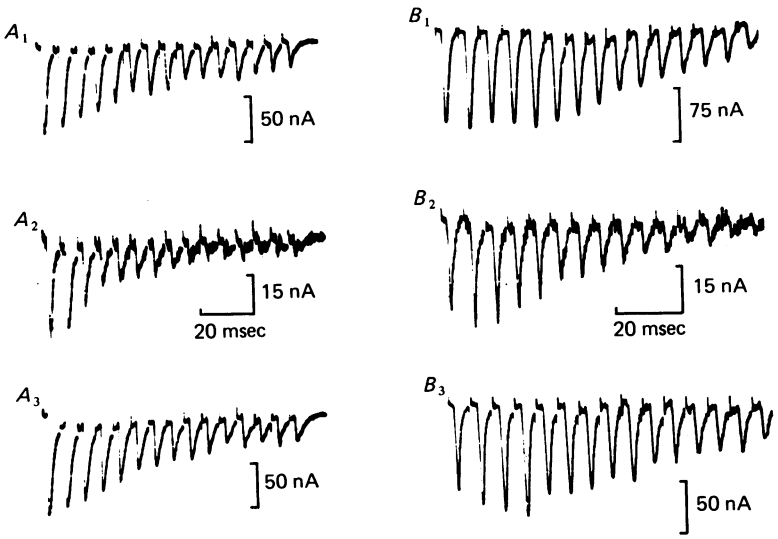


Fig. 1. Effect of tubocurarine (0·5 μM applied in the solution flowing over the muscle) on trains of end-plate currents in reponse to repetitive nerve stimulation at 150/sec recorded from rat diaphragm (A_1, A_2, A_3) or mouse omohyoid (B_1, B_2, B_3) muscle. Each set of trains recorded in sequence from the same muscle fibre: A_1, B_1, no drugs; A_2, B_2, during tubocurarine; A_3, B_3, after washout. Membrane potentials: -50 mV (A_1 to A_3) -60 mV (B_1 to B_3). 24 °C.

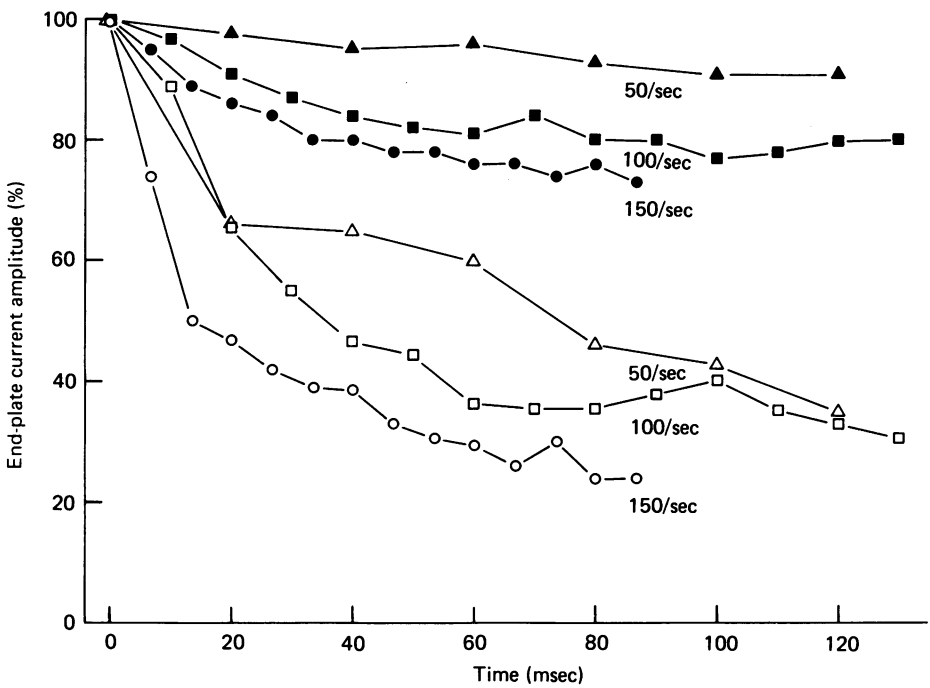


Fig. 2. Effect of tubocurarine (1 μM) on trains of end-plate currents in one fibre at three rates of nerve stimulation: 50/sec (triangles); 100/sec (squares); and 150/sec (circles). Amplitudes of end-plate currents expressed as a percentage of the first end-plate current in the train are plotted against time from the start of the train: in each case filled symbols represent trains in the absence of drugs and open symbols trains in the presence of 1 μM-tubocurarine. All records at -60 mV. 36 °C.

The effects of tubocurarine were still present at temperatures and stimulation rates that might be expected under more physiological conditions. This is shown in Fig. 2 which presents plots of amplitudes of successive end-plate currents (expressed as a percent of the first in each train) recorded in the presence and absence of 1 μM tubocurarine at 36 °C for three stimulation rates. It can be seen that the greater rundown of end-plate current amplitudes in tubocurarine was still present at a stimulation rate of 50 impulses/sec, which falls within the physiological range (Adrian & Bronk, 1928).

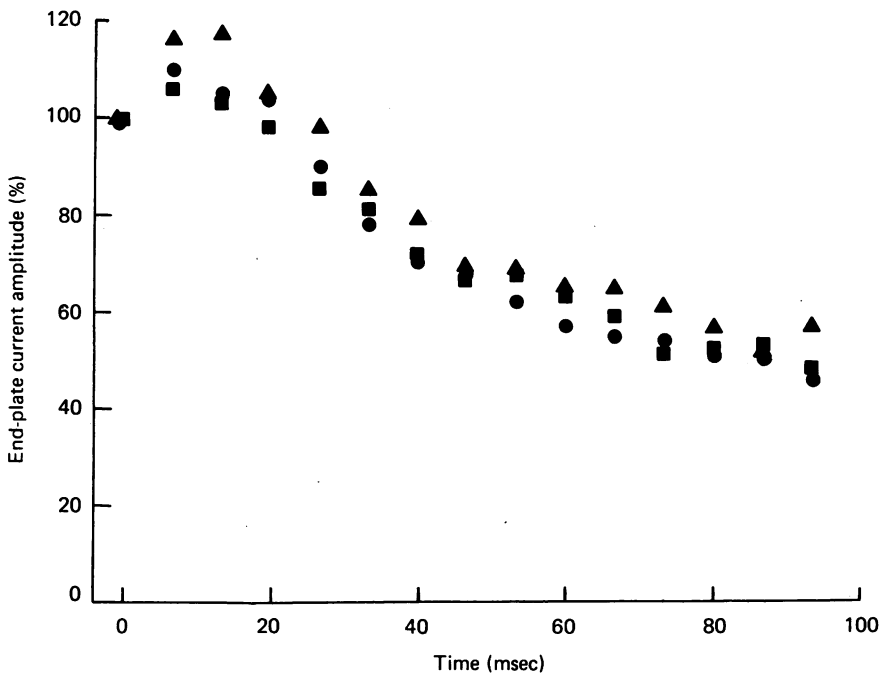


Fig. 3. Effect of membrane potential on rundown of end-plate current amplitudes during repetitive stimulation (150/sec). Amplitudes of end-plate currents, expressed as a percentage of the first end-plate current in the train, are plotted against time from the start of the train at −20 mV (▲) −40 mV (■), or −60 mV (●). Each series of points from averages of four to six trains. Rat diaphragm. 24 °C.

The greater rundown of end-plate current amplitudes in the presence of tubocurarine is not an artifact

A series of experiments were done to determine if the faster rundown of end-plate currents in the presence of tubocurarine is a recording artifact arising from the reduced amplitudes of the end-plate currents in the presence of tubocurarine. For example, in the absence of tubocurarine the end-plate currents are large and it is possible that the voltage clamp may not be able to properly control all parts of the motor end-plate. If this were the case, then depolarization of the muscle cell by partially clamped end-plate potentials might lead to active responses arising from activation of voltage-dependent Na channels. These active responses might obscure

a possible rundown of end-plate currents in the absence of tubocurarine. Nonlinear summation (Martin, 1955) of partially clamped end-plate potentials could also obscure a possible rundown of end-plate currents in the absence of tubocurarine when the potentials would be largest and non-linear summation the greatest. Since the influence of active responses and nonlinear summation would be expected to vary with membrane potential the possible effects of these two factors on the rundown of end-plate currents can be tested by comparing trains collected at various holding potentials.

Such a test is shown in Fig. 3 which presents plots of amplitudes of successive end-plate currents recorded in the absence of tubocurarine. End-plate current amplitudes were determined from the average of six trains at each of three holding potentials, and are expressed in the terms of the amplitude of the first end-plate current in the train.

It can be seen that there was little or no effect of holding potential on the rundown of the trains in the critical range -20 mV (triangles), -40 mV (squares), -60 mV (circles). It can therefore be concluded that there is little or no contribution of active responses and nonlinear summation to the end-plate currents recorded in the absence of tubocurarine. Consequently, the greater rundown observed in the presence of tubocurarine cannot be due to the fact that tubocurarine removes or reduces these factors.

Does channel blockade by tubocurarine lead to greater rundown of end-plate currents?

In addition to its well known competitive blocking action of post-synaptic receptors, it also appears that tubocurarine can block open ion channels associated with the receptors (Manalis, 1977; Katz & Miledi, 1978; Colquhoun, Dreyer & Sheridan, 1978, 1979); in this connexion Colquhoun *et al.* (1979) have suggested that blockade of open channels might contribute to the deepening of paralysis that is often observed during high frequency stimulation in the presence of competitive neuromuscular blocking agents.

If channel blockade by tubocurarine were to contribute significantly to rundown, the forward rate constant for binding to the channel blocking sites would have to be fast enough for blockade of a significant fraction of the open channels, and the dissociation rate constant sufficiently slow during the train so that the effect would become cumulative.

The blockade caused by substances which were thought to bind to open ion channels has been shown to be voltage dependent (e.g. Marty, Neild & Ascher, 1976; Adams, 1977; Ascher, Marty & Nield, 1978; Albuquerque, Eldefrawi, Eldefrawi, Mansour & Tsai, 1978; Ascher, Large & Rang, 1979; Adler, Oliveira, Eldefrawi, Eldefrawi & Albuquerque, 1979) and in the case of tubocurarine blocking of end-plate channels in frog muscle changing the holding potential from -60 to -120 mV should lead to an approximately e-fold increase in the rate of association with open channels and an e-fold decrease in the rate of dissociation from blocked channels (Colquhoun *et al.* 1979).

The effect of membrane potential on possible channel blocking in the mouse omohyoideus and rat diaphragm muscles is not known, but if it is similar to that in the frog, and if cumulative blockade of open channels were a major factor underlying

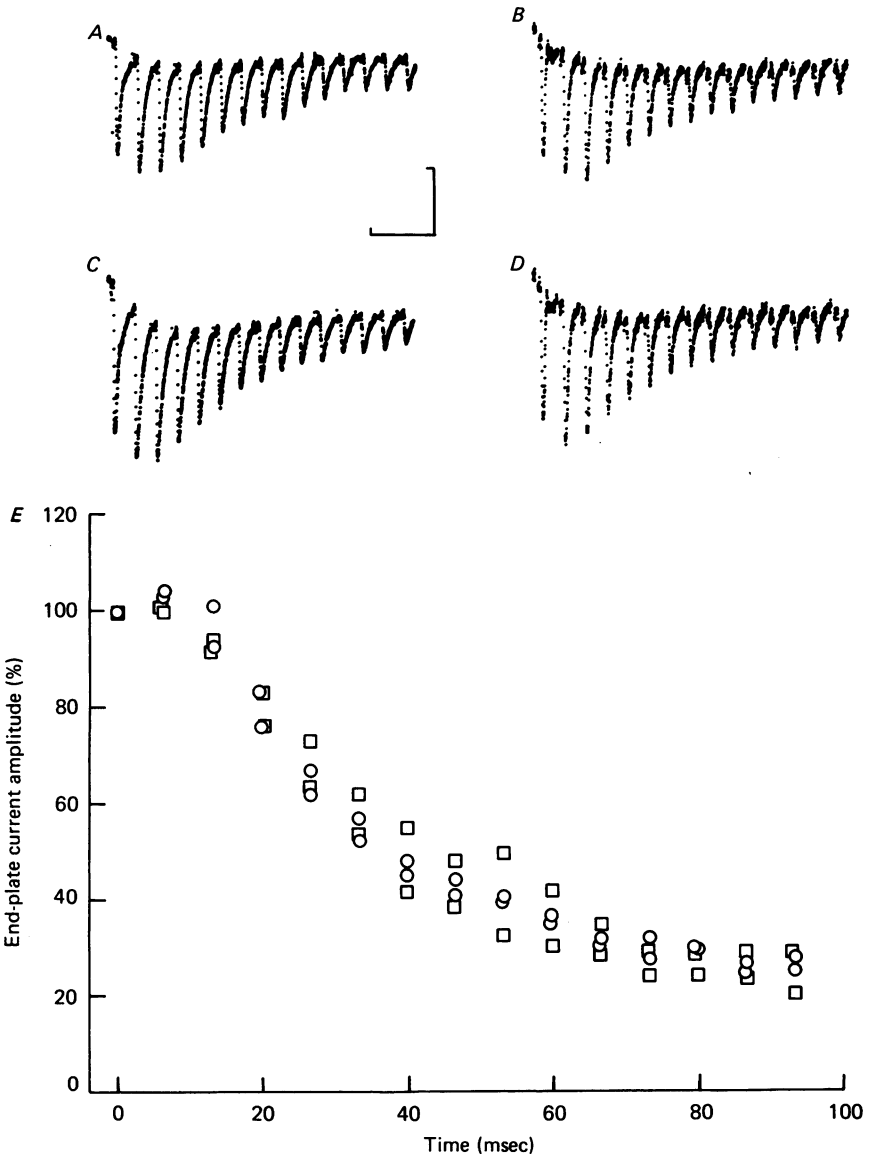


Fig. 4. Effect of membrane potential on the rundown of end-plate current amplitudes during repetitive stimulation (150/sec) in rat diaphragm in the presence of tubocurarine (1 μM). Computer averages sets of six trains recorded in sequence at -120 mV (*A*), -60 mV (*B*), -120 mV (*C*), -60 mV (*D*) Horizontal bar: 20 msec. Vertical bar: *A* and *C*, 10 nA; *B* and *D*, 5 nA. *E* shows the amplitude of end-plate currents expressed as a percentage of the first end-plate current in the train plotted against time from the start of the train at -120 mV (□) or -60 mV (○) 24 °C.

rundown of trains of end-plate currents in the rat and mouse, the rundown would be more extensive at −120 mV than at −60 mV. Fig. 4 illustrates an experiment to test this possibility; currents were recorded in the presence of 1 μM-tubocurarine from rat diaphragm in the sequence −120 mV (*A*), −60 mV (*B*), −120 mV (*C*), and −60 mV (*D*). It can be seen that there was little or no effect of membrane potential on the rate of rundown of the end-plate current amplitudes during the trains. This is more clearly demonstrated in Fig. 4*E* which shows plots of peak amplitudes of end-plate currents in each of the trains, expressed as a percent of the first end-plate current.

The data shown in Fig. 4, were collected at 24 °C. A similar lack of effect of membrane potential on the rate of rundown of end-plate current amplitudes in the presence of tubocurarine was also found for experiments performed at 36 °C. Thus, voltage dependent blockade by tubocurarine is not a significant factor contributing to the greater rundown of the trains of end-plate currents observed in Fig. 1.

Effect of tubocurarine on end-plate current amplitudes during repetitive stimulation at the frog neuromuscular junction

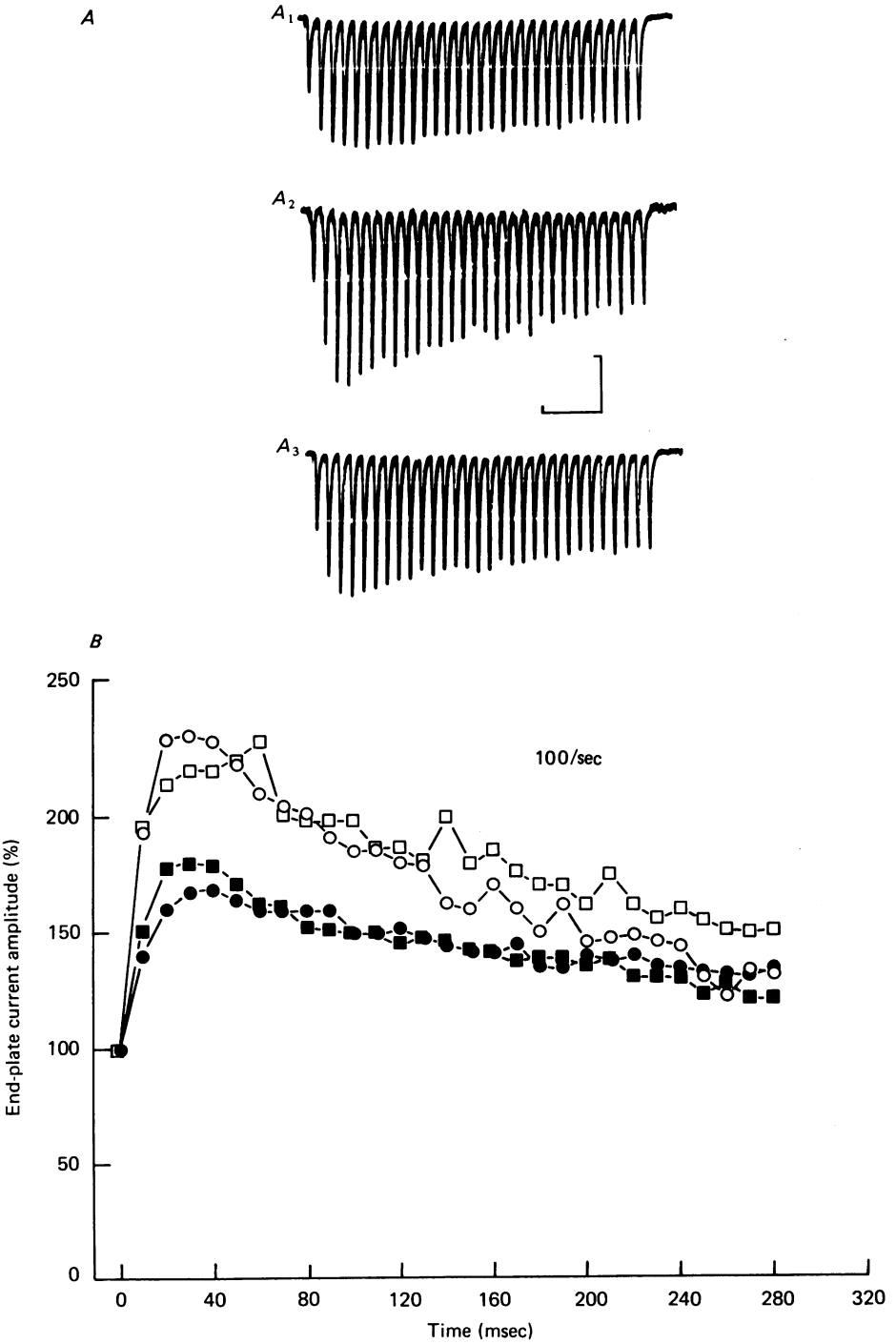
The previous sections established that tubocurarine has an effect on end-plate current amplitudes during repetitive stimulation in the rat diaphragm and mouse omohyoideus.

In this part of the paper we examine whether tubocurarine has similar effects in the frog sartorius nerve-muscle preparation.

Fig. 5*A* shows end-plate currents recorded from a voltage clamped frog sartorius muscle during repeated nerve stimulation at 100/sec. Movement of the muscle was prevented by treatment with glycerol (which is then washed from the muscle before the experiment) which prevents action potentials in the muscle fibres from causing contraction (Gage & Eisenberg, 1967; Howell, 1969). Trains of end-plate currents obtained before (Fig. 5A_1), during (A_2) and after washout (A_3) of 3 μM-tubocurarine added to the solution flowing over the muscle are shown. It can be seen that tubocurarine reduces the amplitudes of the end-plate currents and changes the shape of the response during the trains.

In the presence of tubocurarine end-plate current amplitudes rise rapidly at the start of the train to about 2·2 times the amplitude of the first end-plate current, while in the absence of tubocurarine end-plate current amplitudes rise more slowly to about 1·6 times the amplitude of the first end-plate current. Successive end-plate current

Fig. 5. Effect of tubocurarine (3 μM) on trains of end-plate currents in response to repetitive nerve stimulation at 100/sec recorded from frog sartorius muscle. Trains recorded in sequence from the same muscle fibre: A_1, no drugs; A_2 during exposure to tubocurarine applied in the solution flowing over the muscle; A_3 after washout. Horizontal bar: 50 msec. Vertical bar: 100 nA, A_1; 15 nA, A_2; 50 nA, A_3. *B* shows amplitudes of end-plate currents from this experiment expressed as a percentage of the first end-plate current in the train plotted against time from the start of the train; the trains were recorded in sequence: no drugs (●), in the presence of 3 μM-tubocurarine (○), wash from tubocurarine (■), 3 μM-tubocurarine reapplied (□). The influence of frequency of stimulation on the effects of tubocurarine is shown in the same fibre at 150/sec (*C*) and 50/sec (*D*); in each case filled circles represent trains in the absence of drugs and open circles trains in the presence of 3 μM-tubocurarine. All records at −60 mV.



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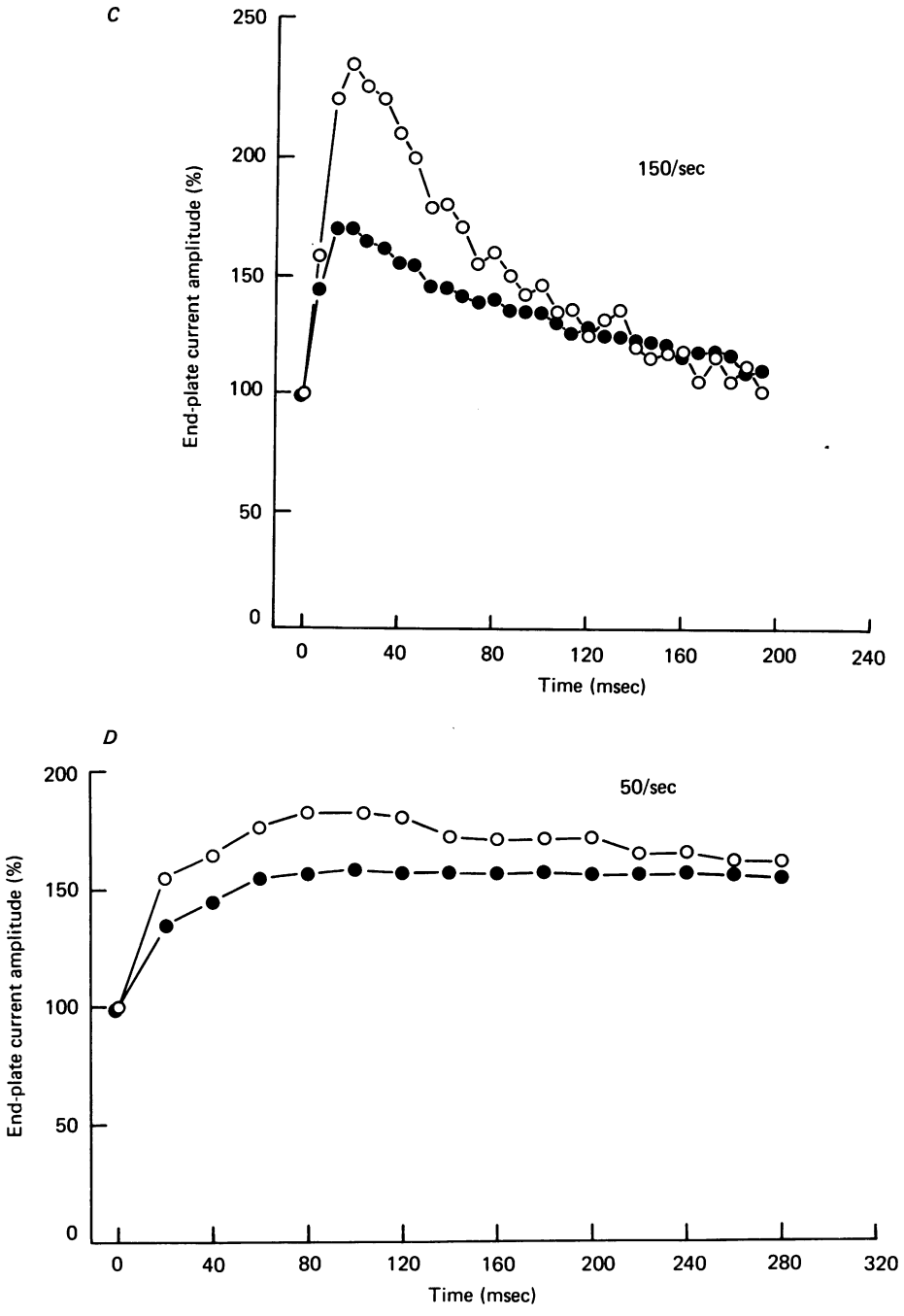
C
250
200
150
100
50
0
End-plate current amplitude (%)
150/sec
0
40
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200
240
Time (msec)
D
200
150
100
50
0
End-plate current amplitude (%)
50/sec
0
40
80
120
160
200
240
280
320
Time (msec)

amplitudes then decrease or rundown more rapidly from this peak value in the presence of tubocurarine than in its absence.

These effects of tubocurarine are seen more clearly in Fig. 5*B*, where the time course of the end-plate current amplitudes during the trains are plotted in terms of the amplitude of the first end-plate currents. Each plotted point represents the average response from 2 to 4 trains. The filled symbols represent the responses obtained in the absence of tubocurarine and the open symbols represent the responses obtained in its presence. This effect of tubocurarine of leading to a more rapid increase and then decline in end-plate current amplitudes during repetitive stimulation was readily reversible. The filled circles represent the control response obtained in the absence of tubocurarine; adding tubocurarine led to the effect (open circles). Washing the preparation (15 min) then reversed the effect (filled squares) which could be obtained again by readding tubocurarine (open squares). The effect of tubocurarine on trains of end-plate currents depended on the stimulation rate. Increasing the stimulation rate usually led to a greater initial increase and then more rapid decline in end-plate current amplitudes during the trains of impulses.

This can be seen in Fig. 5 by comparing data recorded at 150 impulses/sec (*C*), 100 impulses/sec (*B*), and 50 impulses/sec (*D*).

Under conditions of normal (1·8 mM-Ca^{2+}) or elevated (3·6 mM-Ca^{2+}) quantal contents an effect of tubocurarine on the response during trains of impulses was always observed under the conditions of our experiments (50–150 impulses/sec, 2–3 μM-tubocurarine).

The effect of tubocurarine was usually similar to that shown in Fig. 5, but in some experiments the initial increase in end-plate current amplitudes during repetitive stimulation was less than in this Figure and the subsequent decline much greater. An example of such an experiment is shown in Fig. 6 where the stimulation rate was 100/sec. In those frog preparations that gave the type of response shown in Fig. 6 then, the effect of tubocurarine was more like that observed in the rat and mouse.

The failure of Auerbach & Betz (1971) to find a readily apparent effect of tubocurarine on trains of end-plate currents in Fig. 1 of their paper (a small response does appear to be present in this Figure) probably arises from the fact that they changed the concentration of tubocurarine between 1·25 and 3 μg/ml. in this experiment rather than between 0 and 3 μg/ml.

The effect of tubocurarine on trains of end-plate currents in the frog does not appear to be due to a voltage clamping artifact

In the previous part of the paper we established that the greater rundown of end-plate currents at the mammalian end-plate was not due to a voltage clamp artifact arising from the reduced currents in the presence of tubocurarine. Since the frog neuromuscular junction is distributed along the surface of the muscle, instead of being concentrated as the mammalian motor end-plate (Peper & McMahan, 1972; Dreyer, Muller, Peper & Sterz, 1976) the possibility arises that some of the effects of tubocurarine in the frog may be due to lack of control of the membrane potential over the distributed area of the neuromuscular junction. To test this possibility trains of end-plate currents were recorded at various holding potentials, as in the previous section.

End-plate current amplitudes recorded at a holding potential of −20 mV in the presence and absence of tubocurarine are plotted as squares in Fig. 6 and amplitudes recorded at −60 mV are plotted as circles. Although there is some difference in the two records obtained at the two holding potentials in the absence of tubocurarine, the difference is not sufficient to account for the observed effects of tubocurarine.

Similar results were found in additional experiments done to test whether the effect of tubocurarine on the response during trains of end-plate currents results from a

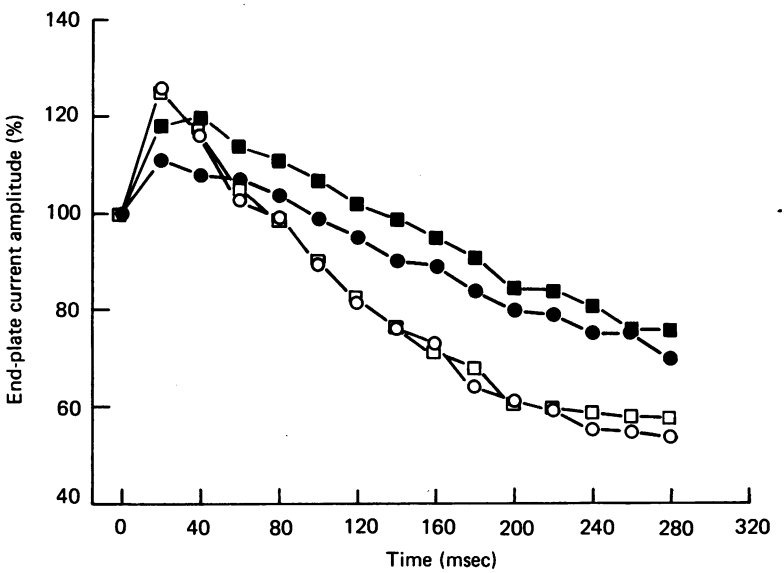

Fig. 6. Effect of tubocurarine (2 μM) on trains of end-plate currents recorded from frog sartorius muscle in response to repetitive nerve stimulation at 50/sec. Amplitudes of end-plate currents, expressed as a percentage of the first end-plate current in the train, are plotted against time after the start of the train either before (●, ■) or during (○, □) application of tubocurarine. Trains recorded at either −60 mV (circles) or −20 mV (squares).

possible voltage clamping artifact due to inadequate control of the membrane potential in the absence of tubocurarine when the end-plate currents are large. For example, for the experiment shown in Fig. 5*B*, trains of end-plate currents were also recorded (in the absence of tubocurarine) at membrane potentials of −100 and −30 mV. Reducing the amplitudes of the end-plate currents more than three times by changing the holding potential over this range had little effect on the pattern of response (similar to the response at −60 mV, filled symbols) when compared to the dramatic effect observed when end-plate currents were reduced with tubocurarine. Thus, as in the rat and mouse, the effect of tubocurarine in the frog (Figs. 5 and 6) is unlikely to have arisen from errors due to the voltage clamping technique.

The effect of tubocurarine on trains of end-plate currents in frog is not due to voltage dependent channel blockade

In the first part of this paper it was shown that the greater rundown of end-plate currents in mammalian end-plates does not appear to be due to a voltage dependent channel blocking by tubocurarine. Fig. 7 presents a similar test for the frog muscle. Trains of end-plate current amplitudes (100/sec stimulation) recorded in the presence of tubocurarine at holding potentials of −60 and −120 mV are plotted against time.

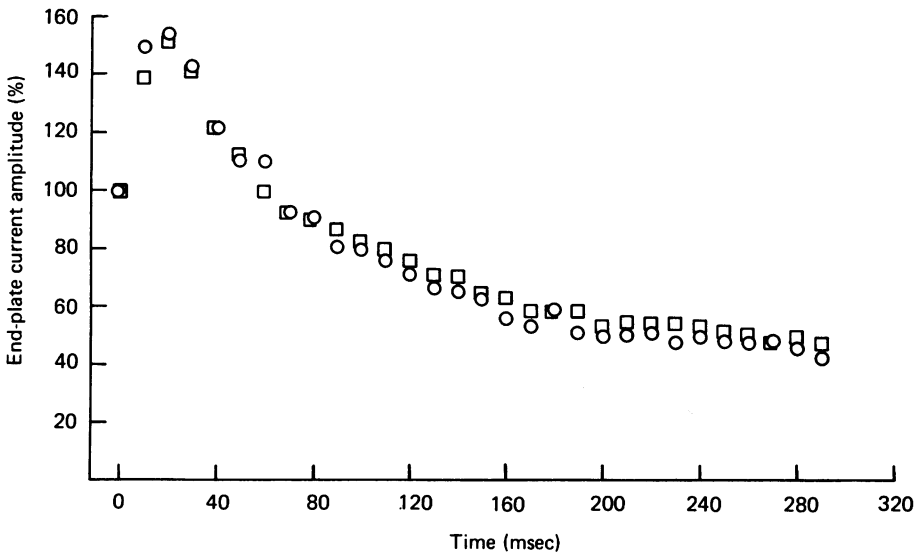

Fig. 7. Effect of membrane potential on trains of end-plate currents in response to repetitive stimulation at 100/sec recorded from frog sartorius muscle in the presence of tubocurarine (3 μM). The amplitudes of end-plate currents expressed as a percentage of the first in the train are plotted against time (msec) from the start of the train at −120 mV (□) or −60 mV (○).

It can be seen that the pattern of response during the trains did not change with voltage; therefore, the effects of tubocurarine on the pattern of response during trains of impulses cannot be attributed to a voltage dependent blocking action of tubocurarine.

DISCUSSION

It is well established that tubocurarine reduces the amplitude of end-plate currents by binding to acetylcholine receptors on the postsynaptic membrane (Jenkinson, 1960; Adams, 1975; Colquohoun, Dreyer & Sheridan, 1979). This paper examines whether tubocurarine also affects neuromuscular transmission by additional mechanisms.

The major findings of this study can be summarized as follows. The presence of

tubocurarine in the bathing solution increased the rundown of the amplitudes of successive end-plate currents during repetitive stimulation in the rat diaphragm and mouse omohyoideus muscles. In the frog sartorius muscle, tubocurarine caused an increase in apparent facilitation of end-plate current amplitudes during the first few impulses of repetitive stimulation; this increase was typically followed by a more rapid rundown of end-plate current amplitudes. These effects of tubocurarine were usually more pronounced at high stimulation rates, and appeared not to be an artifact arising from the voltage clamp technique. Since the greater rundown of end-plate current amplitudes in the presence of tubocurarine showed little or no voltage sensitivity we conclude that blockade of post-synaptic channels by tubocurarine is not a major factor contributing to the greater rundown.

In view of the observation that tubocurarine appears to have a post-synaptic channel blocking action which is voltage dependent (Katz & Miledi, 1978; Colquhoun, Dreyer & Sheridan, 1978, 1979) it is of some interest why channel blocking by tubocurarine does not significantly contribute to the greater rundown observed in the presence of tubocurarine. Since the rate constants for channel blockade by tubocurarine are known for the frog, it is possible to estimate the contribution of channel blockade to the observed rundown. Taking the rate constant for association of tubocurarine with open channels at the membrane potential of -120 mV to be about 0·012/msec in 1 μM-tubocurarine (calculated from Fig. 15 in Colquhoun *et al.* 1979) and assuming 200,000 post-synaptic channels to be open for 1 msec during an end-plate current, approximately 2400 post-synaptic channels would be blocked by tubocurarine during the first end-plate current; thus, assuming no recovery during the train, less than 1 % of the 10 million post-synaptic channels (see Colquhoun, Dreyer & Sheridan, 1979, p. 273) would be blocked by tubocurarine during trains of 15–30 impulses. The rate constant for association of tubocurarine with open channels in the rat and mouse is not known but if it is similar to that observed in the frog then channel blockade would not be expected to be a major factor contributing to rundown in the rat and mouse under the conditions of our experiments.

It seems unlikely that the greater rundown of end-plate currents in the presence of tubocurarine is due to desensitization since desensitization has been shown to be voltage dependent (Magazanik & Vyskocil, 1970; Fiekers *et al.* 1980).

In view of the above considerations, it appears that the greater rundown of end-plate current amplitudes in the rat and mouse during repetitive stimulation and the apparent facilitation and subsequent rundown of end-plate current amplitudes in the frog are not a consequence of the known post-synaptic receptor and channel blocking effects of tubocurarine. Thus, excluding any as yet unknown post-synaptic effects, it appears that tubocurarine also has a presynaptic action on transmitter release as has been suggested previously (Lilleheil & Naess, 1961; Hubbard *et al.* 1969; Blaber, 1970, 1973; Hubbard & Wilson, 1972; Glavinović, 1979).

In the absense of tubocurarine a greater rundown of end-plate current amplitudes during repetitive stimulation typically occurs when the average rate of transmitter release is increased either by increasing $[Ca^{2+}]_o$ or by stimulating faster; this is shown for increasing total transmitter release by stimulating faster in Fig. 5 (filled symbols) and for increasing quantal content by increasing Ca^{2+} in Fig. 8 of Hubbard, Jones & Landau (1971). In those experiments where tubocurarine increases apparent

facilitation, the observed greater rundown might then arise as a consequence of the increased transmitter release at the start of the trains. This explanation, however, cannot account for the greater rundown observed in the presence of tubocurarine in experiments in which there was no initial facilitation. If tubocurarine increased transmitter release by increasing quantal content in these experiments, then this could also lead to a greater rundown.

An increase in quantal content induced by tubocurarine at high levels of release has been reported by Blaber (1970, 1973) but not by Hubbard, Wilson & Miyamoto (1969) and Hubbard & Wilson (1972). Auerbach & Betz (1971) found a small increase in quantal content determined from the ratio of end-plate currents to minature end-plate currents but rejected this finding since they felt it arose from voltage clamp error and estimates of quantal content determined from the coefficient of variation were decreased in curare. Thus, whether the greater rundown of end-plate current amplitudes observed in those experiments in which there was not an initial facilitation is associated with an increase in quantal content in the presence of tubocurarine must await further investigation.

A number of investigators have reported an increased fractional release of transmitter by each impulse in the presence of tubocurarine (Hubbard *et al.* 1969; Blaber, 1970, 1973; Hubbard & Wilson, 1972; Glavinović, 1979) or a decreased 'mobilization' of transmitter (Hubbard *et al.* 1969; Maeno & Nobe, 1970; Hubbard & Wilson, 1972; Blaber, 1973). An increased fractional release or decreased mobilization of transmitter could lead to a greater rundown of end-plate current amplitudes, as was typically observed in the rat and mouse, but neither of these processes would be expected to also facilitate transmitter release before the greater rundown, as was typically observed in the frog.

Although the presynaptic effects of tubocurarine studied in this paper are less pronounced than the well established postsynaptic blocking action of tubocurarine, under the appropriate conditions (low concentrations of tubocurarine and repetitive stimulation) the presynaptic effects of tubocurarine could have an important influence on neuromuscular transmission.

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