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PRESYNAPTIC EFFECTS OF *d*-TUBOCURARINE ON NEUROTRANSMITTER RELEASE AT THE NEUROMUSCULAR JUNCTION OF THE FROG

BY H. MATZNER, H. PARNAS AND I. PARNAS

From The Otto Loewi Center for Cellular and Molecular Neurobiology, The Hebrew University, Jerusalem, Israel

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SUMMARY

1. Presynaptic effects of *d*-tubocurarine on neurotransmitter release were examined at the frog neuromuscular junction, using intracellular and extracellular recording techniques.

2. *d*-Tubocurarine in concentrations of 10^{-7}–10^{-6} M decreased the quantal content (m) measured by the coefficient of variation and failure methods.

3. *d*-Tubocurarine produced a shift to the right of the curve relating log quantal content to log $[Ca^{2+}]_o$ without changing the slope.

4. The duration of twin-impulse facilitation was not affected by 5×10^{-7} M-*d*-tubocurarine. Early facilitation was higher in *d*-tubocurarine.

5. *d*-Tubocurarine altered the synaptic delay histogram. The peak of the histogram was shifted to longer delays. Prolongation of the minimal delay was seen in most but not all experiments.

6. These results suggest that *d*-tubocurarine inhibits release of neurotransmitter by affecting a stage in the process of release, which occurs after the entry of Ca^{2+} ions.

INTRODUCTION

The postsynaptic effects of *d*-tubocurarine at the neuromuscular junction are well characterized. It competes with acetylcholine on the postsynaptic receptors and thereby reduces the end-plate potential (EPP) (Eccles, Katz & Kuffler, 1941; Fatt & Katz, 1951; Jenkinson, 1960; Adams, 1975). In addition, *d*-tubocurarine can block open postsynaptic channels (Manalis, 1977; Katz & Miledi, 1978; Colquhoun, Dreyer & Sheridan, 1979).

Presynaptic effects of *d*-tubocurarine are less clear. In some cases, the reduction in EPP amplitude could be accounted for by postsynaptic mechanisms (Beranek & Vyskocil, 1967; Chang, Cheng & Chen, 1967; Auerbach & Betz, 1971). In others, a presynaptic effect of *d*-tubocurarine was suggested. Reduction in quantal content upon application of *d*-tubocurarine and stimulation at high frequency has been reported by several investigators (Hubbard, Wilson & Miyamoto, 1969; Galindo, 1971; Hubbard & Wilson, 1973). However, Wilson (1982) showed an increase in the secretion of acetylcholine upon application of *d*-tubocurarine in the neuromuscular

junction of the rat. The faster decline of post-tetanic potentiation in curarized preparations was also taken to indicate a presynaptic effect of *d*-tubocurarine (Galvinovic, 1979; Magleby, Pallotta & Terrar, 1981). Finally, Galindo (1971) demonstrated a reduction in spontaneous release after application of *d*-tubocurarine.

In the present work, we re-examined the presynaptic effects of *d*-tubocurarine by measuring the quantal contents elicited by single and twin impulses. In addition, we studied synaptic delay histograms (Katz & Miledi, 1965*a*, *b*), in order to elucidate possible mechanisms involved in the presynaptic effect of *d*-tubocurarine. We found that *d*-tubocurarine reduced the quantal content and shifted the delay histogram to the right. We conclude that *d*-tubocurarine acts to reduce release at a stage which occurs after the stimulus-induced entry of Ca^{2+} ions.

METHODS

Experiments were made on sartorius and cutaneous pectoris muscles of the Israeli frog, *Rana ridibunda*.

Muscles were kept in small lucite chambers (5 ml) in which Ringer solution was continuously flowing. Rapid flow of the solution (10 ml/min) was obtained by a circulation pump. Temperature was controlled by a Peltier feed-back device and kept constant to an accuracy of ± 1 °C. Normal Ringer solution was composed of: 115 mM-NaCl, 2 mM-KCl, 2 mM-$MgCl_2$, 1·8 mM-$CaCl_2$ and 1 mM-$NaHCO_3$. Muscle movements were abolished by low Ca^{2+} (0·4 mM) or high Mg^{2+} (10 mM). Changes in Ca^{2+} or Mg^{2+} concentrations were compensated for by appropriate change in Na^+ concentration to keep osmolarity constant. pH was adjusted to 7·2. The nerve was stimulated at a frequency of 0·5 Hz with a suction electrode. Conventional intracellular recording techniques were used. The microelectrodes were filled with 3 M-KCl and had a resistance of 10–20 MΩ. Extracellular recording from restricted release regions was done using the 'macropatch' technique (Dudel, 1981). The same 'macropatch' electrode was used to pass current and directly depolarize nerve terminals in TTX-treated preparations (Dudel, 1981). The macropatch electrodes (20 μm inner diameter, 40 μm outer diameter) had resistances of about 0·5 MΩ when filled with Ringer solution. Recordings were taken in parallel on an FM tape-recorder (Hewlett–Packard 3964A) and later analysed on a Nicolet digital oscilloscope (20 μs per address).

The quantal content was calculated either by the coefficient of variation method (Martin, 1965) or by the failure method (Del Castillo & Katz, 1954). Since amplitudes of EPPs were not higher than 10 mV, no correction was made for non-linear summation (McLachlan & Martin, 1981). With the 'macropatch' recording, single quanta could be clearly discerned. Even at the higher Ca^{2+} concentration, direct counting of the number of failures was possible.

Facilitation was determined for twin impulses at different intervals. Facilitation was taken as the ratio between the averaged amplitude of the second response and that of the first response. At each interval 128–512 sweeps were averaged on a Nicolet 1174 signal averager. The duration of facilitation is defined as the interval between the two pulses at which facilitation was 10%.

Synaptic delay was measured, from the digitized data, as the time from the negative peak of the nerve terminal potential to the beginning of the single-quantum event (Katz & Miledi, 1965*a*). In cases where the terminal was directly depolarized, the delay was measured from the beginning of the depolarizing pulse. Two millisecond pulses were used and the single-quantum responses were well out of the range of the artifact.

RESULTS

Effect of d-tubocurarine on the quantal content, m

Postsynaptic responses (voltage and current) in controls and in the presence of *d*-tubocurarine are shown in Figs 1 and 2. Figure 1 demonstrates that at 5×10^{-7} M-*d*-tubocurarine, the postsynaptic potential is clearly reduced (note differ-

ences in calibration amplitude between controls and treated preparations) but easily identified and can be used in order to determine the effect of *d*-tubocurarine on the quantal content and facilitation. Figure 2 shows examples of synaptic currents before and after application of *d*-tubocurarine. The synaptic current is reduced in *d*-tubocurarine but the detection of failures is possible. At 10^{-7} M, *d*-tubocurarine had no or only a weak presynaptic effect. At 10^{-6} M, the postsynaptic effect was dominant. We therefore used in most of the experiments 5×10^{-7} M-*d*-tubocurarine.

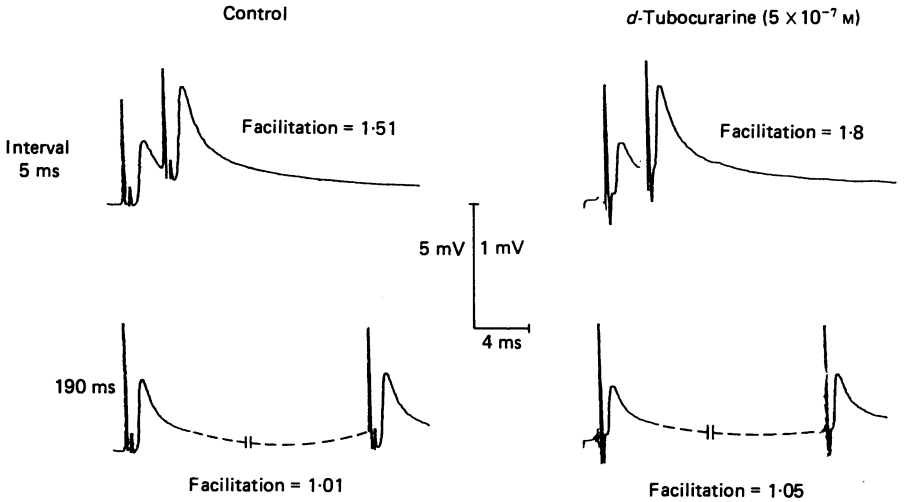

Fig. 1. Average synaptic potentials in controls (left) and after application of 5×10^{-7} M-*d*-tubocurarine (right). Note differences in amplitude of calibration bar (5 mV for left panel and 1 mV for right panel). The responses to twin impulses are given. Top, interval between impulses was 5 ms; facilitation was 1·51 in the control and 1·8 with *d*-tubocurarine. Bottom, the interval between impulses was 190 ms. No facilitation. The control response is an average of 64 sweeps, after *d*-tubocurarine, average of 128 sweeps.

Table 1 shows results of ten experiments in which the quantal content was determined by the coefficient of variation method (Martin, 1965). In each of the experiments the quantal content was reduced after application of *d*-tubocurarine. In six experiments, where prolonged wash with normal Ringer solution was attempted, recovery was complete. In experiments done in two $[Ca^{2+}]_o$ concentrations (0·4 and 0·45 mM), the average quantal content was $16{\cdot}3 \pm 1{\cdot}1$ (average $\pm$ S.E.M.) and $10{\cdot}8 \pm 1$ in controls and *d*-tubocurarine-treated preparations, respectively. The difference is highly significant (*t* test, $P = 0{\cdot}005$).

In a different set of experiments synaptic currents were recorded with a 'macropatch' electrode (Dudel, 1981) and the quantal content was determined by the failure method. The 'macropatch' electrode records synaptic currents from only a small portion of the junction reflecting activity of relatively few release sites. This may explain much of the variability in the quantal content seen in the controls (Table 2) (Trussell & Grinnell, 1985). Despite this variability the results were unambiguous. In twenty-two preparations in which $[Ca^{2+}]_o$ concentration varied between 0·15 and 0·4 mM, the quantal content in controls varied between 0·2 and 1·5,

with an average of $0{\cdot}52 \pm 0{\cdot}07$. After *d*-tubocurarine, release was reduced in every experiment and the average quantal content was $0{\cdot}3 \pm 0{\cdot}03$. In eight experiments the preparations were washed for a long period (> 45 min) and recovery was seen in all cases. The difference between the quantal content in controls and *d*-tubocurarine-treated preparations is highly significant (*t* test, $P = 0{\cdot}005$).

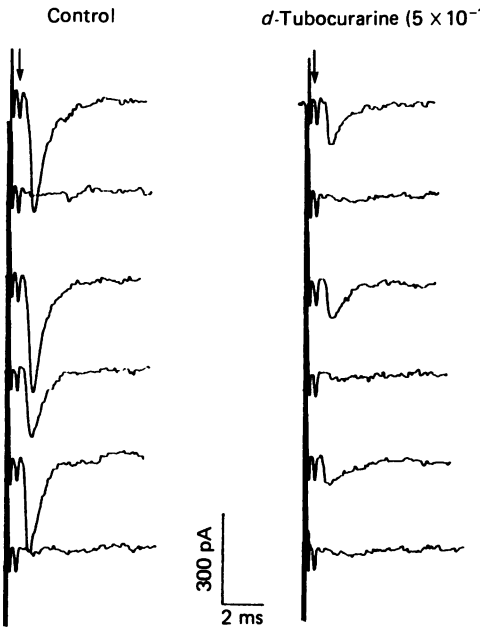

Fig. 2. Samples of synaptic currents in controls and after application of *d*-tubocurarine (5×10^{-7} M). Arrows mark nerve terminal current. The responses are smaller in *d*-tubocurarine, but clearly identifiable. Note that in some sweeps there are failures.

Effect of d-tubocurarine on the dependence of transmitter release on $[Ca^{2+}]_o$

Figure 3 shows the EPSP amplitude (*A*) and the quantal content (*B*) plotted as a function of $[Ca^{2+}]_o$, in controls and in *d*-tubocurarine. In both cases *d*-tubocurarine shifted to the right the line relating log EPSP or log *m* to log $[Ca^{2+}]_o$. In Fig. 3*A* the slope was 3·25 for the control and 3·2 in the presence of *d*-tubocurarine. In Fig. 3*B* the slopes were 3·7 and 3·6 in the control and *d*-tubocurarine respectively. Similar results were obtained in three more experiments where the EPSP amplitude was measured and in three other experiments where quantal content was determined. The average slope in the controls of these eight experiments was $3{\cdot}2 \pm 0{\cdot}15$ and $3{\cdot}3 \pm 0{\cdot}24$ in *d*-tubocurarine.

Effect of d-tubocurarine on facilitation

Figure 4 shows facilitation curves in controls and after application of 5×10^{-7} M-*d*-tubocurarine. In the experiment of Fig. 4*A* the bathing solution contained 0·4 mM $[Ca^{2+}]_o$ and 2 mM $[Mg^{2+}]_o$. In Fig. 4*B* the concentrations of $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ were 2 and 10 mM respectively. In both cases early facilitation increased but the duration of facilitation was similar in controls and in *d*-

TABLE 1. Effect of *d*-tubocurarine on the quantal content, m, using intracellular recordings

$[Ca^{2+}]_o$ (mM)	Control (m_0)	+*d*-Tubocurarine (m_c)	$\frac{m_c}{m_0}$	Wash (m_w)
0·4	14	10	0·71	14
0·4	16	6	0·38	16
0·4	14	9	0·64	15
0·45	21	9	0·43	23
0·45	21	16	0·76	19
0·45	13	8	0·61	11
0·45	15	12	0·80	—
0·45	18	14	0·78	—
0·45	11	9	0·81	—
0·45	20	15	0·75	—
Mean ±S.E.M.	16·3±1·1	10·8±1	0·67±0·14	16·3±1·7

m is calculated from: $m = E^2/\text{var } E$, where E is the average EPP amplitude, and var E is the variance in the EPP amplitudes. 512 pulses were given with and without *d*-tubocurarine. $[Mg^{2+}]_o = 2$ mM. 5×10^{-7} M-*d*-tubocurarine was added in all but the first experiment, in which its concentration was 10^{-7} M. m_0 is the quantal content of the control and m_c that of the curarized preparation. m_w is the quantal content after washing. Values of the quantal contents were rounded to the nearest integer.

TABLE 2. Effect of *d*-tubocurarine on m using the 'macropatch' technique

$[Ca^{2+}]_o$ (mM)	Control (m_0)	+*d*-Tubocurarine (m_c)	$\frac{m_c}{m_0}$	Wash (> 45 ms) (m_w)
0·15	0·4	0·2	0·50	0·4
0·3	0·3	0·2	0·66	0·3
0·3	0·6	0·4	0·66	0·5
0·3	0·2	0·1	0·50	0·2
0·3	0·2	0·1	0·50	—
0·3	0·2	0·1	0·50	—
0·3	0·3	0·2	0·66	—
0·3	0·9	0·5	0·55	—
0·3	0·6	0·4	0·66	—
0·3	0·5	0·3	0·60	—
0·35	0·9	0·5	0·55	1·0
0·35	0·2	0·1	0·50	0·2
0·4	1·5	1·0	0·66	1·4
0·4	0·2	0·1	0·50	0·2
0·4	0·5	0·4	0·80	—
0·4	0·4	0·3	0·75	—
0·4	0·7	0·5	0·71	—
0·4	0·5	0·4	0·80	—
0·4	1·0	0·5	0·50	—
0·4	0·2	0·1	0·50	—
0·4	0·5	0·3	0·60	—
0·4	0·5	0·3	0·60	—
Mean ±S.E.M.	0·52±0·07	0·30±0·03	0·60±0·02	0·52±0·02

m is calculated from $m = \ln N/N_0$, where N is the number of pulses and N_0 the number of failures. N in all cases was 512. m was rounded to the first decimal point. For 0·15 and 0·3 mM $[Ca^{2+}]_o$, 1 mM-$MgCl_2$ was used. For the experiment with 0·35 and 0·4 mM $[Ca^{2+}]_o$ 2 mM-$MgCl_2$ was used.

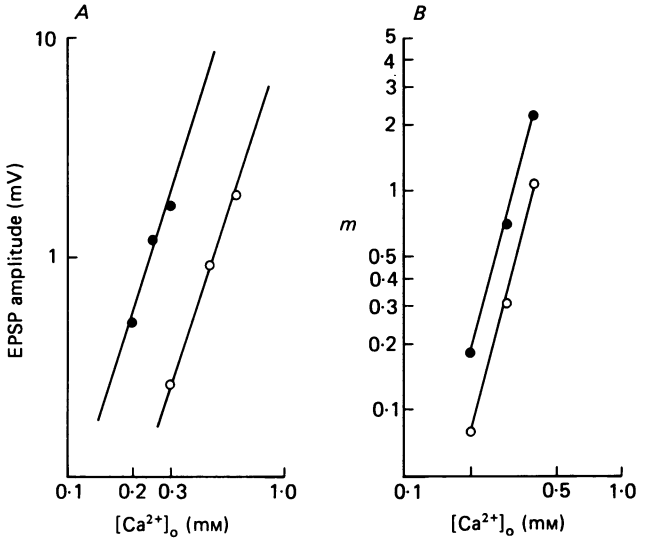

Fig. 3. EPSP amplitude (*A*) and quantal content (*B*) as a function of extracellular Ca^{2+} concentration in controls (●) and after *d*-tubocurarine (5×10^{-7} M) (○); log–log plots. In *A* the slope is 3·25 in control and 3·2 in *d*-tubocurarine. In *B*, the slope is 3·7 in control and 3·6 in *d*-tubocurarine.

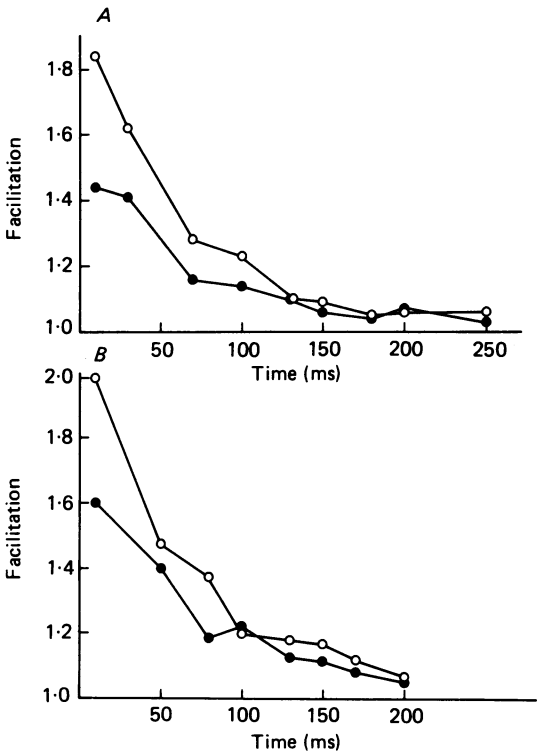

Fig. 4. Facilitation curve obtained for twin-impulse facilitation in controls (●) and in *d*-tubocurarine (○). The duration of facilitation is the same. *A*, 0·4 mM $[Ca^{2+}]_o$, 2 mM $[Mg^{2+}]_o$. *B*, 2 mM $[Ca^{2+}]_o$, 10 mM $[Mg^{2+}]_o$.

tubocurarine. In six experiments where the bathing solution contained 0·4 mM $[Ca^{2+}]_o$ and 2 mM $[Mg^{2+}]_o$ early facilitation (10 ms) was $1·4 \pm 0·09$ in controls and $1·8 \pm 0·07$ in *d*-tubocurarine. Duration of facilitation, T_F, was 130 ± 7 ms in controls and 135 ± 9 ms in *d*-tubocurarine. Three experiments were done in 2 mM $[Ca^{2+}]_o$ and 10 mM $[Mg^{2+}]_o$. Here, early facilitation was $1·6 \pm 0·09$ in controls and $2·0 \pm 0·14$ in *d*-tubocurarine. T_F was 150 ± 9 ms in controls and 160 ± 7 ms in *d*-tubocurarine. For each of these experiments the complete facilitation curve in the control solution and in *d*-tubocurarine were established at the same synapse.

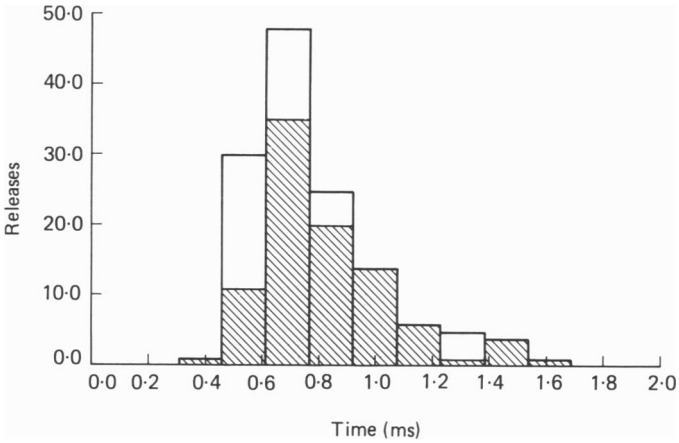

Fig. 5. Synaptic delay histogram in Ringer solution with 1 mM (open bars) and 10 mM (shaded bars) $[Mg^{2+}]_o$. The delay was measured from the negative peak of the nerve terminal potential to the beginning of the quantum event. Release was reduced in high Mg^{2+} but there was no change in the beginning or peak of the histogram. $[Ca^{2+}]_o = 0·4$ mM. Quantal content in low Mg^{2+} was 0·23 and in high Mg^{2+}, 0·16. Temperature 22 °C.

Effects of d-tubocurarine on the kinetics of release

Another tool that can be used to sort out mechanisms of a drug acting presynaptically, is the synaptic delay histogram which reflects the kinetics of release (Katz & Miledi, 1965*a*). As a control, we checked for the effects of Mg^{2+}, which is known to compete with Ca^{2+} entry, on the kinetics of release. Figure 5 shows synaptic delay histograms obtained at a low (1 mM) and a higher (10 mM) $[Mg^{2+}]_o$. It is obvious that fewer quanta were released at the higher Mg^{2+} concentration since the whole area of the histogram is smaller. However, the kinetics of release were the same at the two ionic conditions.

The effect of *d*-tubocurarine on the synaptic delay histogram was different from that of Mg^{2+}. When the same number of pulses was given in controls and in *d*-tubocurarine-treated preparations (Parnas & Parnas, 1987), the delay histogram in *d*-tubocurarine showed a shift of the peak to the right, with a possible shift of the start of the histogram (Fig. 6*A*). The area of the histogram in *d*-tubocurarine is smaller due to the smaller quantal content. The finding that fewer quanta appear at the early part of the histogram indicates a lower probability of release during this period (Katz & Miledi, 1965*a*; Parnas & Parnas, 1987). Even when a larger number of pulses was given to the *d*-tubocurarine-treated preparation such that the number

of quanta released was the same as in the control, there was still a shift to the right, both in the minimal latency and in the peak of the histogram (Fig. 6*B*). Such delay histograms were established in fourteen experiments (22 °C). 512 (eleven experiments) or 1024 impulses were given and the number of quanta released in *d*-tubocurarine was over 100. Of the fourteen experiments the minimal delay shifted

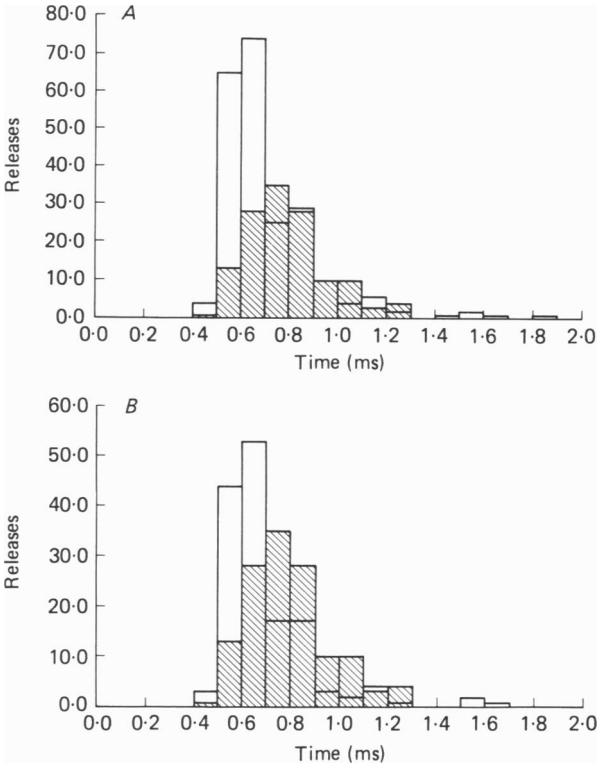

Fig. 6. Delay histogram in controls (open bars) and in 5×10^{-7} M-*d*-tubocurarine (shaded bars). *A*, the same number of pulses (512) applied in control and in *d*-tubocurarine. Quantal contents of control and *d*-tubocurarine were 0·52 and 0·29, respectively. *B*, the same number of releases (132) collected in both control and *d*-tubocurarine. Temperature 22 °C.

to the right in eight. In the remaining six experiments the delay did not change. The average minimal delay in controls was $0{\cdot}31 \pm 0{\cdot}02$ and $0{\cdot}38 \pm 0{\cdot}03$ ms in *d*-tubocurarine (*t* test, $P = 0{\cdot}025$). Of the fourteen experiments, the peak shifted to the right in eleven and there was no change in three experiments (one experiment showed no change in minimal delay and peak). The average time to peak was $0{\cdot}62 \pm 0{\cdot}03$ ms in controls and $0{\cdot}73 \pm 0{\cdot}03$ ms in *d*-tubocurarine (*t* test, $0{\cdot}01 < P < 0{\cdot}02$). Two points should be emphasized regarding these experiments. In all fourteen experiments the quantal content was reduced (including those that showed no change in minimal delay or peak) and in no case was there a shift to the left of the minimal delay or peak in the presence of *d*-tubocurarine. We therefore conclude that *d*-tubocurarine prolongs the minimal delay for release and shifts the peak of the delay histogram to the right.

In order to test the possibility that curare might have been acting by reducing the amplitude of the action potential, synaptic delay histograms were measured also for direct nerve terminal depolarization, using the 'macropatch' electrode in preparations treated with TTX. Here too (Fig. 7), the delay histogram was shifted to the right.

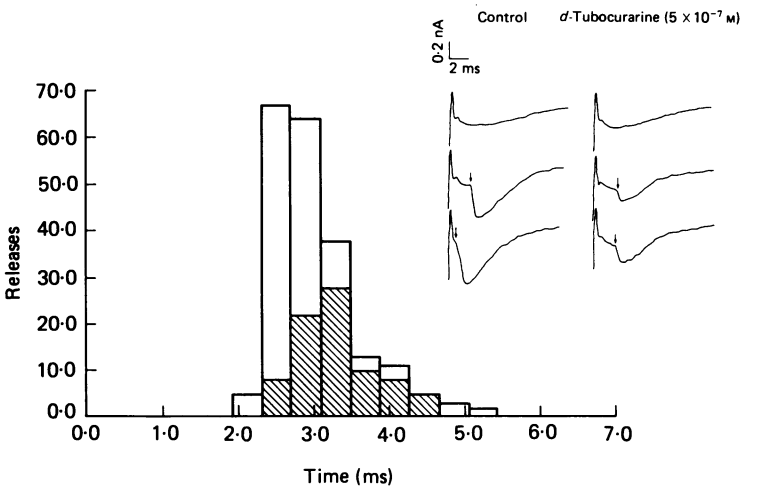

Fig. 7. Synaptic delay histogram in controls (open bars) and in 5×10^{-7} M-*d*-tubocurarine (shaded bars). The terminal was depolarized directly with the macropatch electrode. The delay was measured from the beginning of the depolarizing pulse (2 ms, 1 μA). Note the shift of the peak of the histogram to the right in the *d*-tubocurarine, and that the area of the histogram is smaller. Inset, samples of quantal events, in controls and in *d*-tubocurarine. Upper traces, failures. Arrows mark the beginning of a quantum event. 2 mM-$CaCl_2$, 1 mM-$MgCl_2$. 512 pulses. The quantal content was 0·41 in the control and 0·16 in *d*-tubocurarine. Temperature 10 °C.

DISCUSSION

The presynaptic effects of *d*-tubocurarine, which we found, are summarized as follows. *d*-Tubocurarine: (1) reduced the quantal content, (2) shifted the curve relating log release to log $[Ca^{2+}]_o$ to the right without changing the slope, (3) had no effect on the duration of facilitation, (4) increased early facilitation, (5) affected the synaptic delay histogram of both action potential and direct depolarization, shifting the peak of the histograms to longer delays and prolonging the minimal delay.

With respect to the first point, the reduction of the quantal content in *d*-tubocurarine shows directly that curare acts presynaptically to reduce release. The quantal content does not depend on postsynaptic mechanisms, and the use of the macropatch recording technique enables determination of the number of quanta in controls and in the presence of *d*-tubocurarine (Table 2).

Block of transmitter release may be brought about by a reduced entry of Ca^{2+} ions during the action potential. Such is the case with Mg^{2+} ions (Jenkinson, 1957; Dodge & Rahamimoff, 1967). Indeed, Dodge & Rahamimoff (1967) showed that Mg^{2+} shifts the curve relating log release to log $[Ca^{2+}]_o$ to the right without a change in slope. They concluded that Mg^{2+} competes with Ca^{2+} in the process of entry. This

conclusion is based on an equation relating release to extracellular Ca^{2+} concentration. Curare had a similar effect (Fig. 3). The question is whether such a finding is sufficient to indicate that curare competes with Ca^{2+} entry. Parnas & Segel (1982) pointed out that the same result may be obtained by other mechanisms, if release is related to intracellular Ca^{2+} concentration. In particular it can be brought about by inhibition of the release process at a step following the entry of Ca^{2+} ions.

Since Ca^{2+} currents cannot be measured easily at the neuromuscular junction of the frog, we have used the duration of facilitation as an indicator for Ca^{2+} entry. The duration of facilitation, which depends on residual calcium (Katz & Miledi, 1968), reflects the amount of Ca^{2+} entering during the first impulse (Parnas & Segel, 1982; Parnas, Parnas & Dudel, 1982). The duration of facilitation was not altered by *d*-tubocurarine (Fig. 4), indicating that the same amount of Ca^{2+} entered during the first impulse in controls and in *d*-tubocurarine. Therefore, the possibility that *d*-tubocurarine blocks Ca^{2+} entry is less likely. It may be argued that the duration of facilitation is not a sensitive enough parameter to detect changes in Ca^{2+} entry, but in another case where release was blocked by reduced Ca^{2+} entry, the duration of facilitation was clearly reduced (Miller, Parnas & Parnas, 1985). This conclusion is further supported by recent measurements of Ca^{2+} action potentials in *Aplysia* neurones, where curare did not have an effect on their amplitude or duration but reduced the quantal content (Baux & Tauc, 1987).

The synaptic delay histogram, which reflects the kinetics of release (Katz & Miledi, 1965*a*), is insensitive to changes in extracellular Ca^{2+} concentration (Datyner & Gage, 1980) to repetitive stimulation (Barrett & Stevens, 1972; Datyner & Gage, 1980; Parnas, Dudel & Parnas, 1986*a*) or to Ca^{2+} ionophore (H. Parnas, I. Parnas, H. L. Atwood & J. M. Wojtowicz, unpublished observations). Indeed, increasing $[Mg^{2+}]_o$ concentration reduced the quantal content without affecting the beginning, place of peak or end of the synaptic delay histogram (Fig. 5). It is interesting to note that in the neuromuscular junction of the prawn *Macrobrachium*, dopamine reduced the quantal content, but it did shorten the duration of facilitation and it had no effect on the synaptic delay histogram (minimal delay, peak and end). It was concluded that dopamine reduced release by blocking calcium entry (Miller *et al.* 1985). In contrast *d*-tubocurarine shifted the synaptic delay histograms to longer delays. This can be seen in Fig. 8 where the results of eleven experiments (same experimental conditions: 512 pulses, 22 °C, 5×10^{-7} M-*d*-tubocurarine) were pooled. In each experiment the number of releases per bin was first expressed as a percentage of the total number of quanta for that given experiment. The averages of percentage release per bin were then calculated and are presented in Fig. 8*A*. As the data were normalized, the area of the histogram in controls and in *d*-tubocurarine is the same, even though the average quantal content in the controls was 0·37 and in *d*-tubocurarine was 0·22.

Parnas, Parnas & Segel (1986*b*) suggested a new method to obtain the value of the co-operativity of the release process from synaptic delay histograms. The slope of the line relating log rate of release to log time, for the initial part of the synaptic delay histogram, is the co-operativity. Figure 8*B* shows, in a different way than Fig. 3, that *d*-tubocurarine did not affect the co-operativity. The slope was 3·9 both in the control and in the *d*-tubocurarine. Figure 8 shows that the initial decay of the histogram is the same in controls and in *d*-tubocurarine.

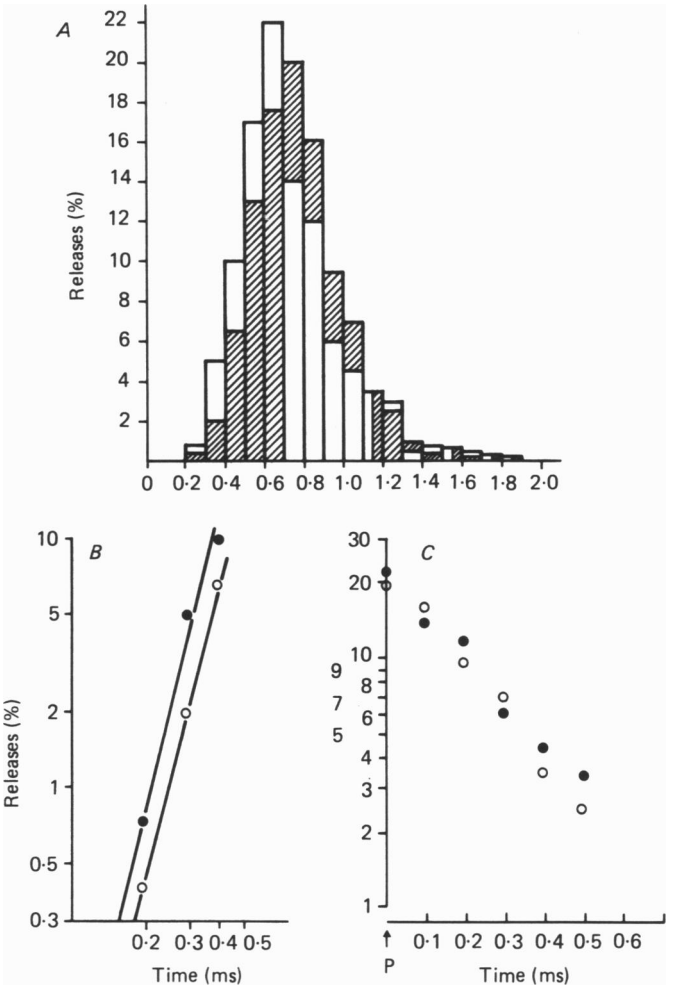

Fig. 8. *A*, pooled data of synaptic delay histograms of eleven experiments. In each experiment 512 pulses were given, and 5×10^{-7} M-*d*-tubocurarine was used. Temperature 22 °C. For every experiment the number of releases per bin was normalized as a percentage of the total number of quanta for that given experiment. The bars indicate the average of percentages of release for each bin for the eleven experiments. Open bars, control; shaded bars, in *d*-tubocurarine. The average quantal content was 0·37 in the controls and 0·22 in *d*-tubocurarine. *B*, slope of log percentage release as a function of log time. The slope of both lines is 3·9. *C*, semilogarithmic plot of the initial decay of the histogram as measured from the peak (P in abscissa) of each histogram. In *B* and *C*: filled circles, control; open circles, *d*-tubocurarine.

Since the delay histogram is not sensitive to changes in intracellular or extracellular $[Ca^{2+}]_o$, we conclude that curare affects the release process by a mechanism different from that of Mg^{2+}, possibly at a step following the entry of Ca^{2+}. The chain of events leading to release after the entry of Ca^{2+} is not known. It is reasonable to conclude that curare interferes in one or more of the slower steps, thus slowing the process of release and reducing the quantal content. Such an effect can also explain the increase in early facilitation, which can now be postulated to result not only from 'residual

Ca^{2+}' but also because of a 'residual factor' which is involved in the slowest step in the release process.

A more-detailed interpretation of the mechanisms of action of *d*-tubocurarine on release of neurotransmitter would be model dependent and is not justified at this time. The effects of *d*-tubocurarine on the synaptic delay histogram clearly put constraints on such possible models.

Our finding that curare blocked transmitter release might have some bearing on the mechanisms reported to be involved in feed-back inhibition by transmitter substances (Wilson, 1982; Michaelson, Avissar, Kloog & Sokolovsky, 1979; Kloog, Galron & Sokolovsky, 1986). In synaptosome preparations, it was shown that postsynaptic agonists blocked release without affecting Ca^{2+} entry, and it was concluded that these compounds block release at a stage which occurs after the entry of Ca^{2+} (Michaelson *et al.* 1979; Dunant & Walker, 1982). If curare acts in the same way, then a postsynaptic antagonist can also act as a presynaptic agonist at the neuromuscular junction.

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