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IONIC MECHANISM OF POST-TETANIC POTENTIATION AT THE NEUROMUSCULAR JUNCTION OF THE FROG

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SUMMARY

1. Transmitter release at the frog neuromuscular junction was studied after sodium influx in nerve and muscle was abolished by tetrodotoxin (TTX).

2. In the presence of TTX, transmitter release evoked by electrotonic depolarization of the nerve terminal was potentiated following presynaptic stimulation by a train of depolarizing pulses.

3. Post-tetanic potentiation (PTP) in the presence of TTX appeared no different from that observed in control (TTX-free) muscles. The magnitude as well as the time course of PTP was dependent on the number of tetanic stimuli and on temperature of the medium when sodium influx was inhibited by TTX.

4. When external sodium was replaced by an isotonic calcium chloride solution PTP was still present. Ionophoretic application of calcium during tetanic nerve stimulation and increase in the intensity of the depolarizing pulse of the train, which presumably enhances calcium movements into nerve endings, caused a large increase in the duration of PTP.

5. It is concluded that PTP does not require sodium but depends on movement of calcium from the external medium into the nerve terminal.

INTRODUCTION

The end-plate potential (e.p.p.) may be increased in amplitude for several seconds to several minutes following repetitive stimulation of the motor nerve (Feng, 1941; Liley & North, 1953; Liley, 1956; Braun, Schmidt & Zimmermann, 1966; Gage & Hubbard, 1966; Rosenthal, 1969). This post-tetanic potentiation (PTP) is associated with an increase in the

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amount of transmitter release (Hutter, 1952; Liley, 1956). It has been proposed that PTP is related to an increase in presynaptic spike amplitude that follows repetitive nerve stimulation (Lloyd, 1949; Hubbard, 1963; Hubbard & Schmidt, 1963). However, the occurrence of PTP is not contingent on enhancement of terminal spike amplitude (Martin & Pilar, 1964; Braun & Schmidt, 1966). Birks (1963) and Birks & Cohen (1965, 1968) have suggested that accumulation of intracellular sodium in nerve terminals during a train of nerve impulses might be the mechanism involved in PTP. They further postulated that the action of sodium in increasing transmitter release is related to calcium influx; the increase of sodium within nerve terminals would displace calcium from a carrier site at the inner membrane surface, which would in turn increase calcium influx and thereby enhance transmitter release. Several recent findings appear to support this hypothesis: (1) extracellular sodium can compete with calcium at the outer surface of the terminal membrane to reduce transmitter release (Birks & Cohen, 1965; Gage & Quastel, 1966; Colomo & Rahamimoff, 1968; Kelly, 1968), (2) the rate of calcium influx into squid axoplasm is accelerated when intraaxonal concentration of sodium is increased (Baker, Blaustein, Hodgkin & Steinhardt, 1967) and (3) calcium-dependent release of catecholamines from the adrenal medulla is potentiated when intracellular sodium concentration is raised (Banks, Biggins, Bishop, Christian & Currie, 1968).

On the other hand, Gage & Hubbard (1966) reported that, in the presence of ouabain, PTP was depressed. Since a progressive intracellular accumulation of sodium is one effect associated with this agent, this result seems to be incompatible with the involvement of sodium in PTP.

From the above discussion the following questions may be raised. First, is accumulation of sodium in nerve endings an essential requirement for the generation of PTP? Secondly, is sodium action mediated through its effect on calcium fluxes, or is calcium itself the only determinant of PTP? The present investigation was undertaken to answer these questions. A preliminary report has been published elsewhere (Weinreich, 1970).

METHODS

The extensor digitorum longus IV or the sartorius muscle of the frog (*Rana pipiens*) was used in all experiments. Normal Ringer solution contained (mM): NaCl 115·6; KCl 2·0; $CaCl_2$ 1·8. In some experiments neuromuscular transmission was partially depressed by addition of magnesium to minimize muscle movements. For this series of observations, the composition of the normal Ringer solution was modified as follows (mM): NaCl 102·0; KCl 2·0; $CaCl_2$ 0·9; $MgCl_2$ 6·0. Further addition of magnesium was occasionally necessary to reduce muscle contraction, particularly in summer frogs. Sodium chloride concentration was reduced in these modified solutions to maintain tonicity at approximately 240 m-osmole. Neostigmine methylsulphate,

10^{-6} g/ml., was added in most experiments to increase the amplitude of the spontaneous miniature potentials (Fatt & Katz, 1951). In the experiments in which all external sodium was replaced by calcium, the bathing medium had the following composition (mM): $CaCl_2$ 83·0; KCl 2·0; neostigmine methylsulphate 10^{-6} g/ml. The recording chamber was constructed of Plexiglass and surrounded by a jacket through which alcohol was circulated from a thermostatically controlled reservoir. Temperature of the bathing solution in the chamber was normally kept between 20 and 24° C but could be lowered to 0° C.

The method of electrotonic depolarization of nerve terminals was similar to that described by Katz & Miledi (1967*a*). Action potentials were abolished by addition of tetrodotoxin (TTX, $1–10 \times 10^{-6}$ g/ml.; see Kao, 1966) to the medium or by substituting the medium with sodium-free Ringer solution. One of the intramuscular branches of the nerve was dissected from the underlying connective tissue up to within a millimetre from its entry into the muscle fibre. The preparation was covered by mineral oil and the dissected nerve was placed on a pair of chloridized silver electrodes. The cathode of the stimulating electrodes was positioned near the motor nerve terminals close to the muscle/oil interface; the distal anode supported the remainder of the nerve twig. In most experiments, low intensity current pulses (1–20 μA and 1·0 msec in duration) were capable of evoking end-plate responses of 15–20 mV in amplitude. Trains of strong electrical pulses applied to the nerve terminal often produced local muscle contraction which resulted in displacement of the recording micro-electrode. To avoid contractions, some muscles were bathed for 1 hr in a Ringer solution containing glycerol (400 mM). Subsequently, when this solution was replaced by a Ringer solution containing TTX, muscle contraction produced by tetanic motor nerve stimulation was abolished. This procedure prevents contraction by disrupting the transverse tubular system (Howell & Jenden, 1967; Eisenberg & Eisenberg, 1968) but does not impair neuromuscular transmission (Gage & Eisenberg, 1967; Kordaš, 1969).

End-plate responses were recorded intracellularly with glass micro-electrodes filled with 2 M potassium citrate solution. The artifact produced by stimulation of the nerve terminal was reduced by differential recording with reference to a suitably placed external micro-electrode (Katz & Miledi, 1967*a*). A 10 kΩ resistor was placed in series with the terminal stimulating electrode to monitor the intensity of the applied current pulse.

For experiments where calcium concentration was locally varied, the muscle was bathed in a solution containing (mM): NaCl 115·6; $CaCl_2$ 0·3; $MgCl_2$ 5·0; KCl 2·0. A micropipette filled with 1 M-$CaCl_2$ was then placed near the nerve terminal, while the efflux of calcium was controlled by an electrical bias applied through the pipette (del Castillo & Katz, 1955).

RESULTS

Effects of tetrodotoxin on PTP

In muscles treated with tetrodotoxin (TTX), the end-plate potential (e.p.p.) was elicited by depolarizing pulses applied electrotonically to the nerve terminal (see Methods). Fig. 1 illustrates changes in the amplitude of e.p.p.s so produced before and at various intervals following a tetanic stimulation of 1000 pulses at 50/sec. End-plate responses evoked by constant depolarizing pulses at 1 sec intervals showed a significant enhancement in amplitude following the tetanus (Fig. 1*A*). The time course and

magnitude of potentiation are represented graphically in Fig. 1*B*. The amplitude of five e.p.p.s after tetanization is averaged and plotted at various intervals as a percentage of the control e.p.p. amplitude. Post-tetanic increases in e.p.p. amplitude persisted for approximately 40 sec (Fig. 1*B*). The depolarizing pulses used before and after tetanic stimulation were identical in magnitude and duration. This precludes the possibility

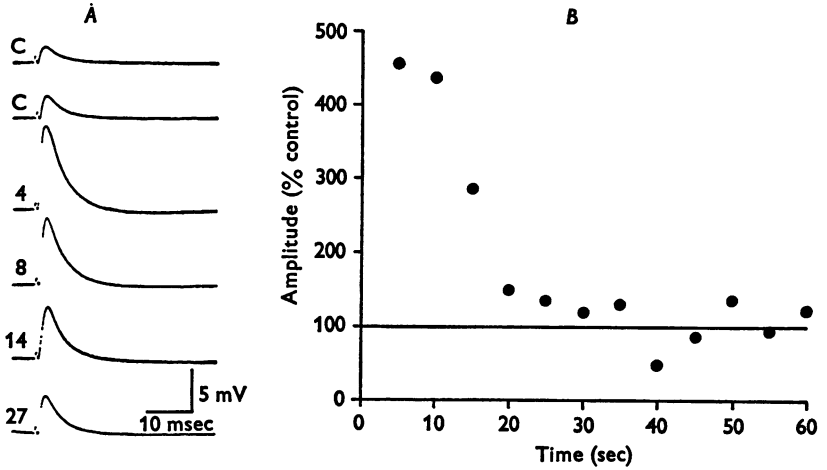

Fig. 1. PTP in TTX-treated muscle. End-plate responses recorded from a single junction before and after tetanic stimulation applied to the motor nerve terminal. Pulses were 1·0 msec in duration and 3·5 μA in intensity. *A*. Sample records of e.p.p.s before (C) and at indicated intervals (sec) after the tetanus at 50/sec for 20 sec. *B*. Time course of potentiation. Each point represents the mean amplitude of five post-tetanic e.p.p.s and refers to time (sec) of the fifth response. Ordinate, the average amplitude of post-tetanic e.p.p.s as a percentage of the control e.p.p. amplitude shown by horizontal line. The control e.p.p. amplitude was computed by averaging about 50 pre-tetanic responses. Discrepancy between sample records (*A*) and averaged response (*B*) is due to the e.p.p. amplitude fluctuation. Temperature was 19° C.

that PTP may be due entirely to changes in amplitude and/or configuration of action potentials of the presynaptic terminal following tetanic stimulation (cf. Lloyd, 1949; Hubbard, 1963; Hubbard & Schmidt, 1963).

Alterations of post-junctional sensitivity to acetylcholine (ACh) can be reflected by changes in miniature end-plate potential (m.e.p.p.) amplitude (Katz, 1962). In some experiments, the amplitude of m.e.p.p.s was relatively large so that changes in their amplitude (quantal size) could be readily detected. The results in Fig. 2 show a large increase in the frequency of m.e.p.p.s following 500 depolarizing pulses at 50/sec with little change in quantal size. Representative records shown in Fig. 2*A* illustrate

m.e.p.p.s before (C) and at various times (sec) following tetanic stimulation. Immediately following the tetanus, m.e.p.p. frequency was almost 25 times the control rate, which then gradually returned to the pre-tetanic level in approximately 60 sec (Fig. 2*B*). In this particular experiment the quantal size appeared to be slightly decreased after tetanic stimulation

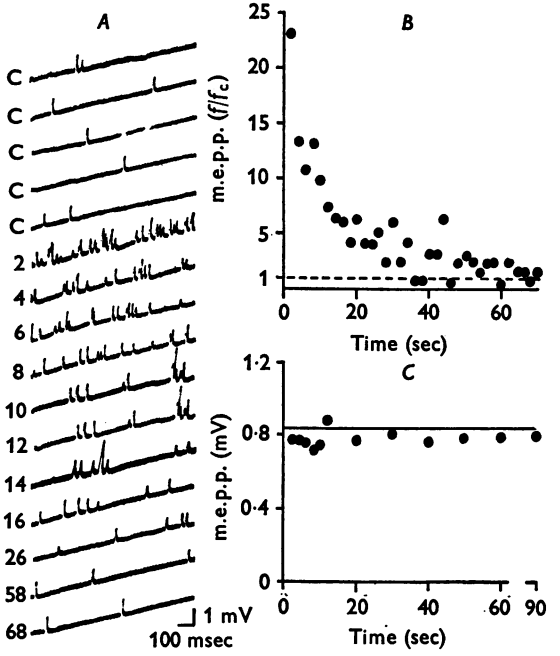

Fig. 2. Variation of m.e.p.p. amplitude and frequency following a train of 500 pulses at 50/sec. Tetanic depolarizing pulses were applied to the motor nerve terminal in a TTX-treated preparation; pulses were 1·5 msec in duration and 3·3 μA in intensity. *A*. Sample records of m.e.p.p.s before (C) and at indicated intervals (sec) after the tetanus. *B*. Changes in m.e.p.p. frequency with time after the train. Ordinate is the ratio of m.e.p.p. frequency, f, to control, f_c. Abscissa is time (sec) after tetanic stimulation. *C*. Each point represents the centre of 2–10 sec periods over which ten to twenty post-tetanic m.e.p.p.s were averaged; average pre-tetanic m.e.p.p. amplitude shown by horizontal line. Ordinate is m.e.p.p. amplitude in mV. Temperature was 18° C.

(Fig. 2*C*). This change was, however, not statistically significant. The alteration in m.e.p.p. amplitude may probably have resulted from a slight displacement of the recording electrode by muscle movement during the tetanus. In five other experiments there was no detectable change in quantal size after tetanic stimulation. These results are in agreement with previous observations in Mg^{2+}-treated muscles (Liley, 1956) and in curarized muscles (Elmqvist & Quastel, 1965). It is concluded that the

increase in e.p.p. size following repetitive nerve-terminal stimulation in TTX-treated muscles is due to an increase in the number of quanta of ACh. It is also clear that PTP of neuromuscular transmission persists when sodium influx into nerve endings is blocked by TTX. However, the question arises as to whether the PTP observed in the presence of TTX is identical in nature with impulse-induced PTP in TTX-free solutions.

TABLE 1. Duration of PTP following different numbers of tetanic stimuli in Mg^{2+}- and TTX-treated muscles. *Glycerol pre-treatment (see Methods)

	Control muscle (Mg^{2+})			Contralateral muscle (TTX)		
	PTP duration (sec)			PTP duration (sec)		
	Number of tetanic stimuli at 40/sec			Number of tetanic stimuli at 40/sec		
Fibre no.	200	800	Ratio 800/200	200	800	Ratio 800/200
1	13	22	1·7	20	40	2·0
2	12	20	1·7	19	43	2·2
3	28	120	4·2	15	39	2·5
4*	17	57	3·3	23	40	1·7
5*	10	54	5·4	7	24	3·4
6*	29	84	2·8	5	27	5·3
Mean	18	59	3·2	15	36	2·9
	Number of tetanic stimuli at 75/sec			Number of tetanic stimuli at 75/sec		
Fibre no.	375	1500	Ratio 1500/375	375	1500	Ratio 1500/375
1*	20	54	2·6	15	43	2·8
2*	39	112	2·8	30	89	2·9
3*	60	116	1·9	115	150	1·3
Mean	40	94	2·5	53	94	2·4

Variation with different stimulating parameters. When transmitter release is evoked by nerve impulses, an increase in the number of tetanic stimuli greatly enhances the time course, but not the magnitude, of PTP (Larrabee & Bronk, 1947; Liley & North, 1953; Curtis & Eccles, 1960; Braun *et al.* 1966; Rosenthal, 1969). If sodium were involved in the mechanism of PTP, the increase in the time course associated with increased number of tetanic stimuli would be insignificant in muscles treated with TTX.

Table 1 summarizes the changes in the duration of PTP associated with different numbers of tetanic stimuli. In these experiments, the number of tetanic stimuli was increased from 200 to 800 pulses at a frequency of 40/sec or from 375 to 1500 at 75/sec. The duration of PTP was taken as the time required for the average e.p.p. amplitude to decline to pre-tetanic control values after a tetanus. The duration so measured might have in-

cluded an error of ± 15 % in both Mg^{2+} and TTX-treated muscles. The data are expressed as ratio of PTP duration produced by the longer tetanus to that by the shorter. In nine fibres from six control (TTX-free) muscles, the ratio ranged from 1·7 to 5·4 when the number of stimuli was increased. Similar results (1·3–5·4) were obtained in the six contralateral muscles bathed in TTX solution. The average duration of PTP in muscles treated with TTX, for a given number of tetanic stimuli, was similar to that obtained from the contralateral Mg^{2+}-treated muscles (Table 1). This agreement was particularly evident with tetanizations consisting of large numbers of stimuli delivered at high frequency (1500 stimuli at 75/sec). From the data in Table 1, it appears that the duration of PTP elicited by different numbers of tetanic stimuli is essentially the same in Mg^{2+}-treated and in TTX-treated preparations.

Effects of temperature on PTP. The time course of PTP is known to have a high temperature coefficient (Feng, 1941; Rosenthal, 1969). With a temperature change from 25 to 10° C, the duration of PTP increases almost threefold. The magnitude of this temperature effect suggests that some part of the mechanism underlying PTP is metabolically dependent. Because intracellular sodium concentration is regulated by the activity of a sodium pump (Hodgkin & Keynes, 1955), a prolongation in the time course of PTP at low temperatures might be a consequence of retarded extrusion of sodium induced by depression of the temperature-sensitive pump mechanism.

Fig. 3 shows the changes in the duration of PTP in TTX- treated muscles when temperature was decreased by about 10° C. PTP in Fig. 3*A* was obtained from two different junctions in the same muscle following a tetanus of 1000 stimuli at 50/sec. The amplitude of e.p.p.s in response to a given depolarizing pulse was reduced by decreasing the temperature. The duration and intensity of the depolarizing pulses in this experiment, however, were adjusted so as to produce pre-tetanic e.p.p.s with similar amplitude at the two temperature levels. The results from these two junctions show that the onset and decay time of PTP were prolonged at the lower temperature (filled circles). In Fig. 3*B*, the effects of 500 stimuli at 50/sec were observed from the same junction at 21° C (open circles) and at 10° C (filled circles). In this experiment, the depolarizing pulses were of equal intensity and duration at both temperatures; nevertheless, the results were similar to those in Fig. 3*A*. It is concluded that the prolongation of PTP time course normally observed at low temperature remains unaltered when sodium influx is inhibited by TTX.

Effects of calcium on PTP

It may be argued that motor nerve terminals have different properties from those of the motor axon. Consequently, the sodium influx associated with depolarization may not be completely inactivated by TTX and, as such, some sodium ions may enter the nerve terminal during a train of depolarizing pulses. This possibility seems unlikely since ionophoretic application of TTX to the terminal arborization prevents release of ACh

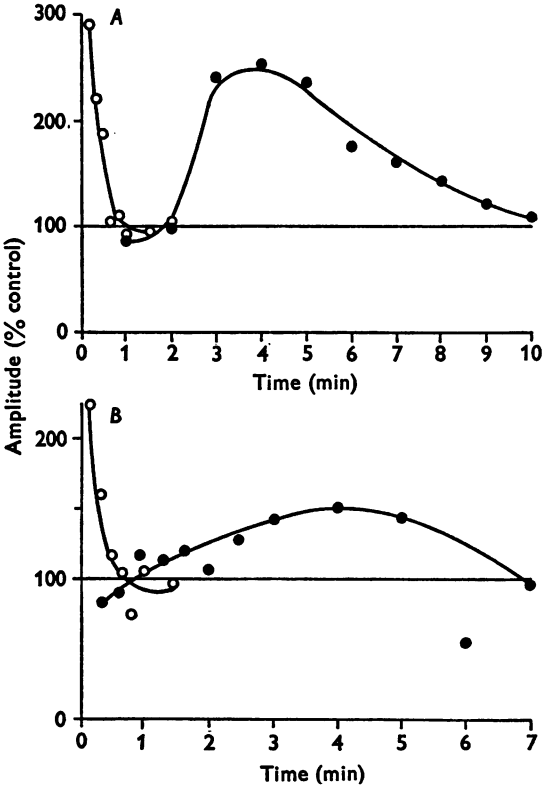

Fig. 3. Effects of temperature on PTP in muscles treated with TTX. *A* and *B*. Ordinates are amplitude of e.p.p. as a percentage of the control amplitude. Abscissae are time (min) after the tetanus. Each point represents the mean size of five to ten e.p.p.s. Depolarizing pulses were applied to nerve terminals every 2 sec before and after tetanization. *A*. Changes in e.p.p. amplitude following 1000 stimuli at 50/sec at two junctions in the same muscle. Depolarizing pulses were 0·6 msec in duration and 10 μA in intensity at 24° C (○); 3·0 msec in duration and 9·0 μA in intensity at 12° C (●). *B*. Changes in e.p.p. amplitude following 500 pulses at 50/sec at the same junction in a different muscle. Pulses, 1·8 msec in duration and 5·1 μA in intensity, remained constant at both temperatures. Temperature was 21° C (○) and 10° C (●).

in response to a nerve impulse beyond the point of application (Katz & Miledi, 1968*b*). However, to test this possibility further, the effects of tetanic stimulation were studied in nerve–muscle preparations in which the external sodium had been replaced by a solution of isotonic (83 mM) calcium chloride. Under these conditions, action potentials cannot be generated in nerve and muscle, but the end-plate region can respond to applied ACh (Takeuchi, 1963). Moreover, Katz & Miledi (1969*b*) have shown that spontaneous m.e.p.p.s are still present and that brief current pulses applied locally to motor nerve endings can evoke e.p.p.s.

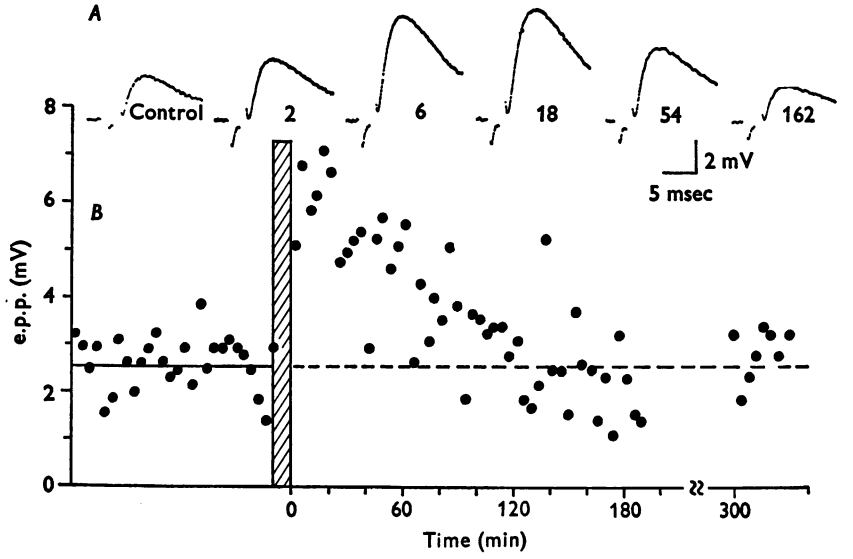

Fig. 4. The effects of 500 depolarizing pulses at 50/sec on e.p.p. amplitude in isotonic (83 mM) calcium chloride. *A*. Sample records of evoked e.p.p.s before (control) and at indicated intervals (sec) following the train. *B*. Amplitude of e.p.p.s before and after the tetanus (hatched bar). Test pulses, 1·5 msec in duration and 1·9 μA in intensity, were applied at 2 sec intervals before and following tetanic stimulation; for clarity, amplitudes (filled circles) are plotted at 4 sec intervals. Ordinate is e.p.p. size in mV. Abscissa is time (sec) after the tetanus. Temperature was 9° C.

Sample records in Fig. 4*A* depict changes in e.p.p. amplitude at various intervals following tetanic stimulation of 500 pulses at 50/sec in a muscle bathed in isotonic calcium chloride solution. End-plate responses were evoked by application of constant depolarizing pulses electrotonically to the nerve terminal at 2 sec intervals. The time course of the e.p.p. change following the tetanus is illustrated in Fig. 4*B*. It can be seen that the e.p.p. amplitude is significantly larger than the control values for nearly 2 min after the train. From these results, it is clear that PTP of neuromuscular

transmission is not directly associated with sodium entry into nerve terminal or with the resultant intracellular sodium accumulation during a train of nerve stimuli.

Effects of calcium during tetanization. Rosenthal (1969) has demonstrated that the time course of PTP increased when the concentration of calcium in the bathing medium was raised. Moreover, she found that the effects of calcium on PTP were due to its presence only during the tetanus and not

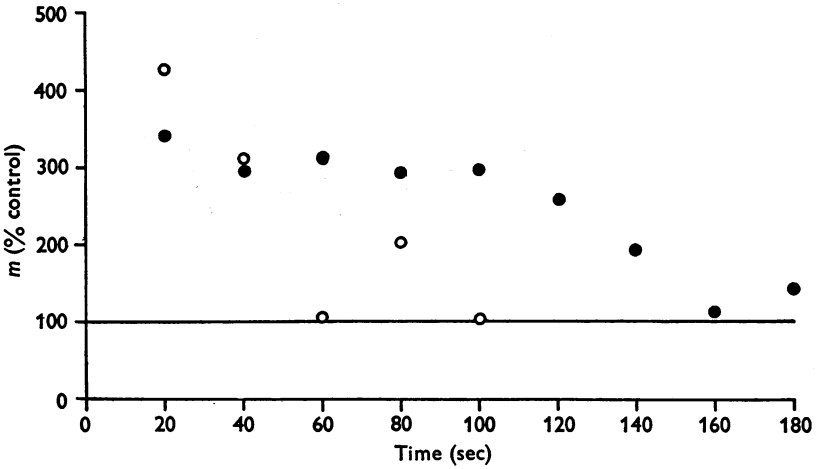

Fig. 5. Effects of ionophoretically applied calcium during a tetanus. Tetanic stimulation by 800 nerve impulses at 40/sec. Open circles show the effects of the train on transmitter release when calcium efflux was held constant at pre-tetanic levels. Filled circles indicate the effects of transiently increased calcium efflux during the time of the tetanus only. Each point represents the mean quantum content (m) estimated from ten to twenty e.p.p.s after the train as a percentage of pre-tetanic quantum content (ordinate). Abscissa is time (sec) after the train. Temperature was 23° C.

to increased calcium entry during the post-tetanic period. Thus, PTP may be associated with an enhanced entry of calcium during tetanic stimulation. This possibility can be further supported by the results shown in Fig. 5. In a bathing solution containing 0·3 mM-$CaCl_2$, motor nerve stimulation at a rate of 1/sec failed to evoke ACh release. When a calcium pipette was located near the nerve terminal and calcium efflux was suitably adjusted, e.p.p.s were occasionally evoked in response to nerve stimulation. Without altering the efflux of calcium, tetanic stimulation by 800 nerve impulses at 40/sec produced an increase in transmitter release persisting for approximately 100 sec (open circles in Fig. 5). Transmitter release was measured by the mean quantum content (m), calculated from the ratio of the mean e.p.p. amplitude to the mean m.e.p.p. amplitude before and

following the tetanus (del Castillo & Katz, 1954). The same procedure was subsequently repeated; but here calcium efflux was transiently increased *during* tetanic stimulation and then reset immediately to pre-tetanic levels at the end of the tetanus. This calcium application resulted in a marked increase in the time course of PTP (filled circles in Fig. 5). In view of the rapid onset and decline of calcium efflux (Katz & Miledi, 1965, 1967*c*), the effect on PTP produced by locally applied calcium was restricted to the time of the tetanus only. These results are in accord with those of Rosenthal (1969). It is concluded that the post-tetanic enhancement of transmitter release depends only on the presence of external calcium during tetanus.

Effects of the intensity of tetanic depolarizing pulses. There is considerable evidence that depolarization of the presynaptic membrane causes calcium ions to enter into the nerve terminal and that the amount of transmitter release depends on the amount of calcium entry (Katz & Miledi, 1965, 1967*a*, *b*, *c*, *d*; Hubbard, Jones & Landau, 1968; Katz & Miledi, 1969*a*, *b*, 1970). If PTP is associated with calcium entry during tetanic stimulation, increasing the intensity or duration of presynaptic depolarization during a tetanus should increase calcium influx and thereby enhance PTP.

To test this possibility, transmitter release was evoked by applying depolarizing pulses to motor nerve endings in the presence of TTX. With this technique, intensity and/or duration of the applied pulse can be altered *during* the time of tetanic stimulation and immediately reset to pre-tetanic levels at the end of the train. The effect of varying intensity of a train of 400 depolarizing pulses at 40/sec on PTP in a single muscle fibre is shown in Fig. 6. Responses were evoked at 1-sec intervals before and following tetanic stimulation. The average amplitude of five to ten e.p.p.s is plotted at various intervals after tetanization (filled circles). The control (pre-tetanic) e.p.p. was computed by averaging the amplitudes of about fifty e.p.p.s. The e.p.p. amplitude following the tetanus was potentiated for approximately 30 sec (Fig. 6*A*). Fig. 6*B* shows the effect of increasing pulse intensity *during* tetanic stimulation at the same junction as shown in *A*. Under these conditions, e.p.p. amplitude following the tetanus increased and remained above control level for almost 11 min. In Fig. 6*C* the same procedure as that illustrated in *A* was repeated. PTP again persisted only for approximately 30 sec. These findings are consistent with the view that PTP is related to calcium movement into the nerve terminal which occurs dependent on the amount of terminal depolarization.

Although the time course of PTP was dramatically increased by increasing the intensity of depolarizing pulses during tetanic stimulation, the magnitude of PTP was relatively unchanged. A similar tendency was noted previously when the tetanic stimulation period was increased (Table 1) and when temperature was lowered (Fig. 3). Rosenthal (1969)

also noted that the magnitude of PTP remained constant when the PTP duration was altered under a variety of conditions at the curarized neuromuscular junction. The basis for this phenomenon is not known, but it does not appear to be due to saturation of the end-plate response, since increasing the intensity of the depolarizing pulses applied during the maximum PTP further increased the end-plate responses.

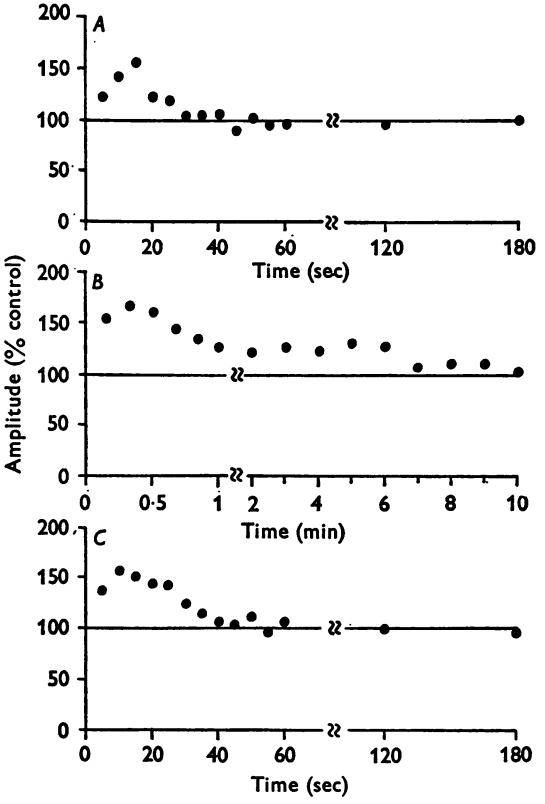

Fig. 6. Effects of increasing pulse intensity during tetanic stimulation by 400 stimuli at 40/sec. *A–C*. Responses recorded intracellularly at a single junction from a TTX-treated muscle. Ordinates are amplitude of e.p.p. as a percentage of control e.p.p. amplitude. Abscissae are time (sec and min) after the tetanus. Each point represents the mean amplitude of five e.p.p.s except in *B* where ten e.p.p.s were averaged for each point. Temperature was maintained at 19° C. *A*. Control run. Depolarizing pulses were 1·0 msec in duration and 2·0 μA in intensity before, during, and after the tetanus. *B*. Pulse intensity was increased to approximately 6·0 μA during tetanic stimulation only; pulse duration was held at 1·0 msec. Note that the abscissa is in min. *C*. Control run. Pulse intensity and duration same as in *A*.

DISCUSSION

The present results provide evidence that sodium entry or the accumulation of sodium in motor nerve endings is not a prerequisite for the increased transmitter release following tetanic stimulation. PTP of the end-plate potential was still evident when sodium influx was blocked by TTX (Figs. 1 and 2) or when all external sodium was replaced by a solution of isotonic calcium chloride (Fig. 4). The PTP observed under TTX appeared to be similar to impulse-induced PTP. Lowering of temperature (Fig. 3) or increasing the number of tetanic stimuli (Table 1) produced an increase in the time course of PTP in the presence of TTX, in the same manner as observed in a TTX-free solution.

Miledi & Thies (1967) have shown that the post-tetanic increase in m.e.p.p. frequency can still be obtained when the extracellular concentration of calcium is drastically reduced by a calcium-chelating agent. They suggested that calcium may still mediate this response if the tetanic stimulus releases some residual calcium from a bound membrane site. As an alternative interpretation Muchnik & Venosa (1969) proposed that tetanic stimulation increased intracellular concentration of sodium within the nerve terminal to produce the post-tetanic response. However, the present results show that the presence of TTX does not alter the increased m.e.p.p. frequency that follows tetanic stimulation. Since there can be no appreciable rise in intracellular sodium concentration under these conditions, an increase in cellular sodium does not appear to account for the potentiation of m.e.p.p. frequency.

It appears unlikely that either potassium or chloride ions are involved in PTP. Alterations in the chloride equilibrium potential or substituting methylsulphate for chloride have no apparent effect on transmitter release (Muchnik & Gage, 1968). In addition, changes in concentration gradient of potassium across motor nerve ending membrane are without effect on PTP (Takeuchi & Takeuchi, 1961; Gage & Hubbard, 1966).

The present study is in agreement with the previous suggestions that PTP may be dependent on accumulation of calcium within nerve terminals (Elmqvist & Quastel, 1965; Gage & Hubbard, 1966; Rosenthal, 1969). The persistence of PTP in an isotonic solution of calcium chloride strengthens this view. In addition, all those procedures which tend to increase the inward movement of calcium during a tetanus, such as increase in the number of tetanic stimuli, elevation of external calcium concentration, or increase in the intensity of depolarizing pulses, prolonged the time course of PTP. It might be argued that these effects on PTP could be related to an increased release of ACh during a tetanus rather than to an enhanced

entry of calcium. PTP, however, is independent of the amount of transmitter liberated during tetanic stimulation (Rosenthal, 1969).

It is possible that the time course of PTP may be determined by a mechanism which inactivates or removes the intraterminal calcium accumulated during a tetanus. Rosenthal (1969) suggested that the calcium extrusion may involve an exchange carrier for which sodium and calcium compete. However, the present results with TTX and with isotonic calcium chloride solution may exclude such possibility. Katz & Miledi (1968*a*) have shown that facilitation of transmitter release following a single conditioning stimulus also results from an accumulation of calcium within the terminal membrane. Thus, it appears that both facilitation and PTP are consequences of essentially the same mechanism, although their time courses are distinctly different. It is interesting to note that PTP may be eliminated by metabolic inhibitors, whereas facilitation is still present under these conditions (Gage & Hubbard, 1966).

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