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On the neuromuscular paralysis produced by procaine

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Summary

1. The effects of procaine on neuromuscular transmission in the rat phrenic nerve diaphragm preparation have been examined using intracellular recording techniques.

2. The paralysis produced by procaine resembled that produced by tubocurarine and it is concluded that this effect of procaine results from a change in post-junctional sensitivity to transmitter; at low rates of stimulation procaine did not depress the transmitter release.

3. During high frequency stimulation of the phrenic nerve a distinct form of prejunctional failure was observed. It is suggested that this depression results from a combination of local anaesthesia and anoxia and that it would explain reports of a reduction in the amount of transmitter released when assayed in a conventional manner.

Introduction

Procaine is able to produce, *in vivo* and *in vitro*, paralysis of mammalian nerve-muscle preparations (Harvey, 1939; Jaco & Wood, 1944; Straughan, 1961; Matthews & Quilliam, 1964). The mechanism of this paralysis is not fully understood. Jaco & Wood (1944) after examining the interactions between procaine and adrenaline and prostigmine suggested that a reduction in transmitter output may contribute to the paralysis. This suggestion was supported and extended by Straughan (1961) who showed that procaine reduced the amount of transmitter released during periods of tetanic stimulation of the motor nerve of the rat-diaphragm preparation; it was suggested that the paralysis arose from anaesthesia of the fine motor nerve terminals. The concentrations of procaine which reduced transmitter output had no effect on conduction by the motor nerve trunk (Matthews & Quilliam, 1964).

In contrast, electrophysiological studies on the actions of procaine on amphibian nerve-muscles suggested that the site of paralysis might be post-junctional (Feng, 1941; Furukawa, 1957). No evidence for a prejunctional site of action has been presented: Furukawa (1957) and del Castillo & Katz (1957) showed that procaine was able to reduce the sensitivity of the end-plate to acetylcholine applied iontophoretically. Maeno (1966) found that concentrations of procaine which reduced the amplitude of spontaneous miniature end-plate potentials (m.e.p.ps) had no effect on the release of transmitter from the motor nerve terminals.

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The present study was undertaken to obtain further information on the mechanism of procaine paralysis in rat phrenic nerve-diaphragm preparations. Preliminary accounts of some of these experiments have already appeared (Hirst & Wood, 1969a, b).

Methods

Isolated phrenic nerve-diaphragms (Bulbring, 1946) of male rats (Wistar strain, 150–250 g) were used in all experiments. The preparations, stretched to approximately 130% of their resting lengths, were mounted in an organ bath similar in design to that of Boyd & Martin (1956). A modified Krebs solution was used of ionic composition: (mM) Na^+, 143·1 ; K^+, 1·22 ; Mg^{2+}, 1·175 ; Ca^{2+}, 2·5 ; Cl^-, 127·8 ; SO_4^{2-}, 1·175 ; PO_4^{3-}, 1·22 ; HCO_3^-, 24·9 ; the potassium concentration was reduced to one-fifth so as to increase the threshold potential (Beránek & Vyskočil, 1967). The solution was gassed with 95% O_2: 5% CO_2, and was allowed to flow through the organ bath at a rate of 200–300 ml/hour. The fluid volume was maintained at 20 ml by continuous suction through a capillary tube. All drug concentrations refer to the final bath concentration. Concentrated drug solutions were first added to the bath via a baffle and after allowing 2 min for them to mix, Krebs solution containing the same final concentration of drug was allowed to flow through the bath. Drugs were washed from the bath by allowing a rapid flow of 'drug-free' Krebs solution. In the experiments where quantal content was to be determined, sufficient crystalline magnesium sulphate was added to the Krebs solution to give a bath concentration of 12–16 mM ; no compensation for the change in osmotic pressure of the solution was made. The temperature of the preparation was maintained at 31° C by partial immersion of the organ bath in a thermostatically controlled water bath (Copeland, 1962).

Glass microelectrodes filled with 3 M KCl, having resistance 10–20 MΩ and tip-potentials less than 10 mV, were used to record membrane potentials using conventional techniques (Fatt & Katz, 1951). The phrenic nerve was stimulated supramaximally with pulses of width 0·05–0·1 ms with the aid of suction electrodes (Furshpan & Potter, 1959). End-plate regions could not be localized precisely by microscopic examination but m.e.p.ps and end-plate potentials (e.p.ps) following stimulation of the phrenic nerve could only be recorded from areas near the nerve trunks. When recording from end-plate regions the position of the microelectrode was adjusted until m.e.p.ps (recorded from non-curarized tissues) had maximum amplitude and e.p.ps (recorded from curarized tissues) had short rise times (Liley, 1956a). All recordings where the resting potential did not remain stable during the addition and subsequent washout of procaine were discarded: in preliminary experiments it was found that procaine (0·1–0·8 mM) had no effect on muscle resting potential. The mean amplitudes and frequencies of m.e.p.ps were determined from continuous photographic recordings over 15–60 seconds. E.p.p. amplitudes and characteristics were determined from several single exposures.

Results

Effects of low concentrations of procaine on spontaneous miniature end-plate potentials (m.e.p.ps)

Following penetration of the muscle membrane, stable resting potentials (range 75–90 mV, internal negative) were recorded. When recorded from end-plate foci,

m.e.p.ps had mean amplitudes in the range 0·1–1·2 mV and frequencies usually in the range 1–150 Hz. Occasionally end-plate foci with higher rates of m.e.p.p. discharge were located but these were discarded as the m.e.p.ps characteristics could not be determined accurately.

On addition of procaine (0·025–0·1 mM) to the Krebs solution the mean size of the m.e.p.ps was rapidly reduced whether the discharge frequencies were high or low. The reduction in m.e.p.p. amplitude was dependent on the bath concentration of procaine (Fig. 1). With concentrations of procaine higher than 0·1 mM the amplitudes of the m.e.p.ps were reduced so much that they were not easily distinguished from electrode noise; occasionally 'giant m.e.p.ps' were detected. The reduction in m.e.p.p. amplitude was not associated with a change in either the m.e.p.p. fre-

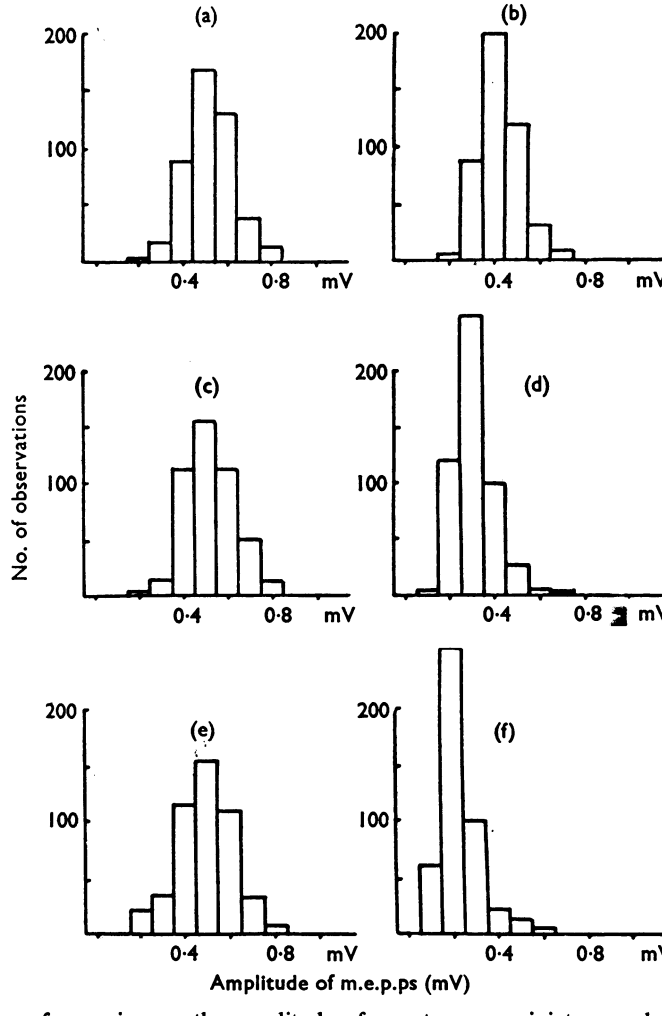

FIG. 1. Action of procaine on the amplitude of spontaneous miniature end-plate potentials. Each recording was made from the same end-plate; m.e.p.ps were recorded for 30 s before and 10 min after the procaine. Controls are shown in Fig. 1(a), (c) and (e); procaine concentrations are Fig. 1(b), 0·025 mM; Fig. 1(d), 0·05 mM; and Fig. 1(f), 0·1 mM.

quency or the muscle resting potential. After washing with 'drug-free' Krebs solution the m.e.p.p. amplitude rapidly returned to its control value.

Effect of procaine on end-plate potentials (e.p.ps)

Following stimulation of the phrenic nerve e.p.ps were recorded from preparations paralysed by tubocurarine (0·0015 mM) or by a high concentration of magnesium (12–16 mM). Procaine (0·05–0·4 mM) reduced the amplitude of 'tubocurarine e.p.ps' in a dose dependent manner (Hirst & Wood, 1970) (Fig. 2).

Similarly, the mean amplitudes of e.p.ps recorded from preparations paralysed by high magnesium concentration were reduced by the addition of procaine (0·1 mM). In these experiments the mean quantal content per nerve impulse (m) was calculated from the relationship $m=\frac{\text{mean amplitude of e.p.ps}}{\text{mean amplitude of m.e.p.ps}}$ (del Castillo & Katz, 1954); at least 180 end-plate responses and sixty spontaneous m.e.p.p.s were recorded for each determination. If the quantal content was low a second

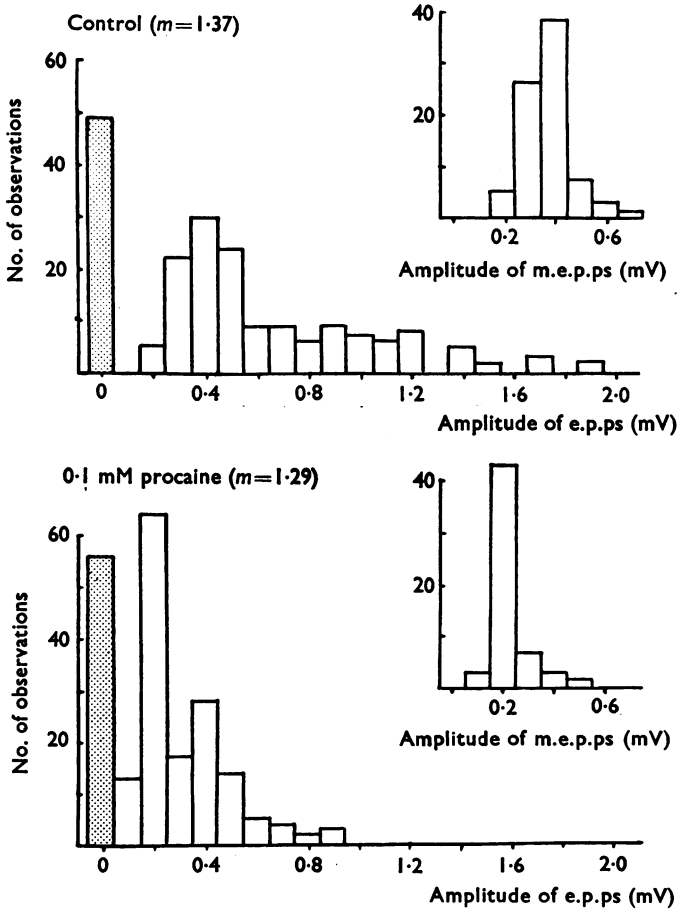

FIG. 2. Histograms of e.p.p. and m.e.p.p. amplitudes, recorded from a muscle paralysed by high magnesium concentration (15 mM). The procaine e.p.ps and m.e.p.ps were recorded 10 min after addition of procaine (0·1 mM) to the bathing fluid. The values for m were calculated as described in text.

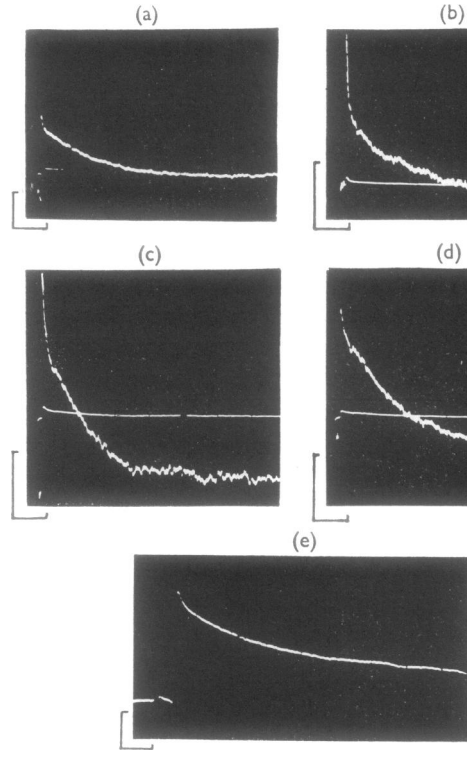

FIG. 3. E.p.ps recorded from nerve muscle preparations paralysed by procaine (0·8 mM). Calibration bars for (a), (b), (c) and (d), 1 mV and 10 ms ; for (e), 2 mV and 2 ms.

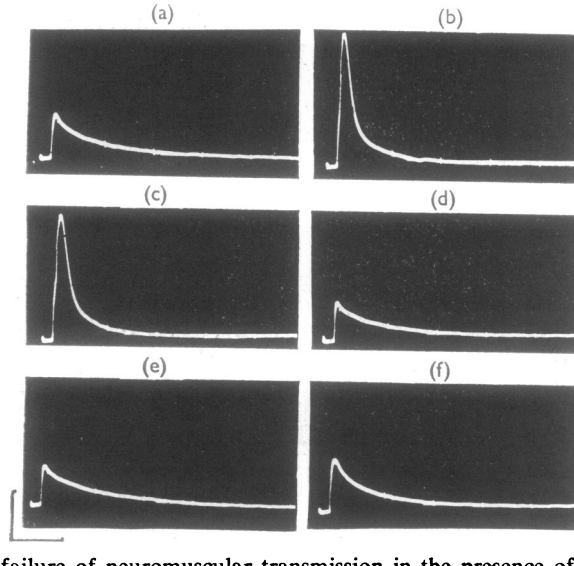

FIG. 4. Irregular failure of neuromuscular transmission in the presence of procaine (0·7 mM). Successive recordings from an end-plate region where the e.p.p. just reached threshold. Calibration bars, 20 mV and 10 ms.

value of the mean quantal content was determined using the relationship $m = \frac{ln \text{ number of impulses}}{\text{number of failures}}$ (del Castillo & Katz, 1954). The results obtained in one of five similar experiments are illustrated in Fig. 2. Clearly, the change in e.p.p. amplitude was not associated with any change in quantal content. However, the concentration of procaine acting alone was insufficient to have caused neuromuscular paralysis and it can be argued that in higher concentrations procaine had additional effects.

Nature of the neuromuscular block produced by high concentrations of procaine

To investigate the possibility that procaine concentrations which produce paralysis act by anaesthetizing the motor-nerve terminal, recordings were made from end-plate regions, starting 10–15 min after the addition of procaine, 0·8 mM. On stimulation of the phrenic nerve e.p.ps were recorded. Evidently the nerve terminal had remained excitable. Typical records are shown in Fig. 3. Similar observations were made with the preparation bathed in normal Krebs solution (see **Methods**), in which the potassium concentration was 5·9 mM. In lower concentrations of procaine (0·6–0·7 mM) or when stimuli were given less than 10 min after applying the drug, transmission was partially impaired and the action potential recorded at end-plate regions had a step in its rising phase; if the e.p.p. just reached threshold, muscle action potentials were initiated on some occasions (Fig. 4).

In several respects the e.p.ps recorded from nerve-diaphragms paralysed by procaine were similar to those recorded from tissues paralysed by tubocurarine; at low rates of stimulation (six/min) successive e.p.ps had the same amplitude; at higher

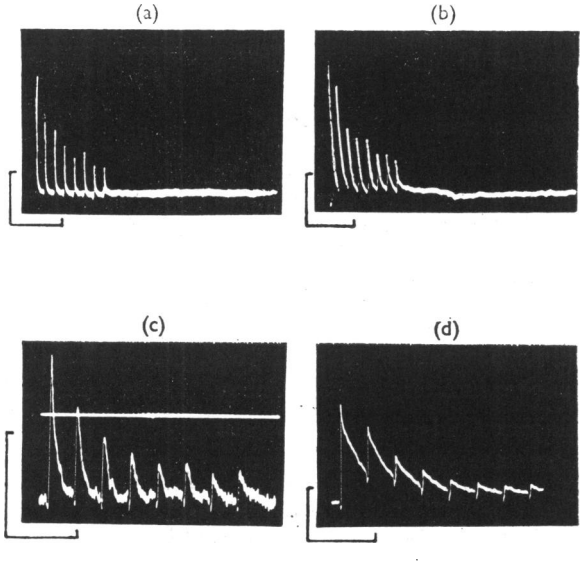

FIG. 5. **Repetitive end-plate responses recorded during high frequency stimulation of the phrenic nerve. (a) and (c) trains of e.p.ps recorded from two separate preparations paralysed by 0·0015 mM tubocurarine; (b) and (d) trains of e.p.ps from two separate preparations paralysed by 0·8 mM procaine. ((a) and (c) have been retouched). Calibration bars for (a) and (b), 2 mV and 100 ms; for (c) and (d), 2 mV and 50 ms. The stimulation frequency in each was 50 Hz.**

rates of stimulation (10–150 Hz) successive e.p.ps decreased to an equilibrium level suggesting that there was not much effect on output. However, it should be noted that the time course of the procaine e.p.ps was distinct from that of the tubocurarine e.p.ps (Hirst & Wood, 1971).

Comparison between action of tubocurarine and procaine

In some experiments e.p.ps were recorded from preparations in which the concentrations of either procaine or tubocurarine were cautiously increased until the twitches to nerve stimulation were just abolished. Thus the concentrations were adjusted so that similar degrees of blockade were achieved with the two drugs. End-plate foci were located by recording the e.p.p. from a given fibre at various distances along that fibre to obtain the maximum response. The mean amplitudes of the 'procaine e.p.ps' (5·8 mV) and of the 'tubocurarine e.p.ps' (5·9 mV) were similar. The ranges of the e.p.ps and in particular the maximum values were also similar. If one supposes that the largest e.p.ps represent the threshold for the initiation of an action potential by the e.p.p. the results suggest that a change in threshold makes no significant contribution to the blocking action of procaine at this concentration.

Effect of procaine on repetitive end-plate responses

It has been shown by Otsuka, Endo & Nonomura (1962) that if the phrenic nerves of preparations paralysed by tubocurarine were stimulated with short trains of impulses (trains of eight at 50 Hz repeated not more frequently than every 20 s) the depression on amplitude of e.p.ps was not related to any change in post-junctional sensitivity to acetylcholine. A comparison of the end-plate potentials recorded on stimulation of the phrenic nerve with similar trains of impulses from

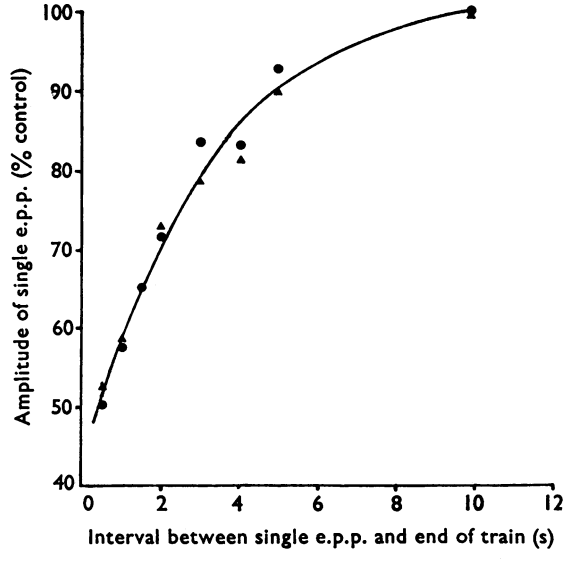

FIG. 6. Recovery from pre-synaptic depression following tetanic stimulation of the phrenic nerve (trains of eight impulses at 50 Hz). The amplitude of the single e.p.p. is plotted as a percentage of the first e.p.p. of the train. (●), Recordings from preparations paralysed by 0·0015 mM tubocurarine; (▲), readings from preparations paralysed by 0·8 mM procaine. Each symbol is the mean of five recordings.

preparations paralysed by tubocurarine (0·0015 mM) or procaine (0·8 mM) was made. In these experiments successive e.p.ps recorded in the presence of either tubocurarine (0·0015 mM) or procaine (0·8 mM) decreased in amplitude; the amplitude of the eighth e.p.p. was reduced to about 30% of that of the first e.p.p. (Ratio of amplitude of eighth e.p.p. to first e.p.p.—after tubocurarine 0·34, $n=8$; after procaine, 0·30, $n=8$). Typical recordings are shown in Fig. 5. Moreover a single stimulus at various intervals after a train showed that the e.p.ps were depressed for up to 5 seconds. When the amplitude of the single e.p.p. was compared with the amplitude of the first e.p.p. in the train, it was apparent that the rates of recovery from depression in either drug solution were similar (Fig. 6). In each of the experiments described above no irregular failures of transmission were observed at this stimulation frequency (that is 50 Hz). However, when the recordings were made from preparations paralysed with procaine irregular failures in transmission occurred frequently (Fig. 7).

At these end-plates, each stimulus initiated an e.p.p. during low frequency stimulation. As the frequency of stimulation was increased there were irregular failures

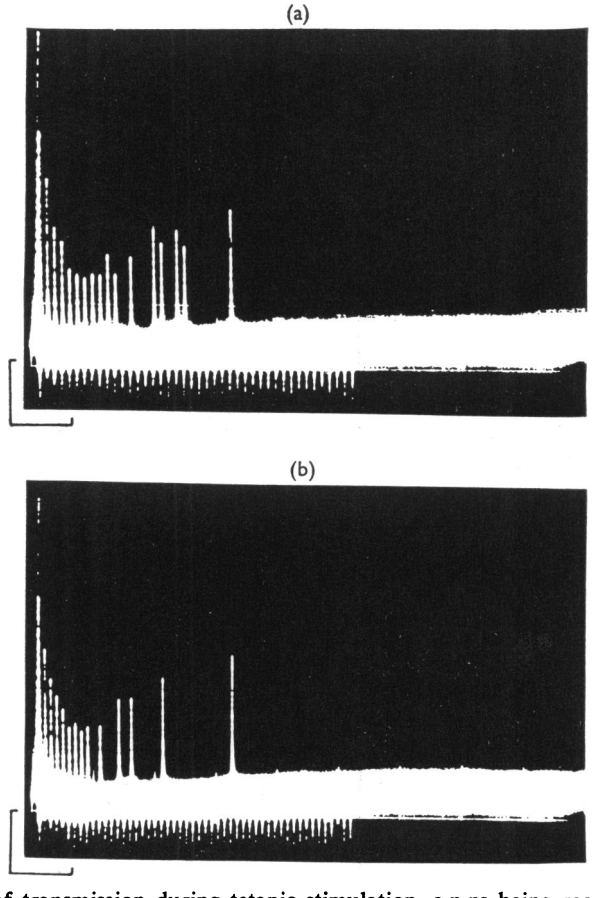

FIG. 7. Failure of transmission during tetanic stimulation, e.p.ps being recorded from a fibre paralysed by procaine (0·8 mM). (a) and (b) were recorded from same end-plate, (b) being recorded 5 min after 'resting' the nerve diaphragm. Stimulation frequency (a), 40 Hz; (b), 50 Hz. Calibration bars, 1 mV and 0·2 seconds.

in transmission and an increase in the number of failures per train ; at high frequencies usually only the first impulse initiated an e.p.p. (Fig. 8). Similar observations were made using trains of impulses of fixed duration (300–400 ms).

In preparations paralysed by tubocurarine no failures of transmission were observed at stimulating frequencies up to 200 Hz if only short trains of stimuli were used. Moreover, in each of three experiments, e.p.ps were initiated regularly when the phrenic nerve was stimulated at 50 Hz for 10 minutes.

Discussion

The results described indicate that at low frequencies of stimulation, procaine blocks neuromuscular transmission by reducing the post-junctional sensitivity to acetylcholine. However, when the phrenic nerve is stimulated at high frequencies a further effect is revealed, namely a reduction in the electrical excitability of the nerve terminal which intermittently caused a complete failure of the response. This is the probable explanation for the reduction in output of acetylcholine detected by assay (Straughan, 1961 ; Matthews & Quilliam, 1964).

The failures in transmission recorded at some end-plates in the presence of procaine during high frequency stimulation are clearly a distinct form of prejunctional depression. Unlike the failures of transmission observed in the presence of high magnesium they are not random, as the first impulse invariably initiates an end-plate potential (Fig. 8) ; the frequency of occurrence of failures increased as the frequency of stimulation was increased (Fig. 8) ; any successive e.p.ps which were initiated decreased in amplitude. Recordings of the nerve action potentials indicate that the failures did not arise from failure of conduction down the nerve trunk

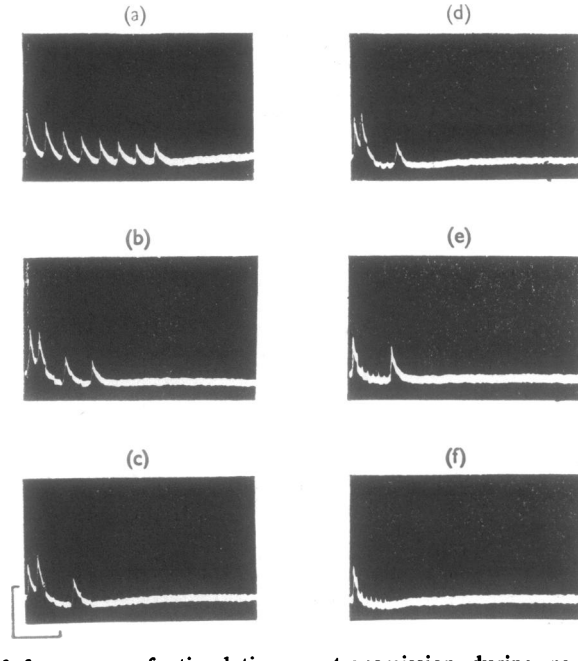

FIG. 8. Effect of frequency of stimulation on transmission during paralysis produced by procaine (0·8 mM). Stimulation frequency (a), 40 Hz ; (b), 50 Hz ; (c), 60 Hz ; (d), 80 Hz ; (e), 100 Hz and (f), 150 Hz. Calibration bars 1 mV and 40 ms.

(Hirst, 1969). Krnjević & Miledi (1958, 1959) reported similar failures of transmission when the phrenic nerve to non-paralysed diaphragms was stimulated at moderately high frequencies for long periods. The pre-junctional failures became more frequent if the oxygen tension of the Krebs solution was decreased (Krnjević & Miledi, 1959) but if the nerve diaphragms were paralysed the failures only occurred after long periods of stimulation at very high frequencies. From the similarities between the failures reported by Krnjević & Miledi (1958, 1959) and those recorded in the presence of procaine (that is, all-or-none failure at high frequencies ; reversal of failure by ' resting ' the nerve diaphragms and the frequency dependence of the failure) it appears likely that a similar form of depression is involved. It is suggested that during tetanic stimulation of paralysed nerve-diaphragms, anoxia of the motor nerves is not an important factor, but that the additional depression by procaine may be sufficient to cause obvious transmission failures. In the conditions used for assay of normal transmitter release, anoxic depression would not be expected to occur since the mechanical response of the diaphragm is only brief (Matthews & Quilliam, 1964 ; Collier, 1964). In the present work all recordings were made from surface fibres and it is to be expected that their associated nerves would be less susceptible to anoxia than the nerves to the deeper muscle fibres, which would be contributing to the assayed amount of transmitter released.

The most direct evidence for the reduction in sensitivity to acetylcholine by procaine is the depression in amplitude of the m.e.p.ps. This was not associated with a change in resting potential either at the end-plate region or at nerve-free regions. As the frequency of the m.e.p.ps was unchanged it can be concluded that the polarization of the nerve terminals and the availability of calcium in the terminals were unaltered (Liley, 1956b ; Hubbard, 1961 ; Katz, 1962). The rapid onset of depression and its ready reversibility make it unlikely that there is any change in the size of quanta of transmitter released, as it has been shown that the effects of agents which depress synthesis of transmitter only appear after long periods of tetanic stimulation (Birks & MacIntosh, 1957). Moreover, del Castillo & Katz, (1957) and Furukawa (1957) have shown that procaine is able to reduce the amplitude of end-plate responses to iontophoretically applied acetylcholine.

Procaine also decreased the amplitude of e.p.ps recorded from nerve-diaphragms paralysed by either tubocurarine or high extracellular magnesium concentration. The concentration of procaine which reduced the mean amplitude of the ' magnesium ' e.p.ps by approximately 50% had no effect on the mean amount of transmitter released per nerve impulse. This suggests that procaine in low concentrations does affect transmitter stores or the probability of release of transmitter or causes significant reduction in the amplitude of the nerve impulse in the nerve terminals (Edwards & Ikeda, 1962 ; Katz, 1962 ; Martin, 1966).

The evidence for the fact that procaine in high concentrations can cause paralysis solely by a post-junctional effect is that it affects neuromuscular transmission in the same way as does tubocurarine (except the time course of the e.p.p.). The post-junctional effect might be attributed either to an interference with the receptor occupying the transmitter or alternatively to a change in the ' equilibrium potential of the transmitter '. The present results throw no light on this (see however, Maeno (1966)) although it is known that tubocurarine causes no change in the transmitter equilibrium potential (Takeuchi & Takeuchi, 1960).

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