Br. J. Pharmac. (1971), **41**, 105–112

Changes in the time course of transmitter action produced by procaine

G. D. S. HIRST* AND D. R. WOOD

Department of Pharmacology, University of Leeds, School of Medicine, Leeds, LS2 8NL

Summary

1. In addition to reducing the sensitivity of the end-plate to transmitter, procaine produced changes in the time course of the end-plate potential. The time to peak potential was reduced by procaine, whether recording from fibres paralysed by tubocurarine or from fibres paralysed by high magnesium concentration. The rate of decay of potential was also markedly reduced. Similar observations were made recording spontaneous miniature end-plate potentials.

2. Procaine did not produce any marked change in the electrical constants of muscle fibres.

3. In the presence of procaine the accumulation of charge at the end-plate was prolonged; the rate of decay of charge was reduced. It is suggested that the prolonged end-plate potentials arise from a prolongation of the end-plate current.

Introduction

A characteristic action of procaine and some other local anaesthetics at the neuromuscular junction is a prolongation of the end-plate potential (Feng, 1941; Furukawa, 1957; Maeno, 1966; Steinbach, 1968a, b). This effect has been attributed to an anticholinesterase action of procaine (Hunt & Kuffler, 1950), to a prolonged release of transmitter probably arising from a prolongation of the nerve action potential (Takeuchi & Takeuchi, 1959), to a change in time constant of the muscle (Takeuchi & Takeuchi, 1959), or to changes in the post-junctional time course of the transmitter and its associated changes in ionic permeability (Furukawa, 1957; Maeno, 1966; Steinbach, 1968a, b); in each case the information was obtained using frog sartorius preparations.

More information on this effect has been sought using the rat phrenic nerve diaphragm. A report of some of these experiments has been given to the British Pharmacological Society (Hirst & Wood, 1969).

Methods

Isolated rat phrenic nerve diaphragms were used in all experiments using apparatus and procedures as previously described (Hirst & Wood, 1971). In some experiments to determine cable constants of the muscle fibres in the presence and absence of procaine a second microelectrode was inserted close to the potential recording

* Present address: Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ.

electrode. Using a circuit similar to that described by Fatt & Katz (1951), currents of varying intensity were passed through this second electrode. The diameter of muscle fibres was measured microscopically using a $\times 50$ water immersion lens and $\times 15$ eyepiece, the preparations being stretched as described previously.

The accumulation and decay of end-plate charge was followed using the method of Fatt & Katz (1951).

Results

Effects of procaine on time course of end-plate potentials

As well as reducing the mean amplitude of spontaneous miniature end-plate potentials, procaine (0·1 mM) altered the time course of these potentials. The rise time of the m.e.p.ps, that is, the time from the onset of potential change to the attainment of maximum potential, was consistently reduced but the rate of decay of potential was also reduced. A typical experiment is illustrated in Fig. 1. The rise time of e.p.ps recorded from nerve-muscles paralysed by either tubocurarine or high extracellular magnesium concentration was also reduced by procaine (0·05–0·2 mM) (Fig. 2); again the rate of decay of potential was reduced. This pattern of behaviour was observed when recording 'magnesium e.p.ps' which presumably consisted of only a few quanta; that is, no evidence of an asynchronous release of transmitter was detected (Ginsborg & Hamilton, 1968).

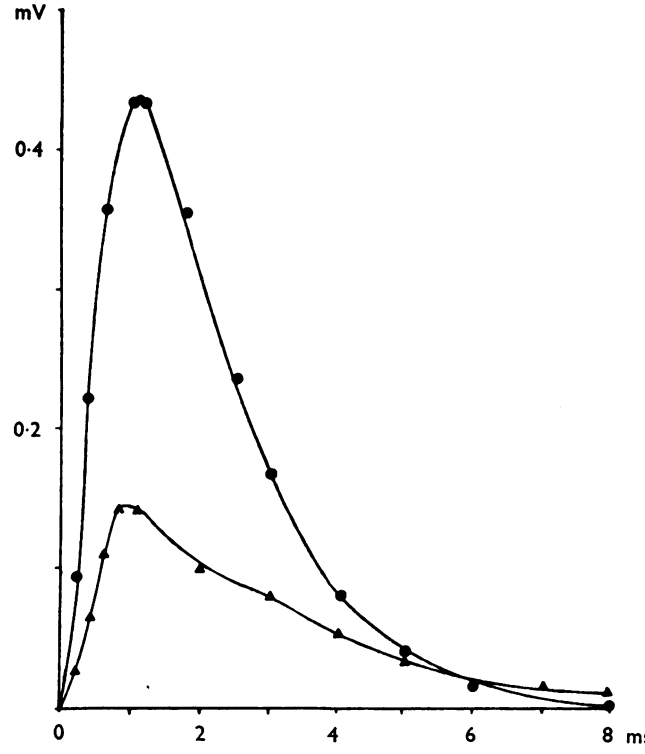

FIG. 1. Effect of procaine on the time course of spontaneous miniature end-plate potentials. (●), Control m.e.p.ps; (▲), 'procaine' m.e.p.ps, recorded from the same end-plate 10 min after addition of procaine (0·1 mM) to the bath. Each curve is the mean of ten m.e.p.ps.

Moreover when neuromuscular transmission was blocked, either by procaine alone (0·7–0·8 mM) or by tubocurarine (0·0015 mM), recordings made from end-plate foci (as judged by the amplitude and rise time of the potentials recorded at various distances along the same fibre) showed that the time course of the ' procaine e.p.ps ' was clearly distinct from the ' tubocurarine e.p.ps '. The rise time of e.p.ps recorded from tissues paralysed by procaine (1·0 ms) was significantly shorter than that (1·2 ms) recorded from tissues paralysed with tubocurarine ($P=0·02$, ten observations each). The decay of potential of the procaine e.p.ps was occasionally complex, an initial rapid decay of potential being followed by an extremely slow decay of potential (Maeno, 1966). More frequently only the slow decay of potential was observed (procaine, half decay time 4·9 ms ; $n=10$; tubocurarine, half decay time 2·4 ms ; $n=10$; $P=0·001$). The possibility that the change in time course of the end-plate potentials arose from changes in the electrical constants of the muscle fibres was then investigated.

Effect of procaine on passive electrical properties of muscle fibres

A second microelectrode, through which ' square ' pulses of current of varying intensity could be passed, was inserted as close as possible to the recording electrode. The insertion of this electrode was usually attended by a fall in the resting

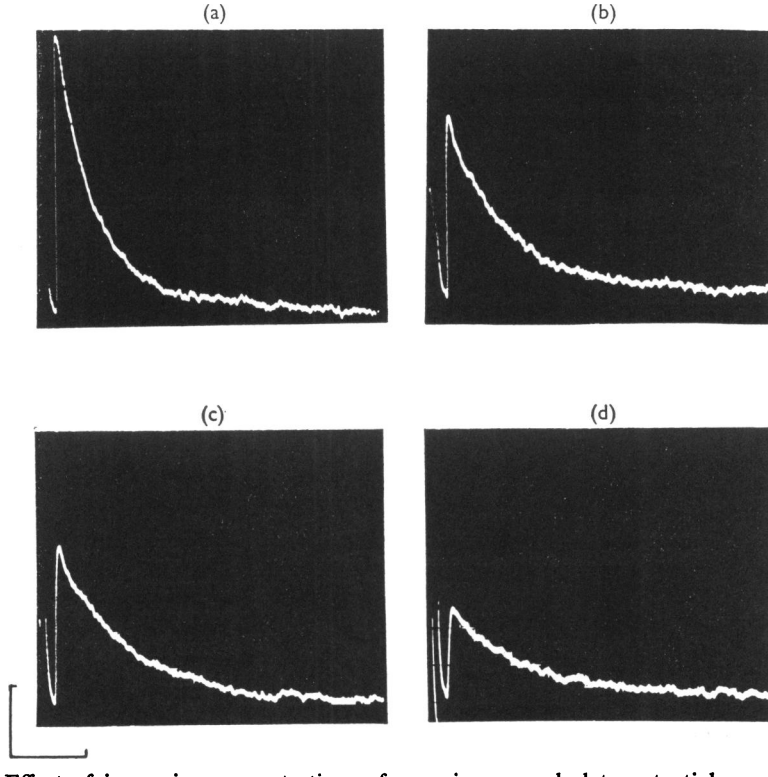

FIG. 2. Effect of increasing concentrations of procaine on end-plate potentials recorded from nerve-diaphragm paralysed by tubocurarine (0·0015 mM). (a), Control e.p.p. Procaine concentrations in (b), (c) and (d) are 0·05 mM, 0·1 mM and 0·2 mM, respectively. Between each addition of the procaine, the preparation was washed with ' fresh tubocurarine ' Krebs' solution ; the control responses after each washing was as in (a). Calibration bars, 2 mV and 10 ms.

potential of 5–10 mV. Recordings were only made at assumed zero electrode separation as the muscle fibre was damaged by repeated insertion of the second electrode. The local potentials and stimulating currents were recorded. The effective resistance of the fibre was determined from a plot of the potential change at the break of current flow (pulse width 30 ms) against current intensity ; T_m, the time constant, was the time taken for the potential to rise to 84% of its steady maximum value (assuming zero electrode separation, Table 1) (Hodgkin & Rushton, 1946). From the recordings summarized in Table 1, it will be seen that the effective resistance of the fibres was not altered by the addition of procaine (0·1 mM) to the Krebs solution (Inoue & Frank, 1962). A slight reduction in T_m was found. The model assumed for this analysis is adequate for the present problem but it will be appreciated that a more realistic model has been described by Falk & Fatt (1964).

Effect of procaine on the accumulation and decay of end-plate charge

It is already clear from the results in Table 1 that the slow decay of e.p.p. is not due to changes in the time constant of the muscle fibres. Information on the prolonged e.p.p. was sought using the method of Fatt & Katz (1951) in which the rate of accumulation and decay of end-plate charge at the end-plate region is determined. In a single fibre, recordings were made of the e.p.p. at various distances from the end-plate focus, the electrode being moved along the fibre until the end-plate focus had been passed and the e.p.p. declined in amplitude. The resting potential was checked after each recording. In a high proportion of experiments only two or three recordings could be obtained before the fibres were torn. The results described are from experiments where a complete set of recordings was obtained. The potential at time t, was determined from the e.p.p. traces and a graph of potential against distance from the end-plate focus was plotted for various values of t. The area under the curve of potential against distance from the end-plate focus is a measure of the charge displaced at t. The results from two experiments are shown in Figs. 3 and 4.

When neuromuscular transmission was blocked by tubocurarine (0·0015 mM), the end-plate charge reached a maximum in 2–3 ms and decayed exponentially with a time constant of 5·5 ms (5·4–5·6 ms) (Fig. 3). When procaine (0·1 mM) and tubocurarine (0·0015 mM) were both present the active phase was prolonged, the

TABLE 1. *Effect of procaine* (0·1 *mM*) *on muscle fibre cable constants*

	Effective resistance (MΩ)	Time constant (ms)	(*N*)	$R_m(\Omega \times Cm^2)$	$C_m(\mu F/cm^2)$
Control	1·04	10·0	18	2,080	4·8
0·1 mM procaine	1·09	8·4	19	2,270	3·7

R_m and C_m were calculated from the equations:

$$V = \tfrac{1}{2} I \sqrt{(r_m . r_i)} \exp [-X/\sqrt{(r_m/r_i)}]$$

where V= potential change produced by steady current I
r_m=transverse resistance of unit length of fibre membrane,
r_i=longitudinal resistance per unit length of fibre,
x=electrode separation;
and $T_m = C_m . R_m$
where C_m=membrane capacity per unit area,
R_m=transverse resistance of unit area of fibre (Fatt & Katz, 1951).

Zero electrode separation was assumed; the mean fibre diameter was determined on three separate diaphragms using a water immersion lens, measuring thirty fibres on each diaphragm; the mean fibre diameter was 30×10^{-4} cm. The value used for the specific resistivity of the myoplasm was that given by Boyd & Martin (1959) corrected to 31° C.

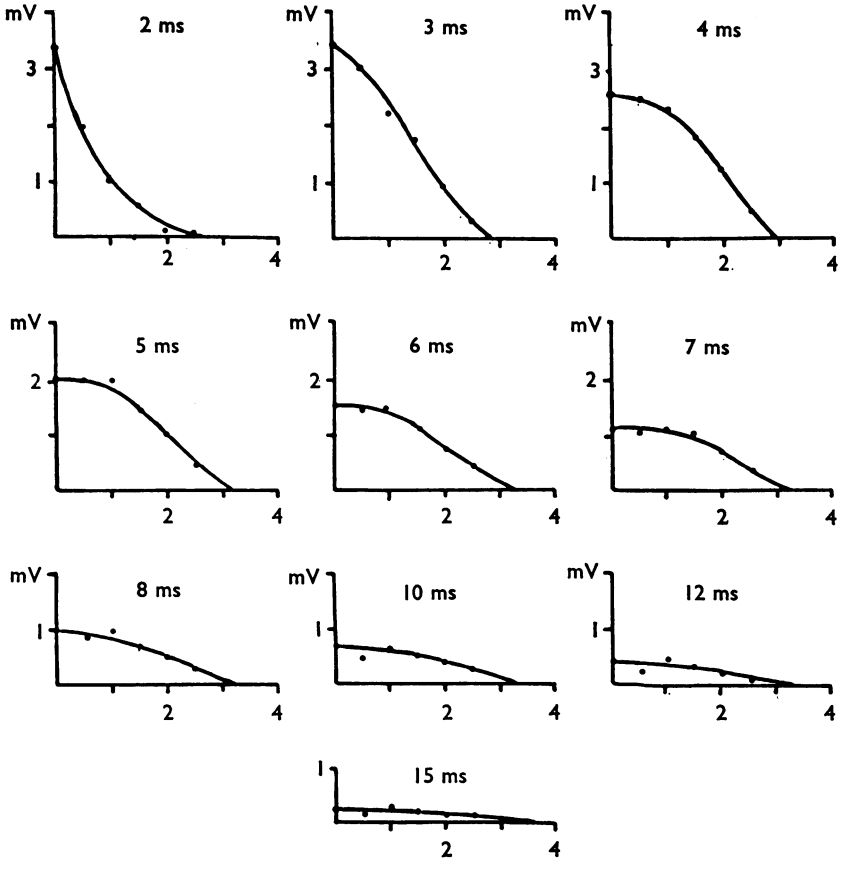

FIG. 3. (a), Spatial distribution of e.p.p. at various times after stimulus artifact in the presence of tubocurarine (0·0015 mM). The curves are constructed from e.p.ps recorded from the same fibre at various distances from the end-plate focus (1 = 0·345 mm). (b), Accumulation and decay of end-plate charge. The end-plate charge reached a maximum after 2–3 ms and decayed exponentially with a time constant of 5·4 ms.

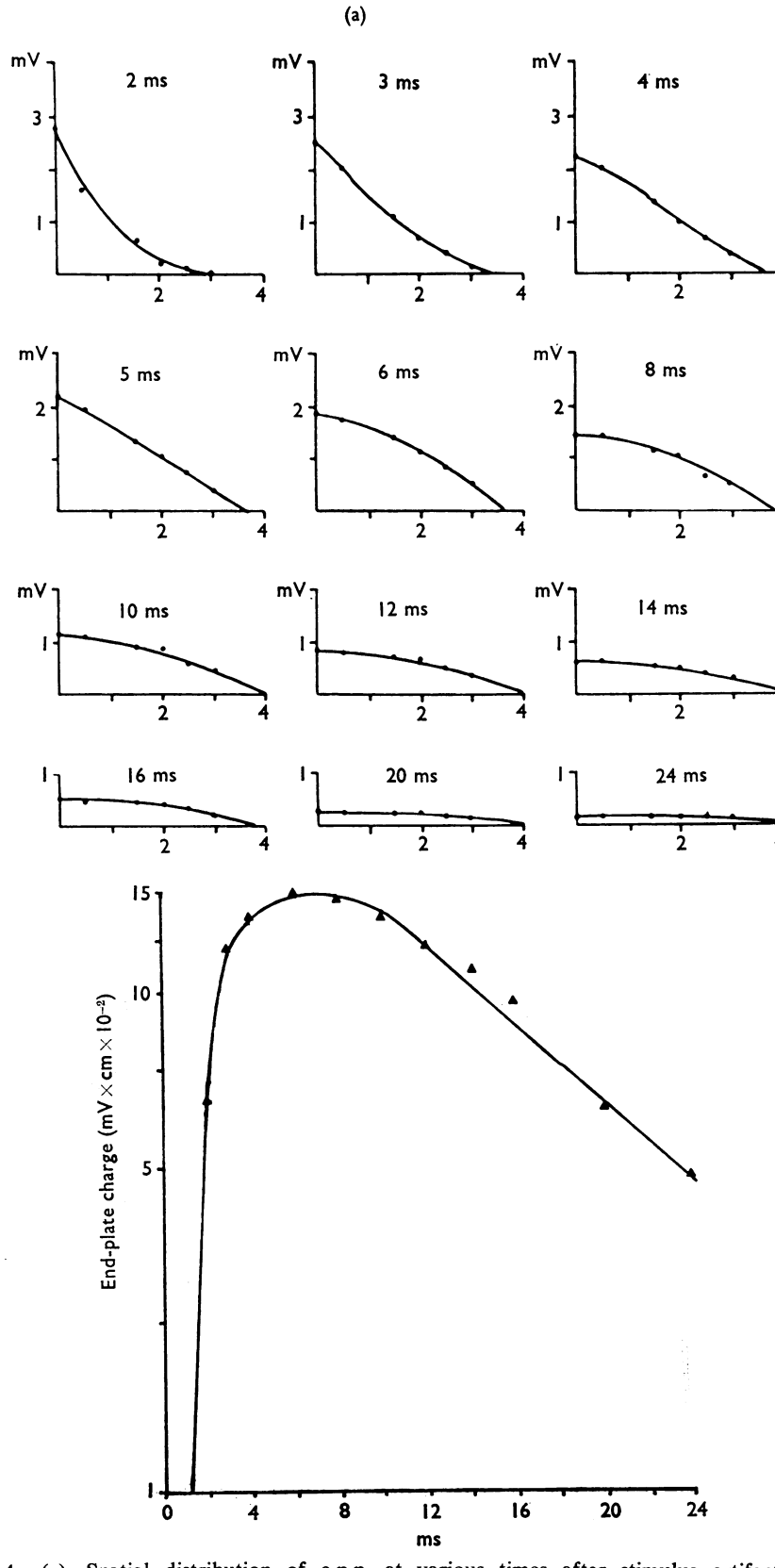

FIG. 4. (a), Spatial distribution of e.p.p. at various times after stimulus artifact in the presence of procaine (0·1 mM) and tubocurarine (0·0015 mM). (b), Accumulation and decay of end-plate charge (procaine 0·1 mM ; tubocurarine 0·0015 mM). The end-plate charge reached a maximum after 4–6 ms and decayed exponentially with a time constant of 13·4 ms.

maximum charge being displaced in 4–6 ms. The exponential decay of charge was also slowed, the time constant being 12·2 ms (10·7–13·4 ms) (Fig. 4).

Discussion

Procaine shortened the rise time of e.p.ps recorded from fibres paralysed by either tubocurarine or high extracellular magnesium concentration. Similar observations were made from recordings of spontaneous m.e.p.ps but the differences in time course, although consistent, were not always statistically significant. In contrast, the rate of decay of potential in each of the three series of experiments was much reduced. These observations are incompatible with the suggestion that local anaesthetics prolong the time during which transmitter is released by prolonging the duration of the nerve action potential (Takeuchi & Takeuchi, 1959). Such an effect would cause little change in the time course of the spontaneous m.e.p.ps but would prolong the rising phase of e.p.ps recorded from fibres paralysed by magnesium where it is assumed that only two or three quanta of transmitter are released (Ginsborg & Hamilton, 1968). The failure of procaine to prolong the rise times of either m.e.p.ps or e.p.ps implies that procaine does not cause changes in time course as a result of an anticholinesterase action (Hunt & Kuffler, 1950).

Procaine had no effect on muscle membrane resistance (Inoue & Frank, 1962). The muscle membrane capacity appeared to be slightly reduced ; the result may be due to experimental error in the determination of the apparent membrane capacity. Clearly, the prolongation of e.p.p. and m.e.p.p. does not result from changes in muscle cable constants.

When the time course of the accumulation and decay of end-plate charge was determined for muscles paralysed by tubocurarine alone, the maximum charge was found to be displaced approximately 1·5 ms after the start of the e.p.p. Subsequently the charge decayed exponentially with a time constant of about 5·5 ms ; the time constant for the decay of end-plate charge was approximately half that of the electric time constant. In the presence of procaine charge accumulated for up to 5 ms and then decayed with a time constant greater than the electrical time constant. The slow rate of decay of end-plate charge indicates that in the presence of procaine the reduction in fibre resistance brought about by transmitter action persists for several milliseconds after the normal cessation of transmitter action. It can be concluded that the prolongation of e.p.p. in the presence of procaine results from a prolongation of e.p.c. A similar conclusion was reached by Armstrong & Gage (1969).

We are indebted to Dr. B. L. Ginsborg for his advice during the preparation of the draft. G. D. S. H. was supported by a grant from the Medical Research Council.

REFERENCES

ARMSTRONG, C. M. & GAGE, P. W. (1968). Miniature end-plate currents in voltage clamped muscle fibres. *Nature, Lond.*, **218**, 363–365.

BOYD, I. A. & MARTIN, A. R. (1959). Membrane constants of mammalian muscle fibres. *J. Physiol., Lond.*, **17**, 450–457.

FALK, G. & FATT, P. (1964). Linear properties of striated muscle fibres observed with intracellular electrodes. *Proc. R. Soc. B.*, **160**, 69–123.

FATT, P. & KATZ, B. (1951). An analysis of the end-plate potential recorded with an intra-cellular electrode. *J. Physiol., Lond.*, **115**, 320–370.

FENG, T. P. (1941). The local activity around the skeletal n–m junctions produced by nerve impulses. *Biol. Symp.*, **3**, 121–136.

FURUKAWA, T. (1957). Properties of the procaine end-plate potential. *Jap. J. Physiol.*, **7**, 199–212.

GINSBORG, B. L. & HAMILTON, J. T. (1968). The effect of caesium ions on neuromuscular transmission in the frog. *Q. Jl exp. Physiol.*, **53**, 162–169.

HIRST, G. D. S. & WOOD, D. R. (1969). The action of procaine on transmission at the mammalian neuromuscular junction. *Br. J. Pharmac.*, **35**, 353*P*.

HIRST, G. D. S. & WOOD, D. R. (1971). On the neuromuscular paralysis produced by procaine. *Br. J. Pharmac.*, **41**, 94–104.

HODGKIN, A. L. & RUSHTON, W. A. H. (1946). The electrical constants of a crustacean nerve fibre. *Proc. R. Soc. B.*, **133**, 444–479.

HUNT, C. C. & KUFFLER, S. W. (1950). Pharmacology of the neuromuscular junction. *Pharmac) Rev.*, **2**, 96–120.

INOUE, F. & FRANK, G. B. (1962). Action of procaine on frog skeletal muscle. *J. Pharmac. exp. Ther.*, **136**, 190–196.

MAENO, T. (1966). Analysis of sodium and potassium conductances in the procaine end-plate. potential. *J. Physiol., Lond.*, **183**, 592–606.

STEINBACH, A. B. (1968a). Alteration by Xylocaine (lidocaine) and its derivatives of the time course of the end-plate potential. *J. gen. Physiol.*, **52**, 144–161.

STEINBACH, A. B. (1968b). A kinetic model for the action of Xylocaine on receptors for acetylcholine. *J. gen. Physiol.*, **52**, 162–180.

TAKEUCHI, A. & TAKEUCHI, N. (1959). Active phase of frog's end-plate potential. *J. Neurophysiol.*, **22**, 395–411.

(*Received August*, 24, 1970.)