Short communications

Prostaglandin E_1 and noradrenaline at the neuromuscular junction

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The effects of prostaglandin E_1 (PGE_1) on neuromuscular transmission in the frog nerve-sartorius muscle preparation were studied with intracellular recording techniques. PGE_1 was inactive in all concentrations examined; both spontaneous miniature end-plate potentials and evoked end-plate potentials were unchanged after preparations were bathed in up to $2{\cdot}2\times10^{-5}$M (8 μg/ml) PGE_1. PGE_1 did not prevent the increase in end-plate potential amplitude which frequently occurred on addition of noradrenaline to the bathing solution.

Although it has been reported that PGE_1 is released from rat phrenic-nerve diaphragms on nerve stimulation (Ramwell, Shaw & Kucharski, 1965), little is known of its action at the neuromuscular junction. It has been suggested that transmitter release reflects the concentration of adenosine 3',5'-monophosphate (cyclic-AMP) in the nerve terminal (Goldberg & Singer, 1969): if this were so, PGE_1 might be expected to modify transmitter output by affecting adenyl cyclase activity (Horton, 1969). Furthermore PGE_1 might be expected to interfere with the action of noradrenaline, which is known to increase the output of transmitter (Jenkinson, Stamenović & Whitaker, 1968).

Methods.—Isolated sciatic nerve sartorius muscle preparations of autumn *Rana temporaria* were used. The bathing solution contained (mM) Na^+, 123·3; K^+, 2·5; Mg^{++}, 6·0; Ca^{++}, 0·9; Cl^-, 136·6 and phosphates, 2·0; pH, 6·8, temperature, 20–25° C. Mg^{++} was occasionally adjusted within the range 5·0–7·0 mM by replacement of Na^+ to give end-plate potentials (e.p.p.) of suitable amplitude. The bath volume was 5 ml.

Intracellular techniques were conventional (Fatt & Katz, 1951). Miniature end-plate potentials (m.e.p.p.) were recorded on continuously moving film; evoked e.p.ps were averaged after amplification, with a Biomac 1000. Stimuli were either single or paired impulses, separated by 20 ms, at intervals of 3·2 seconds. Stimulation was begun at least 20 min before recording.

Drugs were added to the bath as concentrated solutions with a syringe, the bath being stirred. Care was taken to ensure that the drug was dissolved in physiological fluid with a similar composition to that of the bath. Fresh preparations were used in each experiment to test the effects of PGE_1 or noradrenaline.

Drugs used were noradrenaline bitartrate (B.D.H.) and PGE_1, kindly supplied by Dr. J. E. Pike, Upjohn Company, Kalamazoo, Michigan, U.S.A.

Results.—PGE_1 was added to the bath to give final concentrations of up to $2{\cdot}2\times10^{-5}$M (8 μg/ml). This produced neither an immediate change in the resting potential of impaled muscle fibres nor one of more than a few millivolts during periods of up to 90 minutes.

Muscle fibres were selected where the amplitude of the focally recorded e.p.ps were greater than 5 mV so that their coefficient of variation might be relatively small. Sixty-four successive sweeps were averaged and the mean e.p.p. recorded. Successive mean e.p.ps were examined to make certain that the mean e.p.p. was stable to within 5%, before the addition of PGE_1. Mean e.p.ps were photographed for up to 1 h after the addition of the prostaglandin. In seven experiments, the final concentration of PGE_1 was $1{\cdot}1\times10^{-5}$M. In four no change (<5%) was observed after its addition, in the three remaining the changes were +5%, −6% and −6%. No change in either the frequency or mean amplitude of the m.e.p.ps was observed.

In a few experiments where the nerve was stimulated by a pair of impulses (see **Methods**) the prostaglandin did not affect the mean amplitude of either the first or the second, facilitated, e.p.p. (Fig. 1A).

Figure 1B illustrates the fact that PGE_1 does not prevent noradrenaline causing an increase in the mean e.p.p. amplitude. In seven preparations the effect on the mean e.p.p. (see above) of noradrenaline alone (final bath concentration 1×10^{-5}M) was examined; the observed changes were −10%, −8%, <5%, +8%, +10%,

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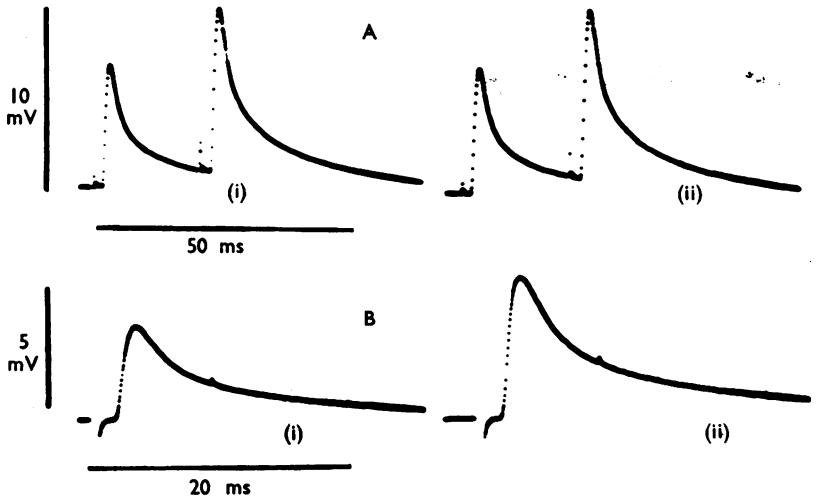

FIG. 1. Averaged end-plate potentials recorded from two muscle fibres in preparations paralysed by reduced extracellular calcium and increased extracellular magnesium concentrations. Figure 1A shows paired mean e.p.ps recorded (i) before, and (ii), 6 min after the addition of PGE_1 ($1{\cdot}1\times10^{-5}$M). Figure 1B shows the mean e.p.ps in the presence of $1{\cdot}1\times10^{-5}$M PGE_1 (i) before, and (ii), 6 min after the addition of noradrenaline (1×10^{-5}M).

+17%, +33%. In the nine experiments in which PGE_1 ($1{\cdot}1\times10^{-5}$M) was present for periods of up to 1 h before the addition of the noradrenaline the changes were −8%, <5% (five experiments), +12%, +20%, +44%. The mean increase caused by noradrenaline happened to be about 7% whether or not the prostaglandin was present.

Discussion.—The absence of any change either in the amplitude of miniature end-plate potentials or of the mean end-plate potential shows that PGE_1, even in the relatively high concentration used in these experiments, has no effect either on the postjunctional sensitivity to the transmitter or on its release.

PGE_1 does not appear to affect the increase in output of the transmitter caused by noradrenaline. As has been previously found (Jenkinson *et al.*, 1968), this increase is itself somewhat variable. In our experiments it occurred in four out of seven preparations; in the presence of PGE_1 an increase occurred in three out of nine. Although there is no significant difference between these proportions ($P>0{\cdot}1$, 2×2 contingency tables), to establish that there is no interaction at all would require a greater number of experiments than the supply of PGE_1 permitted.

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