

Short communications

Action of adenosine triphosphate on endplate potentials recorded from muscle fibres of the rat-diaphragm and frog sartorius

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Evoked and spontaneous endplate potentials (e.p.ps) were recorded with intracellular electrodes from fibres of the rat diaphragm and the frog sartorius muscle. The amplitudes of the evoked e.p.ps were reduced to about one-half their control values in the presence of 0.1 mM and 0.2 mM of adenosine triphosphate (ATP). The amplitude of the spontaneous endplate potentials (miniature e.p.ps) was unaffected by ATP but their frequency was reduced.

Silinsky & Hubbard (1973) have reported that stimulation of the phrenic nerve of the rat may cause the release of ATP from the nerve terminals. It seems of interest therefore to investigate the action of ATP on neuromuscular transmission. That ATP might have some effect is already

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suggested by the fact that the release of the transmitter is reduced by adenosine (Ginsborg & Hirst, 1972) and adenosine monophosphate (AMP) (Ginsborg, Hirst, Maizels & Walker, 1973).

Methods.—Phrenic nerve rat diaphragm preparations were taken from rats of the Piebald Viral Glaxo or Wistar strains. The bathing solution contained (mM) NaCl 117, KCl 5, NaHCO₃ 25, NaH₂PO₄ 1.2, glucose 11 and the concentrations of Ca²⁺ and Mg²⁺ were adjusted to prevent twitches of the muscle in response to nerve stimulation. The solution was aerated with 95% O₂:5%CO₂. Frog nerve-sartorius muscle preparations were taken from *Rana temporaria*. The bathing solution contained (mM) NaCl 117, KCl 2.5, NaH₂PO₄ 1, Na₂HPO₄ 1. The concentrations of Ca²⁺ and Mg²⁺ were adjusted as for the rat preparations. In both preparations the bath volume was 3 ml and the solutions flowed continuously at a rate of 2 to 3 ml/minute. The motor nerves were stimulated supramaximally at 0.5 Hz. Intracellular techniques were conventional (Fatt & Katz, 1951). Miniature endplate potentials (min. e.p.ps) were recorded on continuously moving film: evoked e.p.ps were averaged after amplification, with a Biomac 1000.

Results.—Figure 1 illustrates the reduction caused by two concentrations, 0.02 mM and 0.1 mM, of ATP in the averages of 64 consecutive responses to nerve stimulation of a muscle fibre in the rat diaphragm. Miniature e.p.ps were also recorded in this experiment and their frequency was reduced to about 75% and

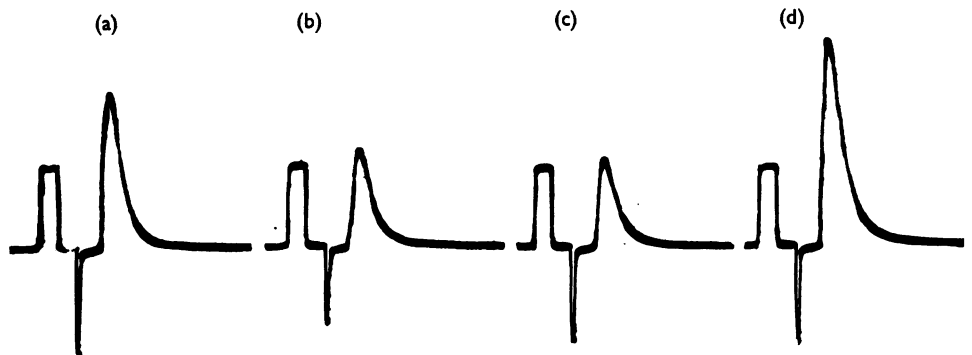


FIG. 1. Effect of ATP on computed averages of 64 evoked endplate potentials from a muscle fibre of the rat diaphragm. Each trace is preceded by a 2 mV calibration pulse of 2 ms duration. (a) Pre-control, quantum content calculated to be 18; (b) 0.02 mM ATP, quantum content 11; (c) 0.1 mM ATP, quantum content 9; (d) Post-control, quantum content 20. Time from start of recording in minutes are respectively 5, 15, 35 and 65 in (a) to (d). The solution contained 3 mM Ca²⁺, and 30 mM Mg²⁺.

65% of the control value by 0.02 mM and 0.1 mM of ATP, respectively. Their amplitudes were unchanged in the presence of either concentration of ATP. Thus the effect of ATP consisted of a reduction in the quantal content of the e.p.ps, to about 50% to 60% of the control value. Similar reductions in output of transmitter in the presence of ATP were observed in 11 separate experiments on rat preparations and 9 on frog preparations. The control quantal content varied from about 1 to about 20 and the concentrations of ATP from 0.1 mM to 0.2 mM. Although no significant effect on the amplitude of the miniature e.p.ps was seen, their frequency was reduced in the rat preparation to about 30% to 70% of the control values in 0.2 mM ATP. In the frog the effects on frequency were more variable and in some fibres no reduction was seen.

In a single experiment in an innervated frog sartorius preparation, where the twitch was prevented by 2 μ M (+)-tubocurarine, ATP 0.2 mM reduced the amplitude of the e.p.p. to 40% of the control value.

Discussion.—ATP, 0.01 mM to 0.2 mM had no post-synaptic effect, since the amplitude of the miniature e.p.ps was unchanged. However, ATP had a consistent presynaptic effect, reducing the output of the transmitter to nerve stimulation in both the rat and the frog. The reduction in spontaneous transmitter release in the frog was not so clear cut as in the rat: small effects were however more difficult to establish in the frog since the control frequency of miniature e.p.ps was itself subject to such large variations.

A point of interest in these results is the recent finding of Silinsky & Hubbard (1973) that ATP is released from motor nerve terminals of the rat apparently together with acetylcholine. If the two substances are released with the same time course from the same region of the nerve terminal the local concentrations of ATP around it, may transiently be of the same order as that of the released acetylcholine (Silinsky, personal communication), and thus the ATP concentrations may be of the same order or possibly greater than the concentrations of ATP used in the present experiments. Thus if the same region of the nerve terminal is that affected by ATP, it is conceivable that the release of ATP may contribute to the depression of the response to repetitive nerve stimulation at high frequency and high outputs of transmitter (see e.g. Otsuka, Endo & Nonomura, 1962).

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(Received July 11, 1973)