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THE EFFECTS OF ADENOSINE TRIPHOSPHATE AND ADENOSINE DIPHOSPHATE ON TRANSMISSION AT THE RAT AND FROG NEUROMUSCULAR JUNCTIONS

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1 The effects of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) were investigated on evoked end-plate potentials (e.p.ps) and on miniature end-plate potentials (min. e.p.ps) recorded from muscle fibres of the rat diaphragm and the frog sartorius.

2 ATP and ADP decreased the quantum content of the e.p.ps and the frequency of the min. e.p.ps. The maximum effects produced by the two substances were similar.

3 The potency of ATP was found to be similar to that of adenosine. In the presence of adenosine, in a concentration producing its maximum effect, the addition of ATP had no further effect. This is compatible with the idea that ATP acts in the same way as adenosine.

Introduction

It has been shown that adenosine and adenosine monophosphate (AMP) decrease the amount of transmitter released from nerve endings at the neuromuscular junction in the rat diaphragm (Ginsborg & Hirst, 1972; Ginsborg, Hirst, Maizels & Walker, 1973). At many sites where these substances act it is found that adenosine triphosphate (ATP) and adenosine diphosphate (ADP) are also effective, and frequently more potent than adenosine (see e.g. Burnstock, 1972). It therefore seemed of interest to compare the effects of ATP and ADP with those of adenosine. A brief account of some of the results has already appeared (Ribeiro & Walker, 1973).

Methods

The experiments were carried out, at room temperature, on isolated preparations of the rat phrenic nerve-diaphragm or of the frog nerve-sartorius. The rats used were Piebald Viral Glaxo or Wistar strains, of either sex and of not more than 200 g in weight; the frogs were *Rana temporaria*. The preparations were mounted in a Perspex chamber through which the solutions flowed continuously at a rate of 2 to 3 ml/min via a roller pump (Watson Marlow); the bath volume was 3 ml, the level being kept constant by suction. Solutions were changed by transferring the inlet tube of the pump from one flask to another.

End-plate potentials (e.p.ps) were recorded in the conventional way (Fatt & Katz, 1951) with intracellular electrodes filled with 3M KCl and of 10 to 20 MΩ resistance; the bath electrode was of the kind described by Beranek, Martin & Wickelgren (1970). Evoked responses to 64 or 128 stimuli were averaged, after amplification, with a Biomac 1000 computer. The output of the computer was coupled to a pen recorder. The nerve was stimulated at a constant rate throughout the experiments and at least 30 min to one hour before recording was begun. Miniature end-plate potentials (min. e.p.ps) were recorded on continuously moving film throughout the period required for obtaining an average evoked response. Quantum contents were estimated as the ratio of the average amplitude of the evoked e.p.ps to the average amplitude of the min. e.p.ps recorded during the same period. Where necessary in the calculation of quantum contents, correction was made for 'non-linear summation' by applying Martin's (1955) formula $m = E\bar{U}/\bar{u}(E - \bar{U})$, where $\bar{U}$ is the average amplitude of the evoked e.p.p., $\bar{u}$ is the mean amplitude of the min. e.p.ps and E is the driving force for the action of the transmitter. E was assumed to be 55 mV in the rat and 75 mV in the frog (see Hubbard, Llinás & Quastel, 1969; Ginsborg & Hirst, 1972).

The usual procedure was to continue to record averages in the same solution until a stable value was obtained, i.e. until two successive averages differed by less than 5%.

The bathing solution for the rat diaphragm contained (mM) NaCl 117, KCl 5, $NaHCO_3$ 25,

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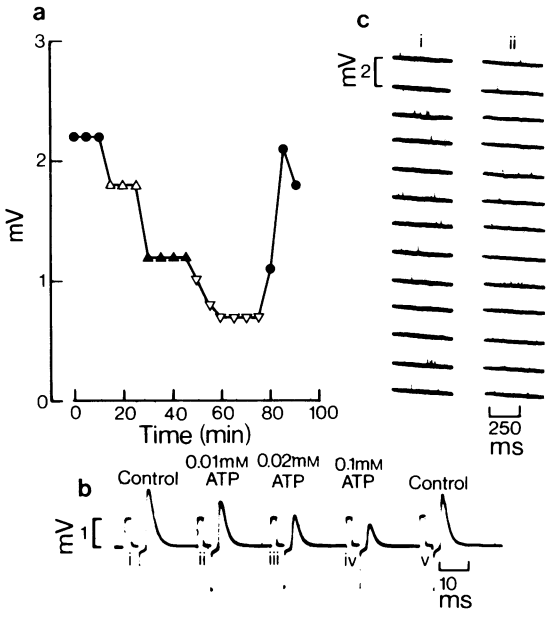

Figure 1 Effect of ATP on the average amplitude of end-plate potentials (e.p.ps) and the frequency of miniature end-plate potentials (min. e.p.ps) (rat diaphragm). Solutions contained 3 mM Ca^{2+} and 30 mM Mg^{2+} which prevented muscle action potentials and twitches. (a) The ordinates are the computed averages of 128 successive e.p.ps at 0.5 Hz and the abscissae are the times the averaging began. Control (•); ATP 0.01 mM (△); 0.02 mM (▲); 0.1 mM (▽). (b) Pen-recorder traces of averages corresponding to (i) 5 (ii) 20 (iii) 40 (iv) 70 (v) 85 min for time in (a). Each response is preceded by a 1 mV calibration pulse. (c) Samples of min. e.p.ps: they correspond to (i) 5 and (ii) 70 minutes.

NaH_2PO_4 1.2, glucose 11, and the concentrations of Ca^{2+} and Mg^{2+} were adjusted to prevent twitches of the muscle in response to nerve stimulation. The bathing solution for the frog sartorius contained (mM) NaCl 117, KCl 2.5, NaH_2PO_4 1 and Na_2HPO_4 1. The concentrations of Ca^{2+} and Mg^{2+} were adjusted as for the rat diaphragm. In a few experiments the muscle twitches were prevented by addition of tubocurarine to a solution containing 1.8 mM $CaCl_2$ and 1 mM $MgCl_2$. The drugs used were ATP, ADP, adenosine (Sigma) and tubocurarine (Burroughs Wellcome).

Results

The effects of ATP on transmitter release

Figure 1 illustrates the effect of ATP in concentrations of 0.01, 0.02 and 0.1 mM on the amplitude of the e.p.ps recorded from a muscle fibre of the rat diaphragm. The e.p.ps (Figure 1b) were evoked by stimulation of the phrenic nerve at the rate of 0.5 Hz. Groups of 128 successive e.p.ps were averaged automatically and the averages recorded at intervals of about 5 minutes. The min. e.p.ps (Figure 1c) were photographed during the averaging period (i) before, and (ii) at about 70 min after the perfusion solution had been changed to one containing ATP. As can be seen, ATP reduced the amplitude of the e.p.ps and the frequency of the min. e.p.ps. The average amplitude of the min. e.p.ps was not affected, indicating that ATP had no post-junctional effect. The reduction in the e.p.p. amplitude must thus be attributed to a reduction in quantum content, i.e. to a reduction in the amount of transmitter released.

Similar experiments were carried out on 15 fibres and the results are summarized in Table 1. It can be seen that 0.2 mM causes no greater reduction in quantum content than 0.1 mM ATP. There thus appears to be a maximum effect (corresponding to an approximately 50% reduction). This was confirmed in a subsequent experiment (see Figure 2) in which 4 mM had no greater effect than 0.2 mM ATP. From the experiments where several concentrations were tested on the same fibre, it appeared that the maximum effect requires between 0.02 and 0.1 mM ATP, although, exceptionally, it was found that in a few fibres 0.01 mM was sufficient. The reduction in the frequency of min. e.p.ps appears also to be concentration dependent.

No tachyphylaxis to the action of ATP was observed. Thus no diminution was seen in its effect on the e.p.p. during a period of 3 h perfusion by 0.2 mM ATP (cf. Mihich, Clarke & Philips, 1964).

Table 2 summarizes the results from 16 end-plates of the frog sartorius preparation, exposed to ATP in the same concentrations as used for the rat diaphragm. At all the end-plates, ATP reduced the amplitude of the e.p.ps. Min. e.p.ps were not recorded in the majority of experiments, but in those in which they were recorded, their mean amplitude was unchanged by ATP. It was therefore assumed that the amplitudes of the e.p.ps (Table 2, col. 2b) were directly proportional to the quantum contents. There were too few results for a detailed analysis, but it appeared that, as in the rat, the lower concentrations of ATP, i.e. 0.01 and 0.02 mM have submaximum effects on the quantum content.

The results so far described suggest that the percentage reduction caused by ATP of the quantum content of the e.p.p. is independent of its initial level up to a quantum content of about

Table 1 Effect of ATP on the quantum content of the end-plate potential (e.p.p.) and on the frequency of miniature end-plate potentials (min. e.p.ps) recorded from rat diaphragm fibres

Concentration of ATP (mM)	*Reduced quantum content as fraction of control value* *Mean, range, (number of expts)*	*Reduced frequency of min. e.p.ps as fraction of control value* *Mean, range, (number of expts)*
0.01	0.69 0.46 to 0.80 (7)	0.80 0.66 to 0.96 (5)
0.02	0.63 0.51 to 0.77 (5)	0.78 0.59 to 1.0 (5)
0.1	0.48 0.35 to 0.63 (9)	0.71 0.64 to 0.78 (2)
0.2	0.50 0.44 to 0.60 (7)	0.57 0.31 to 0.73 (7)

Neuromuscular transmission was blocked by bathing the preparation in solutions containing either low calcium or high magnesium concentrations. The ranges were from 0.2 mM Ca^{2+}, zero Mg^{2+} to 4 mM Ca^{2+}, 30 mM Mg^{2+}. The mean amplitude of the e.p.ps varied from about 0.3 to 4 mV with corresponding mean quantum contents from 0.5 to 20. In some of the experiments where the quantum content was low it was estimated by counting 'failures' (see del Castillo & Katz, 1954). For any one ATP concentration no obvious differences were seen between the results with the different concentrations of Ca or Mg or with different initial quantum contents.

Table 2 Effect of ATP on the quantum content of the end-plate potential (e.p.p.) and on the frequency of miniature end-plate potentials (min. e.p.ps) recorded from frog sartorius fibres

(1)	(2)		(3)
Concentration of ATP (mM)	*Reduced quantum content as fraction of control value* *Mean, range, (number of expts)*		*Reduced frequency of min. e.p.ps as fraction of control value* *Mean, range, (number of expts)*
	a	*b*	
0.01	0.68 0.61 to 0.74 (2)	0.71 0.59 to 0.88 (8)	0.79 0.69 to 0.87 (3)
0.02	0.60 0.48 to 0.72 (2)	0.71 0.53 to 0.81 (5)	0.71 0.69 to 0.73 (2)
0.1	– –	0.50 0.40 to 0.60 (2)	– –
0.2	0.50 0.38 to 0.62 (8)	0.51 0.35 to 0.76 (21)	0.71 0.62 to 0.83 (3)

Neuromuscular transmission was blocked by bathing the preparation in solutions containing either low calcium or high magnesium concentrations. The ranges were from 0.2 mM Ca^{2+}, zero Mg^{2+} to 4 mM Ca^{2+}, 32 mM Mg^{2+}. The mean amplitude of the e.p.ps varied from about 0.7 to 4.8 mV with mean quantum contents from 1.4 to 15.3. The values in column 2a were obtained by direct measurement or by counting 'failures'. In column 2b the values were obtained from the amplitudes of the e.p.ps.

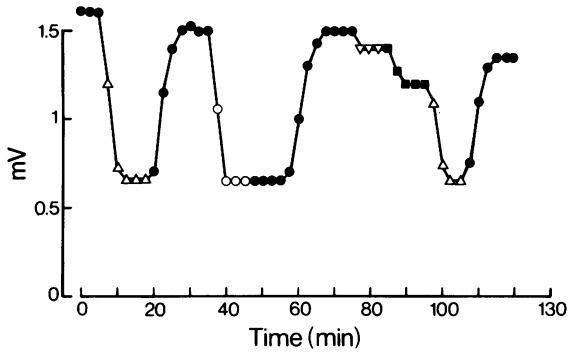

Figure 2 Effect of ATP in the presence of tubocurarine (rat diaphragm). The ordinates are the averages of 128 successive e.p.ps at the times shown. Solutions contained 2.5 mM Ca^{2+}, 1.2 mM Mg^{2+} and 2×10^{-6}M tubocurarine. Control (●); ATP 0.2 mM (△), 4.0 mM (○), 0.05 mM (■), 0.02 mM (▽).

20 (see Table 1). It was of interest to see if ATP was equally effective at higher quantum contents. Figure 2 shows that this was the case. In two experiments where tubocurarine (2×10^{-6}M in the rat and 2×10^{-5}M in the frog) was present, in solutions containing normal concentrations of calcium and magnesium, reductions to about 0.4 of the control value were produced by ATP. These results are similar to those obtained with the lower quantum contents.

Comparison between the effects of ADP, ATP and adenosine

The effects of ADP were investigated at 4 end-plates of the rat diaphragm and 5 end-plates of the frog sartorius. The concentrations used were the same as those of ATP and the results obtained were similar. It is already known that the magnitude of the effect of adenosine (Ginsborg & Hirst, 1972) is also similar to that of ATP and it seemed of interest to make a closer comparison of the maximum effects produced by the three substances. In view of the large scatter in individual results (see e.g. Tables 1 and 2), ATP and ADP were each compared directly with adenosine. From Figures 3 and 4 it can be seen that the three substances produce identical effects.

Comparison of the potencies of ATP and adenosine

The potencies of ATP and adenosine were compared by finding the concentrations necessary to produce approximately equal submaximum effects on e.p.ps recorded from the same endplate. In an experiment on the rat diaphragm bathed in a solution containing 3 mM Ca^{2+} and 30 mM Mg^{2+} it was found that 0.005 mM adenosine caused a smaller reduction than 0.01 mM ATP and that 0.005 mM ATP caused a smaller reduction than 0.01 mM adenosine. Hence adenosine must be less than twice as potent as ATP and ATP must be less than twice as potent as adenosine. Thus ATP and adenosine do not differ in potency by more than a factor of 2.

The effect of ATP in the presence of adenosine

The similarities in the potencies and in the maximum effects of ATP, ADP and adenosine strongly suggest that these substances all act on the nerve terminal in the same way. Further evidence which supports this idea is shown in Figure 5. In the experiment illustrated, adenosine in a concentration producing its maximum effect was first applied. The addition of ATP in a similar concentration now produced no further effect. This is consistent with the idea that the two substances act by a common saturable mechanism.

Discussion

The effect on transmitter release from motor-nerve terminals of a number of nucleotides and nucleosides has now been tested. Of these adenine, inosine, guanosine, uridine and cytidine (cytidine, not cystine as misprinted in the original communication) (Ginsborg *et al.*, 1973) were inactive; adenosine (Ginsborg & Hirst, 1972), AMP (Ginsborg *et al.*, 1973) ATP and ADP (present results) were active to the same extent and in the same concentrations. The same specificity obtains for substances which increase the 3′,5′-cyclic AMP content of cerebral cortex slices (Sattin & Rall, 1970). This is of interest in view of the idea frequently put forward that acetylcholine output requires the presence of cyclic AMP in the nerve terminals (e.g. Rasmussen & Tenenhouse, 1968). Whether there is any connection between transmitter release and cyclic AMP is an open question. Thus the similar specificities might only be coincidental; on the other hand a common receptor might mediate both an increase in cyclic AMP and whatever process is ultimately involved in reduction of transmitter release (Ginsborg & Hirst, 1972). There seems at present however no reason to believe that cyclic AMP is necessarily involved in the process of transmitter release (see also Miyamoto & Breckenbridge, 1974).

The present results also show that the receptors associated with the effect of adenosine and its derivatives on transmitter release from motor

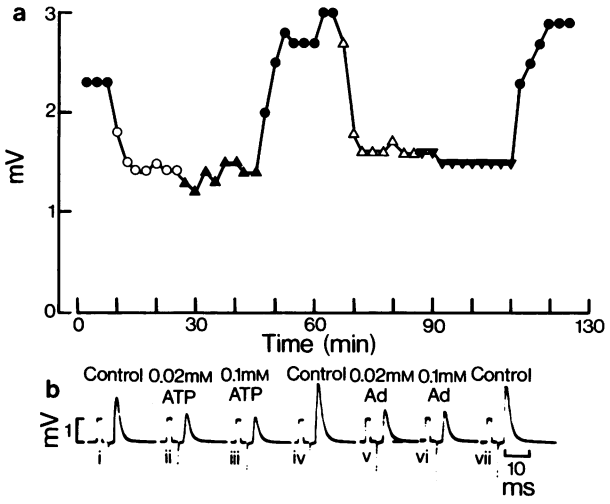

Figure 3 Comparison of effects of ATP with adenosine (Ad), (rat diaphragm). In (a) the ordinates are the averages of 64 successive e.p.ps. Control (●); ATP 0.02 mM (○), 0.1 mM (▲); adenosine 0.02 mM (△), 0.1 mM (▼). (b) Pen-recorder traces corresponding to 5, 17.5, 30, 52.5, 77.5, 97.5 and 122.5 min respectively (i-vii) in (a). For details see Figure 1.

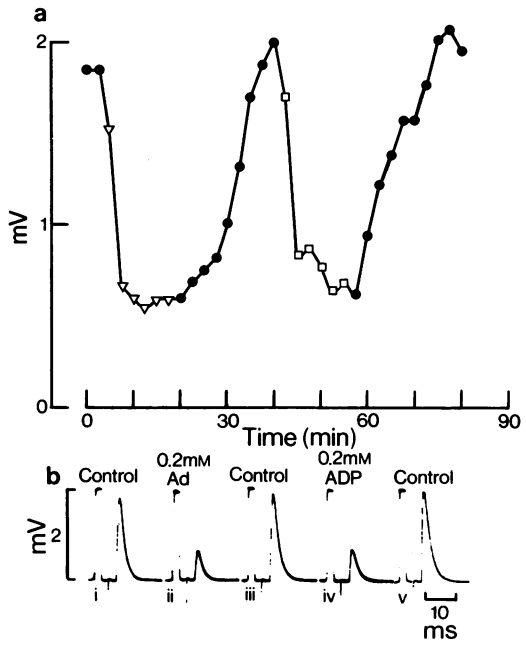

Figure 4 Comparison of effects of ADP with adenosine (Ad), (rat diaphragm). In (a) the ordinates are the averages of 64 successive e.p.ps. Control (●); adenosine 0.2 mM (▽); ATP 0.2 mM (□). (b) Pen-recorder traces corresponding (i-v) to 2.5, 12.5, 37.5, 52.5 and 75 min respectively in (a). For details see Figure 1.

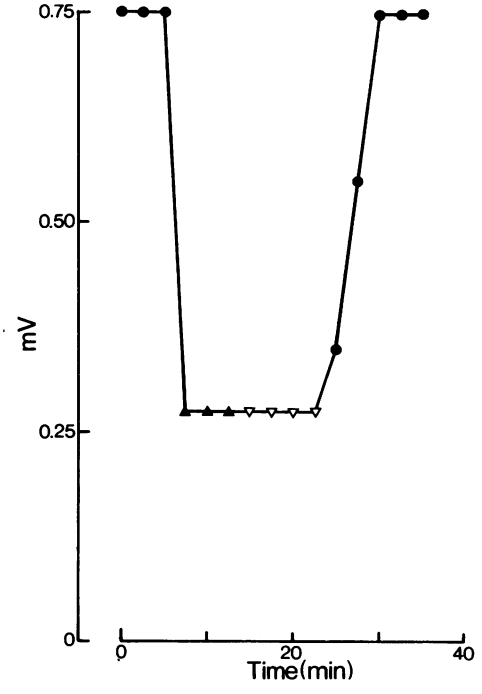

Figure 5 Effect of ATP in the presence of adenosine. Each substance was used in a concentration that alone produced a maximum effect (rat diaphragm). Ordinates are the averages of 128 successive e.p.ps. Solutions contained 2 mM Ca^{2+} and 18 mM Mg^{2+}. Control (●); adenosine 0.2 mM (▲); adenosine 0.2 mM plus ATP 0.2 mM (▽).

nerve are different from the smooth muscle receptors. For example, as inhibitors of spontaneous activity of taenia coli (Axelsson & Holmberg, 1969) ATP and ADP are 3 to 4 times more potent than adenosine and as inhibitors of coronary vessels (Winbury, Papierski, Hemmer & Hambourger, 1953; Wolf & Berne, 1956) they are about 100 times more potent than adenosine. As inhibitors of potassium induced contractures of taenia coli (Axelsson & Holmberg, 1969) adenosine, ATP and ADP are, as in the present experiments, equipotent; but in that situation adenine is also a potent inhibitor whereas it has no effect on transmitter release from the motor nerve.

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References

AXELSSON, J. & HOLMBERG, B. (1969). The effects of extra-cellularly applied ATP and related compounds on the electrical and mechanical activity of the smooth muscle of taenia coli of the guinea-pig. *Acta physiol. scand.*, **75**, 149-156.

BERANEK, R., MARTIN, A.R. & WICKELGREN, W.O. (1970). Effects of iontophoretically applied drugs on spinal interneurones of the lamprey. *J. Physiol., Lond.*, **207**, 653-665.

BURNSTOCK, G. (1972). Purinergic nerves. *Pharmac. Rev.*, **24**, 509-581.

DEL CASTILLO, J. & KATZ, B. (1954). Quantal components of the end-plate potential. *J. Physiol., Lond.*, **124**, 560-573.

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

GINSBORG, B.L. & HIRST, G.D.S. (1972). The effect of adenosine on the release of the transmitter from the phrenic nerve of the rat. *J. Physiol., Lond.*, **224**, 629-645.

GINSBORG, B.L., HIRST, G.D.S., MAIZELS, J.V. & WALKER, J. (1973). Specificity of adenosine on transmitter output at the neuro-muscular junction. *Br. J. Pharmac.*, **47**, 637P.

HUBBARD, J.I., LLINÁS, R. & QUASTEL, D.M.J. (1969). *Electrophysiological analysis of synaptic transmission*, p. 135, London: Arnold.

MARTIN, A.R. (1955). A further study of the statistical composition of the end-plate potential. *J. Physiol., Lond.*, **130**, 114-122.

MIHICH, E., CLARKE, D.A. & PHILIPS, F.S. (1964). Effect of adenosine analogs on isolated intestine and uterus. *J. Pharmac. exp. Ther.*, **111**, 335-342.

MIYAMOTO, M.D. & BRECKENRIDGE, B.M. (1974). A cyclic adenosine monophosphate link in the catecholamine enhancement of transmitter release at the neuromuscular junction. *J. gen. Physiol.*, **63**, 609-624.

RASMUSSEN, H. & TENENHOUSE, A. (1968). Cyclic adenosine monophosphate, Ca^{++} and membranes. *Proc. natn. Acad. Sci. U.S.A.*, **59**, 1364-1370.

RIBEIRO, J.A. & WALKER, J. (1973). Action of adenosine triphosphate on end-plate potentials recorded from muscle fibres of the rat diaphragm and frog sartorius. *Br. J. Pharmac.*, **49**, 724-725.

SATTIN, A. & RALL, T.W. (1970). The effect of adenosine and adenine nucleotides on the cyclic adenosine 3'-5'-phosphate content of guinea-pig cerebral cortex slices. *Molec. Pharmac.*, **6**, 13-23.

WINBURY, M.M., PAPIERSKI, D.H., HEMMER, M.L. & HAMBOURGER, W.E. (1953). Coronary dilator action of the adenine-ATP series. *J. Pharmac. exp. Ther.*, **109**, 252-260.

WOLF, M.M. & BERNE, R.M. (1956). Coronary vasodilator properties of purine and pyrimidine derivatives. *Circ. Res.*, **4**, 343-348.

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