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INCREASE OF ACETYLCHOLINE-RECEPTOR SENSITIVITY BY ADENOSINE TRIPHOSPHATE: A NOVEL ACTION OF ATP ON ACh-SENSITIVITY

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1 The sensitivity of the nicotinic acetylcholine (ACh)-receptor, measured as the amplitude of ACh-current induced by iontophoretic application of ACh to the frog skeletal muscle endplate, was increased by the action of adenosine triphosphate (ATP).

2 This potentiation was not due to the effect of ATP on ACh-esterase, since the increase of the sensitivity could also be demonstrated by use of carbachol (CCh).

3 Kinetic analysis of the effect of ATP on the dose-response curve of CCh-current suggests that ATP increases the ACh-sensitivity by acting on the allosteric site of receptor-ionic channel complex without changing the affinity of ACh for its recognition site.

4 The equilibrium potential and the life-time of the endplate current (e.p.c.) are not altered by the presence of ATP.

5 These results suggest that ATP increases the ACh-sensitivity by increasing either the conductance of unit channels or the total number of available channels.

Introduction

An interesting possibility that postjunctional cholinergic sensitivity may be potentiated by a direct action of high-energy-phosphate adenine nucleotides in rat diaphragm muscle has been suggested by Ewald (1976). The present paper describes experimental evidence which demonstrates that these nucleotides do potentiate the sensitivity of nicotinic cholinoceptors. The mechanism underlying such a potentiating effect of adenosine triphosphate (ATP) on the sensitivity of frog skeletal muscle endplate to acetylcholine (ACh) was analysed by use of voltage-clamp methods in the present experiments.

Methods

Sartorius muscles together with the sciatic nerve isolated from frog (*Rana nigromaculata*) were used for the experiments. Sartorius muscles were perfused continuously with Ringer solution. The voltage-clamp method was employed for recording the endplate current (e.p.c.), acetylcholine-induced current (ACh-C) and carbachol-induced current (CCh-C). Microelectrodes filled with 3 M KCl with tip resistance of 5 to 10 Ω or with 1 M K-citrate with tip resistance of 3 to 5 Ω were used for recording membrane potential and injecting voltage-clamping current, respectively. When measuring ACh-C or CCh-C, ACh or CCh was applied iontophoretically from a micropipette filled with 2 M ACh or CCh, respectively. The e.p.c. was recorded in the presence of (+)-tubocurarine (2 μM). The muscle preparation treated with 400 mM glycerol for 1 h was used for investigating the reversal potential of the e.p.c. The ionic composition of the Ringer solution was as follows (mM): NaCl 112, KCl 2, $CaCl_2$ 1.8 and $NaHCO_3$ 2.4. All experiments were carried out at room temperature (20 to 24°C). Drugs used were as follows: carbamylcholine chloride (Aldrich chemical Co. Inc.), the disodium salt of ATP, the sodium salts of adenosine diphosphate (ADP) and adenosine-monophosphate (AMP), adenosine, ACh chloride (Wako Pure Chemical Industries Ltd), and (+)-tubocurarine chloride (Sigma).

Results

ACh-C was measured at the holding potential of −100 mV, as the parameter of sensitivity of ACh-receptors. When 1 mM ATP was added to the perfusate, the ACh-C gradually increased for 3 to 4 min until it showed a maximum amplitude with a mean 154.8 ± 10.8% ($n = 6$) (mean ± s.d., n = number of samples) of the control, while a small steady inward current was recorded (Figure 1A). The potentiating effect of ATP on the ACh-C disappeared after removal of the drug from the perfusate (Figure 1A).

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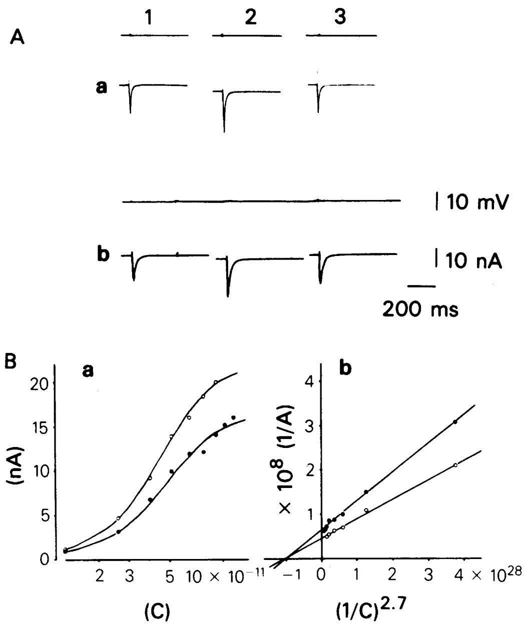

Figure 1 Effects of ATP on the acetylcholine-induced current (ACh-C) and carbachol-induced current (CCh-C) (A) and on the dose-response relationship of CCh-C (B), recorded from frog sartorius muscle endplate by voltage-clamp method (holding potential = −100 mV). (A) (a) and (b) show ACh-C and CCh-C, respectively, and records (1), (2) and (3) are before, during and 10 min after an application of 1 mM ATP for 10 min, respectively. Upper traces in each record indicate membrane potential levels. (B) The effect of 1 mM ATP on the dose-response relationship between relative quantities of CCh applied iontophoretically and CCh-C (a) and the kinetic analysis by Lineweaver-Burk's plot constructed from (a) by assuming Hill number (n_H) = 2.7 (b). (●) Before ATP; (○) during the effect of ATP.

The potentiating effects of ATP in concentrations of 100 and 10 μM were 124 ± 7.7% (n = 3) and 114.8 ± 2.5% (n = 3), respectively.

The effect of ATP on the CCh-C was examined in order to exclude the possibility that ATP potentiated the ACh-C by inhibiting the ACh-esterase activity. The CCh-C was reversibly potentiated to 132.7 ± 10.1% (n = 3) of control by applications of 1 mM ATP to the perfusate (Figure 1A).

In order to determine the mechanism of the potentiating effect of ATP on the ACh-receptor sensitivity, the effect of ATP on the dose-response curve for CCh-C was studied (3 experiments). The dose-response curve for CCh-C was obtained by plotting the amplitude of CCh-C against the logarithm of relative CCh quality used for iontophoresis. ATP 1 mM applied to the perfusate shifted the S-shaped dose-response curve of CCh-C upward. Kinetic analysis of the effect of ATP on the dose-response curve by Lineweaver-Burk's plot suggested that ATP increased V_{max} without changing K_m significantly. An example of these determinations is shown in Figure 2, in which the value of V_{max} estimated from these dose-response curves (Hill number = 2.7) was increased from 16.7 to 22.8 in the presence of 1 mM ATP, while K_m remained almost unchanged. V_{max} returned to the near control value (17.4) after removal of the drug.

Any potentiation of CCh-C was not observed in the presence of 1 mM tartrate or citrate added to Ringer solution, suggesting that the potentiating effect of ATP on ACh-C or CCh-C was not simply due to its possible chelating effect. Effects of derivatives (1 mM) of ATP, i.e. ADP, AMP and adenosine, on the ACh-sensitivity were examined, in order to

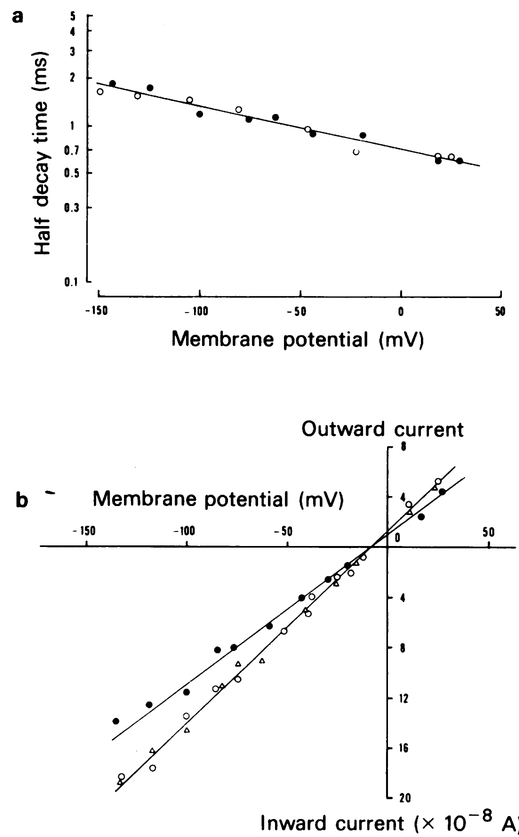

Figure 2 Effects of ATP on the time course of falling phase (a) and on the equilibrium potential (b) of the endplate current (e.p.c.) recorded by voltage-clamp method from glycerol-treated frog sartorius muscle. Ordinates indicate the half decay time (a) and the peak amplitude (b) of the e.p.c. Abscissae indicate the membrane potential in both. (○) Before, (●) during and (△) after application of 300 μM ATP.

compare their effects with those of ATP. The comparison was made with ACh-C recorded from a single preparation. The results obtained from these experiments ($n = 3$) were as follows: ATP $\gg$ ADP > AMP > adenosine (adenosine showed no appreciable effect).

The effect of ATP on the e.p.c. was studied for the purpose of determining whether the life-time and the reversal potential of e.p.c. were altered by this drug. These experiments ($n = 5$) demonstrated that both the time course of the falling phase and the reversal potential of the e.p.c. showed no detectable changes, although the amplitude of the e.p.c. was decreased in the presence of 1 mM ATP. These results are shown in Figure 2.

Discussion

Ewald (1976) has suggested that ATP may increase the sensitivity of the ACh-receptor in rat diaphragm muscle, on the basis of the experimental findings that ATP potentiated the depolarization induced by iontophoretic application of ACh and neither the magnitude nor the time course of the potentiations corresponded to the changes in resting potential or membrane resistance. The present results clearly demonstrate by voltage-clamp experiments that ATP does indeed increase the sensitivity of the ACh-receptor. Furthermore, the fact that ATP potentiated the amplitude of the CCh-C indicates that the increase in ACh-sensitivity is not simply due to inhibition of ACh-esterases. The potentiating effect on the ACh-sensitivity was not observed with adenosine, suggesting that the energy-rich phosphate bonds of ATP may be responsible for its potentiating effect. Indeed, the potency of the sensitizing action correlates with the numbers of phosphate bonds.

If one assumes that the combination of ACh with receptors in the endplate membrane leads to the opening of ionic channels, being coupled with receptors, with a given life-time and conductance (Katz & Miledi, 1972; Anderson & Stevens, 1973), an increased ACh-sensitivity would be due to an increase of either one or both of these channel characteristics, or to an increase in the affinity of ACh for the receptor. The present results demonstrating the effect of ATP on the dose-response relationship between CCh concentration and amplitudes of CCh-C, and Lineweaver-Burk's plot constructed from these results, suggest that the ATP dose not increase the affinity of ACh for the receptor but increases the maximum response by acting on the allosteric site of the receptor-ionic channel complex. Presumably, ATP increases either one or both of the life-time and conductance of opening channels. The fact that the time course of the falling phase of the e.p.c. was not changed by ATP suggests that the increase of ACh-C may be mainly due to an increase of the channel conductance. Presumably, the conductance of unit channel is increased by the action of ATP. The possibility that ATP may increase the total number of available channels cannot be dismissed. Further analysis of the action of ATP on the ACh receptor-ionic channel complex in relation to Ca^{2+}, which controls the conductance of the channel (Lambert & Parsons, 1970), may provide the key to understanding the mechanism of the potentiating effect of ATP on the ACh-sensitivity.

Interestingly, the amplitude of e.p.c. evoked by nerve stimulation was decreased by the effect of ATP, although the sensitivity of nicotinic ACh receptors was increased by the drug. This indicates that the evoked release of neurotransmitter from motor nerve terminals is reduced by the effect of ATP. Presumably, ATP decreases the transmitter release by depolarizing the motor nerve terminals (cf. Akasu, Hirai & Koketsu, 1981).

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