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Effect of adenosine triphosphate on the sensitivity of the nicotinic acetylcholine-receptor in the bullfrog sympathetic ganglion cell

T. Akasu & K. Koketsu

Department of Physiology, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830, Japan

1 The effects of adenosine triphosphate (ATP) and related compounds on the sensitivity of the nicotinic acetylcholine (ACh)-receptor of bullfrog sympathetic ganglion cells were analysed electrophysiologically.

2 ATP in concentrations between 0.05 and 2 mM increased the amplitudes of the potentials and currents induced by ACh, and carbachol-induced currents.

3 Compared with ATP, ADP was less potent in producing augmentation of the carbachol-induced current by one order of magnitude. AMP, cyclic AMP and adenosine had no appreciable effect.

4 Analysis of this ATP effect, based on Michaelis-Menten type kinetics, revealed that ATP increased the maximum response (V_{max}) of the dose-response curve of ACh currents without an appreciable effect on the affinity (K_m) of ACh for its receptor.

5 It is suggested that ATP increased the receptor sensitivity by acting on an allosteric site of the nicotinic ACh receptor-ionic channel complex which, thus, may be linked to an ATP receptor, probably of the P_2-receptor type (Burnstock, 1981).

Introduction

Adenosine and adenine nucleotides have been found to be neurotransmitter or neuromodulator candidates in the central and peripheral nervous systems (Burnstock, 1981; Phillis & Wu, 1981; Stone, 1981).

In amphibian sympathetic ganglia, adenosine triphosphate (ATP) produces a slow depolarizing response of postganglionic neuronal membranes, which is due to the suppression of K^+ conductance (Siggins *et al.*, 1977; Akasu *et al.*, 1981). Presynaptic effects of ATP have also been demonstrated. Exogenously applied ATP, but not adenosine, inhibits ACh release from preganglionic nerve terminals in amphibian sympathetic ganglia (Akasu *et al.*, 1982; 1983a; Silinsky & Ginsborg, 1983).

At the skeletal muscle endplate, it has been found that ATP augments ACh-induced endplate responses by increasing the receptor sensitivity to ACh (Ewald, 1976a; Akasu *et al.*, 1981). The purpose of this paper is to investigate the possibility that ATP modulates the sensitivity of the nicotinic receptor of sympathetic ganglia. Our results suggest that ATP increases the sensitivity of the nicotinic ACh-receptor of the ganglion cell in a non-competitive manner and that this facilitatory effect may be mediated by a P_2-purinoceptor (Burnstock, 1981).

Methods

The 8th or 9th paravertebral sympathetic ganglia were isolated from bullfrogs (*Rana catesbeiana*). The technique used for intracellular recording of membrane potential of the ganglion cells followed the method of Nishi & Koketsu (1960). Glass microelectrodes filled with 3 M KCl with tip resistances of 30–70 MΩ were used. The ACh sensitivity of the cell membrane was measured by recording fast (nicotinic) ACh-induced potentials (and currents) and carbachol-induced postsynaptic currents produced by iontophoretic administration of these drugs through microelectrodes. The composition of the Ringer solution was as follows (mM): NaCl 112, KCl 2, $CaCl_2$ 1.8 and $NaHCO_3$ 2.4. Fast excitatory postsynaptic currents (e.p.s.cs) were elicited by preganglionic nerve stimulation in a Ringer solution containing (+)-tubocurarine. The method used for voltage-clamp measurements was essentially similar to that described previously (Kuba & Nishi, 1979; Akasu & Koketsu, 1981). A microelectrode filled with 1 M K-citrate was used for injecting feedback current and was inserted as a second electrode into the ganglion cells.

All experiments were carried out at room tem-

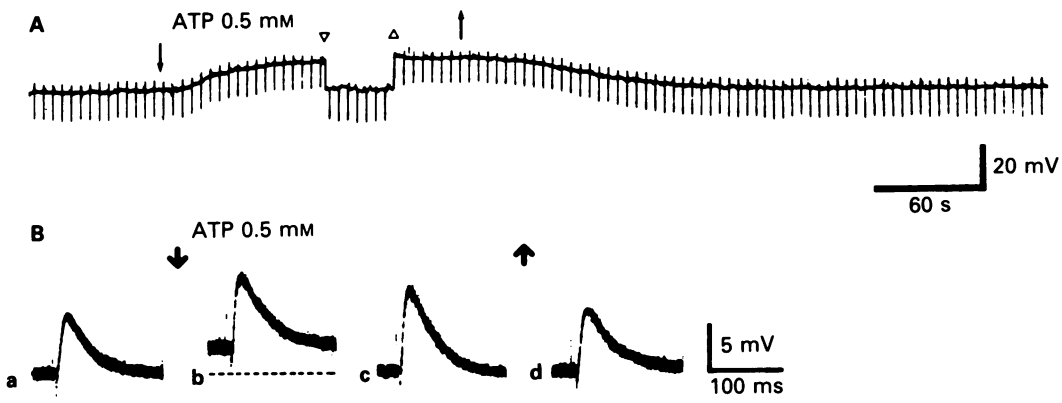

Figure 1 Effects of ATP (0.5 mM) on the membrane potential and resistance (A) and on the potential induced by acetylcholine (ACh) (B). (A) ATP was added to the superfusing solution between arrows. Electrotonic potentials were elicited by applying anodal currents (5 nA) to the ganglion cell through the recording electrode. The membrane potential was returned to original resting potential level (−50 mv) between triangles by applying constant anodal current. (B) ACh potentials were obtained by iontophoretic application of ACh pulses (15 nA for 30 ms) to the ganglion cell. Record (a) is control ACh potential obtained in Ringer solution. Records (b) and (c) were obtained in the presence of ATP (0.5 mM). Record (c) was obtained at the original resting membrane potential level (−62 mv). Record (d) was obtained 5 min after the removal of ATP from the superfusing solution.

perature (22–24°C). Drugs used were (+)-tubocurarine chloride (Sigma), carbamylcholine chloride (carbachol; Merck), adenosine 3′–5′ triphosphate disodium (ATP; Sigma), adenosine diphosphate disodium (ADP; Calbiochem.), adenosine monophosphate sodium (AMP; Sigma), adenosine (Sigma), dibutyryl cyclic AMP (Sigma) and theophylline (Calbiochem.).

Results

Figure 1 shows the effect of ATP (0.5 mM) on the membrane potential and ACh-induced postsynaptic potential (ACh potential) in a bullfrog sympathetic ganglion cell. Bath-administration of ATP (0.5 mM) to the ganglion cell produced a slowly developing depolarization associated with an increase in the membrane resistance (Figure 1A). ACh-induced potentials of constant amplitude were recorded by applying ACh iontophoretically to the ganglion cell at a rate of 0.2 Hz, so that no obvious desensitization (Katz & Thesleff, 1957) was observed. ATP at a concentration of 0.5 mM increased the amplitude of an ACh potential during the ATP-induced depolarization (Figure 1B(b)). The ACh potential obtained at the original membrane potential level was increased to about 150% of the control value by ATP (0.5 mM) (Figure 1B(c)). The minimum concentration of ATP required to increase the amplitude of the ACh potential was 0.05 mM.

Voltage-clamp experiments were carried out to avoid the effects of membrane depolarization and resistance changes during the effect of ATP. ATP (0.5 mM) produced a 2–7 nA inward current in ganglion cells voltage-clamped at −50 mV. ATP-induced inward currents were associated with a decrease in membrane conductance (Figure 2A). We have shown previously (Akasu *et al.*, 1983a, b) that the depression of conductance is due to suppression of the M current (Brown & Adams, 1980). Figure 2B shows the effect of 0.5 mM ATP on carbachol-induced currents (carbachol current) resulting from iontophoretic application to the ganglion cell held at −60 mV. We observed that either no or only a quite small inward current was produced by ATP when the potential was held at −60 mV and this current was smaller than that obtained at −50 mV (Figure 2). The reason for this is that the ATP-induced membrane current was produced by the voltage-sensitive conductance system (M-channels) (Akasu *et al.*, 1983b).

Carbachol currents obtained from 8 cells were increased to 163.3 ± 8.1% (mean ± s.d.) of the control value by ATP at a concentration of 0.5 mM (Figure 2B). Augmentation of the amplitude of carbachol currents reached a maximum level within 1 min of the addition of ATP; this augmentation remained constant thereafter. These actions of ATP were reversible as the inward current and potentiation of carbachol currents were restored to their control levels within 2 min after wash-out of ATP.

The potentiating effect of ATP was concentration-dependent. Figure 3 shows the relationship between various ATP concentrations and their % increase of the

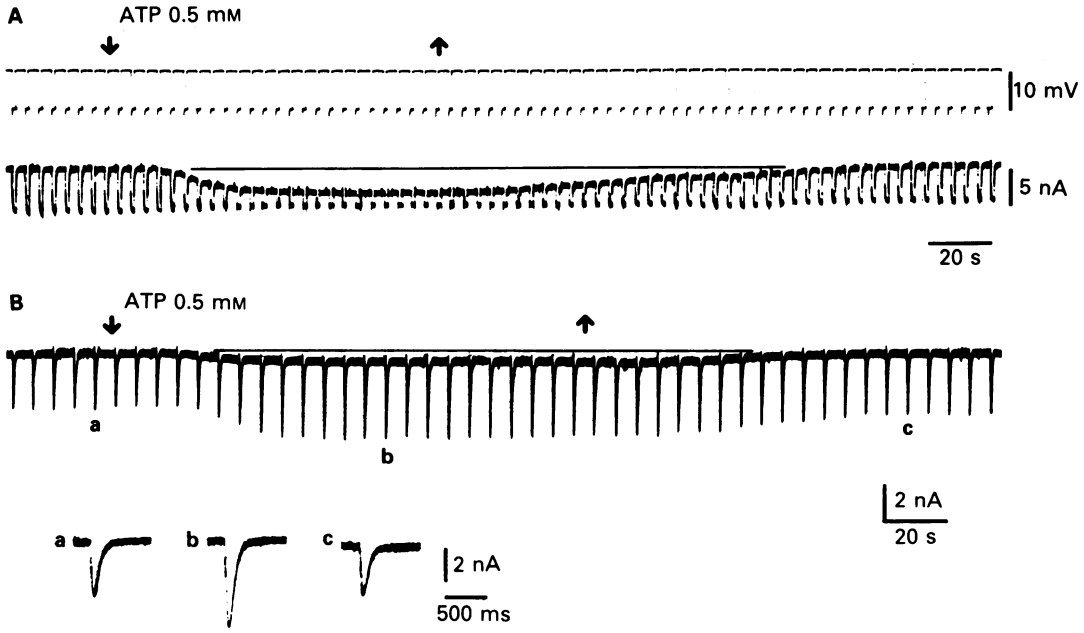

Figure 2 Effect of ATP (0.5 mM) on the membrane conductance (A) and the nicotinic receptor sensitivity (B) of voltage-clamped ganglion cells. Records were obtained from the same ganglion cell held at −50 mV (A) and −60 mV (B). (A) ATP was applied to the superfusing solution between arrows. Instantaneous hyperpolarizing command potentials (upper trace) were applied to measure conductance changes during ATP application. Lower trace indicates the membrane current. (B) Carbachol-induced currents were produced by repeated iontophoretic application of carbachol pulses (20 nA for 40 ms) at a rate of 0.2 Hz to the ganglion cell. Records (a), (b) and (c) are actual oscilloscope tracings and correspond to the time described on recording (B).

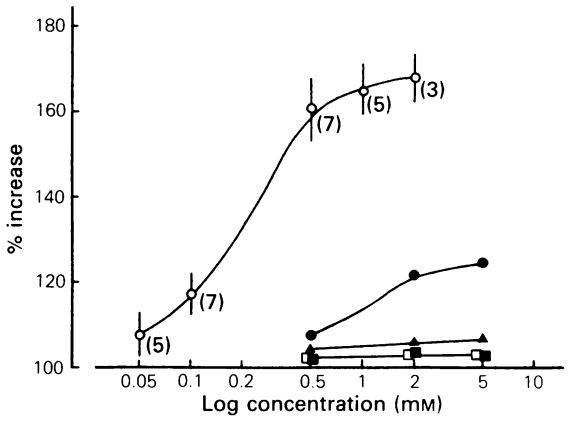

Figure 3 Concentration-response relationship between the % increase of carbachol current (ordinate scale) and log concentration of ATP (○), ADP (●), AMP (△), dibutyryl cyclic AMP (□) and adenosine (■) (abscissa scale). Vertical lines on the ATP curve indicate mean ± s.e. mean. Number of experiments are shown in parentheses. The average of three experiments with ADP, AMP, cyclic AMP and adenosine are shown.

carbachol current. Effects of ADP, AMP, cyclic AMP and adenosine were also compared for their ability to increase the sensitivity of the nicotinic ACh-receptor (Figure 3). Although ADP enhanced the sensitivity of the nicotinic ACh-receptor, it was less potent than ATP by one order of magnitude. AMP, dibutyryl cyclic AMP and adenosine had no significant effect.

The methylxanthines are widely recognized as antagonists of a type of adenosine receptor (P_1-subtype) (Burnstock, 1978; 1981). However, theophylline (2 mM) did not block the ability of ATP to increase the sensitivity of the nicotinic receptor. Thus it seems that the receptor responsible for the potentiating effect of ATP is not the P_1-purinoceptor, but may be a P_2-receptor (Burnstock, 1981). Effects of ATP on the amplitude of miniature excitatory postsynaptic potentials (m.e.p.s.ps) were also investigated. ATP (0.5 mM) produced a slight increase in the mean amplitude of m.e.p.s.ps to 125.6 ± 7.2% ($n = 6$) of control.

To analyse the mechanism for the potentiating effect of ATP on the sensitivity of the nicotinic receptor on ganglion cells, the dose-response curve of ACh-induced currents (Dreyer *et al.*, 1978) was examined. The amplitude of ACh current was plotted

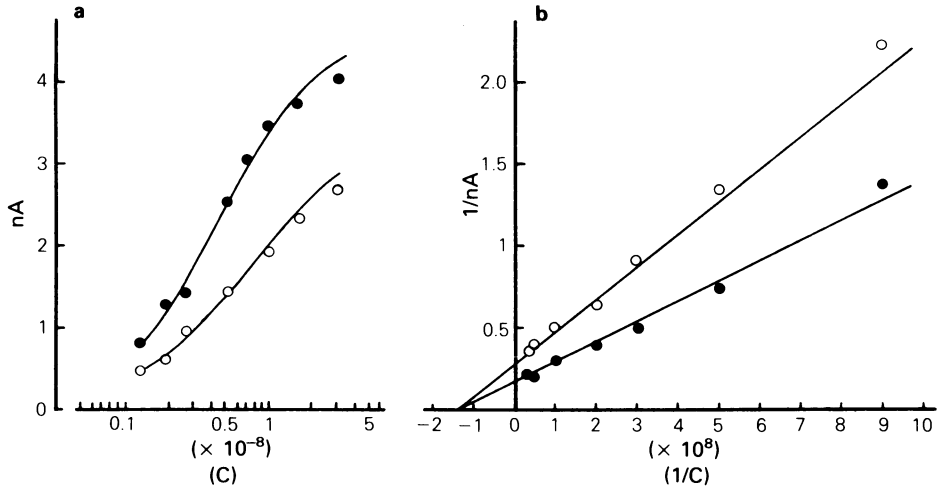

Figure 4 (a) The log-dose relationship between the amount of acetylcholine (ACh) applied iontophoretically to the ganglion cell (abscissa scale) and peak amplitude of ACh current obtained by voltage-clamp technique (ordinate scale). The amount of ACh applied is expressed as electrical charge in coulombs (C). Results obtained before (○) and during (●) the bath-application of ATP (0.5 mM). (b) The kinetic analysis by double reciprocal plot (Lineweaver-Burk) constructed from (a) by assuming Hill number (n_H) = 1.0.

against the log of charge used for iontophoresis of ACh. Figure 4 shows the S-shaped dose-response curve of ACh current and the effect of ATP (0.5 mM). ATP (0.5 mM) increased the amplitude of ACh current and shifted the dose-response curve upward (Figure 4). A linear Lineweaver-Burk plot was obtained from the dose-response curve, assuming a Hill number (n_H) of 1.0. ATP increased the maximum response (V_{max}) of the dose-response curve from 3.3×10^{-9} A to 5.4×10^{-9} A (Figure 4b). On the other hand, ATP did not significantly affect the dissociation contant (K_m: Figure 4b). These results suggest that ATP increases nicotinic receptor-sensitivity in a non-competitive manner.

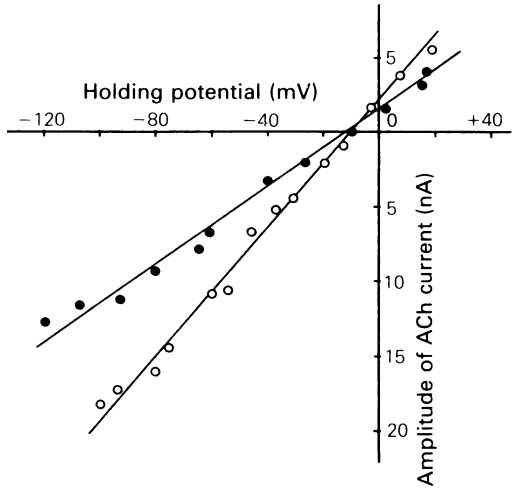

Figure 5 Effect of ATP (0.5 mM) on the equilibrium potential of acetylcholine (ACh)-induced current recorded from a voltage-clamped ganglion cell. Ordinate and abscissa scales indicate the peak amplitude of ACh current and the holding membrane potential, respectively. Results obtained before (●) and during (○) the bath-application of ATP (0.5 mM).

The effect of ATP on the reversal potential of ACh currents obtained from voltage clamped ganglion cells was also studied (Figure 5). The reversal potential of ACh currents obtained in control Ringer solution and in the presence of ATP (0.5 mM) were -5.1 ± 3.6 mV ($n = 7$) and -7.3 ± 4.2 mV ($n = 7$), respectively. These data show that the ATP-induced augmentation of ACh and carbachol responses is not due to changes in the conductance ratio of Na^+ and K^+ at the nicotinic receptor-channel complex. The effect of ATP on the time course of fast e.p.s.cs was investigated to test the possibility that the potentiation of the amplitude of ACh potentials and carbachol currents was due to an increase in the life time of these responses. Figure 6a illustrates the effect of ATP (0.5 mM) on the duration of the falling phase of fast e.p.s.cs recorded in a Ringer solution containing 0.03 mM (+)-tubocurarine. ATP (0.05–0.5 mM) did not change the exponential time course of the falling phase of fast e.p.s.cs (Kuba & Nishi, 1979) (Figure 6a). The half-decay times of fast e.p.s.cs obtained from 9 cells in the control solution and a solution containing ATP were 4.3 ± 1.2 ms and 4.7 ± 1.6 ms, respectively.

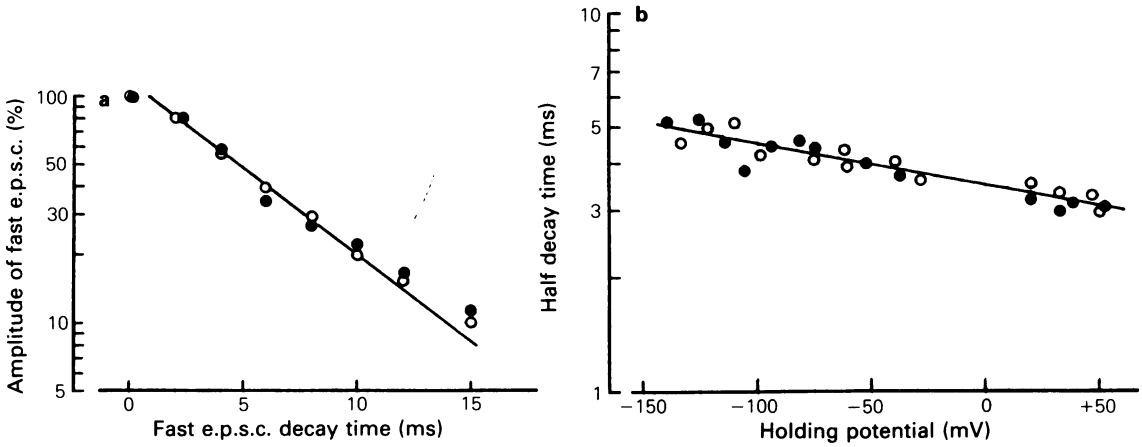

Figure 6 Effect of ATP (0.5 mM) on the fast excitatory postsynaptic currents (e.p.s.cs) elicited by stimulation of preganglionic nerve fibres in the presence of (+)-tubocurarine (30 μM). (a) Effect of ATP on the time course of the falling phase of fast e.p.s.c.. Percentage amplitudes of the falling phase of fast e.p.s.c. (ordinate scale) are plotted against time (abscissa scale). Maximum amplitude of fast e.p.s.c. is expressed as 100%. Results obtained before (●) and during (○) bath-application of ATP. (b) Effect of ATP on the half-decay times of falling phase of fast e.p.s.c. recorded at various holding potential levels. Abscissa scale indicates the holding membrane potentials. Ordinate scale indicates the half-decay time. Results obtained before (●) and during (○) application of ATP.

Figure 6b shows the half-decay time of the falling phase of a fast e.p.s.c. recorded at various holding potentials. The half-decay time was increased and decreased at hyperpolarized and depolarized potential levels, respectively (Kuba & Nishi, 1979). This potential dependency of the half-decay time is known to be due to the voltage-dependent property of closing ionic channels (Magleby & Stevens, 1972; Gage, 1976; Kuba & Nishi, 1979). However, ATP (0.5 mM) did not change the potential dependency of the half-decay time of the fast e.p.s.c. (Figure 6b).

The peak amplitude of fast e.p.s.cs was frequently decreased by the addition of ATP (0.5–1 mM); this may be due to the inhibitory effect of ATP on the release of ACh from preganglionic nerve terminals (Akasu *et al.*, 1982; 1983a; Silinsky & Ginsborg, 1983).

Discussion

Our results demonstrate clearly that ATP increases the amplitude of ACh responses obtained from neurones in bullfrog sympathetic ganglia. Since ATP also increased the amplitude of carbachol currents, the ATP-induced augmentation of ACh responses cannot be due to inhibition of cholinesterase activity. Kinetic analysis revealed that ATP increased the maximum response (V_{max}) of the dose-response curve of ACh-induced currents without affecting the affinity of ACh for its receptor. Thus, the site at which ATP acts may be an allosteric site of the nicotinic ACh receptor-ionic channel complex. The results also show that ATP produces no changes in the life time of fast e.p.s.cs. Therefore, non-competitive facilitation of the receptor sensitivity by ATP is unlikely to be due to an increase in the life time of the channel associated with the nicotinic receptor, but is probably due to an increase in conductance as a result of an increase in the number of available channels. These results are consistent with those obtained at the frog sartorious muscle endplate (Akasu *et al.*, 1981; 1983a).

Adenosine is very much less potent than ATP as a facilitator of nicotinic receptor-sensitivity. Theophylline, known to be an antagonist of the adenosine P_1-receptor, did not block the action of ATP. Thus it appears that the ATP-induced increase in receptor sensitivity may be mediated by a P_2-receptor as has been proposed by Burnstock (1981). Silinsky & Ginsborg (1983) reported that the purinoceptor which mediates the inhibitory action of purine compounds by decreasing the release of ACh from nerve terminals in frog sympathetic ganglion is also the ATP type of purinoceptor. Recently, Henon & McAfee (1983) showed that the Ca^{2+}-dependent action potential in mammalian (rat) sympathetic ganglion cells is depressed by adenosine. In the amphibian sympathetic ganglion cell, adenosine, unlike ATP, did not produce either a significant change in resting membrane potential or depression of Ca^{2+} spikes (Akasu *et al.*, 1983b). These purinoceptors in the bullfrog sympathetic ganglia may also be P_2-receptors.

Recently, Kumamoto & Kuba (1983) found a long-

lasting potentiation of ACh sensitivity after brief stimulation of postganglionic nerve trunks in bullfrog sympathetic ganglia. They suggested that the Ca^{2+} influx after brief excitation of the ganglion cell must underlie the postsynaptic potentiation of ACh potentials. Ewald (1976a, b) found that the amplitude of ACh potentials was increased by ATP, as well as its related compounds, at the endplate of rat diaphragm muscles, as the result of an increase in intracellular Ca^{2+}. However, ATP-induced facilitation of nicotinic receptor sensitivity disappeared within a few minutes after withdrawal of ATP from the superfusing solution. Furthermore, ATP did not increase, but rather suppressed, the voltage-dependent Ca^{2+} current in bullfrog sympathetic ganglion cells (Akasu *et al.*, 1983b). Thus, the facilitation of nicotinic ACh-receptor sensitivity by ATP is not likely to be due to the same mechanism demonstrated by Kumamoto & Kuba (1983). It was recently suggested that divalent cations modulate the coupling between 5-hydroxytryptamine receptors and ion channels, an increase in $[Ca^{2+}]_o$ reducing the coupling or stabilizing the ion channel in the closed conformation (Nash & Wallis, 1981). It may, therefore, be possible that ATP-induced modulation of the nicotinic receptor sensitivity is due to an interaction with a similar Ca^{2+}-dependent mechanism which controls the efficiency of the coupling between receptor and ion channel in the bullfrog sympathetic ganglion cell.

Holck & Marks (1978) reported that a purinoceptor might be associated with the α-adrenoceptor in guinea-pig vas deferens. They suggested that the release of a purine from nerve terminals might be important for the maintenance of α-receptor sensitivity. Recently, Morita *et al.* (1984) found that ATP produced a slow depolarization in sensory afferent neurones of frog dorsal spinal ganglia and that the γ-aminobutyric acid (GABA)-induced depolarization of the spinal dorsal ganglion cell was augmented by ATP in a non-competitive manner.

During cholinergic transmission, ATP is released from motor nerve terminals in association with ACh (Silinsky, 1975). Such ATP may modulate the nicotinic receptor sensitivity at the endplate (Ewald, 1976a, b; Akasu *et al.*, 1981). Although ATP release from preganglionic nerve terminals has not been demonstrated in bullfrog sympathetic ganglia, ATP might also play an important role in maintaining normal receptor function of the ganglion cell.

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