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Effects of prolonged exposure to α,β-methylene ATP on non-adrenergic, non-cholinergic excitatory transmission in the rectum of the chicken

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1 Effects of prolonged exposure to α,β-methylene ATP (α,β-Me ATP) on contractions and excitatory junction potentials (e.j.ps) evoked by non-adrenergic, non-cholinergic (NANC) excitatory nerve stimulation have been investigated in the chicken isolated rectum and longitudinal muscle strip from chicken rectum pretreated with atropine (0.5 μM), methysergide (2 μM) and pyrilamine (3 μM).

2 α,β-Me ATP (20 nM–4 μM) caused a rapid rise in tension of the longitudinal muscle of the isolated rectum preparation which returned to the baseline levels after a few minutes. The magnitude of the contractile response to NANC nerve stimulation was reduced after exposure to the drug. The inhibitory effect was related to the drug concentration; at 4 μM the nerve-mediated contraction was abolished and frequently converted to a relaxation.

3 Adenosine 5′-triphosphate (ATP, 100 μM), bovine neurotensin (2.5 nM) and K^+-rich solutions (30 mM and 60 mM) all produced a transient contraction of the isolated rectum preparation. The exposure to α,β-Me ATP (0.2 and 4 μM) also rendered the preparation less sensitive to these stimulant substances.

4 α,β-Me ATP (0.2 and 4 μM) caused a membrane depolarization in cells of the longitudinal muscle strip. The depolarization reached a peak within 2–3 min after application and then decayed to a steady level that was still more positive than the baseline level. The electrotonic potentials were reduced in amplitude to 44 ± 8% ($n = 7$) of the normal amplitude if measured at the peak depolarization produced with 0.2 μM α,β-Me ATP, and to 62 ± 10% ($n = 7$) if measured at the steady-state depolarization. With 4 μM, the corresponding percentages were 33 ± 7% ($n = 8$) and 55 ± 7% ($n = 8$), indicating a decrease in membrane resistance.

5 The e.j.ps in response to field stimulation of the intramural nerves and Remak's nerve stimulation were decreased in amplitude and duration during exposure to α,β-Me ATP (0.2 and 4 μM).

6 The smooth muscle cells regained normal membrane resistance and sensitivity to ATP on washout of α,β-Me ATP (4 μM) more rapidly than the responses to NANC nerve stimulation.

7 It can be argued from the results that the suppression by α,β-Me ATP of the contraction and e.j.p. evoked by NANC nerve stimulation in the chicken rectum, unlike the mammalian preparations described previously, is due mainly to a change in the electrical properties of the membrane of the smooth muscle cells, rather than being due, or only partly due, to desensitization of the purine receptor.

Introduction

Non-adrenergic, non-cholinergic (NANC) nerves mediating excitatory responses of contractions and excitatory junction potentials (e.j.ps) have been demonstrated to be present in the rectum of the chicken (Bartlet & Hassan, 1971; Takewaki & Ohashi, 1977; Takewaki *et al.*, 1977). Recently, Meldrum & Burnstock (1985) suggested that ATP is the chemical transmitter linking the NANC excitatory nerve with the smooth muscle. Their argument rests on the observations that prolonged exposure of the tissue to α,β-methylene ATP (α,β-Me ATP), a stable ATP analogue, which rendered the smooth muscle insensitive to adenosine 5′-triphosphate (ATP), but not to carbachol, also abolished the con-

tractile responses of the smooth muscle mediated by the NANC nerves when intramural nerves were stimulated. However, they did not measure changes in membrane potential and membrane resistance of the smooth muscle cells produced by α,β-Me ATP, which undoubtedly reflect more directly the activity of the purine receptors. Membrane depolarization responsible for the contractile response to α,β-Me ATP has been described for other smooth muscles (Sneddon & Burnstock, 1984; 1985; Ishikawa, 1985; Byrne & Large, 1986). On the other hand, the electrical membrane properties of the chicken rectal muscle have been investigated using the microelectrode technique (Komori & Ohashi, 1982).

The present study was aimed at confirming the inhibitory effect of α,β-Me ATP on the contractile responses of the smooth muscle of the chicken rectum mediated by the NANC nerves when Remak's nerve or intramural nerves are stimulated. We also measured the drug-induced changes in the membrane properties of the smooth muscle cells, to obtain corroborative evidence that the abolition of the NANC nerve-mediated contraction is attributable to ongoing desensitization of the purine receptors. The experiments were carried out in the presence of atropine, methysergide and pyrilamine, and if necessary, guanethidine, to avoid the effects of acetylcholine, 5-hydroxytryptamine, histamine and catecholamines which might be released from the tissue. A preliminary account of some of these results has been published previously (Ohashi *et al.*, 1987).

Methods

White Leghorn chickens of either sex, aged more than one week, were obtained from commercial sources. Birds were stunned and bled to death. The rectum was excised together with the vessels and Remak's nerve.

Measurement of mechanical responses

The rectum preparation isolated from chickens aged less than two weeks was used to measure changes in tension of the longitudinal muscle. The rectum was mounted vertically in a 2.5 ml polypropylene organ bath in such a way that it was passed through a pair of platinum ring electrodes (5 mm in diameter and about 1 cm apart between the two rings) with one end of it fixed and the other attached by thread to a transducer. The ring electrodes were used for electrical field stimulation of the intramural nerves. In experiments in which Remak's nerve was stimulated, the anal end of the nerve trunk was drawn into a bipolar suction electrode for stimulation and the electrode was immersed together with the rectum preparation in a 5 ml polypropylene organ bath. These organ baths were filled with Tyrode solution.

Trains of square wave pulses of 0.3 ms duration for stimulation of intramural nerves and of 0.8 ms duration for stimulation of Remak's nerve were supplied at various frequencies and at supramaximal intensity from a stimulator (Nihon Kohden, SEN-3013). Mechanical responses of the smooth muscle to nerve stimulation and drugs were measured isometrically by a force-displacement transducer (Nihon Kohden, TB 612T) and recorded on a pen-recorder (Hitachi, 056).

Measurement of e.j.ps and electrotonic potentials

The rectum preparation isolated from 100 or more day old chickens was sectioned lengthwise, from which muscle strips (about 1.5 mm wide and 20 mm long) were dissected along the long axes of the longitudinal muscle cells. The muscle strip was mounted in an organ bath (2 ml) consisting of two chambers, one for recording and the other for stimulating (Abe & Tomita, 1968), through which Tyrode solution flowed continuously at the rate of $3\,ml\,min^{-1}$. In order to suppress mechanical activities of the preparation, isoprenaline (1.3 μM) and methoxyverapamil (D-600, 2 μM), alone or in combination (which inhibit spontaneous electrical activity without changing the membrane resistance and neuromuscular transmission (Komori *et al.*, 1980)) were added to the bathing solution. Glass microelectrodes filled with 3 M KCl, with resistances of 40–80 MΩ were used to record intracellularly the changes in the membrane potential. Records were taken from smooth muscle cells located 0.2–0.3 mm from the partition between the two chambers of the organ bath. To evoke electrotonic potentials, current pulses of 800 or 1000 ms duration were applied to the tissue through the large stimulating plates. Intramural nerves of the tissue were stimulated electrically with square wave pulses of 0.08–0.1 ms duration at a voltage of around 10 V delivered through a pair of Ag-AgCl electrodes (1 mm in diameter). One electrode, which was insulated with Araldite (Nagase) except for the tip, was placed at the centre of the tissue 1.5–2.0 mm away from the stimulating partition in contact with it. The other electrode, which was uninsulated, was placed in the bathing solution. This permitted electrotonic potentials and e.j.ps to be recorded from the same cell (Komori & Ohashi, 1982). The potential changes were displayed on an oscilloscope and photographed.

Solutions and drugs

Tyrode solution was of the following composition (mM): NaCl 136.9, KCl 2.7, NaH_2PO_4 0.4, $CaCl_2$ 1.8,

$MgCl_2$ 2.5, $NaHCO_3$ 11.9 and glucose 5.6, bubbled with air and kept at 31 ± 1°C, because at temperatures higher than this vigorous spontaneous activity occurred and this prevented stable measurements of responses of the tissue. Atropine (0.5 μM), methysergide (2 μM), pyrilamine (3 μM) and guanethidine (2 μM) were added to the solution to eliminate effects of acetylcholine, 5-hydroxytryptamine, histamine and catecholamines which may possibly be released from the tissue. The following drugs were used: atropine sulphate (Tanabe), methysergide hydrogen maleate (Sandoz), pyrilamine maleate (Sigma), guanethidine sulphate (Ciba-Geigy), methoxyverapamil hydrochloride (D-600, Knoll), indomethacin (Wako), (−)-isoprenaline sulphate (Merck), bovine neurotensin (Peptide Institute Inc., Osaka), adenosine 5'-triphosphate (Sigma) and α,β-methylene adenosine 5'-triphosphate (Sigma). The stock solutions of all drugs except for indomethacin were dissolved in distilled water, made up at 1000 or more times higher concentrations than those used for the experiments, and stored at −20°C. Indomethacin was dissolved in ethanol. Final dilutions were made in distilled water just before use. Drugs were injected into the bathing medium or the reservoir in a volume less than 0.8% of the bath and reservoir volumes, and they were washed away by replacing the bathing medium with fresh medium.

Results

Effects of prolonged exposure to α,β-Me ATP on the contractile responses to nerve stimulation

Electrical field stimulation of the intramural nerves and stimulation of Remak's nerve elicited contractions of the longitudinal muscle of the isolated rectum preparation. The responses were mediated by non-adrenergic, non-cholinergic (NANC) neurones, since they were evoked in the presence of atropine (0.5 μM), tetrodotoxin (0.5 μM) completely abolished the responses, and neither noradrenaline nor adrenaline had excitatory effects on the smooth muscles (Komori *et al.*, 1980). Figure 1 shows the stimulus frequency-response relationships for stimulation of both the extrinsic and intrinsic nerves; they are plotted separately. It can be seen from this figure that there is no noticeable difference in the relationship between the stimulating sites. Stimulation at 20 Hz of Remak's nerve or of the intramural nerves elicited the maximal contractile response, but above 20 Hz the size of the responses was decreased.

α,β-Me ATP produced a concentration-dependent contraction of the isolated rectum preparation (Figure 2). The contractions developed rapidly,

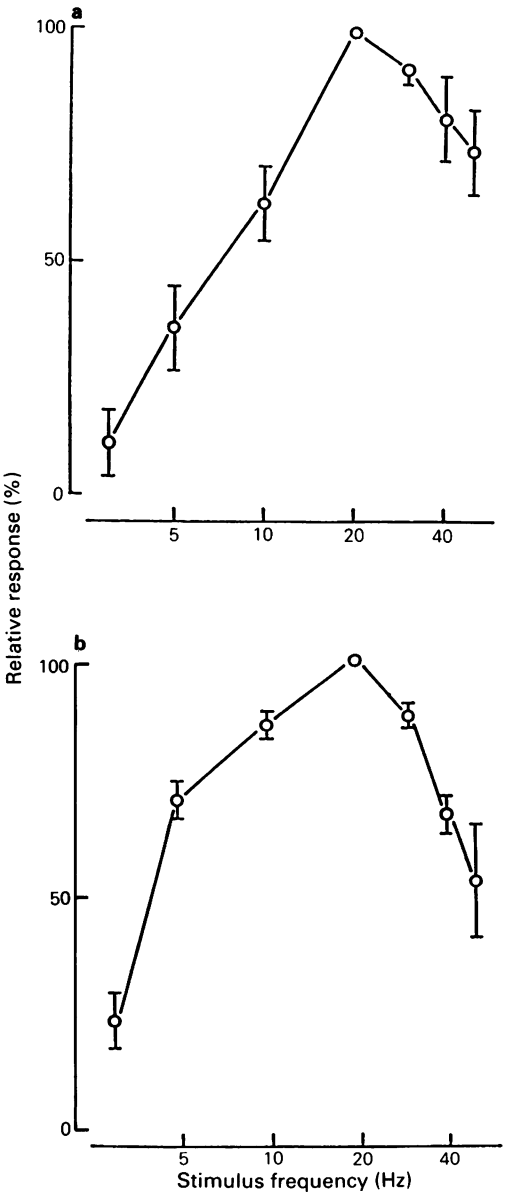

Figure 1 Frequency-response curves for contractions of the isolated rectum preparation to Remak's nerve stimulation (square wave pulses of 0.8 ms duration for 3 s) (a) and field stimulation of the intramural nerves (square wave pulses of 0.3 ms duration for 5 s) (b). Abscissa scale: log stimulus frequency; ordinate scale: % change in the size of the contractile responses (the size of the contractile responses at 20 Hz was taken as 100%). Each point represents the mean of 6–17 measurements; vertical lines indicate s.e. mean. A solution containing atropine (0.5 μM), methysergide (2 μM) and pyrilamine (3 μM) was used throughout the experiment.

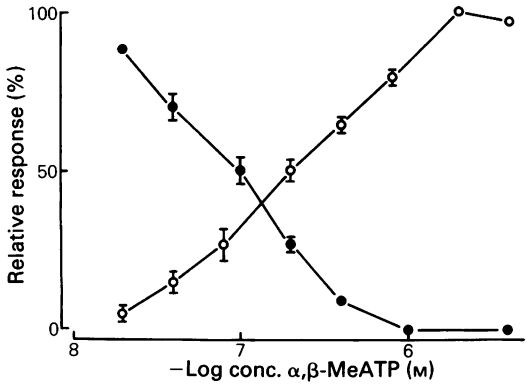

Figure 2 Concentration-response curves for the contractile effect of α,β-Me ATP (○) and for the inhibitory effect of α,β-Me ATP on the contractile response to field stimulation of the intramural nerves (●, square wave pulses of 0.3 ms duration for 5 s at 5 or 10 Hz) in the isolated rectum preparation. Abscissa scale: −log concentration of α,β-Me ATP; ordinate scale: % change in the size of the contractile responses (the size of the contraction induced by 2 μM α,β-Me ATP (○), and of the nerve-mediated contraction for the immediately preceding period in normal solution (●) were taken as 100%). Each point represents the mean of 6–8 measurements; vertical lines indicate s.e. mean. A solution containing atropine (0.5 μM), methysergide (2 μM) and pyrilamine (3 μM) was used throughout the experiment.

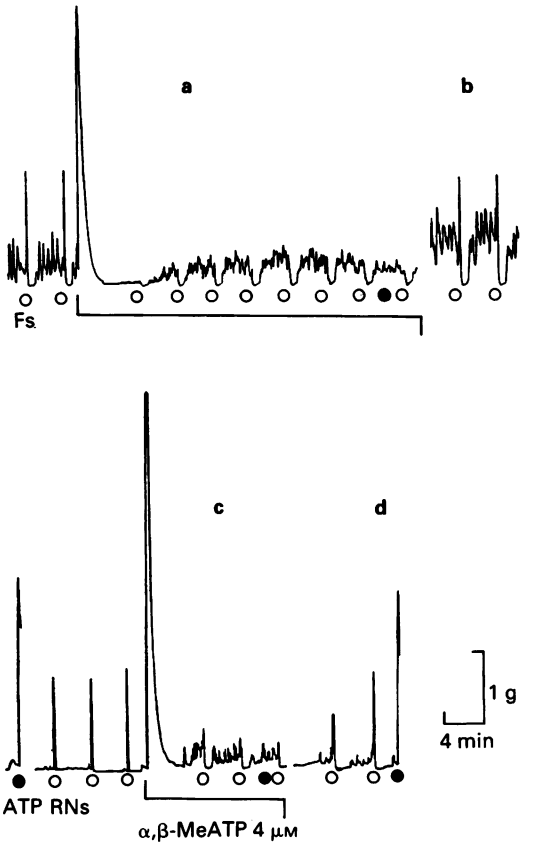

Figure 3 The effects of α,β-Me ATP (4 μM) on the contractile responses in the isolated rectum preparation to field stimulation of the intramural nerves with square wave pulses of 0.3 ms duration at 5 Hz for 5 s (○, Fs) and ATP (●, 100 μM) (a), and stimulation of Remak's nerve with square wave pulses of 0.8 ms duration at 10 Hz for 3 s (○, RNs) and ATP (●, 100 μM) (c). (b) and (d) Recovery from the inhibitory effect 20 min and 30 min after washout of α,β-Me ATP. A solution containing atropine (0.5 μM), methysergide (2 μM), pyrilamine (3 μM) and indomethacin (3 μM) was used throughout the experiment.

reached a peak in several seconds and faded rapidly to the baseline tension, or to a new tension level which was slightly higher than the baseline level especially when the drug was used at concentrations higher than 1 μM. The mean EC_{50} value from six preparations was 0.21 ± 0.03 μM.

After the contraction produced by α,β-Me ATP faded, the muscle was found to be less sensitive to NANC nerve stimulation at 5 or 10 Hz, regardless of the stimulating site. This stimulus frequency was just high enough to produce contractions with submaximal sizes in normal solution (see Figure 1). The inhibitory effect increased in a concentration-dependent manner as the drug concentration was increased (Figure 2). In most preparations, the solution containing 4 μM α,β-Me ATP converted the nerve-mediated contraction to a relaxation (Figure 3). The inhibitory effect of 4 μM α,β-Me ATP reversed slowly over a period of 20 to 60 min after washing, and it was reproducible if an interval of at least 60 min was allowed between applications.

Effects of α,β-Me ATP on contractions produced by ATP, bovine neurotensin and high $[K^+]_o$

The effect of prolonged exposure to α,β-Me ATP on the sensitivity of the longitudinal muscle of the isolated rectum preparation to other stimulant substances was studied. Contractile responses to ATP (100 μM), bovine neurotensin (neurotensin) (2.5 nM) and high K^+ (30 and 60 mM) were recorded 5 min and 10 min after exposure to the solution containing α,β-Me ATP in a medium (0.2 μM) or high (4 μM) concentration, and the magnitudes of the responses were compared with the corresponding values for the immediately preceding period in normal solution. The results are summarized in Figure 4. In every experiment, this treatment rendered the muscle less

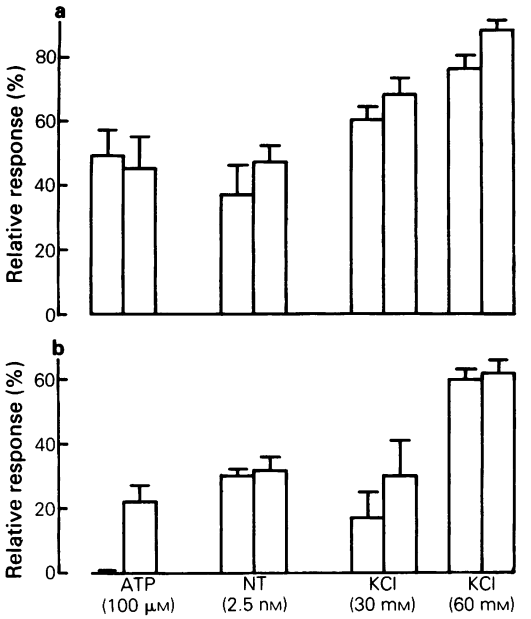

Figure 4 The inhibitory effects of α,β-Me ATP 0.2 μM (a) and 4 μM (b) on the contractile responses of the isolated rectum preparation to ATP (100 μM), bovine neurotensin (NT, 2.5 nM) and high K^+ (KCl, 30 mM and 60 mM). Ordinate scale: % change in the size of the contractile responses (the size of the control response was taken as 100%) measured 5 min (left in each pair) and 10 min (right in each pair) after exposure to α,β-Me ATP. Each column represents the mean of 6 measurements. Vertical lines indicate s.e. means. In fact, 5 min after the exposure to this drug the ATP-induced response was zero % for 5 measurements, but it was 3% for one measurement. A solution containing atropine (0.5 μM), methysergide (2 μM) and pyrilamine (3 μM) was used throughout the experiment.

sensitive to all three stimulant substances. Both neurotensin and ATP caused a transient and concentration-dependent contraction of the muscle (Komori *et al.*, 1986), and their contractile effects remained almost unaltered in the presence of tetrodotoxin (0.25 μM) which abolished contractile responses to field stimulation of the intramural nerves. The concentrations of ATP (100 μM) and neurotensin (2.5 nM) were slightly high to produce their half-maximal effect. When the external K^+ concentration was increased to 30 mM or 60 mM, by adding a concentrated solution of KCl to the bathing solution, the muscle immediately contracted and faded rapidly to the baseline tension. The 30 mM K^+-induced contraction was slightly smaller and the 60 mM K^+-induced contraction larger, than that produced by 2.5 nM neurotensin. Tetrodotoxin (0.25 μM) reduced the high K^+-induced contractions by only 10% or less.

Effects of α,β-Me ATP on membrane properties and e.j.ps evoked by stimulation of NANC neurones

In this series of experiments, α,β-Me ATP in the same concentrations (0.2 μM and 4 μM) as those selected to inhibit the contractile responses to NANC nerve stimulation was used. Figure 5 shows the effects of 0.2 μM α,β-Me ATP on membrane potential, electrotonic potentials (e.ps) and e.j.ps in the longitudinal muscle strip. These effects evidently increased when 4 μM α,β-Me ATP was used, as illustrated in Figure 6. Exposure of the tissue to the solution containing α,β-Me ATP caused membrane depolarization of the smooth muscle cells which reached a peak in 2–3 min after the exposure and within the next 20 s, began to decay to an almost steady level which was still more positive than the initial membrane potential in normal solution. The steady membrane potential was usually reached within 7 min after the peak depolarization and sustained unless the solution was replaced with normal solution. The peak depolarization at 0.2 μM was approximately 10 mV and that at 4 μM approximately 30 mV. The respective steady levels were a few mV and 10 mV or so more positive than the membrane potential in normal solution. The α,β-Me ATP-induced depolarization was accompanied by an increase in membrane conductance, as demonstrated by the decrease in both the amplitude and the time course of the e.ps, recorded from the cells at a distance less than one-fifth of the length constant of the tissue from the stimulating partition (Figures 5 and 6). The reduction of the e.p. amplitude varied from one preparation to another especially when the tissue was exposed to 0.2 μM α,β-Me ATP. The average amplitude obtained from seven different preparations was reduced to 44 ± 8% (±s.e.) at the peak depolarization and to 62 ± 10% at the steady membrane potential (expressed as a % of the control, recorded in the immediately preceding period in normal solution). The corresponding values with 4 μM α,β-Me ATP were 33 ± 7% ($n = 8$) and 55 ± 7% ($n = 8$), respectively. It was calculated from these values that the ATP analogue decreased the membrane resistance to 19% and 38% at 0.2 μM, and to 11% and 30% at 4 μM (Ohashi, 1970). In three preparations, at the peak depolarization produced by 4 μM α,β-Me ATP, electronic potentials were not evoked by current pulses even when applied with stronger potential fields, probably because of a profound increase in membrane conductance.

E.j.ps, evoked by field stimulation of the intramural nerves with a train of pulses at 1 Hz, were

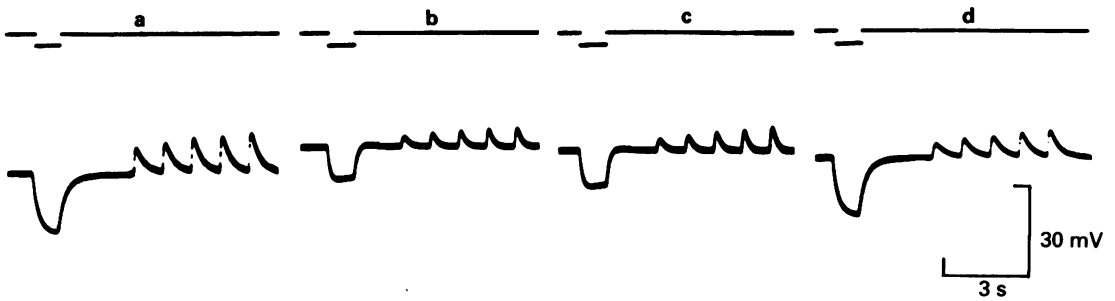

Figure 5 The effects of α,β-Me ATP (0.2 μM) on the intracellularly recorded membrane potential, electrotonic potentials and e.j.ps in the longitudinal muscle of chicken rectum. Electrotonic potentials were evoked by hyperpolarizing current of 1000 ms duration and e.j.ps were evoked by field stimulation of the intramural nerves (square wave pulses of 0.08 ms at 1 Hz). (a) Control; (b) 2.5 min and (c) 10 min after the exposure to α,β-Me ATP; (d) 30 min after washout of this drug. (a)–(d) Records from the same cell at 0.3 mm distance from the nearest stimulating partition (less than one-fifth of the length constant of the tissue). The horizontal line corresponds to the zero potential of the cell and the applied current strength. A solution containing atropine (0.5 μM), methysergide (2 μM), pyrilamine (3 μM), isoprenaline (1.3 μM) and D-600 (2 μM) was used throughout the experiment.

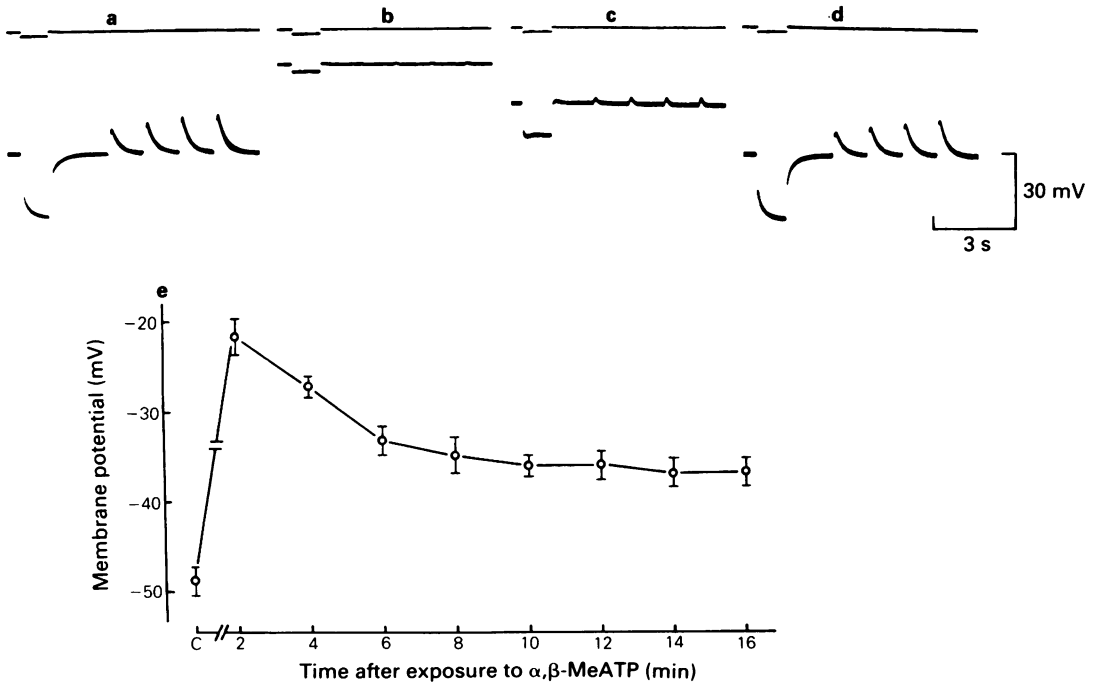

Figure 6 The effects of α,β-Me ATP (4 μM) on the intracellularly recorded membrane potential, electrotonic potentials and e.j.ps in the longitudinal muscle of chicken rectum. Electrotonic potentials were evoked by hyperpolarizing current of 1000 ms duration and e.j.ps were evoked by field stimulation of the intramural nerves (square wave pulses of 0.08 ms duration at 1 Hz). (a) Control; (b) 2 min and (c) 10 min after exposure to α,β-Me ATP; (d) 30 min after washout of this drug. (a)–(d) Records from the same cell at 0.2 mm distance from the nearest stimulating partition (less than one-fifth of the length constant of the tissue). The horizontal line corresponds to the zero potential of the cell and the applied current strength. A solution containing atropine (0.5 μM), methysergide (2 μM), pyrilamine (3 μM), isoprenaline (1.3 μM) and D-600 (2 μM) was used throughout the experiment. (e) Change in the membrane potential plotted against the time after exposure to α,β-Me ATP. C: the membrane potential before exposure to this drug. Each point represents the mean of measurements from 4 cells in 4 different preparations; vertical lines indicate s.e. mean.

Table 1 The amplitude and total duration of excitatory junction potentials (e.j.ps) in the longitudinal muscle strip from the chicken rectum measured at the peak and steady levels of the membrane depolarization produced by α,β-methylene ATP (4μM)

	E.j.p. amplitude (%)	*E.j.p. total duration* (ms)
Control	100	892 ± 23 (n = 8)
At peak depolarization	18 ± 5 (n = 9)*	291 ± 48 (n = 8)*
At steady depolarization	20 ± 3 (n = 9)*	284 ± 51 (n = 8)*

Values show mean ± s.e. mean. * Values significantly different from control values ($P < 0.001$).

decreased in amplitude and duration during the period of exposure to α,β-Me ATP. The magnitude of the effect also varied within a wide range from one preparation to another. The decrease in duration was strictly correlated with the change in membrane potential. However, the decrease in amplitude occurred with a certain delay after the change in membrane potential, so that the inhibition reached its maximum a few minutes after the peak membrane depolarization. This relationship was true in all preparations. The amplitude and total duration of the e.j.ps were measured at the peak depolarization and at the steady level of the membrane potential and the results are summarized in Table 1. Depolarization of the membrane, by injecting current to the same level as the steady membrane potential induced by 4 μM α,β-Me ATP, caused a decrease in the amplitude of e.j.ps of less than 10%, but no change in the total duration or a slight shortening. Therefore, the reduction in the amplitude and duration of the e.j.ps during exposure to α,β-Me ATP is due mainly to the action of this drug and is not as a result of voltage-dependent changes in membrane conductance.

The inhibitory effect of 4 μM α,β-Me ATP on the amplitude of e.j.ps survived after restoration of normal membrane properties by removal of the drug (Figure 7), and thus, there was a period during which e.j.ps were still reduced in amplitude but not in duration. After a period of exposure to 4 μM α,β-Me ATP for 10 min, the time required for complete recovery of e.j.ps from the inhibitory effect was 40 min or more. To see whether or not the prolonged inhibition of the amplitude of e.j.ps reflects desensitization of the purine receptors on the effector cells, ATP (100 μM) was applied to the isolated rectum preparation at various intervals after termination of exposure for 10 min to 4 μM α,β-Me ATP. The sizes of the ATP-induced contractions were plotted against the time after washout of α,β-Me ATP (Figure 8). Figure 8 clearly shows that the longitudinal muscle of the isolated rectum preparation regains normal sensitivity to ATP more rapidly than is seen with the e.j.ps (see Figure 7).

Discussion

The present results showed clearly that in the chicken rectum, NANC contractions and e.j.ps were reduced or abolished in the presence of α,β-Me ATP in concentrations of 0.02 to 4 μM. This was in agreement with previous results obtained using 10 μM α,β-Me ATP and recording only tension in the longitudinal muscle strip of chicken rectum (Meldrum & Burnstock, 1985). Meldrum & Burnstock (1985) concluded that the inhibitory effect of α,β-Me ATP was due to a specific desensitization of the type 2 purine receptor, since there was little effect on the carbachol-induced contraction. However, the present results showed that the presence of the stable ATP analogue resulted in a reduction of the contractile responses to bovine neurotensin and high K^+ as well as ATP. The apparently conflicting data regarding the specificity of desensitization produced by α,β-Me ATP may result from the different stimulant substances employed. In support of this suggestion, it is important to note that bovine neurotensin and high K^+ produced a phasic contraction, a very rapid but transient increase in muscle tension, whereas the carbachol-induced contraction presented by Meldrum & Burnstock was a biphasic response, a rapid phasic contraction immediately followed by a tonic contraction. Inhibition of biphasic contractions could not be readily evaluated. The rapid phase of the carbachol-induced contraction (Meldrum & Burnstock, 1985) was reduced to about half of the control size by α,β-Me ATP, but then the tension gradually increased up to a level as high as the maximal tension of the control contraction: i.e. the ATP analogue inhibited the phasic component of the carbachol-induced contraction leaving the final tension level unaffected.

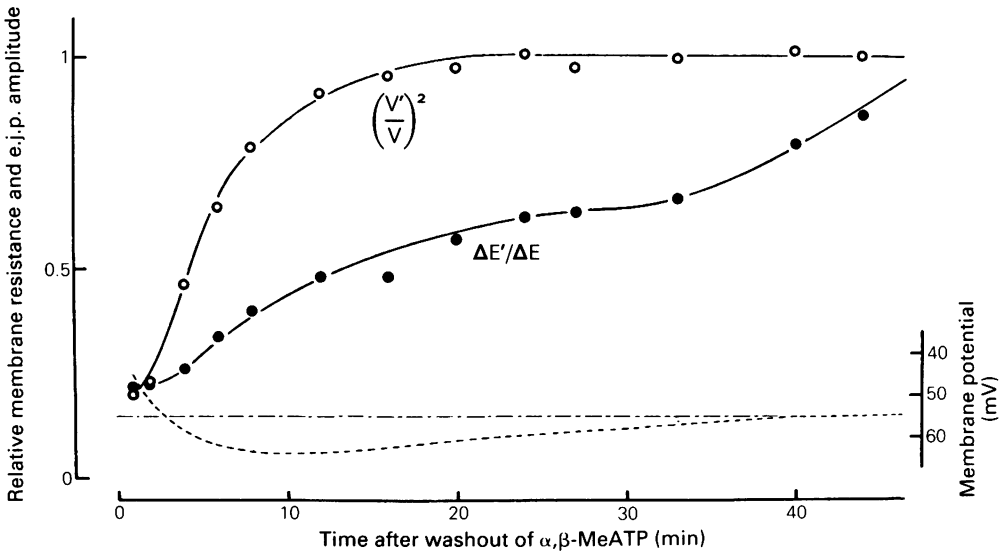

Figure 7 Changes in the membrane potential (– – –), membrane resistance (○) and amplitude (●) of e.j.ps recorded from the same smooth muscle cell in the longitudinal muscle, 0.3 mm from the nearest stimulating partition, after washout of a desensitizing concentration (4 μM) of α,β-Me ATP. Abscissa scale: time after washout of α,β-Me ATP. Ordinate scales: (left), relative membrane resistance in terms of the square of the amplitude ratio of electrotonic potentials (V') to the control electrotonic potential (V) evoked by hyperpolarizing current of 1000 ms duration and relative amplitude of e.j.ps (ΔE') to the control e.j.p. (ΔE) evoked by field stimulation of the intramural nerves (square wave pulses of 0.08 ms duration at 1 Hz); (right), membrane potential. A solution containing atropine (1 μM), guanethidine (2 μM) and D-600 (2 μM) was used throughout the experiment. The lines were drawn to fit the points by eye. The horizontal line corresponds to the control resting membrane potential. See the faster recovery of $(V'/V)^2$ compared to ΔE'/ΔE.

The mechanism underlying the contractile effect of α,β-Me ATP is an increase in Ca^{2+} entry into the smooth muscle cells by membrane depolarization. The depolarization of the smooth muscle cells was accompanied by a profound increase in membrane conductance which could not be attributed to voltage-dependent membrane rectification but was due to a prolonged action of α,β-Me ATP itself. Hence it is evident that the fading of the contractile response to α,β-Me ATP does not simply reflect ongoing desensitization of the purine receptors. The tension fading results from a depolarization block of action potential discharge, which seems to be especially important in the longitudinal muscle, a freely spiking smooth muscle (Komori & Ohashi, 1982), and from a time- and voltage-dependent inactivation of voltage-sensitive Ca channels (Hurwitz, 1986). On the basis of this idea, the reduction in the responses to bovine neurotensin and high K^+, which both activate mainly voltage-sensitive Ca channels (Donoso *et al.*, 1986; Ohashi & Komori, unpublished observations), can be explained. Furthermore, the prolonged increase in membrane conductance is a problem when explaining the inhibition of e.j.ps. The e.j.p., if mediated by a transmitter which increases membrane conductance, would theoretically be expected to become smaller under the decreased membrane resistance. Therefore, the inhibition of the contractions and e.j.ps during exposure to α,β-Me ATP proved to be unsatisfactory in this preparation for qualification of the transmitter as ATP.

Unlike the situation in this smooth muscle, α,β-Me ATP appears to produce selective desensitization of P_2-purinoceptors in other smooth muscles (vas deferens, Meldrum & Burnstock, 1983; Sneddon & Burnstock, 1984; Sneddon & Westfall, 1984; Stjärne & Åstrand, 1985; urinary bladder, Kasakov & Burnstock, 1983; Hoyle & Burnstock, 1985; Moss & Burnstock, 1985; Katsuragi *et al.*, 1986; blood vessel, Sneddon & Burnstock, 1985; Kügelgen & Starke, 1985; Muramatsu, 1986). Hence, it has been used as an investigative tool to demonstrate the roles of noradrenaline and ATP as cotransmitters.

The findings that there was a short delay between the peak inhibitory effect on the e.j.p. and the peak membrane depolarization produced by the ATP analogue, and that the membrane potential and membrane conductance returned to the control level on washout of α,β-Me ATP more rapidly than did the amplitude of the e.j.p. suggest the involvement of

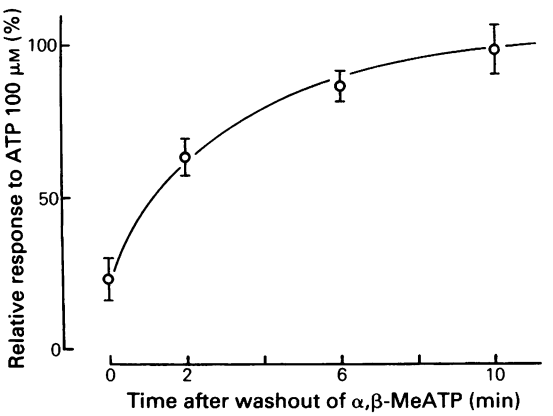

Figure 8 Changes in the size of contractile responses in the isolated rectum preparation to ATP (100 μM) against the time after washout of a desensitizing concentration (4 μM) of α,β-Me ATP. Abscissa scale: time after washout of α,β-Me ATP. Ordinate scale: % change in the size of contractile response. Each point represents the mean of 6–7 measurements; vertical lines indicate s.e. mean. A solution containing atropine (1 μM), methysergide (2 μM), pyrilamine (3 μM) and indomethacin (3 μM) was used throughout the experiment. The line was drawn to fit the points by eye.

unidentified factor(s) in the inhibition of e.j.ps. This might be a prejunctional action of α,β-Me ATP inhibiting transmitter release through activation of a slower process. If the NANC nerves mediate the e.j.p. by releasing ATP, this could be desensitization of the purine receptors. The faster recovery of the contractile effect of ATP after washout of the high concentration (4 μM) of α,β-Me ATP makes the latter possibility unlikely. Therefore, further electrophysiological experiments using local application, by pressure ejection from micropipettes or by ionophoresis, of ATP and other depolarizing drugs need to be undertaken for the period during which the amplitude of the e.j.ps remain reduced; however, the membrane properties returned to normal levels after washout of α,β-Me ATP.

In the guinea-pig vas deferens it has been suggested that the e.j.p. is produced by ATP released from adrenergic fibres as a cotransmitter with noradrenaline (Sneddon *et al.*, 1982; Sneddon & Westfall, 1984). However, the chicken rectum receives adrenergic inhibitory innervation so that noradrenaline, whether applied exogenously or released from the nerve, produces membrane hyperpolarization of the longitudinal muscle (Komori *et al.*, 1980; Komori & Ohashi, 1987). Stimulation of Remak's nerve elicits the NANC excitatory responses followed by the adrenergic inhibitory responses and the former, but not the latter, can be blocked by hexamethonium (Kanazawa *et al.*, 1980). The simultaneous addition of phentolamine and propranolol blocked the residual adrenergic-mediated responses, but did not appear to cause any membrane depolarization (Komori & Ohashi, 1987). The e.j.p. remained unaffected in the presence of guanethidine. Thus, the NANC responses in the chicken rectum do not appear to be mediated by ATP released from the adrenergic nerves as a cotransmitter with noradrenaline.

Much less is known about the coexistence of ATP with acetylcholine within cholinergic nerve terminals in the autonomic nervous system. The shortest latency of the e.j.p. was less than 10 ms and spontaneous e.j.ps have been recorded from the smooth muscle cells in the chicken rectum (Komori & Ohashi, 1982), indicating that the NANC nerve terminals are closely adjacent to the smooth muscle membranes. However, the e.j.p. was not followed by any atropine-sensitive change in membrane potential in normal solution or even in the presence of physostigmine. This makes it difficult to assume that a substance responsible for the e.j.p. is ATP released as a cotransmitter with acetylcholine from cholinergic nerves supplying this organ. If ATP mediated the e.j.p., then the NANC neurones innervating the rectum of the chicken would be purinergic neurones that contain the nucleotide as the principal transmitter in their storage vesicles (Burnstock, 1972).

It can be argued from the present results that the suppression by α,β-Me ATP of the contraction and e.j.p. evoked by excitation of NANC nerves in the chicken rectum, unlike the mammalian preparations described previously, is due mainly to a change in the electrical properties of the membrane of the smooth muscle cells, rather than being due, or only partly due, to desensitization of the purine receptor. In addition, α,β-Me ATP is not necessarily a satisfactory tool in this preparation for investigating whether or not ATP is the transmitter.

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