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Membrane potential responses to ATP applied by pressure ejection in the longitudinal muscle of chicken rectum

Seiichi Komori & Hidenori Ohashi

Laboratory of Pharmacology, Department of Veterinary Science, Faculty of Agriculture, Gifu University, Gifu 501-11, Japan

1 Changes in membrane potential in response to local application of ATP by pressure ejection from a micropipette were recorded intracellularly from smooth muscle cells of the longitudinal muscle strip of chicken rectum.

2 The local application of ATP produced a membrane depolarization. The depolarizing response increased to a plateau of 33 mV with prolongation of the duration of pressure pulses which determines the amount of ATP ejected. The latency and the time required to reach a peak depolarization were not related to the pulse duration, and the shortest latency was 50 ms.

3 When the application of ATP was repeated at a short interval, the second and subsequent depolarizing responses were suppressed, and their latency and time to reach a peak were also increased; the muscle cells became desensitized to ATP. Recovery from the desensitization occurred slowly over a period of 60 s.

4 Electrotonic potentials decreased in amplitude and time course during an ATP-induced depolarization, indicating a decrease in membrane resistance.

5 The ATP-induced depolarization was longer in the latency than an excitatory junction potential (e.j.p.) elicited by electrical field stimulation of the intramural nerves. The other variables such as amplitude, time to reach the peak and duration could not be matched with those of the e.j.p. at the same time.

6 The e.j.p. decreased in amplitude and duration during the ATP-induced depolarization, and its initial amplitude and duration were restored immediately after termination of the ATP-induced depolarization, as in the case of electrotonic potentials. The e.j.ps were recorded in the same manner as the control from the muscle cell which was in a state of desensitization to ATP.

7 The present results provide an argument against the hypothesis that ATP mediates the e.j.p. in the rectum of the chicken.

Introduction

Electrophysiological studies (Ohashi *et al.*, 1977; Takewaki & Ohashi, 1977; Komori & Ohashi, 1982; 1988a) have provided evidence for a dense, excitatory innervation by non-adrenergic, non-cholinergic (NANC) nerves in chicken rectum. Excitatory junction potentials (e.j.ps), which are pharmacologically NANC in nature, can be recorded from cells in the longitudinal muscle layer, irrespective of the sites of nerve stimulation, the extrinsic nerve (Remak's nerve) and intrinsic nerve (intramural nerves). Recently, adenosine 5'-triphosphate (ATP) (Meldrum & Burnstock, 1985) and chicken neurotensin (Komori *et al.*, 1986) have been proposed as candidates for the chemical transmitter linking the nerve with the smooth muscle.

The ATP hypothesis rests on the basis of the observations that ATP produced a rapid contraction like the nerve-mediated contraction in the longitudinal muscle strip of the rectum, and α, β-methylene ATP, which is capable of desensitizing purinoceptors in many smooth muscle preparations, abolished both ATP- and nerve-mediated contractions without change in carbachol-induced contraction. On the other hand, Komori *et al.* (1988) observed that α, β-methylene ATP produced a decrease in membrane resistance of the smooth muscle cells of chicken

rectum; this effect did not disappear unless the drug was removed, and the time taken for recovery of ATP-induced contraction from the inhibition after removal of α, β-methylene ATP was much shorter than that of the e.j.p. From these observations, they argued that the inhibitory effect of α, β-methylene ATP on the nerve-mediated contraction is not merely due to its ability to desensitize the purinoceptor. Thus, many questions remain to be solved, before ATP can be accepted as the transmitter.

To obtain further evidence pertinent to these questions, the present study was attempted to investigate electrical membrane responses to ATP applied locally by means of pressure ejection from a micropipette, in the longitudinal muscle strip of chicken rectum.

Methods

Sixteen White Leghorn chickens (*Gallus domesticus*) of either sex, aged more than 30 days, weighing 0.2–2.0 kg, were stunned and bled to death. The rectal region of the intestine was removed and flushed clean with Tyrode solution (composition, mM): NaCl 137.0, KCl 2.7, NaH_2PO_4 0.4, $NaHCO_3$ 12.0, $MgCl_2$ 1.0, $CaCl_2$ 1.8 and glucose 5.6). Strips of the intestinal wall (1.5–2.0 mm wide, 15–20 mm long) were obtained by cutting along the longitudinal axis of the isolated rectum, from which the longitudinal muscle layer was carefully dissected free of other tissues.

The muscle preparation was mounted serosal side up in an organ bath (about 2 ml) consisting of two chambers, as described by Abe & Tomita (1968). The organ bath was irrigated at a flow rate of 2–3 ml min^{-1} with Tyrode solution preheated to 30°C. At temperatures higher than this, stable measurements of membrane responses could not be obtained. A pair of Ag wire-electrodes (1 mm in diameter) was used for electrical stimulation of intramural nerves of the tissue. One electrode, which was insulated with Araldite 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 the tissue surface, and the other electrode, which was uninsulated, was placed in the bath solution.

For local application of ATP to the tissue, glass micropipettes (3–10 μm in tip diameter) filled with ATP solution (8–20 mM) made up in Tyrode solution were used. The drug-containing pipette was connected by polyethylene tubing to a nitrogen tank via a three-way solenoid valve (CKD corp., UMGI-T1) so that the drug solution could be ejected from the pipette by pressure (2.5–3.5 kg cm^{-2}). Ejected amounts of the drug solution (doses) can be controlled by varying the duration of current pulses (8–100 ms) delivered from a stimulator (Nihon Kohden, MES-3) to turn on the solenoid valve. The relationship between the pulse duration and the drug amount was examined by the following procedures: when a drug-containing micropipette was placed in the air and the drug solution was ejected by pressure, the ejected drug solution was obtained as a water drop on the outer surface of the pipette. The diameter of the water drop was measured under a binocular microscope. When pressure ejection was repeated with current pulses of a fixed duration at an interval of 5 s (the shortest interval tested) or longer, the water drops obtained were virtually constant in their diameter. As the pulse duration was increased the drop diameter increased, and it was found that there is a roughly linear relationship between the logarithm of the drop volume and the logarithm of the pulse duration. If the pulse duration was shortened to less than 8 ms, it was ineffective in ejecting the drug solution, probably because of loss of pressure somewhere in the ejection device.

The experimental apparatus used is capable of recording electrotonic potentials, excitatory junction potentials (e.j.ps) and responses to pressure ejection of ATP from the same cell. Electrotonic potentials were elicited by applying current pulses (0.3 or 0.5 s duration) via the large extracellular electrodes, and e.j.ps by field stimulation of intramural nerves with square-wave pulses of a short duration (0.2 or 0.3 ms) at an appropriate intensity (10–30 V). The stimulus pulses were delivered by a stimulator (Nihon Kohden, SEN-3013).

Glass microelectrodes filled with 3 M KCl solution, with a resistance which varied from 30 to 80 MΩ, were used to record intracellularly changes in membrane potential. When stable penetration of the recording electrode was obtained, the drug-containing micropipette was manipulated to place the tip in a position as close as possible to the tissue surface and 50 μm away from the recording electrode. Stable penetration was accepted if e.j.ps or electrotonic potentials were recorded of sufficiently large amplitude. Potential changes were recorded from smooth muscle cells in a restricted region of the tissue midway between the nerve stimulating electrode and the partition plate (one of the current applying electrodes), which were displayed on a cathode-ray oscilloscope (Nihon Kohden, VC-9) and recorded with a film-movie camera.

Tyrode solution was bubbled with air in the reservoir. To Tyrode solution were added atropine (0.5 μM), isoprenaline (1.3 μM) and methoxyverapamil (D 600, 10 μM) throughout the course of the experiments. Atropine served to block effects of acetylcholine possibly released from the tissue, and isoprenaline and D 600 served to suppress electrical spike activity and development of tension in the

smooth muscle. The concentrated solutions of these drugs were made up and stored at −20°C. Final dilutions of the stock solution were made in Tyrode solution just before use and the solutions were frequently renewed.

Drugs used were atropine sulphate (Tanabe), (−)-isoprenaline sulphate (Merk), adenosine 5'-triphosphate (ATP, Wako) and D 600, (Knoll).

The experimental values obtained were expressed as mean ± s.e. mean. The regression lines were calculated by the least squares method. Statistical significance was tested by Student's t test, and probabilities of less than 0.05 ($P < 0.05$) were considered significant.

Results

Membrane responses to ATP

Local application of ATP by means of pressure ejection from a micropipette produced a transient membrane depolarization of smooth muscle cells of the longitudinal muscle preparation (number of preparations: $n = 27$). With pressure pulses of various durations (8–100 ms) which determine the amount of the drug ejected, ATP-induced depolarizations were found to be 2–35 mV in amplitude, 0.1–3.0 s in the time required to reach a peak and 1–15 s in the total duration. Even with a given pressure pulse, these characteristics of the responses to ATP showed a wide variation from one preparation to another and between cells in one preparation. Because of this, mean values were not given for any of these variables. The ATP response occurred with a delay from the beginning of the current pulse which was monitored as a sharp deflection of the beam on each record. This delay, the latency for the ATP response, varied with a range from 50 ms up to 350 ms in different cells, but in most cells it fell within 100–200 ms. However, when a series of responses to ATP was recorded continuously from the same cell, the amplitude and total duration of ATP responses were reproducible and increased as the duration of pressure pulses was increased. The time to reach a peak and latency seemed not to be pulse duration-dependent. Figure 1 shows the records of the depolarizing responses to ATP ejected with different pressure pulses from two cells in different preparations and the graph plotting changes in the amplitude of the ATP-induced depolarizations against the pulse duration on a semilogarithmic scale. It can be seen that the curve is roughly linear up to 20 mV, but with larger amplitudes than 20 mV it bends towards the abscissa axis. Similar results were obtained with three other preparations and the amplitude of the response to ATP was found to

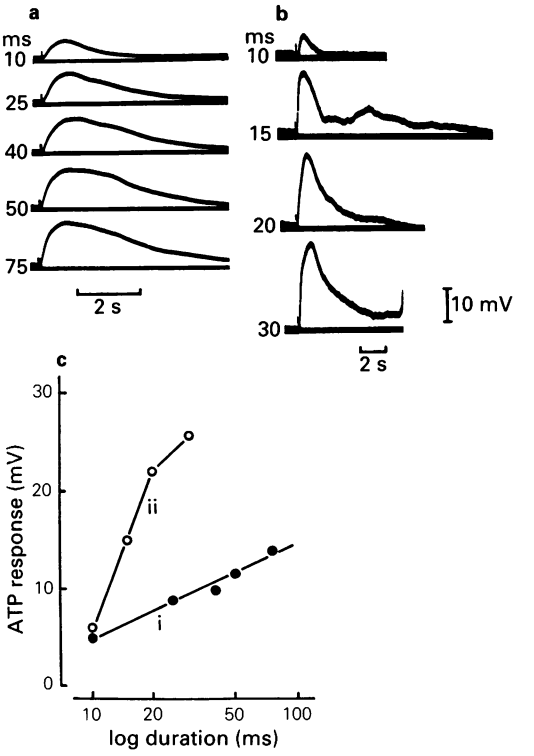

Figure 1 Dose-related membrane responses to local application of ATP. The responses to ATP ejected from a micropipette filled with 8 mM ATP solution by pressure pulses of varied duration (10–75 ms, shown in each record) were recorded intracellularly from two cells (a and b) of different longitudinal muscle preparations of chicken rectum. The sharp upward deflection preceding each ATP-induced response represents the start of pressure ejection. (c) Plots of changes in the amplitude (mV) of responses to ATP against the duration of pressure pulses on a semilogarithmic scale. (i) and (ii): Curves obtained from the records in (a) and (b). In this and subsequent figures, atropine (0.5 μM), isoprenaline (1.3 μM) and D 600 (10 μM) were present in the bath perfusing solution.

increase to a plateau of 33 ± 1.4 mV (number of cells: $n = 6$).

Figure 2 shows the successive responses to ATP applied repeatedly at intervals of 8 s by ejection with pressure pulses of 20 ms duration. The amplitude of the responses decreased and the latency increased for the second and subsequent applications. In four preparations, responses to ATP ejected by a pair of pressure pulses of a fixed duration at varied intervals were recorded from a total of 27 cells. When the interval between the two applications was of such a short period that discrete ATP responses were just

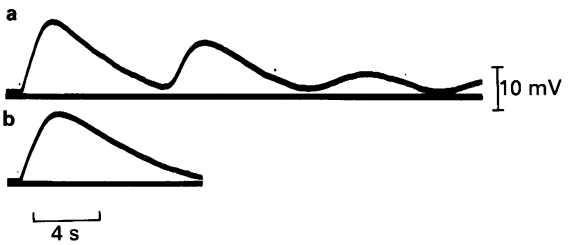

Figure 2 Membrane responses to repeated local applications of ATP. (a) Successive responses to ATP ejected from a micropipette filled with 25 mM ATP solution with a train of 4 pressure pulses of 20 ms duration at 8 s intervals; (b) 50 s after the train. (a and b) Records from the same cell of the longitudinal muscle preparation of chicken rectum.

obtained, the second ATP response was smaller in amplitude in most cells (22 cells). The latency and time to reach the peak for the second response were invariably prolonged to some extent. These effects on the second response evidently decreased with longer application intervals. When a period of 60 s or so elapsed between two successive applications, the ATP responses matched each other in amplitude and time course. The pooled data are summarized in Figure 3, in which the ratio of the amplitude of the second response to the first response is plotted against the interval between the two ATP applications and to avoid complication, the intervals used are grouped into bins every 10 s. The inhibitory response to ATP showed desensitization, and the desensitization subsided slowly with a half decay time of about 20 s and disappeared in around 60 s.

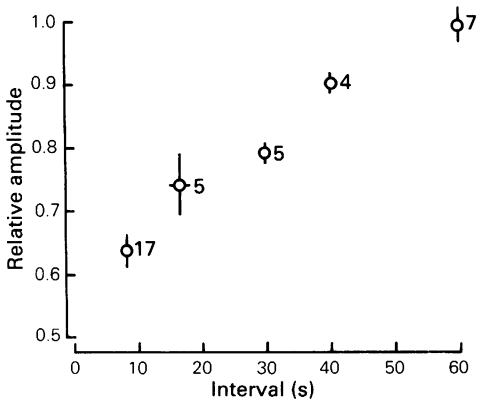

Figure 3 Relationship between amplitude of ATP responses and interval of ATP applications. ATP was ejected from a micropipette filled with 20 mM ATP solution with paired pressure pulses of 20 ms duration at different intervals. The used intervals are grouped into bins every 10 s. The amplitude of the second ATP response is expressed as a ratio to the amplitude of the first ATP response. Each point represents mean of a number of observations (figures by each point) in four different preparations; s.e. shown by vertical lines.

Changes in membrane resistance during depolarization produced by ATP

ATP was ejected with pressure pulses of varied duration and during the depolarizing responses to ATP, electrotonic potentials were evoked every 3 s by passing inward or outward currents (0.3 or 0.5 s in duration). The electrotonic potentials were decreased in amplitude and time course during the membrane depolarization. The effects were maximal with the electrotonic potential evoked almost at the peak depolarization of the ATP response, and evidently decreased with the decay of the ATP response (Figure 4a). Furthermore, the reduction in amplitude of the electrotonic potentials seemed to be increased as the degree of the ATP-induced depolarization increased. This relationship was examined by plotting the percentage reduction of the amplitude of electrotonic potentials (Y) against the amplitude of the ATP responses (X) (Figure 4b). The plotted data were obtained from 30 cells in three different preparations. It was found that there is a positive correlation between the two variables ($n = 30$, $r = 0.769$, $P < 0.001$), giving a regression line of $Y = 2.4\ X + 7.7$. Thus, the membrane depolarization in response to ATP ejection appears to be accompanied by an increase in membrane conductance.

Two discrete ATP responses with markedly different amplitudes were recorded from some cells ($n = 6$) by repetition of ATP ejection with a constant pulse duration at an appropriate interval, while electrotonic potentials were evoking every 3 s. The electrotonic potentials evoked between the two ATP responses were much the same as those evoked before the first ATP application in all of the cells. This indicates that the reduction of the second ATP response is not attributable to any remaining effect of the preceding application of ATP on the membrane resistance.

Comparison of the ATP response and e.j.ps

Electrical field stimulation of the intramural nerves of the muscle preparation with single square-wave pulses (0.2 or 0.3 ms duration) at an intensity of 10–30 V produced e.j.ps in all cells. The amplitude varied from 3 to 15 mV, giving a mean value of 5.7 ± 0.3 mV ($n = 25$), the latency was approximately 10 ms, the time to reach a peak depolarization varied

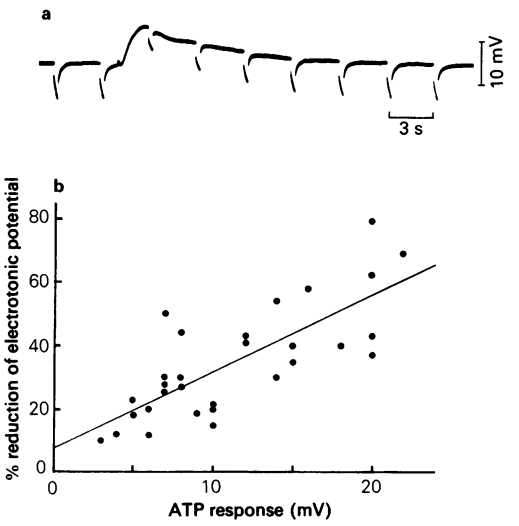

Figure 4 Change in membrane conductance during depolarization produced by local application of ATP. (a) Response to ATP ejected from a micropipette filled with 20 mM ATP solution with a single pressure pulse of 50 ms duration, during which electrotonic potentials were evoked every 3 s by passing an inward current of 300 ms duration. (b) Percentage reduction in amplitude of electrotonic potentials which were evoked at the peak depolarization of ATP responses, plotted against the amplitude of the ATP responses. The amplitude of electronic potentials evoked before ATP application was taken as 100%. The plotted electrotonic potentials and ATP responses were recorded from 30 cells in three different preparations. The calculated regression line is given as $Y = 2.4X + 7.7$ ($n = 30$, $r = 0.769$, $P < 0.001$), where Y is percentage reduction of electrotonic potential amplitude and X is the amplitude of response to ATP.

from 50 to 200 ms, giving a mean value of 68.3 ± 2.0 ms, and a total duration of about 1 s, comparable with those previously reported (Takewaki & Ohashi, 1977; Komori & Ohashi, 1982; 1984). The latency for the e.j.p. was around one-fifth of the shortest latency for the ATP response (50 ms or so). Both the e.j.p. and the ATP response could be matched to each other in any two of the three parameters such as amplitude, time to reach a peak depolarization and total duration, but it was not possible to match all of them at the same time. Some ATP responses reached their peak as rapidly as did the e.j.ps; the time to reach a peak depolarization ranged between 130 and 200 ms ($n = 6$), which fell within the corresponding values for the e.j.ps. However, the amplitude of the ATP responses was larger and their total duration was longer, compared to the e.j.ps. When repetitive field stimulation was carried out, evoked e.j.ps gave a summed depolarization as large as one of the ATP responses. The depolarization resulting from summed e.j.ps differed in duration from the ATP response. The ATP responses (number of cells in different preparations: $n = 6$) with the same amplitude and total duration as the e.j.ps invariably required a longer time to reach peak depolarization (300–500 ms).

Changes of e.j.ps during the ATP response

Figure 5a shows an e.j.p. elicited during the membrane depolarization in response to ATP ejection. It can be seen that the e.j.p. is small in amplitude and short in duration. Plots of the percentage reduction

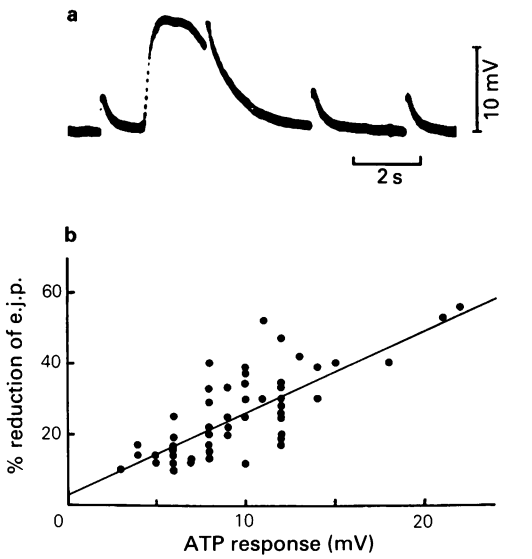

Figure 5 Change in amplitude and time course of e.j.ps elicited by field stimulation of intramural nerves with square-wave pulses of 0.3 ms duration during depolarization produced by local application of ATP in the longitudinal muscle preparation of chicken rectum. (a) The depolarization produced by ATP ejected from a micropipette filled with 20 mM ATP solution by a single pressure pulse of 10 ms duration, and the e.j.ps elicited every 3 s. (b) Percentage reduction in amplitude of e.j.ps which were elicited almost at the peak depolarization of ATP responses plotted against the amplitude of the ATP responses. The amplitude of e.j.ps elicited before ATP application was taken as 100%. All the points were obtained from records in four different preparations. The calculated regression line is given as $Y = 2.3X + 3.1$ ($n = 51$, $r = 0.755$, $P < 0.001$), where Y is the percentage reduction of e.j.p. amplitude and X is the amplitude of the ATP response.

of the e.j.p. amplitude against the amplitude of the ATP response are presented in Figure 5b. All the plotted points were obtained from e.j.ps evoked nearly at the peak of the ATP response in four different preparations, and the amplitude of e.j.ps evoked immediately before each ATP application was taken as 100%, which ranged from 4 to 11 mV with the mean of 6.2 ± 0.2 mV ($n = 51$). It was found that there was a positive correlation between the two variables ($n = 51$, $r = 0.755$, $P < 0.001$), giving a regression line expressed by the equation, $Y = 2.3X + 3.1$, where Y is the percentage reduction of the e.j.p. amplitude and X is the ATP response amplitude. On termination of the ATP response, the e.j.p. was restored to its initial amplitude and duration (see also Figure 5a). It seems unlikely that the inhibitory effect of ATP on the e.j.ps resulted from desensitization to ATP of the muscle cells. This view was corroborated by the following findings. As shown in Figure 6a, when application of ATP was repeated twice at 8 s intervals, the second ATP response was reduced to about half of the first one, whereas the e.j.p. evoked just before the second ATP response was much the same as the e.j.p. before the first ATP response. Qualitatively similar results were obtained repeatedly and consistently from four other preparations. Figure 6b illustrates a typical result of experiments in which e.j.ps were elicited by a train of 5 pulses at 1 Hz before and after desensitization of the cell to ATP. Application of ATP was repeated ten times every 15 s. The amplitude of 15 mV in the first ATP response (not shown in this figure) decreased to about 3 mV in the tenth response. The e.j.ps evoked during the tenth ATP response were very similar to those evoked before the first application of ATP.

Figure 6 Influence of desensitization to ATP on e.j.ps evoked by field stimulation of intramural nerves with square-wave pulses of 0.2 ms duration in the longitudinal muscle cells of chicken rectum. (a) Responses to ATP ejected from a micropipette filled with 20 mM ATP solution by pressure pulses of 10 ms duration, and e.j.ps elicited every 3 s: (i) ATP responses at 8 s interval; (ii) ATP response 60 s later from the second ATP response in (i). Note a decrease in amplitude and time course of e.j.ps evoked during the ATP responses, but no change in the e.j.p. between the ATP responses in (i), (b) E.j.ps elicited by trains of five stimuli at 1 Hz: (i) control; (ii) during the tenth response of successive responses to ATP ejected by a train of ten pressure pulses of 10 ms duration at 15 s interval. (i) and (ii), Records from the same cell. Dashed lines, the basal level of membrane potential of the cell. The amplitude of the ATP responses was decreased from 15 mV to about 3 mV, exhibiting desensitization. Note no appreciable change in the e.j.ps during the desensitization of the cells to ATP.

Discussion

Properties of the ATP-induced response

The present results indicate that local application of ATP by pressure ejection produced a membrane depolarization of the longitudinal muscle of the chicken rectum, which was accompanied by an increase of membrane conductance, as demonstrated by the decrease in both the amplitude and time course of electrotonic potentials. With increase in the amount of ATP applied, the amplitude of the depolarizing response increased to a plateau of 33 mV. The response may result from interaction of ATP with the P_2 type of receptors, since this type of receptor seems to be present in most smooth muscle tissues and responsible for producing the excitatory effects (Kasakov & Burnstock, 1983; Meldrum & Burnstock, 1983).

The shortest latency for the ATP-mediated depolarizing response was 50 ms. Considering the possibility that there are time lags introduced by some factors inherent in this method of application, a delay between the moment at which ATP reaches the cell surface and the onset of the ultimate effect (the membrane depolarization) should be reasonably less than 50 ms. Byrne & Large (1984) applied ATP and noradrenaline ionophoretically to the rat anococcygeus muscle, and found the latency for ATP-mediated depolarization to be approximately 30 ms, which was much shorter than that for the noradrenaline-mediated depolarization via activation of the α-adrenoceptor. The depolarization produced by acetylcholine or carbachol, when applied ionophoretically, has been shown to be delayed by 100 ms or more in intestinal smooth muscle of the guinea-pig, and the delay seems to be mainly required for the sequence of reactions after its binding to the muscarinic receptor (Purves, 1974;

Bolton, 1976). Therefore, in many smooth muscles including the rectal muscle of the chicken, it seems likely that the P_2 type of receptor gives a depolarizing response in a relatively short time after its activation, compared with the α-adrenoceptor and muscarinic receptor.

When ATP was applied repeatedly at short intervals (up to 5 s), the amplitude of the depolarizing response to ATP decreased in all of the cells from which the responses were recorded, i.e. the muscle cells, resulting in a state of desensitization to ATP. Moreover, latency and time to reach peak depolarization were prolonged. All these changes occurred without any remaining effect of the preceding ATP on the electrical membrane properties, as monitored by electrotonic potentials. Further, the amount of ATP, if ejected repeatedly at an interval of up to 5 s from a micropipette, was kept constant (see Methods). Therefore, the desensitization to ATP may be attributable to a receptor alteration or a post-receptor phenomenon. Substantially similar desensitization to ATP has been described for the rabbit ear artery (Suzuki, 1985). Recovery from the desensitization occurs slowly over a period of 70 s for the rabbit ear artery and 60 s for the chicken rectum; the rate of recovery is very similar in both smooth muscle tissues. These characteristics may be true of other smooth muscle tissues, at least if ATP is applied locally and interacts with the P_2-purinoceptors. A similar repetitive application of neurotensin produced successive membrane depolarizations in the rectal muscle of the chicken, which remained almost unchanged in amplitude and time course (Komori & Ohashi, 1988b).

Does ATP mediate the e.j.p.?

It has been shown that in the chicken rectum, the e.j.p. is associated with a transient increase in membrane conductance, and its reversal potential is about −15 mV (Komori & Ohashi, 1988a). The present results show that the ATP-induced depolarization was accompanied by an increase in membrane conductance. As the amount of ATP ejected was increased, the amplitude of the depolarization increased to a maximum of about 35 mV, suggesting the reversal potential for the ATP response is much more positive than the resting membrane potential (about −47 mV). Such a similarity in the actions on the muscle of ATP and a substance mediating the e.j.p. is superficially consistent with the hypothesis that ATP is the neutrotransmitter of the NANC excitatory nerve in this region of the intestine (Meldrum & Burnstock, 1985).

The response to a single local application of ATP can be viewed as having four characteristic variables: the latency, time to reach peak depolarization, amplitude of peak depolarization and duration. Thus, these variables were compared with the corresponding variables of the e.j.p. The latency is longer in the ATP response. With the other three variables, all of them could not be fitted to those of the e.j.p. at the same time. Occasionally, ATP elicited depolarizations which reached a peak as rapidly as the e.j.p., but these responses were much larger in amplitude and duration than the e.j.p. However, since the variables such as amplitude and duration are a function of the amount of ATP applied (see Figure 1), it would be possible to obtain a depolarizing response just like the e.j.p. by reducing the amount of ATP applied. If local application of ATP mimicked the e.j.p. it would provide a necessary basis, but not totally convincing evidence for identification of ATP as the neurotransmitter. In fact, a similar local application of neurotensin, which has also been considered as a candidate for the neurotransmitter (Komori *et al.*, 1986), produces much the same effect as ATP on the rectal muscle (Komori & Ohashi, unpublished observation).

The present results provide an argument against the ATP hypothesis. The inhibition of the e.j.p. during the depolarization produced by ATP could not be attributed to any desensitization of the muscle cell to ATP. After one exposure of a muscle cell to ATP, the cell became desensitized to ATP, a state which lasted for a period of about 60 s whereas the amplitude and total duration of the e.j.p. returned to the control level immediately after termination of the depolarizing response to ATP lasting for less than 15 s. After a muscle cell was rendered nearly insensitive to ATP, the e.j.ps recorded from the cell remained almost unchanged. A plausible interpretation is that the inhibition of the e.j.p. during an ATP response resulted from an increase in the membrane conductance and/or a decrease in the driving force for the e.j.p. with the depolarized membrane.

It can be argued from the present results that the NANC nerves in the rectal muscle of the chicken mediate the e.j.p. by releasing a substance other than ATP.

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