Effects of Adenosine Triphosphate and Its Related Compounds on the Phasic Contraction of the Guinea-pig Vas Deferens and Ureter Smooth Muscle

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(Received November 6, 1972)

In general, extracellularly applied ATP and its related nucleotides showed an inhibitory action on the mechanical and electrical activity of smooth muscle (Axelsson et al, 1969; Burnstock et al, 1970; Moulton et al, 1957; Bueding et al, 1967). Recently Burnstock et al (1970) reported the possibility that adenosine triposphate or related nucleotides is the transmitter substance relaesed by the non-adrenergic inhibitory neurones in the gut. However it cannot excluded that ATP or related compounds has a direct action to smooth muscle cell membrane. The purpose of the present experiment is to find out a direct action of ATP and related compounds to smooth muscle. The following experiments were designed; (1) effect of ATP and its related nucleotides on vas deferens and ureter, (2) barium ion and ATP effects on them and (3) quinidine and ATP effects on them.

METHODS

Guinea-pigs, weighing 300–500g, were used. The animal was stunned with a blow and bled out. Segments of vas deferens and ureter were removed. The length of the preparation was 2–3 cm. The experimental methods in the present study were the same as those described in the previous paper (Ohkawa, 1972). The following compounds were used ; adenosine, adenosine-5'-monophosphate disodium (AMP), adenosine-5'-diphosphate trisodium (ADP), adenosine-5'-triphosphate disodium (ATP), tetrodotoxin, quinidine sulfate and cysteine hydrochloride. Electrical stimulation was applied through two silver-ring electrodes which were placed at both ends of the preparation in the organ bath. Single square pulse, 20 sec interval, was usually used. The electrical stimulation was supplied by MSE-40 (Nihon Kohden).

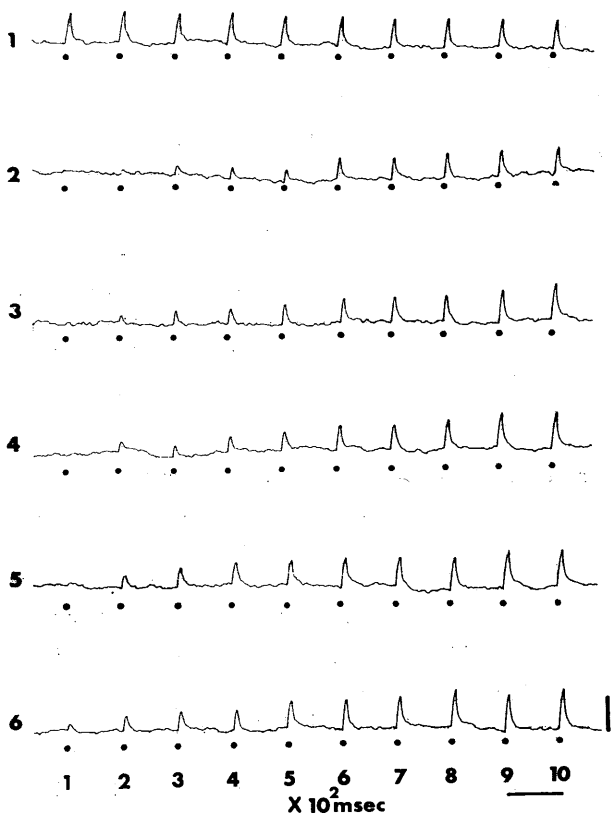

Fig. 1. Effects of various concentrations of ATP on the phasic contraction of vas deferens. 1. control in normal solution ; the electrical stimulation (1000msec duration and 20sec interval) was applied at the dot. 2. control in normal solution; the duration of the single pulse was ranged from 100 msec to 1000msec as indicated below. The graded response was observed. 3. ATP 10^{-7} g/ml. 4. ATP 10^{-6} g/ml. 5. ATP 10^{-5} g/ml and 6. ATP 10^{-4} g/ml, calibration, 20sec and 1 g.

RESULTS

Effects of ATP and related compounds

The preparation of vas deferens had no spontaneous activity in normal solution. When the preparation was stimulated by single square pulse, the phasic contraction was produced. The magnitudes of the phasic contraction were gradually reduced by successive stimulations but it became to be nearly constant after a few stimulation. All the drugs were applied into the organ bath after obtaining the constant magnitude of phasic contraction. The tension development was depended on the duration of the pulse and the external ATP concentrations. The ATP concent-

rations ranged from 10^{-7} to 10^{-4} g/ml. The duration of the single pulse was changed from 100 msec to 1000 msec (the interval and intensity were constant). Fig. 1 shows the effects of various sinlge pulse on the tension development of the vas deferens in the normal solution and various ATP solutions. As shown in Fig. 1, the increase in the tension development was observed with increasing the duration of pulse and the external applied ATP concentrations. The maximum tension was obtained at the concentration of ATP 10^{-4}g/ml.

The similar experiment was carried out on the ureter preparation. Segments of ureter usually not showed the spontaneous mechanical activity in normal solution. When single pulse was applied, the phasic contraction occurred. The tension of phasic contraction was gradually decreased by the continuous simulation which had short interval below 20 sec while it was nearly constant by longer interval over 20sec. When the tension of the phasic contraction became nearly constant, ATP or its related compounds was applied.

The duration of the single pulse was changed from 60 msec to 1000 msec but the interval and intensity were constant. In ureter preparation, the graded responses with increasing the pulse duration were not occurred. When the excitability of the preparation was over a critial level, the maximum response was generated. The inhibitory action of ATP was depended on the external concentrations of ATP. In ATP 10^{-4}g/ml solution, no response was observed even by the maximum duration of the pulse. Fig. 2 shows the inhibitory effect of ATP on the response of the ureter preparation.

When ATP 10^{-5}g/ml was applied to the vas deferens, the tension of phasic contraction was increased. The mean magnitude which was measured from the constant phasic contractions was 159% (n=9). ADP 10^{-5}g/ml had a potentiatory action on the phasic contraction. The mean value in magnitude was 222% (n=10). AMP 10^{-5}g/ml and adenosine 10^{-5}g/ml showed also excitatory effects on the phasic contraction. The mean values were 137% (n=14) and 122% (n=12) respectively. That is, the order of the potentiation on phasic contraction was ADP>ATP>AMP>adenosine. Fig. 3-b shows the effect of ATP and its related compounds on the response of phasic contraction of vas deferens. The effects were summarized in Table I.

Adenosine 10^{-5}g/ml produced slight decrese in the tension development of the phasic contraction of ureter. The mean value of the magnitude of phasic contraction was 90% (n=20). AMP 10^{-5}g/ml, ADP 10^{-5}g/ml and ATP 10^{-5}g/ml also exhibited the inhibitory effect on the tension of phasic contraction. The mean values were 81% (n=20) by AMP, 81% (n=14) by ADP and 80% (n=16) by ATP. The order of inhibitory action was ATP>ADP>AMP>adenosine. The effects of ATP and related nucleotides on the response of ureter were shown in Fig. 3-a and summarized in Table I.

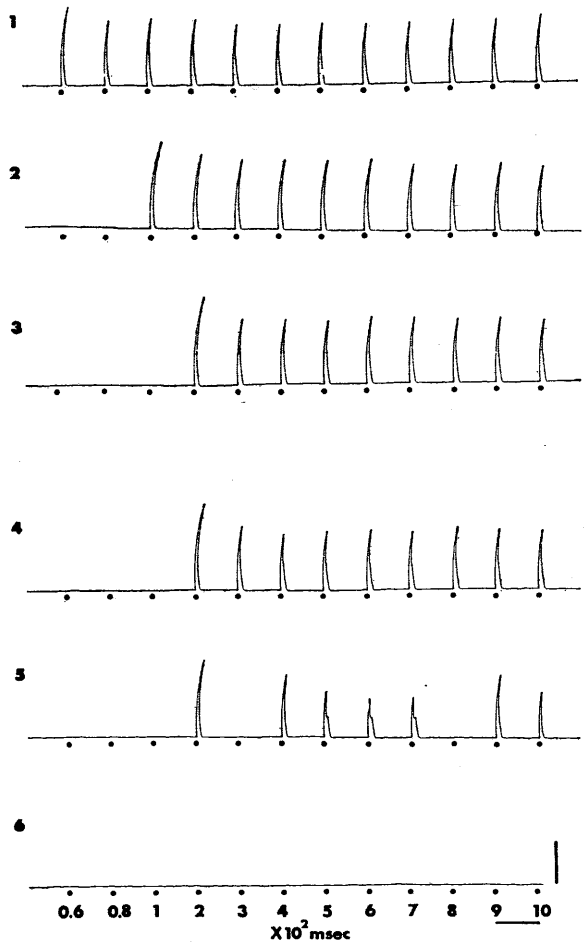

Fig. 2. Effects of various concentrations of ATP on the phasic contraction of ureter. 1. control in normal solution; the electrical stimulation (300msec duration and 20sec interval) was applied at the dot. 2. control in normal solution; the duration of the single pulse was ranged from 60msec to 1000msec as indicated below. 3. ATP 10^{-7} g/ml. 4. ATP 10^{-6} g/ml. 5. ATP 10^{-5} g/ml and 6. ATP 10^{-4} g/ml. No response was observed in ATP 10^{-4} g/ml even the maximum duration of the pulse. Calibration, 20sec and 1g.

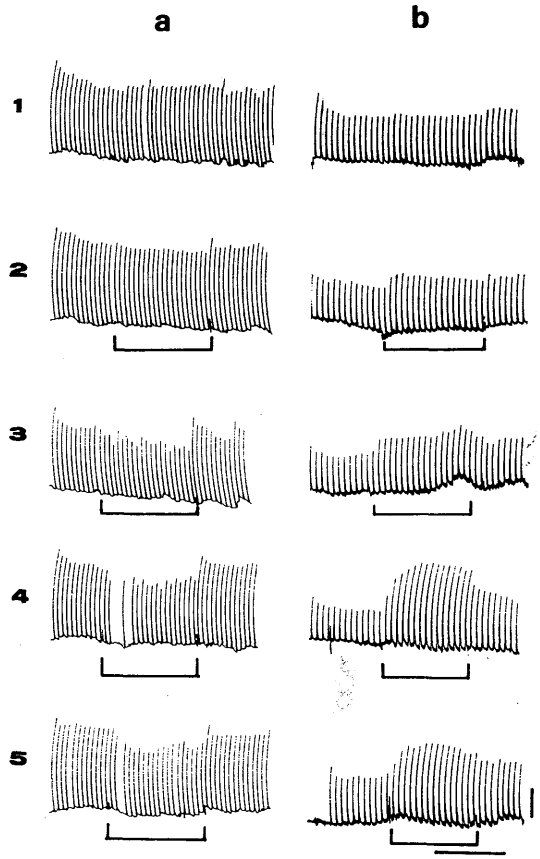

Fig. 3. Effects of ATP and its related compounds on the phasic contraction of vas deferens and ureter.
a, ureter; b, vas deferens
1. (a and b), control ; The constant tension development of the phasic contraction was obtained after few stimulations. The following drugs were applied during the underbar. 2. adenosine 10^{-5} g/ml ; 3. AMP 10^{-5} g/ml; 4. ADP 10^{-5} g/ml and 5. ATP 10^{-5} g/ml. The phasic contraction was produced by external electrical stimulation. Calibration, 5min and 1g.

Table I. Effects of ATP and its related compounds on the phasic contraction of vas deferens and ureter preparation.

Drugs	Mean value in the tension of phasic contraction (±S.D.)	
	vas deferens (%)	ureter (%)
Adenosine 10^{-5} g/ml	121.8 ± 6.2 (n=12)	90.4 ± 3.9 (n=20)
AMP 10^{-5} g/ml	137.1 ± 3.9 (n=14)	81.3 ± 8.4 (n=20)
ADP 10^{-5} g/ml	222.2 ± 0.5 (n=10)	81.5 ± 5.6 (n=14)
ATP 10^{-5} g/ml	159.6 ± 2.6 (=9)	79.9 ± 5.0 (n=16)

Ba^{++} and ATP effects

The vas deferens preparation had no spontaneity in the normal solution. Only the phasic contraction was generated by the electrical stimulation. However the normal solution was replaced by the Krebs solution containing Ba^{++} 1mM, the spontaneous mechanical activity was produced. The pattern of the spontaneous activity was irregular. When the active preparation was stimulated, large complicated contraction was elicited. After exposure to Ba^{++} 1mM, ATP 10^{-5}g/ml increased the spontaneous activity and the electrical stimulation in ATP solution produced considerable, large complicated contraction. That is, ATP accerelated the spontaneous mechanical activity of vas deferens preparation, contraversely to the results obtained from taenia coli (Axelsson et al, 1969). Fig. 4 shows the effects of Ba^{++} and ATP on the contractile activity of the vas deferens.

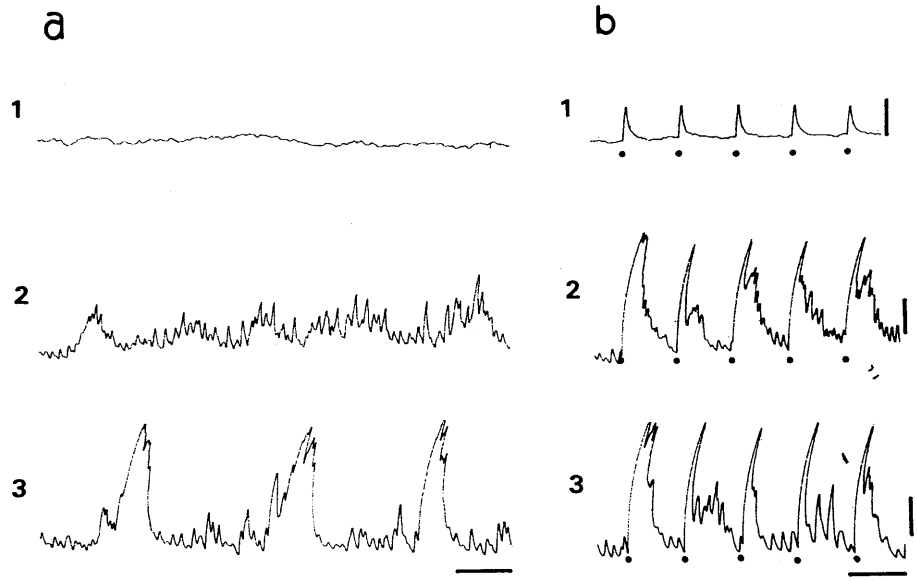

Fig. 4. Effects of Ba^{++} and ATP on the mechanical activity of vas deferens. a-1, control; a-2, Ba^{++} 1mM, The spontaneous mechanical activity was produced. a-3. ATP 10^{-5} g/ml increased the spontaneous mechanical activity obtained in Ba^{++} 1mM. b-1, control, The phasic contraction was produced by the single pulse stimulation. b-2, Large complicated contraction was generated by electrical stimulation in Ba^{++} 1mM. b-3. The contraction produced by electrical stimulation was promoted in the additional application of ATP 10^{-5} g/ml. (Note the magnitude of phasic contraction). The electrical stimulation (300msec duration and 20sec interval) was applied at the dot. Calibration, 20sec and 1g.

In the present experiment, the ureter preparations did not exhibited the spontaneity. Ba^{++} 1mM could not elicited a continuous active phasic contraction but only sporadic, phasic contraction was generated spontaneously. The tension of the phasic contraction was increased in Ba^{++} solution. The inhibitory action of ATP on response could not be observed up to 3×10^{-5}g/ml.

Tetrodotoxin and ATP effects

Tetrodotoxin is known a blocking drug on the nerve activity. To except the stimulating action of ATP and its related compounds on the nerve or nerve ending which may remain in the tissue, the pretreatment with tetrodotoxin was made. During the pretreatment with tetrodotoxin 1.6×10^{-7}g/ml, the phasic contraction of vas deferens was reduced slightly. The additional application of ATP 10^{-5}g/ml still increased the magnitude of phasic contraction. The mean value in increase by ATP was 134% (n=7).

Tetrodotoxin 1.6×10^{-7}g/ml also reduced the magnitude of the response of the ureter preparation. The tension was slightly reduced by the addition of ATP 10^{-5}g/ml after tetrodotoxin.

Quinidine and ATP effects

Burnstock et al (1970) discovered that quinidine had a prevent action on ATP effect. However it is unknown where or how quinidine antagonises the action of ATP. When quinidine 10^{-5}g/ml was applied, the magnitude of the phasic contraction of vas deferens not changed. After 5-10min exposure to quinidine 10^{-5}g/ml, ATP 10^{-5}g/ml had a potentiation effect on the phasic contraction. However the increase in the magnitude was smaller than that in the ATP 10^{-5}g/ml only. The mean value in tension of the phasic contraction in ATP 10^{-5}g/ml with the treatment by quinidine 10^{-5}g/ml was 125% (n=10). In high concentration of quinidine (10^{-4}g/ml), the mean value in the magnitude of phasic contraction was increased up to 165% (n=16). ATP 10^{-5}g/ml during exposure to quinidine 10^{-4}g/ml still potentiated the phasic contraction. However the mean value of the tension of phasic contraction in ATP 10^{-5}g/ml with quinidine 10^{-4}g/ml was 120% (n=10), the control value was taken from the mean value in quinidine 10^{-4}g/ml. Fig. 5 shows the comparison of the potentiatory actions of ATP 10^{-5}g/ml in quinidine 10^{-5}g/ml and 10^{-4}g/ml.

Quinidine 10^{-5}g/ml did not altered the tension of phasic contraction of the ureter preparation. The magnitude of the response was not changed in ATP 10^{-5}g/ml with quinidine 10^{-5}g/ml. Namely, the inhibitory action of ATP on the ureter preparation was prevented by quinidine.

Cysteine and ATP effects

It is known that cysteine increases ATPase activity. In the present study, cysteine was used to test whether or not ATPase is related with the effect of ATP on the phasic contraction. When the vas deferens preparation was immersed

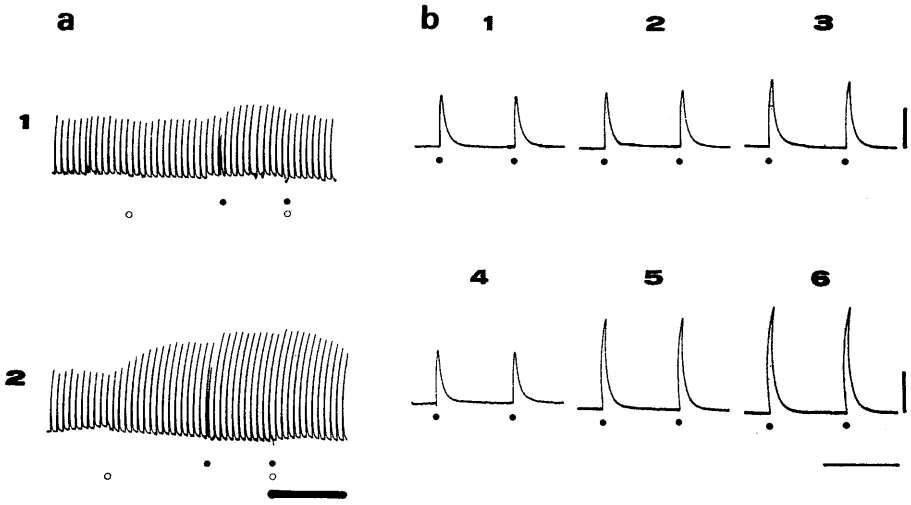

Fig. 5. Effects of quinidine and ATP on the phasic contraction of vas deferens. a-1, quinidine 10^{-5} g/ml was applied between two white circles and ATP 10^{-5} g/ml was additional between two black circles. a-2, quinidine 10^{-4} g/ml was applied between two white circles and ATP 10^{-5} g/ml was additional between two black circles. b-1, control; b-2, quinidine 10^{-5} g/ml and b-3, ATP 10^{-5} g/ml during quinidine 10^{-5} g/ml. b-4, control; b-5, quinidine 10^{-4} g/ml and b-6, ATP 10^{-5} g/ml during quinidine 10^{-4} g/ml. The electrical stimulation (300msec duration and 20sec interval) was applied at the dot (b-1——b-6). Calibration 5min in a and 20sec in b; 1g.

in cysteine 10^{-5}g/ml, the phasic contraction was nearly no change or slightly reduced. The mean value of the tension of phasic contraction was increased after exposure to cysteine 10^{-5}g/ml. The mean value 172% (n=9) was obtained in ATP 10^{-5}g/ml after exposure to cysteine. ADP 10^{-5}g/ml also exhibited the potentiatory effect on the phasic contraction of vas deferens which was exposured to cysteine 10^{-5}g/ml. However the increase by ADP was rather small than that by ATP, i.e., the mean value in tension was 131% (n=9).

DISCUSSION

It reported that extracellularly applied ATP and its related nucleotides had a direct inhibitory action on the spontaneous spike discharge and the mechanical activity of taenia coli smooth muscle (Axelsson et al 1969). ATP also showed an inhibitory effect on the mechanical activity of intestinal smooth muscle (Axelsson et al, 1965) and vascular smooth muscle (Hashimoto et al, 1965). Forrester et al (1951) had examined the effect of ATP on the contractile activity of the skeletal

muscle and atrial muscle. The twitch tension of skeletal muscle was diminished by the treatment with ATP (Forrester et al, 1951). As above mentioned, the relaxant and inhibitory effect of ATP or its related nucleotides had reported on various tissues. In general, ATP and related compounds seem to have a direct inhibitory action on the mechanical activity of intestinal smooth muscle. In some tissues, ATP and related compounds showed an excitatory effect on the mechanical activity (Forrester et al, 1970; Brecht et al, 1952; Naess et al, 1957).

It is well known that gastrointestinal tract includes the enteric plexus which contains excitatory, inhibitory and non-adrenergic inhibitory neurones. Recently Burnstock et al (1970) pointed out that ATP or its related nucleotides is a transmitter substance from nonadrenergic inhibitory neurones. In fact, they showed that ATP or its related nucleotides had a relaxant effect on the contractile activity of smooth muscles from various animals and gastrointestinal organs included non-adrenergic inhibitory neurones.

Above results might indicate a dual action of ATP and related compounds, i.e., the possibility as a transmitter substance and a direct action to smooth muscle without via nervous factors. Therefore a direct action of ATP and related drugs to smooth muscle cannot excluded. In the present study, for the purpose to find out a direct action of ATP and related drugs to smooth muscle, the vas deferens and ureter preparation smooth muscles were used since they not include such intrinsic inhibitory neurones.

The results of ATP and other nucleotides on the vas deferens preparation were very different from that obtained from gastrointestinal smooth muscles. That is, the potentiation in the phasic contraction of the vas deferens smooth muscle was observed. In contrast, the tension of phasic contraction of the ureter smooth muscle was reduced by ATP or related nucleotides, similar to the intestinal smooth muscle. From above results, it is thought the presence of two different processes for direct action of ATP to smooth muscle. Tetrodotoxin did not block the response of the vas deferens and ureter smooth muscles. It had reported that tetrodotoxin did not block the spike activity of smooth muscle (Kuriyama et al, 1966). In these preparations, the spike may generate by the external electrical stimulation in tetrodotoxin. However the tension were slightly reduced in tetrodotoxin. The reduction in the response by tetrodotoxin was not clear. The decrease in tension may due to a direct action of phenol which is a solvent substance to tetrodotoxin. The effects of ATP on vas deferens and ureter smooth muscle were still observed in tetrodotoxin. Therefore the potentiation on vas deferens and the inhibitory action on ureter may be direct action to smooth muscle cell membrane.

Intestinal smooth muscle generally exhibits a spontaneous activity. When ATP or related compounds applied, the spontaneous contractile activity was inhibited. The vas deferens smooth muscle was quiescent in normal solution. To find out

the effect of ATP on the spontaneous mechanical activity of the vas deferens smooth muscle, Ba^{++} was added into the external solution. Ba^{++} induced the considerable spontaneous activity of vas deferens. Further activation of the spontaneous activity was observed by ATP. That is, ATP had the potentiatory effect on the spontaneous activity of vas deferens.

Burnstock et al (1970) showed the antagonistic action of quinidine and ATP on the contractile activity of ileum and taenia coli. The effect was considered to effect on the activity of non-adrenergic inhibitory nerves in the gut. However, as described above, quinidine showed the antagonistic effect to ATP on the vas deferens smooth muscle. This result suggests that quinidine prevents the ATP action at the smooth muscle cell membrane.

The mechanism of the direct ATP effect to smooth muscle is unknown. However, Forrester et al (1970), Axelsson et al (1966) and Axelsson et al (1969) discussed on the ATP action with a relation on the adenyl cyclase system and the changes in the cell membrane permeability. In the present study, cysteine, ATPase activator, changed the effect of ATP on the response of vas deferens and ureter smooth muscles. These results suggest that the direct action of ATP to smooth muscle cell membrane may relate with ATPase system in the cell membrane.

It is concluded from these studies that extracellularly applied ATP and its related nucleotides act to smooth muscle directly and quinidine prevents the ATP action at the cell membrane.

SUMMARY

1. The effects of ATP and its related nucleotides on the phasic contraction of vas deferens and ureter smooth muscle were examined.
2. ATP and related compounds increased the tension of phasic contraction in vas deferens while inhibited in ureter.
3. The potentiatory and inhibitory action of ATP was observed in tetrodotoxin. ATP potentiated the spontaneous mechanical activity of vas deferens produced by Ba^{++}.
4. Quinidine prevented the effects of ATP on both preparations and cysteine changed the effects of ATP and ADP.
5. It is concluded that extracellularly applied ATP and its related nucleotides act to smooth muscle directly. Quinidine prevents the ATP action at the cell membrane.

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