Relaxant Effect of ATP on the Mechanical Activity of the Cat Small Intestine

Hiromichi OHKAWA
Department of Physiology, Yamaguchi University School of Medicine
(Received February 23, 1973)

INTRODUCTION

The existence of the non-adrenergic inhibitory nerves in the gut wall had been reported by many authers. Recently Burnstock et al[1] suggested the possibility that adenosin triphosphate (ATP) or a related nucleotide is the transmitter substance released by the non-adrenergic inhibitory neurones in the gut. However, many problems on the possibility are present. One of the against evidences is that ATP still increased the membrane potential of the taenia coli smooth muscle in which the inhibitory junction potential had been blocked by quinidine (Saito).[2] Another evidence is that the relaxant effect of ATP was not affected by tetrodotoxin (Burnstock et al).[1]

Moreover the results of the effect of ATP obtained from the guinea-pig ileum were little complex. That is, the effects of ATP on the mechanical response of the guinea-pig ileum were excitatory or diphasic (Burnstock et al,[1] Burnstock et al).[3] To understand the effect of ATP on the response of intestinal smooth muscle, this experiment was carried out.

METHODS

Small intestine from cats anaesthetized by an intraperitoneal injection of alpha-chloralose was removed. Segments of ileum were mainly used but some experiments were made by segments of duodenum or jejunum. The experimental methods in the present study were the same as those described in the previous paper (Ohkawa).[4] The following drugs were used; atropine sulfate, adenosine-5'-monophosphate disodium (AMP), adenosine-5'-diphosphate trisodium (ADP), adenosine-5'-triphosphate (ATP), guanin, imidazole, noradrenaline bitartrate, phentolamine hydrochloride, phenoxybenzamine hydrochloride, quinidine sulfate, hexamethonium bromide, procaine hydrochloride and tetrodotoxin (TTX). The final concentrations in the bath are expressed as salt.

RESULTS

Effects of ATP and its related compounds

Segments of the small intestine of cat usually showed the spontaneous mechanical activity in normal solution but the magnitudes and intervals of successive phasic contractions were irregular. When atropine 10^{-6} g/ml was applied, the phasic contraction was slightly inhibited in its magnitude and frequency. Usually the preparation was immersed in atropine during 15min or more before applying ATP, its related compounds or other drugs.

Low concentration (10^{-7} -10^{-6} g/ml) of ATP had no considerable inhibitory effect on the contractile activity of atropinized preparation. When ATP (10^{-5}g/ml) was applied, the contractile activity was decreased, i. e., the tone was decreased and the magnitude and frequency of phasic contraction were reduced. At high concentration (10^{-4}g/ml) of ATP, the mechanical activity of atropinized segment was immediately abolished. The tone was also reduced. After washing out, the mechanical activity was recovered but the recovery took long period. In the guinea-pig ileum, a rebound phenomenon was observed after washing out the ATP solution (Burnstock et al).[1] Similar results on the taenia coli had been also reported (Bennett,[5] Campbell[6]). However, the rebound excitation phenomenon was not seen in the segment of cat ileum. The concentration of ATP 10^{-5} g/ml was used in the following experiments. Fig. 1 shows the relaxant effect of ATP on the mechanical activity of atropinized segment.

Effects of ADP or other related drugs were also examined. ADP produced the relaxant effect on the mechanical activity of the atropinized preparation. The tone was gradually and slightly reduced and the magnitude of phasic contraction was also decreased slightly in the low concentration of ADP (10^{-6} g/ml). After washing out, the activity was recovered immediately. However, strong inhibition on the contractile activity was produced by ADP 10^{-4} g/ml as shown in Fig. 2. Adenosine (10^{-5} g/ml) and AMP (10^{-5} g/ml) also exhibited the relaxant effects on the spontaneous mechanical activity of the atropinized preparation (Fig. 2 B & C). However, guanin (10^{-5} - 10^{-4} g/ml) had no relaxant effect (Fig. 2 D).

Effects of TTX and hexamethonium on ATP

In TTX (1.6×10^{-7} g/ml), the contractile activity was no change in the magnitude and frequency. In some preparations, the magnitude was slightly increased during exposure to TTX. After TTX, the addition of ATP (10^{-5} g/ml) caused the relaxation in tone and decreased the magnitude and frequency of the phasic contraction. Hexamethonium (10^{-4}g/ml) produced the small relaxation in tone and the decrease in the magnitude of phasic contraction. Further relaxation in tone and inhibition of the phasic contraction were observed by additional treatment with ATP 10^{-5}g/ml. The inhibitory effects of ATP in TTX or hexamethonium were shown in Fig. 3.

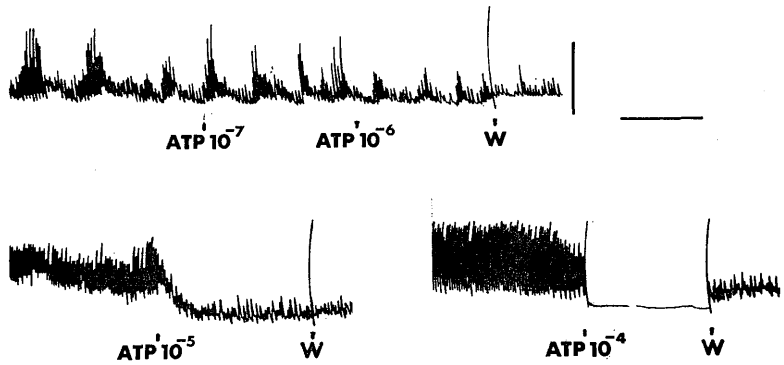

Fig. 1. Effect of ATP on the contractile activity of the cat small intestine. In concentrations ranging between 10^{-7} and 10^{-4} g/ml, ATP was examined. Slight decreases in tone were observed in ATP 10^{-7} and 10^{-6}g/ml. Tone and the magnitude of phasic contraction were reduced by ATP 10^{-5}g/ml. ATP 10^{-4}g/ml inhibited the contactile activity strongly. Calibration, 5 min and lg. W indicates the washing out by normal solution (Fig. 1 -5).

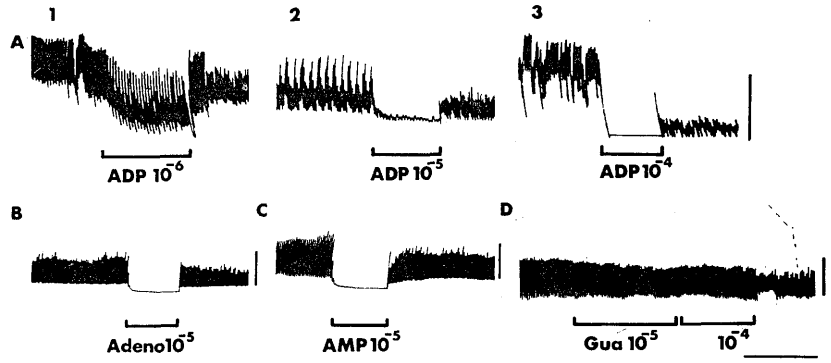

Fig. 2. Effects of ATP related drugs on the contractile activity of the cat small intestine. A; In concentrations ranging between 10^{-6} -10^{-4} g/ml, ADP inhibited the contractile activity with increasing the concentrations of ADP. B and C show the relaxant actions of adenosine 10^{-5}g/ml and AMP 10^{-5}g/ml. D; In concentrations ranging between 10^{-5} -10^{-4}g/ml, guanin did not affect the phasic contraction. The drugs were applied during the period indicated by each underbar. Calibration, 5 min and lg.

Effects of α-adrenergic blocking drugs on ATP

Bueding et al[7] reported that imidazole inhibited the effect of ATP on the guinea-pig taenia coli. In the cat ileum, imidazole (3×10^{-4}g/ml) exhibited the inhibitory effect immediately. The decreases in tone and magnitude of phasic contraction were produced by imidazole. After 15min in imidazole, the addition of ATP (10^{-5}g/ml) still caused the relaxant effect on the mechanical activity (Fig. 4 A). In phenoxybenzamine (10^{-5}g/ml) or phentolamine (3×10^{-5}g/ml), the inhibitory effect by ATP (10^{-5}g/ml) was produced. After the contractile

activity was inhibited by ATP (10^{-5}g/ml), noradrenaline in concentration of 3×10^{-6}g/ml caused further relaxation. The phasic contraction was completely inhibited.

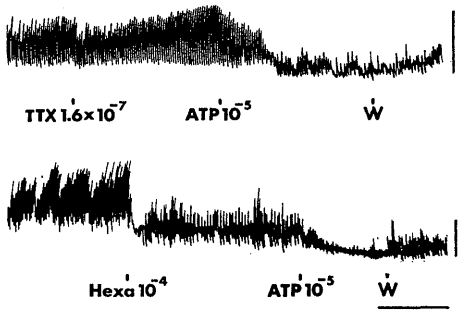

Fig. 3. Effects of TTX and hexamethonium on the relaxant action of ATP. TTX (1.6×10^{-7}g/ml) had no effect on the contractile activity. After TTX, the addition of ATP 10^{-5}g/ml caused the relaxation. Hexamethonium 10^{-4}g/ml exhibits the inhibitory effect on the mechanical activity. ATP 10^{-5}g/ml produced further relaxation after the treatment of hexamethonium. Calibration, 5 min and lg.

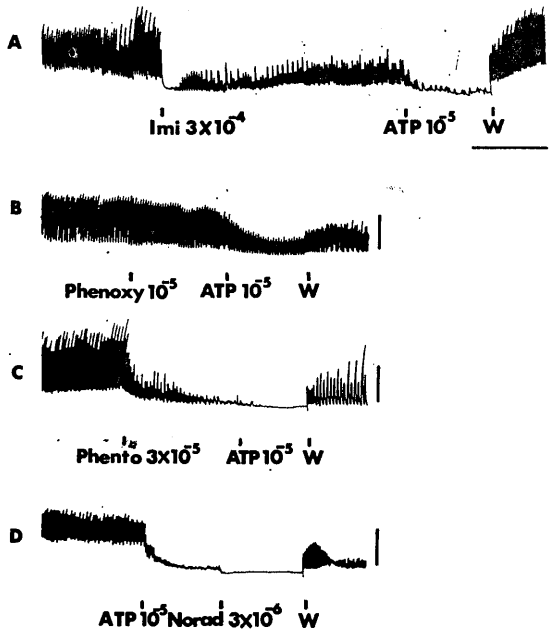

Fig. 4. Effects of α-adrenergic blocking drugs on the relaxant effect of ATP. After imidazole (A ; 3×10^{-4}g/ml), phenoxybenzamine (B ; 10^{-5}g/ml) and phentolamin (C ; 3×10^{-5}g/ml), further relaxations were observed by the addition of ATP 10^{-5}g/ml. After ATP 10^{-5}g/ml, noradrenaline 3×10^{-6}g/ml produced further inhibition on the contractile activity (D). Calibration, 5 mim and lg.

Effects of quinidine and procaine on ATP

Quinidine in concentration of 10^{-5}g/ml showed the inhibitory effect on the spontaneous contractile activity of the atropinized preparation (Fig. 5 A). The magnitude of the contraction was reduced and the frequency was slightly reduced. In the presence of both quinidine (10^{-4}g/ml) and atropine (10^{-6}g/ml) in which the rhythmic contractions were still present, ATP (10^{-5}g/ml) had no effect on the spontaneous contractions, that is, the magnitude and the frequency of the phasic contraction were not changes (Fig. 5 B). Fig. 5 C shows the inhibitory effect of noradrenaline (3×10^{-6}g/ml) in the presence of quinidine (10^{-4}g/ml) and atropine (10^{-6}g/ml). The phasic contractile activity was abolished immediately by the treatment of noradrenaline.

It had been reported that an inhibitory effect of procaine on the inhibitory junction potential of the avian gizzard smooth muscle cell membrane (Furness).[8] After atropine (10^{-6}g/ml), procaine in concentration of 10^{-5}g/ml had nearly no effect on the phasic contractions initially. After 10min in procaine 10^{-5}g/ml, the magnitude of the phasic contraction was slightly reduced. The additional application of ATP 10^{-5}g/ml after atropine and procaine showed the inhibitory effect on the magnitude of the phasic contraction. The tone of the preparation under such conditions was slightly decreased. In high concentration of procaine (10^{-4}g/ml) with atropine (10^{-6}g/ml), the contractile activity was potentiated, i. e., the tone was increased but the frequency of the phasic contraction was

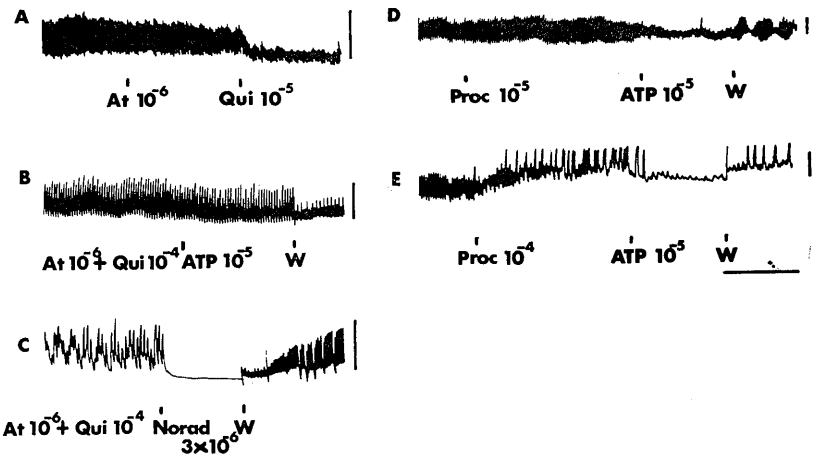

Fig. 5. Effects of quinidine and procaine on the relaxant action of ATP.
A; After atropine 10^{-6}g/ml, quinidine 10^{-5}g/ml produced the relaxation. B; After atropine 10^{-6}g/ml and quinidine 10^{-4}g/ml, ATP 10^{-5}g/ml was applied at the mark. No relaxant effect of ATP was observed. C; After atropine 10^{-6}g/ml and quinidine 10^{-4}g/ml, noradrenaline 3×10^{-6}g/ml produced the strong inhibition on the mechanical activity. D and E; After procaine (10^{-5}–10^{-4}g/ml), ATP 10^{-5}g/ml still exhibited the relaxant action. Calibration, 5 min and lg.

reduced. ATP (10^{-5} g/ml) under the presence of atropine (10^{-6} g/ml) and procaine (10^{-4} g/ml) exhibited the inhibitory effect on the activity. Fig. 5 D and E show the effect of ATP in procaine.

DISCUSSION

In the present experiment, the relaxant effects of ATP or its related compounds were shown on the contractile activity of the cat small intestine. In many intestinal segments, the relaxant effect of ATP had been reported except guinea-pig ileum (Burnstock et al,[3] Satchell et al,[9] Saito,[2] Suzuki et al,[10] Axelsson et al,[11]). Burnstock et al[3] suggested the possibility that ATP or its related nucleotides is the transmitter substance released from non-adrenergic inhibitory nerves in the gut.

Further relaxation produced by the addition of noradrenaline after ATP suggests the different types in the innervation to the intestinal smooth muscles. That is, the presence of different types of receptors in the smooth muscle cell membrane are considered. Similar results were obtained by ATP after phenoxybenzamine, phentolamine and imidazole. However, the possibility that ATP had a direct inhibitory effect to the smooth muscle without affecting to nervous factors is present. TTX is known the inhibitor of nerve activity. It is also known that action potentials in smooth muscle was not blocked by TTX (Kuriyama et al).[12] In the present experiment, the spontaneous mechanical activity of cat ileum was not inhibited but increased slightly in the magnitude of phasic contraction. Similar result was obtained in the previous paper (Ohkawa).[4]

After TTX or hexamethonium which may block the activity of intrinsic neurones, ATP still showed the relaxant effect. These results also suggest that the direct effect of ATP to smooth muscle. However, ATP did not show the relaxant effect in quinidine. The antagonized action of quinidine on ATP had been reported (Burnstock et al).[3] From the results above mentioned, the mechanism of the direct inhibitory action of ATP to smooth muscle without affecting to nervous factors may be unlikely. However, it had been reported that ATP hyperpolarized the cell membrane of taenia coli smooth muscle in the presence of quinidine (Saito).[2] Noradrenaline showed a relaxant effect in quinidine. The presence of different type receptors in the smooth muscle cell membrane is again suggested from this result.

Furness[8] reported that procaine blocks the inhibitory junction potential in smooth muscle of the guinea-pig colon. The increase in tone and phasic contraction may due to the blocking action of procaine to the inhibitory junction potential while the possibility that procaine has a direct excitatory action to smooth muscle is still remained. The relaxant effect of ATP in procaine was obtained in the present

experiment. One possible explanation is that procaine inhibits only the adrenergic inhibitory neurones.

An alternative view is that ATP stimulates the release of transmitter substance from the non-adrenergic inhibitory neurones. It is possible to conclude tentatively that three types of the intrinsic nerves are present in cat small intestine and that ATP could be a transmitter substance released from non-adrenergic inhibitory neurones. Further study on the mechanism of the relaxant effect of ATP or its related compounds is required.

SUMMARY

1. Effects of ATP and its related compounds on the contractile activity of the cat small intestine were examined.
2. ATP, ADP, AMP and adenosine exhibited the relaxant effect on the mechanical activity of atropinized segments but guanin had no inhibitory effect.
3. After TTX or hexamethonium, ATP still had an inhibitory effect on the contractile activity.
4. ATP showed the relaxant action in α-adrenergic blocking drugs and in procaine, but not showed the relaxant action in quinidine.
5. The possibility on the presence of non-adrenergic inhibitory nerves and its transmitter substance was discussed.

REFERENCES

1) Burnstock, G., Campbell, G., Satchell, D. & Smythe, A. : Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. *Br. J. Pharmac.*, **40** : 668-688, 1970.
2) Saito, K. : Effects of extracellularly applied ATP and its related nucleotides on the membrane potential of the guinea-pig taenia coli. *Jap. J. Smooth Muscle Res.*, **8** : 32-39, 1972 (in Japanese)
3) Burnstock, G., Satchell, D. G. & Smythe, A. : A comparison of the excitatory and inhibitory effects of non-adrenergic, non-cholinergic nerve stimulation and exogeneously applied ATP on a variety of smooth muscle preparations from different vertebrate species. *Br. J. Pharmac.*, **46** : 234-242, 1972.
4) Ohkawa, H. : Inhibitory effect of tetrodotoxin on the contractile activity of the guinea-pig ileum and distal colon. *Br. J. Pharmacol.*, (in submit)
5) Bennett, M. R., Burnstock, G. & Holman, M. E. : Transmission from intramural inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. *J. Physiol.*, **182** : 541-558, 1966.
6) Burnstock, G., Campbell, G. & Rand, M. J. : The inhibitory innervation of the taenia of the guinea-pig caecum. *J. Physiol.*, **182** : 504-526, 1966.
7) Bueding, E., Bulbring, E., Gercken, G., Hawkins, J. T. & Kuriyama, H. : The effect of adrenaline on the adenosine triphosphate and creatine phosphate content of intestinal smooth muscle. *J. Physiol.*, **193** : 187-212, 1967.

8) Furness, J. B.: An electrophysiological study of the innervation of the smooth muscle of the colon. *J. Physiol.*, **205**: 549-562, 1969.
9) Satchell, D. G., Lynch, A., Bourke, P. M. & Burnstock, G. : Potentiation of the effects of exogeneously applied ATP and purinergic nerve stimulation on the guinea-pig taenia coli by dipyridmole and hexobendine. *Europ. J. Pharmacol.*, **19**: 343-350, 1972.
10) Suzuki, T., Fukushi, Y. & Rikimaru, A. : Relaxant effect of adenosine triphosphate and its related nucleotides on the guinea-pig taenia coli. *Jap. J. Smooth Muscle Res.*, **7**: 207-212, 1971.
11) Axelsson, J. & Holmberg, B.: The effects of extracellularly applied ATP and related compounds on electrical and mechanical activity of the smooth muscle taenia coli from the guinea-pig. *Acta Physiol. Scand.*, **75**: 149-156, 1969.
12) Kuriyama. H., Osa, T. & Toida. N. : Effects of tetrodotoxin on smooth muscle of the guinea-pig taenia coli. *Br. J. Pharmacol.*, **27**: 366-376, 1966.