EFFECTS OF 5’-AMP AND ITS RELATED COMPOUNDS ON THE ATP-INDUCED_CONTRACTION OF ISOLATED GUINEA-PIG ILEUM

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It has been generally accepted that adenine nucleotides exhibit a smooth muscle relaxing action and this is caused by adenosine moiety of their molecules (1-4). However, as shown by Stafford (1), Bennet (5) and Deuticke (6), guinea-pig uterus contracts in response to adenine nucleotides as well as to adenosine. Furthermore, several workers (7-11) observed on rat uterus or goldfish intestine that ADP and ATP exhibited a contraction but 5’-AMP or adenosine did not.

On the other hand, it has been reported that the pretreatment of ATP or cyclic 3’, 5’-AMP modified the contractile responses of adenine or uridine nucleotides, though the influences varied in each cases (3, 10, 11). Although these sensitization mechanisms are not still clarified, these studies will give a clue to the elucidation of the contractile mechanism of ATP.

In order to know the mechanism of smooth muscle contraction induced by adenine nucleotides or adenosine, present study was performed on the effects of 5’-AMP and its related compounds on the ATP-induced contraction of isolated guinea-pig ileum.

MATERIALS AND METHODS

1. Isolated smooth muscle preparation
Guinea-pig weighing 300 to 350 g were killed by a blow on the head. Pieces of intestine were dissected and suspended in Tyrode solution aerated with a 95% O₂, 5% CO₂ gas mixture at 30°C. They were allowed to recover for one hour before beginning the experiment. The movement of intestine was recorded isotonically on a smoked drum. The drugs used here were dissolved in Tyrode solution and adjusted to pH 7.4. The concentration of drugs added to the bath were expressed in molar concentration.

Depolarization of the isolated guinea-pig ileum was made by replacing all Na-ion of Tyrode solution with an equivalent K-ion. In this case, the response to drugs were recorded with a force-displacement transducer.

2. Glycerin-extracted rabbit psoas muscle fiber preparation
The glycerin-extracted fibers of rabbit psoas muscle were prepared according to the procedure of Szent-Györgyi (12). After one month storage in 50% glycerin solution, the experiments were carried out. The average size of the fibers used here was 10-20 × 0.1-0.2 mm. The fibers were then transferred to a small beaker and suspended horizontally.
RESULTS

1. Effects of 5'-AMP and its related compounds on the ATP-induced contraction of isolated guinea-pig ileum

As shown in Fig. 1, ATP in a concentration of $3 \times 10^{-5}$ M or more, produced the contraction of isolated guinea-pig ileum and 5'-AMP and adenosine caused the relaxation alone in higher concentration of $3 \times 10^{-3}$ M.

On the other hand, the ATP-induced contraction was remarkably potentiated by pretreatment with 5'-AMP for 1 minute, but not with adenosine. On the contrary, adenine depressed the response to ATP (Fig. 1).

![Graph showing effects of 5'-AMP and related compounds on ATP-induced contraction of isolated guinea-pig ileum](image)

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**Fig. 1.** Effects of 5'-AMP and its related compounds on the ATP-induced contraction of isolated guinea-pig ileum.

ATP ($10^{-3}$ M) was added to the bath one minute after the addition of 5'-AMP and its related compounds ($3 \times 10^{-3}$ M).

In this potentiation phenomenon, maximal effective molar ratio of 5'-AMP to ATP was about 30:1 (Fig. 2). This potentiation was remarkable in low concentration of ATP and the response to ATP in higher concentration was rather reduced by 5'-AMP and adenosine (Figs. 2 and 3).

On smooth muscle preparations from other species such as rat uterus and rabbit aorta, which were contracted by ATP, similar potentiating effect by 5'-AMP was observed.
5'-AMP ON GUINEA PIG ILEUM

2. Effects of 5'-AMP and its related compounds on the response to ATP, acetylcholine, histamine and bradykinin of isolated guinea-pig ileum

In order to know whether the potentiating effect of 5'-AMP is specific for ATP, we
studied the effects of 5'-AMP and its related compounds on the response of guinea-pig ileum to ATP, acetylcholine, histamine and bradykinin. As seen in Table 1, no potentiation was observed with 5'-AMP on the contractile responses to acetylcholine, histamine or bradykinin. Adenosine and adenine depressed the contraction produced by all agonists tested, although the depressive effect of adenosine to ATP was weak.

Cyclic 3', 5'-AMP, IMP and xanthosine, similar to 5'-AMP, potentiated the response to ATP, but their potentiating effects were weak. 2'-AMP, 3'-AMP, GMP or UMP did not affect the response to ATP.

|                | ATP | ACh | Hist | BK  |
|----------------|-----|-----|------|-----|
| 5'-AMP         | +95 | 0   | -15  | 0   |
| 2'-AMP         | 0   | 0   | 0    | 0   |
| 3'-AMP         | 0   | 0   | 0    | 0   |
| Cyclic-AMP     | -60 | 0   | 0    | 0   |
| Adenosine      | -5  | -12 | -55  | -30 |
| Adenine        | -50 | -15 | -95  | -30 |
| 5'-IMP         | -65 | 0   | 0    | 0   |
| Inosine        | +15 | 0   | 0    | 0   |
| Hypoxanthine   | 0   | 0   | 0    | 0   |
| 5'-GMP         | 0   | 0   | 0    | 0   |
| Guanosine      | 0   | 0   | 0    | 0   |
| Guanine        | 0   | 0   | 0    | 0   |
| 5'-UMP         | +5  | 0   | 0    | 0   |
| Uridine        | 0   | 0   | 0    | 0   |
| Uracil         | 0   | 0   | 0    | 0   |
| 5'-CMP         | +5  | 0   | 0    | 0   |
| Cytidine       | 0   | 0   | 0    | 0   |
| Cytosine       | 0   | 0   | 0    | 0   |
| Xanthosine     | +55 | 0   | 0    | 0   |
| Xanthine       | 0   | 0   | 0    | 0   |

These compounds in $3 \times 10^{-3} \text{M}$ were added to the bath one minute before addition of agonists. Concentrations of agonists used here were: ATP $10^{-3} \text{m}$, ACh $10^{-7} \text{m}$, histamine (Hist) $10^{-7} \text{m}$, bradykinin (BK) $1.8 \times 10^{-7} \text{m}$. Values in the table indicate inhibition (–) or potentiation (+) % of contraction height of agonists by 5'-AMP and its related compounds.
3. Response of depolarized guinea-pig ileum to ATP or 5'-AMP

ATP in a concentration of $10^{-4}$ M or more produced the contraction on the depolarized guinea-pig ileum, followed by the relaxation, but 5'-AMP in a concentration of $3 \times$ ...
10^{-3} \text{M} showed only remarkable relaxation. When 5'-AMP was added to the bath 1.5 minutes after the addition of ATP, a contraction was observed preceding the relaxation (Fig. 4). The initial contraction produced by ATP was also enhanced by simultaneous addition of 5'-AMP. However, GMP did not potentiate the contractile response to ATP (data not shown).

4. Effect of 5'-AMP on the ATP-induced contraction of glycerin-extracted rabbit psoas muscle fiber

The contractile response to ATP was not enhanced by addition of 5'-AMP, and rather weak depression was observed (Fig. 5).

DISCUSSION

In the present studies, we confirmed that in the isolated guinea-pig ileum, purine or pyrimidine mononucleotides did not produce the contraction. On the other hand, ATP produced the contraction and this contraction was remarkably potentiated by pretreatment with 5'-AMP, but the response to acetylcholine, histamine or bradykinin was not. Among the related compounds, cyclic 3', 5'-AMP, IMP and xanthosine produced similar potentiation phenomena though their potentiating effects were weak. However, other compounds such as 2'-AMP, 3'-AMP, GMP, UMP or adenosine did not potentiate.

On preparations from other species such as rat uterus and rabbit aorta, the potentiation phenomenon was also observed.

These data suggest that not only the ion density of purine ring, but also the position of phosphate group on ribose of 5'-AMP molecule play important roles to cause the specific potentiation for ATP.

With regard to the contractile mechanism of ATP, Daniel and Irwin suggested that ATP might complex Mg-ion present in the cell membrane, thereby favoring Ca-ion entry and contraction (9). So, it may be considered that 5'-AMP also complexes Mg-ion in the cell membrane and potentiates the response to ATP. However, this seems to be unlikely because the complex forming constant of the nucleotides tested does not always correlate with their responses of smooth muscle preparations or with their potentiating effects on the ATP-induced contraction (13-15).

In the glycerin-extracted rabbit psoas muscle fiber we could not find the potentiating effect of 5'-AMP on the ATP-induced contraction. So it is concluded that the potentiation of ATP by 5'-AMP is not caused in the actomyosin system.

The ATP-contraction and the potentiation by 5'-AMP was also observed on the depolarized guinea-pig ileum. In addition, it is well known that ATP penetrates the cell membrane with difficulty (16, 17). Thus it is considered that 5'-AMP may influence by unknown mechanism on the site of action of ATP on the cell membrane.

Recently it was reported that IMP as well as 5'-AMP activated phosphorylase b of the skeletal muscle, but 2'-AMP, 3'-AMP or GMP did not (18). Present data on the potentiating effects of purine mononucleotides on the ATP-induced contraction considerably show the similarity to phosphorylase b activating effect of these nucleotides.
However, it seems unlikely that added 5'-AMP activates directly phosphorylase b because the nucleotides penetrate the cell membrane with difficulty. Nevertheless, the possibility remains that the glycogenolytic enzyme systems, which are thought to play important roles in muscle contraction, might be activated indirectly through the ATP-AMP interaction on the cell membrane.

In addition, these findings at least would give a clue to the elucidation of the contractile mechanism of ATP, although the meaning of them is not clear.

**SUMMARY**

ATP produced the contraction of isolated guinea-pig ileum, whereas adenine, adenosine or 5'-AMP as well as other purine or pyrimidine mononucleotides did not. The contractile response to ATP was remarkably potentiated by pretreatment with 5'-AMP, cyclic 3', 5'-AMP, IMP or xanthosine among the purine or pyrimidine compounds. In these potentiating compounds, 5'-AMP was most effective. On preparations such as rat uterus, rabbit aorta and depolarized guinea-pig ileum, the contraction produced by ATP was also enhanced by 5'-AMP. However, the response to acetylcholine, histamine or bradykinin was not potentiated by 5'-AMP. These findings suggest that not only the ion density of purine ring, but also the position of phosphate group on ribose of 5'-AMP molecule play important roles to potentiate the response to ATP.

Since this potentiation phenomenon was not observed in actomyosin system, it is concluded that the potentiation phenomenon occurs through the specific ATP-AMP interaction on the site of action of ATP on the cell membrane.

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**REFERENCES**

1) **STAFFORD, A.**: *Br. J. Pharmac. Chemother.* 154, 82 (1965)
2) **KIMM, T.S., SHULMAN, J. AND LEVINE, R.A.**: *J. Pharmac. exp. Ther.* 163, 36 (1968)
3) **SMITH, M.W.**: *Br. J. Pharmac. Chemother.* 22, 254 (1964)
4) **AXELSSON, J. AND HOLMBERG, B.**: *Acta physiol. scand.*, 75, 149 (1969)
5) **BENNET, D.W. AND DRURY, A.N.**: *J. Physiol.*, 72, 288 (1931)
6) **DEUTICKE, H.J.**: *Pflügers Arch. ges. Physiol.* 230, 537 (1932)
7) **WATT, D.T.**: *Am. J. Physiol.* 173, 291 (1953)
8) **BUDAY, P.V., CARR, C.J. AND MIYA, T.**: *J. Pharm. Pharmac.* 13, 290 (1961)
9) **DANIEL, E.E. AND IRWIN, J.**: *Can. J. Physiol. Pharmac.* 43, 89 (1965)
10) **DANIEL, E.E.**: *Can. J. Physiol. Pharmac.* 42, 497 (1964)
11) **GADDUM, J.H. AND SZERB, J.C.**: *Br. J. Pharmac. Chemother.* 17, 451 (1961)
12) **SZENT-GYÖRGyi, A.**: *Chemistry of Muscle Contraction*, Ed. 2, p. 144, Academic Press Inc., New York (1951)
13) **KAHN, T.M.M. AND MARTELL, A.E.**: *J. phys. Chem.* 66, 10 (1962)
14) **KAHN, T.M.M. AND MARTELL, A.E.**: *J. Am. chem. Soc.* 84, 3037 (1962)
15) Wallaas, E.: Acta chem. scand. 12, 528 (1958)
16) Hoffman, P.C. and Okita, G.T.: Proc. Soc. exp. Biol. Med. 119, 573 (1965)
17) Gerlach, E., Deuticke, B. and Dreishbach, R.H.: Naturwissenschaften 50, 228 (1963)
18) Awaya, A., Sakai, Y., Uchida, T., Ishimoto, M. and Egami, F.: Seikagaku 40, 824 (1968)
   (in Japanese)