Relaxation and contraction induced by ATP *via* P2 receptors in longitudinal muscle strips of the rat proximal colon

REN Lei-Ming[1,2*], WANG Miao[1]

(1. *Department of Pharmacology, School of Pharmacy, Hebei Medical University, Shijiazhuang* 050017, *China*;
2. *Hebei Professional College in Chemical & Pharmaceutical Sciences, Shijiazhuang* 050031, *China*)

Abstract: **AIM** Action of adenosine triphosphate(ATP) on longitudinal muscle strips of the rat distal colon has been reported, however, that of the rat proximal colon remains to be clarified. In this study we investigated the effects of ATP on longitudinal muscle strips isolated from the rat proximal colon and the receptors involved in the effects. **METHODS** Isometric relaxant and contractile responses to ATP ($0.1\ \mu mol \cdot L^{-1} - 1\ mmol \cdot L^{-1}$) and adenosine ($1 - 100\ \mu mol \cdot L^{-1}$) in longitudinal muscle strips of the rat proximal colon were observed. **RESULTS** ATP ($0.1\ \mu mol \cdot L^{-1} - 1\ mmol \cdot L^{-1}$) produced a complicated response including an inhibition of rhythmic contraction and a weakly transient decrease in basic tone (0.05 – 0.08 g) followed by a concentration-dependent contraction (0.04 – 0.44 g) in longitudinal muscle strips of the rat proximal colon at resting tension. Tetrodotoxin ($0.1\ \mu mol \cdot L^{-1}$) did not influence the responses to ATP. Adenosine ($1 - 100\ \mu mol \cdot L^{-1}$) did not produce an obvious contractile response in the preparation at resting tension. Concentration-dependent relaxant responses to ATP ($1\ \mu mol \cdot L^{-1} - 1\ mmol \cdot L^{-1}$) in the preparation precontracted with 5-hydroxytryptamine or with acetylcholine were 23.2% – 94.6% or 24.8% – 92.4%, however the relaxant responses to adenosine were much weaker than those to ATP. **CONCLUSION** ATP produces contractile responses mainly *via* purine and pyrimidine(P)2 receptors and relaxant responses partially *via* P1 receptors in longitudinal muscle strips of the rat proximal colon.

Key words: adenosine triphosphate; adenosine; receptor, purinergic; muscle contraction; muscle relaxation; colon

LCC number: R962
Document code: A

Arctile ID: 1000-3002(2005)05-0321-06

Received date: 2004-12-22 **Accepted date**: 2005-04-14
Foundation item: The project supported by Natural Science Foundation of Hebei Province(302481)

Biography: REN Lei-Ming(1956 –), male, native of Hebei Province, professor, main research field is cardiovascular pharmacology.
* Corresponding author. E-mail: ren-leiming@263.net
Tel: (0311)6266004 Fax: (0311)5110024

It has been demonstrated that the distal colon contains P1 receptors[1], while circular muscle strips of the proximal colon contain P2 receptors[2] in the guinea pig. Venkova, *et al*[3] suggested that in the cat colon circular muscle strips ATP induce contraction mediated by P2X receptors and relaxation mediated by both P2Y and P1 receptors. Bailey and Hourani[4] reported that ATP and adenosine produced only relaxant responses *via* P1 receptors in longitudinal muscle strips of the rat distal colon. In preliminary study, we observed the effects of ATP on longitudinal and circular muscle strips of the rat distal and proximal colon. The result obtained from our preliminary study in longitudinal muscle strips of the rat distal colon was consistent with that reported by Bailey and Hourani[4], but the effect of ATP on longitudinal muscle strips of the rat proximal colon was obviously different from that of the distal colon. Although previous studies have shown that ATP plays an important role in the motility of the colon smooth muscle, there has been no report about the effects of ATP on longitudinal muscle strips of the rat proximal colon. The purpose of this study was to investigate the effects of ATP on longitudinal muscle strips of the rat proximal colon under normal or precontracted conditions.

1 MATERIALS AND METHODS

1.1 Animals

Male Wistar rats (230 – 260 g) were obtained from the Experimental Animal Center of Hebei Medical University (Certificate №. SCXK

(J)2003-1-003).

1.2 Reagents

Adenosine triphosphate (ATP), adenosine and 5-hydroxytryptamine (5-HT) were all purchased from Sigma Chemical Co. Acetylcholine (ACh) was obtained from Institute of Pharmacology and Toxicology in Academy of Military Medical Sciences, and tetrodotoxin (TTX) was the product of Hebei Institute of Aquatic Products.

1.3 Longitudinal muscle preparation

Rats were anesthetized with an injection of urethane (1.5 $g \cdot kg^{-1}$, ip) and then exsanguinated. After the abdomen was opened, a segment (4 cm in length) of the proximal colon (~1 cm from the ileocecal sphincter) was excised and cleaned of excess connective tissue. Along the root of the mesentery, the segment of colon was cut and washed in Krebs-Henseleit (K-H) solution of the following composition ($mmol \cdot L^{-1}$): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2 and 5% CO_2 at 37℃ (pH 7.2). The tissue was pinned out in a dish containing K-H solution, and the mucosal layer, facing up, was removed under a magnifying glass, and then two full-thickness strips (approximately 8 mm in length and 3 mm in width) were cut along the longitudinal axis of the colon tissue[5]. Silk ligatures were tied to each end of the muscle strip, one end was attached to a holder and the other to an isometric tension transducer coupled to a polygraph (ERT-884, Youlin Electron Co, Kaifeng) to record responses of the preparations. The longitudinal muscle strips were mounted in a 10 mL organ bath containing K-H solution. An initial resting tension of 1 g was applied to the longitudinal muscle preparations[4,6], which were then left to equilibrate for 1 h. At the end of this period the tension on the strip was taken as the resting tension and no further mechanical adjustment was made during experimentation. Before experiments, the preparation was exposed to 1 $\mu mol \cdot L^{-1}$ ACh for several times until the responses became constant.

1.4 Drug administration

Cumulative concentration-response curves for 5-HT (0.01 – 100 $\mu mol \cdot L^{-1}$), ACh (0.001 – 10 $\mu mol \cdot L^{-1}$) and KCl (1 – 80 $mmol \cdot L^{-1}$) were constructed in the preparations at resting tension, and EC_{50} values of each agent mentioned above were calculated. On the other hand, a selective P2 receptor agonist ATP (0.1 $\mu mol \cdot L^{-1}$ – 1 $mmol \cdot L^{-1}$) and a selective P1 receptor agonist adenosine (1 – 100 $\mu mol \cdot L^{-1}$) were added non-cumulatively at 30 min intervals to prevent the rapid desensitization of P2 receptors. Only one concentration-response curve for ATP or adenosine was generated per preparation. TTX (0.1 $\mu mol \cdot L^{-1}$) to analyze the neurogenic influence was added to the organ bath 10 min before the administration of ATP (100 $\mu mol \cdot L^{-1}$).

To investigate the relaxant responses to ATP and adenosine, the longitudinal muscle strips were precontracted with EC_{50} of 5-HT (1 $\mu mol \cdot L^{-1}$), ACh (0.3 $\mu mol \cdot L^{-1}$) or KCl (25 $mmol \cdot L^{-1}$)[7-9], and then ATP (1 – 1000 $\mu mol \cdot L^{-1}$) or adenosine (1 – 100 $\mu mol \cdot L^{-1}$) was administered in a non-cumulative manner.

1.5 Statistical analysis

Changes in basic tone and contractile responses induced by ATP and contractile responses to 5-HT, ACh or KCl in longitudinal muscle strips of the rat proximal colon at resting tension were expressed as g ($\bar{x} \pm s$). EC_{50} values were calculated with the equation: $\log (E/(E_{max} - E)) = \log C - \log K$ (E, response; E_{max}, maximal response; C, agonist concentration; K, equilibrium dissociation constant). When preparations were precontracted with 5-HT, ACh or KCl, relaxant response to each concentration of ATP and adenosine was calculated as percentage of the control value obtained immediately before giving ATP or adenosine. A paired *t*-test was used to evaluate the significant difference between the data before and after the treatment with TTX. Two-way ANOVA was used to evaluate any differences between two concentration-response curves and a Dunnett multiple comparisons test (with GraphPat InStat V2.05a)[10] was used to evaluate the significant difference in other experiments. *P* values less than 0.05 were considered statistically significant.

2 RESULTS

2.1 Effects of 5-HT, ACh and KCl on longitudinal muscle strips of the rat proximal colon

In the preparations without treatment, the rhythmic contraction (at a speed of 1.3 – 2.2 min^{-1} and an amplitude of 0.4 – 0.8 g) was observed in longitudinal muscle strips of the rat proximal colon. 5-HT (0.01 – 100 $\mu mol \cdot L^{-1}$), ACh (0.001 – 10 $\mu mol \cdot L^{-1}$) and KCl (1 – 80 $mmol \cdot L^{-1}$) produced contractile responses in a concentration-dependent manner in longitudinal muscle strips of the rat proximal colon at resting tension, respectively (Fig 1). EC_{50} values for 5-HT, ACh and KCl were approximately 1 $\mu mol \cdot L^{-1}$, 0.3 $\mu mol \cdot L^{-1}$ and 25 $mmol \cdot L^{-1}$, and the maximal contraction induced by them was (1.06 ± 0.05), (1.12 ± 0.20) and (1.07 ± 0.12)g, which were not significantly different from each other ($n = 5$, $P > 0.05$, Fig 1).

2.2 Effects of ATP and adenosine on longitudinal muscle strips of the rat proximal colon

ATP at 1 $\mu mol \cdot L^{-1}$ produced a complicated response including an inhibition of rhythmic contraction and a weakly transient decrease in basic tone[(0.05 ± 0.02)g, $n = 8$] followed by a contractile response in longitudinal muscle strips of the rat proximal colon at resting tension. The contractile responses to ATP became large in a concentration-dependent manner with the increase in the drug concentration from 1 $\mu mol \cdot L^{-1}$ to 1 $mmol \cdot L^{-1}$(Fig 1A and Fig 2A), however, the transient decrease in basic tone did not change along with the increasing concentrations [(0.05 ± 0.02) – (0.08 ± 0.06)g, $n = 8$]. The maximal contraction induced by ATP at 1 $mmol \cdot L^{-1}$ was (0.44 ±

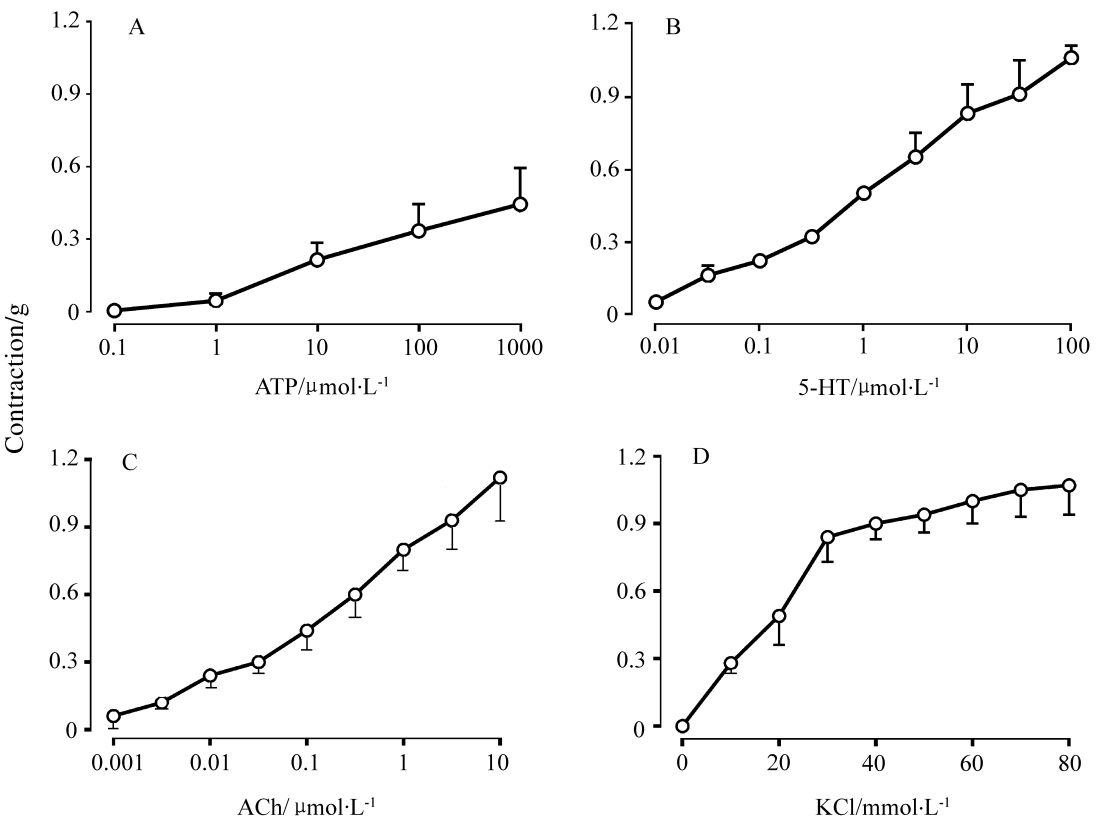

Fig 1. Concentration-response curves of contractile response to ATP (A), 5-HT (B), ACh (C) and KCl (D) in longitudinal muscle strips of the rat proximal colon. A Dunnett multiple comparisons test was used to evaluate any significant differences among the maximal contractile responses to the four agents. $\bar{x} \pm s$, $n = 5 - 8$.

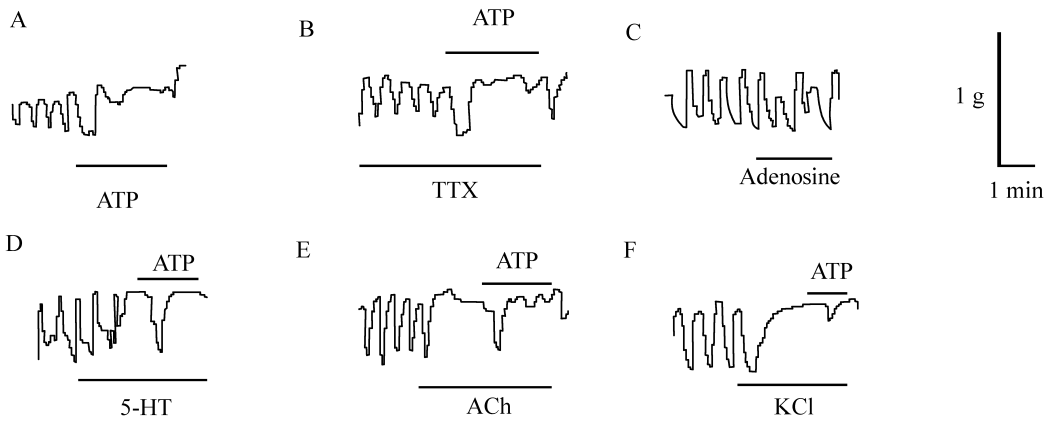

Fig 2. Responses to ATP or adenosine in longitudinal muscle strips of the rat pronimal colon. Responses to 100 μmol·L^{-1} ATP before (A) and after a treatment with 0.1 μmol·L^{-1} TTX (B), or to 100 μmol·L^{-1} adenosine (C) at resting tension and responses to 100 μmol·L^{-1} ATP in the preparations precontracted with 1 μmol·L^{-1} 5-HT (D), 0.3 μmol·L^{-1} ACh (E) and 25 mmol·L^{-1} KCl (F), respectively.

0.15) g which was significantly smaller than that by 5-HT, ACh or KCl ($P < 0.01$, Fig 1). Before and after the treatment with TTX (0.1 μmol·L^{-1}), the decrease in basic tone by ATP (100 μmol·L^{-1}) was (0.06 ± 0.03) g and (0.07 ± 0.04) g ($n = 8$, $P > 0.05$), respectively, whereas contractile response to the same concentration of ATP was (0.34 ± 0.11) g and (0.29 ± 0.15) g ($n = 8$, $P > 0.05$) (Fig 2A and Fig 2B). Adenosine at 1 – 100 μmol·L^{-1} did not induce obviously contractile responses in the preparation (Fig 2C, data not shown).

2.3 Effects of ATP and adenosine on longitudinal muscle strips of the rat proximal colon precontracted with 5-HT, ACh or KCl

Constant contractile responses to 5-HT (1 μmol·L^{-1}), ACh (0.3 μmol·L^{-1}) and KCl (25 mmol·L^{-1}) were (0.59 ± 0.13) g, (0.54 ± 0.10) g and (0.66 ± 0.26) g, respectively, and there were no significant differences among the responses in the three groups ($n = 8$, $P > 0.05$). In the preparations precontracted with 5-HT and ACh, ATP (1 μmol·L^{-1} – 1 mmol·L^{-1}) produced obviously relaxant responses in a concentration-dependent manner (Fig 2D, E). There was no significant difference between the two response-curves for ATP (Fig 3A, $n = 8$, $P > 0.05$). On the other hand, in the preparations precontracted with KCl (25 mmol·L^{-1}) that produced a

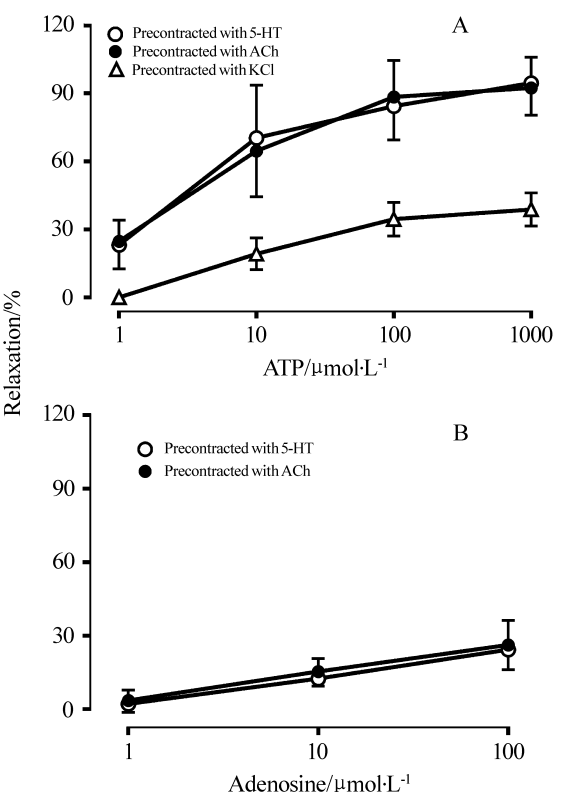

Fig 3. Concentration-response curves of relaxant responses to ATP (A) in longitudinal muscle strips of the rat proximal colon precontracted with 5-HT (1 μmol·L^{-1}), ACh (0.3 μmol·L^{-1}) and KCl (25 mmol·L^{-1}), and those to adenosine (B) in the preparations precontracted with 5-HT (1 μmol·L^{-1}) and ACh (0.3 μmol·L^{-1}). Two-way ANOVA was used to evaluate any differences between two concentration-response curves, and a Dunnett multiple comparisons test was used to evaluate any differences among relaxant responses to the agents at the same concentration. $\bar{x} \pm s$, $n = 8$.

contractile response to a similar extent like 5-HT (1 μmol·L^{-1}) and ACh (0.3 μmol·L^{-1}), ATP produced much smaller relaxation (Fig 3A). Adenosine (1 – 100 μmol·L^{-1}) produced much weaker relaxation in the preparations precontracted with 5-HT and ACh (Fig 3B).

3 DISCUSSION

In longitudinal muscle strips of the rat proximal colon under resting tension, ATP produced obviously contractile responses in a concentration-dependent manner, but adenosine could not induce muscle contraction. When the preparation was precontracted with 5-HT or ACh, ATP produced relaxant response concentration-dependently, and the relaxant response to adenosine (100 μmol·L^{-1}, precontracted with 5-HT) was only 28.8% of that to ATP (100 μmol·L^{-1}, precontracted with 5-HT). The results indicate that ATP produces muscle contraction mainly *via* P2 receptors and a relaxant response partially *via* P1 receptors in longitudinal muscle strips of the rat proximal colon.

It is well-known that P receptors are divided into two categories, adenosine is a selective agent to P1 receptors, and ATP but not adenosine is an active agent to P2 receptors[11,12]. ATP itself exerts physiologic and pharmacologic effects *via* P2 receptors in many organs and tissues of different animals even human being, and it also can be rapidly metabolized to adenosine that acts on P1 receptors[13]. It was reported that both ATP and adenosine produced only relaxation *via* P1 receptors in longitudinal muscle strips of the rat distal colon[4], and we also confirmed the same response to both ATP and adenosine in the rat distal colon in the present study (data not shown). Furthermore, we found that ATP induced a small relaxation followed by a concentration-dependent contraction in longitudinal muscle strips of the rat proximal colon, and TTX at a concentration enough to block neurogenic response[14,15] did not affect both relaxant and contractile responses to ATP. These results indicate that the longitudinal muscle responses to nucleoside and nucleotide in the rat proximal colon are distinctly different from that in the rat distal colon, and the contractile responses to ATP are mainly regulated through P2 receptors.

When the preparation was precontracted with 5-HT or ACh, ATP induced relaxant responses in a concentration-dependent manner. ATP at 100 μmol·L^{-1} produced relaxant responses by (84.4 ± 15.0)% (precontracted with 5-HT) and (88.4 ± 16.1)% (pretcontracted with ACh), however, adenosine at the same concentration produced relaxant responses by only (24.3 ± 8.2)% (precontracted with 5-HT) and (26.2 ± 10.0)% (precontracted with ACh). Therefore, the most part of relaxant responses to ATP in longitudinal muscle strips of the rat proximal colon is mediated through P2 receptors, and the response to ATP and its mechanism are extremely different from those in longitudinal muscle strips of the rat distal colon. In the present study, we also conclude that KCl is not a suitable agent for producing precontraction in longitudinal muscle strips of the rat proximal colon to observe the relaxant responses induced by nucleoside and nucleotide. A study for analyzing the contribution of P2X or P2Y receptor subtypes to the relaxant and contractile responses to ATP in the rat proximal colon will be needed in further.

4 REFERENCES:

[1] Kadowaki M, Takeda M, Tokita K, Hanaoka K, Tomoi M. Molecular identification and pharmacological characterization of adenosine receptors in the guinea-pig colon[J]. *Br J Pharmacol*, 2000, **129**(5):612 – 616.

[2] Zagorodnyuk V, Maggi CA. Pharmacological evidence for the existence of multiple P2 receptors in the circular muscle of guinea-pig colon[J]. *Br J Pharmacol*, 1998, **123**(1): 122 – 128.

[3] Venkova K, Milne A, Krier J. Contractions mediated by α_1-adrenoceptors and P_2-purinoceptors in a cat colon circular muscle[J]. *Br J Pharmacol*, 1994, **112**(4):1237 – 1243.

[4] Bailey SJ, Hourani SM. Effects of purines on the longitudinal muscle of the rat colon[J]. *Br J Pharmacol*, 1992, **105**(4):885 – 892.

[5] Percy WH, Christensen J. Pharmacological characterization of opossum distal colonic muscularis mucosae *in vitro*[J].

Am J Physiol, 1986, **250**(1):G98 – G102.

[6] Yang YL, Zheng TZ, Qu SY, Li W, Xie DP. Action of cimetidine and ranitidine on contractile activity of isolated small intestinal longitudinal strips in rats[J]. *Chin J Pharmacol Toxicol*(中国药理学与毒理学杂志), 2000, **14**(6):467 – 469.

[7] Curro D, Preziosi P. Involvement of vasoactive intestinal polypeptide in nicotine-induced relaxation of the rat gastric fundus[J]. *Br J Pharmacol*, 1997, 121(6):1105 – 1112.

[8] Tamaoki J, Yamauchi F, Chiyotani A, Yamawaki I, Takeuchi S, Konno K. Atypical beta-adrenoceptor-(beta 3-adrenoceptor) mediated relaxation of canine isolated bronchial smooth muscle[J]. *J Appl Physiol*, 1993, **74**(1):297 – 302.

[9] Clemens A, Katsoulis S, Nustede R, Seebeck J, Seyfarth K, Morys-Wortmann C, *et al*. Relaxant effect of xenin on rat ileum is mediated by apamin-sensitive neurotensin-type receptors[J]. *Am J Physiol*, 1997, **272**(1): G190 – G196.

[10] Niu CQ, Zhao D, Jia XM, Ren LM. α_1-Adrenoceptor antagonist profile of doxazosin and its enantiomers in isolated rabbit blood vessels[J]. *Chin J Pharmacol Toxicol*(中国药理学与毒理学杂志), 2003, **17**(5):354 – 359.

[11] Ren LM, Zhang M. Physiological roles of the receptors for purines and pyrimidines[J]. *Chin Pharmacol Bull*(中国药理学通报), 2002, **18**(1):20 – 22.

[12] Fredholm BB, Abbracchio MP, Burnstock G, Daly JW, Harden TK, Jacobson KA, *et al*. Nomenclature and classification of purinoceptors[J]. *Pharmacol Rev*, 1994, **46**(2):143 – 156.

[13] Abbracchio MP, Burnstock G. Purinoceptors: are there families of P2X and P2Y purinoceptors[J]? *Pharmacol Ther*, 1994, **64**(3):445 – 475.

[14] Helm JF, Bro SL, Dodds WJ, Sarna SK, Hoffmann RG. Myogenic mechanism for peristalsis in opossum smooth muscle esophagus[J]. *Am J Physiol*, 1992, **263**(6):G953 – G959.

[15] Zhao D, Ren LM. Non-adrenergic inhibition at prejunctional sites by agmatine of purinergic vasoconstriction in rabbit saphenous artery[J]. *Neuropharmacology*, 2005, **48**(4):597 – 606.

ATP 通过 P2 受体调节大鼠近端结肠纵行肌的舒张与收缩反应

任雷鸣[2], 王　秒[1]

(1. 河北医科大学药学院, 河北 石家庄　050017; 2. 河北化工医药职业技术学院, 河北 石家庄　050031)

摘要: 目的　腺苷三磷酸(ATP)对大鼠离体远端结肠纵行肌运动的影响已明确,对近端结肠纵行肌的影响可能不同,但未有报告,为此对此进行观察并探讨其受体机制。**方法**　观察静息张力时或预收缩时 0.1 μmol·L^{-1} ~ 1 mmol·L^{-1} ATP 和 1 ~ 100 μmol·L^{-1} 腺苷对大鼠近端结肠纵行肌的抑制和兴奋作用。**结果**　在静息张力下,1 μmol·L^{-1} ~ 1 mmol·L^{-1} ATP 对大鼠近端结肠纵行肌产生 3 种效应,即抑制自发性收缩反应,一过性轻度降低基础张力(0.05 ~ 0.08 g),随后产生浓度依赖性收缩反应(0.04 ~ 0.44 g)。0.1 μmol·L^{-1}河豚毒素不影响 ATP 的上述作用。在静息张力下,1 ~ 100 μmol·L^{-1}腺苷对近端结肠纵行肌未产生明显的收缩反应。应用 5-羟色胺(5-HT)或乙酰胆碱(ACh)预收缩标本时,1 μmol·L^{-1} ~ 1 mmol·L^{-1} ATP 产生明显的浓度依赖性舒张反应(23.2% ~ 94.6%, 5-HT 预收缩; 24.8% ~ 92.4%, ACh 预收缩),而腺苷引起的舒张反应明显小于 ATP。**结论**　在大鼠离体近端结肠纵行肌,ATP 主要通过嘌呤嘧啶(P)2 受体介导收缩反应,部分通过 P1 受体介导舒张反应。

关键词: 腺苷三磷酸; 腺苷; 受体, 嘌呤; 肌收缩; 肌舒张; 结肠

基金项目: 河北省自然科学基金(302481)

(本文编辑　乔　虹)