

흰쥐 하대정맥에서 퓨린수용체활성화에 의한 혈관반응

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Vascular Reactivity by Purinoceptor Activation in Rat Inferior Vena Cava

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ABSTRACT

Background : Extracellular ATP, released from platelets and nerve endings, plays significant roles in the regulation of circulation. The effects of ATP depend on the location of the vessels and the species of experimental animals. Until now, studies were limited to arteries, so we compared the effects of ATP in rat vena cava with those in the aorta and attempted to identify the characteristics of their receptors. **Methods :** Vascular rings were isolated from the rat inferior vena cava and descending thoracic aorta. Endothelial cells were preserved or removed by gentle rubbing. The isometric contractions were recorded on polygraph using a force transducer. **Results :** In the vena cava ring precontracted by 100 nM norepinephrine (NE), ATP elicited relaxations in a dose-dependent manner. These effects were abolished by removal of the endothelium or pretreatment with a nitric oxide synthase inhibitor. Relaxations to ATP in the vena cava (EC_{50} : 9.9 μ M) were less potent than those in the aorta (1.7 μ M). The relative order of potencies was ADP > ATP > AMP > adenosine, but the maximal relaxation to ADP was smaller than to ATP. ATP-induced vasorelaxation was blocked by suramin, a nonselective antagonist for P_2 purinoceptor and reactive blue-2, a P_{2Y} blocker. At basal tension, ATP contracted the vena cava dose-dependently and these effects were potentiated by endothelium-removal. Contractions in the vena cava were also less potent than in the aorta, and the order of potencies was $UTP > ATP > ADP > AMP =$ adenosine. ATP-induced vasoconstriction was blocked by suramin and α -MeATP, a desensitizing antagonist of P_{2X} purinoceptor, and potentiated by pretreatment with UTP. **Conclusion :** These results suggest that ADP and ATP acts on P_{2Y1} - and P_{2Y2} -purinoceptor in the endothelium, respectively and induces vasorelaxation of the vena cava, which is mediated by nitric oxide. Since ATP and UTP induced vasoconstriction in endothelium-denuded condition, it may be mediated by the activation of the P_{2X} and $P_{2Y4,6}$ purinoceptor on smooth muscles, respectively. (Korean Circulation J 2000;30(9):1156-1164)

KEY WORDS : Adenosine 5'-triphosphate (ATP) · α -methylene adenosine 5'-triphosphate (α -MeATP) · Inferior vena cava · Purinoceptor · Vasorelaxation · Vasoconstriction.

서 론

1)2) 3)4) 5) 6)

adenine

adenosine

7)

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adenine	adenosine	ATP	가	CaCl ₂ 1.91, KH ₂ PO ₄ 1.19, MgSO ₄ 1.44, NaHCO ₃ 24.8 glucose 5.5 mM , 95% O ₂ - 5% CO ₂ pH 7.4 . Adenosine(AD), adenosine 5' - monophosphate(AMP), adenosine 5' - diphosphate(ADP), adenosine 5' - triphosphate(ATP), , - methylene adenosine 5' - triphosphate(, - MeATP), uridine 5' - triphosphate(UTP), theophylline, reactive blue - 2(RB - 2), N - nitro - L - arginine methylester(L - NAME), acetylcholine(ACh) norepinephrine(NE) Sigma (Sigma Chemical Co., St. Louis, MO, USA) , suramin RBI (Research Biochemicals International, MA, USA) . Adenosine theophylline DMSO , .
enosine	P ₂	P ₁ ATP	8)	
P ₁ A ₁ , A ₂ , A ₃ , P ₂	P _{2X1 - 7} P _{2Y1 - 11}	ATP	adenine	
9)10)	P _{2X} P _{2Y}		11 - 13)	
ATP	14)			
	가			
	P _{2Y}			1 N NaOH pH가 7.0 20
8)15)16)				
nitric oxide(NO)	prostacy -			대정맥 또는 대동맥 적출 및 장력측정 sodium thiopental(50 mg/kg) heparin(500 IU/kg)
17)18)				
adenine	adenosine	ATP	가	
5 mm	KRB			
가 forcep				
KRB	organ bath	L		
가 stainless steel	force transducer(Grass FT03C)			
polygraph				
300 gm	(Sprague -	(Grass Model 7E)		KRB 37
Dawley rat)				(95% O ₂ - 5% CO ₂)
				0.5 gm,
실험용액 및 약물		1 gm		, 60
Krebs - Ringer bicarbonate(KRB)				KRB
NaCl 117, KCl 4.7,	3 4			, Furchgott

19) 100 nM NE
가 1
M ACh 80%
(E⁺) , 1 μM ACh
가
(E⁻) .

퓨린수용체 활성화에 의한 장력변화 측정
가 100 nM
NE , ATP, ADP,
AMP, AD 100 nM
1 mM 가

NE
,
50%
(EC₅₀)
ATP
KCl(40 mM)
가 NO가
NO L - NAME(1 mM)

퓨린수용체 차단제에 의한 영향
ATP
, 가 NE
100 μM ATP
P₁
theophylline(50 μM), P₂
suramin(1 mM), P_{2X}
, - MeATP(100
μM),²⁰⁾²¹⁾ P_{2Y} RB - 2(10 μM)
20 ATP
P_{2Y2} /P_{2Y4}
UTP(50 μM) ATP
(desensitization)²²⁾
ATP
가 2 mM ATP

자료 분석
± ,
Student paired t - test p 0.05

결 과
정맥 및 동맥에서 ATP에 의한 혈관이완반응
100 nM NE
ATP 가 ,
Fig. 1A B
100 nM NE
가 , ATP
EC₅₀(9.9 μM)
가 (1.7 μM)
30 μM 100% 가
, 300 μM 80%
(Fig. 1C).

내피세포 제거 및 NO 생성 차단에 의한 영향
가
ATP 가
, 30 μM
L - NAME
NO
(Fig. 2).
Adenine 뉴클레오티드 및 adenosine에 의한 혈관이완
반응
NE ATP, ADP, AMP
adenosine 30 nM 1 mM
가
ADP(EC₅₀ ; 0.52 μM)
ATP . AMP(153 M)

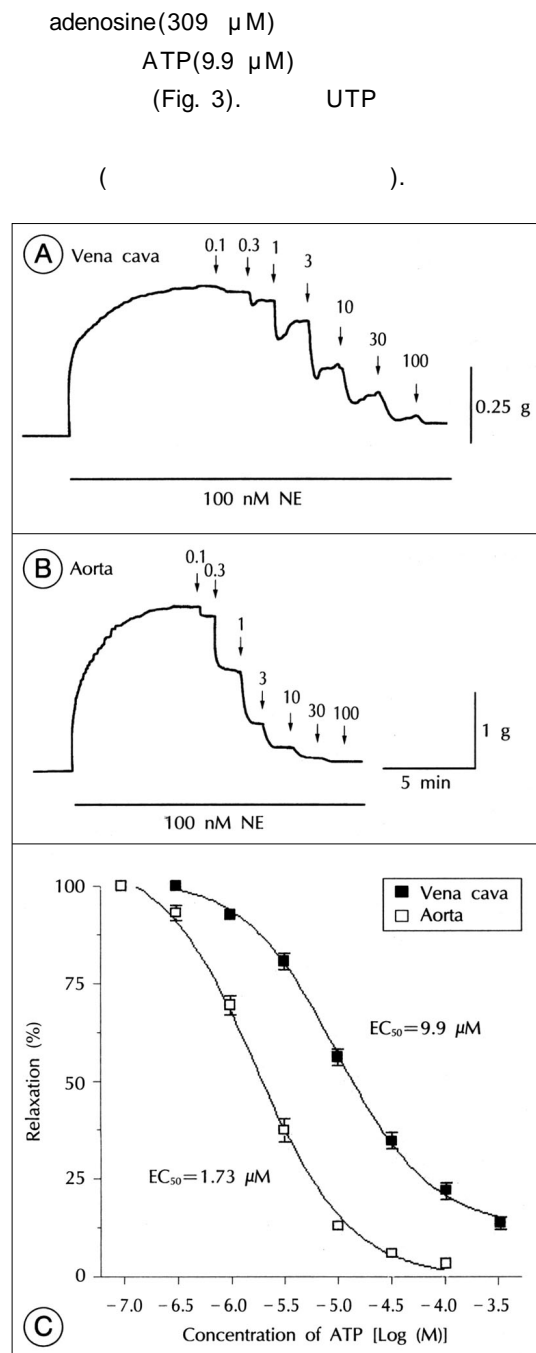
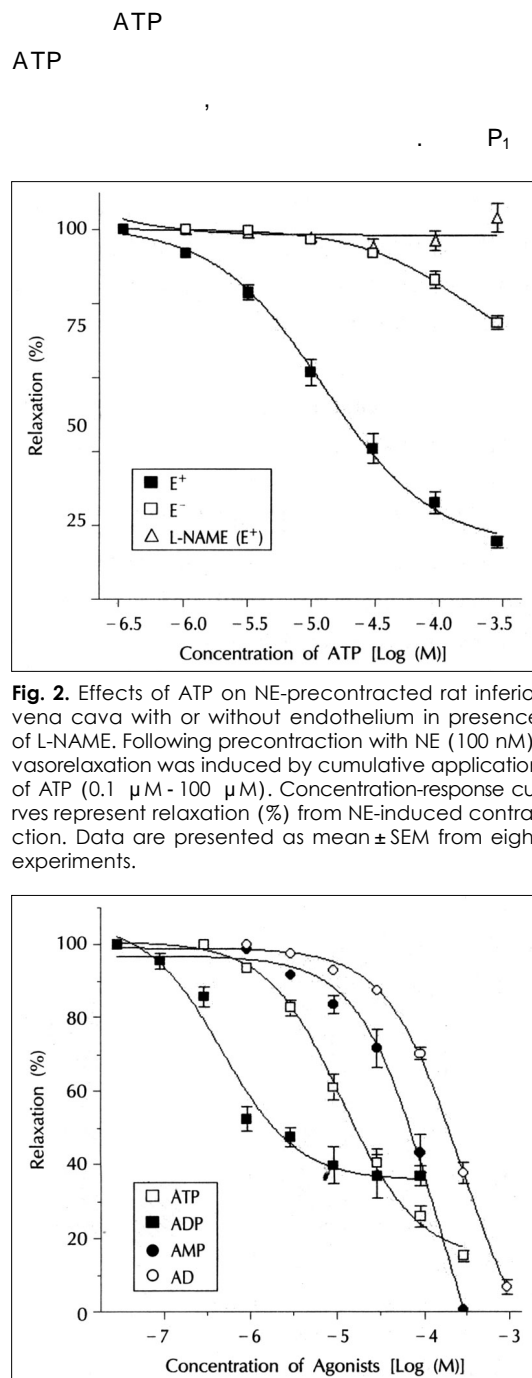


Fig. 1. Comparison of ATP-induced vasorelaxation in NE-precontracted rat inferior vena cava and aorta. Following precontraction with NE (100 nM), vasorelaxation was induced by cumulative application of ATP (0.1 μ M - 100 μ M) in inferior vena cava (a) and aorta (b) with intact endothelium. (c) Concentration-response curves represent relaxation (%) from NE-induced contraction. Data are presented as mean \pm SEM from six experiments.



theophylline(50 μ M) ATP
(2.8 \pm 1.9%), P₂ suramin(1 mM)
(80.0 \pm 2.0%). P_{2Y2}
/P_{2Y4} UTP(50 μ M) P_{2Y}
RB-2(10 μ M) 69.6 \pm 8.0%
18.0 \pm 3.0% P_{2X}
- MeATP(100 μ M)
(- 2.9 \pm 9.2%)(Fig. 4).

정맥 및 동맥에서 ATP에 의한 혈관수축반응
ATP 10 μ M
M 3 mM 가 ,
Fig. 5 A B .

(Fig. 5). ATP
NO 가 가
(Fig. 6).

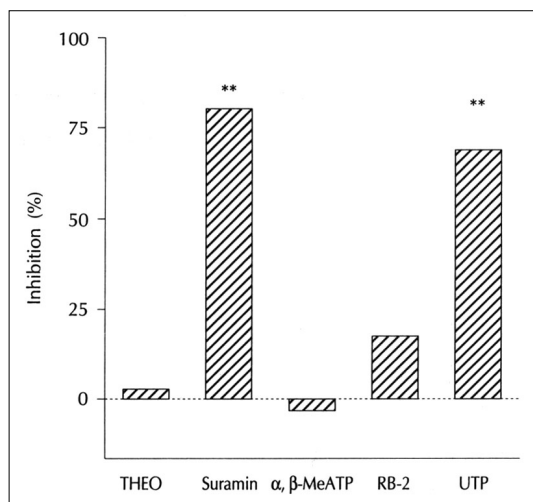


Fig. 4. Effects of purinergic agonists and antagonists on ATP-induced relaxations in NE-precontracted rat inferior vena cava with intact endothelium. Following pretreatment with theophylline (THEO, 50 μ M), suramin (1 mM), RB-2 (10 μ M), α , β -MeATP (100 μ M) or UTP (50 μ M) for 20 min, ATP (100 μ M)-induced relaxation (%) was acquired in NE (100 nM)-precontracted preparation. The value below zero indicates more contraction than control. Data are presented as mean \pm SEM from six experiments and denotes $p < 0.01$.

여러 뉴클레오타이드 및 adenosine에 의한 정맥 수축반응
- MeATP, UTP, ATP,
ADP, AMP adenosine 10 μ M 3 mM
가
- MeATP 가
UTP, ATP, ADP , AMP
adenosine (Fig. 7).
ATP
ATP

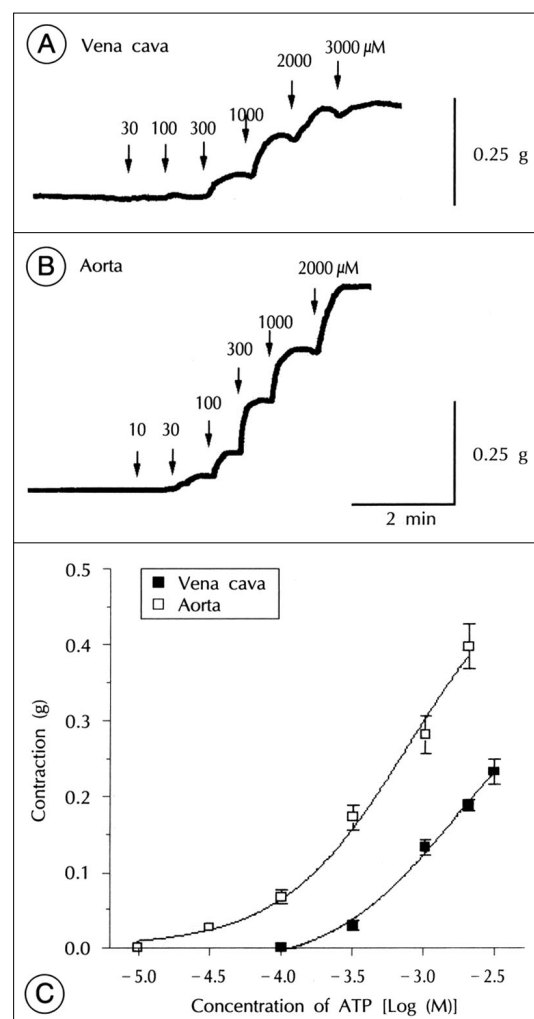


Fig. 5. Comparison of ATP-induced vasoconstriction in rat inferior vena cava and aorta as basal tension. Vasoconstriction was induced by cumulative application of ATP (10 μ M - 3 mM) in inferior vena cava (a) and aorta (b) with intact endothelium. (c) Cumulative concentration-response curves represent changes in vascular tension. Data are presented as mean \pm SEM from five experiments.

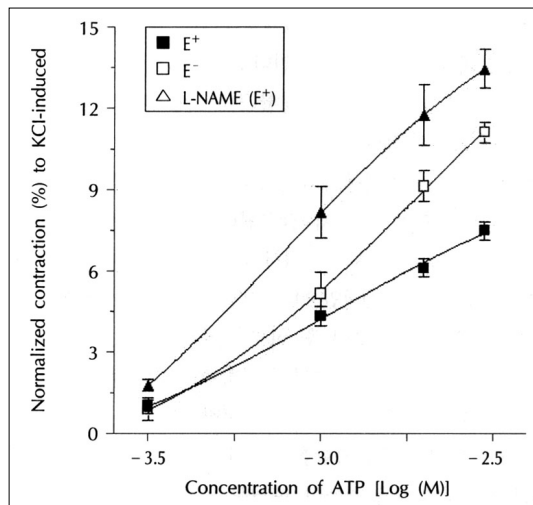


Fig. 6. Comparison of ATP-induced contraction in rat inferior vena cava with or without endothelium in presence of L-NAME. ATP (300 μ M - 2 mM) was added cumulatively in inferior vena cava. Contraction (%) was normalized to the maximal tension developed by high KCl (40 mM). Data are presented as mean \pm SEM from five experiments.

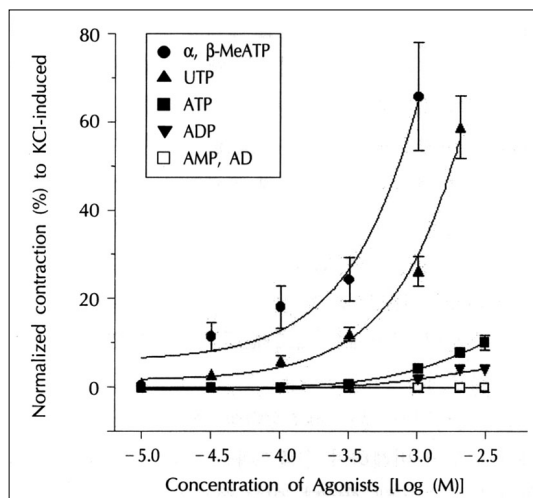


Fig. 7. Effects of adenine nucleotides and adenosine on basal tension of rat inferior vena cava. Contraction was induced by a cumulative application of different purinergic receptor agonists (α , β -MeATP, UTP, adenine nucleotides and adenosine (10 μ M - 3 mM)) from endotheliumdenuded preparation. Contraction (%) was normalized to the maximal tension developed by high KCl (40 mM). Data are presented as mean \pm SEM from six experiments.

ophylline (50 μ M) (5.4 \pm 2.9%), suramin(1 mM ; 74.1 \pm 7.4%) , - Me -

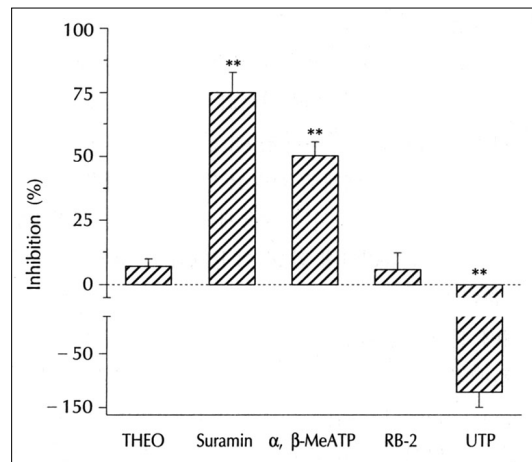


Fig. 8. Effects of purinergic agonists and antagonists on ATP-induced contraction in rat inferior vena cava. Following pretreatment with theophylline (THEO, 50 μ M), suramin (1 mM), RB-2 (10 μ M), α , β -MeATP (100 μ M) and UTP (50 μ M) for 20 min., then ATP (100 μ M) was applied to endotheliumdenuded preparation. Result represents as percentage changes of ATP-induced contraction. The value below zero indicates a further contraction than those in the absence of purinergic agonists. Data are presented as mean \pm SEM from six experiments and ** denotes $p < 0.01$.

ATP (100 μ M ; 48.7 \pm 3.1%)
RB-2(10 μ M) ATP
(9.3 \pm 7.5%), UTP
가 (- 127.7 \pm 25.5%) (Fig. 8).

고 안

ATP norepinephrine(NE)

(23 - 25)

vesicle ATP가 600 mM

(13)26)

ATP가

ATP

가

(14)27)28)

ATP

가 , (35)(36) NO가

endothelium derived relaxing factor(EDRF) . (19) P_{2Y}

ATP가 EDRF , EDRF가 (agonist sensitivity) (33)

EDRF , EDRF ATP ADP

가 , nitric UTP

oxide(NO) , (29)(30) NO (

cyclooxygenase prostacyclin). P_{2Y2}/P_{2Y4}

prostacyclin , NO UTP⁽²²⁾ P_{2Y2}/P_{2Y4}

L - NAME ATP

ATP P_{2Y2}

NO ADP P_{2Y1} ,

Adenine ADP ATP ATP UTP

ADP P_{2Y2} P_{2Y1} P_{2Y2} 가 ,

ADP , (9)

ADP 60% ATP

ATP AMP adeno - sine 가

AMP adenosine ATP adenosine P₁

가 ,

(). Adenosine NO synthase inhibitor

P₁ 가 (15)

ATP

가 ,

가

P₁ theophylline 가 (37)

P₂ suramin ATP NO

가 P₂ , ATP 가 P₂ P_{2X} NO 가

가

가

, - methylene adenosine 5' - triphosphate(, - MeATP)⁽²⁰⁾⁽²¹⁾⁽³²⁾ , P_{2Y} 가

reactive blue - 2⁽³³⁾ 18.0%,

UTP⁽²²⁾⁽³⁴⁾ 69.6%가 ATP

adenosine

, - MeATP>UTP>ATP>ADP>AMP=

adenosine , P₂

가 Ca²⁺ IP₃ (38) ATP

theophylline

RB - 2³³⁾

UTP

ATP

ATP

ATP

연구목적 :

adenosine 5' - triphosphate(ATP)

요 약

ATP

방 법 :

가

lygraph

결 과 :

100 nM norepinephrine(NE)

ATP

ATP

suramin reactive blue - 2

MeATP>UTP>ATP>ADP>AMP>adenosine

. ATP

결 론 :

중심 단어 : Adenosine 5' - triphosphate (ATP) ,
- methylene adenosine 5' - triphosphate (, -
MeATP) .

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