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Colforsin or imidazolidione potentiates cAMP elevation caused by endothelin-1 in rat aorta

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ABSTRACT Endothelin-1 (ET-1) caused slow-developing and stable vasoconstrictions in isolated rings of rat thoracic aortae with a pD_2 value of 7.55 ± 0.10 compared to pD_2 values of 9.30 ± 0.10 and 8.36 ± 0.30 for angiotensin II and norepinephrine, respectively. Although the potency of ET-1 was somewhat lower than those of norepinephrine and angiotensin II, the maximal tension generated by ET-1 was comparable to that of norepinephrine and considerably greater than that of angiotensin II. Incubation of aortic rings in the absence of extracellular Ca^{2+} or in the presence of the Ca^{2+} channel blocker nifedipine (100 nmol $\cdot L^{-1}$) greatly attenuated ET-1-induced vasoconstriction. ET-1 (20 nmol $\cdot L^{-1}$, approximately the ED_{50} for vasoconstrictions) also caused elevation of cAMP levels in aortic rings after 15 and 25 min of exposure. The cAMP phosphodiesterase inhibitor imidazolidione (Imi, Ro 20-1724, 100 μmol $\cdot L^{-1}$) potentiated the cAMP responses to ET-1. Rings incubated for 25 min with ET-1 (20 nmol $\cdot L^{-1}$) showed much larger cAMP elevations caused by colforsin (Col, forskolin 1 μmol $\cdot L^{-1}$), a direct adenylate cyclase activator and potentiator, than with Col or ET-1 alone. Therefore, ET-1 may utilize at least 2 signal transduction mechanisms, one involving the opening of nifedipine-sensitive Ca^{2+} channels and the other involving the elevation of cAMP levels, to produce the unusually slow-delevoping and stable vasoconstrictions in rat aortae.

KEY WORDS endothelins; thoracic aorta; vascular smooth muscle; calcium; nifedipine; adenosine cyclic monophosphate; forskolin; imidazoles

Endothelin-1 (ET-1) is an endothelium-derived vasoconstrictor factor, which was isolated from porcine aortic endothelium as 21 amino acid peptide[1]. ET-1-induced vasoconstriction involves the influx of extracellular Ca^{2+} through a voltage-dependent Ca^{2+} channel[2,3], of which the blocker, however, had little effect on the contraction induced by ET-1 in rabbit aortae[3] and human internal mammary arteries[4] indicating that ET-1 does not produce contraction by an interaction with voltage-dependent Ca^{2+} channels.

Cyclic AMP is involved in the regulation of intracellular Ca^{2+} concentrations and contractile force generated in vascular smooth muscles. ET-1-induced contraction was not associated with an alteration in the levels of cAMP in rabbit aortic rings[3] and in cultured rat thoracic aorta smooth muscle cells[5]. ET-1 stimulated cAMP formation in embryonic bovine tracheal cells[6], rat mesangial cells[7], and iris sphincter of varies mammals[8]. In contrast, ET-1 reduced isoproterenol or Col-induced cAMP accumulation in adult rat cardiac myocytes[9]. The present study was designed to determine whether ET-1 has an effect on cAMP levels in rat aortic

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rings and to explain how this cAMP response affects the ET-1-induced vasoconstriction.

MATERIALS AND METHODS

Mechanical responses Sprague-Dawley rat (♂, 220—250 g) were injected ip with heparin (1000 IU) before decapitation[10]. The thoracic aortae were placed in ice-cold Krebs-Ringer-bicarbonate solution (KRB) at pH 7.4 containing NaCl 118.5, KCl 4.74, $MgSO_4$ 1.18, $CaCl_2$ 2.5, KH_2PO_4 1.18, $NaHCO_3$ 24.9, glucose 10 mmol·L^{-1} and gassed with 95 % O_2 + 5 % CO_2. After removing the adhering fat and connective tissue, the aortae were cut transversly into rings 4 mm wide. Care was paid to protect the endothelium lining from damage. The rings were mounted in organ baths containing 5 ml of the KRB at 37 ℃ under 1 g of resting tension. The KRB was refreshed every 15 min during 1-h equilibration. Contractile responses were measure isometrically using force-displacement transducer (Grass Model FT-03) and a polygraph recorder (Beckman, Model R611).

Cyclic AMP measurement Rat aortic rings were freeze-clamped between metal blocks precooled in liquid nitrogen and immediately homogenized in ice-cold 10 % trichloroacetic acid (1 ml). The homogenates were centrifuged (3000 × *g* for 10 min) to precipitate protein. Cyclic AMP amounts in supernatants were determined by radioimmunoassay[20].

Drugs ET-1 was obtained from Peninsula Laboratories (Belmont CA, Lot 016563). Imi was a gift from Dr Theodore J Torphy (SmithKline Beecham, King of Prussia PA). Col was purchased from Calbiochem Corp (San Diego CA). Adenosine 3′5′-cyclic phosphoric acid 2′-O-succinyl-3-[^{125}I]iodotyrosine methyl ester (74 PBq·mol^{-1}) were obtained from Biomedical Technologies Inc (Stoughton MA). Anti-cAMP antibody was obtained from Research Products International Corp (Mount Prospect IL). Other chemicals and drugs were purchased from Sigma Chemical Co (St Louis MO). All drugs were dissolved in water, except Imi, nifedipine and Col, which were dissolved in Me_2SO. The final concentrations for these 3 drugs in the tissue bath were 0.1 %, 0.1 % and 0.0001 % (vol/vol), respectively.

Statistical analysis Results were analyzed by Dunnett's test and *t* test.

RESULTS

ET-1-induced vasoconstriction ET-1 caused concentration-dependent vasoconstriction in rings of rat thoracic aortae. The maximal tension of the contraction was 4.6±0.7 g ($n=6$). Those of norepinephrine- and angiotensin II-induced contraction were 4.1 ± 0.4 g ($n=4$, $P>0.05$) and 2.5±0.6 g ($n=4$, $P<0.001$), respectively. Therefore, the efficacy of ET-1-induced vasoconstriction was comparable to that of norepinephrine-induced vasoconstriction and considerably larger than that of angiotensin II-induced vasoconstriction. The estimated pD_2 value of ET-1 was 7.55±0.10 ($n=6$). The pD_2 values of norepinephrine and angiotensin II were 8.36 ± 0.30 ($n=4$) and 9.30±0.10 ($n=4$), respectively. Hence, the potency order was angiotensin II > norepinephrine > ET-1. The ET-1-induced vasoconstriction was slow-developing, long-lasting, and difficult to wash out.

Extracellular Ca^{2+}-dependency of ET-1-induced vasoconstriction ET-1 (20 nmol·L^{-1}, approximately EC_{50}) produced a contractile force of 3.3±0.7 g ($n=11$) in rat aortic rings. When rings were equilibrated for 15 min in Ca^{2+}-free (+egtazic acid 1 mmol·L^{-1}, ethylene glycol bis (β-aminoethyl ether)-*N*, *N*, *N*′, *N*′-tetra-acetic acid), the forces developed by ET-1 20 nmol·L^{-1} were decreased to 0.31±0.02 g ($n=4$, $P<0.001$), only 9.6± 3.8 % of the vasoconstriction in the presence of extracellular Ca^{2+} 2.5 mmol·L^{-1}. Pretreatment with nifedipine 100 nmol·L^{-1} for 10 min greatly attenuated the contractile force developed to only 0.59±0.18 g ($n=4$, $P<0.001$), thus reducing the contraction to only 18.0±2.6 % of the control.

Col inhibited ET-1-induced vasconstriction Pretreatment with Col (1 μmol·L^{-1}) for

10 min reduced the ET-1-induced force generation to 0.61±0.24 g ($n=4$, $P<0.001$), representing a 81±4 % decrease in contractile response (Fig 1). During the stable contraction Col (100 nmol·L^{-1} and 1 μmol·L^{-1}) added to ET-1 (20 nmol·L^{-1}) induced relaxation of 40±12 % ($n=5$) and 99.5±0.5 % ($n=5$), respectively.

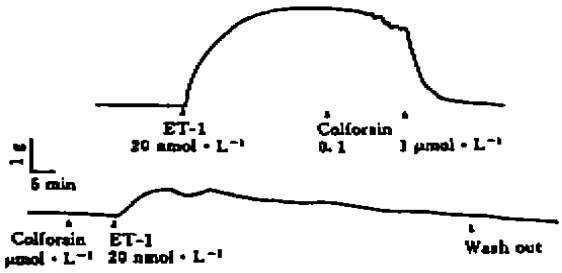

Fig 1. Effect of colforsin on endothelin-1-induced contractions in rat aorta. Col (100 nmol·L^{-1} and 1 μmol·L^{-1}) added after ET-1 (20 nmol·L^{-1}) relaxed the ET-1-precontracted rings and Col (1 μmol·L^{-1}) added 10 min before ET-1 (20 nmol·L^{-1}) inhibited ET-1-induced vasoconstricition.

ET-1 increased cAMP levels Exposure of aortic rings in ET-1 20 nmol·L^{-1} (the EC_{50} value for causing vasoconstriction) produced a time-dependent increase in cAMP content. But this response was so small and slow-developing that significant elevation of cAMP levels was not achieved until 15—25 min after ET-1 addition (from a control value of 2.9±0.3 to 4.2±0.9 or 4.7±0.9 pmol/mg protein with ET-1 added, respectively for 15 or 25 min, both $P<0.01$).

Imi potentiated the cAMP responses to ET-1 Imi (100 μmol·L^{-1}) per se did not significantly elevate cAMP levels in rat aorta. The combination of Imi (100 μmol·L^{-1}, 10 min pretreatment) and ET-1 (20 nmol·L^{-1} for 5 min) markedly elevated cAMP levels more than Imi or ET-1 alone. When rings were equilibrated for 15 min in Ca^{2+}-free KRB (+ egtazic acid 1 mmol·L^{-1}), Imi did not potentiate the cAMP responses to ET-1 (Tab 1).

Tab 1. Effects of imidazolidlone (Imi) 0.1 nmol·L^{-1} or endothelin-1 (ET-1) 20 nmol·L^{-1} on cAMP elevation induced by ET-1 20 nmol·L^{-1} or colforsin (Col) 1 μmol·L^{-1} in rat aortae. $n=6—8$ rats, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* appropriate control.

Time of exposure to ET-1	Time of exposure to Imi	Time of exposure to Col	n	cAMP, pmol/mg protein KRB	cAMP, pmol/mg protein Ca^{2+}-free KRB
0	0		8	3.9±0.6	
5 min	0		6	3.7±0.7^a	
0	15 min		6	5.5±0.9^a	
5 min	15 min		8	8.5±1.4^b	5.6±1.4^a
0		0	8	2.9±0.3	
25 min		0	8	4.7±0.9^c	
0		10 min	6	8.2±1.5^c	
25 min		10 min	8	14.6±3.3^c	

ET-1 potentiated Col-induced elevation of cAMP levels Although ET-1 by itself caused only a small increase in cAMP levels, ET-1 (20 nmol·L^{-1}) clearly potentiated the Col (1 μmol·L^{-1})-induced increase in cAMP levels. The cAMP levels are significantly higher for the Col plus ET-1 group than for the Col or ET-1 only group ($P<0.01$) (Tab 1).

DISCUSSION

Equilibration in Ca^{2+}-free medium or addition of the Ca^{2+} channel blocker nifedipine inhibited ET-1-induced vasoconstrictions by 80—90 %. In contrast, pretreatment of aortic rings with nifedipine (100 nmol·L^{-1}) for 10 min inhibited norepinephrine-induced vasoconstrictions by only 31 ± 6 % (data not shown). These data suggest that in rat aortae ET-1-induced vasoconstriction, more than norepinephrine-induced vasoconstriction, is dependent on extracellular Ca^{2+}.

The efficacy and potency of ET-1-induced vasoconstricition obtained in the present study were different from the data of others[1,11,12]. In addition, on cAMP our results are in sharp

contrast to the results described in the literatures[3,5-9]. These discrepancies may be related to the different species, and/or the studied tissues.

The mechanism of the ET-1-induced cAMP response is likely to involve activation of adenylate cyclase, because of the potentiating actions of the Col, a known potentiator of adenylate cyclase activation, and the phosphodiesterase inhibitor Imi. One possible explanation for the cAMP elevation to ET-1 is that, elevation of intracellular Ca^{2+} concentration induces vasoconstriction and stimulation of adenylate cyclase. In supporting this hypothesis, Imi does not potentiate the cAMP response to ET-1 in aortae when incubated in Ca^{2+}-free KRB (Tab 1).

In vasular smooth muscles, increases in cAMP levels usually lead to relaxation of smooth muscles. However, besides present study, some reports showed that a few agonists caused both vasoconstriction and elevation of cAMP levels[13,14]. We find that Col inhibits ET-1-induced vasoconstrictions and 8-bromo-cAMP (100 $\mu mol \cdot L^{-1}$) added to ET-1-precontracted aortic rings relaxes the vessels by 65 % (data not shown). These results suggest that the cAMP elevations caused by ET-1 may be a negative feedback signal, which is responsbile for the slow development of ET-1-induced vasoconstriction. In other words, cAMP at the early stages of ET-1-induced contraction may attenuate, and thereby slow down the progress of contraction. The cAMP elevations induced by ET-1 in rat thoracic aortae may also be responsible for the lower potency of ET-1-induced contraction in this blood vessels than in coronary and renal arteries[1,11].

In conclusion, ET-1 may utilize at least 2 signal transduction mechanisms, one involving the opening of Ca^{2+} channels and the other involving the generation of cAMP, to cause the unusually slow-developing and stable vasoconstriction in rat aorta.

REFERENCES

1 Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, *et al*. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988; **332**: 411-5.

2 Iijima K, Lin L, Nasjletti A, Goligorsky MS. Intracellular signaling pathway of endothelin-1. J Cardiovasc Pharmacol 1991; **17** Suppl 7: SI46-SI49.

3 Ohlstein EH, Horohonich S, Hay DWP. Cellular mechanisms of endothelin in rabbit aorta. J Pharmacol Exp Ther 1989; **250**: 548-55.

4 Yang Z, Bauer E, Segesser LV, Stulz P, Turina M, Lüscher TF. Different mobilization of calcium in endothelin-1-induced contractions in human arteries and veins: effects of calcium antagonists. J Cardiovasc Pharmacol 1990; **16**: 654-60.

5 Sugiura M, Inagami T, Hare GMT, Johns JA. Endothelin action: inhibition by a protein kinase C inhibitor and involvement of phosphoinositols. Biochem Biophys Res Commun 1989; **158**: 170-6.

6 Oda K, Fujitani Y, Watakabe T, Inui T, Okada T, Urade Y, *et al*. Endothelin stimulates both cAMP formation and phosphatidylinositol hydrolysis in cultured embryonic bovine tracheal cells. FEBS Lett 1992; **299**: 187-91.

7 Simonson MS, Dunn MJ. Endothelin-1 stimulates contraction of rat glomerular mesangial cells and potentiates β-adrenergic mediated cyclic adenosine monophosphate accumulation. J Clin Invest 1990; **85**: 790-7.

8 Abdel-Latif AA, Zhang Y. Species differences in the effects of endothelin-1 on myo-inositol triphosphate accumulation, cyclic AMP formation and contraction of isolated iris sphincter of rabbit and other species. Invest Ophthalmol Vis Sci 1991; **32**: 2432-8.

9 Hilal-Dandan R, Urasawa K, Brunton LL. Endothelin inhibits adenylate cyclase and stimulates phosphoinositide hydrolysis in adult cardiac myocytes. J Biol Chem 1992; **267**: 10620-4.

10 Zhou H-L, Fiscus RR. Brain natriuretic peptide (BNP) causes endothelium-independent relaxation and elevation of cyclic GMP in rat thoracic aorta. Neuropeptides 1989; **14**: 161-9.

11 Tomobe Y, Miyauchi T, Saito M, Yanagisawa S, Kimura S, Goto K, *et al*. Effects of endothelin on the renal

artery from spontaneously hypertensive and Wistar Kyoto rats. Eur J Pharmacol 1988; 152: 373—4.
12 Brain SD, Tippins JR, Williams TJ. Endothelin induces potent microvascular constriction. Br J Pharmacol 1988; 95: 1005—7.
13 Fiscus RR, Dyer DC. Cyclic nucleotides and contractility in human and sheep umbilical arteries. Eur J Pharmacol 1981; 73: 283—91.
14 Vegesna RVK, Diamond J. Effects of prostaglandin E_1, isoproterenol and forskolin on cyclic AMP levels and tension in rabbit aortic rings. Life Sci 1986; 39: 303—11.

考福新或咪唑二酮增强内皮素-1升高大鼠主动脉腺苷环一磷酸

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摘要 硝苯啶(100 nmol·L^{-1})或无细胞外钙抑制内皮素-1(ET-1)收缩大鼠胸主动脉80%以上. ET-1(20 nmol·L^{-1}; 15、25 min)增加血管环腺苷酸(cAMP)含量, 该作用被咪唑二酮(100 μmol·L^{-1})增强. ET-1还能增强福斯科林的增加cAMP作用. 本文证明ET-1收缩大鼠胸主动脉至少涉及两种信息转导机制, 即开放硝苯啶敏感性钙通道和增加cAMP.

关键词 内皮素; 胸主动脉; 血管平滑肌; 钙; 硝苯啶; 腺苷环一磷酸; 福斯科林; 咪唑类

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Three drugs inhibit phospholipase A_2-induced high permeability of endothelial monolayers[1]

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ABSTRACT The permeability of aortic endothelial monolayers to fluid and albumin increased 13.5 and 16.1 times respectively after pretreatment with phospholipase A_2 (PLA_2, 100 U·ml^{-1}) for 30 min. 1-(*p*-Chlorobenzoyl)-5-methylindole-3-acetic acid (1.16 mmol·L^{-1}), SRI 63-441 (30 nmol·L^{-1}), and 1,25-dihydroxycholecalciferol (0.1 μmol·L^{-1}) decreased PLA_2-induced high permeability. PLA_2 did not damage the endothelial cells significantly. Our results indicate that the action of PLA_2 to increase the permeability of endothelial monolayers is mainly due to PLA_2-induced lipid mediators released from endothelial cells.

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KEY WORDS phospholipases A; capillary permeability; vascular endothelium; prostaglandins; platelet activating factor; cholecalciferols

Phospholipase A_2 (PLA_2) increased the pulmonary vascular permeability and caused pulmonary injury in isolated perfused guinea pig lungs[1]. Extracellular PLA_2 is associated with many inflammatory diseases[1,2]. It causes the damages by generating proinflammatory products such as platelet activating factor (PAF), arachidonate and their derivatives[1,3]. PLA_2 also increased the membrane permeability of endothelial cells (EC) and the releases of lactate dehydrogenase (LDH), kininase II and malondialdehyde from EC in high concentrations[4]. Pulmonary responses in-