

난소절제 고혈압 백서에서 에스트로겐 투여가 혈관반응성에 미치는 영향

서봉관^{1,2} · 최동주^{1,2} · 황진용^{1,2} · 천일석¹ · 이유판¹

Effect of Estrogen Replacement on Vascular Responsiveness in Ovariectomized Spontaneously Hypertensive Rat

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ABSTRACT

Background : Although postmenopausal estrogen replacement therapy is known to reduce cardiovascular mortality, the mechanism is not clear yet. Furthermore, the effect of estrogen on vascular tonus is reportedly variable according to the animal models, vascular beds and agonists used. **Materials and Method :** Bilateral ovariectomies were performed in 12 week-old, 18 spontaneously hypertensive rats (SHR) and 18 normotensive Wistar-Kyoto rats (WKY). Rats were divided into three groups according to the dose of 17 β -estradiol (E₂) pellets implanted subcutaneously two weeks after ovariectomy ; control (no implantation), low-dose (0.5 mg) and high-dose (5 mg) E₂ replacement group. Two weeks after pellet implantation, organ bath experiments were performed using descending thoracic aortae. For endothelium-dependent relaxation, acetylcholine (10⁻⁹ - 3 \times 10⁻⁶ M) was cumulatively added into the vessels precontracted with 10⁻⁷ M norepinephrine (NE). For vasoconstrictor responses, cumulative concentration-contraction curves were constructed in quiescent vessels using NE (10⁻⁹ - 10⁻⁵ M), U46619 (10⁻⁹ - 3 \times 10⁻⁶ M), endothelin-1 (10⁻¹⁰ - 10⁻⁷ M). In addition, contraction to angiotensin (10⁻⁷ M) was also obtained. Serum 17 β -estradiol levels were measured by radioimmunoassay. Blood pressure was measured by tail-cuff method in some SHRs before ovariectomy and after placebo/E₂ replacement. **Results :** Endothelium-dependent relaxation to acetylcholine was impaired in WKY treated with 5 mg E₂ (pIC₅₀ ; control vs 5 mg E₂ ; 7.75 \pm 0.13 vs 7.27 \pm 0.16 ; n = 6 ; p<0.05). No significant effect was noted in SHR. Contraction to angiotensin was inhibited by low-dose E₂ in WKY and high-dose E₂ in SHR (% of the contraction to 60 mM KCl ; WKY ; control vs 0.5 mg E₂ ; 39 \pm 5 vs 25 \pm 2 ; SHR ; control vs 5 mg E₂ ; 34 \pm 4 vs 22 \pm 2 ; n = 6 and p<0.05 in WKY and SHR). In contrast, NE-induced contraction was enhanced by E₂ replacement (both low- and high-dose) in WKY and SHR (WKY ; control vs 0.5 mg E₂ vs 5 mg E₂ ; AUC ; 280 \pm 24 vs 387 \pm 26 vs 374 \pm 25 ; maximal contraction ; 137 \pm 8 vs 166 \pm 8 vs 162 \pm 3 ; pD₂ ; 7.63 \pm 0.11 vs 8.17 \pm 0.13 vs 8.13 \pm 0.13 ; SHR ; control vs 0.5 mg E₂ vs 5 mg E₂ ; AUC ; 265 \pm 17 vs 349 \pm 16 vs 406 \pm 19 ; maximal contraction ; 152 \pm 6 vs 181 \pm 9 vs 203 \pm 16 ; pD₂ ; 7.45 \pm 0.13 vs 7.91 \pm 0.08 vs 8.04 \pm 0.04 ; n = 6 and p<0.05 between control and treated groups in WKY and SHR for all parameters). Contraction to U46619 was enhanced by E₂ replacement in SHR (control vs 0.5 mg E₂ ;

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AUC ; 478 ± 30 vs 574 ± 23 ; maximal contraction ; 181 ± 9 vs 230 ± 10 ; $n = 6$; $p < 0.05$ for both parameters). Maximal contractile response to endothelin-1 was also enhanced in SHR (control vs 0.5 mg E_2 vs 5 mg E_2 ; maximal contraction ; 165 ± 7 vs 189 ± 7 vs 199 ± 8 ; $n = 6$ and $p < 0.05$ between control and treated groups) but not in WKY. Blood pressure was not different between placebo and E_2 treated SHR (171 ± 2 vs 174 ± 4 mmHg). **Conclusion** : In WKY, chronic high-dose estrogen replacement impairs endothelium-dependent relaxation to acetylcholine. ; low-dose estrogen replacement does not affect endothelium-dependent relaxation in SHR and WKY. Estrogen replacement enhances the contraction to most of the contractile agonists tested except angiotensin II in both WKY and SHR. These results suggest that estrogen replacement affect the vascular tonus differently according to the vasoactive substances and/or hormones without significant effect on blood pressure. **(Korean Circulation J 2000;30(4):528-539)**

KEY WORDS : Estrogen · Endothelium-dependent relaxation · Vascular tonus · Spontaneously hypertensive rat.

relaxation) ¹⁶⁾¹⁷⁾

서 론

가

50%

¹⁾²⁾

(low - density lipoprotein ; LDL)

(high - density lipoprotein ; HDL)

³⁾ LDL oxidized LDL

가 ⁴⁾ fibrinogen⁵⁾⁶⁾

plasminogen activator inhibitor(PAI) - ¹⁷⁾

thromboxane analogue U - 46619 ¹⁸⁾

angiotensin II ¹⁹⁾

()

가

(vas -

cular tonus)

^{4)8 - 15)} nitric oxide prosta -

cyclin (endothelium - derived

relaxing factor ; EDRF)

가

가

nitric oxide

가

^{11 - 15)}

가

가

(endothelium - independent vaso -

재료 및 방법

난소 절제 및 에스트로겐(17β -estradiol; 이하 E_2) 투여
12 SHR() Wi -
star - Kyoto (WKY) ketamine xylazine(
)
2
(6 SHR, 6 WKY) E_2
, E_2 21
 E_2 0.5 mg(E_2 0.5 mg ; 6 SHR, 6
WKY) 5 mg(E_2 5 mg ; 6 SHR, 6 WKY) pellet
10 gauge trochar
2 가
 E_2
modi -
fied Krebs - Ringer [NaCl 118, KCl 4.7, $CaCl_2$
2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, EDTA
0.026, glucose 1.1(; mmol/L) ; control]
4

에스트로겐이 내피세포의존성 혈관이완에 미치는 효과

가
3 mm (ring)
organ bath(37 , 95% O_2 /
5% CO_2)
organ bath ,
force transducer physiograph
isometric tension
organ bath
(preload ; 2 g) 30
60 mM KCl
control
norepinephrine(10^{-7} M)
tension
nitric oxide ace -

tylcholine (10^{-9} - 3×10^{-6} M)

여러 agonist에 의한 혈관수축에 미치는 에스트로겐의
영향

E_2 가 가
norepinephrine(10^{-9}
- 10^{-5} M), U46619(10^{-9} - 3×10^{-6} M), endothelin
- 1(10^{-10} - 10^{-7} M) angiotensin II(10^{-7} M)
 E_2
Angiotensin II

angiotensin 가
60 mM KCl
60mM KCl
norepine -

phrine, endothelin - 1, U46619, angiotensin II
가

Krebs - Ringer

60 mM KCl

혈중 E_2 농도의 측정

E_2
 E_2
radioimmunoassay E_2
 E_2 가

SHR E_2

사용 시약

E_2 pellet(0.5 mg 5 mg pellet) Innovative
Research of America (Sarasota, Fa, USA)
norepinephrine, acetylcholine, an -
giotensin II, U46619 endothelin - 1 Sig -
ma (St Louis, Mo, USA) Endo -
thelin - 1 0.1% bovine serum albumin ,

혈압의 측정
 가
 8 SHR
 E₂ 0.5 mg(3)
 tail - cuff
 가
 2
 E₂ 0.5 mg
 가
 5

결과분석 및 통계처리
 pIC₅₀
 (norepinephrine 10⁻⁷ M
 acetylcholine - log)
 (area under the curve ;
 AUC),
 50%
 - log)
 Student's unpaired t - test

rank test ,
 Mann - Whitney U - test
 p<0.05
 Wilcoxon signed

결과
 에스트로겐이 내피세포의존성 혈관이완에 미치는 효과
 (Table 1, Fig. 1)
 (SHR) E₂

Table 1. Effect of 17 β -estradiol (E₂) replacement on endothelium-dependent relaxation to acetylcholine in Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR)

		Maximal relaxation	pIC ₅₀
WKY (n = 6)	Control	90.2 \pm 3.5	7.75 \pm 0.13
	0.5 mg E ₂	92.5 \pm 3.1	7.64 \pm 0.15
	5 mg E ₂	80.5 \pm 5.9	7.27 \pm 0.16*
SHR (n = 6)	Control	100 \pm 5.1	7.83 \pm 0.12
	0.5 mg E ₂	91.1 \pm 5.1	7.57 \pm 0.08
	5 mg E ₂	91.1 \pm 3.7	7.48 \pm 0.11

The pIC₅₀ denotes the negative log molar concentrations of acetylcholine needed to relax the vessels down to 50% of the precontraction induced by norepinephrine (10⁻⁷ M). Data are expressed as mean \pm SEM. * ; p<0.05 versus control

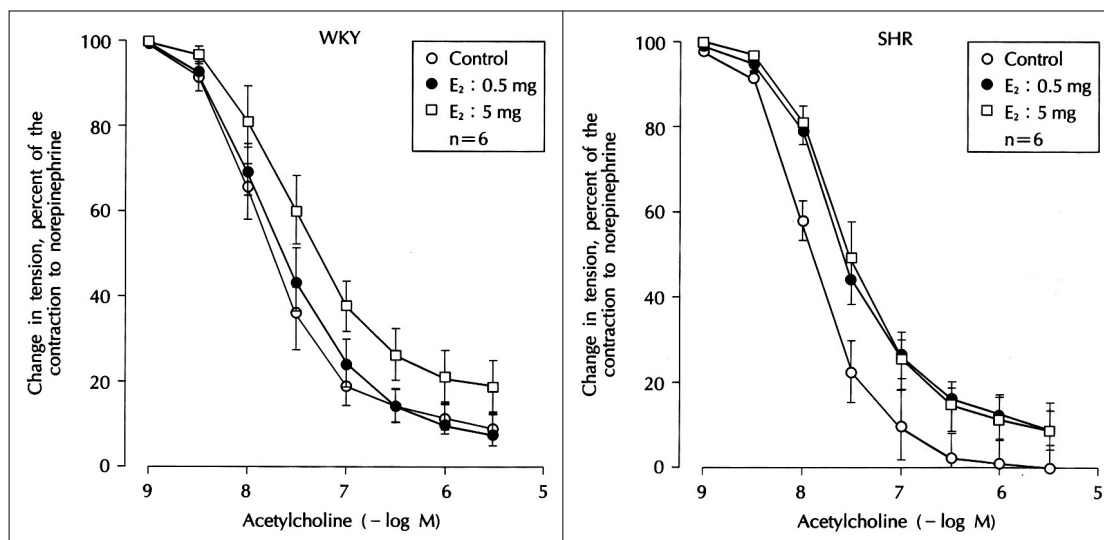


Fig. 1. Effect of 17 β -estradiol replacement on endothelium-dependent relaxation to acetylcholine in Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Data are expressed as mean \pm SEM.

(WKY) 여러 agonist에 의한 혈관수축에 미치는 에스트로겐의 영향

E₂ (pIC₅₀ ; 7.75 ± 0.13,

E₂ 7.27 ± 0.16 ; n = 6 ; p < 0.05), E₂ Norepinephrine (Table 2, Fig. 2)

SHR E₂ norepineph -

rine (AUC ; 265

Table 2. Effect of 17 β -estradiol (E₂) replacement on the contractile responses to various agonists in Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR)

Agonists	Group	W K Y			S H R		
		AUC	Max	pD ₂	AUC	Max	pD ₂
NE	Control	280 ± 24	137 ± 8	7.63 ± 0.11	265 ± 17	152 ± 6	7.45 ± 0.13
	0.5 mg E ₂	387 ± 26*	166 ± 8*	8.17 ± 0.13*	349 ± 16*	181 ± 9*	7.91 ± 0.08*
	5 mg E ₂	374 ± 25*	162 ± 3*	8.13 ± 0.13*	406 ± 19*	203 ± 16*	8.04 ± 0.04*
U46619	Control	389 ± 18	162 ± 6	8.22 ± 0.06	478 ± 30	181 ± 9	8.51 ± 0.08
	0.5 mg E ₂	431 ± 12	181 ± 4*	8.26 ± 0.09	574 ± 23*	230 ± 10*	8.49 ± 0.06
	5 mg E ₂	449 ± 15*	177 ± 8	8.43 ± 0.08	525 ± 28	211 ± 10	8.42 ± 0.05
ET-1	Control	217 ± 19	157 ± 9	8.52 ± 0.09	253 ± 10	165 ± 7	8.73 ± 0.06
	0.5 mg E ₂	231 ± 12	171 ± 8	8.55 ± 0.07	291 ± 17	189 ± 7*	8.76 ± 0.08
	5 mg E ₂	246 ± 12	182 ± 10	8.57 ± 0.09	307 ± 16*	199 ± 8*	8.79 ± 0.10
AT II	Control		39 ± 5			34 ± 4	
	0.5 mg E ₂		25 ± 2*			29 ± 4	
	5mg E ₂		33 ± 6			22 ± 2*	

Abbreviations : AUC ; area under the curve ; Max ; maximal contraction ; NE ; norepinephrine ; ET-1 ; endothelin-1 ; AT II ; angiotensin II. pD₂ denotes the negative log molar concentrations of agonists needed to contract the vessels up to 50% of the contractions induced by 60 mM KCl. Data are expressed as mean ± SEM. * ; p < 0.05 versus control

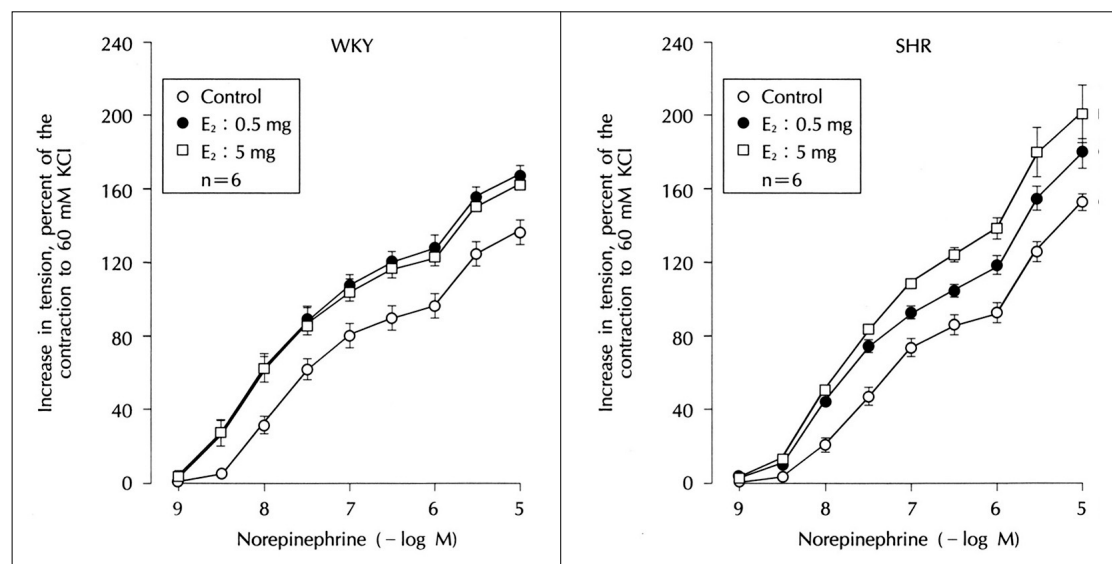


Fig. 2. Effect of 17 β -estradiol replacement on the contraction to norepinephrine in Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Data are expressed as mean ± SEM.

± 17 , E_2 349 ± 16 , E_2 406 ± 19 ; 7.63 ± 0.11 , E_2 8.17 ± 0.13 , E_2
 $152 \pm 6\%$, E_2 $181 \pm 9\%$, 8.13 ± 0.13 ; $n=6$; E_2
 E_2 $203 \pm 16\%$; pD_2 ; 7.45 ± 0.13 , E_2 $p<0.05$), E_2
 7.91 ± 0.08 , E_2 8.04 ± 0.04 ; $n=6$; $p<$
 E_2 E_2 $p<$
 0.05).
 Angiotensin II (Table 2, Fig. 3)
 WKY E_2 SHR E_2
 norepinephrine angiotensin II (
 (AUC; 280 ± 24 , E_2 $387 \pm$ $34 \pm 4\%$, $22 \pm 2\%$; $n=6$; $p<0.05$).
 26 , E_2 374 ± 25 ; $137 \pm 8\%$, E_2
 E_2 $166 \pm 8\%$, E_2 $162 \pm 3\%$; pD_2 ;

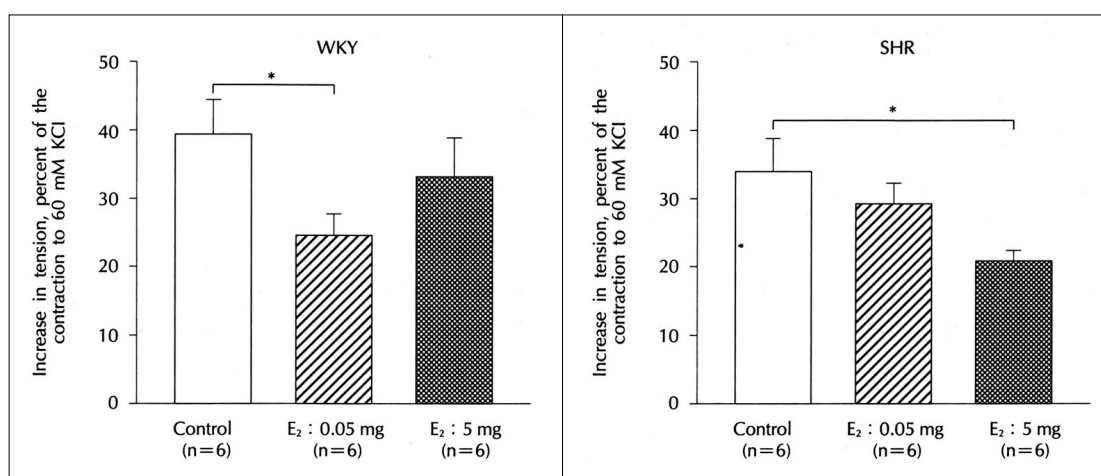


Fig. 3. Effect of 17β -estradiol replacement on the contraction to angiotensin II in Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Data are expressed as mean \pm SEM. *; $p<0.05$

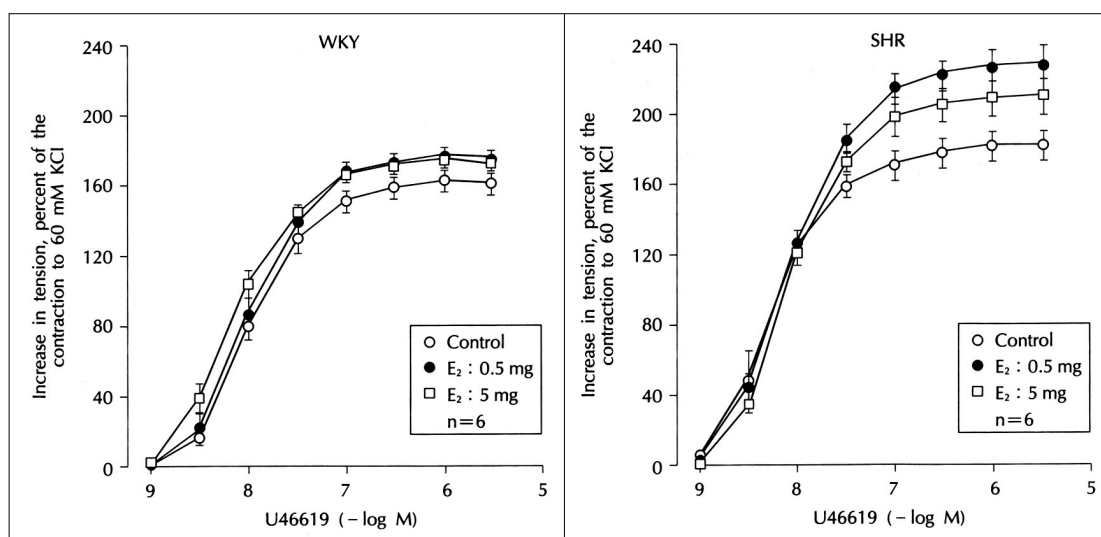


Fig. 4. Effect of 17β -estradiol replacement on the contraction to U46619 in Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Data are expressed as mean \pm SEM.

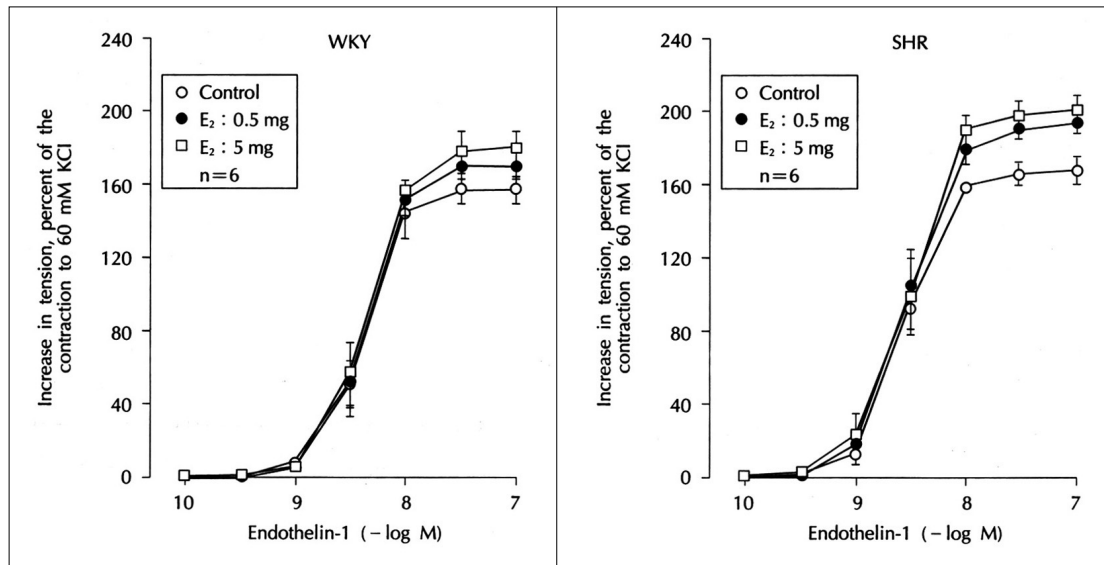


Fig. 5. Effect of 17 β -estradiol replacement on the contraction to endothelin-1 in Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Data are expressed as mean \pm SEM.

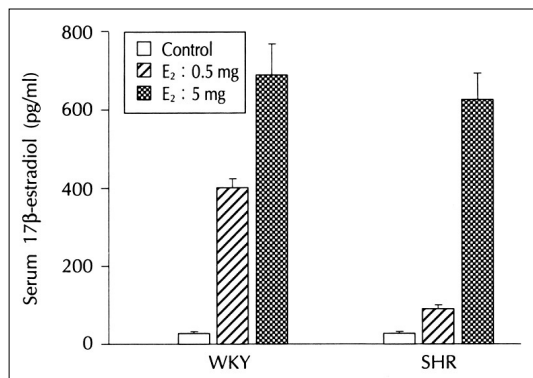


Fig. 6. Serum 17 β -estradiol (E_2) concentrations in Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats according to the doses of E_2 . Data are expressed as mean \pm SEM.

WKY E_2 (39 \pm 5%, 25 \pm 2%; n = 6; p < 0.05).

U46619 (Table 2, Fig. 4)

SHR E_2 (AUC ; 478 \pm 30, E_2 574 \pm 23 ; 181 \pm 9%, E_2 230 \pm 10% ; n = 6 ; p < 0.05).

pD₂ E_2

E_2 가

WKY U46619 (AUC ; 389 \pm 18, E_2 449 \pm 15 ; n = 6 ; p < 0.05). (181 \pm 4%) (161 \pm 6%) 가 (n = 6 ; p < 0.05), AUC pD₂ 가

Endothelin - 1 (Table 2, Fig. 5)

SHR E_2 (AUC ; 253 \pm 10, E_2 307 \pm 16 ; 165 \pm 7%, E_2 199 \pm 8% ; n = 6 ; p < 0.05). pD₂ 가 (189 \pm 7%) (n = 6 ; p < 0.05).

WKY E_2 endoth - elin - 1 가

혈중 에스트로겐 농도 (Fig. 6)

E_2 WKY SHR

E_2 (pg/ml) WKY ; 24 ± 3, E_2 8 -
 403 ± 19, E_2 698 ± 87 ; SHR ; ¹⁵⁾가
 21 ± 4, E_2 76 ± 6, E_2 ¹⁶⁾⁽¹⁷⁾⁽²²⁾
 641 ± 78 ; p<0.05).
 , E_2 가 SHR
 WKY 가
 SHR E_2 acetylcholine
 77 ± 17 pg/ml (n=4), SHR 0.5 mg
 E_2
 WKY 5 mg 17 - estradiol
 혈압의 변화
 (n=5) 157
 ± 3 mmHg, E_2 (n=5) 158 ± 3 mmHg
 . Pellet 가
 2 (4)
 171 ± 2 mmHg, E_2 174 ±
 4 mmHg
 가(p<0.05)가
 가
 고 찰
 nitric oxide
 mRNA
 가 ¹⁻³⁾ 가
 가 ²³⁾ 가
 가 ²⁴⁻²⁶⁾
 가
 LDL
 HDL 가
 가
 가
³⁾ fibrinogen PAI -
 1(plasminogen activator inhibitor - 1) 가
 가 Miller ²⁷⁾
¹⁾⁵⁻⁷⁾ 가
²⁰⁾ arachidonic acid , thromboxane
 A_2
 가
 , nitric oxide synthase

가 nitric oxide 가 . Norepinephrine
 가 SHR norepinephrine
 cyclooxygenase 가 가
 가 SHR acetylcholine norepinephrine 가
 가 28) 가
 가 Norepinephrine
 angiotensin II(10^{-7} M) SHR WKY
 E₂ E₂ 가 , SHR
 SHR E₂ WKY 가
 angio -
 tensin II
 acetyl -
 choline nitric oxide angiotensin II -
 agonist , A23187, ADP, serotonin, bradykinin angiotensin II
 (2×10^{-8} M) 30)가 angio -
 tensin II (10^{-7} M) 가
 가
 Angiotensin II
 가 19)31)
 angiotensin II
 (downregulation) 가
 32) angiotensin II
 , Ca -
 31) estradiol 가
 angiotensin II
 angiotensin II
 가
 norepinephrine
 가 WKY SHR
 SHR
 가
 WKY SHR norepinephrine
 SHR norepinephrine
 U46619
 , SHR U46619
 () 가
 , norepinephrine
 U46619

pD₂ U46619 (abluminal release) endothe-
 (sensitivity) 가 lin - 1
 . WKY . WKY SHR
 endothelin - 1 가
 , 가
 가 .
 SHR WKY 가
 U46619 가 .
 . Thromboxane A₂
 SHR WKY
 , .
 가 . 가 throm - norepinephrine
 boxane A₂ analog U46619 , U46619
 가 endothelin - 1
 가 angiotensin II
 Farhat ¹⁸⁾ 1 .
 U46619
 angiotensin II 가
 . indomethacin ³⁶⁾
 U46619 가 . 가
 cyclooxygenase 가
¹⁸⁾ thromboxane A₂ SHR 가
 가 가 가
 E₂ thromboxane A₂ .
 가 ³³⁾
 thromboxane A₂ 요 약
 가 .
 가 endothelin - 1 서 론 :
 SHR WKY
 . SHR 가
 endothelin - 1
 가 . , U46619 pD₂ , 재료 및 방법 :
 가 . Endothelin - 1 12 spontaneously hypertensive rat(SHR)
 Wistar - Kyoto rat(WKY)
 2 3 17 - estr -
 adiol pellet(E₂ ; 0.5 mg 5 mg)
 2 organ bath
 . norepi -
 endothelin - 1 nephrine(10⁻⁷ M) acetyl -
 가 . endot - choline(10⁻⁹ - 3 × 10⁻⁶ M)
 helin - 1 , norepinephrine(10⁻⁹ - 10⁻⁵

M), angiotensin II(10^{-7} M), U46619(10^{-9} - 3×10^{-6} M) endothelin - 1(10^{-10} - 10^{-7} M) 가 .

결 과 :

SHR WKY E₂ E₂
(pIC₅₀ ; vs 5 mg E₂ ; 7.75 ± 0.13 vs 7.27 ± 0.16 ; n=6 ; p<0.05). Angiotensin II WKY SHR E₂
(60 mM KCl % ; SHR ; vs 5 mg E₂ ; 34 ± 4 vs 22 ± 2 ; n=6 ; p<0.05 ; WKY ; vs 0.5 mg E₂ ; 39 ± 5 vs 25 ± 2 ; n=6 ; p<0.05). norepinephrine E₂ (SHR ; vs 0.5 mg E₂ vs 5 mg E₂ ; ; 265 ± 17 vs 349 ± 16 vs 406 ± 19 ; (60 mM KCl %) ; 152 ± 6 vs 181 ± 9 vs 203 ± 16 ; pD₂ ; 7.45 ± 0.13 vs 7.91 ± 0.08 vs 8.04 ± 0.04 ; n=6 ; p<0.05). U46619 E₂ (SHR ; vs 0.5 mg E₂ ; ; 478 ± 30 vs 574 ± 23 ; ; 181 ± 9 vs 230 ± 10 ; n=6 ; p<0.05). Endothelin - 1 SHR E₂ (vs 0.5 mg E₂ vs 5 mg E₂ ; 165 ± 7 vs 189 ± 7 vs 199 ± 8 ; n=6 ; p<0.05). E₂ (174 ± 4 mmHg) (171 ± 2 mmHg) 가 .

결 론 :

E₂

중심 단어 :

감사문

1997

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