ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5): 440 - 444
http://www.chinaphar.com

17β-Estradiol inhibits carotid sinus baroreceptor activity in anesthetized male rats

WANG Sheng, FAN Zhen-Zhong, HE Rui-Rong[1]
(*Department of Physiology, Institute of Basic Medicine, Hebei Medical University, Shijiazhuang 050017, China*)

KEY WORDS 17β-estradiol; baroreflex; carotid sinus; efferent pathways; tamoxifen; nitric oxide

ABSTRACT

AIM: To study the effect of 17β-estradiol (E_2) on carotid baroreceptor activity (CBA). **METHODS**: The functional curve of carotid baroreceptor (FCCB) was constructed and the functional parameters of carotid baroreceptor were measured by recording sinus nerve afferent discharge in anesthetized male rats with perfused isolated carotid sinus. **RESULTS**: E_2 3, 10, and 30 μmol/L shifted FCCB to the right and downward, with a marked decrease in peak slope (PS) and peak integral value of carotid sinus nerve discharge (PIV) in a concentration-dependent manner, indicating the inhibitory effect of E_2 on CBA. Pretreatment with tamoxifen (TAM) 10 μmol/L, an inhibitor of estrogen receptor, did not block the effect of E_2 on CBA. Preperfusion with *L*-NAME 100 μmol/L, an inhibitor of NO synthase, could completely abolish the effect of E_2 on CBA. NO donor SIN-1 10 μmol/L could potentiate the inhibitory effect of E_2. **CONCLUSION**: E_2 inhibits CBA via endothelial NO release.

INTRODUCTION

Estrogen exerts a protective effect on the cardiovascular system[1]. It exhibits antiatherosclerotic[2], antiarrhythmic[3], and vasodilatory effects[4]. It is well known that arterial baroreflex plays a critical role in short-term regulation of arterial blood pressure, while the studies concerning the effect of estrogen on carotid barareceptor activity (CBA) are somewhat controversial. Saleh and Connell have found that acute intravenous injection of 17β-estradiol (E_2) in male rats induced an increase in baroreflex sensitivity[5]. He *et al* showed that chronic treatment with E_2 in ovariectomized rats also increased baroreflex sensitivity[6], while their subsequent experiment demonstrated that chronic administration of E_2 had no effect on the functional curve of baroreflex[7]. Recently, our lab has shown that E_2 inhibited carotid baroreflex[8]. Whether E_2 affects CBA remains to be clarified as yet. The aim of the present study was, therefore, to observe the effects of E_2 on CBA in anesthetized male rats with perfused isolated carotid sinus, and to elucidate the mechanism(s) involved.

MATERIALS AND METHODS

Sprague-Dawley rats (♂, 310 g ± 40 g, Grade Ⅱ, Certificate № 04036), obtained from the Experimental Animal Center of Hebei Province, were anesthetized with ip urethane 1.0 g/kg. The trachea was cannulated for ventilation.

Perfusion of left carotid sinus The method of isolating the carotid sinus has been described in our previous study[9]. Briefly, the left carotid sinus area was fully exposed after turning rostrally the trachea and esophagus. Sternohyoideus muscles and superior laryngeal nerves were sectioned. The bilateral aortic nerves, right carotid sinus nerves, cervical sympathetic nerves, and recurrent laryngeal nerves were all sectioned. The common, external, and internal carotid arteries and smaller arteries originating from these vessels were exposed and ligated, while carefully leaving the left carotid sinus nerve undisturbed. Ligation of the occipital artery at its origin from the external carotid artery excluded chemoreceptors from the isolated carotid sinus, thereby preventing chemoreceptor activation secondary to decreased carotid sinus pressure. Plastic catheter introduced into the left common carotid artery in the anterograde way (served as inlet tube) was attached to a peristaltic pump (1210, Harvard) which controlled the intrasinus pressure (ISP). The intrasinus pressure was monitored by a pressure trans-

[1] Correspondence to Prof HE Rui-Rong. Phn 86-311-604-4121, ext 5566. Fax 86-311-604-8177. E-mail syho@hebmu.edu.cn
Received 2000-09-28 Accepted 2001-02-21

ducer (MPU-0.5A, Nihon Kohden, Japan) connected with inlet tube. A plastic catheter inserted into the external carotid artery served as an outlet tube. The carotid sinus was then perfused wiuh warm oxygenated modified Krebs-Henseleit (K-H) solution (mmol/L: NaCl 118.0, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 5.6, pH 7.35 – 7.45).

Recording of sinus nerve afferent discharge The carotid sinus nerve was cut near the glossopharyngeal nerve and desheathed. The isolated sinus nerve and surrounding structures were immersed in warm (37 ℃) liquid paraffin to avoid drying of the tissues. The sinus nerve was placed on a bipolar platinum electrode connected to a bioelectrical amplifier (AB-621G, Nihon Kohden, Japan). Bioelectrical signal was fed to a bioelectrical integrator (EI-610G, Nihon Kohden, Japan), with an integral time of 5 s. ISP and integral of sinus nerve activity (ISNA) were recorded synchronously by 4-channel physiologic recorder (RM-6200, Nihon Kohden, Japan) and monitored by oscilloscope (VC-22, Nihon Kohden, Japan).

Protocols According to the computer controlled program, ISP was altered in a stepwise manner by perfusing left carotid sinus with K-H solution. After ISP was lowered from 13.3 kPa to 0 kPa, it began to increase slowly to 32.0 kPa in a staircase manner, and then decreased to 0 kPa in the same manner, and again stabilized at 13.3 kPa. Each step of the staircase changed the ISP by 4.0 kPa and lasted for 15 s. The functional curve for ISP-ISNA relation was constructed, and the functional parameters of carotid baroreceptor such as peak slope (PS), peak integral value (PIV), threshold pressure (TP), saturation pressure (SP), and operation range (OR) were determined. TP was the ISP at which ISNA began to increase by 15 % in response to increase in ISP. SP was the ISP at which ISNA just showed no further increase with an increase in ISP. OR was calculated as the difference between SP and TP.

On perfusing carotid sinus with K-H solution, the functional curve of carotid baroreceptor (FCCB) was drawn, obtaining the control parameters of TP, SP, OR, PS, and PIV. ISP was then fixed at 13.3 kPa for 20 min, and K-H solution containing E_2 3, 10, 30 μmol/L was then perfused to examine the changes in ISNA, followed by measurement of the parameters again. Finally the carotid sinus was perfused with K-H solution as a postcontrol.

The effect of tamoxifen (TAM) on the response to E_2 was examined. After the control parameters of CBA were obtained, the isolated carotid sinus was perfused with K-H solution containing TAM 10 μmol/L for 30 min, and the above parameters were measured. Then E_2 10 μmol/L was added to perfuse the sinus area. The parameters were measured within 30 min, and then the drugs were washed out with K-H solution. To determine whether the effect of E_2 10 μmol/L was caused by endothelium-dependent nitric oxide release, one group in the experiment was pretreated with *L*-NAME 100 μmol/L for 20 min before E_2 was added. To further determine the involvement of NO in the effect of E_2 on CBA, pretreatment with a NO donor SIN-1 (3-morpholino-sydnonimine) 10 μmol/L was done.

Drugs 17β-Estradiol (purity 99 %, Sigma) and tamoxifen (purity 99 %, Sigma) were dissolved in diluted ethanol (1∶2000). *L*-NAME (Sigma) and SIN-1 (Cassella, Germany) were dissolved in saline. The same amount of diluted ethanol was also added to normal K-H solution as control. No changes in ISNA were observed during perfusion with ethanol (1∶2000).

Statistics All data are presented as $\bar{x} \pm s$. The significance of group differences was determined by one way ANOVA and q test.

RESULTS

Effect of E_2 on carotid baroreceptor activity By perfusing carotid sinus with K-H solution and elevating ISP from 0 to 32.0 kPa in a stepwise manner, ISNA was increased. There was no difference in CBA parameters among controls. As compared with control groups, E_2 concentration-dependently decreased PIV and PS, and increased TP and SP, shifting FCCB to the right and downward (Tab 1, Fig 1). The above effects occurred within 10 min after perfusing carotid sinus with E_2, and reached the peak during 20 – 40 min. Fig 2 is an original tracing showing the effects of E_2 on ISNA.

Effect of TAM on E_2 responses TAM 10 μmol/L did not induce any change in functional parameters of carotid baroreceptor, and also did not block the effect of E_2 10 μmol/L (Tab 2).

Effect of *L*-NAME on E_2 responses *L*-NAME 100 μmol/L *per se* did not produce any change in functional parameters of carotid baroreceptor, but markedly blocked the most effects of E_2 except OR (Tab 2).

Effect of SIN-1 on E_2 responses SIN-1 10 μmol/L did not change CBA, while it potentiated the effects of E_2 on CBA (Tab 2).

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

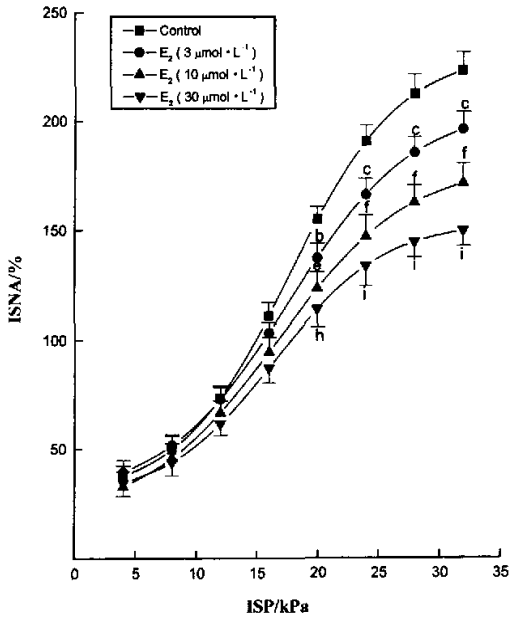

Fig 1. Effect of different concentrations of E_2 on the functional curves of carotid baroreceptor in rats. $n = 6$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^eP < 0.05$, $^fP < 0.01$ *vs* E_2 3 μmol·L^{-1}. $^hP < 0.05$, $^iP < 0.01$ *vs* E_2 10 μmol/L. ISP: intrasinus pressure; ISNA: integral of sinus nerve activity.

DISCUSSION

The main finding of the present study is that E_2 inhibited CBA in a concentration-dependent manner. In our experiment, E_2 shifted FCCB to the right and downward, with reduction of PIV and PS, indicating the inhibitory action of E_2 on CBA. This was in accordance with the inhibitory effect of E_2 on carotid baroreflex observed in our previous study[8]. Saleh and Connell have found that acute administratio of E_2 enhances baroreceptor sensitivity[5]. While He *et al* drew a contradictory conclusion in their successive studies[6,7]. According to their experiments, intravenous injection of phenylephrine and sodium nitroprusside was used to induce the changes in blood pressure, resulting in overloading and unloading of the baroreceptor respectively. Under such conditions, the two drugs had direct effects on cardiovascular and central nervous systems, thereby interfering with the effects of E_2. In addition, on systemically injecting E_2 it was not possible to define the precise location where E_2 acted. In the present study, the technique of perfusing the isolated carotid sinus was used and CBA was altered by increasing or decreasing ISP. Under such conditions, administration of the active agent was restricted to the local sinus area and the direct actions of E_2 on cardiovascular or central nervous system were avoided.

The α and β forms of estrogen nuclear receptor have been cloned from human and rats[10,11], which have been detected in a wide range of tissues including the cardiovascular system[12]. It is generally accepted that estrogen has genomic and non-genomic actions on target tissues; the former are mediated through activation of estrogen nuclear receptor, and the latter suggested to be associated with membrane receptor. In our experiment, the inhibitory effects of E_2 on CBA appeared within 20 – 40 min after acutely perfusing sinus area with different concentrations of E_2. In addition, pretreatment with TAM, an inhibitor of estrogen nuclear receptor, could not block the effects of E_2. Accordingly, the inhibitory effects of E_2 on CBA may be attributed to its non-genomic action mediated through activation of membrane receptor, but not to genomic action.

Evidence has been presented that endothelium-dependent NO release induced by E_2 could in part be responsible for its non-genomic action[13]. In our study, administration of *L*-NAME, an inhibitor of NO synthase, completely abolished the inhibitory effects of E_2. Furthermore, SIN-1, a donor of NO, could potentiate the action of E_2 on CBA. Based on these results, it is conceivable that the effects of E_2 on CBA could be ascribed to NO release from sinus area. Li *et al* have shown that NO suppressed action potential discharge of baroreceptor via regulation of the sodium currents[14]. Bolotina *et al* have also reported that the activation of calcium-dependent K^+ channels localized in vascular smooth muscle by NO may hyperpolarize baroreceptor neurons, thereby decreasing their activity[15]. Both these mechanisms may account for our results.

In summary, E_2 inhibits CBA, an effect of which may be mediated by NO release from endothelial cells of the sinus area.

REFERENCES

1 Farhat MY, Lavigne MC, Ramwell PW. The vascular protective effects of estrogen. FASEB J 1996; 10: 615 – 24.
2 Martin C, Barturen K, Martinez R, Lacort M, Ruiz-Larrea MB. *In vitro* inhibition by estrogens of the oxidative modifications of human lipoproteins. J Physiol Biochem 1998; 54: 195 – 202.
3 Nakajima T, Iwasawa K, Oonuma H, Morita T, Goto A,

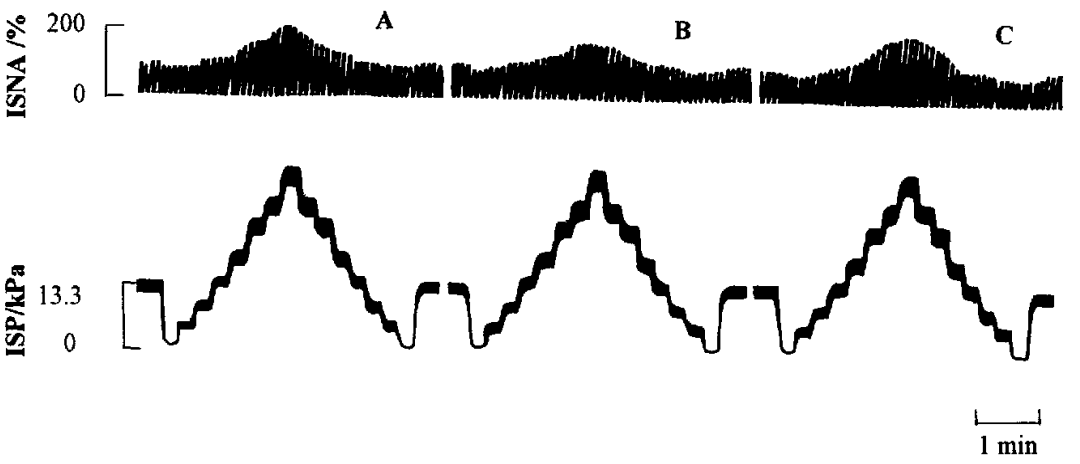

Fig 2. Original receording showing the responses of ISNA to intrasinus perfusion with E_2. A: Control; B: Perfusion with E_2 10 μmol/L; C: Washing out.

Tab 1. Effects of E_2 on functional parameters of carotid baroreceptor in male reats. $n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^dP > 0.05$, $^fP < 0.01$ *vs* E_2 3 μmol·L^{-1}. $^gP > 0.05$, $^hP < 0.05$, $^iP < 0.01$ *vs* E_2 10 μmol/L.

Drugs	TP/kPa	SP/kPa	OR/kPa	PS/%·kPa^{-1}	PIV/%
Control	8.01±0.09	26.0±0.4	18.4±0.4	19.9±0.3	217±9
E_2 3 μmol/L	8.5±0.5^b	27.1±0.9^b	18.6±1.0^a	18.0±0.4^c	189±7^c
E_2 10 μmol/L	9.36±0.29^{cf}	27.9±0.9^{cd}	19.3±0.8^{ad}	16.2±0.4^{cf}	172±10^{cf}
E_2 30 μmol/L	10.60±0.07^{ci}	29.2±0.6^{ch}	19.4±0.9^{ag}	13.6±0.5^{ci}	148±8^{ci}

TP: threshold pressure; SP: saturation pressure; OR: operation rage; PS: peak slope; PIV: peak intergral value of sinus nerve activity.

Tab 2. Effects of TAM 10 μmol/L, *L*-NAME 100 μmol/L, and SIN-1 10 μmol/L on the responses of carotid baroreceptor to E_2 10 μmol/L. $n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* E_2 10 μmol·L^{-1}.

Drugs	TP/kPa	SP/kPa	OR/kPa	PS/%·kPa^{-1}	PIV/%
Control	8.0±0.3	26.3±0.8	8.6±0.4	19.82±0.13	220±6
E_2	8.9±0.8^a	27.4±1.1^a	18.6±0.5^a	16.0±0.3^c	175±9^c
TAM + E_2	9.0±0.7^{ad}	27.5±1.0^{ad}	18.9±0.7^{ad}	16.1±0.3^{cd}	180±8^{cd}
Control	8.42±0.24	26.1±0.5	18.5±0.7	19.88±0.24	218±9
E_2	9.18±0.16^c	28.1±0.3^c	18.7±0.3^a	16.2±0.6^c	174±9^c
L-NAME + E_2	8.24±0.22^{af}	26.3±0.4^{af}	18.2±0.8^{ad}	19.7±0.6^{af}	222±7^{af}
Control	8.1±0.4	25.8±0.5	18.9±0.4	20.1±0.4	214±8
E_2	9.1±0.4^b	27.9±0.4^c	19.1±0.6^a	16.2±0.3^c	171±9^c
SIN-1 + E_2	9.8±0.3^{ce}	28.9±0.7^{ce}	19.2±0.8^{ad}	15.3±0.5^{ce}	158±8^{ce}

TP: threshold pressure; SP: saturation pressure; OR: operation rage; PS: peak slope; PIV: peak intergral value of sinus nerve activity.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Wang Y, *et al*. Antiarrhythmic effect and its underlying ionic mechanism of 17β-estradiol in cardiac myocytes. Br J Pharmacol 1999; 127: 429 – 40.
4 Hugel S, Neubauer S, Lie SZ, Ernst R, Horn M, Schmidt HH, *et al*. Multiple mechanisms are involved in the acute vasodilatory effect of 17β-estradiol in the isolated perfused rat heart. J Cardiovasc Pharmacol 1999; 33: 852 – 8.
5 Saleh TM, Connell BJ. Centrally mediated effect of 17β-estradiol on parasympathetic tone in male rats. Am J Physiol 1999; 276: R474 – R481.
6 He XR, Wang WH, Crofton JT, Share AL. Effects of 17β-estradiol on sympathetic activity and pressor response to phenylephrine in ovariectomized rats. Am J Physiol 1998; 275: R1202 – R1208.
7 He XR, Wang WH, Crofton JT, Share AL. Effects of 17β-estradiol on the baroreflex control of sympathetic activity in conscious ovariectomized rats. Am J Physiol 1999; 277: R493 – R498.
8 Wang S, Fan ZZ, He RR. 17β-Estradiol inhibites carotid sinus baroreflex in male rats. *Acta Physiol Sin* 2000; 52: 445 – 9.
9 Zhao G, He RR. The facilitating effect of atrial natriuretic peptide on the carotid sinus baroreflex function. Chin J Physiol Sci 1993; 9: 68 – 75.
10 Korke S, Sakai M, Muramatsu M. Molecular cloning and characterization of rat estrogen receptor. Nucl Acids Res 1987; 15: 2499 – 513.
11 Kuiper GG, Enmark E, Peltor-Huikko M, Nilsson S, Gustafsson JA. *Cloning of a novel receptor expressed in rat prostate* and ovary. Proc Natl Acad Sci USA 1997; 138: 863 – 70.
12 Venkov CD, Rankin AB, Vaughan DE. Identification of authentic estrogen receptor in cultured endothelial cells. A potential mechanism for steroid hormone regulation of endothelial function. Circulation 1996; 94: 727 – 33.
13 Kim HP, Lee JY, Jeong JK, Bae SW, Lee HK, Jo I. Nongenomic stimulation of nitric oxide release by estrogen is mediated by estrogen receptor α localized in caveolae. *Biochem Biophys Res Commun* 1999; 263: 257 – 62.
14 Li Z, Chapleau MW, Bates JN, Bielefeldt K, Lee HC, Abboud FM. Nitric oxide as an autocrine regulator of sodium currents in baroreflex neurons. *Neuron* 1998; 20: 1039 – 49.
15 Bolotina VM, Najibi S, Palacine PJ, Pagano JL, Cohen RA. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. Nature 1994; 368: 850 – 3.

17β-雌二醇抑制麻醉雄性大鼠颈动脉窦压力感受器的活动

王 升，范振中，何瑞荣[1]（河北医科大学基础医学研究所生理室，石家庄 050017，中国）

关键词 17β-雌二醇；压力反射；颈动脉窦；传出通路；他莫昔芬；一氧化氮

目的：观察 17β-雌二醇对颈动脉窦压力感受器活动的影响. **方法**：在麻醉雄性大鼠隔离灌流颈动脉窦条件下记录窦神经传入放电，观察 17β-雌二醇(E_2)对压力感受器机能曲线和机能参数的影响. **结果**：E_2 3，10 和 30 μmol/L 使压力感受器机能曲线向右下方移位，曲线最大斜率和窦神经传入放电积分最大值均明显减小，表明 E_2 可抑制颈动脉窦压力感受器活动. 雌激素受体抑制剂他莫昔芬 10 μmol/L 不能阻断 E_2 的效应. 一氧化氮合酶阻断剂 *L*-NAME 100 μmol/L 完全阻断 E_2 对压力感受器活动的抑制效应. 一氧化氮供体 SIN-1 10 μmol/L 能加强 E_2 的效应. **结论**：E_2 抑制雄性大鼠颈动脉窦压力感受器的活动，此效应系其使内皮细胞释放一氧化氮所致.

（责任编辑 朱倩蓉）