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Oxidative Stress Attenuates Phenylephrine-Induced Contractile Responses in Rat Aorta

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Introduction

Free radical-induced oxidative stress has been involved in the pathogenesis of a number of human diseases such as diabetes mellitus[1], hypertension[2] and atherosclerosis[3]. In these diseases, it has been generally accepted that the vascular system is the first target of free radicals[4]. Recent findings indicating that free radicals interfere with endothelium-dependent function in experimental models[5, 6] has turned to our attention to the effect of free radicals on the contractile responses of vasculature. Although several investigators have shown that free radicals interfere with the normal contractile function in vascular smooth muscle, the results of these studies are quite contradictory. In this context, Mizukawa and Okabe[7] reported that singlet oxygen depresses noradrenaline-induced contractions in rabbit mesenteric artery. In contrast, Gumusel et al.[5] reported that in isolated rat aortic rings, the contractile response to phenylephrine was potentiated when the bathing solution was subjected to electrolysis. Thus, in spite of the accumulating body of evidence indicating free radicals impair the endothelium-dependent relaxation[8], their effects on the contractile responses on the vasculature still need to be evaluated.

Taking the above debate into consideration, the aim of this study was to investigate the effects of oxidative stress on PE- induced contractions. Conditions of oxidative stress were established in isolated rings of rat thoracic aorta by inactivating endogenous Cu/Zn superoxide dismutase (Cu/Zn SOD) with DETCA and with the superoxide anion generating system (XO/HX) (9, 10).

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Materials and Methods

The animal experiments were carried out in accordance with guidelines described by the Ethics Committee of the Faculty of Pharmacy, Ege University.

Preparations of the arterial rings

Thoracic aortas were obtained from rats of either sex (200-300 g) that were exanguinated. After careful removal of loose connective tissue, 4 adjacent pairs of rings 2-3 mm long from thoracic aorta were cut. Care was taken not to cause damage to the endothelium. Then the rings were suspended in organ chambers filled with physiological salt solution (Krebs) at 37 ÐC, continuously oxygenated with 95% O2 - 5% CO2. The composition of Krebs solution was (in mM): NaCl, 118; KCl, 4.7; CaCl2.2H20, 2.5; KH2PO4, 1.20; MgSO4.7H20, 1.17; Glucose, 11.1; NaHCO3, 25. A resting tension of 2 g was applied to the rings which were then allowed to equilibrate for 45 minutes before experimental procedures were initiated. In this period tissues were washed out with Krebs solution for every 15 minutes. Isometrical changes in tension were displayed on IOS Lab software (version 3.23 MS8) via Grass FT03 transducer.

Experimental protocol

The rings were contracted with potassium chloride (KCl; 60 mM). Maximal contractions for each ring were obtained by reaching the stable plateau value. Two of the rings were then treated with NÐ-nitro-L-arginin (LNA; 10-4 M). Agonist stimulated activity of NO was determined by assessing acetylcholine (ACh)- induced relaxation and cumulative concentration-dose response curves to ACh (10-9-10-4 M) were constructed on all rings following pre-contraction with phenylephrine (PE; 3x10-6 M). After each concentration-response curve, the organ baths were repeatedly washed out and the tissues were allowed to re-equilibrate for 30 min before further experimentation.

In order to investigate the effects of irreversible endogenous superoxide dismutase (SOD) inhibition on PE- induced contractions, cumulative concentration-response curves were constructed after a 30 min pretreatment with DETCA (3 mM; 10,11) in presence and absence of catalase. The effects of superoxide anion generation by XO (16 mu ml-1)/ HX (1 mM)[9] on PE contractions were also examined in other experiments. XO

was added to aortic rings for 15 minutes to allow it permeate to tissue. HX was then added and experiments were performed in the presence of catalase (1000 u/ml) to safeguard against accumulation of hydrogen peroxide (H2O2).

As a consequence of experimental protocol, four groups of rings were established to investigate the effects of DETCA and XO/HX on PE contractions. Experimental groups were as follows: Group A (control), Group B (only LNA treatment), Group C (only oxidative stress), and Group D (LNA treatment + oxidative stress).

Materials

Acetylcholine hydrochloride, catalase (bovine liver), diethyldithiocarbamic acid (DETCA), hypoxanthine, NĐ-nitro-L-arginin (LNA), phenylephrine hydrochloride and xanthine oxidase (butter-milk) were obtained from Sigma. Potassium chloride (KCl) was obtained from Merck. All the drugs were dissolved in saline (0.9 NaCl %) except for hypoxanthine which was dissolved in 0.1 % sodium hydroxide.

Analysis of data

All data are expressed as mean ± SE; n indicates the number of animals. The negative logarithm of the concentration (pD2) that produced half of the maximal effect (Emax) of that agonist was calculated using linear regression analysis (Polywin95, 1.0, Commat, Ankara, Turkey). Means of pD2 and Emax values were compared. Acetylcholine-induced relaxations were normalized to the initial phenylephrine contraction. Contractile responses to phenylephrine are normalized to the maximum tension induced by KCl for each ring. Statistical comparisons were made by Student's t test. A value of $p < 0.05$ was considered significant.

Results

Endothelium-dependent relaxations

Acetylcholine (ACh) induced concentration-dependent relaxations in all groups of rings precontracted with phenylephrine (3 x10-6 M) in the absence of LNA. Treatment with LNA completely inhibited ACh- induced relaxations (Figure 1a and b).

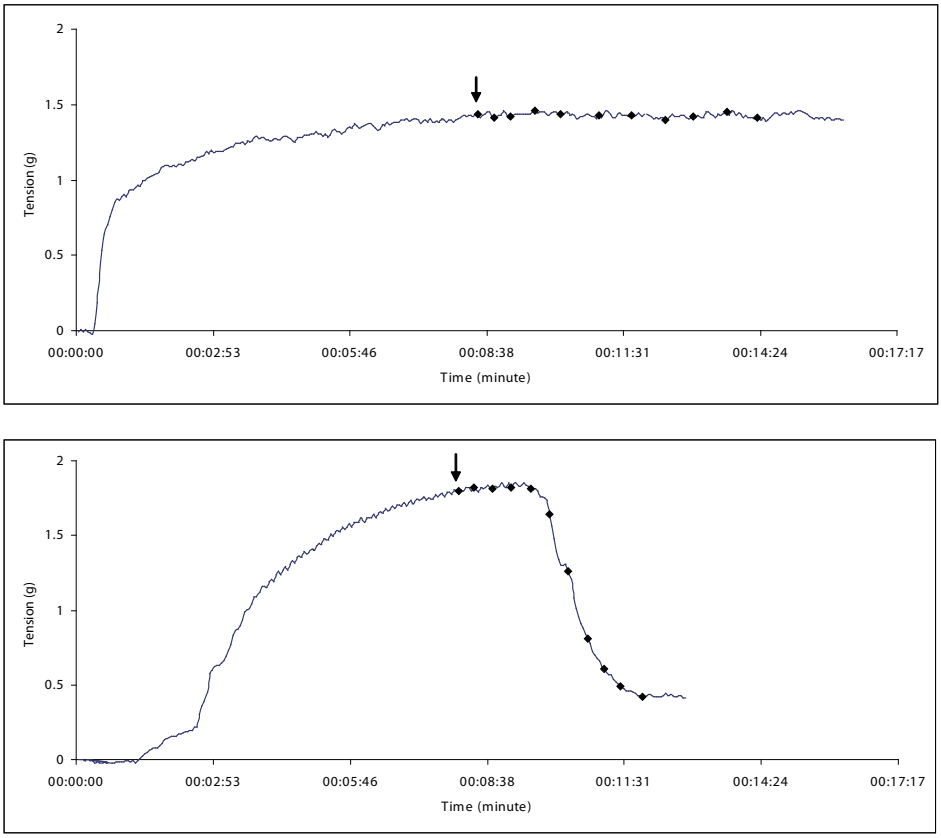

Figure 1

Typical traces of ACh- induced concentration-response curves in PE- precontracted (3 x 10-6 M) rings in the presence (a) and absence of LNA (a).

Contractions

Effects of DETCA

Phenylephrine induced concentration-dependent contractions in rat aortic rings in both presence and absence of an intact endothelium. Incubation with DETCA significantly attenuated contractile responses (Emax) to PE and shifted concentration-response curve to the right (Figure 2, Table I). Inhibition of nitric oxide synthase by LNA did not affect the inhibitory action of DETCA on PE contractions and the sensitivity (Figure 2, Table I).

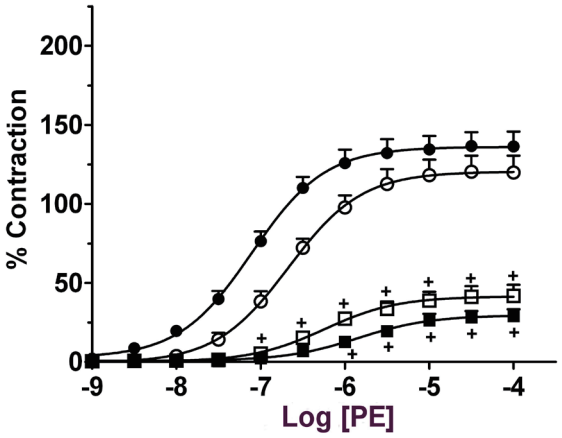

Figure 2

Effects of DETCA pretreatment on PE- induced contractions. Concentration-response curves obtained from Group A (control) (Ð); Group B (LNA) (Ð); Group C (DETCA) (Ð) and Group D (LNA + DETCA) (Ð) are shown. Data are expressed as mean ± SE (+$p < 0.01$, Group A vs. Group C and Group B vs. Group D, Student's t for paired data, n = 5).

Effects of DETCA + catalase

DETCA, in the presence of catalase, inhibited PE-induced contractions and reduced the sensitivity. Neither the Emax nor the pD2 values of PE were affected by LNA in rings under oxidative stress (Figure 3, Table 1).

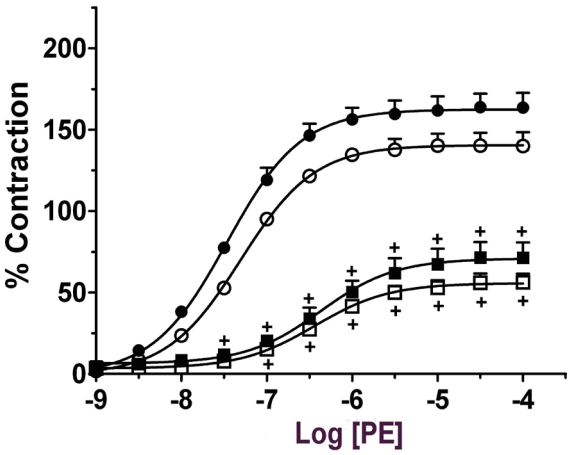

Figure 3

Effects of DETCA + catalase pretreatment on PE- induced contractions. Concentration-response curves obtained from Group A (control) (Ð); Group B (LNA) (Ð); Group C (DETCA + catalase) (Ð) and Group D (LNA + DETCA + catalase) (Ð) are shown. Data are expressed as mean ± SE (+$p < 0.01$, Group A vs. Group C and Group B vs. Group D, Student's t for paired data, n = 5).

Effects of XO/HX + catalase

Superoxide anion generation by XO/HX decreased the Emax values without affecting the pD2 values. Treatment with LNA reversed the diminished contractions to PE in oxidatively damaged rings but it did not affect the pD2 values in these rings (Figure 4, Table I).

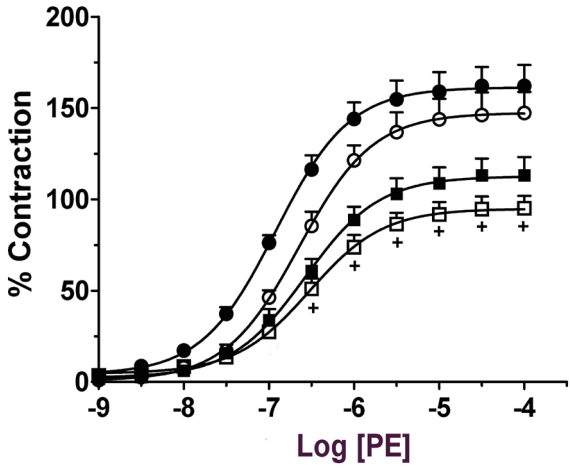

Figure 4

Effects of XO/HX + catalase pretreatment on PE- induced contractions. Concentration-response curves obtained from Group A (control) (Ð); Group B (LNA) (Ð); Group C (XO/HX + catalase) (Ð) and Group D (LNA + XO/HX + catalase) (Ð) are shown. Data are expressed as mean ± SE (+p < 0.01, Group A vs. Group C and Group B vs. Group D, Student's t for paired data, n = 5).

Discussion

The results of the present study demonstrated that oxidative stress, either generated by inhibition of SOD or produced by accumulation of excessive amounts of superoxide anions, attenuated the phenylephrine-induced contractile responses in rat aorta. Impaired endothelium-dependent vasodilator relaxations in response to reactive oxygen species are well documented[8,12]. However, several investigators have suggested that free radicals interfere with normal contractile function in vascular smooth muscle[7]. In contrast with our findings, Wolin & Belloni[13] reported the ineffectiveness of xanthine oxidase-derived oxygen metabolites in phenylephrine-induced contractions pointing out the possibility that the smooth muscles or Ð1-mediated responses are not the site of action of the reactive oxygen metabolites. In the present study, the finding that free

TABLE I

Effects of oxidative stress induced by DETCA and XO/HX on pD2 and Emax values of phenylephrine. Data are expressed as mean ± SE. n represents the number of the animals in each group (+p < 0.01, Group A vs. Group C and Group B vs. Group D, Student's t for paired data).

DETCA TREATMENT				
	Group A (control) (n = 5)	Group B (LNA) (n= 5)	Group C (DETCA) (n = 5)	Group D (LNA + DETCA) (n= 5)
% E_{max} (ACh)	77,4 ± 3,61	-	78,6 ± 4,21	-
% E_{max} (PE)	119,9 ± 10,69	136,4 ± 9,34	41,9 ± 7,0 +	29,2 ± 4,09 +
pD_2 (PE)	6,66 ± 0,02	7,11 ± 0,07	6,27 ± 0,11+	5,84 ± 0,13 +
DETCA + catalase TREATMENT				
	Group A (control) (n = 5)	Group B (LNA) (n = 5)	Group C (DETCA + catalase) (n =5)	Group D (LNA + DETCA + catalase) (n = 5)
% E_{max} (ACh)	69,7 ± 4,76	-	73,5 ± 3,97	-
% E_{max} (PE)	140,1 ± 8,48	163,1 ± 9,06	56,3 ± 5,32+	71,3 ± 9,45+
pD_2 (PE)	7,31 ± 0,08	7,47 ± 0,04	6,45 ± 0,06+	6,32 ± 0,07+
XO/HX + catalase TREATMENT				
	Group A (n = 5)	Group B (LNA) (n = 5)	Group C (XO/ HX + catalase) (n = 5)	Group D (LNA + XO/HX + catalase) (n = 5)
% E_{max} (ACh)	82,3 ± 4,35	-	79,2 ± 5,27	-
% E_{max} (PE)	147,3 ± 11,56	162,1 ± 11,58	95,1 ± 6,81+	113,04 ± 10,11
pD_2 (PE)	6,65 ± 0,05	6,92 ± 0,03	6,51 ± 0,08	6,59 ± 017

radicals attenuate the contractile force development to phenylephrine-induced conractions in rat aortic rings is also inconsistent with the results of Gao & Lee (14). These investigators have shown that hydrogen peroxide, probably a more harmful reactive oxygen species than superoxide anion because having the ability of diffusing across plasma membrane, induces a greater contraction in mesenteric arteries of spontaneously hypertensive rats. Similarly, the finding of an enhanced responsiveness to

free radicals in isolated vessels came from the study of Gumusel et al.[5]. However, in 1997, Mizukawa & Okabe[7] have reported that exogenous singlet molecular oxygen depresses noradrenaline- induced contractions in rabbit mesenteric artery suggesting that oxygen-derived free radicals may be linked to the loss of contractile function of the vessels.

In the present study, regardless of the mechanisms (differences and/or similarities) inducing the in vitro oxidative stress, the attenuated contractile responses to phenylephrine suggests the participation of free radicals in physiological (and/or pathological) regulation of vascular function in rat aortic rings[14]. In this study, the finding that incubation with DETCA significantly decreased contractile responses (Emax) and pD2 values of phenylephrine has given rises the question whether DETCA blocks the Ð1-adrenergic receptor-mediated responses in the vessels. However, the attenuated responses of serotonin under the same conditions, has been ruled out this possibility[15]. In DETCA- induced oxidative stress conditions, the persistent attenuation of phenylephrine- induced contraction in rat aorta in the presence of NO inhibition by LNA strongly suggests that nitric oxide does not interfere with the diminished contractile force development to phenylephrine in rat aortic rings. However, the finding that inhibition of nitric oxide by LNA reversed the decreased Emax values of phenylephrine caused by superoxide anions generated by XO/HX suggests a role of nitric oxide, either alone or in combination with superoxide anion, in the impaired vascular contractile function. In conclusion, in the present study, the data presented here, show that free oxygen radicals depress phenylephrine- induced contractile responses in rat aorta. However the mechanism of this effect needs to be evaluated.

Summary

The effects of diethyldithiocarbamic acid (DETCA)- and xanthine oxidase/hypoxanthine (XO/HX)- generated oxidative stress on phenylephrine- induced contractile responses were investigated in isolated rat thoracic aorta. The rings were subjected to reactive oxygen species by incubation with DETCA or XO/HX. Contractions of the ring preparations by phenylephrine were significantly attenuated by oxidative stress in the presence of an intact endothelium. Inhibition of nitric oxide synthesis by NÐ-nitro-L-arginin (LNA, 10-4 M) prior to this chemically generated oxidative stress did not affect the decreased contractile responses of phenylephrine (PE). Moreover, the sensitivity of PE- induced contractions was

significantly reduced by oxidative stress in either presence or absence of nitric oxide synthase inhibition. Our results suggest that reactive oxygen radicals may be involved in the vascular incompetence.

Key Words: In vitro oxidative stress; contractile response; nitric oxide; rat

Özet

Oksidatif Stresin Sıçan Aortunda Fenilefrinin Kasılma Yanıtlarını Azaltması

Dietilditiyokarbamik asit ve ksantin oksidaz / hipoksantin ile oluşturulan oksidatif stresin fenilefrin kasılma yanıtları üzerine olan etkileri, izole sıçan torasik aorta ring preparatlarında incelenmiştir. Ringler DETCA veya XO/HX ile inkübe edilerek oksidatif strese maruz bırakılmıştır. Oksidatif stres, fenilefrin kasılma yanıtlarını endoteli işlem görmemiş dokularda belirgin derecede azaltmıştır. Oluşturulan kimyasal oksidatif stres öncesinde nitrik oksit sentezinin NÐ-nitro-L-arginin (LNA, 10-4 M) ile inhibe edilmesi fenilefrin kasılma yanıtlarındaki azalmayı etkilememiştir. Bunlara ek olarak, nitrik oksit sentez inhibisyonu varlığında ve yokluğunda, oksidatif stres fenilefrin duyarlığını belirgin olarak azaltmıştır. Sonuçlarımız reaktif oksijen radikallerinin vasküler yetmezlik sürecinde rol oynayabileceğini düşündürmektedir.

Anahtar Kelimeler : In vitro oksidatif stres; kasılma yanıtı; nitrik oksit; sıçan

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