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Modulation by hydrogen peroxide of noradrenaline-induced contraction in aorta from streptozotocin-induced diabetic rat

Tsuneo Kobayashi, Katsuo Kamata*

Department of Physiology and Morphology, Institute of Medicinal Chemistry, Hoshi University, Shinagawa, Tokyo 142-8501, Japan

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Abstract

Hydrogen peroxide (H_2O_2) is known to modify vascular tone in various preparations and its production is elevated in the diabetic aorta. We have investigated its possible involvement in regulation of the noradrenaline-induced contractile response in aorta from streptozotocin-induced diabetic rats. In diabetic but not in control aorta, the noradrenaline-induced contraction was significantly enhanced by catalase and significantly inhibited by polyethylene-glycolated superoxide dismutase. Adding catalase to the superoxide dismutase prevented the latter's attenuation of the contraction. In the presence of N^G-nitro-L-arginine, the noradrenaline-induced contraction of aorta from diabetic rats, but not from controls, was inhibited by catalase treatment. Noradrenaline increased the nitrite and nitrate levels in the perfusates from control and diabetic aortic strips. In the latter, the noradrenaline-induced nitrite and nitrate level was significantly enhanced by incubation with superoxide dismutase but not by incubation with catalase plus superoxide dismutase. Thus, endogenously produced H_2O_2 may be an important factor in the regulation of aortic tone in diabetic rats. Enhanced production of H_2O_2 in the aorta from diabetic rats may seem contribute to the endothelial generation of nitric oxide and vasoconstrictor prostanoids.

Keywords: H_2O_2; Diabetes; Contraction; Catalase; Nitric oxide (NO); Streptozotocin

1. Introduction

Oxygen-derived free radicals are involved in mediating the effects of vascular injury in various disease states, such as diabetes mellitus (Poston and Taylor, 1995; Pieper, 1998). As an example, we and other investigators have shown that superoxide dismutase activity is reduced in aorta from diabetic rats and that an increased production of superoxide anion (O_2^-) may cause an impairment of endothelium-dependent relaxation (Hattori et al., 1991; Kamata and Kobayashi, 1996; Kobayashi and Kamata, 1999, 2001; Kobayashi et al, 2000). Indeed, the production of both superoxide and hydrogen peroxide (H_2O_2) is elevated in aorta from diabetic rats (Pieper, 1995; Kobayashi and Kamata, 2001). It is well known that O_2^- is the primary radical formed by the reduction of molecular oxygen and that its formation may be followed by the production of secondary radicals or reactive oxygen species, such as H_2O_2. The principal sources of H_2O_2 in the vascular wall are oxidative metabolic pathways (such as the lipoxygenase, cytochrome P450 monooxygenase and xanthine/xanthine oxidase systems, mitochondrial respiration and superoxide dismutase-catalyzed superoxide anion dismutation) (Panus et al., 1993; Marin and Rodriquez-Martinez, 1995).

There is an accumulating body of evidence to indicate has been reported that H_2O_2 causes relaxation of the canine coronary artery (Rubanyi and Vanhoutte, 1986), bovine pulmonary artery (Wolin and Burke, 1987), rat aorta (Thomas and Ranwell, 1988; Pieper and Gross, 1988; Mian and Martin, 1995a) and rabbit aorta (Zembowicz et al., 1993). Further, the H_2O_2-induced relaxation in the rabbit aorta is completely endothelium-dependent and is mediated by nitric oxide (NO) (Bharadwaj and Prasad, 1995). In contrast, H_2O_2 has been shown to produce contraction in the isolated rat aorta (Rodriguez-Martinez et al., 1998; Yang et al., 1998; Sotonikova, 1998; Shen et al., 2000), porcine pulmonary artery (Pelaez et al., 2000) and rabbit aorta (Iesaki et al., 1994). It has also been reported that H_2O_2 can stimulate both cyclooxygenase (Katusic et al., 1993) and cytochrome P450-dependent enzymes (Yang et al., 1998) as well as phosholipase A_2 (Rao et al., 1995) in vascular smooth muscle cells.

* Corresponding author. Tel./fax: +81-3-5498-5856.
E-mail address: kamata@hoshi.ac.jp (K. Kamata).

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Abnormal functioning of the vascular smooth cell has been implicated as one of the mechanisms underlying vascular disease in diabetes. The ability of H_2O_2 to modify vascular tone has been studied in a number of different vascular preparations. However, to our knowledge, there have been no studies concerning the possible modulating influence of endogenously produced H_2O_2 over noradrenaline-induced contractile responses and acetylcholine-induced relaxation responses in the diabetic state.

Our objective in the present study was therefore to assess the influence of H_2O_2, mainly by examining the effect of catalase, on the noradrenaline-induced contraction and acetylcholine-induced relaxation seen in aortic strips isolated from control and streptozotocin-induced diabetic rats.

2. Materials and methods

2.1. Animals and experimental design

Male Wistar rats, 8 weeks old and 180–250 g in weight, received a single injection via the tail vein of streptozotocin 65 mg/kg dissolved in a citrate buffer. Age-matched control rats were injected with buffer alone. Food and water were available ad libitum. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (which is accredited by the Ministry of Education, Science, Sports and Culture, Japan).

2.2. Measurement of plasma glucose

Ten weeks after the streptozotocin injection, plasma glucose was determined using a commercially available enzyme kit (Wako, Osaka, Japan).

2.3. Measurement of isometric force

Rats were anaesthetized with diethyl ether and killed by decapitation 10 weeks after treatment with streptozotocin or buffer. A section of the thoracic aorta from between the aortic arch and the diaphragm was then removed and placed in oxygenated, modified Krebs–Henseleit solution. The solution consisted of (mM): NaCl 118.0, KCl 4.7, $NaHCO_3$ 25.0, $CaCl_2$ 1.8, NaH_2PO_4 1.2, $MgSO_4$ 1.2, dextrose 11.0. The aorta was cleaned of loosely adhering fat and connective tissue and cut into helical strips 3 mm in width and 20 mm in length. The tissue was placed in a well-oxygenated (95% O_2, 5% CO_2) bath of 10 ml Krebs–Henseleit solution at 37 °C with one end connected to a tissue holder and the other to a force-displacement transducer (Nihon Kohden; TB-611T). The tissue was equilibrated for 60 min under a resting tension of 1.0 g (determined to be optimum in preliminary experiments). The relaxation response to acetylcholine was expressed as a percentage of the contractile force induced by 10^{-7} M noradrenaline. For the relaxation studies, the aortic strips, which were weighed at the end of each experiment, were precontracted with an equieffective concentration of noradrenaline (5×10^{-8}–3×10^{-7} M). When the noradrenaline-induced contraction had reached a plateau level, acetylcholine (10^{-9}–10^{-5} M) was added in a cumulative manner. For the contraction studies, noradrenaline (10^{-9}–10^{-5} M) was added cumulatively to the bath until a maximal response was achieved. After the addition of sufficient aliquots of the agonist to produce the chosen concentration, a plateau response was allowed to develop before the addition of the next dose of the same agonist. To investigate the influence of 1800 U/ml catalase, 41 U/ml polyethylene-glycolated superoxide dismutase (a cell-permeant superoxide anion scavenger) (Beckman et al., 1988), 10^{-4} M N^G-nitro-L-arginine and 10^{-5} M indomethacin on the noradrenaline-induced contractile or acetylcholine-induced relaxant responses, the strip was incubated for 30 min in medium containing one or more of the above agents before the cumulative addition of the agonist.

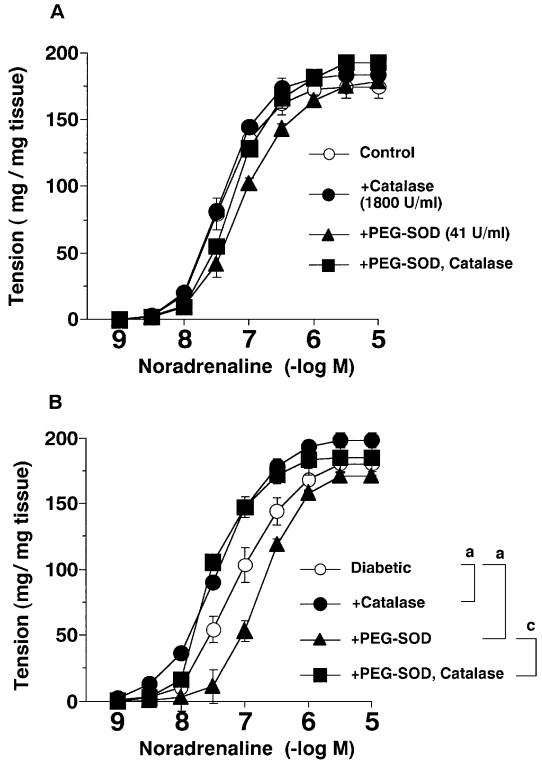

Fig. 1. Effects of catalase and polyethylene-glycolated superoxide dismutase on noradrenaline-induced contractile response in aorta from controls and streptozotocin-induced diabetic rats. Aortic strips from (A) age-matched controls, (B) diabetic rats were treated with catalase (1800 U/ml), polyethylene-glycolated superoxide dismutase (PEG-SOD) (41 U/ml) or catalase (1800 U/ml) plus PEG-SOD (41 U/ml). Ordinate shows increase in tension (expressed in mg tension/mg tissue) measured at peak. Each data point represents mean ± S.E.M. of six to eight experiments; the S.E.M. is included only when it exceeds the dimension of the symbol used. $^aP<0.05$, catalase vs. diabetic or (PEG-SOD) vs. diabetic; $^cP<0.001$, PEG-SOD vs. PEG-SOD plus catalase.

2.4. Measurement of nitrite (NO_2^-) and nitrate (NO_3^-)

The concentration of nitrite and nitrate in the effluent from each type of tissue was assayed by the method described by Singer et al. (1977) and Yamada and Nabeshima (1997). Briefly, the NO_2^- and NO_3^- in the perfusate were separated by means of a reverse-phase separation column packed with polystyrene polymer (NO-PAK; 4.6 × 50 mm; Eicom), then NO_3^- was reduced to NO_2^- in a reduction column packed with copper-plated cadmium filings (NO-RED; Eicom). NO_2^- was mixed with a Griess reagent to form a purple azo dye in a reaction coil. The separation and reduction columns and the reaction coil were placed in a column oven set at 35 °C. The absorbance of the colour of the product dye at 540 nm was measured by a flow-through spectrophotometer (NOD-10; Eicom). The mobile phase, which was delivered by a pump at a rate of 0.33 ml/min, was 10% methanol containing 0.15 M NaCl/NH4Cl and 0.5 g/l 4 Na-ethylenediamino-*N*,*N*,*N*′,*N*′-tetraacetic acid. The Griess reagent, which was 1.25% HCl containing 5 g/l sulpfanilamide with 0.25 g/l *N*-naphthylethylenediamine, was delivered at a rate of 0.1 ml/min. The concentration of NO_2^- and NO_3^- in the Krebs–Henseleit solution and the reliability of the reduction column were examined in each experiment. For the determination of NO_2^- and NO_3^-, samples was collected over a 0- or 40-min period during the response to 10^{-7} M noradrenaline. When the effects of catalase (1800 U/ml) and polyethylene-glycolated superoxide dismutase (41 U/ml) on the noradrenaline response were to be examined in control or diabetic aorta, the catalase or/and polyethylene-glycolated superoxide dismutase was added to the bath 30 min before the administration of 10^{-7} M noradrenaline.

2.5. Drugs

Streptozotocin, (−) noradrenaline hydrochloride, catalase, polyethylene-glycolated superoxide dismutase, N^G-nitro-L-arginine and indomethacin were purchased from Sigma (St. Louis, MO, USA). Acetylcholine chloride was purchased from Daiichi Pharmaceuticals (Tokyo, Japan). All drugs were dissolved in saline, except where otherwise noted. All concentrations are expressed as the final molar concentration of the base in the organ bath.

2.6. Statistical analysis

The contractile force developed by aortic strips from control and diabetic rats is expressed in mg tension/mg tissue. Data are expressed as the mean ± S.E.M. In some experiments, statistical differences were assessed using Dunnett's test for multiple comparisons after a one-way analysis of variance, a probability level of $P<0.05$ being regarded as significant. Statistical comparisons between concentration–response curves were made using a two-way analysis of variance (ANOVA) with Bonferroni's correction performed post hoc to correct for multiple comparisons. A two-tailed value of $P<0.05$ was considered significant.

Table 1
Maximal response and EC_{50} values for noradrenaline-induced contraction of aortic strips in controls and STZ-induced diabetic rats

	Control rats, max. response (mg/mg tissue)	− log EC_{50}	Diabetic rats, max. response (mg/mg tissue)	− log EC_{50}
Untreated	174.4 ± 8.1 (8)	7.42 ± 0.07 (8)	178.4 ± 8.7 (9)	7.21 ± 0.12 (9)
Catalase	183.3 ± 4.3 (8)	7.44 ± 0.07 (8)	198.8 ± 1.0 (9)[a]	7.57 ± 0.13 (9)[a]
PEG-SOD	189.5 ± 13.3 (8)	7.16 ± 0.05 (8)[b]	171.1 ± 6.4 (6)	6.91 ± 0.12 (6)
PEG-SOD + Catalase	189.2 ± 2.2 (6)	7.37 ± 0.10 (6)	185.9 ± 11.1 (8)	7.59 ± 0.02 (8)[a,c]
L-NOARG	210.3 ± 5.8 (6)[d]	7.67 ± 0.04 (6)	226.1 ± 9.9 (8)[e]	7.93 ± 0.06 (8)[f,g]
L-NOARG + Catalase	206.9 ± 3.3 (6)[d]	7.57 ± 0.07 (6)	192.7 ± 4.2 (8)[a]	7.69 ± 0.01 (8)[e,h]
L-NOARG + Ind	248.3 ± 19.4 (6)[d]	7.48 ± 0.07 (6)	228.5 ± 12.1 (6)[e]	7.81 ± 0.03 (6)[e,i]
L-NOARG + Ind + Catalase	N.T.	N.T.	222.7 ± 13.8 (6)[e]	7.83 ± 0.03 (6)[e]
Ind	183.3 ± 15.6 (4)	7.13 ± 0.07 (4)[b]	182.8 ± 8.4 (8)	6.75 ± 0.05 (6)[e,j]
Ind + Catalase	184.6 ± 6.7 (6)	7.41 ± 0.08 (6)	199.3 ± 1.1 (8)	7.56 ± 0.15 (8)[k]

Values are means ± S.E.M. Number of determinations is shown in parenthesis. Catalase (1800 U/ml); PEG-SOD, polyethylene-glycolated superoxide (41 U/ml); L-NOARG, N^G-nitro-L-arginine (10^{-4} M); Ind, indomathacin (10^{-5} M). N.T., Not tested.

[a] $P<0.05$ vs. untreated diabetic.
[b] $P<0.05$ vs. untreated.
[c] $P<0.01$ PEG-SOD.
[d] $P<0.01$ vs. untreated.
[e] $P<0.01$ vs. untreated diabetic.
[f] $P<0.001$ vs. untreated diabetic.
[g] $P<0.01$ L-NOARG-treated control.
[h] $P<0.01$ vs. L-NOARG.
[i] $P<0.01$ L-NOARG + Ind-treated control.
[j] $P<0.01$ vs. Ind-treated control.
[k] $P<0.01$ vs. Ind.

3. Results

3.1. Plasma glucose levels

As previously reported in studies using the same procedure to induce diabetes (Kamata and Kobayashi, 1996; Kobayashi and Kamata, 1999, 2001; Kobayashi et al., 2000), 10 weeks after treatment with STZ, the concentration of glucose in plasma was elevated significantly, from 108.7 ± 4.2 (mg/dl) in age-matched controls to 518.2 ± 13.7 (mg/dl) in diabetic rats, respectively.

3.2. Contraction response to noradrenaline

Cumulative administration of noradrenaline (10^{-9}–10^{-5} M) induced a dose-dependent contraction in aortic strips from both controls and streptozotocin-induced diabetic rats. There were no significant differences, in terms of either maximum contractile force or sensitivity, between control and diabetic rats (Fig. 1A,B and Table 1). The noradrenaline-induced dose-dependent contractile response was significantly enhanced by catalase (1800 U/ml) in diabetic-rat aorta but not in the controls. In diabetic rats, catalase shifted the dose–response curve for the noradrenaline-induced vasoconstriction to the left and increased the

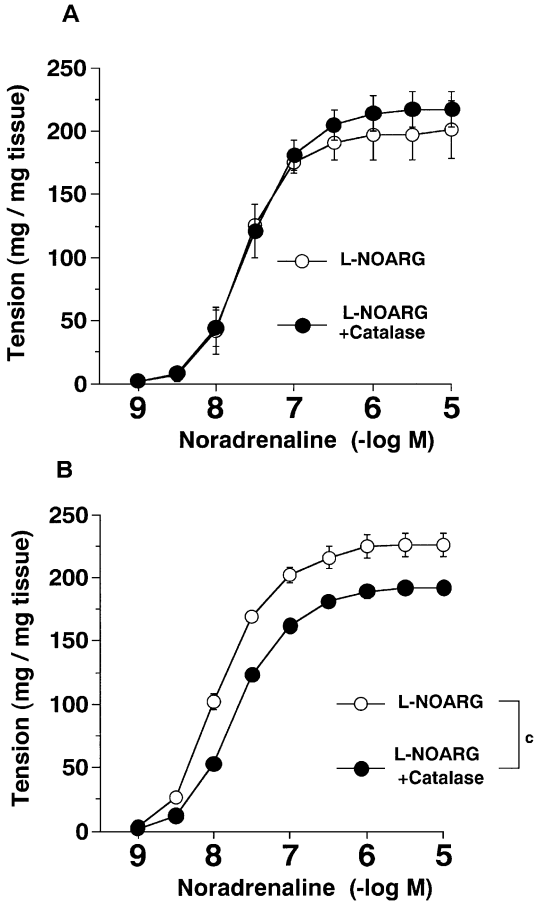

Fig. 2. Effects of N^G-nitro-L-arginine and catalase on concentration–response curves for noradrenaline-induced contraction in aorta from controls and streptozotocin-induced diabetic rats. Aortic strips from (A) age-matched controls, (B) diabetic rats were treated with N^G-nitro-L-arginine (10^{-4} M) or N^G-nitro-L-arginine plus catalase (1800 U/ml). Ordinate shows increase in tension (expressed in mg tension/mg tissue) measured at peak. Each data point represents mean ± S.E.M. of six to eight experiments; the S.E.M. is included only when it exceeds the dimension of the symbol used. $^cP<0.001$, N^G-nitro-L-arginine vs. N^G-nitro-L-arginine plus catalase.

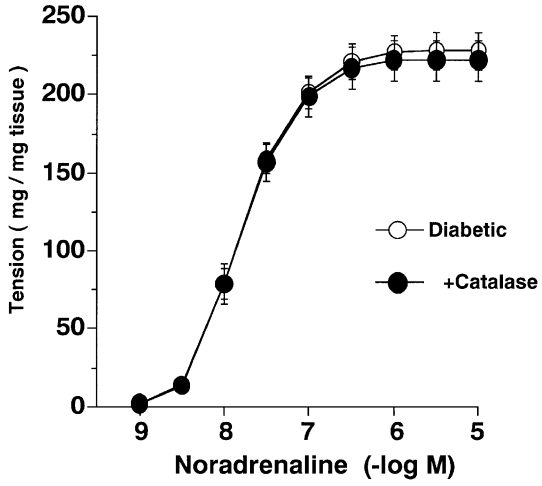

Fig. 3. Effect of catalase (in combined presence of N^G-nitro-L-arginine and indomethacin) on concentration–response curves for noradrenaline-induced contraction in diabetic rat aorta. Treatment was with N^G-nitro-L-arginine (10^{-4} M) plus indomethacin (10^{-5} M) or N^G-nitro-L-arginine plus indomethacin plus catalase (1800 U/ml). Ordinate shows increase in tension (expressed in mg tension/mg tissue) measured at peak. Each data point represents mean ± S.E.M. of six to eight experiments; the S.E.M. is included only when it exceeds the dimension of the symbol used.

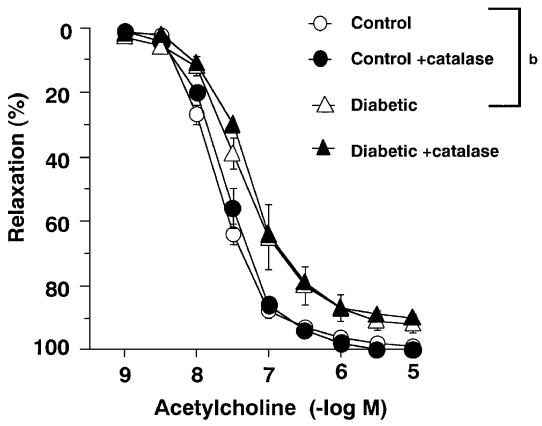

Fig. 4. Effects of catalase on concentration–response curves for acetylcholine-induced relaxation of aorta from controls and diabetic rats, each tissue having been treated or not treated with catalase (1800 U/ml). Ordinate shows relaxation of aortic strips as a percentage of the contraction induced by an equieffective concentration of noradrenaline (5×10^{-8}–3×10^{-7} M). Each data-point represents mean ± S.E.M. of six to eight experiments; the S.E.M. is included only when it exceeds the dimension of the symbol used. $^bP<0.01$, diabetic vs. control.

maximum response (Fig. 1B and Table 1). Polyethylene-glycolated superoxide dismutase (41 U/ml) significantly attenuated the noradrenaline-induced contraction in diabetic-rat aorta, but not in the controls and the attenuated response in the diabetic group was restored by catalase treatment (1800 U/ml). In the presence of the nitric oxide synthase inhibitor N^G-nitro-L-arginine (10^{-4} M), the noradrenaline-induced contractile response was significantly inhibited by catalase (1800 U/ml) in the diabetic group but not in the controls (Fig. 2A and B). Catalase (1800 U/ml) had no effect on the noradrenaline-induced contractile response in diabetic-rat aorta pretreated with both N^G-nitro-L-arginine (10^{-4} M) and the cyclooxygenase inhibitor indomethacin (10^{-5} M) (Fig. 3). Indomethacin (10^{-5} M) significantly attenuated the noradrenaline-induced contraction in aorta from diabetic rats, but not in controls and the attenuated response in the diabetic group was restored by catalase treatment (1800 U/ml) (Table 1).

3.3. Relaxation response to acetylcholine

When the noradrenaline (5×10^{-8}–3×10^{-7} M)-induced contraction had reached a plateau, acetylcholine (10^{-9}–10^{-5} M) was added cumulatively. In aortic strips from age-matched control rats, acetylcholine (10^{-9}–10^{-5} M) caused a concentration-dependent relaxation, with the maximum response at 10^{-5} M. This relaxation was significantly weaker in strips from streptozotocin-induced diabetic rats (Fig. 4). Preincubation with catalase (1800 U/ml) had no effect on the acetylcholine-induced relaxation in either group (controls or streptozotocin-induced diabetic rats) (Fig. 4).

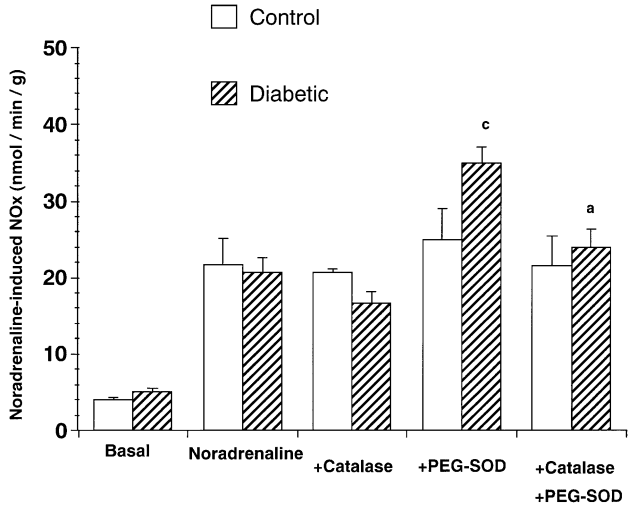

Fig. 5. Effects of catalase (1800 U/ml) and PEG-SOD (41 U/ml) on noradrenaline-stimulated release of NO_x from aorta isolated from control and diabetic rats, as measured in the perfusate. Each column represents mean ± S.E.M. of six to eight experiments; the S.E.M. is included only when it exceeds the dimension of the symbol used. $^cP < 0.001$, diabetic vs. corresponding control. $^aP < 0.05$, PEG-SOD-treated diabetic vs. PEG-SOD plus catalase-treated diabetic.

3.4. Measurement of NO_2^- and NO_3^-

Incubation of aortic strips with noradrenaline (10^{-7} M) increased the NO_x (NO_2^- and NO_3^-) level in the perfusates, the actual level reached being no difference between controls and diabetic rats (Fig. 5). Treatment with catalase (1800 U/ml) tended to produce a slight inhibition of this release in the diabetic group but not in the controls. Treatment with polyethylene-glycolated superoxide dismutase (41 U/ml) enhanced the noradrenaline-induced release of NO_x in the diabetic group, but not in the controls, and this enhancement was inhibited by catalase treatment (1800 U/ml) (Fig. 5).

4. Discussion

It has been reported that H_2O_2 accelerates NO release (Rubanyi and Vanhoutte, 1986), activates soluble guanylate cyclase activity and causes smooth muscle relaxation (Zembowicz et al., 1993). It has also been reported that catalase abolishes the vascular- and endothelium-dependent responses that are elicited both by xanthine oxidase plus xanthine and by H_2O_2 (Dowell et al., 1993; Gao et al., 1994; Mian and Martin, 1995b). In the present study, although we did not examine the effect of H_2O_2 itself on tension in vascular smooth muscle, we found that catalase, which metabolizes H_2O_2 to H_2O, increased the noradrenaline-induced contractile response in diabetic rat aorta but not in the controls. Furthermore, polyethylene-glycolated superoxide dismutase, which dismutates O_2^- to H_2O_2, decreased the noradrenaline response in the diabetic state and this decreased response became blocked one when catalase was added. Taken together, these results suggest that the production or accumulation of H_2O_2 may negatively regulate the noradrenaline-induced contractile response in the diabetic state. Possibly, overproduction of H_2O_2, including superoxide dismutase-production, in the diabetic aorta may stimulate NO synthase, produce NO and thereby inhibit the noradrenaline-induced contraction. This notion is supported by our finding that noradrenaline-stimulated NO_x-release levels in aortic strips from diabetic rats were significantly increased by polyethylene-glycolated superoxide dismutase and tended to be slightly inhibited by catalase. An enhancement of the noradrenaline-induced contraction was produced by catalase only in the diabetic group, strongly suggesting that a production or accumulation of H_2O_2 occurs only in the diabetic state. Interestingly, we recently found that the basal O_2^- level was greater in aortic rings from diabetic rats than in those from control animals (Kobayashi and Kamata, 2001). The increased concentration of O_2^- may be metabolized to yield an elevated H_2O_2 concentration and this in turn may affect (inhibit) the noradrenaline-induced contraction.

In marked contrast, catalase, polyethylene-glycolated superoxide dismutase and polyethylene-glycolated super-

oxide dismutase plus catalase had no effects at all on the noradrenaline-induced contraction in aorta from control rats. These results are consistent with H_2O_2 production or accumulation occurring only in the diabetic state.

In the presence of the nitric oxide synthase inhibitor N^G-nitro-L-arginine, the noradrenaline-induced contraction seen in aortic strips from diabetic rats was inhibited, rather than enhanced, by catalase pretreatment but catalase had no effect in the combined presence of N^G-nitro-L-arginine and the cyclooxygenase inhibitor indomethacin. This surprised us, since it indicates that an enhanced production of H_2O_2 in the diabetic aorta pretreated with N^G-nitro-L-arginine leads to the production of cyclooxygenase-dependent vasoconstrictor prostanoids from the endothelium. Previously, a number of different mechanisms have been proposed to explain the contractile and relaxant effects of H_2O_2. The vascular contraction induced by H_2O_2 has been said to be mediated by activation of cytochrome P450-dependent enzymes and phosholipase A_2 in arteries or vascular smooth muscle cells but it has also been said to be mainly dependent on the products of the cyclooxygenase pathway in some arteries and veins (Tate et al., 1984; Katusic et al., 1993). It has been reported that in vascular cells, oxidative stress is a potent stimulus for the activation of the metabolism of arachidonic acid via the cyclooxygenase pathway (Tate et al., 1984; Mian and Martin, 1995b). Furthermore, prostaglandin H_2 has been proposed as a mediator of endothelium-dependent contraction in the aorta in diabetic rabbits (Tesfamariam et al., 1989). Taken together, the above observations make it seem likely that under conditions in which NO synthase activity is low enhanced production or accumulation of H_2O_2 may stimulate cyclooxygenase and lead to the formation of contractile prostanoids such as prostaglandin H_2. This is consistent with our observation of an attenuation of the noradrenaline-induced contraction by catalase in aortic strips from diabetic rats in the presence of N^G-nitro-L-arginine. On the basis of our results, we propose that (i) the H_2O_2 concentration in the aorta is elevated in the diabetic state, (ii) the increased level of H_2O_2 in the diabetic state decreases the noradrenaline-induced contraction when NO synthase activity is normal and (iii) in contrast, the increased H_2O_2 level in the diabetic state augments the noradrenaline-induced contraction when NO synthase activity is low.

An accumulating body of evidence indicates that the relaxation responses induced in aortic strips by endothelium-dependent agents are weaker in streptozotocin-induced diabetic rats (Oyama et al., 1986; Kamata et al., 1989; Poston and Taylor, 1995; Pieper, 1998; Kobayashi et al., 2000; Kobayashi and Kamata, 2001). When O_2^- reacts with NO, it produces the less potent vasodilators peroxynitrite, NO_2^- and NO_3^- (Beckman et al., 1994). Indeed, it has been reported that an enhanced formation of this radical species may lead to an accelerated inactivation of NO (Gryglewski et al., 1986; Mian and Martin, 1995b; Kobayashi and Kamata, 2001). We therefore examined acetylcholine-induced endothelium-dependent relaxation in aortic strips from diabetic rats and controls. Since preincubation with catalase had no effect on the acetylcholine-induced relaxation in either control or streptozotocin-induced-diabetic aorta, it is unlikely that H_2O_2 has any effect on endothelium-dependent relaxation. In agreement with this, it has been reported that H_2O_2 is not involved in endothelial dysfunction in streptozotocin-induced diabetic rats (Hattori et al., 1991; Kamata and Kobayashi, 1996).

In conclusion, our results suggest that endogenously produced H_2O_2 may be an important factor in the regulation of vascular tone in diabetic rats. We propose enhanced production of H_2O_2 in diabetic blood vessels negatively regulates the noradrenaline-induced contraction via generation of NO when NO synthase activity is normal but enhances it via the formation of vasoconstrictor prostanoids when NO synthase activity is low. If this is the correct, then the level of NO synthase activity may hold key to the nature and extent of the pathological role played by H_2O_2.

Acknowledgements

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