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Chronic or delayed treatment with an oral dithiocarbamate analog decreases glycation and protects diabetic arteries

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Abstract

In the present study, we examined the efficacy of a dithiocarbamate-based compound, denoted as NOX-700, on diabetes-induced endothelial dysfunction and glycosylation of hemoglobin (Hb). Streptozotocin-induced diabetic rats received 3 mg/ml NOX-700 in drinking water beginning at 72 h or 4 weeks and continued to 8 weeks. Oxidative and glycooxidative stress were examined by electrophoretic mobility shift assay (EMSA) for nuclear factor-κB (NF-κB) in nuclear fractions of aortic homogenates and by glycosylated Hb, respectively. Vascular reactivity was examined in aortic ring segments ex vivo. Treatment with NOX-700 inhibited glycosylated Hb formation when given long-term or after delayed administration. NOX-700 improved endothelium-dependent relaxation to acetylcholine but did not alter reactivity to norepinephrine or nitroglycerin, suggesting selective protection of the endothelium. Nuclear factor-κB (NF-κB) nuclear binding activity was significantly increased in diabetic aortas and abrogated by NOX-700. Thus, vascular protection by NOX-700 is believed to be mediated, in part, by an antioxidant mechanism and decreased protein glycation.

Keywords: Diabetes mellitus; Hemoglobin glycated; Endothelium; NF-κB (nuclear factor-κB); Iron chelator

1. Introduction

Endothelial dysfunction in diabetes mellitus is believed to be mediated, in part, by decreased nitric oxide bioactivity as a consequence of increased oxidative stress (Pieper, 1998; Pieper, 2000; Hink et al., 2001) or enhanced protein glycation (Angulo et al., 1996; Vlassara et al., 1992). Thus, chronic treatment with agents that either counteract oxidative stress or limit protein glycation should protect the vascular endothelium.

Recently, there has been active interest in the protective effects of α-lipoic acid in diabetes-associated complications. The parent compound, α-lipoic acid, is a disulfide that is reduced in vivo to a dithiol, its biologically active form (Packer, 1998). This agent is a metal chelator and a powerful antioxidant (Ou et al., 1995) and also displays action to inhibit protein glycation in vitro. In placebo-controlled, clinical trials in diabetic patients, α-lipoic acid significantly improved cardiac and peripheral neuropathy (Ziegler et al., 1995; Ziegler et al., 1997). Studies conducted in experimental diabetic animals suggest that the neuroprotective actions of α-lipoic acid may be related to protection of the endothelium (Keegan et al., 1999).

Antioxidants and iron chelators have been known to inhibit protein glycation in vitro (Hunt et al., 1988). Interestingly, α-lipoic acid has been shown to inhibit hemoglobin (Hb) glycation in vitro (Suzuki et al., 1991; Jain and Lim, 2000). Despite antagonizing protein glycation in vitro, a recent clinical trial on diabetes-induced neuropathy failed to show any improvements in glycation in vivo (Ziegler et al., 1997).

Dithiocarbamic acid-based derivatives (or dithiocarbamates) are another class of agents that are structurally related to α-lipoic acid and its active metabolite, dihydrolipoic acid. Dithiocarbamates also display significant antioxidant and metal chelation activity. Agents such as pyrrolidine dithiocarbamate have been traditionally used to antagonize cytokine-induced activation of the transcription factor, nuclear factor-κB (NF-κB), and formation of NF-κB-

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Table 1
Body weights and blood glucose in experimental group

Groups	*n*	Body weights (g)			Blood glucose (mg/dl)		Glycated Hb (%)
		Initial	Implant	Final	Implant[a]	Final[b]	Final
Control	9	397 ± 2	n.d.	494 ± 18	n.d.	93 ± 6	5.51 ± 0.26
Diabetic (8 weeks)	12	371 ± 5	321 ± 5[c]	335 ± 11[cd]	289 ± 21	410 ± 17[d]	14.68 ± 0.42[d]
Diabetic (NOX-700)	11	384 ± 4	330 ± 10[c]	285 ± 15[cd]	327 ± 24	389 ± 50[d]	11.28 ± 0.38[d]
P value vs. Diabetic		n.s.	n.s.	< 0.05	n.s.	n.s.	< 0.005
Diabetic (4 weeks)	6	352 ± 2	320 ± 3[c]	342 ± 4[cd]	340 ± 26	376 ± 33[d]	10.59 ± 0.49[d]
Diabetic, delayed, NOX-700	10	354 ± 3	324 ± 3[c]	386 ± 5[cd]	346 ± 18	348 ± 16[d]	12.47 ± 0.42[d]
P value vs. 8-weeks Diabetic + NOX-700			n.s.	< 0.01	n.s.	n.s.	< 0.01

[ab]Determined in unanesthetized and pentobarbital-anesthetized animals, respectively.
[cd]Significantly different from initial value within the same group and from nondiabetic control group, respectively. n.d., not determined; n.s., not significant.

dependent genes in vascular cells such as inflammatory cytokines and cell adhesion molecules that lead to endothelial dysfunction in many disease states (Somers et al., 2000). Thus, activation of NF-κB may be an early indicator of oxidative stress in vascular disease states.

Information regarding the activation of NF-κB in diabetic arteries in vivo is extraordinarily rare. We were the first known laboratory to show that NF-κB binding activity is increased by twofold in arteries in vivo within just a few days of onset of diabetes mellitus (Pieper, 2000). Similarly, there is also extraordinarily little information whether agents that counteract reactive oxygen might also impact on activation of this important transcription factor in diabetic arteries in vivo. The efficacy of antioxidants was documented in one of our recent studies in which we showed that chronic treatment with an antioxidant, known as nitric oxide-donating aspirin derivative, NCX4016, was able to prevent activation of NF-κB in diabetic arteries and protect against diabetes-induced endothelial dysfunction (Pieper et al., 2002).

Recently, we have evaluated an oral-formulated compound known as NOX-700 that is related to the dithiocarbamate class of compounds. We found that chronic treatment with NOX-700 diminished NF-κB activation in pancreatic islets and decreased the incidence of hyperglycemia in a genetic model of insulin-dependent diabetes mellitus (Roza et al., 2001b). Interestingly, this action was equipotent to that achieved by low-dose cyclosporine. Furthermore, in a model of cardiac allograft transplant rejection, we were also able to show that NOX-700 alone or in combination with low-dose cyclosporine was able to limit the activation of NF-κB, decrease inflammatory cell infiltration and cytokine gene expression, and enhance graft survival (Roza et al., 2001a).

Despite the current interest in the potential actions of lipoic acid, there has been no known evaluation of the chronic actions of any dithiocarbamates on vascular complications associated with diabetes mellitus. At present, we examined the potential benefits of treatment with NOX-700 in vivo on glycation of hemoglobin (Hb), activation of NF-κB activation, and vascular reactivity in diabetic arteries.

2. Methods

Following anesthesia with 100 mg/kg ketamine, diabetes was induced by tail-vein injection of 65 mg/kg streptozotocin in male Sprague–Dawley rats (age 10–11 weeks). At 48 h, hyperglycemia was verified by using a glucometer and test strips (Medisense, Cambridge, MA, USA). At 72 h, all diabetic animals received a subcutaneous slow-release insulin implant (Linplant, Scarborough, Ontario, Canada). Control animals received an empty implant. Implant size was adjusted to provide a release rate of approximately 0.6 U insulin/day in order to replenish some of the lost insulin but to maintain hyperglycemia throughout the duration of the

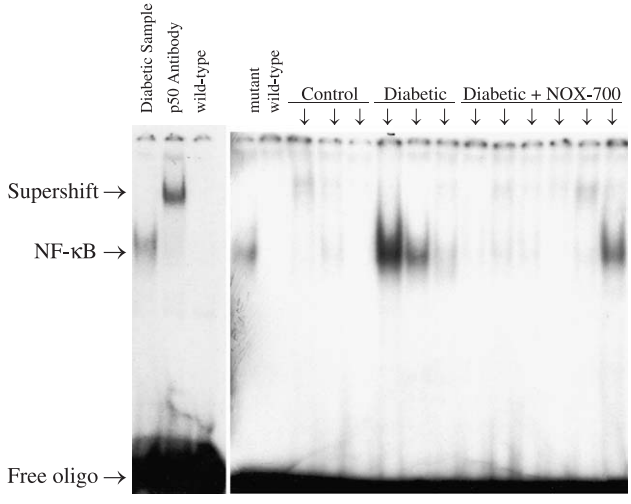

Fig. 1. EMSA showing increased NF-κB binding activity in nuclear fractions of aorta derived from diabetic compared to nondiabetic rats. Treatment with NOX-700 decreased NF-κB binding activity. Specificity for NF-κB binding was shown by supershift by incubation with p50 antibody and elimination by incubation with 100 × excess cold, mutant but not wild-type oligonucleotide.

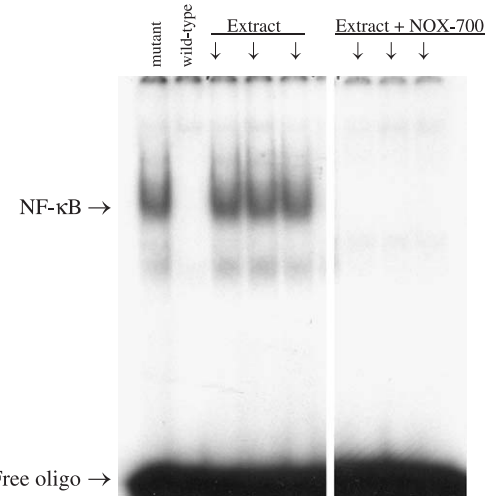

Fig. 2. Incubation of a nuclear extract sample with NOX-700 in the EMSA reaction inhibits NF-κB dimer binding. Results show the binding of three samples without and three samples with incubation of 0.7 μg/ml NOX-700.

study. A portion of diabetic animals received 3 mg/ml NOX-700 (Medinox, San Diego, CA) in drinking water. This concentration was chosen since this concentration was observed by us to reduce the incidence of hyperglycemia in diabetic-prone BB rats (Roza et al., 2001b), limit the activation of NF-κB, and prolong graft survival in a cardiac transplant model (Roza et al., 2001a). Diabetic and age-matched controls were housed for 8 weeks. To assess the role of late intervention, a series of diabetic animals remained untreated but supplemented with NOX-700 during the last 4 weeks of the study.

At the end of this 8-week period, animals were anesthetized with 65 mg/kg pentobarbital. Blood was collected for determination of glucose and total glycosylated Hb (Sigma Diagnostics, St. Louis, MO, USA). Thoracic aortic segments were isolated and cleaned of adventitial tissue and mounted in tissue baths for evaluation of vascular reactivity. Abdominal aortic samples were homogenized and nuclear extracts obtained for analysis of NF-κB binding activity by electrophoretic mobility shift assay (EMSA) as previously described in the laboratory (Pieper and ul-Haq, 1997).

Isometric tension was monitored using Radnoti force-displacement transducers (Monrovia, CA, USA) and a Gould TA6000 recorder (Oxnard, CA, USA) for vascular reactivity studies as previously described (Pieper and Siebeneich, 1997). Aortic rings were subjected to increasing concentrations of norepinephrine, serially washed, equilibrated, and rechallenged with a suboptimal, equipotent concentration of norepinephrine (approximately 1 μM). At peak contraction, rings were challenged with acetylcholine or nitroglycerin to assess endothelium-dependent and -independent relaxation, respectively. For endothelium-independent relaxation, the endothelium was removed prior to mounting in the tissue bath.

Data were calculated as mean values and S.E.M. Data were analyzed by analysis of variance followed by Student–Newman–Keuls test or *t* tests, where appropriate. A value of $P < 0.05$ was selected to denote statistical significance.

3. Results

3.1. Body weights and glycemic control

Body weights were significantly ($P < 0.01$) decreased to equivalent levels in all groups after injection of streptozotocin and just prior to insertion of implants (Table 1). At the end of the study, body weights in all diabetic rats were significantly ($P < 0.01$) lower compared to nondiabetic controls. Body weight in animals treated for 8 weeks with NOX-700 was significantly lower than for untreated diabetic animals or for diabetic animals treated for only the last 4 weeks with NOX-700. In animals with delayed treatment with NOX-700, body weight was significantly ($P < 0.05$) higher than for animals treated for 8 weeks with NOX-700.

At 72 h after streptozotocin, hyperglycemia was observed in all groups (Table 1). Nonfasting, blood glucose concentration in these unanesthetized animals was not different between the diabetic groups. Similarly, at the end of the study in anesthetized animals, blood glucose concentration in all untreated diabetic animals was significantly ($P < 0.01$) elevated compared to nondiabetic controls. Chronic treatment with NOX-700 had no apparent action on blood glucose concentration.

To assess drug-treatment effects on protein glycation, we determined glycated Hb levels. Diabetes produced a

Table 2
Vascular reactivity to norepinephrine

Group	*n*	With endothelium		Without endothelium	
		Maximum		Maximum	
		Developed tension (g)	− log EC_{50} (95% C.L.)	Developed tension (g)	− log EC_{50} (95% C.L.)
Control	9	1.22 ± 0.07	6.88 (6.96–8.81)	3.00 ± 0.06	8.46 (8.66–8.26)
Diabetic, 4 weeks	6	1.74 ± 0.16[a]	6.84 (6.96–6.72)	3.22 ± 0.27	9.18 (9.53–8.83)[a]
Diabetic, 8 weeks	12	1.37 ± 0.15	6.95 (7.03–6.87)	2.62 ± 0.18[a]	8.60 (8.86–8.34)
Diabetic, 8 weeks + NOX-700	11	1.46 ± 0.11	7.13 (7.21–7.05)	2.34 ± 0.10	8.81 (9.13–8.49)
Diabetic, delayed, NOX-700	10	1.27 ± 0.08	6.91 (6.99–6.82)	1.70 ± 0.19[b]	8.43 (8.74–8.11)

[a] $P < 0.05$ vs. nondiabetic control.
[b] $P < 0.05$ vs. untreated diabetic group.

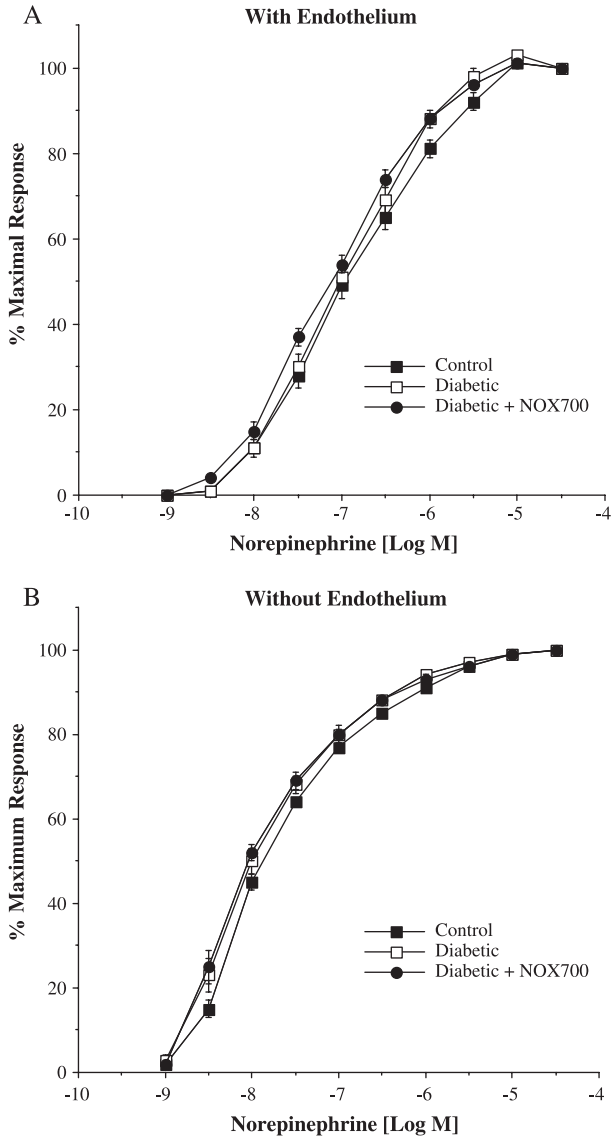

Fig. 3. Sensitivity to contraction with norepinephrine is unchanged in diabetic rats and diabetic rats treated with NOX-700 either in aortic rings with endothelium (Panel A) or without endothelium (Panel B). Each point represents the mean ± S.E.M. of $n = 9–12$ for each group.

significant ($P < 0.01$) increase in glycated Hb (Table 1). Glycated Hb levels were significantly ($P < 0.01$) decreased after 8 weeks of treatment compared to corresponding diabetic rats without NOX-700. If NOX-700 was given to rats for 4 weeks beginning at 4 weeks of diabetes, glycated Hb was significantly ($P < 0.01$) decreased compared to 8-week untreated diabetic rats but not compared to 4-week untreated diabetic rats, owing to the cumulative influence of long-term diabetes mellitus (Table 1).

3.2. Electrophoretic mobility shift assay (EMSA)

EMSA analysis revealed an increase in NF-κB binding activity (i.e. NF-κB activation) in nuclear extracts of aorta derived from 8-week diabetic rats compared to that observed in nondiabetic controls (Fig. 1). Specificity for NF-κB binding was confirmed by the absence of binding in the presence of 100-fold excess wild-type oligonucleotide (lane 2) but not mutant oligonucleotide (lane 1) and by supershift using antibody to NF-κB p50 subunit. In contrast, NF-κB activation was blocked in aortic nuclear extracts of five out of six diabetic animals receiving NOX-700. To examine the potential direct action of NOX-700, we added a low concentration of NOX-700 directly to the nuclear extract within the reaction mixture per se. In this case, NOX-700 significantly inhibited NF-κB binding activity compared to pair-matched control incubation with saline vehicle (Fig. 2).

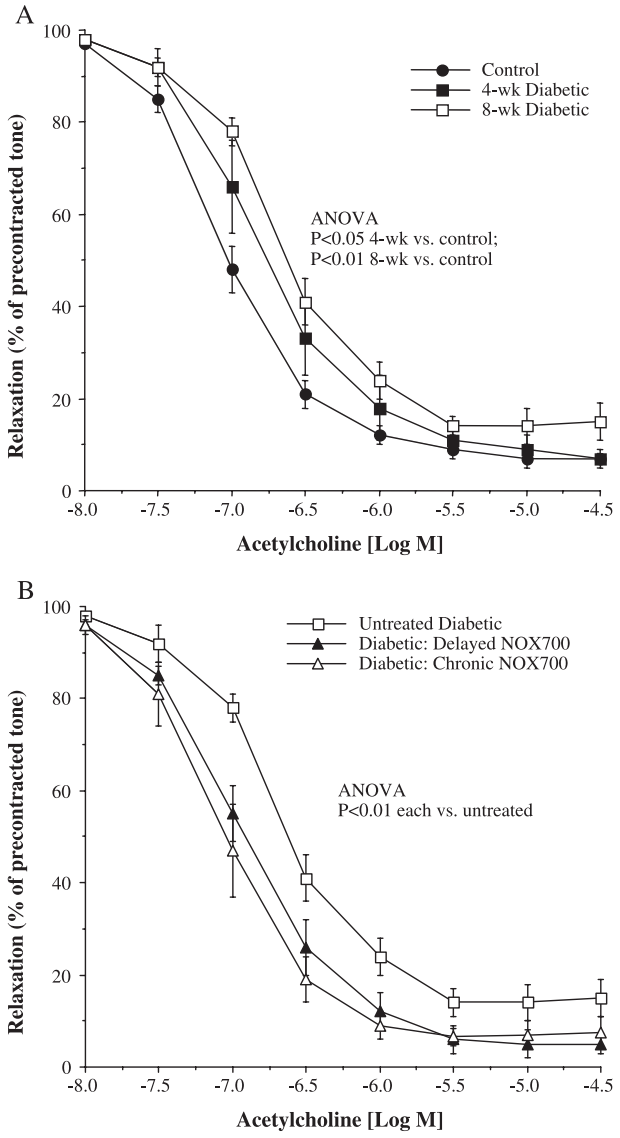

Fig. 4. (A) Diabetes-induced impairment in endothelium-dependent relaxation to acetylcholine. (B) Effect of chronic or delayed treatment of diabetic rats with NOX-700 on endothelium-dependent relaxation to acetylcholine. Each value is the mean ± S.E.M. of $n = 9–12$ for each group.

3.3. Vascular reactivity

In aortic rings with endothelium, diabetes of 4-week duration enhanced maximum developed tone but did not alter sensitivity to norepinephrine relative to controls (Table 2). In contrast, diabetes of 8-week duration did not cause any change in vascular responsiveness to norepinephrine. Indeed, neither maximum contractile tone nor pA_2 was changed. In aortic rings without endothelium, sensitivity to norepinephrine was enhanced after 4 weeks of diabetes (Table 2); however, the change in maximum contractile tone compared to rings with endothelium was decreased relative to controls, suggesting a decrease in basal NO tone (Table 2). In rings with (Fig. 3A) or without endothelium (Fig. 3B), chronic treatment with NOX-700 for 8 weeks did not alter sensitivity to norepinephrine.

Endothelium-dependent reactivity was assessed by challenging preconstricted aortic rings with acetylcholine. There was a progressive impairment in relaxation from 4 to 8 weeks of diabetes that achieved a maximum ($P<0.01$) by 8 weeks compared to nondiabetic controls (Fig. 4A). In contrast, acetylcholine-mediated relaxation in rings from animals treated with NOX-700 was improved relative to rings from untreated diabetic animals, and not impaired compared to rings from control animals (Fig. 4B).

Vascular smooth muscle cell reactivity, as assessed by responses to nitroglycerin, was not altered by chronic treatment with NOX-700. In aortic rings without endothelium, neither maximum relaxation (untreated: $93 \pm 3\%$; NOX-700: $92 \pm 4\%$) nor sensitivity ($-\log EC_{50}$: untreated: 7.10, with 95% confidence limits of 7.30 to 6.90; NOX-700: 6.79, with 95% confidence limits of 6.96 to 6.60) to nitroglycerin was altered by chronic treatment.

4. Discussion

Dithiocarbamate-based derivatives have been proposed for the treatment of a variety of disorders including immune depressive disorder, cancer, atherosclerosis, and endotoxic shock. Little is known about their efficacy in diabetes-induced complications although one study suggested a protective action of pyrrolidine dithiocarbamate against retinopathy (Yoshida et al., 1999). We have previously observed that this agent protects endothelial cells against high-glucose-induced changes in endothelial cell function (Pieper and Dondlinger, 1998) including prevention of glucose-induced activation of NF-κB (unpublished observations).

In the present study, both early and delayed administration of the dithiocarbamate analog, NOX-700, antagonized glycation of Hb, inhibited the activation of NF-κB in diabetic arteries, and inhibited the development of diabetes-induced endothelial dysfunction. In contrast, nitroglycerin-induced vasodilation was unaltered by this treatment. This indicates that the protective action cannot be accounted for by changes in smooth muscle cell reactivity but rather by an action specific to the vascular endothelium.

4.1. Glycation and endothelial dysfunction

Glycation of proteins (e.g. glycated Hb, glycated low-density lipoprotein, and advanced glycation end products or AGE) are known to impair endothelium-dependent relaxation in vitro (Angulo et al., 1996; Galle et al., 1998) and in vivo (Vlassara et al., 1992). The acute action is believed to be related to quenching NO and limiting its bioactivity via enhanced production of reactive oxygen, but the precise molecular events have not been determined. One possibility is that glycated proteins have enhanced capacity to sequester redox-active metal ions such as copper and iron (Qian et al., 1998a; Saxena et al., 1999). This might account for the enhanced copper or iron deposition in diabetic tissue (Qian et al., 1998b; Saxena et al., 2000). Bound metals may serve as source of superoxide anion radical generation to quench NO activity, thereby limiting endothelium-dependent relaxation. Furthermore, these metals may participate in hydroxyl radical formation as demonstrated by EPR spectroscopy with spin trapping (Saxena et al., 2000).

Thus, agents that decrease protein glycation under in vivo conditions are potential candidates to improve endothelial function under diabetic conditions. Indeed, we have found that chronic treatment with diverse types of agents, such as modified deferoxamine and *N*-acetylcysteine that prevent endothelial dysfunction, also partially inhibits the levels of glycated Hb (Pieper and Siebeneich, 1997, 1998). In the present study, chronic treatment of diabetic rats with the experimental dithiocarbamate derivative, NOX-700, also significantly decreased glycated Hb levels. Furthermore, we also found that delayed administration of NOX-700 blocked the progression in Hb glycation. Our findings are consistent with the studies of Rodríguez-Manas et al. (1998) who demonstrated that the degree of glycation of Hb in vivo correlated with the degree of endothelial dysfunction examined ex vivo. Thus, we conclude that the protective actions of NOX-700 are mediated, in part, by inhibiting protein glycation.

While we did not measure tissue AGE in this study, it is possible that NOX-700 might have decreased AGE as well. Indeed, NOX-700 was shown to inhibit glycated Hb and 3-deoxyglucosone, a by-product of AGE, in Zucker diabetic rats (Lai, personal communications). This importance of AGE vs. glycated Hb in development of endothelial dysfunction in chronic diabetes mellitus is not well established; however, Vallejo et al. (2000) showed that pharmacological intervention with acarbose improved endothelial dysfunction and glycated Hb, and that improved endothelial function was not associated with any changes in plasma AGE. In contrast, the level of glycated Hb in diabetes mellitus correlated well to the development of endothelial dysfunction assessed ex vivo (Rodríguez-Manas et al., 1998). The effects of NOX-700 on glycated Hb were independent of

changes in glucose homeostasis as levels of glucose were unaltered by treatment. Thus, we conclude that NOX-700 interferes with glycation.

Interestingly, we also showed that NOX-700 decreased the final body weight compared to untreated animals. The nature of this action is unclear. It is believed to be unrelated to drug toxicity since the treatment levels were well below the known toxic doses of this compound. The effect on body weight is consistent with observations in our laboratory of lower body weights after chronic treatment with either NOX-200 or NOX-700 in genetic, diabetes-prone BB rats to examine changes in diabetogenesis (unpublished observations).

4.2. Interference with glycation by antioxidants/metal chelators

Glycation of protein produced under in vitro conditions can be inhibited by co-incubation with antioxidants or metal chelators, indicating that glycation involves reactive oxygen or metal-catalyzed reactive oxygen (Jain and Palmer, 1997; Hunt et al., 1988). In theory, under in vivo conditions in diabetes mellitus, the beneficial actions of antioxidants might be potentially mediated via limiting glycation. Unfortunately, measurements of protein glycation following antioxidant interventions in vivo have not been routinely examined. In a few isolated reports in experimental diabetes mellitus, glycosylated Hb was decreased by chronic treatment with ascorbate or deferoxamine (Young et al., 1995). In diabetic patients, the evidence is contradictory. For example, vitamin E or other antioxidants modestly decreased glycosylated Hb (Jain et al., 1996) or had no effect (Reaven et al., 1995; Bursell et al., 1999; Ludvigsson et al., 2001). We speculate that the variability in the efficacy of these agents on glycation may be related to the concentration and type of antioxidant chosen. In this regard, it may be more important that antioxidants act indirectly to remove metals since metal chelators are potent inhibitors of glycation in vitro. The action of NOX-700 to reduce protein glycation is consistent with the known actions of α-lipoic acid, a disulfide parent compound that chelates metals and inhibits glycation.

4.3. Effects on activation of NF-κB

We considered other actions of NOX-700. For example, we have noted that various dithiocarbamate-based agents including NOX-700 also have the capacity to limit activation of the transcription factor, NF-κB (Cooper et al., 1998; Roza et al., 2001a,b). Part of this action appeared to coincide with the additional property of NOX-700 and related compounds to limit monocyte/macrophage cell infiltration. In the present study, NF-κB binding activity in nuclear fractions of diabetic aorta was shown to be elevated over nondiabetic controls. This increase was effectively blocked by chronic treatment with NOX-700.

The precise mechanism by which NOX-700 limits activation of NF-κB in vivo is not known with complete certainty but we hypothesize some likely possibilities. The first action is via a direct antioxidant effect. Antioxidants are well known to block NF-κB activation by a variety of stimuli. The antioxidant activity of various dithiocarbamates is known (Mankhetkorn et al., 1994; Somers et al., 2000). The source of reactive oxygen necessary for activation of NF-κB in diabetes mellitus in vivo may be multifactorial. Under in vitro conditions, we were the first to demonstrate that NF-κB in cultured endothelial cells can be activated by elevated glucose levels in a concentration-dependent and time-dependent manner by acute exposure (i.e. hours) (Pieper and ul-Haq, 1997). These findings suggest that hyperglycemia in vivo may be a mediator of activation of this transcription factor in vascular tissue. In contrast, other investigators have shown that NF-κB can be activated in cultured endothelial cells by glycated products (i.e. AGE) acting via AGE receptors (Schmidt et al., 1995). Thus, AGE may be an additional stimulant for activation of NF-κB during chronic diabetes mellitus in vivo. In addition to AGE, glycated of extracellular proteins such as albumin also have the capacity to activate NF-κB in cultured vascular cells (Hattori et al., 1999).

Activation of NF-κB is potentially important to the development of vascular disease. Indeed, there are known NF-κB binding domains in the promoter region for various inflammatory genes including cytokines and cell adhesion molecules. The latter are known to be up-regulated in response to hyperglycemic conditions and facilitate monocyte adhesion to endothelium (Kim et al., 1994). It has been generally regarded that activation of NF-κB by various stimuli occurs via a reactive oxygen signaling pathway.

The precision mechanism on how NOX-700 limits activation of NF-κB is unclear. The first possibility is that NOX-700 may act directly as an antioxidant in vivo although NOX-700 per se has no known direct, intrinsic antioxidant efficacy in cell-free conditions. A second possibility is that antioxidant efficacy may arise secondarily from the action of NOX-700 to sequester and remove redox-active metal ions that may participate as sources for reactive oxygen necessary to cause activation of NF-κB. Indeed, the iron-chelator, α-lipoic acid, inhibited activation of NF-κB in vitro by elevated glucose concentration or by AGE in cultured endothelial cell preparations (Du et al., 1999; Bierhaus et al., 1997). Finally, a third possibility is that the effect of NOX-700 on transcription factor activation in diabetic aortas may be mediated, in part, by a more direct action on DNA binding. In this context, we have found that the addition of 0.7 μg/ml NOX-700 to the reaction mixture in the gel shift assay was able to significantly inhibit DNA binding of NF-κB dimers. Since iron was not added to this system, this indicates that NOX-700 may also have effects that influence oxidative pathways that may not require iron. This observation suggests a potential direct interference with NF-κB dimer binding to DNA. Whether this concentration

of NOX-700 is achieved in the nuclear fraction under in vivo conditions cannot be determined by current techniques.

4.4. Summary

Lipoic acid, a disulfide, has been used for many years in Europe for the treatment of diabetes-induced neuropathy. In this study, we show a vascular protective action of a new orally available, dithiocarbamate-derivative, NOX-700, in diabetes mellitus. Protection was associated with interference with oxidative signaling of the redox-sensitive transcription factor, NF-κB, and inhibiting protein glycation.

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