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CARBOXYEBSELEN A POTENT AND SELECTIVE INHIBITOR OF ENDOTHELIAL NITRIC OXIDE SYNTHASE

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Ebselen (Ebs) a glutathione peroxidase like agent has been recently described as an inhibitor of nitric oxide synthase (NOS). Presently, we report that carboxyebselen (HOOC-Ebs), a hydrophyllic derivative of Ebs inhibits NOS present in enzymatic preparations from bovine endothelium, porcine cerebella, and murine spleen, however, it is both more potent and more selective for the constitutive endothelial NOS than Ebs. Unlike Ebs, HOOC-Ebs (0.1—30 μM) causes a concentration-dependent endothelium-independent relaxations of rings of rabbit aorta. The mechanism of this relaxation remains unknown and it is attenuated by glutathione (GSH, 30—300 μM) and N-acetyl-L-cysteine (NAC, 30—300 μM). The vasorelaxant activity of acetylcholine (Ach, 0.1—1 μM) in aortic rings exposed to low concentrations of HOOC-Ebs (0.1—1μM) or rings exposed to 10 μM HOOC-Ebs after their pretreatment with GSH or NAC (30—300 μM) remained unchanged. The lack of activity of HOOC-Ebs as a NOS inhibitor in intact endothelial cells contrasts the effectiveness of Ebs in this respect.

Key words: *nitric oxide synthase inhibitors, ebselen, carboxyebselen, L-arginine, EDRF.*

INTRODUCTION

Ebselen (Ebs, 2-phenyl-1,2-benzisoselenazol-3-(2H)-one) is a non-toxic, highly lipophilic selenoorganic compound with antioxidant and anti-inflammatory activity (1). Many of the actions of Ebs can be explained by

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Abbreviations: Ebs, ebselen (2-phenyl-1,2-benzisoselenazol-3-(2H)-one); DiEbs, diselenide of ebselen (2,2-diselenobis-N-phenyl-benzamide); HOOC-Ebs, carboxyebselen (2-(4-carboxyphenyl) -1,2-benzisoselenazol-3-(2H)-one); HOOC-DiEbs, diselenide of HOOC-Ebs (2,2-diselenobis-N-phenyl-4-carboxybenzamide); NOS, nitric oxide synthase; GSH, glutathione; NAC, N-acetyl-L-cysteine; Phe, phenylephrine; Ach, acetylcholine.

its unique glutathione (GSH) peroxidase-like activity. In the presence of thiols, including GSH or N-acetyl-L-cysteine (NAC), Ebs reduces H_2O_2 and other peroxides (2). As shown in *Fig. 1,* the peroxidase activity of Ebs depends upon

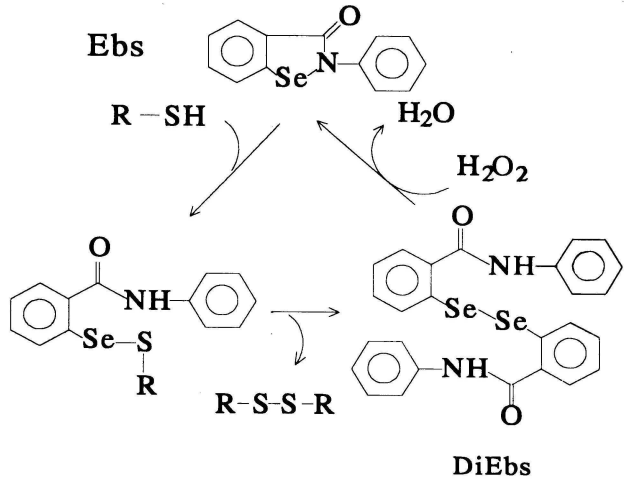

Fig. 1. The mechanism of catalytic reduction of peroxides by ebselen (Fischer & Dereu, 1987). Ebselen (Ebs) reacts with thiols forming selenyl sulfides. Selenyl sulfides react further to form diselenide of Ebs (DiEbs) and disulfides. The formation of DiEbs is a rate limiting step in this cycle. Subsequently, DiEbs is oxidized by peroxides, with the regeneration of Ebs.

its ability to react with thiols forming selenyl sulfides, which further react to form diselenide of Ebs (DiEbs) and disulfides. Subsequently, DiEs is oxidized by peroxides, with the regeneration of Ebs (3). Consistent with this mechanism of action of Ebs is its antioxidant action against lipid peroxidation (4) and its cytoprotective action, both dependent on intracellular levels of GSH and potentiated by GSH or NAC (5, 6). Interestingly, Ebs has also been shown to inhibit the activity of flavoproteins involved in electron transport, such as NADPH oxidase in human and guinea pig polymorphonuclear leukocytes (7, 8), rat microsomal NADH cytochrome b_5 reductase (9) and, importantly, murine microsomal NADPH cytochrome P-450 reductase (9—11).

As nitric oxide synthase (NOS) is structurally related to cytochrome P-450 reductase we investigated the effects of Ebs on NOS activity. Our initial work demonstrated an irreversible and relatively selective inhibition by Ebs of endothelial NOS that is independent of the GSH-peroxidase-like activity of Ebs and can be prevented but not reversed in a concentration dependent manner by GSH or NAC (12). We suggested that the inhibitory activity of Ebs is mediated through the inactivation of a thiol group on NOS which is essential for the catalytic activity of the enzyme. Our work builds on the observation by

Wang et al. (13) that Ebs inhibits nitric oxide release from activated rat Kuppfer cells and extends the understanding of the mechanism through which this activity is mediated.

Ebs is the prototype of a new class of NOS inhibitors. Previous studies on NOS have relied primarily on the use of the arginine derivaties N-monomethyl-L-arginine (L-NMMA) and N-nitro-L-arginine methyl ester (L-NAME) when experimental protocols call for the abolition of NOS activity. Ebs-like compounds (which, like the parent compound, may act as irreversible inhibitors of NOS) are alternatives to the arginine derivatives (which display the kinetics of competitive inhibition) of NOS. Ebs-like compounds are also promising tools which may serve for exploration of the importance of thiol moieties in a variety of enzymatic reactions. Because of that we undertook the study on a series of Ebs-derived benzisoselenazolones and their corresponding diselenides. Now we present the first part of this study.

MATERIALS AND METHODS

Materials

Ebselen (Ebs; 2-phenyl-1,2-benzisoselenazol-3-(2H)-one), its diselenide (DiEbs; 2,2-diselenobis-N-phenyl-benzamide), carboxyebselen (HOOC-Ebs; 2-(4-carboxyphenyl)-1,2-benzisoselenazol-3-benzisoselenazol-3-(2H)-one), and its diselenide (HOOC-DiEbs; 2,2-diselenobis-N-phenyl-4-carboxybenzamide) were synthesized by Prof. Jacek Młochowski at the Institute of Organic and Physical Chemistry of Politechnika Wrocławska (Wrocław, Poland). Reduced glutathione (GSH) was purchased from Biomed (Kraków, Poland). 5,5'-dithiobis-2-nitrobenzoic acid (Ellman's reagent) was from BDH (Germany). Inorganic salts were obtained from Polskie Odczynniki Chemiczne (Gliwice, Poland). All other reagents were purchased from Sigma.

Organ bath experiments

Segments of thoracic aorta were obtained from New Zealand rabbits of either sex (2—2.5 kg), stripped of attached fascia and connective tissue, cut into rings of approximately 2 mm width, suspended between stainless steel hooks and mounted in 5 ml organ baths filled with warmed (37° C) and oxygenated (95% O_2, 5% CO_2) Krebs buffer containing 5.6 μM indomethacin. The Krebs' buffer had the following composition: 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.17 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, and 5.6 mM glucose. Denuded rings were prepared by gently rotating the rings on a stainless steel wire prior to mounting. Isometric force was measured with Biogestab K30 type 351 transducers (Hugo Sachs Electronic, Germany). A tension of 4 g was applied and the rings were equilibrated (30—60 minutes) adjusting the preload to 4 g every 15 min. After a stable baseline was obtained (ca. 1 hr), the rings were contracted with KCl (60 mM) followed by approximately 30 min. equilibration in fresh Krebs' buffer. The rings were then contracted with phenylephrine (Phe, 0.2 μM), typically producing 60—80% of maximal contraction and acetylcholine (Ach, 0.3 μM) was added to the baths to test the functional integrity of endothelium. Only tissues which relaxed more than 40% of the Phe-induced tone after addition of Ach were considered to have undamaged endothelium. Ach (0.3 μM) did not change the tone of endothelium-denuded rings. After extensive washing with fresh buffer the rings were contracted with Phe (0.2 μM) and cumulative concentration response curves induced by Ach (0.01—1 mM) in the absence or presence of HOOC-Ebs (10 μM) were recorded. The slow, endothelium-

-independent relaxation of arterial smooth muscle in the presence of HOOC-Ebs was allowed to proceed for 30 minutes before concentration response curves were determined. In most experiments, NAC (30—300 μM) or GSH (30—300 μM) were added to the baths 15 min. before addition of HOOC-Ebs. Following the addition of Ach, the tissues were equilibrated for 1 hr in fresh Krebs' buffer (changed every 10 min.) either with or without NAC (300 μM) or GSH (300 μM). Phe (0.2 μM) was again added to the tissues and the cumulative concentration-response curve induced by Ach (0.01—1 μM) repeated.

Determination of free thiols

GSH peroxidase-like activity of Ebs, DiEbs, HOOC-Ebs, and HOOC-DiEbs was assayed by monitoring, in the absence or presence of these compounds (100 μM), the disappearance of free SH groups in 1 mM solutions of NAC induced by H_2O_2 (1 mm). After 5 min. of incubation at 37°C, concentrations of free SH groups were quantified by the method of Ellman (14), using NAC as a standard.

Preparation of homogenates of bovine aortic endothelium, porcine cerebellum and murine spleen

Bovine aortas and porcine cerebellum were obtained from a local slaughterhouse. The endothelial cells of 6—10 bovine aortas were scraped with a scalpel blade into ice-cold phosphate buffered saline (pH 7.5) containing EDTA (0.1 mM). Endothelial cells were collected by centrifugation at 2000 g for 10 min. Male Wistar rats weighing 200—300 g were injected with E. coli lipopolysaccharide (serotype 0127 B8, 20—30 mg/kg). After 6 hr, the rats were killed by cervical dislocation and their spleens were removed after perfusion of the vascular bed with saline for 5 min.

The endothelial cells, porcine cerebella, and murine spleens were homogenized using an Omni-mixer homogenizer (Camlab, UK.), set at the maximal speed, in 1 volume of Tris buffer (0.05 M, pH 7.5) containing EDTA (0.1 mM) and the protease inhibitors leupeptin (40 μg/ml), phosphoramidon (40 μg/ml) and aprotinin (200 U/ml).

Determination of NOS activity

The activity of NOS in the endothelial, cerebellar and splenic homogenates was assayed by monitoring the conversion of L-arginine-[2,3-^{3}H] to L-citrulline [2,3-^{3}H] (15). Incubations of endothelial and cerebellar homogenates were performed in 100 μl of assay mixture containing 0.5 mM NADPH, 250 U/ml of calmodulin and 2 mM $CaCl_2$ in Tris buffer (0.05 M, pH 7.41 at 37°C). Calmodulin and $CaCl_2$ were not included in incubations of splenic homogenate (the inducible NOS is calcium- and calmodulin-independent). Stock solutions of Ebs, HOOC-Ebs, and their diselenide derivatives were prepared in 50% DMSO/H_2O and the final concentration of DMSO in the incubations was 0.5%. Assays of endothelial NOS contained 30 μl of homogenate while those of cerebellar and splenic NOS contained 20 μl of the supernate obtained by centrifuging the tissue homogenates at 9000 g for 3 min. L-NAME (0.3 mM) or the Ebs compounds (0.3—300 μM) were preincubated with the homogenates for 5 min. before addition of L-arginine-[2,3-^{3}H] (approximately 200 000 dpm). Reactions were stopped after 30 min. with 1 ml of ice-cold Tris buffer (pH 5.0) containing L-NAME (0.3 mM) and EDTA (100 mM) and the samples were then applied to chromatography columns containing 1 ml of wet bed volume of AG 50WX-8 (Na^+ form, Bio-Rad) followed by 1 ml of Tris buffer (pH 5.0 containing 100 mM EDTA). Radioactivity present in 2 ml of column effuent was quantified by liquid scintillation counting and was considered to be indicative of NOS-dependent formation of L-citrulline-[2,3-^{3}H]. Total NOS activity was defined as the difference between controls and the L-NAME (0.3 mM) blanks; this definition explains the

seemingly paradoxical observation of greater than 100% NOS activity at low concentrations and the greater than 100% inhibition of NOS activity at high concentrations of Ebs-like compounds in certain experiments.

Statistical analysis

Results are expressed as means ±SEM of n observations. In organ bath experiments, n is the number of arterial rings used in a particular protocol from separate rabbits. In NOS assays, n is the number of observations made at a particular concentration of an Ebs-like compound form. Statistical differences between means were assessed using unpaired two-tailed Student's t test or, in the case of multiple means, one-way analysis of variance followed by *post hoc* Bonferroni test. A p value less than 0.05 was considered statistically significant. IC_{50} values were determined by computer generated linear regression of data from the NOS assays.

RESULTS

Glutathione peroxidase-like activity of ebselen-like compounds

The incubation of 1 mM solution of NAC with H_2O_2 (1 mM) resulted in decreased concentrations of free SH groups (*Fig. 2*). Ebs, DiEbs, HOOC-Ebs, and HOOC-DiEbs (all at 30 μM) markedly and equipotently enhanced the rate

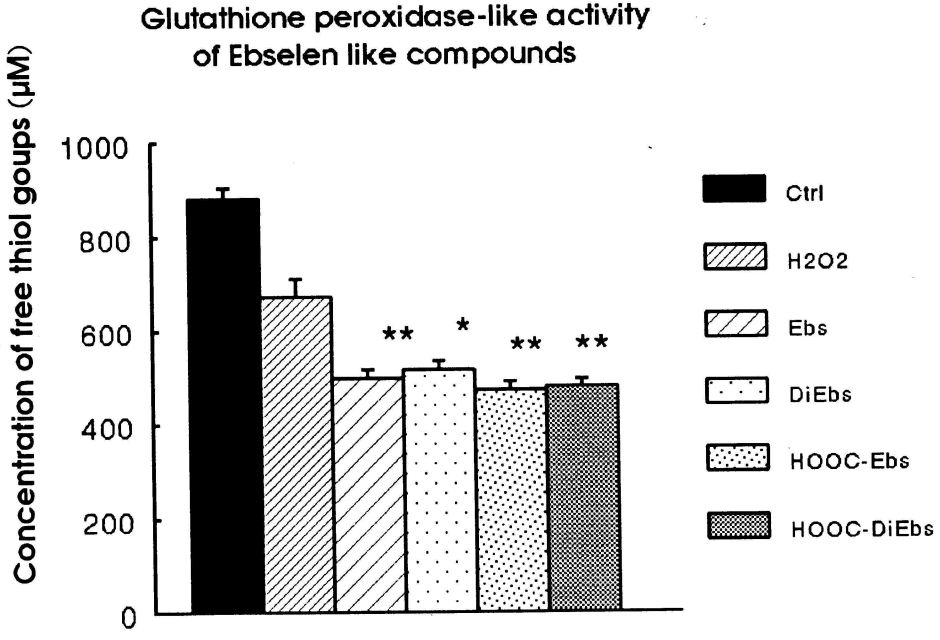

Fig. 2. Glutathione peroxidase-like activity of ebselen-like compounds. The incubation of 1 mM solution of N-acetyl-L-cysteine with 1 mM H_2O_2 resulted in a decreased concentration of free SH groups (H_2O_2) when compared with controls. In the presence of ebselen (Ebs, 30 μM), carboxyebselen (HOOC-Ebs, 30 μM), or their diselenide derivatives (DiEbs, HOOC-DiEbs, both at 30 μM) the rate of oxidation of thiols by H_2O_2 was markedly enhanced. The four compounds were equipotent. Values represent means° +/− SEM of 4 determinations. ** Bonferroni $p < 0.01$, * $p < 0.05$, as compared to H_2O_2.

of oxidation of thiols by H_2O_2 (*Fig. 2*). This observation is consistent with the proposed mechanism of action of ebselen-like compounds and indicates that the glutathione peroxidase-like activity of ebselen is not influenced by its transformation into a carboxylic acid or their (Ebs or HOOC-Ebs) transformation to diselenides.

Carboxyebselen inhibits selectively the endothelial isoform of NOS and is more potent than ebselen

The activities of NOS present in 30 µl of endothelial, 20 µl of cerebellar and 20 µl of splenic homogenates did not differ and averaged 17947+/2063 (n = 12), 22997+/−3416 (n = 16) and 17034+/−3397 (n = 13) dpm of L-citrulline-[2,3-^{3}H]/30 min., respectively. As shown in *Fig. 3* and 4, both Ebs (0.3—30 µM) and HOOC-Ebs (0.2—30 µM) dose-dependently inhibit the activity of endothelial NOS (n = 5—6). In contrast to their effects on

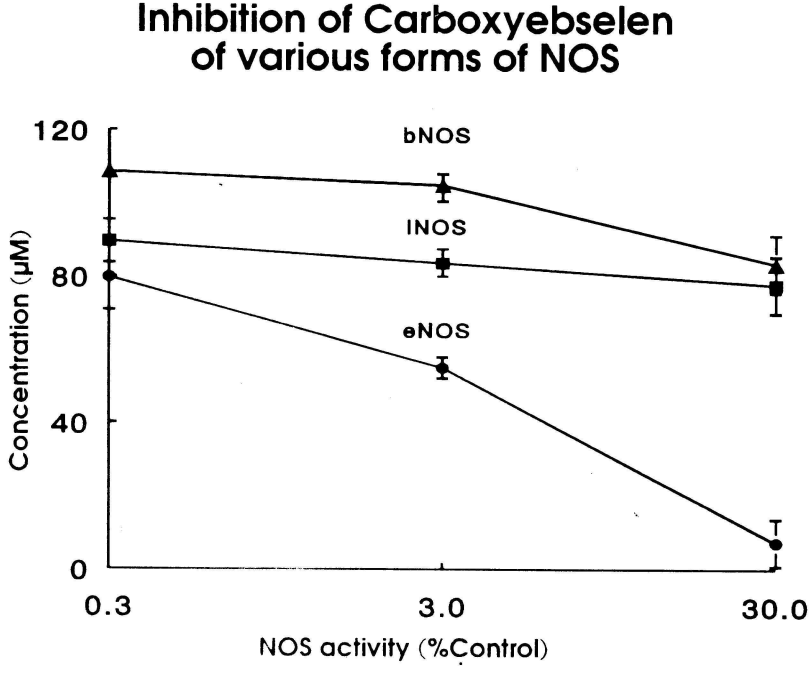

Fig. 3. Selective inhibition of endothelial NOS by carboxyebselen. Figure shows the effect of increasing concentrations of carboxyebselen on the activity of endothelial NOS (eNOS) present in homogenates of bovine aortic endothelium, brain NOS (bNOS) present in homogenates of porcine cerebellum and inducible NOS (iNOS) present in homogenates of spleen obtained from lipopolysaccharide-treated rats. NOS activity was expressed as L-NO_2Arg(300 µM)-inhibitable conversion of L-arginine-[2,3-^{3}H] to L-citrulline-[2,3-^{3}H]. Values represent means +/− SEM of n = 6—10 determinations from n/2 homogenates which were also used for construction of *Fig. 4*.

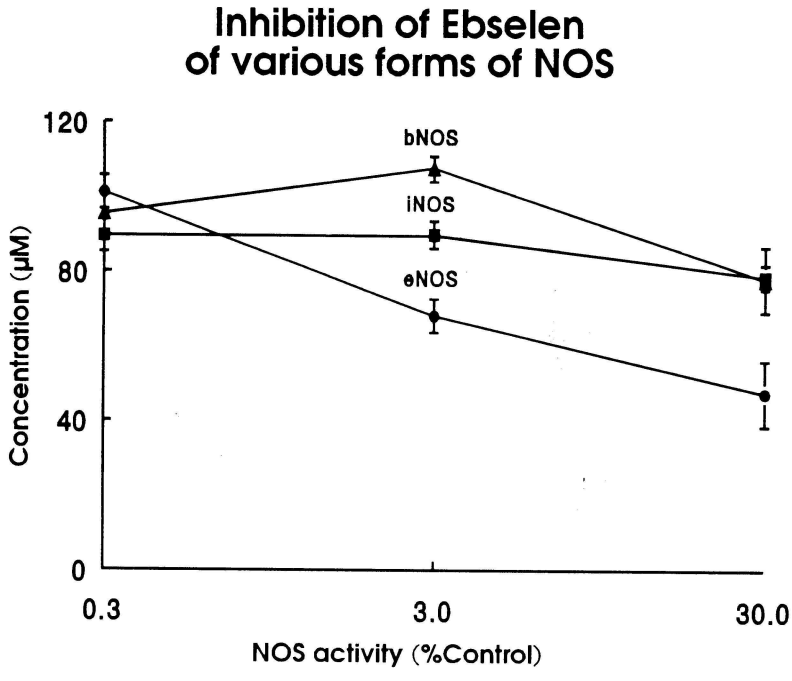

Fig. 4. Selective inhibition of endothelial NOS by ebselen. Figure shows the effect of increasing concentrations of ebselen on the activity of endothelial NOS (eNOS) present in homogenates of bovine aortic endothelium, brain NOS (bNOS) present in homogenates of porcine cerebellum and inducible NOS (iNOS) present in homogenates of spleen obtained from lipopolysaccharide-treated rats. NOS activity was expressed as L-NO_2Arg (300°μM)-inhibitable conversion of L-arginine-[2,3-^{3}H] to L-citrulline-[2,3-^{3}H]. Values represent means +/−SEM of n = 6—10 determinations from n/2 homogenates which were also used or construction of *Fig. 3.*

endothelial NOS, Ebs and HOOC-Ebs were only weak inhibitors of the constitutive NOS present in homogenates of porcine cerebellum and the inducible NOS present in homogenates of spleen obtained from lipopolysaccharide-treated rats (*Figs. 3* and *4*, n = 6—10). The endothelial, cerebellar, and inducible isoforms of NOS were inhibited more potently by HOOC-Ebs than by Ebs. In particular HOOC-Ebs at concentration of 30 μM, was both a more potent and a more selective inhibitor of endothelial NOS than Ebs (*Figs. 3* and *4*). At a concentration of 300 μM HOOC-EbS inhibited eNOS by 100%, bNOS by 46±12% and iNOS by 70±15% (n = 16, not shown in *Fig. 3*).

Carboxyebselen causes an endothelium-independent relaxation of rabbit aorta

Increasing concentrations of HOOC-Ebs (1.0—30 μM) reduced the tone of rabbit aorta rings precontracted with Phe (0.2 μM) in a dose-dependent manner. The presence of endothelium is not required for these relaxations.

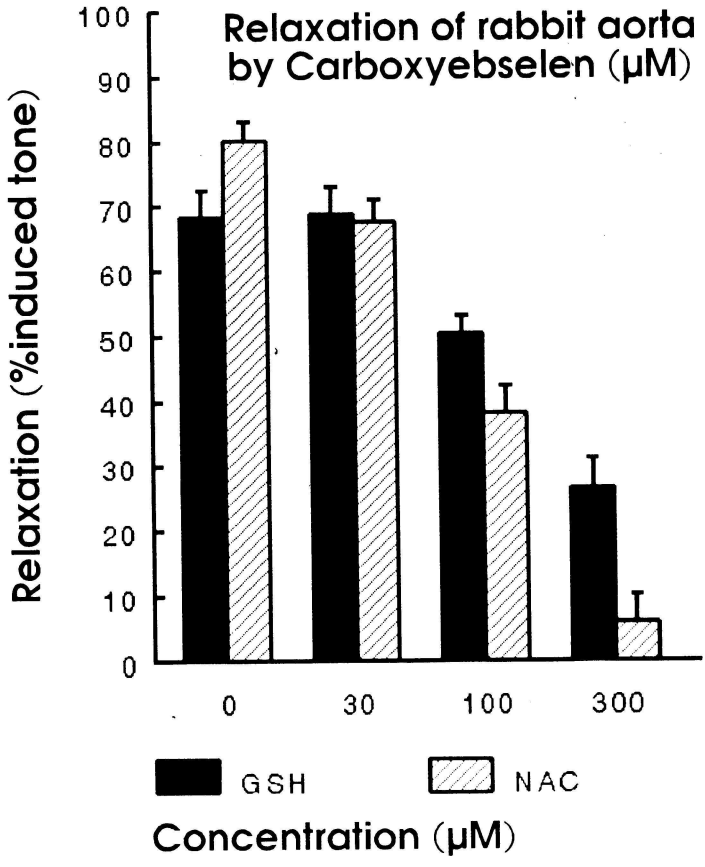

Fig. 5. Attenuation of the endothelium-independent relaxation induced in rings of rabbit aorta by carboxyebselen (10 μM) in the presence of increasing concentrations of glutathione (GSH) or N-acetyl-L-cysteine (NAC). Values represent means +/−SEM of n = 5—6 separate experiments.

Inhibition by GSH and NAC of carboxyebselen-induced endothelium-independent relaxation of rabbit aorta

Neither NAC nor GSH at concentrations of 30—300 μM has any affect on either the basal tone of rings of rabbit aorta or the Ach-induced relaxation of rings with intact endothelium (12). However, both NAC and GSH (30—300 μM) attenuated, in a concentration-dependent manner, the vasorelaxant action of HOOC-Ebs in aortic rings (*Fig. 5*). The efficacies of NAC and GSH were significanty different only at a concentration of 300 μM, with NAC being a more potent compound ($n = 5—6$, $p < 0.05$ by *post hoc* Bonferroni test).

Carboxyebselen does not inhibit the vasorelaxant activity of Ach in rings of rabbit aorta

Direct measurement of the vasorelaxant activity of Ach in rings of rabbit aorta after the addition of HOOC-Ebs (10 μM) was impossible because of the simultaneous endothelium-independent relaxation induced by HOOC-Ebs. The indirect methods which we devised for overcoming this technical difficulty, while perhaps being less convincing than direct demonstration, strongly support the hypothesis that HOOC-Ebs does not inhibit the NOS of intact endothelial cells.

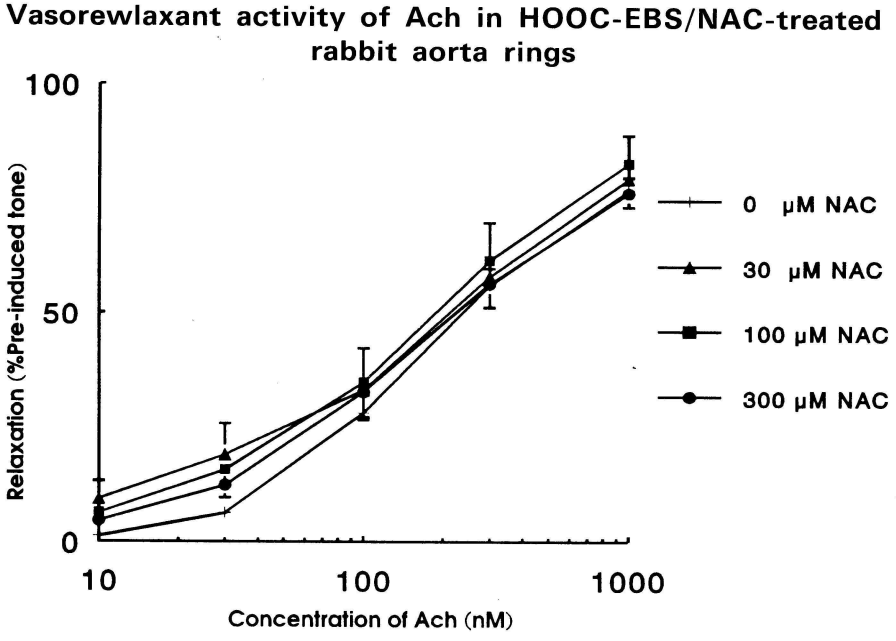

Fig. 6. Carboxyebselen (HOOC-Ebs) does not inhibit NOS of intact endothelial cells. As shown in Fig. 5, HOOC-Ebs (10 μM) causes an endothelium-independent relaxation of rings of rabbit aorta that is attenuated in a concentration-dependent manner by N-acetyl-L-cysteine (NAC, 30—300 μM). The vasorelaxant activity of acetylcholine (Ach, 0.01—1 μM) in these rings was unchanged when compared with that in tissues not exposed to HOOC-Ebs (Control). Values represent means +/−SEM of n = 5 separate experiments. Identical results were obtained with glutathione (n = 6).

Direct measurement of the vasorelaxant activity of Ach on rings of rabbit in the presence of low concentrations of HOOC-Ebs (0.1—1.0 μM) having minimal effects on the smooth muscle tone showed no inhibition of endothelium-dependent relaxation when compared with controls.

Direct measurement of the vasorelaxant activity of Ach on rings of rabbit aorta in which the endothelium-independent relaxation by HOOC-Ebs was

attenuated by increasing concentrations of NAC (30—300 μM) showed that the endothelium-dependent relaxations remained unchanged when compared with controls (*Fig. 6*). Similar results were obtained with GSH (n = 6).

DISCUSSION

Here we report that carboxyebselen (HOOC-Ebs) a carboxylated analogue of ebselen (Ebs, 2-phenyl-1,2-benzisoselenazol-3-(2H)-one), and the diselenide derivatives of both compounds (HOOC-DiEbs, DiEbs), possess mild glutathione peroxidase-like properties as it was earlier described for Ebs (2). This enzyme-like activity is equally distributed among all four compounds, which at concentrations of 30 μM each increase the reduction of H_2O_2 by NAC by 25%. However, the major finding of this paper is that at the same concentrations of 30 μM Ebs inhibits endothelial NOS by 50% while HOOC-Ebs by 95%. HOOC-Ebs is a more potent inhibitor of endothelial NOS as compared to Ebs. It is also a more selective inhibitor of NOS from bovine aortic endothelium as compared to other isoforms of NOS from porcine cerebellum or murine spleen. Unlike Ebs, HOOC-Ebs seems to fail to inhibit NOS in intact endothelial cells of rabbit aortic rings, possibly owing to reduced lipophilic properties of the molecule of HOOC-Ebs as compared to Ebs. Another difference between Ebs and its carboxylated derivative is that this last shows an endothelium-independent vasorelaxant action on rabbit aortic rings. The mechanism of this reversible phenomenon remains unexplained, apart from the fact that thiols such as GSH and NAC prevent HOOC-Ebs induced relaxations.

Wang et al. (13) were the first to report the inhibition of NOS by Ebs, specifically the NOS present in cultures of LPS-pretreated rat Kuppffer cells. Their results, while provocative, required clarification and confirmation in that the technique by which the production of NO was assessed, (spectrophotometric measurement of the conversion of oxyhemoglobin to methemoglobin) is indirect and reliable only in cell-free systems (16). Thus, the findings of Wang et al. (13) are subjected to the criticism that they do not prove that the diminished oxidation of oxyhemoglobin in the presence of Ebs can be accounted solely for inhibition of the formation of NO. Our initial studies with Ebs demonstrated the irreversible and relatively selective inhibition by Ebs of the endothelial isoform of NOS (12). We reported the concentration-dependent inhibition by Ebs (3—30 μM) of the vasorelaxant effects of Ach (0.01—3 μM) as well as of calcium ionophore A23187 (0.01—1 μM), a receptor-independent activator of endothelial NO biosynthesis, and the lack of inhibition by Ebs of relaxations induced by nitroglycerin (0.01—3 μM), an exogenous donor of NO. The mechanism of inhibition of NOS by Ebs, we claim, is independent on its

GSH peroxidase-like activity and therefore of any putative "removal of peroxide tone". On contrary the inhibitory action of Ebs on NOS can be prevented but not reversed by GSH and NAC (30—500 μM). Finally, in an enzyme assay system in which the conversion of L-arginine-[2,3-^{3}H] to L-citrulline-[2,3-^{3}H], of NO, was measured, we found Ebs to be a relatively selective inhibitor of the endothelial isoform of NOS as compared with the constitutive enzyme found in porcine cerebellum and inducible enzyme harvested from the spleens of LPS-treated rats. On the basis of these and other findings we suggested that the inhibitory activity of ebselen is mediated through a covalent modification of NOS, and possibly through the inactivation of a thiol group essential to the catalytic activity of the enzyme.

Wang et al. (13) showed that both the sulfur-containing analog of ebselen (2-phenyl-1,2-benzisothiazol-3[2H]one; 10 μM) and a methylated derivative of ebselen-(methylselenobenzanilide; 10 μM) produced only slight inhibitory effects on TPA-induced luminol chemiluminescence (an indicator of the activity of NADPH oxidase) and LPS-induced NO formation in Kuppffer cells, findings consistent with the hypothesis of Cotgreave et al. (6) that the mechanism through which Ebs inhibits NADPH oxidase may involve reaction with critical thiol/disulfide groups in that enzyme. The carboxyl group of HOOC-Ebs, however, is attached to the phenyl side chain of Ebs at the *para* position and thus could be expected, in the first place, to have relatively little impact on the redox status of the selenium atom and, in the second, to enhance the solubility while adversely affecting the lipophilicity of Ebs.

In our previous study we suggested that the isoform-selective inhibition of NOS by Ebs may be due either to differences in the accessibility to Ebs of the functionally critical thiol in various isoforms of NOS or to pharmacokinetic factors of Ebs peculiar to the assay systems we employed. While the second consideration cannot be ruled out entirely with respect to the more potent and more selective inhibition by HOOC-Ebs of the enzymic preparation of endothelial NOS as compared with Ebs, the more plausible explanation is that the altered physicochemical characteristics of the molecule enhance its affinity for the region of endothelial NOS in which the putative critical thiol is located. If this is the case, it would indicate that the lipophilicity of Ebs is of relatively less importance vis-a-vis the endothelial NOS an their putative thiol is probably not sequestered in a lipophilic pocket of the NOS.

Of course, the transmembrane passage of a molecule of Ebs-like NOS inhibitors strongly depends on their lipophilicity. Our experiments with rings of rabbit aorta support this line of reasoning in that we could demonstrate no evidence whatsoever of inhibition by HOOC-Ebs of the NOS of intact endothelial cells. That HOOC-Ebs had no effect at all, in light of its proved superior potency when compared with Ebs in crude enzyme preparations, strongly suggests that in this system NOS is completely or almost completely

inaccessible to HOOC-Ebs. The simplest explanation under these circumstances is that HOOC-Ebs, by virtue of its greater hydrophilicity, is much less capable than Ebs of diffusing through the cell membrane and is therefore unable to interact with and inactivate the membrane-bound NOS of endothelial cells.

The above findings demonstrate that ebselen founded a new class of NOS inhibitors different from that of reversible arginine metabolites like N^G-monometyl-L-arginine or N^G-nitro-L-arginine and structural changes in the molecule of ebselen may increase its potency and selectivity towards constitutional endothelial NOS, but also abolish inhibitory activities on NOS in intact cell systems.

We conclude that HOOC-Ebs, in comparison with Ebs, is a more potent and more selective inhibitor of endothelial NOS in open cell systems but that its activity is limited with respect to intact endothelial cells. We suggest that the abolition of the activity of HOOC-Ebs in intact endothelial cells can be explained in terms of the comparatively reduced lipophilicity of the molecule.

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