Augmented BH4 by gene transfer restores nitric oxide synthase function in hyperglycemic human endothelial cells

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Abstract

Objective: Endothelial dysfunction in diabetes is characterized by decreased nitric oxide (NO) bioactivity and increased superoxide (SO) production. Reduced levels of tetrahydrobiopterin (BH4), an essential cofactor of endothelial NO synthase (eNOS), appear to be associated with eNOS enzymatic uncoupling. We sought to investigate whether augmented BH4 biosynthesis in hyperglycemic human aortic endothelial cells (HAEC) by adenovirus-mediated gene transfer of GTP cyclohydrolase I (GTPCH, the rate-limiting enzyme for the de novo BH4 synthesis), would be sufficient to rescue eNOS activity and dimerization. HAEC were cultured in media with low glucose (5 mM) or high glucose (30 mM).

Methods: After 5 days, the cells with/without GTPCH gene transfer (AdeGFP as a control) were prepared for assays of (1) NO with electron paramagnetic resonance (EPR); (2) SO with cytochrome c reduction and dihydroethidine (DHE) fluorescence; (3) BH4 with high-performance liquid chromatography (HPLC); (4) eNOS expression and dimerization with immunoblotting.

Results: We found that high glucose decreased HAEC NO and increased SO production, in association with reductions in both total biopterin and BH4 levels. High glucose increased total eNOS protein levels in HAEC 1.5-fold, but this was present principally in the monomeric form. GTPCH gene transfer increased cellular biopterin levels and NO production but decreased SO production. Furthermore, augmenting BH4 increased the eNOS dimer:monomer ratio 2.6-fold.

Conclusion: This study demonstrates a critical role for BH4 in regulating eNOS function, suggesting that GTPCH is a rational target to augment endothelial BH4 and recover eNOS activity in hyperglycemic endothelial dysfunction states.

Keywords: Endothelial; Nitric oxide; Superoxide; Gene therapy

This article is referred to in the Editorial by J. Bauersachs and A. Schäfer (pages 768–769) in this issue.

1. Introduction

Nitric oxide (NO) generated by endothelial nitric oxide synthase (eNOS) is an important regulator of vascular homeostasis. In vascular disease states such as diabetes, endothelial dysfunction is characterized by a decrease in NO bioactivity and a concomitant increase in superoxide (SO) formation [1], despite the observation that eNOS mRNA and protein levels are maintained or even increased [2–4]. Previous data suggest that enzymatic ‘uncoupling’ of eNOS may in part account for both reduced NO production and increased SO production [5].

Tetrahydrobiopterin (BH4) is an essential cofactor for activity of all NOS enzymes [5,6]. GTP cyclohydrolase I (GTPCH; EC 3.5.4.16) is the rate-limiting enzyme for BH4 de novo biosynthesis by catalyzing GTP to dihydroneopterin triphosphate. BH4 is finally produced through further steps catalyzed by 6-pyruvol-tetrahydrop-
The role of BH4 in NOS regulation remains unclear, but it appears to facilitate electron transfer from the eNOS reductase domain and maintains the heme prosthetic group in its redox active form [8–10]. Moreover, intracellular BH4 is required to promote and possibly stabilize eNOS protein in the active homo-dimer form [11–13]. Incubation of human aortic endothelial cells in high glucose inhibits eNOS-derived NO production [14,15], and BH4 supplementation can recover NO activity [16]. Similarly, endothelial dysfunction in vessel rings from models of diabetes is improved by pharmacologic supplementation of BH4 [3,17]. Hyperglycemia is associated with reduced intracellular BH4 bioavailability [18] and recent data suggest that the loss of BH4 in the hyperglycemic environment is the consequence of oxidative stress [1,19]. In humans, BH4 administration appears to augment NO-mediated effects on forearm blood flow in patients with diabetes [20]. However, the effects of BH4 on NO bioactivity observed in these studies may not be entirely mediated through direct modulation of eNOS activity, because high concentrations of extracellular BH4 may result in non-specific anti-oxidant effects that indirectly increase NO bioactivity by SO scavenging [19]. Conversely, BH4 supplementation using the precursor sepiapterin may have unpredictable pro-oxidant effects in vascular disease states that augment SO release from eNOS [21,22]. Thus, the role and mechanisms of BH4 in regulating eNOS activity and protein dimerization in diabetes remain controversial based on studies using pharmacologic interventions.

Accordingly, we sought to investigate (1) the impact of hyperglycemia on BH4 levels with eNOS activity and protein dimerization; (2) the role of intracellular BH4 in regulating eNOS activity and protein dimerization, using adenovirus-mediated gene transfer of GTPCH in hyperglycemic human aortic endothelial cells.

2. Experimental procedures

2.1. Cell cultures

The investigation conforms with the principles outlined in the declaration of helsinki (Cardiovascular Research 1997:35:2–4).

Human aortic endothelial cells (HAEC) and cell culture media were obtained from TCS Cellworks (Botolph Claydon, UK). The cells were grown in large vessel endothelial cell growth medium at 37 °C in a 5% CO2 atmosphere, and used for experiments between passages 3 and 6. Prior to experiments, the cells were incubated in medium containing either low glucose (5 mM) or high glucose (10–30 mM) for 5 days. For adenoviral infection, cells were grown to confluence and inoculated with virus at a multiplicity of infection (MOI) of 20 for 1 h, then replaced with the medium for 24 h.

2.2. Construction of adenovirus vectors

The adenovirus (AdGCH) encoding human GTPCH, incorporating a C-terminal HA epitope tag, under the control of the CMV immediate-early promoter was constructed as described previously [13]. The recombinant adenoviruses, AdeGFP and AdβGal encoding eGFP or β-galactosidase, respectively, were used as controls for viral infection.

2.3. NOS activity assays by electron paramagnetic resonance

NO production in cells was assayed using colloid Fe(DETC)2 spin-trapping and EPR spectroscopy [23,24]. The cells were passaged into 6-well plates (1×106/well) and incubated with low or high glucose medium. After 5 days of incubation, the cell monolayers were washed three times with Krebs-HEPES buffer (KHB) (NaCl 99 mM, KCl 4.7 mM, MgSO4 1.2 mM, KH2PO4 1.0 mM, CaCl2 1.9 mM, NaHCO3 25 mM, HEPES 20 mM). Colloid Fe(DETC)2 was made by separately dissolving 3.6 mg NaDETC (Alexis) and 2.25 mg FeSO4·7H2O (BDH Laboratory Supplies) in 10 ml ice-cold KHB under argon gas bubbling, and mixing immediately before use. Triplicate wells were incubated for 2 h in 700 μl KHB containing 0.4 mM colloid Fe(DETC)2, calcium ionophore (A23187; 1 μM) and L-arginine (100 μM). The cell monolayers were harvested, pelleted and resuspended in 100 μl KHB, and then frozen using liquid nitrogen. EPR spectra were obtained using a benchtop X-band EPR spectrometer (Miniscope MS 200, Magnettech, Germany), equipped with a rectangular resonator cavity H102. Instrument settings were: center-field (B0) 3276 G, sweep 115 G, microwave power 10 mW, modulation frequency 100 kHz, amplitude modulation 8000 mG, sweep time 60 s, and number of scans 4. Signals were quantified by measurement of NO-Fe(DETC)2 EPR signals from the cell extracts. Data are shown as mean±SD of two sets of triplicates determination and expressed as arbitrary unit/hour/106 cells. (*p<0.05, n=6).

Fig. 1. Decreased NO production in hyperglycaemic human aortic endothelial cells. HAEC were cultured with either low glucose (5 mM) or high glucose (30 mM) media. After 5 days, HAEC monolayers were stimulated with A23187 (1 μM) for 2 h. eNOS activity was determined by measurement of NO-Fe(DETC)2 EPR signals from the cell extracts. Data are shown as mean±SD of two sets of triplicates determination and expressed as arbitrary unit/hour/106 cells. (*p<0.05, n=6).
Fig. 2. Increased SO production in hyperglycaemic human aortic endothelial cells. HAEC were measured for SO production after 5 days incubation with either low glucose (5 mM) or high glucose (30 mM) medium. (A) HAEC monolayers were incubated with cytochrome c (50 μM), with or without SOD (350 u/ml) for 30 min. SO production was spectrophotometrically determined by calculating the absorbance differences between the cell extracts with or without SOD. Data are shown as mean±SD of two sets of triplicates and expressed as arbitrary unit/10^6 cells (*p<0.01, n=6). (B) The cell monolayers were incubated with DHE (2.5 μM) in the presence or absence of PEG-SOD (500 u/ml) for 2 min. Fluorescence intensity (red) of cells in high glucose medium increased compared with cells in low glucose medium, and were abolished in the presence of PEG-SOD. Each experiment was repeated three times. (C) HAEC monolayers were incubated with cytochrome c (50 μM), with or without SOD (350 u/ml) in the presence of L-NAME (100 μM), DPI (50 μM), or Chelerythrine (10 μM), respectively, for 30 min. SO production was spectrophotometrically determined by calculating the absorbance differences between the cell extracts with or without SOD. Data are shown as mean±SD of two sets of triplicates and expressed as arbitrary unit/10^6 cells (*p<0.001; **p<0.001; ***p<0.05, n=6).
measuring the total amplitude, after correction of baseline, and expressed as arbitrary units/h/10⁶ cells.

2.4. SO production assays

Two different methods were used to determine SO levels in HAEC; ferricytochrome c reduction [25] and dihydroethidine (DHE) fluorescence. For cytochrome c reduction assays, cells were passaged in 12-well plates (5×10⁵ cells/well) in the growth medium containing either low or high glucose. After 5 days, the cell monolayers were washed once with KHB and incubated at 37 °C for 30 min with KHB containing 50 μM cytochrome c (Sigma) in the presence or absence of superoxide dismutase (SOD) (350 units/ml) (Sigma), or in the presence of 100 μM N⁵-nitro-L-arginine (L-NAME), 50 μM diphenyleneiodonium (DPI) or 10 μM chelerythrine (Sigma). The absorbance of the medium was read spectrophotometrically at 550 nM. Reduction of cytochrome c in the presence of SOD was subtracted from the values without SOD. For detection of DHE fluorescence, cells were grown in chamber slides (2×10⁵ cells/well) (Nalge Nunc International) in medium containing low or high glucose. After 5 days, cells were incubated at 37 °C for 2 min with KHB containing 2 μM dihydroethidine (Molecular Probes) in the presence or absence of PEG-SOD (500 units/ml) (Sigma), and were washed immediately with ice-cold KHB and imaged using a Bio-Rad MRC1024 confocal microscope.

2.5. Determination of biopterins in cell lysates

Determination of biopterin levels in cell lysates was carried out as described previously [13]. Briefly, cell pellets from 12-well plates were lysed in cold extract buffer (50 mM Tris–HCl, pH 7.4, 1 mM DTT, 1 mM EDTA). Protein concentration was measured using the Bio-Rad protein assay. Proteins were removed by adding 10 μl of a 1:1 mixture of 1.5 M HClO₄ and 2 M H₃PO₄ to 90 μl of extract, followed by centrifugation. To determine total biopterins (BH₄, BH₂, biopterin) by acid oxidation 10 μl of 1% iodine in 2% KI solution was added to 90 μl of extracts, followed by centrifugation. Alkaline-oxidation samples were then acidified with 20 μl of 1 M H₃PO₄. Iodine was reduced by adding 5 μl of fresh ascorbic acid (20 mg/ml). HPLC was performed using a Spherisorb ODS-1 column (Waters, Elstree, UK) with a 10% methanol/90% water mobile phase run at 0.4 ml/min. Fluorescence detection (350 nm excitation, 450 nm emission) was performed using an FP-2020 detector (Jasco UK, Essex, UK). BH₄ concentration, expressed as pmol/mg protein, was calculated by subtracting BH₂+biopterin from total biopterins. All samples were determined in triplicate.

2.6. Western blot analysis

Western blot analysis was performed as described previously [13]. Briefly, cells were lysed in 50 mM Tris–HCl pH 8, containing 0.2% Nonidet P-40, 180 mM NaCl, 0.5 mM EDTA, 100 mM phenylmethylsulfonyl fluoride, 1 M DTT and protease inhibitors (Boehringer Complete). Equal amounts of cellular proteins were resolved by 6% SDS-PAGE and transferred to PVDF membranes. To investigate eNOS homodimer formation in endothelial cells, low-temperature SDS-PAGE was employed as described previously [13]. Membranes were incubated with either a 1:2000 dilution of mouse anti-eNOS monoclonal antibody (Transduction Laboratories), or 1:1000 dilution of rat anti-HA high affinity monoclonal antibody (Roche). Bands were visualized using chemiluminescence, and quantified using NIH Image software.

2.7. Statistics

Results are expressed as mean±SD. Statistical significance of differences between means was assessed using ANOVA single factor. A p value <0.05 was considered statistically significant.

3. Results

3.1. NO production in hyperglycemic HAEC

Initially, we examined the effects of experimental hyperglycemia on NO production in HAEC using EPR. Following culture in medium containing low glucose (5 mM) or high glucose (30 mM), cells were stimulated for 2 h with A23187 in the presence of colloid Fe(DETC)_2. EPR quantification of NO-Fe(DETC)_2 adduct revealed a

Fig. 3. Reduced BH4 in hyperglycaemic human aortic endothelial cells. HAEC were cultured in either low glucose (5 mM) or high glucose (30 mM) medium. After 5 days incubation, total biopterin and BH4 levels were measured in cell pellets by differential oxidation in acid and base followed by the reverse phase HPLC. Data are shown as mean±SD of two sets of triplicates determination and expressed as pmol/mg of cell protein. (*p<0.01, n=6).
30% (n=6, p<0.05) decrease in NO production in high glucose cultures (Fig. 1).

3.2. Superoxide production in hyperglycemic HAEC

We next determined the effects of hyperglycemia on SO production in HAEC, using two complementary methods. Cytochrome c reduction revealed a 40% increase in SO production in high glucose cultures (n=6, p<0.01) (Fig. 2A). In line with this finding, we also observed significantly increased DHE fluorescence in cells cultured in high glucose (Fig. 2B). The signal was abolished when incubated with PEG-SOD, demonstrating specificity of the signal for SO.

In order to investigate sources of SO production in HAEC, we incubated the cells with various oxidase inhibitors, including L-NAME to inhibit eNOS, DPI to inhibit NADPH oxidase and chelerythrine to inhibit the PKC signaling pathway (Fig. 2C). Cytochrome c reduction assays revealed significant inhibition of SO production in high glucose cultures in response to L-NAME, DPI and chelerythrine. These results suggest that the important sources of SO production in HAEC are from uncoupled eNOS and NADPH oxidases. It also appears that PKC signaling plays a role in regulating SO production.

3.3. BH4 levels in hyperglycemic HAEC

We determined the impact of hyperglycemia on total cellular biopterin and BH4 levels using differential oxidation and HPLC (Fig. 3). Hyperglycemia reduced levels of total biopterin and BH4 by 30% and 60%, respectively (n=6, p<0.01).

3.4. eNOS dimerization in hyperglycemic HAEC

Table 1
Effect of GTPCH gene transfer on cellular biopterin production in hyperglycemic human endothelial cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total biopterin (pmol/mg)</th>
<th>BH4 (pmol/mg)</th>
</tr>
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<tbody>
<tr>
<td>Cells alone</td>
<td>3.14±3.38</td>
<td>1.01±0.73</td>
</tr>
<tr>
<td>Ad/GAL</td>
<td>3.41±2.03</td>
<td>2.02±2.42</td>
</tr>
<tr>
<td>AdGCH</td>
<td>109.33±33.5*</td>
<td>79.63±35.76*</td>
</tr>
</tbody>
</table>

HAEC were incubated with medium alone or infected with Ad/Gal, or AdGCH. After 24 h, cell pellets were harvested and biopterin levels were determined by HPLC using both acid-base oxidation with fluorometric detection. Values shown are means±SD of triplicate determinations (n=6, *p<0.001).

To further evaluate the relationship between BH4 levels and eNOS activity, we investigated whether eNOS protein levels and dimerization were altered by hyperglycemia. In high glucose medium, total eNOS protein levels were increased 1.5-fold (n=6, p<0.05) relative to low glucose (Fig. 4A and B). In contrast, the ratio of eNOS dimers to monomer (*p<0.05, n=6).

Fig. 4. eNOS protein expression and dimerization in hyperglycaemic human aortic endothelial cells. (A) HAEC were harvested after 5 days incubation in media containing either low glucose (5 mM) or high glucose (30 mM). Boiled or non-boiled cell lysates were fractionated by SDS-PAGE or low temperature SDS-PAGE, respectively, and immunoblotted with monoclonal antibody to eNOS. (B) Plot of eNOS band intensity (p<0.05, n=6). (C) Plot of eNOS band intensity: ratio of dimer vs. monomer (*p<0.05, n=6).

Fig. 5. Recombinant GTPCH expression increases NO production in hyperglycemic human aortic endothelial cells. HAEC were cultured in high glucose (30 mM) media for 5 days, and were further incubated with high glucose media in the presence or absence of AdGCH (20 MOI) (AdGFP as control). After 24 h of infection, the cells were stimulated with A23187 (1 µM) for 2 h. eNOS activity was determined by measurement of NO-Fe(DETC) EPR signals from the cell extracts. Data are shown as mean±SD of two sets of triplicates determination and expressed as arbitrary unit/h/10^6 cells. *p<0.05 vs. corresponding control conditions (n=6).
monomers, assayed by low-temperature SDS-PAGE and immunoblotting, was significantly lower in high glucose than in low glucose ($n=6$, $p<0.05$). Thus, increased total eNOS protein levels in hyperglycemia is associated with a relative reduction in the abundance of the active dimeric form.

Taken together, these data show that experimental hyperglycemia in human endothelial cells results in decreased NO production, and increased SO production. Moreover, in hyperglycemic condition BH4 levels are reduced and eNOS protein is present mainly in the inactive monomeric form, despite an overall increase in total eNOS protein levels.

### 3.5. Effect of GTPCH gene transfer on hyperglycemic HAEC

We next set out to determine whether the effects of hyperglycemia on eNOS function could be rescued by augmenting intracellular BH4 synthesis, using GTPCH gene transfer. HAEC monolayers, incubated in high glucose medium, were infected with AdGCH (MOI 20) or with AdβGal as a control. Biopterin levels in cell lysates were determined by using differential oxidation followed by fluorometric detection with HPLC. As shown in Table 1, cells alone or cells after AdβGal infection had very low levels of total biopterin and BH4, which were increased more than 40-fold by AdGCH infection ($n=6$, $p<0.001$).

To investigate the effects of GTPCH overexpression by gene transfer on eNOS activity, we measured NO production by calcium ionophore (A23187)-stimulated HAEC in the presence of colloid Fe(DETC)$_2$. AdGCH infection increased eNOS activity by approximately 30% ($n=6$, $p<0.05$) (Fig. 5). In contrast, eNOS activity in cells alone or infected with AdeGFP remained at low levels. Thus, recombinant GTPCH expression in endothelial cells by...

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**Fig. 6.** Recombinant GTPCH expression reduces SO production in hyperglycaemic human aortic endothelial cells. HAEC were cultured with high glucose (30 mM) media for 5 days, and incubated with the media in the presence or absence of AdGCH (20 MOI) (AdβGal as control) for another 24 h. (A) HAEC monolayers were incubated with cytochrome c (50 μM), with or without SOD (350 u/ml) for 30 min. SO production was spectrophotometrically determined by calculating the absorbance differences between the cell extracts with or without SOD. Data are shown as mean±SD of two sets of triplicates determination and expressed as arbitrary unit/min/10⁶ cells ($*p<0.01$, $n=6$). (B) The cell monolayers were incubated with DHE (2.5 μM) in the presence or absence of PEG-SOD (500 u/ml) for 2 min. Cells infected with AdGCH had reduced fluorescence intensity (red) compared with cells alone or cells infected with AdeGFP. The signals were abolished in the presence of PEG-SOD. Each experiment was repeated three times.
adenoviral gene transfer is sufficient to increase both intracellular biopterin levels and eNOS activity.

We next compared SO production in hyperglycemc HAEC, and cells after AdGCH or AdβGal gene transfer. Using cytochrome c reduction, we observed a 40% reduction in SO after AdGCH infection (Fig. 6A). Furthermore, DHE fluorescence was almost abolished in the cells infected with AdGCH but remained unchanged with AdβGal (Fig. 6B).

Finally, we investigated the effects of GTPCH gene transfer on eNOS dimerization in hyperglycemc HAEC. In control cells, eNOS was present mainly as the 135 kDa monomer (n=6, p<0.05). In contrast, eNOS protein in cells infected with AdGCH was predominantly in the active homodimeric form, which corresponded with enhanced eNOS activity (Fig. 5). Densitometric quantification of blots from two individual experiments showed that in AdGCH infected cells, the total quantity of eNOS protein was similar, but the dimer/monomer ratio was almost doubled compared with control cells (n=6, p<0.05) (Fig. 7B, C).

4. Discussion

In this study, we investigated the impact of experimental hyperglycemia on eNOS regulation by BH4 in human endothelial cells. We found that hyperglycemia results in BH4 deficiency and eNOS dysfunction characterized by decrease in NO and a concomitant increase in SO production. Furthermore, increased total eNOS protein was nevertheless associated with a relative reduction in the abundance of the active dimeric form. Using adenovirus-mediated GTPCH gene transfer, we showed that augmenting intracellular BH4 biosynthesis was sufficient to rescue the abnormalities in NO production, SO production and eNOS dimerization.

Our work adds further evidence to previous studies that high glucose culture reduces NO activity [14,15], increases SO production [2,4] and is associated with reduced eNOS dimerization [4] in endothelial cells. However, the relationship between these features and intracellular BH4 availability remains unexplored. Our study shows that hyperglycemia results in significant reductions in both total biopterins and BH4. BH4 bioavailability is postulated to be limiting in several vascular disease states [26]. Loss of BH4 appears to be one factor in eNOS uncoupling [18], whereby electron transfer from the reductase domain to the oxygenase domain of eNOS are diverted to molecular oxygen rather than L-arginine [8,27]. This leads to preferential SO production over NO production. Increased SO not only scavenges NO, but also forms peroxynitrite [4], a potent oxidant that can rapidly oxidize BH4 to BH3, and subsequently to BH2 [16,19]. BH2 may compete with L-arginine for eNOS, resulting in further impaired eNOS bioactivity [22].

Dimerization is an absolute requirement for all NOS catalytic activity [28–30]. Structural studies suggest a role for BH4 in NOS dimerization based on purified recombinant proteins in reconstituted cell-free systems [31,32]. A study based on bovine eNOS expressed in E. coli suggested that BH4 affected eNOS homodimerization [29]. In another study of recombinant eNOS purified from a baculovirus system, exogenously added BH4 increased both eNOS activity and dimerization [33]. Our observation of increased eNOS protein expression in response to high glucose culture in endothelial cells is consistent with other reports [4,14]. However, using low-temperature SDS-PAGE, we found that eNOS protein was mainly present in an inactive monomeric form. Thus, changes in eNOS dimerization may in part underlie the apparent discordance between total eNOS protein levels and eNOS activity in hyperglycemia.
The role of BH4 in the regulation of eNOS activity and dimerization remains controversial. A detailed structural study based on recombinant eNOS and native eNOS of bovine aortic endothelial cells (BAEC) cultured in high glucose suggested that dissociation of eNOS dimers to monomers was due to peroxynitrite oxidation on the zinc-thiolate cluster where the BH4 binding site is located [4], and nitrosylating the zinc-thiolate cluster at the dimeric interface [34]. After peroxynitrite oxidation the zinc-thiolate cluster, supplementation with high dose BH4 (100 μM) did not prevent dimer dissociation or reassemble the eNOS monomers [4]. However, another study found that dysfunctional eNOS in BAEC subjected to peroxynitrite was fully restored by supplementation of BH4, when EPR was used to measure NO production [16]. Indeed, these experiments based on supplementation of high doses of extracellular BH4 might lead to some unpredictable effects, such as superoxide generation that reduces NO bioactivity [21,35]; as well as anti-oxidant by simple removal of free radical species rather than any direct specific effect on NOS activity or regulation [8,16,19,36].

We investigated the effects of BH4 on eNOS function by targeting intracellular BH4 biosynthesis rather than by pharmacologic supplementation. GTPCH overexpression by adenoviral gene transfer is highly effective in augmenting endothelial cell BH4 levels with the consequence of allosteric effects on eNOS biological function and protein dimerization [13]. This intracellular increase in BH4 in relation to other cellular bipterins is specific to GTPCH gene transfer [13]. Our data thus support a view that the principal action of BH4 in HAEC cultured in high glucose is to restore eNOS normal enzymatic activity. This is accompanied by promoting eNOS homodimerization. It may suggest that the stability of integrity of the zinc-thiolate complex of eNOS requires the presence of BH4 coordination.

Our study also has important implications for therapeutic strategies aimed at restoring eNOS activity. eNOS uncoupled in diseased endothelium appears to be an important aspect of endothelial dysfunction in vascular disease states, including hypercholesterolemia, hypertension, and atherosclerosis [19]. In several of these conditions, BH4 deficiency is postulated to contribute to abnormal endothelial function. Increasing BH4 synthesis has been shown to restore eNOS function in diabetes [37] and atherosclerosis [38]. Our studies in human endothelial cells now provide proof-of-principle that strategies targeting the intracellular BH4 biosynthetic pathway are a rational and effective approach to augment BH4 levels in endothelial cells, and that this has specific effects on eNOS activity and regulation.

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References


