Calmodulin binding to inducible nitric-oxide synthase may play an important role in its Ca$^{2+}$-independent activity. Studies of inducible nitric-oxide synthase chimeras containing the calmodulin binding sequence of neuronal or endothelial nitric-oxide synthases show that the calmodulin binding sequence of inducible nitric-oxide synthase is necessary but not sufficient for the Ca$^{2+}$-independent activity. The mutations at lysine 525 located at the C terminus of the calmodulin binding sequence of inducible nitric-oxide synthase were examined for the effects on the Ca$^{2+}$-independent activity with chimeras containing the oxygenase or reductase domains of inducible or neuronal nitric-oxide synthases. Results show that the Ca$^{2+}$-independent binding of calmodulin is not solely responsible for maximal Ca$^{2+}$-independent activity of inducible nitric-oxide synthase. Lysine 525 of inducible nitric-oxide synthase may also play an important role in coordinating the maximal Ca$^{2+}$-independent activity.

All NOS$^1$ isoforms contain three similar functional domains, an oxygenase domain in the N-terminal half and a reductase domain in the C-terminal half with an intervening CaM-binding domain. NOSs require heme, BH$_4$, CaM, FMN, and FAD as essential cofactors and oxygen and NADPH as essential co-substrates for activity. Dimerization necessary for NOS activity occurs mainly through the intersubunit interactions of the oxygenase domains to form the active sites and is independent of the reductase domain (1). The enzymes catalyze the conversion of L-arginine to L-citrulline and nitric oxide in a stepwise reaction. During NO synthesis, NADPH-derived electrons pass through the flavins and across domains to the heme iron in the reaction. During NO synthesis, NADPH-derived electrons pass through the flavins and across domains to the heme iron in the reaction. During NO synthesis (2–4). Although CaM also binds mono-

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The abbreviations used are: NOS, nitric-oxide synthase; CaM, calmodulin; BH$_4$, 8,9,10,11-tetrahydro-5,6,7,8-tetrahydropteridine; nNOS, neuronal NOS; eNOS, endothelium NOS; iNOS, inducible NOS; NO, nitric-oxide, L-NAME, N′-nitro-L-arginine methyl ester; TFP, trifluoperazine.

**MATERIALS AND METHODS**

**Construction of Mutant NOSs**—Residue lysine 525 of iNOS, a conserved residue at the C terminus of the CaM-binding sequence, and the corresponding residues in chimeras nNOS-I$^{1-533}$, nNOS-I$^{504-1144}$ and nNOS-I$^{504-1144}$ were mutated to glutamate or alanine in cDNAs of K/ E iNOS, K/A iNOS, K/E nNOS-I$^{504-1144}$, K/A nNOS-I$^{504-1144}$.
Fig. 1. A, domain scheme for nNOS, iNOS, and chimeric NOSs. NOS enzymes contain oxygenase and reductase domains separated by a CaM binding sequence. The CaM-binding segment can be subdivided into the N-terminal flanking, canonical CaM-binding, and C-terminal flanking regions. NADPH, FAD, and FMN consensus-binding regions are located in the reductase domain, whereas heme, Arg, O₂, and BH₄ bind to the oxygen domain. Additionally, nNOS contains a PDZ domain located at its N-terminal end, which is a protein interaction module and is involved in cellular localization. Chimeric and mutant NOSs were constructed as described under “Materials and Methods.” Shown in the figure are nNOS, iNOS, nNOS-I₁–₅₃₃, (nNOS residues 726–1429 replaced with iNOS residues 504–1144), nNOS-I₁–₅₃₃ (nNOS residues 1–755 replaced with iNOS residues 1–533), nNOS-I₅₀₃–₅₃₃ (nNOS residues 275–755 replaced with iNOS residues 503–533), and point mutants (K/E nNOS, K/A iNOS, K/E nNOS-I₁–₅₃₃, K/A nNOS-I₁–₅₃₃, K/E nNOS-I₅₀₄–₁₁₄₄, and K/E nNOS-I₅₀₃–₅₃₃ derived from iNOS, nNOS-I₅₀₄–₁₁₄₄, and nNOS-I₅₀₃–₅₃₃ with the corresponding lysine 525 of iNOS replaced by glutamate (K/E) or alanine (K/A), respectively). B, comparison of CaM binding sequences among NOS isoforms. The +3 residue is indicated outside a 12-amino acid core sequence with a pair of aromatic or long chain hydrophobic amino acids (indicated by a pair of connecting arrows) that bind to hydrophobic pockets in the N and C domains of CaM. A lysine residue at the +3 position from the C-terminal hydrophobic residue in the CaM-binding sequence is conserved in iNOS (bold and underlined) from mammalian and chicken versions (36–42). This is different from the corresponding residues Thr or Gln in mammalian eNOS and nNOS as well as Arg from Drosophila nNOS. The representative sequences for mammalian NOSs are bovine eNOS, rat nNOS, and murine iNOS. There are variations in mammalian iNOS CaM-binding sequences (listed under the corresponding residue in lower case), but no variations occur in mammalian eNOS and nNOS.

RESULTS

Mutations in nNOS-I₁–₅₃₃—nNOS-I₁–₅₃₃ comprises the oxygenase domain and the CaM-binding region of iNOS and the reductase domain of nNOS (Fig. 1A). nNOS-I₁–₅₃₃ has 50–70% activity in the absence of Ca²⁺ (2–2.5 mM EGTA) compared with the presence of Ca²⁺ (Fig. 2). This Ca²⁺-independent activity of nNOS-I₁–₅₃₃ is inhibited by TFP (a CaM antagonist) in a concentration-dependent manner and is not associated with high affinity binding of CaM (see Figs. 7 and 8 in Ref. 13). The equivalent residue of iNOS lysine 525 in nNOS-I₁–₅₃₃ resides in the C-terminal end of the CaM-binding region was mutated to glutamate in K/E nNOS-I₁–₅₃₃ (Fig. 1). Interestingly, K/E nNOS-I₁–₅₃₃ had no significant NOS activity in the presence of 10 mM of fresh medium. At 16–24 h post-transfection, the transfection media were replaced with fresh medium with addition of 2 mM L-NAME, 200 μM BH₄, or 10 μM hemin in combination for expressing active enzymes. The transfected cells were harvested as described previously (13). Co-immunoprecipitation, trifluoperazine (TFP) treatment, and Western blot analysis were also performed as described previously (13). Anti-CaM monoclonal antibody was purchased from Upstate Biotechnology (Catalog no. 05–173).

| nNOS | nNOS-I₁–₅₃₃ | K/E nNOS-I₁–₅₃₃ | K/A nNOS-I₁–₅₃₃ | nNOS-I₅₀₄–₁₁₄₄ | K/E nNOS-I₅₀₄–₁₁₄₄ | K/A nNOS-I₅₀₄–₁₁₄₄ | nNOS-I₅₀₃–₅₃₃ | K/E nNOS-I₅₀₃–₅₃₃ | K/A nNOS-I₅₀₃–₅₃₃ |
|------|-------------|-----------------|-----------------|----------------|-----------------|-----------------|----------------|-----------------|-----------------|
| nNOS | 696 | 725 | 755 | 797 |
| nNOS-I₁–₅₃₃ | 765 | 753 | 753 | 753 |
| K/E nNOS-I₁–₅₃₃ | 415 | 696 | 796 | 1429 |
| K/A nNOS-I₁–₅₃₃ | | | | |
| nNOS-I₅₀₄–₁₁₄₄ | | | | |
| K/E nNOS-I₅₀₄–₁₁₄₄ | | | | |
| K/A nNOS-I₅₀₄–₁₁₄₄ | | | | |
| nNOS-I₅₀₃–₅₃₃ | | | | |
| K/E nNOS-I₅₀₃–₅₃₃ | | | | |
| K/A nNOS-I₅₀₃–₅₃₃ | | | | |

| nNOS/bovine | 474KK.LVPVEVKAYVQWKISQSTMTCMLMA |
| nNOS/rat | 474KRAIGFNYKLAEEVSVKSFSGMGQAN |
| nNOS/murine | 474RKEIRFPAVKVQVFASLMRKEVMA |
| nNOS/variants | plk a cl t v |
| Drosophila-nNOS | 454KREIKLSILKAKAVLALLLLQTKMTA |

with 10 ml of fresh medium. At 16–24 h post-transfection, the transfection media were replaced with fresh medium with addition of 2 mM L-NAME, 200 μM BH₄, or 10 μM hemin in combination for expressing active enzymes.
abscance of Ca$^{2+}$ (2–2.5 mM EGTA) and required a higher concentration of Ca$^{2+}$/CaM for half-maximal activation (Fig. 2). To determine whether the loss of activity of K/E nNOS-I1–533 in the absence of Ca$^{2+}$ was due to the introduction of a negative charge or the loss of a positive charge, K/A nNOS-I1–533 was constructed. Although K/A nNOS-I1–533 had a lower concentration of Ca$^{2+}$/CaM required for half-maximal activation than K/E nNOS-I1–533 (Fig. 2B), it was also mainly dependent on Ca$^{2+}$ for NO production with marginal activity (10–30%) in the absence of Ca$^{2+}$ (Fig. 2, A and B). Because the specific activities of K/A nNOS-I1–533 and K/E nNOS-I1–533 in the presence of Ca$^{2+}$ were comparable with nNOS-I1–533, the alanine or glutamate replacements at the corresponding residue of iNOS lysine 525 of iNOS residue lysine 525 with glutamate (K/E) or alanine (K/A) changed the dependence of NOS activity on [Ca$^{2+}$]. NOS activities at different [Ca$^{2+}$] in lysates of COS-7 cells transfected with wild type nNOS (C), wild type iNOS (A), nNOS-I1–533 (B), K/A nNOS-I1–533 (C), and K/E nNOS-I1–533 (D) were expressed as a percent of their respective activity in the presence of 300 μM Ca$^{2+}$. Data are means ± S.D. of at least three experiments. Error bars smaller than symbols are not shown.

**Fig. 2. Mutation in nNOS-I1–533.** A, replacement of the corresponding lysine 525 of iNOS in nNOS-I1–533, with glutamate or alanine resulted in loss of activity in the presence of 2.5 mM EGTA. NOS activities in lysates of COS-7 cells transfected with nNOS, iNOS, nNOS-I1–533, K/A nNOS-I1–533, and K/E nNOS-I1–533 were measured, and the respective Ca$^{2+}$–independent activity of each enzyme activity in the absence of Ca$^{2+}$ with 2.5 mM EGTA was expressed as a percent of activity found in the presence of 100 μM Ca$^{2+}$. Data are means ± S.D. of at least three experiments. B, replacement of the corresponding lysine 525 of iNOS in nNOS-I1–533, with glutamate (K/E) or alanine (K/A) changed the dependence of NOS activity on [Ca$^{2+}$]. NOS activities at different [Ca$^{2+}$] in lysates of COS-7 cells transfected with wild type nNOS (C), wild type iNOS (A), nNOS-I1–533 (B), K/A nNOS-I1–533 (C), and K/E nNOS-I1–533 (D) were expressed as a percent of their respective activity in the presence of 300 μM Ca$^{2+}$. Data are means ± S.D. of at least three experiments. Error bars smaller than symbols are not shown.

**Fig. 3. Mutation of lysine 525 iNOS residue.** A, replacement of iNOS residue lysine 525 with glutamate (K/E) or alanine (K/A) attenuated Ca$^{2+}$–independent NOS activity. NOS activities in lysates of COS-7 cells transfected with iNOS, K/A iNOS, and K/E iNOS were measured, and the respective activity of each enzyme in the presence of 2.5 mM EGTA was expressed as a percent of activity found in the presence of 100 μM Ca$^{2+}$. Data are means ± S.D. of at least three experiments. B, replacement of iNOS residue lysine 525 with glutamate (K/E) or alanine (K/A) changed the dependence of NOS activity on [Ca$^{2+}$]. NOS activities at different [Ca$^{2+}$] in lysates of COS-7 cells transfected with wild type nNOS (C), wild type iNOS (A), K/E iNOS (B), and K/A iNOS (D), were expressed as a percent of their respective activity found in the presence of 300 μM Ca$^{2+}$. Data are means ± S.D. of at least three experiments. Error bars smaller than symbols are not shown.
action of the CaM-binding region and the oxygenase domain of iNOS.

nNOS-I503-533 consists of nNOS with its CaM-binding region (residues 725–755) substituted for that of iNOS (residues 503–533) (Fig. 1A) and is dependent on Ca2+ for activation but with a lower concentration of Ca2+/CaM for half-maximal activation than nNOS (Fig. 4). nNOS-I503-533 and nNOS-I1-533 share the same CaM-binding sequence but differ in the origin of the oxygenase domain. Because the concentration of Ca2+/CaM required for half-maximal activity of K/E nNOS-I1-533 was greater than that for nNOS-I1-533, the corresponding mutation at nNOS-I503-533 was predicted to also increase the concentration of Ca2+/CaM for required half-maximal activity compared with nNOS-I503-533. This proved to be the case (Fig. 4C). Accordingly, lysine 525 of iNOS may be in a position not only to influence activity in the absence of Ca2+ (2–2.5 mM EGTA) but also to facilitate NO production at low Ca2+ concentrations.

**TFP Inhibition and Association of CaM with K/E iNOS, K/E nNOS-I504-1144, and K/E nNOS-I1-533.**—Because the point mutation affects the Ca2+-independent activity differently in iNOS, nNOS-I504-1144, and nNOS-I1-533, the mutational effects on other properties of these enzymes were further studied. TFP (a CaM antagonist) treatment and co-immunoprecipitation with CaM were used to further investigate the properties of K/E iNOS, K/E nNOS-I504-1144, and K/E nNOS-I1-533 compared with their respective parental enzymes. The activity of K/E iNOS was resistant to TFP inhibition in the presence of Ca2+ but more sensitive in the absence of Ca2+ compared with iNOS or with nNOS-I504-1144 (Fig. 5) (see also Fig. 7 in Ref. 13). The activity of K/E nNOS-I504-1144 was resistant to inhibition by TFP treatment in either the presence or the absence of Ca2+ and similar to nNOS-I504-1144 (Fig. 5) (see also Fig. 7 in Ref. 13). The activity of K/E nNOS-I1-533 in the presence of Ca2+ was more sensitive to inhibition by TFP compared with non-mutated nNOS-I1-533 but still more resistant than nNOS (Fig. 5) (see also Fig. 7 in Ref. 13). Thus, the effects of TFP on the activities of these glutamate mutants were similar to the mutational effects on the activities in the absence of Ca2+, i.e., only effective in inhibiting enzymes containing both the CaM-binding region and the oxygenase domain of iNOS.

In the absence of Ca2+, CaM binds to iNOS or nNOS-I504-1144 with higher affinity than to nNOS-I1-533 (see Fig. 8 in Ref. 13). CaM binding properties to K/E iNOS, K/E nNOS-I504-1144, and K/E nNOS-I1-533 were also investigated herein to study the mutational effect on their affinity for calmodulin (Fig. 6). Lysates of transfected COS-7 cells were immunoprecipitated with anti-nNOS or anti-iNOS antibodies in the presence of 100 µM CaCl2 or 2.5 mM EGTA plus 0.5% Triton X-100 for dissociating CaM. Co-immunoprecipitation results showed that CaM associated with K/E iNOS and K/E nNOS-I504-1144 either in the presence or absence of Ca2+. Also, CaM dissociated from K/E nNOS-I1-533, a Ca2+-dependent enzyme, in the absence of Ca2+ as found for nNOS and the non-mutated nNOS-I1-533 (13). Therefore, in contrast to Ca2+-independent activity and TFP inhibition, the mutation had no significant effect on the affinity of these enzymes for CaM.

Collectively, the mutation at iNOS lysine 525 did not significantly change the properties of K/E nNOS-I504-1144 in terms of activity in the absence of Ca2+ (2–2.5 mM EGTA), high affinity association with CaM, and resistance to TFP treatment. In contrast, the mutation decreased activities of K/E iNOS and K/E nNOS-I1-533 in the absence of Ca2+ (2–2.5 mM EGTA) as well as affected the resistance to TFP treatment for both enzymes but had no effect on affinity for CaM.

**DISCUSSION**

Site-directed mutagenesis and crystal structures have provided insight into the functions of the various domains of some NOS enzymes (29–33). Crystal structures of the dimeric oxygenase domains of eNOS and iNOS illustrate the interface interactions for dimerization and the formation of the active sites. The functional binding of heme, BH4, and L-arginine to NOS is thus well depicted and fits in an electron transfer link and relevant spatial interactions for catalysis of NO produc-
Nonmutated chimeric enzymes (13). With anti-nNOS (for K/E nNOS-I1–533) or anti-iNOS (for K/E iNOS and K/E nNOS-I504–1144) were measured in the presence of 2 mM EGTA without or with 300 μM free Ca⁡²⁺ at indicated concentrations of TFP (in μM) and l-NAME. Data shown are means ± S.D. of at least three experiments. Error bars smaller than symbols are not shown.

**Fig. 5. Inhibition of K/E mutant NOSs by TFP and l-NAME.** The activities of K/E iNOS, K/E nNOS-I1–533, and K/E nNOS-I504–1144 in the presence of 2 mM EGTA (open circles) or in the presence of Ca⁡²⁺ (closed circles) were both normalized to 100% in the absence of TFP, respectively. l-NAME (200 μM), an inhibitor of NOS activity, significantly inhibited enzyme activities in the presence of 1 mM TFP either in the presence (open squares) or absence (closed diamonds) of Ca⁡²⁺, indicating that these are NOS activities. NOS activities in lysates of COS-7 cells transfected with K/E iNOS, K/E nNOS-I1–533, and K/E nNOS-I504–1144 were measured in the presence of 2 mM EGTA or without or with 300 μM free Ca⁡²⁺ at indicated concentrations of TFP (in μM) and l-NAME. Data shown are means ± S.D. of at least three experiments. Error bars smaller than symbols are not shown.

**Fig. 6. Association of CaM with NOS glutamate mutants.** Co-immunoprecipitation of CaM with different NOS enzymes was used to evaluate high affinity association of CaM. COS-7 cells were transfected with the indicated NOS, and their lysates were immunoprecipitated with anti-nNOS (for K/E nNOS-I1–533) or anti-iNOS (for K/E iNOS and K/E nNOS-I504–1144) in the presence of 100 μM Ca⁡²⁺ or 2.5 mM EGTA. Immunoprecipitates were subjected to 6 and 15% SDS-polyacrylamide gel electrophoresis for Western blot analysis by anti-NOSs (upper panels) and by anti-CaM antibodies (lower panels), respectively. NOS protein was not detected in mock controls, and CaM levels in mock controls were the same as those in immunoprecipitates of K/E nNOS-I504–1144 in the presence of EGTA (data not shown). Results showed that replacement of lysine 525 in iNOS and the corresponding residues in NOS-I504–1144 and nNOS-I1–533 with glutamate did not significantly change the relative affinities of these enzymes for CaM in the presence or absence of Ca⁡²⁺, which is similar to their respective wild type and nonmutated chimeric enzymes (13).

It was recently shown that a zinc ion coordinates pairs of symmetry-related cysteine residues at the interface of the oxygenase dimer for the FMN domain docking to stabilize BH₄ binding (31, 32). These cysteine residues are from a pair of conserved tetrahedral motifs (CXXanyak) each from alternate oxygenase domains. A highly positively charged interaction surface for the FMN domain docking to the oxygenase domain is also proposed in the vicinity of the zinc-binding site. Given the difference in on-off rates of association of the oxygenase and reductase domain of iNOS (always associated) and nNOS (controlled by Ca²⁺/CaM association), the docking interactions of iNOS may have evolved differently from nNOS and accordingly account for the Ca²⁺-independent activity of iNOS. Replacing iNOS reductase domain with the counterpart of nNOS or vice versa, as in nNOS-I1–533 or nNOS-I504–1144 may change the docking interface interactions. These considerations may account for the inhibitory effect of EGTA on the activities of nNOS-I1–533 and nNOS-I504–1144.

The further loss of activity in K/E nNOS-I1–533 in the absence of Ca²⁺ with EGTA triggered the investigation into the mutational effect of Lys-525 on iNOS activity. The lack of the CaM-binding region in the crystal structures of both the iNOS and eNOS oxygenase domains (30–32) hinders our understanding of how CaM is functionally involved. However, in the iNOS oxygenase structure (Fig. 1 in Ref. 32) the α-12 helix immediately preceding the CaM-binding region projects toward the caved docking interface, and thus the CaM-binding sequence is probably located around the interface proposed for FMN domain docking. Thus, CaM associated with the CaM-binding sequence may be embedded in the docking interface. If this is true, the equivalent lysine 525 in iNOS and nNOS-I1–533 may be located at or close to a solvent-accessible surface at the docking site. Mutation of the equivalent lysine 525 in iNOS and nNOS-I1–533 may change the accessibility of some effective sites to EGTA, e.g. the coordinated zinc, as perhaps occurred with K/A nNOS-I1–533 and K/E nNOS-I1–533, enzymes with activities more sensitive to inhibition by EGTA. Although crystal structures of NOSs are consistent in general with the mutagenesis data, some discrepancy has been noted due to the preparations of proteins and conditions for crystallization (31, 32). The zinc ion center of iNOS was not identified in early crystal studies because of the protein preparation (30–32).

Because EGTA is a divalent metal ion chelator, the inhibitory effect of EGTA may involve other identified metal factors such as the coordinated zinc ion at the interface of oxygenase dimer for FMN domain docking. EGTA has a greater affinity for Ca²⁺ (10⁻¹¹ M) than for zinc (10⁻⁷ M). If the coordinated zinc becomes solvent accessible due to the mutation, EGTA chelation of the zinc ion may occur in the absence of Ca²⁺. Given the proposed role of the coordinated zinc ion in pterin binding and reductase docking, dissociation of the zinc ion from K/E nNOS-I1–533 or K/A nNOS-I1–533 is likely to cause attenuation in electron transfer from FMN domain to the pterin and heme for NO production. This inhibition would be released when the free Ca²⁺ concentration increases because of competition for the EGTA, similar to the inhibition of nNOS and iNOS by high concentrations of zinc through competitive binding to a non-heme iron-binding site (28).

EGTA may inhibit iNOS activity to varied extents. Some investigators have reported previously that murine iNOS can be partially inhibited by EGTA (12, 27, 34) although others found no effect (11, 13, 35). Similar observations were also reported for rat and human iNOS (21–26). Herein, our results show that Lys-525 of iNOS is in a position critical for activities of K/A nNOS-I1–533 and K/E nNOS-I1–533 in the absence of Ca²⁺ with EGTA. It also plays an important role in maintaining maximal activity of iNOS in the absence of Ca²⁺.
REFERENCES

1. Ghosh, D. K., and Stuehr, D. J. (1995) Biochemistry 34, 801–807
2. Siddhanta, U., Presta, A., Fan, B., Wolan, D., Rousseau, D. L., and Stuehr, D. J. (1998) J. Biol. Chem. 273, 18850–18858
3. Masters, B. S., McMillan, K., Sheta, E. A., Nishimura, J. S., Roman, L. J., and Stuehr, D. J. (1999) J. Biol. Chem. 274, 34799–34805

4. Lee, S. J., and Stull, J. T. (1998) J. Biol. Chem. 273, 14631–14635

5. Gachhui, R., Krebs, J., VanBerkum, M. F., Tang, W. J., Maune, J. F., Means, R. A., Stull, J. T., and Beckingham, K. (1993) J. Biol. Chem. 268, 20594–20602

6. Abu-Soud, H. M., and Stuehr, D. J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10769–10772

7. Lee, S. J., and Stull, J. T. (1998) J. Biol. Chem. 273, 14631–14635

8. Chen, Z. P., Mitchelhill, K. I., Michell, B. J., Stapleton, D., Rodriguez-Crespo, I., Witters, L. A., Power, D. A., Ortiz de Montellano, P. R., and Kemp, B. E. (1999) FEBS Lett. 443, 285–289

9. Gachhui, R., Presta, A., Bentley, D. F., Abu-Soud, H. M., McArthur, R., Mayer, B., George, S. E., and Stuehr, D. J. (1996) J. Biol. Chem. 271, 10609–10619

10. Marletta, M. A. (1994) J. Biol. Chem. 270, 5451–5454

11. Ruan, J., Xie, Q., Hutchinson, N., Cho, H., Wolfe, G. C., and Nathan, C. (1996) J. Biol. Chem. 271, 23679–23686

12. Venema, R. C., Sayegh, H. S., Kent, J. D., and Harrison, D. G. (1996) J. Biol. Chem. 271, 6435–6440

13. Lee, S. J., and Stull, J. T. (1998) J. Biol. Chem. 273, 27430–27437

14. Lee, S. J., Stull, J. T., and Beckingham, K. (1993) J. Biol. Chem. 268, 20096–20104

15. Gachhui, R., Krebs, J., VanBerkum, M. F., Tang, W. J., Maune, J. F., Means, R. A., Stull, J. T., and Beckingham, K. (1993) J. Biol. Chem. 268, 29117–29122

16. Stevens-Truss, R., Beckingham, K., and Marletta, M. A. (1997) Biochemistry 36, 12337–12345

17. Lee, S. J., and Stull, J. T. (1998) J. Biol. Chem. 273, 5451–5454

18. Chen, Z. P., Mitchelhill, K. I., Michell, B. J., Stapleton, D., Rodriguez-Crespo, I., Witters, L. A., Power, D. A., Ortiz de Montellano, P. R., and Kemp, B. E. (1999) FEBS Lett. 443, 285–289

19. Venema, R. C., Sayegh, H. S., Kent, J. D., and Harrison, D. G. (1996) J. Biol. Chem. 271, 6435–6440

20. Lee, S. J., Stull, J. T., and Beckingham, K. (1993) J. Biol. Chem. 268, 20096–20104

21. Stuehr, D. J., Cho, H. J., Kwon, N. S., Weise, M. F., and Nathan, C. F. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 7773–7777

22. Venema, R. C., Sayegh, H. S., Kent, J. D., and Harrison, D. G. (1996) J. Biol. Chem. 271, 6435–6440

23. Nishida, C. R., and Ortiz de Montellano, P. R. (1998) J. Biol. Chem. 273, 5566–5571

24. Breit, D. S., Hwang, P. M., Glatt, C. E., Lowenstein, C., Reed, R. R., and Snyder, S. H. (1991) Nature 351, 714–718

25. Lyons, C. R., Orloff, G. J., and Cunningham, J. M. (1992) J. Biol. Chem. 267, 6370–6374

26. Iida, S., Ohshima, H., Oguchi, S., Hata, T., Suzuki, H., Kawasaki, H., and Esumi, H. (1992) J. Biol. Chem. 267, 25385–25388

27. Sherman, P. A., Laubach, V. E., Reep, B. R., and Wood, E. R. (1993) Biochemistry 32, 11600–11605

28. Charles, I. G., Palmer, R. M., Hickery, M. S., Bayliss, M. T., Chubb, A. P., Hall, V. S., Moss, D. W., and Moncada, S. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11419–11423

29. Geller, D. A., Lowenstein, C. J., Shapiro, R. A., Nussler, A. K., Di Silvio, M., Wang, S. C., Nakayama, D. K., Simmons, R. L., Snyder, S. H., and Billiar, T. R. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3491–3495

30. Perry, J. M., and Marletta, M. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11010–11016

31. Stuehr, D. J., Cho, H. J., Kwon, N. S., Weise, M. F., and Nathan, C. F. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 7773–7777

32. Nishida, C. R., and Ortiz de Montellano, P. R. (1998) J. Biol. Chem. 273, 5566–5571

33. Nishida, C. R., and Ortiz de Montellano, P. R. (1998) J. Biol. Chem. 273, 5566–5571

34. Breit, D. S., Hwang, P. M., Glatt, C. E., Lowenstein, C., Reed, R. R., and Snyder, S. H. (1991) Nature 351, 714–718

35. Sessa, W. C., Harrison, J. K., Barber, C. M., Zeng, D., Durieux, M. E., D'Angelo, D. D., Lynch, K. R., and Peach, M. J. (1992) J. Biol. Chem. 267, 15274–15276

36. Sessa, W. C., Harrison, J. K., Barber, C. M., Zeng, D., Durieux, M. E., D'Angelo, D. D., Lynch, K. R., and Peach, M. J. (1992) J. Biol. Chem. 267, 15274–15276

37. Sessa, W. C., Harrison, J. K., Barber, C. M., Zeng, D., Durieux, M. E., D'Angelo, D. D., Lynch, K. R., and Peach, M. J. (1992) J. Biol. Chem. 267, 15274–15276

38. Sessa, W. C., Harrison, J. K., Barber, C. M., Zeng, D., Durieux, M. E., D’Angelo, D. D., Lynch, K. R., and Peach, M. J. (1992) J. Biol. Chem. 267, 15274–15276

39. Sessa, W. C., Harrison, J. K., Barber, C. M., Zeng, D., Durieux, M. E., D’Angelo, D. D., Lynch, K. R., and Peach, M. J. (1992) J. Biol. Chem. 267, 15274–15276

40. Sessa, W. C., Harrison, J. K., Barber, C. M., Zeng, D., Durieux, M. E., D’Angelo, D. D., Lynch, K. R., and Peach, M. J. (1992) J. Biol. Chem. 267, 15274–15276