Glutathiolation of Proteins by Glutathione Disulfide S-Oxide Derived from S-Nitrosoglutathione

MODIFICATIONS OF RAT BRAIN NEUROGRANIN/RC3 AND NEUROMODULIN/GAP-43*

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Junfa Li, Freesia L. Huang, and Kuo-Ping Huang‡

From the Section on Metabolic Regulation, Endocrinology and Reproduction Research Branch, NICHD, National Institutes of Health, Bethesda, Maryland 20892-4510

S-Nitrosoglutathione (GSNO) undergoes spontaneous degradation that generates several nitrogen-containing compounds and oxidized glutathione derivatives. We identified glutathione sulfonic acid, glutathione disulfide S-oxide (GS(O)SG), glutathione disulfide S-dioxide, and GSSG as the major decomposition products of GSNO. Each of these compounds and GSNO were tested for their efficacies to modify rat brain neurogranin/RC3 (Ng) and neuromodulin/GAP-43 (Nm). Among them, GS(O)SG was found to be the most potent in causing glutathiolation of both proteins; four glutathiones were incorporated into the four Cys residues of Ng, and two were incorporated into the two Cys residues of Nm. Ng and Nm are two in vivo substrates of protein kinase C; their phosphorylations by protein kinase C attenuate the binding affinities of both proteins for calmodulin. When compared with their respective unmodified forms, the glutathiolated Ng was a poorer substrate and glutathiolated Nm a better substrate for protein kinase C. Glutathiolation of these two proteins caused no change in their binding affinities for calmodulin. Treatment of [35S]cysteine-labeled rat brain slices with xanthine/xanthine oxidase or a combination of xanthine/xanthine oxidase with sodium nitroprusside resulted in an increase in cellular level of GS(O)SG. These treatments, as well as those by other oxidants, all resulted in an increase in thiolation of proteins; among them, thiolation of Ng was positively identified by immunoprecipitation. These results show that GS(O)SG is one of the most potent glutathiolating agents generated upon oxidative stress.

Protein S-glutathiolation can be induced in cells by mild oxidative stress (1). GSSG has been shown to oxidatively regulate the activity of several purified enzymes including carbonic anhydrase III (2, 3), protein kinase C (PKC)² (4), human aldose reductase (5), and human immunodeficiency virus, type I protease (6), and in each case the effects of glutathiolation can be reversed by reducing agents. As the concentration of reduced GSH in the mammalian cells is in the millimolar range and that of GSSG is less than 5% of GSH, glutathiolation of proteins by GSSG in vivo is not likely an efficient mechanism. More recently, the superoxide-induced glutathiolation of protein (7) and that induced by peroxynitrite, nitric oxide (NO), and nitrosothiol, in particular, S-nitrosoglutathione (GSNO), are thought to be the main avenues leading to protein thiolation (8–12). In mammalian cells, a relatively high concentration of GSH (0.5–10 mM) serves as an NO sink to form GSNO (13–16), which can undergo transnitrosylation with protein sulf hydryl group to form S-nitrosoprotein and GSNO or to form protein-GSH mixed disulfide and nitroxyl (17–20). GSNO can also release NO in the presence of cuprous ion (21), ascorbate (22), or thiols (20, 23) and serves as a possible source of nitrosium or nitroxyl ions (24). In addition, GSNO is unstable in aqueous solution and undergoes decomposition, which is believed to be homolytic cleavage of the S–N bond to give NO and a thyl radical (25, 26). Indeed, the reactions involving GSNO are fairly complex and generate many potential products including ammonia, NO, nitrous oxide, nitrite, sulfamide, hydroxylamine, and several oxidized forms of glutathione (20, 23). Recently, it was found that freshly prepared GSNO was effective in S-nitrosylation of proteins through transnitrosylation, whereas the decomposed GSNO was more effective in S-glutathiolation of proteins (10). It was suggested that glutathione sulfenic acid was the active component for glutathiolation of proteins.

Neurogranin/RC3 (Ng) and neuromodulin/GAP-43 (Nm) are two prominent PKC substrates in the brain. Phosphorylations of both Ng and Nm reduce their binding affinities for calmodulin (CaM) (27, 28). Rat brain Ng contains four, and Nm contains two Cys residues; these Cys residues in Ng form two pairs of intramolecular disulfides upon oxidation by NO and other oxidants (29, 30), and those in Nm undergo palmitoylation (31). Intramolecular disulfide formation renders Ng a poorer substrate of PKC and also reduces its binding affinity for CaM (29). The effect of oxidation of the two Cys residues of Nm has not been elucidated. However, it was shown that treatment of cultured dorsal root ganglion and PC-12 cells with 3-morpholino-sydnonimine, which generates both NO and superoxide, inhibited palmitoylation of Nm (32). Recently, we found that treatment of Ng with GSNO caused oxidation to form intramolecular disulfides, as well as glutathiolation; the extent of glutathiolation was greatly increased upon incubation of Ng with the decomposed GSNO (33). In this study, by using mass spectrometry, high pressure liquid chromatography; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis.

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‡ To whom correspondence should be addressed: Bldg. 49, Rm. 6A36, NIH, 49 Convent Dr., MSC 4510, Bethesda, MD 20892-4510. Tel.: 301-496-7827; Fax: 301-496-7434; E-mail kphuang@helix.nih.gov.
† The abbreviations used are: PKC, protein kinase C; Ng, neurogranin/RC3; Nm, neuromodulin/GAP-43; CaM, calmodulin; GSNO, S-nitrosothiol, GS(O)SG, glutathione sulfenic acid; SNP, sodium nitroprusside; IAM, iodoacetamide; AM, acetylamide; X, xanthine; XO, xanthine oxidase; ES-MS, electrospray ionization mass spectrometry; ACSF, artificial cerebrospinal fluid; red, reduced; ox, oxidized; GS–, glutathiolated residue; NO, nitric oxide; GSOH, glutathione sulfonic acid; GSOOG, glutathione disulfide S-oxide; GS(O)2SG, glutathione disulfide S-dioxide; HPLC, high pressure liquid chromatography; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis.
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Glutathione, we have identified several glutathione derivatives as the degradation products of GSNO, including glutathione sulfonic acid (GSO$_2$H), glutathione disulfide S-oxide (GS(O)SO$_2$G), glutathione disulfide S-dioxide (GS(O$_2$)SO), and GSSG. Among them, GSO$_2$H was the most potent in causing glutathiolation of Ng and Nm. The level of this compound was found to increase upon treatment of rat brain slices with oxidants. Ng had been positively identified as a target of thiolation under oxidative stress.

**EXPERIMENTAL PROCEDURES**

**Materials**—The following materials were obtained from the indicated sources: GSNO was from Calbiochem; sodium nitroprusside (SNP), diamide, bovine serum albumin, GSH, iodoacetamide (IAM), GSSG, xanthine (X), and xanthine oxidase (XO) were from Sigma; H$_2$O$_2$ was from Fisher Scientific; phosphorylase b and dideoxyerythroester were from Avanti Polar Lipids; BCA protein determination reagent was from Pierce; [35S]cysteine was from PerkinElmer Life Sciences; protein A-Sepharose was from Amersham Pharmacia Biotech; and horseradish peroxidase-conjugated goat anti-rabbit IgG was from Bio-Rad.

**Purification of the Various Glutathione Derivatives of GSNO—** GSNO (100 mM) dissolved in water was kept in room temperature for 24–72 h, and the solution was injected into reverse phase HPLC column (connecting two Varian 218TP54, 0.46 × 25 cm) eluted with 0.1% trifluoroacetic acid for 2 h at a flow rate of 0.5 ml/min. Fractions of 0.25 ml were collected, lyophilized, and subjected to electrospray mass spectrometry analysis. The concentrations of the various glutathione derivatives were determined by amino acid composition analysis.

**Preparation of Rat Brain Slices and Treatments with Oxidants—** Brain from adult Sprague Dawley rat (200–250 g) was removed immediately after decapitation and placed in ice-cold artificial cerebrospinal fluid (ACSF; 125 mM NaCl, 2.5 mM KCl, 2 mM CaCl$_2$, 26 mM NaHCO$_3$, 1.25 mM Na$_2$HPO$_4$, 1 mM MgCl$_2$, 25 mM glucose, bubbled with 95% O$_2$/5% CO$_2$). The slices were incubated for 1 h in ACSF containing 200 μm cycloheximide to block protein synthesis and then [35S]cysteine (22 μCi/ml) was added to label the intracellular GSH for 1–2 h. The slices were treated with X (0.5 mM)-XO (80 milliunits) and X-XO plus SNP for 15 min.

**Immunoprecipitation of Thiolated Ng and Nm by Immunoblot—** Immunoblot was used for the identification of Ng and Nm by immunoblot with an antibody recognizing both proteins.

**RESULTS**

**Oxidation of Ng by GSNO and Its Decomposition Products—** Rat brain Ng is susceptible to oxidation by several oxidants resulting in the formation of intramolecular disulfides. The oxidized Ng exhibits an increased electrophoretic mobility in nonreducing SDS-PAGE (29). Incubation of red-Ng with a low concentration (<1 mM) of freshly prepared GSNO resulted in the formation of a fast-migrating oxidized Ng containing intramolecular disulfides. At a higher GSNO concentration (>3 mM) this fast-migrating oxidized Ng was slightly reduced (Fig. 1A). This phenomenon became more pronounced when the red-Ng was treated with a partially decomposed GSNO preparation (Fig. 1B). A decrease in the intramolecular disulfide-bridged form of Ng at a higher concentration of GSNO, especially with the decomposed GSNO, suggests that a different type of modification of Ng sulfhydryl group occurs. To characterize the mechanism involved in the oxidation of Ng by GSNO, the reactions were terminated by addition of 100 mM IAM, purified by HPLC, and analyzed by ES-MS. For controls, treatment of red-Ng (7494.8 Da) with IAM resulted in an incorporation of 4 acetamides (AM/Ng (7723.6 Da) (Fig. 1C), whereas treatment of the air-oxidized Ng (7491.9 Da) with IAM did not result in any incorporation of AM (Fig. 1D). For brevity, we...
referred to the Ng containing intramolecular disulfide(s) as ox-Ng. By ES-MS analysis, the ox-Ng containing one pair of intramolecular disulfides will incorporate 2 AM, and that containing two pairs of intramolecular disulfides will incorporate none. Incubation of red-Ng with 3 mM of the partially decomposed GSNO for 5 s resulted in an extensive glutathiolation (1–3 glutathiolated residues (GS–)/Ng) (Fig. 1E), and after 30 min of incubation a majority of Ng was glutathiolated to 4 GS–/Ng (8718.1 Da) (Fig. 3,5), suggesting that one of the sulfhydryl groups is modified by these compounds (3 mM for 5 min) was analyzed by ES-MS, P-1 (m/z = 356.1) was identified as GSO3H (C) (mass, 337 Da), P-2a and P-2b (m/z = 629.2) were GS/O/SG (C and D); P-3 (m/z = 645.1) was GS(O)2SG (E); P-4 (m/z = 337.0) was GSNO (F); and P-5 (m/z = 613.1) was GSSG (G). In panel F, fragmentation of GSNO (m/z = 337) resulted in the release of NO from GSNO to form an ionic species of m/z = 307.

Predict that P-1 is GSO3H (mass, 356 Da), P-2a and P-2b are stereoisomers of GS/O/SG (mass, 629 Da), P-3 is GS(O)2SG (mass, 645 Da), P-4 is GSNO (mass, 337 Da), and P-5 is GSSG (mass, 613 Da). The multiple ionic species seen in P-4, GSNO, are because of fragmentation of the parent compound during ES-MS, i.e. the m/z = 307 species is the one without NO.

The effects of these compounds on the modification of Ng were analyzed by SDS-PAGE and ES-MS (Fig. 3). GSO3H, GS(O)2SG, GSNO, and GSSG were effective in causing Ng oxidation-forming intramolecular disulfide, whereas both GS(O)SG stereoisomers (P-2a and P-2b) were unique in causing Ng modification of Ng characteristic for glutathiolation. The Ng modified by these compounds (3 mM for 5 min) was analyzed by ES-MS. P-1, GSO3H, caused partial oxidation of Ng generating one intramolecular disulfide bond without glutathiolation (7624.3 Da) (Fig. 3, P-1). This oxidized form of Ng contained one extra oxygen over the expected species, which should have one intramolecular disulfide and two free sulfhydryl groups being modified by IAM with a resulting mass of 7608 Da. We speculate that this is because of modification of the methionine residue to form methionine sulfoxide. P-2, GS(O)2SG, caused extensive glutathiolation of Ng with the majority of the proteins containing 4 GS–/Ng (8717.9 Da) (Fig. 3, P-2). The identity of another modified Ng of 8063.4 Da is unknown. P-3, GS(O)2SG, caused partial oxidation of Ng generating one intramolecular disulfide bond without glutathiolation, such as ox-Ng plus 2 AM (7609.3 Da) (Fig. 3, P-3). It should be noted that the major Ng species after treatment with GS(O)2SG contained 3 AM (7666.8 Da), suggesting that one of the sulfhydryl groups is...
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FIG. 3. Modification of Ng by the various glutathione derivatives. Recombinant Ng reduced with 1 mM DTT was incubated with increasing concentrations of GSO₃H (P-1), GS(O)SG (P-2), GS(O₂)SG (P-3), GSNO (P-4), and GSSG (P-5) at 30 °C for 5 min and terminated the reactions with 100 mM IAM, and samples were analyzed by nonreducing SDS-PAGE (10–20% gradient gel) for protein staining with Coomassie Blue (upper panels). Those samples treated with 3 mM each of the various compounds were purified by HPLC and analyzed by ES-MS. Modified Ng of known structure is indicated.

Red-Ng + Os-Ng

FIG. 4. Dose response of the GS(O)SG-mediated glutathiolation of Ng. Reduced Ng (80 μM) was incubated with increasing concentrations of GS(O)SG at 30 °C for 5 min, and reactions were terminated with 100 mM IAM, purified by HPLC, and analyzed by ES-MS. A, red-Ng alone; B, plus 50 μM GS(O)SG; C, plus 100 μM GS(O)SG; D, plus 200 μM GS(O)SG; E, plus 500 μM GS(O)SG; and F, plus 1 mM GS(O)SG. Modified Ng of known structure is indicated.

Concentration of ~500 μM (with a ratio of GS(O)SG/Ng = ~6 or GS(O)SG/-SH = ~1.5). It should be noted that some of the GS(O)SG-treated Ng samples also contained an odd number of modified –SH groups in the ES-MS spectra, suggesting that this compound can also form Ng-Cys-OH. Because each Ng molecule contains four Cys residues, it requires at least four times the concentrations of GS(O)SG to achieve a complete glutathiolation if only one GS– is transferred to Ng.

We also tested the efficacy of GS(O)SG on the glutathiolation of Nm, which contains two Cys residues. In the control, treatment of red-Nm with IAM resulted in the incorporation of 2 AM into rat brain Nm, which contained multiple phosphorylated species (Fig. 5A). Incubation of red-Nm (25 μM) with an equimolar concentration of GS(O)SG resulted in ~50% of Nm being glutathiolated to 2 GS–/Nm (Fig. 5B). The nonglutathiolated Nm, however, did not contain any AM, suggesting that both –SH groups are modified by this compound to form Nm-Cys-OH. As the concentrations of GS(O)SG were increased to 50 (Fig. 5C), 100 (Fig. 5D), 200 (Fig. 5E), and 500 μM (Fig. 5F) nearly all the Nm contained 2 GS–/Nm. The maximal level of glutathiolation reached at a ratio of GS(O)SG/Nm = ~2 or GS(O)SG/-SH = ~1. These results further confirm that GS(O)SG is indeed a very potent glutathiolating agent.

Effects of Glutathiolation of Ng and Nm on the Phosphoryl-
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**TABLE I**

|                          | red-Ng | GS-Ng | red-Nm | GS-Nm |
|--------------------------|--------|-------|--------|-------|
| $K_m$ (μM)               | 24.1 ± 3.9 | 35.9 ± 4.6 | 20.3 ± 2.5 | 11.2 ± 1.6 |
| $V_{max}$ (unit/mg)      | 2110 ± 120 | 1010 ± 70 | 1390 ± 120 | 1330 ± 87 |
| $V_{max}/K_m$            | 88     | 28    | 70     | 120    |

Phosphorylation of the various substrates by PKC was carried out under the standard assay conditions in the presence of PS/DG/Ca$^{2+}$ as described under “Experimental Procedures.” The concentrations of red-Ng, GS-Ng, red-Nm, and GS-Nm were varied from 2 to 130, 1.3 to 90, 8 to 100, and 2 to 60 μM, respectively. The $K_m$ and $V_{max}$ values were obtained by computer analysis (30) and expressed as mean ± S.D.

**Generation of GS(O)SG and other Oxidized Glutathione Species in Rat Brain Slices Treated with Oxidants**—Rat brain slices were incubated with $[^{35}S]$cysteine to label glutathione in the presence of cycloheximide followed by treatment with X-XO and a combination of X-XO and SNP. These experiments were designed to test whether generation of GS(O)SG was responsive to oxidative and nitrosative stresses. Previously, we have shown that SNP was one of the most potent NO donors tested, including 2-(N,N-diethylamino)-diazenolate-2-oxide and S-nitroso-N-acetylpenicillamine (29, 30, 36), in causing Ng oxidation. The perchoric acid-soluble fractions were analyzed by C_{18} reverse phase HPLC (Fig. 6). Identification of the various glutathione derivatives was based on their retention times as compared with the authentic compounds. GSOH and $[^{35}S]$cysteine had the same retention time; they were not separable by this chromatography. Treatment of the slices with X-XO, which generates superoxide, caused an increase in both GSSG and GS(O)SG (Fig. 6B) as compared with the control (Fig. 6A), and a greater increase in these two compounds was seen with a combination of X-XO and SNP (Fig. 6C). The latter treatment also resulted in a slight increase in GSNO; however, because of its instability quantification of GSNO was not vigorously pursued.

To determine the extent of thiolation of proteins in the $[^{35}S]$cysteine-labeled brain slices, the tissue extracts were resolved by nonreducing (Fig. 7A) and reducing SDS-PAGE (Fig. 7B), and proteins were transferred to nitrocellulose membrane and analyzed by autoradiography. Following treatments of the slices with $H_2O_2$, SNP, diamide, X-XO, and X-XO/SNP several prominent $[^{35}S]$-labeled proteins of 43, 39, 32, 28, 20, 17, and 14 kDa were observed to be thiolated as evidenced by their release of $^{35}S$ in the reducing gel. The overall patterns of protein thiolation by these oxidants were qualitatively similar. However, thiolation of certain proteins was more distinct by treatment with different oxidants; e.g., thiolation of the 43-kDa protein was more prominent by diamide and X-XO, and that of the 36-kDa protein was more prominent by $H_2O_2$, X-XO, and X-XO/SNP.

**Characterization of Ng Thiolation by Immunoprecipitation**—The various oxidant-treated $[^{35}S]$cysteine-labeled brain slices were analyzed for the thiolation of Ng and Nm by immunoprecipitation with antibody number 270, which recognizes both Ng and Nm. Following resolution by nonreducing SDS-PAGE, transfer of the slices with $[^{35}S]$cysteine-labeled brain slices, the tissue extracts were resolved by nonreducing (Fig. 7A) and reducing SDS-PAGE (Fig. 7B), and proteins were transferred to nitrocellulose membrane and analyzed by autoradiography. Following treatments of the slices with $H_2O_2$, SNP, diamide, X-XO, and X-XO/SNP several prominent $[^{35}S]$-labeled proteins of 43, 39, 32, 28, 20, 17, and 14 kDa were observed to be thiolated as evidenced by their release of $^{35}S$ in the reducing gel. The overall patterns of protein thiolation by these oxidants were qualitatively similar. However, thiolation of certain proteins was more distinct by treatment with different oxidants; e.g., thiolation of the 43-kDa protein was more prominent by diamide and X-XO, and that of the 36-kDa protein was more prominent by $H_2O_2$, X-XO, and X-XO/SNP.

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**FIG. 5.** Dose response of the GS(O)SG-mediated glutathiolation of Nm. Reduced Nm (25 μM) was incubated with increasing concentration of GS(O)SG at 30 °C for 5 min, and reactions were terminated with 100 μM IAM, purified by HPLC, and analyzed by ES-MS. A, red-Nm alone; B, plus 25 μM GS(O)SG; C, plus 50 μM GS(O)SG; D, plus 100 μM GS(O)SG; E, plus 200 μM GS(O)SG; and F, plus 500 μM GS(O)SG. Purified rat brain Nm contained multiply phosphorylated species, which were denoted as +1 PO_4, +2 PO_4, etc. Modified Nm of known structure is indicated.

**FIG. 6.** Identification of various glutathione derivatives on reverse phase HPLC. A panel of derivatized glutathione compounds was used to assign identities to various peaks of reduced glutathione derivative (Fig. 6B) as compared with the control (Fig. 6A).

**FIG. 7.** Characterization of Ng thiolation by immunoprecipitation. A panel of oxidized glutathione derivatives was assayed for Ng thiolation by immunoprecipitation with antibody number 270 (Fig. 7B).
prominent by treatments with SNP, X-XO, and X-XO/SNP than those with H₂O₂ and diamide. The increase in thiolation of Ng induced by the former three treatments ranged from 3–4-fold, and those by the latter two treatments ranged from 0.6–1.6-fold more than the untreated control from two separate experiments. Thiolation of Ng detected in the immunoprecipitate was further confirmed by running the SDS-PAGE under reducing conditions, in which all the 35S bound to the protein was removed (Fig. 8C), and all the intramolecular disulfide forms of Ng were reduced (Fig. 8D).

**DISCUSSION**

While testing the effect of GSNO on the oxidation of Ng to form intramolecular disulfide, we found that the freshly prepared GSNO was effective in this modification, but a partially decomposed GSNO was more effective in causing glutathiolation (33). Analysis of the decomposition products of GSNO led us to identify GS(O)SG as one of the most potent glutathiolating agents among the various glutathione derivatives tested, including GSO₃H, GS(O)₂SG, GSNO, and GSSG (Fig. 3). Although these latter compounds can oxidize Ng to form intramolecular disulfide bonds, they are not very effective for glutathiolation of this protein. Glutathiolation of protein by GS(O)SG likely proceeds by the following reactions,

\[
R₁-SH + GS(O)SG \rightarrow R₁-S-SG + GSOH
\]  
**REACTION 1**

\[
R₂-SH + GSOH \rightarrow R₂-S-SG + H₂O
\]  
**REACTION 2**

where \(R₁\) and \(R₂\) are either protein or any sulfhydryl-containing compound. Modification of proteins containing multiple sulfhydryl groups, such as Ng, by GS(O)SG is complicated by two competing reactions, namely, formation of intramolecular disulfide and glutathiolation. Partially glutathiolated Ng can be driven to form intramolecular disulfide, but the intramolecular disulfide form of Ng cannot be glutathiolated. Thus, at a low GS(O)SG concentration Ng forms intramolecular disulfide, and at a high concentration Ng is glutathiolated. When the ratio of GS(O)SG/–SH is equal or greater than one, both Ng and Nm are stoichiometrically glutathiolated.

Protein S-thiolation, especially S-glutathiolation, has been recognized as one of the physiological responses to nitrosative and oxidative stresses. The mechanism by which these stresses induce protein S-thiolation is poorly understood. Several mechanisms have been proposed for protein glutathiolation including the following: 1) thiol–disulfide exchange between protein thiols and GSSG (38); 2) oxidation of protein thiols by oxy-radicals or H₂O₂ to form thyl radicals or sulfenic acids and then to interact with GSH to produce mixed disulfide (39); 3) nucleophilic attack of protein thiolate on GSNO to produce mixed disulfide (9–12); 4) oxidation of GSH to form sulfenic acid and then interact with protein thiols to form mixed disulfides (10); and 5) nitrosation of protein thiols followed by interaction with GSH to form mixed disulfides (9, 10). The present...
study suggests another mechanism that utilizes GS/OG as a potential GS-donor for protein glutathiolation. GS/OG is more potent than GSNO in glutathiolation of protein, and this degradation product of GSNO may account for some of the effects of GSNO. GS/OG is present at a low level in the control rat brain slices labeled with [35S]cysteine and is in —

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fig. 8. Identification of Ng thiolation by immunoprecipitation. Rat cortical slices were labeled with [35S]cysteine and treated with H2O2, SNP, diamide, XOX, and XOX/SNP for 15 min (see text for details). Tissue extracts (600 μg/ml) were incubated with anti-Seraphage. Ng was eluted from protein A-Sepharose with glycine, pH 3, and neutralized with Tris-Cl. Proteins were analyzed by nonreducing (A and B) and reducing (C and D) 10–20% SDS-PAGE and transferred to nitrocellulose membrane. The same membranes were analyzed by autoradiography (A and C) and immunoblot with antibody number 270 (C and D). Note that the levels of Ng among the various treated samples determined by immunoblot were comparable (B), but the extents of thiolation (A), as well as the formation of ox-Ng (B), were increased in the oxidant-treated samples. Both the thiolated Ng (A) and intramolecular disulfide-bridged form of Ng (ox-Ng) (B) disappeared following separation on reducing gel (C and D).
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Glutathiolation of Proteins by Glutathione Disulfide S-Oxide Derived from S-Nitrosoglutathione: MODIFICATIONS OF RAT BRAIN NEUROGRANIN/RC3 AND NEUROMODULIN/GAP-43

Junfa Li, Freesia L. Huang and Kuo-Ping Huang

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