British Journal of Pharmacology (2000) 130, 1060–1064

www.nature.com/bjp

Nitric oxide mediated modulation of norepinephrine transport: identification of a potential target for S-nitrosylation

*,[1]David M. Kaye, [1]Sara Gruskin, [2]A. Ian Smith & [1]Murray D. Esler

[1]Molecular Neurocardiology Laboratory, Baker Medical Research Institute, Commercial Road, Prahran, Victoria 3181, Melbourne, Australia; [2]Peptide Biology Laboratory, Baker Medical Research Institute, Commercial Road, Prahran, Victoria 3181, Melbourne, Australia

1 Carrier mediated uptake (uptake-1) transport of norepinephrine (NE) plays a key role in the regulation of sympathetic neurotransmission. Recent investigations indicate that nitric oxide (NO) may modulate uptake-1 activity, possibly in a cyclic GMP independent manner.

2 Carrier mediated transport of [^{3}H-NE] and [^{3}H-dopamine, DA] was examined in CHO cells transfected with cDNA for the NE and DA transporters (NET, DAT) respectively.

3 While exposure to the NO donor S-nitroso-N-acetylpenicillamine (100 μM, SNAP) significantly reduced [^{3}H-NE] uptake ($P<0.001$), no effect on [^{3}H-DA] transport was apparent.

4 Comparison of the amino acid sequences for NET and DAT identified cysteine residue 351 in NET, which was not present in DAT. Site-directed mutagenesis of Cys 351 to Ser produced a functional NET that was resistant to the inhibitory effects of SNAP.

5 The presence of SNAP mediated nitrosylation of the cysteine residue in an 8-mer model peptide based around Cys 351 in NET was confirmed by both biochemical and mass spectroscopic means.

6 These data indicate the potential regulatory role for NO in modulating sympathetic neurotransmission, and further confirm the importance of non-cyclic GMP dependent mechanisms in mediating the actions of NO.

British Journal of Pharmacology (2000) **130,** 1060–1064

Keywords: Uptake-1; norepinephrine; norepinephrine transporter; nitric oxide; nitrosylation

Abbreviations: CHO, Chinese hamster ovary; Cys, cysteine; NE, norepinephrine; NO, nitric oxide; SNAP, S-nitroso-N-acetyl penicillamine

Introduction

The plasmalemmal norepinephrine transporter (NET) located on the postganglionic sympathetic nerve terminal plays a key role in the rapid removal of neurotransmitter from the sympatho-adrenergic synapse. Together with the plasma membrane transporters for dopamine (DAT) and serotonin, this group of plasmalemmal proteins belong to a family of Na^+ and Cl^- coupled monoamine transporters that are predicted to contain 12 membrane spanning domains (Amara, 1993; Blakely *et al*., 1994; Pacholyczyk *et al*., 1991). Although comparison of NET with DAT reveals moderate structural homology, and functional studies demonstrate that the two transporters exhibit robust transport of both norepinephrine (NE) and dopamine, striking differences in their sensitivity to certain pharmacological agents is also observed (Buck & Amara, 1995; Giros *et al*., 1994).

Despite the identification of certain discrete structural domains that confer sensitivity to cocaine and tricyclic antidepressants, little evidence has accumulated to suggest the presence of physiological mechanisms for the acute regulation of monoamine transport by post-translational modification of plasmalemmal transporter protein (Bonisch & Eiden, 1998). To date, the only mechanism described for acutely modulating NE transport appears to be a protein kinase C-mediated reduction in the cell surface expression of NET, rather than by PKC-mediated phosphorylation of NET itself (Bonisch *et al*., 1998). Recently we examined the influence of nitric oxide (NO) on NE transport in PC12 cells (Kaye *et al*., 1997), given data suggesting that NO plays an important role in modulating neurotransmission (for review see Garthwaite & Boulton, 1995). In our previous study NO significantly depressed the uptake of [^{3}H]-NE in a concentration-dependent manner, which appeared to be mediated by a cyclic GMP independent process, possibly the result of S-nitrosylation of a regulatory site on the plasmalemmal NE transporter (Kaye *et al*., 1997).

NO is now widely recognized as a biological messenger with a diverse range of actions in both normal physiological and pathological states. In broad terms, it can be considered that these effects generally represent the consequences of interactions with thiols or transition metals, and the subsequent formation of S-nitrosothiols or metal nitrosyl products respectively (Stamler *et al*., 1997). Indeed, the classical NO-mediated cyclic GMP signalling cascade is a direct consequence of an interaction with the haeme core of soluble guanylate cyclase (GC), although it has recently been suggested that the presence of an appropriate motif in GC for S-nitrosylation could provide an alternate explanation for NO-mediated activation of GC. Nitrosylation of sulphydryl acceptors has also been proposed to account for many of the biological actions of NO, including interactions with the NMDA receptor, ion channels and a number of enzymes (Stamler *et al*., 1997; Zech *et al*., 1999). Accordingly, the aim of the present study was to specifically examine the role of S-nitrosylation as a mechanism for the acute regulation of NE transport, and to identify the site(s) at which this post-translational modification occurs.

*Author for correspondence; E-mail: david.kaye@baker.edu.au

Methods

Cloning and site-directed mutagenesis NE transporter

The left stellate ganglion was obtained at post-mortem from a 60-year-old male subject. Total RNA was subsequently extracted according to the method of Chomczynski & Sacchi 1987), after tissue homogenization. Reverse transcription of RNA was performed according to standards protocols (Sambrook *et al*., 1989). In brief 1 μg of total RNA was reverse transcribed for 10 min at 42°C in a solution containing 10 mM tris(hydroxymethyl)aminomethane (Tris)-HCl, 50 mM KCl, 5 mM $MgCl_2$, 1 mM deoxynucleotide triphosphates (dNTPs), 2.5 μM oligo d(T), 1 U μl^{-1} RNase inhibitor and 2.5 U μl^{-1} reverse transcriptase (MuLV, Perkin Elmer, Branchburg, NJ, U.S.A.).

Amplification of the resultant cDNA was performed by the polymerase chain reaction (31 cycles; 94°C denaturing for 30 s; 60 C annealing for 30 s; and 72°C extension for 1 min) using the following primers: (sense1) 5′-AGGACCGGTAAAGT TCCTCTCGCC-3′, (antisense1) 5′-CAGAATGTAGATCCA-GACAGGG-3′, (sense2) 5′-CTGCCTTACTTGTGCTGT TCG-3′, (antisense2) 5′-CCTGTGACCTGGACATTGGCA TGG, and which correspond to bp −34 to −10, bp +1250 to +1271, bp +865 to 886 and bp +1942 to +1965 of a human norepinephrine transporter (hNET) previously identified in neuroblastoma cells (Pacholyczyk *et al*., 1991). The resultant PCR products were purified and subcloned into pGEM T Vector (Promega), and used to transfect XL1-Blue *E. coli*. Subsequently the containing 5′ region of hNET was excised at the *Apa*I and *Hinc*II and the resultant product ligated with the product of a *Hinc*II – *Sal*I digest of the vector containing the 3′ portion of hNET, and cloned into pBluescript (Stratagene). The identity of the full-length cDNA was confirmed by automated sequencing (ABI Prism, data not shown). The full length NET cDNA was subsequently cloned into an expression vector (pcDNA3, Invitrogen). Introduction of an amino acid substitution mutation in NET was performed by site-directed mutagenesis (QuikChange, Stratagene), using single strand mutagenic primers designed to introduce three point mutations resulting in a C351S mutation and the creation of a silent *Spe*I restriction site.

Cell culture and transfection

Chinese hamster ovary (CHO) cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% foetal calf serum (FCS) in 24-well plates (Falcon) until 60–70% confluent. Cells were then transiently transfected (Lipofectamine, Gibco) with the expression vectors containing either wild-type NET cDNA, C351S mutant cDNA or with an expressing vector (pcDNA3) containing full-length cDNA for the human dopamine transporter (DAT, kindly provided by Dr J. Javitch, Columbia Univ, NY, U.S.A.). The transfection medium was removed and replaced with DMEM/FCS after 18 h, and experiments were conducted after a further 36 h.

Uptake studies

Prior to uptake studies CHO cells were washed with transport buffer containing (in mM): NaCl, 157; KCl, 2.7; NaH_2PO_4, 11.8; $MgCl_2$, 1; $CaCl_2$, 0.1; pH 7.4, as previously described (Gu *et al*., 1994). Uptake studies were then performed by incubating the cells in transport buffer supplemented with 50 nM [^{3}H]-1-NE (plus unlabelled NE as appropriate) and 100 μM ascorbic acid for 20 min at 37°C. Studies of [^{3}H]-dopamine employed the same protocol, using 85 nM [^{3}H]-dopamine. The cells were then rapidly washed in ice-cold phosphate buffered saline and lysed in 0.1% Triton X. The radioactivity of the cell lysates was determined by liquid scintillation counting. An aliquot of the cell lysate was retained for protein determination, using a commercially available system (Biorad DC Protein assay). Specific uptake of [^{3}H]-NE and [^{3}H]-DA was established by subtracting non-specific uptake in untransfected cells. Data are presented as mean ± s.e.mean, and were obtained from at least three separate experiments.

Protein nitrosation and measurement of nitroso content

Peptide nitrosation was conducted according to two separate methods. First nitrosation using $NaNO_2$ with HCl was performed essentially as described by Zhang *et al*., (1996). In brief, the 8-mer peptides (0.5 ml ml^{-1}) SSINCITS (corresponding to wild-type NET amino acids: 347–354) and SSINSITS (containing the C351S mutation) were combined with an equal volume of 1 mM $NaNO_2$ in 1 N HCl and allowed to react for 30 min at room temperature. The nitroso- content of the resultant samples was determined by means of the Saville assay (Saville, 1958). A 40 μl sample of the S-nitroso derivative was combined with an equal volume of 0.5% ammonium sulphamate in 0.4 N HCl for 2 min. Subsequently 400 μl of a solution containing 2.7% sulphanilamide and 0.25% $HgCl_2$ in 0.4 N HCl was added, followed by 320 μl of a solution containing 0.1% N-(1-napthyl)ethylenediamine dihydrochloride in 0.4 N HCl. The reaction mixture was incubated at room temperature for 10 min and the absorbance read at 544 nm. The nitroso-content was calculated from a standard curve using S-nitroso-acetyl penicillamine (SNAP) as the reference agent over the range 5–50 μM.

To explore whether peptide nitrosation could be detected under conditions that mimicked the cell culture studies, the 8 mer peptides (0.4 mg ml^{-1}) were incubated in phosphate-buffered saline containing 100 μM SNAP for 30 min at 37°C. At the termination of the incubation period, reaction mixtures were loaded onto a Sep-Pak column. After washing with 0.1% trifluoroacetic acid to remove SNAP from the reaction mixture, the bound peptides was eluted using 0.1% trifluoroacetic acid in 80% methanol. Peptide S-nitroso content was then determined as outlined above.

Mass spectroscopy

The two peptides (prior to and post the nitrosation reaction) were analysed using a Hewlett Packard 1100 MSD HPLC system with on-line u.v. and electrospray mass spectrometric detection (Hewlett Packard, Waldbronn, Germany). Aliquots (10 μg of each peptide) were injected onto a C-18 reversed-phase column (Vydac C18, 1 mm × 25 cm, Agilent Technologies, Forest Hill, Victoria, Australia). Separations were achieved using a linear gradient of 0–100% B over 60 min at a flow rate of 100 μl min^{-1} where Solvent A was 0.1% TFA with 0.02% acetic acid and solvent B was 60% acetonitrile containing 0.1% TFA. Absorbance of emerging peaks was monitored at 214 nm.

Statistical methods

Group data are presented as mean ± s.e.mean. Between group comparisons are performed using students *t*-test or analysis of variance for multiple comparisons. A P value less than 0.05 was considered significant.

Results

Nitric oxide inhibits norepinephrine transport

In keeping with our previous studies, the NO-donor S-nitroso-acetyl penicillamine (SNAP) significantly reduced the maximal rate of specific norepinephrine transport in CHO cells transfected with full-length cDNA for the human norepinephrine transporter (Figure 1). Our previous data showed that the inhibitory action of NO on norepinephrine transport is not mediated by a classic NO activated cylic GMP-protein kinase G pathway, but perhaps more likely due to S-nitrosation. Accordingly, to pursue this potential mechanism, we studied the influence of SNAP on catecholamine transport mediated by the dopamine transporter, a protein structurally related to the norepinephrine transporter. However, unlike the effect on norepinephrine transport, SNAP was completely without influence on the transport of [^{3}H]-dopamine by CHO cells expressing the dopamine transporter (Figure 2).

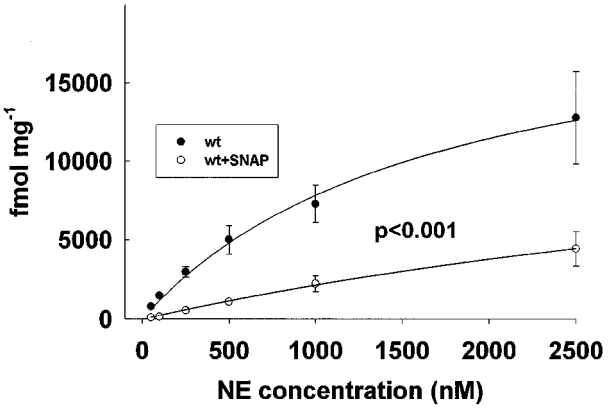

Figure 1 Effect of S-nitroso-N-acetyl penicillamine (SNAP, 100 μM) on the uptake of norepinephrine (NE) in CHO cells transfected with wild-type (wt) norepinephrine transporter.

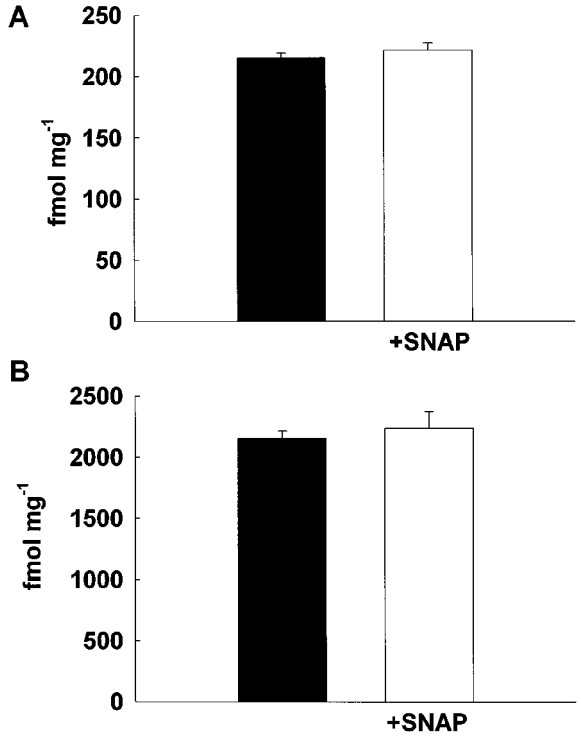

Figure 2 Lack of effect of 100 μM SNAP on the uptake of [^{3}H dopamine] in CHO cells expressing the dopamine transporter, at a dopamine concentration of 85 nM (A) and 1 μM (B) respectively.

To explore the potential mechanism for the apparent difference in the response of the catecholamine transporters to SNAP we compared the distribution of cysteine residues amongst the two proteins. Indeed, cysteine residues were highly conserved, with one notable exception being the presence of a cysteine residue at position 351 in the norepinephrine transporter in contrast to the corresponding position in the dopamine transporter (Figure 3). Site directed mutation of cysteine-351 to serine yielded a functional norepinephrine transporter that was resistant to the effects of SNAP (Figure 4).

Detection of S-nitrosation

To examine whether exposure to the NO donor SNAP could result in nitrosation of cysteine residues, we performed a series of complementary experiments. First, wild-type and NETC351S 8-mers were exposed to the nitrosating effects of $NaNO_2$/HCl. As described in Table 1, nitrosation of the wild-type peptide was readily detectable in contrast to the mutant C351S peptide. Similarly, exposure to 100 μM SNAP resulted in significant S-nitrosation, while no effect of SNAP on the mutant peptide was apparent. Using a similar experimental approach, the presence of wild-type peptide nitrosation was confirmed by RP-HPLC with on-line mass spectrometric detection, whilst the mutant peptide appeared resistant to nitrosation (data not shown).

Table 1 S-Nitrosation of peptides

Nitrosating agent	*Nitroso/peptide (mol/mol)*	
	Wild type NET 8-mer	*C351S NET mutant 8-mer*
$NaNO_2$/HCl	0.11±0.002	ND
SNAP (100 μM)	0.06±0.0003	ND

ND: not detectable.

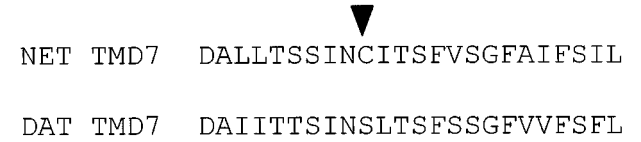

Figure 3 Alignment comparison of the predicted amino acid sequences for transmembrane domain 7 in the norepinephrine and dopamine transporters. Arrow indicates the non-conserved cysteine residue.

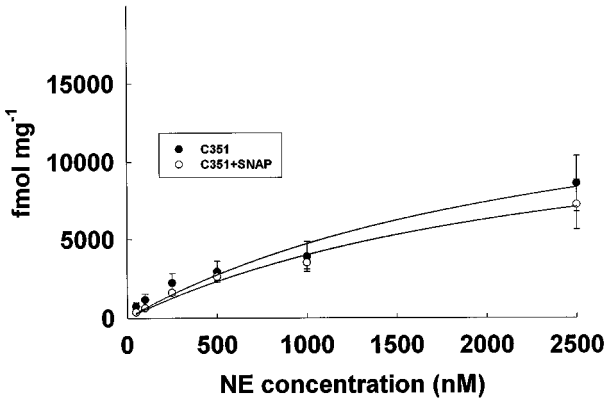

Figure 4 Effect of 100 μM SNAP on norepinephrine uptake in CHO cells expressing the Cys351Ser (C351S) norepinephrine transporter mutant.

Discussion

Nitric oxide (NO) is widely recognized as a biological messenger with a diverse range of actions in both normal physiological and pathological states. In broad terms, it can be considered that these effects generally represent the consequences of interactions with thiols or transition metals, and the subsequent formation of S-nitrosothiols or metal nitrosyl products respectively. Indeed, the classical NO-mediated cyclic GMP signalling cascade is a direct consequence of an interaction with the haeme core of soluble guanylate cyclase (GC), although it has recently been suggested that the presence of an appropriate motif in GC for S-nitrosylation could provide an alternate explanation for NO-mediated activation of GC. Nitrosylation of sulphydryl acceptors has also been proposed to account for many of the biological actions of NO, including interactions with the NMDA receptor, ion channels and a number of enzymes (Friebe *et al*., 1997; Stamler *et al*., 1997).

Recently we examined the influence of NO on norepinephrine (NE) transport in PC12 cells given the key role of NO in modulating neurotransmission (Kaye *et al*., 1997). In that study, NO generated by a NO donor or by endothelial cells expressing the inducible form of nitric oxide synthase significantly depressed the uptake of [^{3}H]-NE, and this appeared to be mediated by a cyclic GMP independent process. Furthermore, our observation that the influence of NO on NE uptake could be caused by both endogenously generated NO and a NO donor raised the possibility that the effect was the result of S-nitrosylation of a regulatory site on the plasmalemmal NE transporter, although not necessarily mediated *via* a trans-nitrosation reaction.

To further address the mechanism by which NO modulates NE transport we have examined the effect of a NO donor S-nitroso-acetylpenicillamine (SNAP), on the uptake of [^{3}H]-NE in CHO cells transfected with a full length cDNA for the human NE transporter. Consistent with our previous observations, SNAP significantly attenuated the uptake of [^{3}H]-NE. In these series of studies, uptake of norepinephrine was determined in the presence of the NO donor, and we did not attempt to examine the potential reversibility of this process, nor its time course.

To identify a possible S-nitrosylation target in the NE transporter, we investigated the effect of SNAP on the uptake of [^{3}H]-dopamine in CHO cells transiently expressing the rat dopamine transporter (DAT). The rationale for these studies was based upon the 66% homology with the NE transporter (Amara, 1993). In DAT transfected CHO cells, SNAP was without effect on [^{3}H]-DA transport. Comparison of the predicted amino acid sequences for NET and DAT reveals a high degree of conservation of cysteine residues (Amara, 1993; Kilty *et al*., 1991; Pacholyczyk *et al*., 1991). The NET transporter contains 10 cysteine residues, of which eight are conserved in DAT. The first of these mismatches is located in the amino terminal intracytoplasmic tail of NET and the other mismatch (C351) is located in the seventh transmembrane domain, which has been previously characterized as a potential binding site for tricyclic antidepressant mediated inhibition of NE transport (Giros *et al*., 1994). On this basis, site-directed mutagenesis was employed to substitute a serine residue for the cysteine residue at position 351. While CHO cells expressing the C351S NET mutant exhibited robust NE transport, a significant attenuation in the sensitivity to the influence of SNAP on NE transport was evident. These findings are therefore consistent with modulation of NE transport *via* nitrosylation of the thiol residue on cysteine 351. In addition to the functional studies, complimentary biochemical and mass spectroscopic studies confirmed the ability of the cysteine residue in an 8-mer peptide covering amino acids 347–354 of the norepinephrine transporter to undergo nitrosylation.

In addition to identifying nitrosylation as a means of rapidly modifying transport activity, our study also supports a key role for transmembrane domain 7 in mediating catecholamine transport by catecholamine transporters. For example Buck & Amara (1995) demonstrated a marked reduction in the V_{max} for [^{3}H]-dopamine transport by a series of chimeric transporters based upon the dopamine and norepinephrine transporters. Similarly, serine residues in the seventh transmembrane domain of the serotonin transporter, have been shown to alter substrate and inhibitor binding (Kitayama *et al*, 1993).

Although extensive data has characterized the pivotal role that the plasmalemmal norepinephrine transporter plays in the regulation of noradrenergic neurotransmission, few mechanisms for acute regulation of transport activity have been identified (Bonisch *et al*., 1998). The present data identify post-translational modification of cysteine-351 by S-nitrosylation as an important mechanism for acutely altering NE transport. Moreover, in this study, we demonstrate a potential mechanism for differential regulation of noradrenergic and dopaminergic neurotransmission by nitric oxide. While these studies specifically address the issue of catecholamine re-uptake, other studies have suggested that nitric oxide may also modulate catecholamine release (Lonart & Johnson, 1994). To this end, the functional effect of neuronally derived nitric oxide would be expected to represent the net balance of the influence of nitric oxide on neurotransmitter release and re-uptake. For example, Choate & Paterson (1999) recently showed that neuronally derived nitric oxide attenuated the positive chronotropic effect of sympathetic nerve stimulation in innervated guinea-pig atria. Further studies are also required to examine whether the influences of neuronally derived NO on release and re-uptake take place in parallel or at differing thresholds. In addition to NO generated by neurons themselves, we have also shown that under certain circumstances paracrine sources of NO may also modulate re-uptake (Kaye *et al*., 1997), raising this as an additional regulatory mechanism.

In conclusion, our data indicate that nitric oxide plays a key role in modulating catecholamine uptake *via* the norepinephrine transporter. Furthermore the application of a site-directed mutagenic approach indicates that the actions of nitric oxide are mediated *via* S-nitrosylation of a key cysteine residue in transmembrane domain 7 of the norepinephrine transporter.

This work was supported by grants from the Wellcome Trust (to D.M. Kaye) and an Institute Grant from the National Health and Medical Research Council of Australia.

References

AMARA, S. (1993). Neurotransmitter transporters. *Annu. Rev. Neurosci.,* **16,** 73–93.

BLAKELY, R.D., DEFELICE, L.J. & HARTZELL, H.C. (1994). Molecular physiology of norepinephrine and serotonin transporters. *J. Exp. Biol*., **196,** 263–281.

BONISCH, H. & EIDEN, L. (1998). Catecholamine reuptake and storage. *Adv. Pharmacol.,* **42,** 149–164.

BONISCH, H., HAMMERMAN, R. & BRUSS, M. (1998). Role of protein kinase C and second messengers in regulation of the norepinephrine transporter. *Adv. Pharmacol.,* **42,** 183–186.

BUCK, K.J. & AMARA, S.G. (1995). Structural domains of catecholamine transporter chimeras involved in selective inhibition by antidepressants and psychomotor stimulants. *Mol. Pharmacol.,* **48,** 1030 – 1037.

CHOATE, J.K. & PATERSON, D.J. (1999). Nitric oxide inhibits the positive chronotropic and inotropic responses to sympathetic nerve stimulation in the isolated guinea-pig atria. *J. Aut. Nerv. Syst.,* **75,** 100 – 108.

CHOMCZYNSKI, P. & SACCHI, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.,* **162,** 156 – 159.

FRIEBE, A., WDEEL, B., HARTENEK, C., FOERSTER, J., SCHULTZ, G. & KOESLING, D. (1997). Functions of conserved cysteines of soluble guanylyl cyclase. *Biochemistry,* **36,** 1194 – 1198.

GARTHWAITE, J. & BOULTON, C.L. (1995). Nitric oxide signaling in the central nervous system. *Ann. Rev. Physiol.,* **57,** 683 – 706.

GIROS, B., WANG, Y.-M., SUTER, S., MCLESKEY, S.B., PIFL, C. & CARON, M.G. (1994). Delineation of discrete domains for substrate, cocaine, and tricyclic antidepressant interactions using chimeric dopamine-norepinephrine transporters. *J. Biol. Chem.,* **269,** 15985 – 15988.

GU, H., WALL, S.W. & RUDNICK, G. (1994). Stable expression of biogenic amine transporters reveals differences in inhibitor sensitivity, kinetics, and ion dependence. *J. Biol. Chem.,* **269,** 7124 – 7130.

KAYE, D.M., WIVIOTT, S.D., KOBZIK, L., KELLY, R.A. & SMITH, T.W. (1997). Regulation of neuronal (uptake-1 mediated norepinephrine transport by nitric oxide: mechanism and influence of autocrine and paracrine sources of nitric oxide. *Am. J. Physiol.,* **272,** H875 – H883.

KITTY, J.E., LORANG, D. & AMARA, S.G. (1991). Cloning and expression of a cocaine-sensitive rat dopamine transporter. *Science,* **254,** 578 – 579.

KITAYAMA, S., WANG, J. & UHL, G. (1993). Dopamine transporter mutants selectively enhance MPP + transport. *Synapse,* **15,** 58 – 62.

LONART, G. & JOHNSON, K.M. (1994). Inhibitory effects of nitric oxide on the uptake of [^{3}H] dopamine and [^{3}H] glutamate by striatal synaptosomes. *J. Neurochem.,* **63,** 2108 – 2117.

PACHOLYCZYK, T., BLAKELY, R.D. & AMARA, S. (1991). Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. *Nature,* **350,** 350 – 353.

SAMBROOK, J., FRITSCH, E.F. & MANIATIS, T. (1989). *Molecular cloning: A laboratory manual*. New York: Cold Spring Harbor.

SAVILLE, B. (1958). A scheme for the colorimetric determination of microgram amounts of thiols. *Analyst,* **83,** 670 – 672.

STAMLER, J.S., TOONE, E.J., LIPTON, S.A. & SUCHER, N.J. (1997). (S)NO signals: translocation, regulation, and a consensus motif. *Neuron,* **18,** 691 – 696.

ZECH, B., WILM, M., VANELDICK, R. & BRUNE, B. (1999). Mass spectrometric analysis of nitric oxide-modified caspase-3. *J. Biol. Chem,.* **274,** 20931 – 20936.

ZHANG, Y.Y,. XU, A.M., NOMEN, M., WALSH, M., KEANEY, J.F. & LOSCALZO, J. (1996). Nitrosation of tryptophan residues in serum albumin and model dipeptides: biochemical characterization and bioactivity. *J. Biol. Chem.,* **271,** 14271 – 14279.

(Received February 11, 2000
Revised March 22, 2000
Accepted April 6, 2000)