Free Rad. Res., Vol. 31, pp. 619–629
Reprints available directly from the publisher
Photocopying permitted by license only

Capturing the Sublimity of a Free Radical Gas

SENG-KEE LEONG

Department of Anatomy, Faculty of Medicine, National University of Singapore, 10, Kent Ridge Crescent, Singapore 119260, Singapore

Accepted by Prof. B. Halliwell

(Received 10 April 1999; In revised form 4 May 1999)

This paper reviews the work related to nitric oxide (NO) done by the author and his postgraduates and colleagues in the past 7 years in the National University of Singapore. Our work shows that (i) NADPH-d and NO synthase (NOS) are often but not always identical; (ii) NO (as indicated by NADPH-d histochemistry and NOS immunohistochemistry) is generated in some endocrine (thyroid, parathyroid and ultimobranchial glands) and immune (thymus and bursa of Fabricius) organs and the cochlea. It is noted from the above studies that NO could possibly regulate blood flow through the various organs via its presence in the vascular endothelial cells and also via nitrergic neurons innervating the blood vessels. It could also regulate the activity of the secretary cells of these organs by being present in them, as well as acting through nitrergic neurons closely related to them. The paper also examines the Janus-faced nature of NO as a neuroprotective and neurodestructive agent, and the apparent non-involvement of peroxynitrite and inducible NOS in neuronal death occurring in the red nucleus and nucleus dorsalis after spinal cord hemisection.

Keywords: Nitric oxide, NADPH-d, NOS, endocrine and immune organs, cochlea, neuroprotective, neurodestructive, peroxynitrite, nucleus dorsalis, red nucleus

INTRODUCTION

Reviews[1–3] of the early history of nitric oxide (NO) reveal that this colourless gas was first generated by heating potassium nitrate (nitre) with glowing charcoal in the absence of air. First named nitrous air because it was regarded as volatile nitre in the air, it was later renamed nitric oxide by Murray in 1806. Subsequent work by chemists showed that the gas could be generated by other methods. Of little compliment to the gas is the finding that it is an atmospheric pollutant, being the by-product of jet and motor engines. However, some sublimity of the gas is noted in its ability to reduce putrefaction. In fact, it has been utilised extensively for preserving meat and vegetables. The discovery by Mitchell *et al.* in 1916[3] that the gas is also produced in biological systems and later verification by biologists further raised the status of the gas. In fact NO started to sublime when a series of studies[4,5] led to the conclusion that it is involved in macrophage-induced cytotoxicity directed against certain tumour cell types. Some of these studies also discovered the substrate required for the production of NO, viz., L-arginine, and the competitive inhibitors of the substrate, the N_G-substituted L-arginine analogues. Later study by Murad *et al.*[6] led to the conclusion that NO released by nitroglycerine can relax smooth muscle cells.

In fact, NO could be the equivalent of endothelium derived relaxing factor or some related substance, from which NO is released.[7,8] Furchgott and Ignarro then presented in a scientific meeting in 1986 their discovery of NO as a signal molecule in the organism. Such landmark discovery of a gas inspired many cell and molecular biologists to study more of its properties, resulting in the capture of more of its sublimity (and ignobility). In fact, the findings of the trio, Furchgott, Murad, and Ignarro, laid the foundation for the discovery of the sensational Viagra, an anti-impotence drug. During the first two weeks on the market in the United States, 36,809 prescriptions of this drug were dispensed. The trio were awarded the Nobel Prize for Medicine in 1998.

While reviews of the genesis, biochemistry, physiology and mechanism of action of nitric oxide are not lacking (e.g. Refs. [9–14]) the anatomical distribution of NO is usually cursorily dealt with. The present review is not an attempt to provide an exhaustive account of the anatomical localisation of NO, rather to put together the findings of our laboratory in the past 7 years. The anatomical review will be followed by an analysis of the Janus-faced nature of NO, which has bewildered NO scientists for many years.

Note on NOS Isoforms

Before reviewing our past work, a brief note on the nitric oxide synthase (NOS) isoforms should be in place. The cell cannot directly regulate the concentration of NO. It has to regulate it by controlling NO synthesis. For this reason the structure of NOS has become a major interest of molecular biologists and has been extensively studied and reviewed, as stated earlier. An understanding of its molecular structure will lay the foundation for the development of drugs for the treatment of diseases related to over- or underproduction of NO. At least 3 isoforms of NOS have been characterised, and these may be either constitutive or inducible. The neuronal (NOS I) and endothelial (NOS III) forms are said to be constitutive as their activation (by calcium/calmodulin) to produce NO does not require new enzyme synthesis. Though normally present in the cell, they may also be induced under conditions of disease and trauma. The inducible (NOS II), now known as the immunological form of NOS, was first detected in macrophages stimulated by a variety of cytokines. It is not normally detectable in the cell and requires new protein for its synthesis, but is not dependent on calcium for its activation. In addition to macrophages, NOS II has also been induced in microglia and astrocytes. Unlike the constitutive NOS that produces NO for short period, NOS II produces large amount of NO for sustained periods. While this could lead to cytotoxic action on micro-organisms and tumour cells, it may also lead to disease states.

ANATOMICAL LOCALISATION OF NO

As NO is a gas, it cannot be captured for visualisation in an anatomical locus. Its precise cellular location is usually indicated by nicotinamide adenine dinucleotide hydrogen phosphate-diaphorase (NADPH-d) histochemistry and/or NOS immunohistochemistry. In the histochemical method, first introduced by Thomas and Pearse[15] and subsequently modified by other workers, oxidative enzymes that possess diaphorase activity reduce tetrazolium dyes in the presence of NADPH to a dark blue formazan precipitant. The first anti-NOS antibodies developed were against purified rat cerebellar nNOS.[16]

Is NADPH-d Always Colocalised with NOS?

It has been claimed that NADPH-d is NOS,[17,18] or that the two will only colocalise when the tissue is fixed in 4% paraformaldehyde.[19] Our findings showed total colocalisation of NADPH-d and NOS in pancreatic ganglion neurons,[20] and in neuronal and non-neuronal cells of some immune

(thymus, ultimobranchial gland and bursa of Fabricius) and endocrine (thyroid and parathyroid) organs fixed by 4% paraformalydehyde.[21–23] The same is also true of the neurons of the nucleus dorsalis after axotomy.[24] There is, however, a discrepancy in localisation between the two enzymes in the facial motoneurons after axotomy.[25] Also, while red nucleus neurons display positive NOS I immunofluorescence, they do not stain for NADPH-d in normal as well as experimental rats subjected to spinal cord hemisection.[24] Other observations made by us also suggest that NADPH-d and NOS are not the same enzyme even though they may be found in the same cell. Thus, while NADPH-d is localised in the membrane of the nucleus, rough endoplasmic reticulum, mitochondria and other subcellular organelles in the chick thymus and guinea pig cochlea,[26,27] and in the membrane of the synaptic vesicle in the rat spinal trigeminal nucleus,[27a] NOS I is diffusely present in the neuronal cytoplasm and axoplasm with no relationship to any subcellular organelle in the spiral ganglion cells and pancreatic neurons in the guinea pig.[27,28] This confirms a previous report[29] of the cytosolic nature of NOS, although particulate fractions of NOS have also been identified[30] and NOS I has been localised to the sarcolemma of fast muscle fibres.[31] In addition, it is well known that NO is produced and released on demand without involving vesicular structures of the nerve endings.[32] Based on these findings, future work using the two enzymes as indicators for the presence of NO should do a colocalisation study before assuming that the results from one enzyme may be applied to the other.

DISTRIBUTION AND FUNCTIONS OF NO

NO, as revealed by NADPH-d histochemistry and NOS immunohistochemistry and also by other methods, has been reported in the vasculature,[7,8,34] macrophages and neutrophils (for review, see Moncada *et al.*[9]) neural tissue[9,11,32–40] including the retina,[41] sensory[42] and autonomic[9,43–46] ganglion neurons, and the nerve plexuses surrounding the carotid body,[47,48] carotid sinus,[49] respiratory tract,[50,51] gastrointestinal tract,[52,53] urinary tract,[54,55] reproductive organs,[56,57] endocrine organs like the adrenal gland,[58] the endocrine pancreas[59] and the pituitary gland,[60] and other tissues like the skeletal muscle.[31,61] Studies related to neurons show that in the brain, NO is a ubiquitous neural messenger involved in a wide variety of brain functions and pathologies. These include noradrenaline, dopamine and glutamate release, wakefulness, morphogenesis, synaptic plasticity, learning, memory, long-term potentiation, regulation of gene expression, circadian rhythm, olfaction, cerebrovascular system regulation, and food intake. It may also be associated with stroke, cerebral ischaemia, Alzheimer's disease and Huntington's disease. NO is most likely the endothelium derived relaxing factor which brings about vasodilation. It also causes relaxation of smooth muscles in other body systems such as the gastrointestinal and respiratory tracts. In addition, it is responsible for the regulation of renal haemodynamics and excretory function, and a host of other key biological functions. Its major biochemical target is the soluble guanylate cyclase, forming the second messenger, cyclic guanosine monophosphate (cGMP), the level of which is thereby raised to act on different classes of enzymes to bring about complex response characteristics. Analysis of the results by various investigators cited in the above literature reveals that many of the responses elicited by NO are of an inhibitory nature. These include inhibition of contraction of smooth muscles in blood vessels, urogenital tract and bronchial tree; force output of skeletal muscle; platelet formation; substance P and cholinergic transmission in ileum; chemoreceptors in carotid body, and sensory nerves in the mesentery. Also, NO depresses the background activity in the dorsal horn, is colocalised with GABA in a number of CNS regions like the lamina II of the spinal cord and the cerebral cortex, brings about long-term depression in the

hippocampus and the cerebellum, is cytostatic on microbes and is tumoricidal, and prevents neuronal differentiation. Lastly, NOS I gene knockout mice show aggressive behaviour and excessive inappropriate sexual behaviour.[62]

Differences between NO and Classical Neurotransmitters

The above and related works have established NO as a neurotransmitter in the peripheral nervous system. Though it is clear that it is a neuronal messenger in the central nervous system, further work needs to be done to establish its neurotransmitter nature in every location it is found. As a neurotransmitter, NO differs from the classical ones in that it is not stored in any synaptic vesicles; rather, it is synthesised on demand by NOS from L-arginine. Judging from the diffuse distribution of NOS, NO may be generated anywhere in a neuron. When released it diffuses across cellular membranes and brings about its action by acting on intracellular targets of adjacent cells. Its diffusion distance in the peripheral tissue is 0.5 mm but has not been established in the brain. Neither is its half-life known, though it is often said to be between 3 and 5 s.

Our Findings Related to the Distribution and Possible Functions of NO

(i) In Immune and Endocrine Organs

Our laboratory was the first to report the presence of a variety of NADPH-d/NOS positive non-neuronal cells at the corticomedullary junction in the chick and rat thymus.[63] Downing[64] subsequently confirmed this finding. The results suggested a role of NO in modulating the function of the labelled undifferentiated, lymphoid, cystic, myoid, endocrine-like and some other epithelial reticular cells. Latter work by Fehsel *et al.*[65] showed that NO could induce apoptosis in thymocytes. We also reported colocalisation of NADPH-d with neuron specific enolase in some cells associated with blood vessels in the interlobular connective tissue and at the corticomedullary junction,[66,67] thus establishing the existence of intrathymic neurons for the first time. Unlike the central nervous system, nitrergic nerves were not demonstrable in the embryonic thymus of the chick until E17, indicating that NO plays no part in early development of the organ. At E17 and later, NO might be required to facilitate the egress of T-lymphocytes from the thymus through the thymic vasculature, as the first mature T-lymphocytes are detected towards the end of the gestation period.

In addition, another group in our laboratory[21–23] reported nitrergic reactivity in neuron-like cell bodies and fibres and endothelial cells of larger blood vessels of the thyroid, parathyroid and ultimobranchial glands and immune organs such as the bursa of Fabricius of chickens. Nitrergic reactivity was detected in the thyroid follicular epithelial cells, parathyroid chief cells, cystic epithelial cells and C cells of the ultimobranchial gland, and interfollicular epithelial cells and lymphocytes of the bursa of Fabricius. Besides the above organs, NADPH-d reactivity has also been demonstrated in pancreatic ganglion neurons and endothelial lining of the vasculature of the pancreas of many mammalian (including monkeys) and avian species.[68]

With regard to the pattern that NO normally regulates the activities of the organ secreting it, two common features emerge from the above studies. (1) Judging from the distribution of NADPH-d and NOS I, it is likely that NO regulates blood flow through the organ via its presence in the vascular endothelial cells, as well as via nitrergic neurons innervating the blood vessels. (2) NO also regulates the activity of the secretary cells of these organs by being present in them, as well as acting through nitrergic neurons closely related to them. This is supported by a previous finding[69] that the nerve fibres, which deviate from blood vessels and appear to terminate in relation to the chief cells of the parathyroid gland, are secretary fibres.

In addition to the above two common features, NO should regulate the activities of thymocytes of the thymus and lymphocytes of the bursa of Fabricius, as nitrergic reactivity has been detected in these cell types, and nitrergic nerve fibres have been found closely related to them. As the thymus and the bursa of Fabricius are the exclusive organs forming T and B lymphocytes respectively in the bird, the NO generated and released may act as a messenger molecule for communication with their microenvironment. Its exact roles in immunoregulation await physiological elucidation.

(ii) In the Cochlea

Our study[27] showed that NO may be involved in the regulation of guinea pig and human cochlear blood flow and in the neurotransmission of the inner hair cells. NOS immunoreactivity exists in both afferent and efferent innervation of the inner hair cells, as demonstrated in the spiral ganglion neurons and the inner spiral and radial nerve fibres.

COEXISTENCE OF NO WITH OTHER NEUROACTIVE CHEMICALS

As stated earlier, NO may coexist with acetylcholine or noradrenaline. It may also coexist with various peptides[70–75] such as vasoactive intestinal peptide (VIP), somatostatin, calcitonin gene related peptide, galanin, neuropeptide Y (NPY), tachychinin and substance P. Our studies[20,76] have also demonstrated nitrergic neurons containing various peptides such as VIP, NPY, substance P, calcitonin gene-related peptide and bombesin, as well as choline acetyltransferase (ChAT), and dopamine-β-hydroxylase (DβH), an enzyme involved in the synthesis of noradrenaline. With double labelling in adjacent sections, we have shown that certain pancreatic neurons may contain as many as 4 neuroactive chemicals: ChAT/NOS/VIP/NPY, ChAT/NOS/VIP/DβH, ChAT/NOS/NPY/DβH. The coexistence of a plethora of neuroactive chemicals in the pancreatic ganglion neurons opens up a new vista to research into the mechanisms of neuronal transmission in the pancreas. Our studies were the first to report the coexpression of NADPH-d and DβH activities in the pancreatic neurons, and also reveal that the same nitrergic pancreatic neurons may contain both ChAT and DβH, suggesting that the same neuron may synthesise both acetylcholine and noradrenaline and that their axon terminals might use both acetylcholine and noradrenaline. It is not impossible that acetylcholine release is controlled by noradrenaline, as noradrenaline release is controlled by acetylcholine.[77] Our study is supported by another report that some pelvic ganglion neurons also contain both ChAT and tyrosine hydroxylase.[78]

COLOCALISATION WITH GLUTAMATE RECEPTORS

Our study[79] has demonstrated the anatomical localisation of glutamate receptor AMPA subunits (Glur 1, Glur 2/3 and Glur 4) in pancreatic islet and ganglion cells. This provides the morphological basis for the hormone secretion mediated by these receptors.[80] We also demonstrated the colocalisation of Glur 2/3 and Glur 4 with NADPH-d in the majority of pancreatic ganglion neurons of the guinea pig. Although there is convincing evidence that some neuronal effects of glutamate in the central nervous system may be mediated by NO,[81,82] one cannot assume that such is the case in the peripheral nervous system. Our study, however, has provided evidence to show that it does occur with pancreatic neurotransmission.

NITRIC OXIDE – FRIEND OR FOE?

Whether NO is a friend rendering neuroprotection or a foe causing neurodestruction has sparked off intense interest and discussion.

Proponents of the neurodestructive role of NO hypothesised that when produced in excessive amounts, as indicated by an upregulation of NADPH-d or NOS, NO might be responsible for the neuronal death observed after sciatic neurectomy in 1-day-old rats,[83] spinal root avulsion[84] or axotomy of the vagus nerve.[85] This is supported by the observation that NOS inhibitors can significantly reduce the death of NOS positive motoneurons induced by spinal root avulsion.[84] They can also protect some cultured cortical neurons from death induced by exposure to glutamate neurotoxicity.[86] NO might exert its neurotoxic effect by inhibiting enzymes involved in DNA synthesis, and might also suppress mitochondrial electron transport as well as the citric acid cycle by binding to iron–sulphur prosthetic groups.[87,88] On the other hand, NOS-containing neurons have been shown to be resistant to neurodegenerative disease such as Huntington's chorea and excitatory amino acid neurotoxicity.[89,90] Also some *in vivo*,[91,92] *in vitro*[93–95] and brain slice[96] studies showed no significant neurodestruction, and in fact, even neuroprotective effects of NO.

Our Findings

A series of our investigations in the above subject has led us to believe that NO has a dual role of neurodestruction and neuroprotection. In one set of experiments, we[97] showed that about 60% of the sciatic motoneurons in BALB/c mice are lost within the first 15 days after sciatic nerve cut at day 5 postnatal. During this period, none of the ventral horn cells expressed NADPH-d reactivity. The fact that neuronal death could occur in the absence of NADPH-d expression indicates that NO is not responsible for motoneuron death induced by neurectomy at the fifth postnatal day. In fact, it is doubtful whether NO is responsible for the neuronal cell death induced by sciatic neurectomy in 1-day-old rats, especially when Li *et al.*[98] and our study have demonstrated no significant difference in the number of cells lost, whether the sciatic nerve is cut at the first or fifth day after birth. NO may, in fact, have a beneficial function as suggested by another study of ours[99] which showed sprouting of NADPH-d positive facial motoneurons in adult rats subjected to facial nerve compression. NO may protect injured neurons from the by-products of increased metabolic activity as a result of its free radical-scavenger function.[100] A related important observation is the increased diaphorase staining of the vascular endothelium on postnatal day 7 when the total soluble nitric oxide synthase activities (measured by radiometric assay) in the facial motor nucleus and surrounding tissue are high.[101] This suggests that the increase in total NOS activities immediately after facial nerve compression may be predominantly endothelial. This could lead to vasodilation and therefore improved blood supply to the region containing the cell bodies of the neurons whose axonal processes had been compressed. L-NAME administration had no significant effect upon the period taken for maximal facial function recovery.[102] Our recent study in the cochlea also showed that the destructive effect on auditory hair cells of an NO donor, sodium nitroprusside, is not due to NO, rather to the cyanide released from the donor.[103] In fact, nitroglycerin, another NO donor, infused into the perilymphatic space could protect nerve endings at the base of inner hair cells from injury.[104] Lastly, NOS immunoreactive neurons in the arcuate nucleus in the mouse appear to be selectively spared after neonatal glutamate treatment, although they appeared less intensely stained for the enzyme.[105] This is consistent with reports demonstrating that nitrergic neurons are relatively spared in the hippocampus of brains obtained from patients who died of Alzheimer's disease[106] and in the striatum of Parkinson and Alzheimer brains.[107]

While all the above studies point to a neuroprotective role of NO, our experiment on the rat after facial nerve avulsion[25] seems to point to a neurodestructive role of the gas. After avulsion

of the facial nerve, the number of NADPH-d and NOS positive neurons increases steadily with increasing survival time. Neuronal death occurs at a time closely parallel to the development of intense NADPH-d/NOS reactivity in the facial motoneurons. Daily administration of L-NAME protects only 17% of the neurons from death. This might be due to the fact that the chemical was introduced intraperitoneally rather than in a concentrated dosage to the site of NO genesis. Also, it was delivered at 24 h interval. Shorter time intervals for its deliverance might be able to maintain an adequate sustained level of the chemical to give a more beneficial effect. However, one cannot rule out the possibility that the small percentage of neurons salvaged by L-NAME could mean that NO might not be that destructive after all.

Our subsequent work[24] on two different central nuclei has also shed light on the subject, especially as it is an *in vivo* study. It showed marked differences in NOS I expression between nucleus dorsalis (ND) and red nucleus (RN) after axotomy at the lower thoracic cord segment of the rat. Neurons of ND, which normally do not express NOS I reactivity, show intense NOS I staining after axotomy and display signs of degenerative changes. Shortly afterwards, there is significant neuronal loss in the ND. L-NAME injection[108] after spinal cord hemisection reduces neuronal loss in the ipsilateral ND, implicating a neurodestructive role of NO. On the other hand, NOS I immunoreactivity is moderately expressed in neurons of the RN in normal rats. It is upregulated on both sides of the nucleus after cord hemisection. Neuronal loss in the contralateral RN is not detected until 4 weeks after NOS I upregulation. No significant neuronal loss is found in the ipsilateral nucleus, even though 10–28% of the RN neurons project to the ipsilateral spinal cord. As there is no apparent difference in NOS I expression between the ipsilateral and contralateral RN, it seems unlikely that NO is responsible for the death of neurons in the contralateral RN. In fact, the administration of L-arginine results in significant reduction of neuronal loss in the contralateral RN, but treatment with L-NAME has no effect.

Despite what has been said[109–116] about the oxidative stress and neurotoxicity of some putative endogenous NO derivatives like peroxynitrite and thiol compounds, beneficial biological effects of *S*-nitrosoglutathione (GSNO) have been reported by not a few investigators in a number of tissues[117–121] and also recently by Rauhala *et al.*,[122] who suggested that the neuroprotective effect of GSNO and/or NO may be due to their potent antioxidative properties in terminating the lipid peroxidation chain reactions caused by redox cycling of iron–oxygen complexes. We also show that peroxynitrite plays no or little role in the neurodegeneration in the ND and RN after axotomy.[24] Most neurons in the RN of normal rats are weakly reactive to nitrotyrosine, a marker for peroxynitrite. After axotomy, neurons in both ipsilateral and contralateral RN show progressive increase in NT immunoreactivity. The increase parallels that of NOS staining in the RN. However, there is no difference in NT immunostaining between the ipsilateral and contralateral nuclei. There is also no NT staining in the ND.

Lastly, the involvement of NOS II has been implicated in the development of such pathological conditions as cerebral ischaemia,[123] experimental allergic encephalitis,[124] multiple sclerosis,[125] and subarachnoid haemorrhage.[126] It is conjectured that the excessive NO produced may cause oxidative injury, leading to the cascade of pathological events seen in these conditions. Our study,[127] however, observed the expression of NOS II only in the supraventricular amoeboid microglial cells after intraperitoneal injections of lipopolysaccharide or interferon-γ in neonatal BALB/c and athymic mice. Also, after lower thoracic spinal cord hemisection in rats, though intense microglial reaction could be observed in the ND and the RN, and a large number of the neurons in these two regions (32% in the ND and 13% in the RN) eventually die, there is no expression of NOS II in the affected nuclei.[24]

References

[1] J.R. Partington (1964) *A History of Chemistry.* Vol. 4. McMillan Company, London.

[2] M. Feelisch and J.S. Stamler (1996) *Methods in Nitric Oxide Research.* John Wiley & Sons, Incorporated, New York, USA.

[3] J.J. Lancaster (1996) *Nitric Oxide: Principles and Actions.* Academic Press, New York, USA.

[4] D.J. Stuehr and M.A. Marletta (1985) Mammalian nitrate biosynthesis: mouse macrophages nitrite and nitrate in response to *Escherichia coli* lipopolysaccharide. *Proceedings of the National Academy of Sciences (USA)*, **82**,7738–7742.

[5] J.B. Hibbs, R.R. Taintor and Z. Vavrin (1987) Macrophage cytotoxicity: role for L-arginine deaminase and imino nitrogen oxidation to nitrite. *Science*, **235**, 473–476.

[6] F. Murad, C.K. Mittal, W.P. Arnold, S. Katsuki and H. Kimura (1978) Guanylate cyclase: activation by azide, nitro compounds, nitric oxide, and hydroxyl radical and inhibition by hemoglobin and myoglobin. *Advances in Cyclic Nucleotide Research*, **9**, 145–158.

[7] R.F. Furchgott and J.V. Zawadzki (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature (London)*, **288**, 373–376.

[8] R.M.J. Palmer, A.G. Ferrige and S. Moncada (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature (London)*, **327**, 524–526.

[9] S. Moncada, R.M.J. Palmer and E.A. Higgs (1991) Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacological Reviews*, **43**, 109–142.

[10] C. Nathan (1992) Nitric oxide as a secretory product of mammalian cells. *FASEB Journal*, **6**, 3051–3064.

[11] H.H.H.W. Schmidt and U. Walter (1994) NO at work. *Cell*, **78**, 919–925.

[12] V.L. Dawson and T.M. Dawson (1995) Physiological and toxicological actions of nitric oxide in the central nervous system. *Advances in Pharmacology*, **34**, 323–342.

[13] E. Aoki, I.K. Takeuchi and R. Shoji (1995) Nitric oxide: an attractive signaling molecule. *Acta Histochemica et Cytochemica*, **28**, 97–106.

[14] Y. Wang and P.A. Marsden (1995) Nitric oxide synthases: gene structure and regulation. *Advances in Pharmacology*, **34**, 71–90.

[15] E. Thomas and A.G.E. Pearse (1964) The solitary active cells: histochemical demonstration of damage-resistant nerve cells with a TPN-diaphorase reaction. *Acta Neuropathologica*, **3**, 238–249.

[16] D.S. Bredt and S.H. Snyder (1990) Isolation of nitric oxide synthase, a calmodulin-requiring enzyme. *Proceedings of the National Academy of Sciences (USA)*, **87**, 682–685.

[17] B.T. Hope, G.J. Michael, K.M. Knigge and S.R. Vincent (1991) Neuronal NADPH-diaphorase is a nitric oxide synthase. *Proceedings of the National Academy of Sciences (USA)*, **88**, 2811–2814.

[18] T.M. Dawson, D.S. Bredt, M. Fotuhi, P.M. Hwang and S.H. Snyder (1991) Nitric oxide synthase and neuronal NADPH-diaphorase are identical in brain and peripheral tissues. *Proceedings of the National Academy of Sciences (USA)*, **88**, 7797–8701.

[19] T. Matsumoto, M. Nakane, J.S. Pollock, J.E. Kuk and U. Förstermann (1993) A correlation between soluble brain nitric oxide synthase and NADPH-diaphorase activity is only seen after exposure of the tissue to fixative. *Neuroscience Letters*, **155**, 61–64.

[20] H.P. Liu, S.S.W. Tay and S.K Leong (1996) Nitrergic neurons in the pancreas of newborn guinea pig: their distribution and colocalization with various neuropeptides and dopamine-β-hydroxylase. *Journal of the Autonomic Nervous System*, **61**, 248–256.

[21] M.A. Syed, S.K Leong and A.S. Chan (1994) Localization of NADPH-diaphorase reactivity in the chick and mouse thyroid gland. *Thyroid*, **4**, 475–478.

[22] M.A. Syed, A.S. Chan and S.K. Leong (1995) Localization of nitrergic neuronal and non-neuronal cells in the ultimobranchial glands of the chicken. *Anatomy and Embryology*, **193**, 161–168.

[23] M.A. Syed, S.K. Leong and A.S. Chan (1996) Histochemical and immuno-histochemical localization of nitrergic neuronal and non-neuronal cells in the bursa of Fabricius. *Cell and Tissue Research*, **285**, 273–279.

[24] M. Xu, Y.K. Ng and S.K. Leong (1998) Induction of microglial reaction and expression of nitric oxide synthase I in the nucleus dorsalis and red nucleus following lower thoracic spinal cord hemisection. *Brain Research*, **808**, 23–30.

[25] R.S. Ruan, S.K. Leong and K.H. Yeoh (1995) The role of nitric oxide in facial motoneuronal death. *Brain Research*, **698**, 163–168.

[26] P. Gulati, A.S. Chan and S.K. Leong (1995) Ultrastructral localisation of NADPH-diaphorase in the chick thymic medulla. *Cell and Tissue Research*, **279**, 405–409.

[27] R.S. Ruan, S.K. Leong and K.H. Yeoh (1997) Localization of nitric oxide synthase and NADPH-diaphorase in guinea pig and human cochleae. *Journal of Brain Research*, **38**, 433–441.

[27a] J.F. Yeo, F.R. Tang and S.K. Leong (1997) Ultrastructural study of NADPH-d positive neurons in Laminae I & II of the rat caudal spinal trigeminal nucleus. *International Journal of Neuroscience*, **91**, 29–43.

[28] H.P. Liu (1997) Innervation of the pancreas. Ph.D. thesis. National University of Singapore.

[29] H.H.H.W Schmidt, J.S. Pollock, M. Nakane, L.D. Gorsky, U. Förstermann and F. Murad (1991) Purification of a soluble isoform of guanylyl cylase-activating-factor synthase. *Proceedings of the National Academy of Sciences (USA)*, **88**, 365–369.

[30] U. Forstermann, J.S. Pollack, H.H. Schmidt, M. Heller and F. Murad (1990) Calmodulin dependent endothelium-derived relaxing factor/nitiric oxide synthase activity is present in the particulate and cytosolic fractions of bovine aortic endothelial cells. *Proceedings of the National Academy of Sciences (USA)*, **88**, 1788–1792.

[31] M.B. Reid (1998) Role of nitric oxide in skeletal muscle: synthesis, distribution and functional importance. *Acta Physiologica Scandinavica*, **162**, 401–409.

[32] T.M. Dawson, V.L. Dawson and S.H. Snyder (1992) A novel neuronal messenger molecule in brain. *Annals of Neurology*, **32**, 297–311.

[33] C.J. Lowenstein, J.L. Dinerman and S.H. Snyder (1994) Nitric oxide: a physiologic messenger. *Annals of Internal Medicine*, **120**, 227–237.

[34] J.S. Gillespie, X. Liu and W. Martin (1989) The effects of L-arginine and N^G-monomethyl L-arginine on the response of the rat anococcygeus muscle to NANC nerve stimulation. *British Journal of Pharmacology*, **98**, 1080–1082.

[35] C.G. Li and M.J. Rand (1989) Evidence for a role of nitric oxide in the neurotransmitter system mediating relaxation of the rat anococcygeus muscle. *Clinical and Experimental Pharmacology and Physiology*, **16**, 933–938.
[36] D.S. Bredt, P.M. Hwang and S.H. Snyder (1990) Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature (London)*, **347**, 768–770.
[37] C.A. Ross, D. Bredt and S.H. Snyder (1990) Messenger molecules in the cerebellum. *Trends in Neuroscience*, **13**, 216–222.
[38] A. Gally, P.R. Montaque, G.N. Reeke and G.M. Edelman (1990) The NO hypothesis: possible effects of a short lived rapidly diffusable NO in the development and function of the nervous system. *Proceedings of the National Academy of Sciences (USA)*, **87**, 3547–3551.
[39] D.S. Bredt (1995) Molecular characterisation of nitric oxide synthase. In: *Nitric Oxide in the Nervous System* (Ed. Vincent, S.). TJ Press, Padstow, Cornwall, pp. 1–19.
[40] S.R. Vincent and B.T. Hope (1992) Neurons that say NO. *Trends in Neuroscience*, **15**, 108–113.
[41] J.H. Sandell (1985) NADPH diaphorase cells in the mammalian inner retina. *Journal of Comparative Neurology*, **238**, 466–472.
[42] Y. Aimi, M. Fujimura, S.R. Vincent and H. Kimura (1991) Localization of NADPH-diaphorase containing neurons in sensory ganglia of the rat. *Journal of Comparative Neurology*, **306**, 382–392.
[43] C.J.S. Hassall, M.J. Saffrey, A. Belai, C.H.V. Hoyle, E.W. Moules, J. Moss, H.H.H.W. Schmidt, F. Murad, U. Förstermann and G. Burnstock (1992) Nitric oxide synthase immunoreactivity and NADPH-diaphorase activity in a subpopulation of intrinsic neurones of the guinea-pig heart. *Neuroscience Letters*, **143**, 65–68.
[44] P. Alm, B. Uvelius, J. Ekstr'm, B. Holmqvist, B. Larsson and K.-E. Anderson (1995) Nitric oxide synthase–containing neurons in rat parasympathetic, sympathetic and sensory ganglia: a comparative study. *Histochemical Journal*, **27**, 819–831.
[45] N.J. Dun, S.L. Dun, S.Y. Wu and U. Förstermann (1993) Nitric oxide synthase immunoreactivity in rat superior cervical ganglia and adrenal glands. *Neuroscience Letters*, **158**, 51–54.
[46] Z. Grozdanovic, H.G. Baumgarten and G. Bruling (1992) Histochemistry of NADPH-diaphorase, a marker for neuronal nitric oxide synthase, in the peripheral autonomic nervous system of the mouse. *Neuroscience*, **48**, 225–235.
[47] N.R. Prabhakar, G.K. Kumar, C.H. Chang, F.H. Agani and M.A. Haxhiu (1993) Nitric oxide in the sensory function of the carotid artery. *Brain Research*, **625**, 16–22.
[48] Z.-Z. Wang, L.J. Stensaas, D.S. Bredt, B. Dinger and S.J. Fidone (1994) Localization and actions of nitric oxide in the cat carotid body. *Neuroscience*, **60**, 275–286.
[49] B. Höhler, B. Mayer and W. Kummer (1994) Nitric oxide synthase in the rat carotid body and carotid sinus. *Cell and Tissue Research*, **276**, 559–564.
[50] P.J. Barnes (1993) Nitric oxide and airways. *European Respiratory Journal*, **6**, 163–165.
[51] P.G. Jorens, P.A. Vermeira and A.G. Herman (1993) L-arginine dependent nitric oxide synthase: a new metabolic pathway in the lung and airways. *European Respiratory Journal*, **6**, 258–266.
[52] M. Costa, J.B. Furness, S. Pompolo, S.J.H. Brookes, J.C. Bernstein, D.S. Bredt and S.H. Snyder (1992) Projections and chemical coding of neurons with immunoreactivity for nitric oxide synthase in the guinea pig small intestine. *Neuroscience Letters*, **148**, 121–125.
[53] J.B. Furness, Z.S. Li, H.M. Young and U. Förstermann (1994) Nitric oxide synthase in the enteric nervous system of the guinea pig: a quantitative description. *Cell and Tissue Research*, **277**, 139–149.
[54] S. Bachmann and P. Mundel (1994) Nitric oxide in the kidney: synthesis, localization and function. *American Journal of Kidney Diseases*, **24**,112–129.
[55] C.H. Wilcox, W.J. Welch, F. Murad, S.S. Gross, G. Taylor, R. Levi and H.H.H.W. Schmidt (1992) Nitric oxide synthase in macula densa regulates glomerular capillary pressure. *Proceedings of the National Academy of Sciences (USA)*, **89**, 11 993–11 997.
[56] L.J. Ignarro, P.A. Bush, G.M. Buga, K.S. Wood, J.M. Fukota and J. Rajfer (1990) Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. *Biochemical and Biophysical Research Communications*, **170**, 843–850.
[57] C. Yallampalli, R.E. Garfei and B. Smith (1993) Nitric oxide inhibits uterine contractility during pregnancy but not during delivery. *Endocrinology*, **133**, 1899–1902.
[58] M. Palacios, R.G. Knowles, R.M.J. Palmer and S. Moncada (1989) Nitric oxide from L-arginine stimulates the soluble guanylate cyclase in adrenal glands. *Biochemical and Biophysical Research Communications*, **165**, 802–809.
[59] H.H.H.W. Schmidt, T.D. Warner, K. Ishii, H. Sheng and F. Murad (1992) Insulin secretion from pancreatic β-cells caused by L-arginine-derived nitrogen oxides. *Science*, **255**, 721–723.
[60] D.J. Wolf and G.A. Datto (1992) Identification and characterization of a calmodulin-dependent nitric oxide synthase from GH3 pituitary cells. *Biochemical Journal*, **285**, 201–206.
[61] M. Nakane, H.H.H.W. Schmidt, J. Pollack, U. Förstermann and F. Murad (1993) Cloned human brain nitric oxide synthase is highly expressed in skeletal muscle. *FEBS Letters*, **316**, 175–180.
[62] P.L. Huang, T.M. Dawson, D.S. Bredt, S.H. Snyder and M.C. Fishman (1993) Targeted disruption of the neuronal nitric oxide synthase gene. *Cell*, **75**, 1273–1286.
[63] P. Gulati, A.S. Chan and S.K. Leong (1993) NADPH-diaphorase positive cells in the rat and chick thymus. *Thymus*, **22**, 117–124.
[64] J.E.G. Downing (1994) Multiple nitric oxide synthase systems in adult rat thymus revealed using NADPH-diaphorase histochemsitry. *Immunology*, **82**, 659–664.
[65] K. Fehsel, K.-D. Kroncke, K.L. Meyer, H. Huber, V. Wahn and B. Kolb-Bachofen (1995) Nitric oxide induces apoptosis in mouse thymocytes. *Journal of Immunology*, **155**, 2858–2865.
[66] P. Gulati, A.S. Chan and S.K. Leong (1997) Nitrergic, peptidergic and substance P innervation of the chick thymus. *Journal of Brain Research*, **38**, 253–264.
[67] P. Gulati, A.S. Chan and S.K. Leong (1998) Ontogeny of NADPH-diaphorase expression in the chick thymic microenvironment. *Cell and Tissue Research*, **294**, 335–343.
[68] H.P. Liu, S.K. Leong and S.S.W. Tay (1994) Localization of NADPH-diaphorase positive neurons in the pancreas of the mouse, rat, chick, kitten and monkey. *Journal of Brain Research*, **35**, 501–510.
[69] H.E. Raybuck (1952) The innervation of parathyroid glands. *Anatomical Record*, **112**, 117–123.

[70] J.B. Furness, J.C. Burnstein, R. Murphy and S. Pompolo (1992) Roles of peptides in transmission in the enteric nervous system. *Trends in Pharmacological Science*, **15**, 66–71.
[71] S.R. Vincent, O. Johansson, L. Skirboll and T. Hökfelt (1982) Coexistence of somatostatin and avian pancreatic polypeptide-like immunoreactivities in striatal neurons which are selectively stained for NADPH-diaphorase activity. *Advances in Biochemical Psychopharmacology*, **33**, 453–462.
[72] A.L. Kirchgessner, M.T. Liu and M.D. Gershon (1994) NADPH-diaphorase (nitric oxide synthase) continuing nerves in the enteropancreatic innervation: sources, co-stored neuropeptides, and pancreatic function. *Journal of Comparative Neurology*, **342**, 115–130.
[73] C. Singaram, A. Sengupta, M.A. Sweet, D.J. Sugarbaker and R.K. Goyal (1994) Nitrergic and peptidergic innervation of the human oesophagus. *Gut*, **35**, 1690–1696.
[74] L. Ny, P. Alm, P. Ekstrom, J. Hannibal, B. Larsson and K.E. Anderson (1994) Nitric oxide synthase-containing, peptide-containing, and acetylcholinesterase positive nerves in the cat lower oesophagus. *Histochemical Journal*, **26**, 721–733.
[75] M. Ziche, L. Morbidelli, E. Masini, S. Amerini, H.G. Granger, C.A. Maggi, P. Geppett and F. Ledda (1994) Nitric oxide mediates angiogenesis *in vivo* and endothelial cell growth and migration *in vitro* promoted by substance P. *Journal of Clinical Investigation*, **94**, 2036–2044.
[76] H.P. Liu, S.S.W. Tay, S.K. Leong and M. Schemann (1998) Colocalization of ChAT, DβH and NADPH-d in the pancreatic neurons of the newborn guinea pig. *Cell and Tissue Research*, **294**, 227–231.
[77] J.H. Burn and M.J. Rand (1965) Acetylcholine in adrenergic transmission. *Annual Review of Pharmacology and Toxicology*, **5**, 163–182.
[78] J.R. Keast, G.B. Luckensmeyer and M. Schemann (1995) All pelvic neurons in male rats contain immunoreactivity for the synthetic enzymes of either noradrenaline or acetylcholine. *Neuroscience Letters*, **196**, 209–212.
[79] H.P. Liu, S.S.W. Tay and S.K. Leong (1997) Localization of glutamate receptor subunits of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) type in the pancreas of newborn guinea pigs. *Pancreas*, **14**, 360–368.
[80] G. Bertrand, R. Gross, R. Puech, M.M. Loubatieres-Mariani and J. Bockaert (1993) Glutamate stimulates glucagon secretion via an excitatory amino acid receptor of the AMPA subtype in rat pancreas. *European Journal of Pharmacology*, **237**, 45–50.
[81] D.W. Brann (1995) Glutamate: a major excitatory transmitter in neuroendocrine regulation. *Neuroendocrinology*, **61**, 213–225.
[82] G. Lonart and K.M. Johnson (1995) Characterization of nitric oxide generator-induced hippocampal [3H] norepinephrine release. 1. The role of glutamate. *Journal of Pharmacology and Experimental Therapeutics*, **275**, 7–13.
[83] G.J. Clowry (1993) Axotomy induces NADPH-diaphorase activity in neonatal but not adult motoneurones. *Neuroreport*, **5**, 361–364.
[84] W.E. Wu and L.X. Li (1993) Inhibition of nitric oxide synthase reduces motoneuron death due to spinal root avulsion. *Neuroscience Letters*, **153**, 121–124.
[85] W.H.A. Yu (1994) Nitric oxide synthase in motor neurons after axotomy. *The Journal of Histochemistry and Cytochemistry*, **42**, 451–457.
[86] X. Vigé, A. Carreau, B. Scatton and J.P. Nowicki (1993) Antagonism by NG-nitro-L-arginine of L-glutamate-induced neurotoxicity in cultured neonatal rat cortical neurons. Prolonged application enhances neuroprotective efficacy. *Neuroscience*, **55**, 893–910.
[87] S.J. Green, C.A. Nacy and M.S. Meltzer (1991) Cytokine-induced synthesis of nitrogen oxides in macrophages: a protective host response to Leishmania and other intracellular pathogens. *Journal of Leukocyte Biology*, **50**, 93–103.
[88] J.B. Hibbs, Z. Vavrin and R. Taintor (1987) L-arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. *Journal of Immunology*, **138**, 550–565.
[89] R.J. Ferante, N.W. Kowall, M.F. Beal, E.P. Richardson Jr., E.D. Bird and J.B. Martin (1985) Selective sparing of a class of striatal neurons in Huntington's disease. *Science*, **230**, 561–563.
[90] J.-Y. Koh, S. Peters and D.W. Choi (1986) Neurons containing NADPH-diaphorase are selectively resistant to quinolinate toxicity. Science, **234**, 73–76.
[91] K.A. Haberny, S. Pou and C.U. Eccles (1992) Potentiation of quinolinate-induced hippocampal lesions by inhibition of NO synthesis. *Neuroscience Letters*, **146**, 187–190.
[92] M. Lerner-Natoli, G. Rondouin, F. de Bock and J. Bockaer (1992) Chronic NO synthase inhibition fails to protect hippocampal neurones against NMDA toxicity. *Neuroreport*, **3**, 1109–1112.
[93] C. Demerlj-Pallardy, M.-O. Lonchampt, P.-E. Chabrier and P. Braquet (1991) Absence of implication of L-arginine/nitric oxide pathway on neuronal cell injury induced by L-glutamate or hypoxia. *Biochemical and Biophysical Research Communications*, **181**, 456–464.
[94] S.J. Hewett, J.A. Corbett, M.L. McDaniel and D.W. Choi (1993) Inhibition of nitric oxide formation does not protect murine cortical cell cultures from N-methyl-D-aspartate neurotoxicity. *Brain Research*, **625**, 337–341.
[95] R.F. Regan, K.E. Renn and S.S. Panter (1993) NMDA neurotoxicity in murine cortical cell cultures is not attenuated by hemoglobin or inhibition of nitric oxide synthesis. *Neuroscience Letters*, **153**, 53–56.
[96] G. Garthwaite and J. Garthwaite (1994) Nitric oxide does not mediate acute glutamate neurotoxicity, nor is it neuroprotective, in rat brain slices. *Neuropharmacoogy*, **33**, 1431–1438.
[97] B.P. He, S.S.W. Tay and S.K. Leong (1996) Motoneuronal death without expression of NADPH-diaphorase activity after sciatic nerve cut in 5-day-old mice. *Brain Research*, **733**, 125–128.
[98] Y. Li, W. Wu, F.P. Schino and G.E. Goode (1993) Sciatic nerve transaction causes expression of nitric oxide synthase (NOS) and death of spinal motoneurons in newborn and earlier postnatal rats. *Society of Neuroscience Abstract*, **19**, 440.
[99] R.-S. Ruan, S.-K. Leong and K.-H. Yeoh (1994) Expression of NADPH-diaphorase activity in the facial motoneurons after compression of the facial nerves in the albino rat. *Brain Research*, **652**, 350–352.
[100] D.A. Wink, I. Hanbauer, M.C. Krishna, W. DeGraff, J. Gamson and J.B. Mitchell (1993) Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. *Proceedings of the National Academy of Sciences (USA)*, **90**, 9813–9817.

[101] P.T.H. Wong, R.S. Ruan, S.K. Leong and K.H. Yeoh (1995) Compression of the facial nerve caused increased nitric oxide synthase activity in the facial motor nucleus. *Neuroscience Letters*, **67**, 697–702.
[102] R.-S. Ruan, S.-K. Leong and K.-H. Yeoh (1994) The role of nitric oxide in facial motoneuronal death and facial function recovery. *Fourth IBRO World Congress of Neuroscience*, p. 134.
[103] R.S. Ruan, S.K. Leong and K.H. Yeoh (1999) Ototoxicity of sodium nitroprusside is not due to nitric oxide. *Experimental Neurology*. (in press).
[104] R.S. Ruan, S.K. Leong and K.H. Yeoh (1999) Nitroglycerin protects afferent synapses of inner hair cells of guinea pig cochlea from ischemic injury. (submitted for publication).
[105] Y.-D. Xue, P.T.H. Wong and S.K. Leong (1997) Nitric oxide synthase-, N-methyl-D-aspartate receptor-, glutamate- and aspartate-immunoreactive neurons in the mouse arcuate nucleus: effects of neonatal treatment with monosodium glutamate. *Acta Neuropathologica*, **94**, 572–582.
[106] B.T. Hyman, K. Marzloff, J.J. Wenniger, T.M. Dawson, D.S. Bredt and S.H. Snyder (1992) Relative sparing of nitric oxide synthase-containing neurons in the hippocampal formation of Alzheimer's disease. *Annnals of Neurology*, **32**, 818–820.
[107] E.J. Mufson and M.M. Brandabur (1994) Sparing of NADPH-diaphorase striatal neurons in Parkinson's and Alzheimer's diseases. *Neuroreport*, **5**, 705–708.
[108] M. Xu, Y.-K. Ng and S.-K. Leong (1999) Neuroprotective and neurodestructive functions of nitric oxide after spinal cord hemisection. (submitted for publication).
[109] S. Przedborski, V. Jackson-Lewis, R. Yokoyama, T. Shibata, V.L. Dawson and T.M. Dawson (1996) Role of neuronal nitric oxide in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopamin-ergic neurotoxicity. *Proceedings of the National Academy of Sciences (USA)*, **93**, 4565–4571.
[110] P. Hantraye, E. Brouillet, R. Ferrante, S. Palfi, R. Dolan, R.T. Matthews and M.F. Beal (1996) Inhibition of neuronal nitric oxide synthase prevents MPTP-induced parkinsonism in baboons. *Nature Medicine*, **2**, 1017–1021.
[111] J.S. Beckman, T.W. Beckman, J. Chen, P.A. Marshall and B.A. Freeman (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proceedings of the National Academy of Sciences (USA)*, **87**, 1620–1624.
[112] D.A. Wink, R.W. Nims, J.F. Darbyshire, D. Chistodoulou, I. Hanbauer, G.W. Cox, F. Laval, J. Laval, J.A. Cook, M.C. Krishna, W. DeGraff and J.B. Mitchell (1994) Reaction kinetics for nitrosation of cysteine and glutathione in aerobic nitric oxide solutions at neutral pH. Insights into the fate and physiological effects of intermediates generated in the NO/O_2 reaction. *Chemical Research in Toxicology*, **7**, 519–525.
[113] N. Högg, R.J. Singh and B. Kalyanaraman (1996) The role of glutathione in the transport and catabolism of nitric oxide. *FEBS Letters*, **382**, 223–228.
[114] A.J. Gow, D.G. Buerk and H. Ischiropoulos (1997) A novel reaction mechanism for the formation of *S*-nitrosothiol *in vivo*. *Journal of Biological Chemistry*, **272**, 2841–2845.
[115] S.S. Gross and M.S. Wolin (1995) Nitric oxide: pathophysiological mechanisms. *Annual Review of Physiology*, **57**, 737–769.
[116] V.L. Dawson and T.M. Dawson (1996) Nitric oxide neurotoxicity. *Journal of Chemical Neuroanatomy*, **10**, 179–190.
[117] D.A. Wink, I. Hanbauer, M.C. Krishna, W. DeGraff, J. Gamson and J.B Mitchell (1993) Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. *Proceedings of the National Academy of Sciences (USA)*, **90**, 9813–9817.
[118] E.A. Konorev, M.M. Tarpey, J. Joseph, J.E. Baker and B. Kalyanaraman (1995) *S*-Nitrosoglutathione improves functional recovery in the isolated rat heart after cardioplegic ischemic arrest-evidence for a cardioprotective effect of nitric oxide. *Journal of Pharmacology and Experimental Therapeutics*, **274**, 200–206.
[119] A.T. Struck, N. Hogg, J.P. Thomas and B. Kalyanaraman (1995) Nitric oxide donor compounds inhibit the toxicity of oxidized low-density lipoprotein to endothelial cells. *FEBS Letters*, **361**, 291–294.
[120] Y.M. Kim, M.E. de Vera, S.C. Watkins and T.R. Billiar (1997) Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor-α-induced apoptosis by inducing heat shock protein 70 expression. *Journal of Biological Chemistry*, **272**, 1402–1411.
[121] H.H. Gutierrez, B. Nieves, P. Chumley, A. Rivera and B.A. Freeman (1996) Nitric oxide regulation of superoxide-dependent lung injury: oxidant-protective actions of endogenously produced and exogenously administered nitric oxide. *Free Radical Biology and Medicine*, **21**, 43–52.
[122] P. Rauhala, A.M.-Y. Lin and C.C. Chiueh (1998) Neuroprotection by *S*-nitrosoglutathione of brain dopamine neurons from oxidative stress. *FASEB Journal*, **12**, 165–173.
[123] C.F. Zhang and S. Xu (1995) Inducible nitric oxide synthase gene expression in brain following cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism*, **15**, 378–384.
[124] W. Zhao, R.G. Tilton and J.A. Corbett (1996) Experimental allergic encephalo-myelitis in the rat is inhibited by aminoguanidine, an inhibitor of nitric oxide synthase. *Journal of Neuroimmunology*, **164**, 123–133.
[125] O. Bagasra, F.H. Michaels and Y.M. Zheng (1995) Activation of the inducible form of nitric oxide synthase in the brains of patients with multiple sclerosis. *Proceedings of the National Academy of Sciences (USA)*, **92**, 12 041–12 045.
[126] T. Sayama, S. Suzuki and M. Fukui (1998) Expression of inducible nitric oxide synthase in rats following subarachnoid haemorrhage. *Neurological Research*, **20**, 79–84.
[127] W.W. Htain, S.K. Leong and E.A. Ling (1997) *In vivo* expression of inducible nitric oxide synthase in supraventricular amoeboid microglial cells in neonatal BALB/c and athymic mice. *Neuroscience Letters*, **223**, 53–56.