Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 4688–4692, May 1996
Medical Sciences

Excitotoxicity in the lung: *N*-Methyl-D-aspartate-induced, nitric oxide-dependent, pulmonary edema is attenuated by vasoactive intestinal peptide and by inhibitors of poly(ADP-ribose) polymerase

(glutamate receptors/acute lung injury/adult respiratory distress syndrome/poly(ADP-ribose) synthetase)

SAMI I. SAID*, HASAN I. BERISHA, AND HEDAYATOLLAH PAKBAZ

Departments of Medicine and Physiology, University Medical Center and Northport Veterans Affairs Medical Center, Stony Brook, NY 11794-8172

Communicated by Mary Ellen Avery, Harvard Medical School, Boston, MA, January 5, 1996 (received for review October 18, 1995)

ABSTRACT **Excitatory amino acid toxicity, resulting from overactivation of *N*-methyl-D-aspartate (NMDA) glutamate receptors, is a major mechanism of neuronal cell death in acute and chronic neurological diseases. We have investigated whether excitotoxicity may occur in peripheral organs, causing tissue injury, and report that NMDA receptor activation in perfused, ventilated rat lungs triggered acute injury, marked by increased pressures needed to ventilate and perfuse the lung, and by high-permeability edema. The injury was prevented by competitive NMDA receptor antagonists or by channel-blocker MK-801, and was reduced in the presence of Mg^{2+}. As with NMDA toxicity to central neurons, the lung injury was nitric oxide (NO) dependent: it required L-arginine, was associated with increased production of NO, and was attenuated by either of two NO synthase inhibitors. The neuropeptide vasoactive intestinal peptide and inhibitors of poly(ADP-ribose) polymerase also prevented this injury, but without inhibiting NO synthesis, both acting by inhibiting a toxic action of NO that is critical to tissue injury. The findings indicate that: (*i*) NMDA receptors exist in the lung (and probably elsewhere outside the central nervous system), (*ii*) excessive activation of these receptors may provoke acute edematous lung injury as seen in the "adult respiratory distress syndrome," and (*iii*) this injury can be modulated by blockade of one of three critical steps: NMDA receptor binding, inhibition of NO synthesis, or activation of poly(ADP-ribose) polymerase.**

The amino acid glutamate, abundantly present in the mammalian central nervous system (CNS), is the major excitatory neurotransmitter, and plays an important role in many functions, including learning, memory, development, and other forms of synaptic plasticity (1–5). Among the classes of glutamate receptors that have been identified, cloned, and characterized (4, 6), the ionotropic *N*-methyl-D-aspartate (NMDA) receptor has been the focus of much attention because of its implication in neuronal cell injury and death in such acute conditions as head injury, strokes, and epileptic seizures, and in chronic neurodegenerative diseases including Alzheimer disease, Huntington disease, Parkinson disease, amyotrophic lateral sclerosis (7–10), and AIDS dementia (11).

Evidence linking NMDA receptor activation to neurotoxicity is based on glutamate toxicity to neuronal cells in culture and upon systemic administration *in vivo* (12–14), and the neuroprotective effect of NMDA receptor antagonists in a variety of settings (9, 13). The view that neuronal injury induced by glutamate or related excitatory amino acids resulted from overexcitation suggested the term "excitotoxicity" (7). An important insight into the mechanisms underlying this phenomenon was the observation that NMDA neurotoxicity was Ca^{2+}-dependent (15, 16). The influx of Ca^{2+} into the cell may lead to cell death by several mechanisms, including the activation of protein kinases, phospholipases, proteases, and nitric oxide synthase (NOS), and the generation of reactive oxygen species (9). Evidence has been presented for a key mediator role for NO in glutamate-stimulated neurotoxicity in primary cultures of rat fetal cortical, striatal, and hippocampal neurons (11, 17–19), although others could not confirm an essential role for NO in NMDA-induced neurotoxicity (20, 21). The generation of reactive oxygen species, including superoxide and hydroxyl radicals and singlet oxygen, together with NO or as a result of its formation, may contribute importantly to NMDA toxicity (22–25). NO itself, produced in high concentrations, can injure neurons and other cells through a variety of toxic actions, including the inhibition of the mitochondrial electron-transport chain and of mitochondrial enzymes, and DNA damage, which in turn triggers the activation of poly(ADP-ribose) polymerase (PARP), also known as poly(ADP-ribose) synthetase (PARS) (26, 27).

Using the isolated perfused and ventilated rat lung as a model, we have investigated the possible occurrence and mechanisms of excitotoxicity outside the CNS. We report that addition of NMDA to lung perfusate, in the presence of L-arginine, elicited an acute lung injury manifested by high-permeability edema. As in central neuronal glutamate toxicity, the lung injury was NMDA receptor-mediated and NO-dependent. Vasoactive intestinal peptide (VIP), which protects the lung and other organs against oxidant injury (28–30) and promotes neuronal survival and differentiation (31, 32), prevented this form of injury, as did inhibitors of PARP activation. The findings suggest that glutamate toxicity is not limited to the CNS, and that activation of NMDA receptors may be a previously unsuspected mechanism of injury of the lung and other peripheral tissues and organs.

MATERIALS AND METHODS

Isolated Lung Preparation. Rats (Sprague–Dawley, male, 300–350 g) were anesthetized with i.p. injection of sodium

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AP-5, DL-2-amino-5-phosphonopentanoic acid; AP-7 (or AP-H), DL-2-amino-7-phosphonoheptanoic acid; BAL, bronchoalveolar lavage; L-NAME, N^{ω}-nitro-L-arginine methyl ester; MK-801, (+)-10,11-dihydro-5H-dibenzo[a,d]cyclo-hepten-5,10-imine maleate or dizocilpine maleate; NMDA, *N*-methyl-D-aspartate; NOS, nitric oxide synthase; PARP, poly(ADP-ribose) polymerase; P_{AW}, peak airway pressure; P_{PA}, mean pulmonary artery pressure; VIP, vasoactive intestinal peptide; W/D, wet/dry lung weight ratio; CNS, central nervous system.

*To whom reprint requests should be addressed at: Pulmonary/Critical Care Medicine, University Medical Center, T17–040, Stony Brook, New York 11794-8172.

pentobarbital (100 mg/kg) and intubated intratracheally. The lungs were ventilated with the aid of a rodent respirator (model 640; Harvard Apparatus) with a humidified mixture of 95% O_2/5% CO_2, at 60 cycles per min and a tidal volume of 5 ml/kg. A positive end-expiratory pressure of 2 cm of H_2O was maintained to reduce atelectasis. The chest was opened, the heart was exposed, and an injection of heparin given (400 United States Pharmacopeia units) into the inferior vena cava. An inflow cannula was then inserted into the main pulmonary artery through the right ventricle, and an outflow cannula in the left atrium through an incision in the left ventricle. The lungs were perfused *in situ* at constant flow with Krebs solution (117.6 mM NaCl/5.4 mM KCl/25.0 mM $NaHCO_3$/1.0 mM NaH_2PO_4/2.5 mM $CaCl_2$) containing 4% bovine serum albumin (Krebs–BSA) and equilibrated with 95% O_2/5% CO_2. As explained below, $MgCl_2$ (1.0 mM) was added to the solution in some experiments and withheld in the others. Pulmonary perfusion was started at a rate of 8 ml/min by a pulsatile circulation pump (model 1405; Harvard Apparatus); the rate was adjusted to reach an initial mean pulmonary artery pressure (P_{PA}) of 5–8 cm of H_20, then was held constant. Initially, the lungs were perfused in an open, nonrecirculating mode for 10 min to wash out residual blood, after which perfusion was changed to a recirculating mode with 100 ml of Krebs–BSA. The lung preparation was allowed to equilibrate for 10 min under constant perfusion and ventilation before any intervention was begun. In this preparation, acute lung injury resulting in high-permeability pulmonary edema is manifested by sharp increases in the peak airway pressure (P_{AW}), in the P_{PA}, and in the wet weight of the lung, as well as by leakage of protein into the airspaces (28, 29).

Physiological Measurements: Assessment of Lung Injury. Peak P_{AW} and P_{PA} were continuously monitored by pressure transducers (Statham P23A; Statham, Hato Rey, PR) attached to the tracheal cannula and to a polyethylene catheter in the main pulmonary artery, respectively, and were recorded (2600S recorder; Gould, Cleveland). Left atrial pressure was maintained at 2 cm of H_2O by placing the tip of the outflow cannula 2 cm above the level of the atrium. To assess the contribution of hydrostatic forces to the induced pulmonary edema, we periodically measured pulmonary microvascular pressure by the double-occlusion method (33). At the conclusion of the experiment, the left lung was lavaged with 3 ml of saline for measurement of bronchoalveolar lavage (BAL) fluid protein content, as an index of protein leakage due to alveolar-microvascular membrane injury. The right lung was removed, gently blotted, and weighed (wet weight, W) and oven-dried to a constant weight (dry weight, D) for determination of dry weight and wet/dry (W/D) lung weight ratio, as measures of the severity of pulmonary edema.

Induction of Lung Injury. NMDA (1 mM) was added to the perfusate beginning at 0 time and for 20 min. L-Arginine (100 μM–10 mM) was added to the perfusate at −10 min.

NMDA Receptor Antagonists. Infusions of competitive NMDA receptor antagonists DL-2-amino-5-phosphonovaleric acid (AP-5, or APV, 100 μM) or DL-2-amino-7-phosphonoheptanoic acid (AP-7, 100 μm), or channel blocker (+)-10,11-dihydro-5H-dibenzo[a,d]cyclo-hepten-5,10-imine maleate or dizocilpine maleate (MK-801, 1, 5, or 10 μM), were begun just after L-arginine and continued for the balance of the experiment.

Inhibitors of NOS. We evaluated the influence of the NOS inhibitors N^{ω}-nitro-L-arginine methyl ester (L-NAME, 0.1 and 2 mM) and N^{ω}-nitro-L-arginine (L-nitroarginine, 1.0 mM) (1) on the occurrence and severity of lung injury and the increased production of NO. The inhibitors were added just after L-arginine and continued for the duration of the experiment. The specificity of the inhibition by L-NAME was confirmed by testing the negative effect of its enantiomer D-NAME (2 mM) in the perfusate.

Assay of NO Synthesis: cGMP levels. As an index of NO production, we measured cGMP levels in lung perfusate by a specific radioimmunoassay, in the presence of the phosphodiesterase inhibitor isobutylmethylxanthine (1 mM). ^{125}I-labeled cGMP was diluted to provide 7000 cpm per 100 μl, and antibody was diluted to yield a maximal binding or 40–50% of total counts. Samples were acetylated to enhance the sensitivity of the assay (34). Bound cGMP was precipitated with excess rabbit nonimmune immunoglobulin G and polyethylene glycol, achieving a recovery of cGMP of >95%. The concentration of cGMP was expressed per ml of lung perfusate or per mg of protein of lung tissue. The NO-dependence of cGMP was confirmed by its attenuation in the presence of NOS inhibitors.

Infusions of VIP and PARP Inhibitors. Infusions of VIP (10 μg/ kg·min^{-1}) and of benzamide (1 mM) or 6(5*H*) phenanthridinone (1 mM) were begun just after the addition of L-arginine, and continued for the balance of the experiment.

Experimental Groups. There were eight major experimental groups, some with subgroups, determined by the particular intervention in each case, i.e., the additions to the perfused lungs:

1) Control group: (no additions, $n = 8$).

2) NMDA + L-arginine, both at different concentrations: a) NMDA (1 mM) + L-arginine (10 mM, $n = 16$); b) NMDA (0.1 mM) + L-arginine (10 mM, $n = 3$); c) NMDA (1 mM) + L-arginine (2 mM, $n = 3$); d) NMDA (1 mM) + L-arginine (0.1 mM, $n = 3$).

3) NMDA (1 mM) + L-arginine (10 mM) + NOS inhibitor L-NAME (0.1 mM, $n = 3$; 2 mM, $n = 4$), or L-nitroarginine [(1 mM, $n = 3$), or inactive analog D-NAME (2 mM, $n = 4$).

4) NMDA (1 mM) + L-arginine (10 mM) + competitive NMDA receptor inhibitor AP5 (100 μM, $n = 3$) or AP7 (100 μM, $n = 3$).

5) NMDA (1 mM) + L-arginine (10 mM) + NMDA channel-blocker MK-801 (1 μM, $n = 3$; 5 μM, $n = 5$ and 10 μM, $n = 3$.

6) NMDA (1 mM) + L-arginine (10 mM) + VIP (10 μM/ kg·min^{-1}, $n = 5$).

7) NMDA (1 mM) + L-arginine (10 mM) + PARP inhibitor benzamide (1 mM, $n = 7$) or 6 (5*H*) phenanthridinone (1 mM, $n = 6$).

8) NMDA (1 mM) + L-arginine (10 mM), with $MgCl_2$ (1 mM) added to the lung perfusate ($n = 3$).

Chemicals and Reagents. The cGMP kit was from New England Nuclear/DuPont; 6(5*H*) phenanthridinone was from

Table 1. Increases in P_{AW} and P_{PA} in isolated rat lungs treated with NMDA

	Untreated (control lungs)	Treatment			
		NMDA	NMDA + MK-801	NMDA + L-NAME	NMDA + VIP
P_{AW}	8.50 ± 0.03 (8)	18.25 ± 3.32* (16)	9.30 ± 0.66 (3)	12.00 ± 1.58 (4)	10.30 ± 0.33 (5)
P_{PA}	7.00 ± 0.32 (8)	13.25 ± 3.98* (16)	8.20 ± 0.16 (3)	9.25 ± 0.62 (4)	7.60 ± 1.20 (5)

Measurements were made at 60 min from beginning of lung perfusion. NMDA was given at 1 mM, together with 10 mM L-arginine; MK-801 was at 10 μM, L-NAME at 2 mM, and VIP at 10 μg/kg·min^{-1}. P_{AW} and P_{PA} are in centimeters of H_2O. Number of determinations is shown in parentheses.

*Significantly greater than control values at $P < 0.01$.

Aldrich, MK-801 was from Leslie D. Iversen (Merck Sharp and Dohme), and VIP was from Viktor Mutt (Karolinska Institute, Stockholm). All other chemicals, including NMDA, L-NAME, D-NAME, L-nitroarginine, AP-5, AP-7, and benzamide, were from Sigma.

Statistical Analysis. The influence of each intervention on functional and biochemical measurements was assessed by analysis of variance followed by Tukey's procedures for intergroup comparison (35). Repeated measurements of P_{AW} and P_{PA} over time were analyzed by multiple-paired *t* tests.

RESULTS

Lung Injury Due to NMDA (Table 1, Fig. 1). In the presence of L-arginine (10 mM), the addition of NMDA (1 mM) to the perfusate induced acute injury in perfused rat lungs within 60 min. P_{AW} increased to 18.25 ± 3.32 from 8.50 ± 0.03 cm H_2O ($n = 16$, $P < 0.01$), P_{PA} increased to 13.25 ± 3.98 from 7.00 ± 0.32 cm H_2O ($n = 16$, $P < 0.01$), W/D lung weight ratio increased to 6.34 ± 0.38 from a control value of 5.34 ± 0.03 ($n = 8$, $P < 0.05$), and protein content of BAL fluid to 1.61 ± 0.42 mg/ml from a control value of 0.58 ± 0.15 ($n = 10$, $P < 0.01$).

The induction of lung injury by NMDA was dose-dependent: a lower concentration (0.1 mM) caused more moderate elevations in P_{AW} (to 12.33 ± 1.01 cm H_2O) and P_{PA} (to 9.2 ± 0.16 cm H_2O), and did not alter W/ D or BAL protein content after 60-min perfusion.

The lung injury was directly related to activation of NMDA receptors, since it was prevented by competitive NMDA receptor antagonists AP-5 or AP-7, at 100 μM: P_{AW}, P_{PA}, W/D, and BAL protein content remained within the normal range ($n = 3$ for each). Similarly, channel blocker MK-801 was fully protective at 10 or 5 μM ($n = 3$ for each, Table 1), but at 1 μM there was a moderate increase in mean P_{AW} (to 11.8 ± 1.1 from 8.1 ± 0.6 cm H_2O), P_{PA} (to 9.2 ± 0.4 from 7.3 ± 0.3 cm H_2O), and W/D was 5.08 ± 0.32 versus 4.73 ± 0.15 with 10 μM MK-801.

Influence of Mg^{2+}. In three experiments in which $MgCl_2$ (1 mM) was added to the lung perfusate, P_{AW} and P_{PA} showed little or no increase after 60 min, and W/D and BAL protein content were within normal limits (data not shown). In these experiments, therefore, the combination of 1 mM NMDA and 10 mM L-arginine failed to induce lung injury.

Requirement of L-Arginine. The presence of L-arginine was necessary for induction of the lung injury by NMDA. The requirement for L-arginine, as for NMDA, was dose-dependent: at 2 mM and 100 μM concentrations ($n = 3$ for each), the injury was progressively reduced in severity, compared with that observed at 10-mM L-arginine.

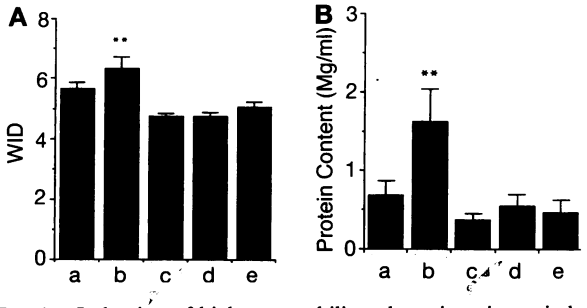

FIG. 1. Induction of high-permeability edema in guinea pig lungs by NMDA and its prevention by NMDA receptor antagonist AP-5, by NOS inhibitor L-NAME, and by VIP. W/D lung weight ratios (*A*) BAL protein content (*B*) in several experimental groups: a = control, untreated lungs ($n = 8$); b = NMDA (1 mM) + L-arginine (10 mM), $n = 16$; c = NMDA + L-arginine + AP-5 (100 μM), $n = 3$; d = NMDA + L-arginine + L-NAME (2 mM), $n = 4$; e = NMDA + L-arginine + VIP (10 μM/kg · min^{-1}), $n = 5$. **, $P < 0.01$ versus control.

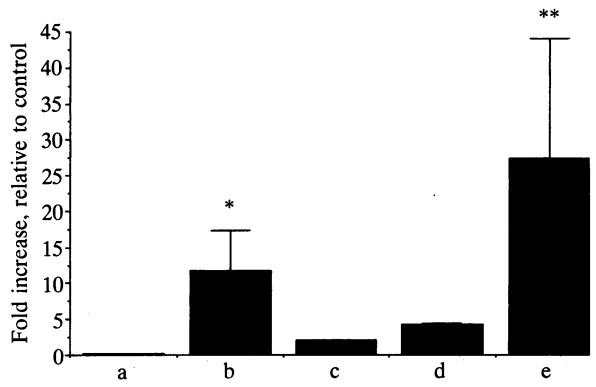

FIG. 2. Increased NO production in NMDA-induced lung injury. cGMP levels (in multiples of basal values) in lung perfusate are shown in the same experimental groups (a-e) as in Fig. 1.

Increased NO Production. cGMP levels in lung perfusate increased almost 12-fold relative to control basal values in NMDA-injured lungs ($P < 0.05$). This increase was markedly attenuated by L-NAME and by AP-5 ($P < 0.05$). VIP, on the other hand, further augmented the cGMP levels ($P < 0.01$, Fig. 2).

Attenuation of Injury by NOS Inhibitors. The presence in the perfusate of either of the two NOS inhibitors, L-NAME (0.1 and 2 mM), or L-nitroarginine (1.0 mM), significantly reduced or nearly prevented all signs of injury (Table 1). P_{AW} and P_{PA} did not increase till the end of the 60 min of perfusion; and W/D and BAL protein content remained in the normal range (Fig. 1). The specificity of the protection by L-NAME and L-nitroarginine was confirmed by the absence of protection when the inactive D-isomer D-NAME (2 mM) was used instead (P_{AW} to 14.2 ± 1.5 from 8.8 ± 0.5 cm H_2O, W/D to 5.86 ± 0.47, and BAL protein to 0.87 ± 0.25 mg/ml).

Prevention of Injury by VIP. Addition of VIP (10 μg/kg·min^{-1}) to the perfusate totally prevented all manifestations of NMDA injury ($n = 5$, $P < 0.01$): P_{AW} and P_{PA} did not increase above control values, and W/D and BAL protein content remained at normal levels (4.91 ± 0.14 and 0.35 ± 0.12 mg/ml, respectively) (Fig. 1, Table 1).

Prevention of Injury by Known PARP Inhibitors. Both benzamide (1 mM, $n = 7$) and 6(5*H*) phenanthridinone (1 mM, $n = 6$) totally protected the lungs against injury due to NMDA (1 mM) plus L-arginine (10 mM) (Table 2).

DISCUSSION

Our findings demonstrate that NMDA, in the presence of L-arginine, induces acute injury of perfused rat lungs. The injury is manifested by a high-permeability pulmonary edema,

Table 2. Protection against lung injury by PARP inhibitors benzamide and phenanthridinone

	Treatment	
	Benzamide (7)	Phenanthridinone (6)
P_{AW}	11.7 ± 0.68	9.7 ± 0.45
P_{PA}	7.8 ± 0.26	8.1 ± 0.35
W/D	4.69 ± 0.11	4.48 ± 0.16
BAL protein	0.24 ± 0.05	0.64 ± 0.18

Measurements of P_{AW} and P_{PA}, both in centimeters of H_2O, of W/D, and of BAL protein concentration (mg/ml), were made at 60 min from beginning of lung perfusion. NMDA was given at 1 mM, together with 10 mM L-arginine; benzamide and phenanthridinone were at 1 mM. Number of determinations is shown in parentheses. All values were not significantly different from those obtained in normal, untreated lungs (Fig. 1 and Table 1).

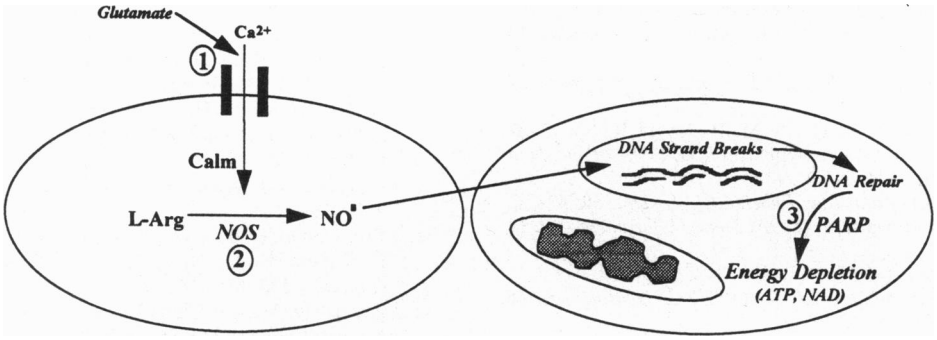

FIG. 3. Postulated sequence of events leading to the production of cell injury. Overactivation of NMDA glutamate receptors leads to influx of Ca^{2+} into the cell, which activates NOS in the presence of calmodulin (calm). Excess NO thus generated diffuses out of the cell, causing cytotoxicity of neighboring cells. DNA repair enzyme PARP is activated by DNA strand breaks, resulting in depletion of mitochondrial energy sources (ATP, NAD) and cell lysis. In addition to NO, reactive oxygen species (ROS) and more glutamate are released from cell upon activation of NMDA receptors (46).

and by increases in the pressures required to ventilate and perfuse the lung, at constant breath volume and vascular flow rate, respectively (28, 29, 33, 36).

That the observed injury was due to NMDA receptor activation was confirmed by attenuation or prevention of the injury by (*i*) competitive receptor antagonists APV and AP7, and by channel blocker MK-801, and (*ii*) by addition of Mg^{2+} (1 mM) to the perfusate (6).

As with NMDA-induced injury in primary cortical cultures (17), and in cerebellar (16) and hippocampal slices (19), the lung injury appeared to be mediated by excessive NO synthesis. This conclusion is supported by several lines of evidence. First, the injury was markedly reduced or totally prevented ($n = 4$, $P < 0.05$) by either of two NOS inhibitors, L-NAME (0.1 and 2 mM), or L-nitroarginine (1 mM): P_{AW} and P_{PA} did not increase significantly until the last few minutes of the perfusion (Table 1); and W/D and BAL protein content remained in the normal range (Fig. 1). The specificity of the protection by these inhibitors was confirmed by the absence of protection when the D-isomer D-NAME (2 mM) was used instead (P_{AW} to 14.2 ± 1.5 from 8.8 ± 0.5 cm H_2O, W/D to 5.86 ± 0.5, and BAL protein to 0.9 ± 0.3 mg/ml). Second, the injury was associated with an 11- to 12-fold increase in cGMP levels in the perfusate ($n = 4$, $P < 0.05$, Fig. 2), and this increase was reduced 64% by L-NAME ($n = 4$), despite the presence of 10 mM L-arginine, and 83% by AP-5 ($n = 2$). Third, as in central neuronal excitotoxicity, the injury was dependent on the presence of L-arginine, the substrate for NOS. Finally, dot-blot hybridization demonstrated increased neuronal NOS mRNA in rat and ferret tracheal ganglia upon addition of NMDA (10 nM) (A. Bandyopadhyay, S. Rattan, and S.I.S, unpublished data; NOS probe courtesy of Solomon H. Snyder).

The neuropeptide VIP, previously shown to prevent or reduce oxidant injury of the lungs (28, 29), including at least one form of injury mediated by excess NO synthesis (36), and to protect the heart against ischemia-reperfusion injury (30) and neuronal cells in culture (31, 32), completely prevented the injury in the present model as well. However, the protective effect of VIP against NMDA injury was not attributable to suppression of NO production. In fact, judging from the augmentation of cGMP levels in lung perfusate, VIP may have enhanced NO production in the present preparation (Fig. 2), as reported for VIP and for other cAMP-promoting agents in other tissues (37, 38). Alternatively, VIP, through an antioxidant effect, may have decreased superoxide anion, resulting in increased half-life of NO and secondary increase in guanylyl cyclase activation and cGMP formation. The absence of lung injury in the face of increased cGMP production with VIP treatment demonstrates a dissociation between NO toxicity and cGMP.

The injury was also totally prevented by either of two PARP inhibitors, benzamide or 6(5*H*) phenanthridinone. PARP activation is likely a key mechanism of NO-mediated NMDA neurotoxicity (26, 27), and PARP inhibitors can prevent such toxicity without inhibiting NO synthesis (27). One may speculate, therefore, that VIP may have acted as a PARP inhibitor, although this conclusion awaits confirmation.

NMDA receptor activation and neurotoxicity have been extensively investigated in the CNS, but little is known about the physiological or pathophysiological significance of these receptors in the peripheral nervous system. To our knowledge, this is the first report of the existence of NMDA receptors in the lung, and of NMDA receptor activation as a possible mechanism of tissue injury outside the CNS. Since this investigation was begun (39), we have become aware of several reports of the expression of functional NMDA receptors in peripheral tissues, including the myenteric plexus (40), sensory and autonomic ganglion neurons (41), adrenal medulla (42), pancreatic islet cells (43), and the gastrointestinal tract (44).

The sites of localization of NMDA receptors in the lungs are at present unknown, but are presumably neuronal. Our results suggest that excessive stimulation of glutamate receptors may be a novel mechanism of neurogenic tissue injury, and may be involved in the pathogenesis of some inadequately understood forms of "neurogenic" pulmonary edema such as that occurring at high altitude (45), and of inflammatory injury of asthmatic airways. If glutamate receptors in the lung are exclusively neuronal, the manner in which neuronal injury caused by their activation leads to acute lung injury, i.e., to damage of the alveolar-microvascular membrane, is not readily apparent. The possible existence of these receptors on other lung cells, e.g., epithelial and endothelial cells, should therefore be considered.

Our findings have important implications for tissue injury not only in the lungs but also in other organs, as well as in the CNS. If, as has now been shown, glutamate receptors exist in multiple peripheral sites, it seems likely that over-activation of these receptors may be a pathogenetic mechanism of injury and inflammation in those sites, as in the brain and the lungs. Endogenous glutamate production can be triggered or stimulated by a variety of inflammatory mediators, including arachidonate metabolites and reactive oxygen species (11). Finally, our data underscore the conclusion that NMDA glutamate neurotoxicity is susceptible to modulation by intervention at one of several levels, including: blockade of the ionotropic receptor, inhibition of NO synthesis, or inhibition of NO-induced activation of PARP (Fig. 3).

1. Nakanishi, S. (1992) *Science* **258,** 597–603.
2. Malenka, R. C. & Nicoll, R. A. (1993) *Trends Neurosci.* **16,** 521–527.

3. McBain, C. J. & Mayer, M. L. (1994) *Physiol. Rev.* **74,** 723–760.
4. Hollmann, M. & Heinemann, S. (1994) *Annu. Rev. Neurosci.* **17,** 31–108.
5. Gasic, G. P. & Hollmann, M. (1992) *Annu. Rev. Neurosci.* **54,** 507–536.
6. Younkin, D. P., Tang, C.-M., Hardy, M., Reddy, U. R., Shi, Q.-Y., Pleasure, S. J., Lee, V. M.-Y. & Pleasure, D. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2174–2178.
7. Olney, J. W. (1990) *Annu. Rev. Pharmacol. Toxicol.* **30,** 47–71.
8. Meldrum, B. & Garthwaite, J. (1990) *Trends Pharmacol. Sci.* **11,** 379–387.
9. Beal, F. M. (1992) *FASEB J.* **6,** 3338–3344.
10. DeBoni, U. & McLachlan, D. R. (1985) *J. Neurol. Sci.* **68,** 105–118.
11. Lipton, S. A. & Gendelman, H. E. (1995) *N. Engl. J. Med.* **332,** 934–940.
12. Olney, J. W. (1969) *J. Neuropathol.* **28,** 455–474.
13. Choi, D. W., Koh, J. & Peters, S. (1988) *J. Neurosci.* **8,** 185–196.
14. Choi, D. W. (1991) in *Excitatory Amino Acid Antagonists*, ed. Meldrum, B. S. (Blackwell Scientific, Oxford), pp. 216–236.
15. Choi, D. W. (1985) *Neurosci. Lett.* **58,** 293–297.
16. Garthwaite, G. & Garthwaite, J. (1986) *Neurosci. Lett.* **66,** 193–198.
17. Dawson, V. L., Dawson, T. M., London, E. D., Bredt, D. S. & Snyder, S. H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6368–6371.
18. Dawson, V. L., Dawson, T. M., Bartley, D. A., Uhl, G. R. & Snyder, S. H. (1993) *J. Neurosci.* **13,** 2651–2661.
19. Izumi, Y., Benz, A. M., Clifford, D. B. & Zorumski, C. F. (1992) *Neurosci. Lett.* **135,** 227–230.
20. Demerlé-Pallardy, C., Lonchampt, M.-O., Chabrier, P.-E. & Braquet, P. (1991) *Biochem. Biophys. Res. Commun.* **181,** 456–464.
21. Zinkand, W. C., Stumpo, R. J., Thompson, C., Patel, J. & Pullman, L. M. (1993) *NeuroReport* **5,** 148–150.
22. Lafon-Cazal, M., Cucasi, M., Gaven, F., Pietri, S. & Bockaert, J. (1993) *Neuropharmacology* **11,** 1259–1266.
23. Hammer, B., Parker, W. D., Jr., & Bennett, J. P., Jr. (1993) *NeuroReport.* **5,** 72–74.
24. Noronha-Dutra, A. A., Epperlein, M. M. & Woolf, N. (1993) *FEBS Lett.* **321.** 59–62.
25. Reynolds, I. J. & Hasting, T. G. (1995) *J. Neurosci.* **15,** 3318–3327.
26. Cosi, C., Suzuki, H., Milani, D., Facci, L., Menegazzi, M., Vantini, G., Kanai, Y. & Skaper, S.D. (1994) *J. Neurosci. Res.* **39,** 38–46.
27. Zhang, J., Dawson, V. L., Dawson, T. M. & Snyder, S. H. (1994) *Science* **263,** 687–689.
28. Berisha, H., Foda, H. D., Sakakibara, H., Trotz, M., Pakbaz, H. & Said, S. I. (1990) *Am. J. Physiol.* **259,** L151–L155.
29. Pakbaz, H., Foda, H. D., Berisha, H. I., Trotz, M. & Said, S. I. (1993) *Am. J. Physiol.* **265,** L369–L373.
30. Kalfin, R., Maulik, N., Engelman, R. M., Cordis, G. A., Milenov, K., Kasakov, L. & Das, D. K. (1994) *J. Pharmacol. Exp. Ther.* **268,** 952–958.
31. Brenneman, D. E., Westbrook, G. L., Fitzgerald, S. P., Ennist, D. L., Elkins, K. L., Ruff, M. R. & Pert, C. B. (1988) *Nature (London)* **335,** 639–642.
32. Pincus, D. W., Di Cicco-Bloom, E. M. & Black, I. B. (1990) *Nature (London)* **343,** 564–570.
33. Sata, T., Kubota, E., Misra, H. P., Mojarad, M., Pakbaz, H. & Said, S. I. (1992) *Am. J. Physiol.* **262,** L147–L152.
34. Harper, J. F. & Brooker, G. (1975) *J. Cyclic Nucleotide Res.* **1,** 207–218.
35. Tukey, J. W. (1968) in *Experimental Design: Procedures for Behavioral Sciences*, ed. Kirk, R. E. (Brooks/ Cole, Belmont, CA), pp. 87–90.
36. Berisha, B., Pakbaz, H., Absood, A. & Said, S. I. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7445–7449.
37. Murthy, K. S., Zhang, K.-M., Jin, J.-G., Grider, J. R. & Makhlouf, G. M. (1993) *Am. J. Physiol.* **265,** G660–G671.
38. Imai, T., Hirata, Y., Kanno, K. & Marumo F. (1994) *J. Clin. Invest.* **93,** 543–549.
39. Said, S. I., Berisha, H. I. & Pakbaz, H. (1995) *Neuroscience* **65,** 943–946.
40. Shannon, H. E. & Sawyer, B. D. (1989) *J. Pharmacol. Exp. Ther.* **251,** 518–523.
41. Shigemoto, R., Ohishi, H., Nakanishi, S. & Mizuno, N. (1992) *Neurosci. Lett.* **144,** 229–232.
42. Yoneda, Y. & Ogita, K. (1986) *Brain Res.* **383,** 387–391.
43. Inagaki, N., Kuromi, H., Gonoi, T., Okamoto, Y., Ishida, H., Seino, Y., Kaneko, T., Iwanaga, T. & Seino, S. (1995) *FASEB J.* **9,** 686–691.
44. Sninsky, C. A., Brooderson, R. J., Broome, T. A. & Bergeron, R. J. (1994) *Gastroenterology* **106,** A569 (abstr.).
45. Simon, R. P. (1984) *Semin. Neurol.* **4,** 490–496.
46. Reynolds, I. J. & Hastings, T. G. (1995) *J. Neurosci.* **15,** 3318–3327.