Pentamidine Is an *N*-methyl-D-aspartate Receptor Antagonist and Is Neuroprotective *in vitro*

Ian J. Reynolds¹ and Elias Aizenman²

Departments of ¹Pharmacology and ²Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

Acquired immunodeficiency syndrome (AIDS) is frequently associated with dementia. The wide spectrum of neurological abnormalities associated with this dementia may involve a neurotoxin that activates the NMDA subtype of glutamate receptor in neurons. We have found that the antimicrobial agent pentamidine, which is prescribed for AIDS patients for the prophylaxis and treatment of *Pneumocystis carinii* pneumonia, is an effective NMDA receptor antagonist. Pentamidine inhibited ³H-dizocilpine binding to the NMDA receptor in rat brain membranes at a site separate from glutamate, glycine, and spermidine, with an affinity near 2 μ M. Similar concentrations of pentamidine block NMDA-induced increases in intracellular Ca^{2+} and NMDA-induced currents in cultured forebrain and cortical neurons, apparently without use dependence or voltage dependence, suggesting that pentamidine may represent a novel chemical class of NMDA receptor antagonist. Finally, pentamidine protects neurons from the lethal effects of acute NMDA exposure *in vitro*. As pentamidine may accumulate in the brain at relevant concentrations following repeated high-dose parenteral administration, these findings suggest that the drug may be neuroprotective *in vivo*.

Infection with the human immunodeficiency virus-1 (HIV-1) is commonly associated with profound disturbances in CNS function (Navia et al., 1986a; Gabuzda and Hirsch, 1987). Most studies have reported that CNS disturbances, collectively termed HIV-1-associated dementia complex (Report of a working group of the American Academy of Neurology AIDS Task Force, 1991), eventually develop in 60% or more of patients with AIDS (Navia et al., 1986a; Gabuzda and Hirsch, 1987). The basis of these neurological alterations is presumably associated with the pathological changes in neuronal as well as non-neuronal cells that are observed in postmortem specimens (Navia et al., 1986b; Gabuzda and Hirsch, 1987; Price et al., 1988). Although loss of cortical neurons has been demonstrated in several studies (Ketzler et al., 1990; Everall et al., 1991; Wiley et al., 1991), HIV-1 generally does not directly infect neurons (Wiley et al., 1986, 1991), and the precise etiologic agent underlying HIV-1-associated dementia complex is not clear. A neurotoxic action

of the HIV-1 coat protein, gp120, has been suggested (Brenne- man et al., 1988; Dreyer et al., 1990). Recently, Lipton et al. (1991) demonstrated that gp120-induced neurotoxicity could be prevented by the NMDA receptor antagonists 2-amino-5-phosphonovaleate (APV) and dizocilpine (MK801). Increased cerebrospinal fluid levels of quinolinic acid, an endogenous neurotoxin that activates NMDA receptors, has also been found in patients afflicted with AIDS (Heyes et al., 1989, 1991). Finally, it was recently demonstrated that HIV-1-infected human monocy- toid cells secrete a neurotoxin (Giulian et al., 1990; Pulliam et al., 1991). This neurotoxin is apparently different from quin- olinic acid, but nevertheless kills cells by a mechanism that can be blocked by NMDA receptor antagonists (Giulian et al., 1990). Although the chemical nature of this neurotoxin remains un- known, these data suggest that an NMDA antagonist may be useful therapeutically in AIDS patients to prevent or delay the onset of the component of dementia that arises from the loss of neurons.

The bisamidine antimicrobial pentamidine is frequently used in AIDS patients in both the treatment and prophylaxis of op- portunistic lung infections by *Pneumocystis carinii*. Recent structure-activity studies in our laboratory with arcaine, which is a competitive antagonist at the polyamine site on the NMDA receptor complex (Reynolds, 1990a,b), suggested that pentam- idine might also act as an NMDA receptor antagonist. Accord- ingly, we investigated possible interactions of pentamidine NMDA receptor and report here that pentamidine is a potent and effective NMDA receptor antagonist. Moreover, pentami- dine also protects neurons against the neurotoxic actions of NMDA, thus demonstrating that pentamidine is a novel neu- roprotective agent *in vitro*.

Materials and Methods

Receptor binding assays. Nonequilibrium ³H-dizocilpine binding and dissociation kinetic assays were performed using well-washed mem- branes from rat brain as previously described (Reynolds and Miller, 1988b; Reynolds, 1990a). Nonspecific binding was defined by the ad- dition of 30 μ M unlabeled dizocilpine. Data were analyzed using the EBDA and KINETIC routines (Elsevier Biosoft, New York, NY).

Cell culture. Primary cultures were prepared from embryonic day 17– 18 rat fetuses as previously described (Aizenman et al., 1990). For $[Ca^{2+}]_i$ recordings, cells were plated on poly-L-lysine-coated 31 mm glass cov- erslips in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (24 U/ml), and streptomycin (24 μ g/ml). After 24 hr the medium was replaced with one containing 10% horse serum and the coverslips were inverted. Cultures were used 2–4 weeks later. For neurotoxicity and electrophysiological studies, cells were plated on 12 mm coverslips coated with collagen and poly-L-lysine in DMEM with 10% F-12, 10% heat-inactivated iron-supplemented calf serum, penicillin (24 U/ml), and streptomycin (24 μ g/ml). This medium was changed on a Monday–Wednesday–Friday schedule. Cells were

Received June 14, 1991; revised Oct. 2, 1991; accepted Oct. 18, 1991.

The technical assistance of Kristi Rothermund and Karen A. Hartnett is greatly appreciated. We thank Drs. William C. de Groat, Jon W. Johnson, Alan M. Palmer, and Paul A. Rosenberg for reading earlier versions of the manuscript.

Correspondence should be addressed to Ian J. Reynolds, Department of Phar- macology, E1354 BST, University of Pittsburgh, Pittsburgh, PA 15261.

Copyright © 1992 Society for Neuroscience 0270-6474/92/120970-06\$05.00/0

Figure 1. Pentamidine inhibits ^3H -dizocilpine binding to NMDA receptors. *A*, Nonequilibrium ^3H -dizocilpine binding was inhibited by pentamidine. The apparent potency of pentamidine was similar in the presence of 0.1 μM glutamate and 0.03 μM glycine (1.82 $\pm$ 0.25 μM ; squares) and 100 μM glutamate and 30 μM glycine (2.59 $\pm$ 0.19 μM ; circles), but was significantly reduced by the addition of 50 μM spermidine (6.85 $\pm$ 0.44 μM ; p < 0.01, Student's t test, triangles). Hill slopes were 0.84 $\pm$ 0.09, 1.63 $\pm$ 0.06, and 1.31 $\pm$ 0.07 respectively. The results represent the mean ($\pm$ SEM) of five experiments performed in duplicate. *B*, Spermidine increased ^3H -dizocilpine binding in a concentration-dependent fashion. Pentamidine clearly reduced both the maximum response and the affinity of spermidine at each concentration tested. The results represent the mean ($\pm$ SEM) of four experiments performed in duplicate. *C*, Pentamidine, like Zn $^{2+}$, slowed the dissociation of ^3H -

treated with 2 μM cytosine arabinoside after 2 weeks, and cells were used 1 week later.

Intracellular Ca^{2+} recordings. [Ca^{2+}]_i was monitored in individual cultured forebrain neurons that had been loaded with 5 μM fura-2 AM (Molecular Probes) in HEPES-buffered salt solution (HBSS) that contained 5 mg/ml BSA for 1 hr as previously described (Reynolds et al., 1990). After rinsing, coverslips were mounted in a chamber (approximately 0.5 ml vol) and perfused with Mg $^{2+}$ -free buffer at a rate of 20 ml/min. Neurons mounted in this way were illuminated using light from a 150 W Hg-Xe lamp that was alternately filtered through 340 and 380 nm narrow band-pass filters and directed to a Nikon Diaphot microscope using a liquid light guide and quartz optics. Fluorescence output from individual neurons was passed through a 510 nm narrow band-pass filter, and light collection was limited to the cell body by means of a rectangular diaphragm placed in the light path. Background fluorescence was subtracted from the signal by moving the coverslip to a cell-free area at the end of the recording period and recording light levels from an identical area. The signal was not otherwise filtered. The ratio of the background-subtracted fluorescence signal was converted to [Ca^{2+}]_i by means of parameters obtained from a calibration curve (typical values: R_{min} , 0.3; R_{max} , 5.5; constant, 600) derived by using 20 μM fura-2 free acid with 10 EGTA-buffered free Ca^{2+} concentrations between 10 and 1500 nM. Drugs were applied by superfusion, typically employing 20 sec exposure to agonists and 3 min pretreatment with antagonists. In the absence of pentamidine, the mean NMDA- and glycine-stimulated increase in [Ca^{2+}]_i was 363 $\pm$ 51 nM (n = 13) above basal levels of 20–50 nM.

Electrophysiological studies. Whole-cell patch-clamp recording conditions were similar to those described earlier (Aizenman et al., 1988). Cells were continuously superfused at a rate of 0.5 ml/min with a physiological solution based on Hank's salts (composition, in mM: NaCl, 137; NaHCO $_3$, 1; NaHPO $_4$, 0.34; KCl, 5.36; KH $_2$ PO $_4$, 0.44; CaCl $_2$, 2.5; HEPES, 5; dextrose, 22.2; phenol red, 0.011 g/liter; adjusted to pH 7.2 with 0.3 N NaOH). One micromolar TTX was routinely added to the superfusate. Glycine (1 μM) was added to minimize variability of this amino acid among dishes (Johnson and Ascher, 1987). The intracellular pipette solution contained (in mM) CsCl, 140; MgCl $_2$, 1; CaCl $_2$, 1; EGTA, 2.25; HEPES, 10; adjusted to pH 7.2 with concentrated TEA-OH. Whole-cell recordings were performed with patch electrodes of 3–5 M Ω , utilizing an Axopatch IC patch-clamp amplifier (Axon Instruments). Data were acquired and analyzed with a PC-based computer/interface system and commercially available software (pCLAMP, Axon Instruments). Drugs were applied via puffer pipettes placed in close proximity to the cells under study.

Toxicity studies. Assessment of cell survival was performed by counting surviving cortical neurons utilizing trypan blue exclusion (Pixley and Cotman, 1985; Aizenman et al., 1990). Cell counts were performed under phase-contrast optics with an inverted microscope at 200 $\times$ magnification. Neurons were identified as phase-bright cells with one to three long, tapering processes. The accuracy of this identification has been confirmed by both tetanus immunocytochemistry and electrophysiology (Rosenberg and Aizenman, 1989). Neurons that had excluded the dye were counted over 30 fields (7.5 mm 2) with the use of a grid-containing eyepiece. Cell counts were performed by a person blind to the arrangement of treatment groups.

Materials. Pentamidine isethionate was obtained from Sigma (St. Louis, MO). ^3H -dizocilpine (22.5 Ci/mmol) was purchased from DuPont/New England Nuclear. Unlabeled dizocilpine was a gift of Dr. Richard Ransom (Merck Sharp and Dohme, West Point, PA). All other materials were from commercial sources.

dizocilpine. Both pentamidine and Zn $^{2+}$ were tested at 10 μM . This concentration was sufficient to reduce the dissociation rate of ^3H -dizocilpine from 0.00354 $\pm$ 0.0009 to 0.00227 $\pm$ 0.0008 min $^{-1}$ in the presence of pentamidine and to 0.00219 $\pm$ 0.00011 min $^{-1}$ in the presence of Zn $^{2+}$ (mean $\pm$ SEM; n = 4). One-way ANOVA revealed a significant difference between groups (F = 64.6; p < 0.0001), and post hoc tests with the Bonferroni correction for comparison of multiple means showed a significant difference between pentamidine and control and also Zn $^{2+}$ and control (p < 0.001).

Figure 2. Pentamidine inhibits NMDA- and glycine-induced $[Ca^{2+}]_i$ increases. *A*, $[Ca^{2+}]_i$ changes in cultured forebrain neurons were monitored using the Ca^{2+} -sensitive fluorescent dye fura-2. NMDA (30 μM) and glycine (1 μM) were added together at the arrowheads. Two control responses were obtained in the absence of pentamidine. Pentamidine was added at the concentrations indicated starting 100–120 sec before addition of NMDA and glycine. A short period of perfusion of pentamidine-free buffer was sufficient to reverse the drug action substantially. The trace represents data from a typical cell. *B*, Concentration–response data for inhibition of NMDA- and glycine-induced $[Ca^{2+}]_i$ increase. The Hill slope of this plot was 0.56 ± 0.093 . The data represent the mean ($\pm$ SEM) of 6–12 determinations per point made in separate cells. *C*, The action of pentamidine is relatively specific for NMDA receptors. A concentration of pentamidine sufficient to inhibit NMDA response

Results

We tested pentamidine for activity at the NMDA receptor complex by monitoring 3H -dizocilpine binding to rat brain membranes. Pentamidine effectively inhibited 3H -dizocilpine binding (Fig. 1*A*) with an apparent potency of $1.82 \pm 0.25 \mu M$ (mean $\pm$ SEM; $n = 5$) in the presence of low concentrations of glutamate and glycine and $2.59 \pm 0.19 \mu M$ in the presence of saturating glutamate and glycine concentrations. The absence of a significant difference in potency under these two conditions suggested that pentamidine did not bind to the glycine or NMDA recognition sites. Sodium isethionate had no effects at concentrations up to 1 mM. The addition of 50 μM spermidine to the high glutamate and glycine condition significantly reduced the potency of pentamidine in inhibiting 3H -dizocilpine binding to $6.85 \pm 0.44 \mu M$ ($n = 5$; $p < 0.01$, Student's *t* test). However, it is unlikely that this was the result of a competitive action at the polyamine site as pentamidine decreased both the affinity and maximal effect of spermidine in stimulating 3H -dizocilpine binding (Fig. 1*B*). In contrast, the competitive polyamine antagonist arcaine predominantly decreases the apparent potency of spermidine (Reynolds, 1990a,b). Moreover, low concentrations of pentamidine decreased the dissociation of 3H -dizocilpine (Fig. 1*C*), an effect mimicked by Zn^{2+} but not arcaine (Reynolds, 1990a,b).

We next examined the actions of pentamidine in more functional assays of NMDA receptor activity. Pentamidine inhibited NMDA/glycine-induced increases in intracellular free Ca^{2+} ($[Ca^{2+}]_i$) in cultured forebrain neurons from fetal rats. Half-maximal effects of pentamidine were observed at about 3 μM , and the effects were both rapid in onset and quickly reversed by washing without subsequent agonist application (Fig. 2). Pentamidine was relatively selective for NMDA receptors, as high concentrations did not alter responses to AMPA or to depolarization with 50 mM KCl, and had only small effects on kainate-induced increases in $[Ca^{2+}]_i$ (Fig. 2*C*). Kainate responses obtained in this way are insensitive to AP5 (I. J. Reynolds and E. Aizenman, unpublished observations), so the effects of pentamidine are thus unlikely to be the result of inhibition of the action of synaptically released glutamate acting at NMDA receptors. Similar concentrations of pentamidine (3–30 μM) also effectively reduced voltage-clamped NMDA/glycine-induced whole-cell currents in cultured cortical neurons from fetal rats. Within the range of concentrations tested (3–30 μM , $n = 11$), we found the block to be fully reversible (Fig. 3*A*) as well as voltage independent (Fig. 3*B*). Similar to the Ca^{2+} experiments, the action of pentamidine could be reversed without subsequent agonist application in the whole-cell recordings. Full reversal of the block at all holding potentials was usually observed by the first application of agonist following pentamidine removal, which usually occurred within 10–30 sec.

Many studies have demonstrated that acute NMDA exposure produces a delayed cell death in culture that is believed to mimic neurotoxic actions of glutamate-like agonists *in vivo* (Choi, 1988). Presumably, for pentamidine to be neuroprotective, it should

by about 80% had no effect on $[Ca^{2+}]_i$ increases elicited by depolarization with 50 mM KCl, or by application of 50 μM AMPA. Pentamidine produced a small but significant ($p < 0.05$, *t* test) decrease in the $[Ca^{2+}]_i$ response produced by 100 μM kainate. These results represent the mean ($\pm$ SEM) of five or six data points obtained in separate neurons.

Figure 3. Effects of pentamidine on NMDA-evoked whole-cell currents in cortical neurons in culture. *A*, Reversibility of block. Whole-cell responses in a cortical neuron in culture after application of 30 μ M NMDA and 1 μ M glycine (GLY) alone (Control & Recovery) or in combination with 30 μ M pentamidine. Note that the large degree of block produced by pentamidine is completely reversible. The neuron was voltage clamped at -60 mV. *B*, Effects of transmembrane potential on pentamidine block: whole-cell responses obtained from a second cortical neuron after application of 30 μ M NMDA and 1 μ M glycine (GLY) alone or in combination with 30 μ M pentamidine. The neuron was voltage clamped at -60 and +30 mV. Note that the extent of block produced by this concentration of pentamidine is equal at both potentials. Thus, in three cells tested, 3 μ M pentamidine produced $67.5 \pm 7.1\%$ inhibition at -60 mV and $63.8 \pm 7.9\%$ inhibition at +30 mV holding potential.

Figure 4. Neuroprotective actions of pentamidine in astrocyte-rich cultures of cerebral cultures. Assessment of neuronal survival in sister coverslip cultures was performed after the following treatments: *Cont*, vehicle alone; *Pent*, 30 min exposure to 5 μ M pentamidine; *NMDA*, 30 min exposure to 200 μ M NMDA; *NMDA + Pent*, 30 min exposure to a combination of 200 μ M NMDA and 5 μ M pentamidine following a 30-min exposure to 5 μ M pentamidine alone. Viability was assessed 18–24 hr after drug treatments with a trypan blue exclusion method. Values shown are pooled from four different experiments from two separate culture dates and represent means $\pm$ SEM; each experiment was performed in triplicate. A one-way ANOVA revealed significant differences between treatment groups ($F = 4.6$; $p = 0.02$). Two post hoc tests among selected groups with a Bonferroni correction for two comparisons revealed a statistical difference between the *Cont* group and the *NMDA* group (** $p = 0.006$) and between the *NMDA* and *NMDA + Pent* groups (* $p = 0.02$).

effectively prevent delayed NMDA-induced cell death. Figure 4 shows that incubating cortical cultures with 200 μ M NMDA for 30 min was sufficient to kill more than 50% of the neurons, assessed by a trypan blue exclusion method. The addition of 5 μ M pentamidine together with the agonist was sufficient to prevent this action of NMDA completely. It is noteworthy that this concentration reduced the NMDA-induced $[Ca^{2+}]_i$ change by 60%. Pentamidine (5 μ M) was also effective in protecting neurons when included for 24 hr after NMDA exposure (data not shown). In preliminary studies we have found that overnight incubation with pentamidine at concentrations above 30 μ M produces a marked change in the morphological features of the neurons and glia in our culture system. These changes include a loss of phase brightness by the neurons as well as the apparent retraction of glial processes (Fig. 5), although the cells still excluded trypan blue. Removal of pentamidine did not result in recovery of cell morphology 24 hr later. We are currently investigating the potential toxic properties of pentamidine. It is noteworthy, however, that these morphological changes were not produced by concentrations of pentamidine (5 μ M) that were completely neuroprotective in our toxicity assay, even after a prolonged (24 hr) exposure.

Discussion

This study has demonstrated that pentamidine is an effective NMDA antagonist *in vitro* and can prevent NMDA-induced cell death in primary cultures of cortical neurons. This finding is of particular significance because pentamidine is currently in clinical use for the treatment of *Pneumocystis carinii* pneumonia in patients with AIDS. As described earlier, the HIV-1-associated dementia complex that eventually develops in the majority of AIDS patients is a complex phenomenon associated with changes in both neuronal and nonneuronal cells in the brain. The neuronal component of this dementia complex may

Figure 5. High concentrations of pentamidine are toxic. Cortical neurons were incubated for 24 hr in the absence (*A*) or presence (*B*) of 30 μM pentamidine. Similar observations were made in four separate culture preparations. The pentamidine-treated cells still excluded trypan blue, but the change in morphology of the cells made it difficult to determine whether there was a significant loss of neurons or glia. Scale bar, 40 μm .

arise from the action of a range of possible neurotoxins (Giulian et al., 1990; Pulliam et al., 1991) that either mimic NMDA or enhance the neurotoxic effects of NMDA (Heyes et al., 1989, 1991; Giulian et al., 1990) or, in the case of gp120, synergize with glutamate at the NMDA receptor complex (Lipton et al., 1991). As an NMDA receptor complex-based mechanism seems to be a final common pathway for the action of most of these toxins, an NMDA receptor antagonist would appear to be the optimal therapeutic agent. In this study we have shown that pentamidine prevents NMDA-induced neurotoxicity. This suggests that pentamidine will also inhibit the action of these neurotoxins, although this remains to be directly demonstrated. If the loss of neurons that is observed in AIDS patients (Ketzler et al., 1990; Everall et al., 1991; Wiley et al., 1991) is a critical event in the development of HIV-1-associated dementia complex, then parenteral pentamidine use might be associated with a lower incidence of neurological dysfunction. However, we are unaware of any clinical study that has directly addressed this possibility.

The relevance of our findings to the clinical situation depends in part on the bioavailability and pharmacokinetic characteristics of pentamidine in humans. A recent study of the distribution of pentamidine in AIDS patients postmortem suggested that repeated high doses of pentamidine given intravenously or intramuscularly results in measurable accumulation of the drug in brain in about 35% of patients (Donnelly et al., 1988). The mean measurable concentration in the brain was 2.4 $\mu\text{g/gm}$ tissue. Assuming equal distribution between compartments and assuming that 1 gm is equivalent to 1 ml, this would correspond to a concentration of 7.2 μM pentamidine, which is clearly an effective concentration based on our results. It is also noteworthy that the limit of detection in the study of Donnelly et al. (1988) corresponds to 2.4 μM . Thus, even brains that did not contain

measurable pentamidine concentrations may still have pharmacologically relevant levels. It is also notable that brain pentamidine could be detected in one patient over a year after the last dose was administered. This suggests that pentamidine may accumulate slowly and be eliminated slowly, which represents ideal characteristics for a drug to treat a slowly progressive disorder like dementia. More recently, pentamidine has been administered by aerosol in order to deliver the drug directly to the intended site of action and to avoid systemic toxicity (e.g., Hirschel et al., 1991; Murphy et al. 1991). This route of administration will presumably result in lower accumulation in the brain.

The mechanism of action of pentamidine has not been fully resolved. It is unlikely that pentamidine acts directly at the NMDA or glycine recognition site as the addition of high concentrations of either agonist did not significantly alter the apparent potency of pentamidine in the ^3H -dizocilpine binding assay. Although the action of pentamidine could modulate the effects of spermidine, this interaction did not appear to be competitive in nature. Thus, the shift in the affinity of pentamidine (2.59 to 6.85 μM) produced by the addition of 50 μM spermidine (10-fold above the EC_{50} for spermidine) is less than the 10-fold change predicted for a competitive antagonist. It is also clear that the profile of the interaction of pentamidine with the spermidine concentration-response curve (Fig. 1*B*) is distinct from that observed with arcaine, the prototypical competitive polyamine antagonist (Reynolds, 1990b). In addition, pentamidine slowed the dissociation of ^3H -dizocilpine, which has previously been observed with Zn^{2+} , but not the polyamine antagonists arcaine or diaminodecane (Reynolds and Miller, 1988b; Reynolds, 1990a). Moreover, no functional inhibition of NMDA response with arcaine has been observed (I. J. Reynolds, unpublished observations). Thus, the principal effects of pentam-

idine are apparently not related to competitive antagonism of the polyamine site on the NMDA receptor complex. The absence of voltage and use dependency apparently precludes an action at either the Mg^{2+} or phencyclidine sites (Mayer et al., 1984; Nowak et al., 1984; Honey et al., 1985; Huettner and Bean, 1988; MacDonald and Nowak, 1990). If pentamidine acts at a previously described site, the Zn^{2+} site appears to be the most likely site of action (Peters et al., 1987; Westbrook and Mayer, 1987; Forsythe et al., 1988; Reynolds and Miller, 1988a), although with the data presented in this study we cannot determine whether pentamidine is actually acting at the Zn^{2+} site or simply mimicking the actions of Zn^{2+} by binding to a distinct site elsewhere on the receptor complex. Nonetheless, an action at the Zn^{2+} site would be consistent with the excellent agreement of potency in the various assays, the lack of competition between pentamidine and glutamate, glycine, and spermidine, the slowing of the dissociation of 3H -dizocilpine, the voltage independence of its action, and its neuroprotective actions, all of which have been observed with Zn^{2+} .

References

- Aizenman E, Frosch MP, Lipton SA (1988) Responses mediated by excitatory amino acid receptors in solitary retinal ganglion cells from rat. *J Physiol (Lond)* 396:75–91.
- Aizenman E, Hartnett KA, Reynolds IJ (1990) Oxygen free radicals regulate *N*-methyl-D-aspartate receptor function via a redox modulatory site. *Neuron* 5:841–846.
- Brenneman DE, Westbrook GL, Fitzgerald SP, Ennist DL, Elkins KL, Ruff MR, Pert CB (1988) Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. *Nature* 335:639–642.
- Choi DW (1988) Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1:623–634.
- Donnelly H, Bernard EM, Rothkotter H, Gold JWM, Armstrong D (1988) Distribution of pentamidine in patients with AIDS. *J Infect Dis* 157:985–989.
- Dreyer EB, Kaiser PK, Offermann JT, Lipton SA (1990) HIV-1 coat protein neurotoxicity prevented by calcium channel antagonists. *Science* 248:364–367.
- Everall IP, Luthert PJ, Lantos PL (1991) Neuronal loss in the frontal cortex in HIV infection. *Lancet (N Am Ed)* 337:1119–1121.
- Forsythe ID, Westbrook GL, Mayer ML (1988) Modulation of excitatory synaptic transmission by glycine and zinc in cultures of mouse hippocampal neurons. *J Neurosci* 8:3733–3741.
- Gabuzda DH, Hirsch MS (1987) Neurologic manifestations of infections with human immunodeficiency virus. *Ann Intern Med* 107:383–391.
- Giulian D, Vaca K, Noonan CA (1990) Secretion of neurotoxins by mononuclear phagocytes infected with HIV-1. *Science* 250:1593–1596.
- Heyes MP, Rubinow D, Lane C, Markey SP (1989) Cerebrospinal fluid quinolinic acid concentrations are increased in acquired immunodeficiency syndrome. *Ann Neurol* 26:275–277.
- Heyes MP, Brew BJ, Martin A, Price RW, Salazar AM, Sidtis JJ, Yergey JA, Mouradian MM, Sadler AE, Keilp J, Rubinow D, Markey SP (1991) Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: relationship to clinical and neurological status. *Ann Neurol* 29:202–209.
- Hirschel B, Lazzarin A, Chopard P, Opravil M, Furrer H-J, Rüttimann S, Vernazza P, Chave J-P, Ancarani F, Gabriel V, Heald A, King R, Malinverni R, Martin J-L, Mermillod B, Nicod L, Simoni L, Concetta Vivirito M, Zerboni R, Swiss Group for Clinical Studies on AIDS (1991) A controlled study of inhaled pentamidine for primary prevention of *Pneumocystis carinii* pneumonia. *N Engl J Med* 324:1079–1083.
- Honey CR, Miljkovic Z, MacDonald JF (1985) Ketamine and phencyclidine cause a voltage-dependent block of responses to L-aspartic acid. *Neurosci Lett* 61:135–139.
- Huettner JE, Bean BP (1988) Block of NMDA-activated current by the anticonvulsant MK-801: selective binding to open channels. *Proc Natl Acad Sci USA* 85:1307–1311.
- Johnson JW, Ascher P (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* 325:529–531.
- Ketzler S, Weis S, Haug H, Budka H (1990) Loss of neurons in the frontal cortex in AIDS brains. *Acta Neuropathol (Berl)* 80:92–94.
- Lipton SA, Sucher NJ, Kaiser PK, Dreyer EB (1991) Synergistic effects of HIV coat protein and NMDA receptor mediated neurotoxicity. *Neuron* 7:111–118.
- MacDonald JF, Nowak LM (1990) Mechanisms of blockade of excitatory amino acid receptor channels. *Trends Pharmacol Sci* 11:167–172.
- Mayer ML, Westbrook GL, Guthrie PB (1984) Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* 309:261–263.
- Murphy RL, Lavelle JP, Allan JD, Gordin FM, Dupliss R, Boswell SL, Waskin HA, Davies SF, Graziano FM, Saag MS, Walter JB, Crane LR, MacDonell KB, Hodges TL, Pierce PF (1991) Aerosol pentamidine prophylaxis following *Pneumocystis carinii* pneumonia in AIDS patients: results of a blinded dose-comparison study using an ultrasonic nebulizer. *Am J Med* 90:418–426.
- Navia BA, Jordan BD, Price RW (1986a) The AIDS dementia complex: I. Clinical features. *Ann Neurol* 19:517–524.
- Navia BA, Cho E-S, Petito CK, Price RW (1986b) The AIDS dementia complex: II. Neuropathology. *Ann Neurol* 19:525–535.
- Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307:462–465.
- Peters S, Koh J, Choi DW (1987) Zinc selectively blocks the action of *N*-methyl-D-aspartate on cortical neurons. *Science* 236:589–593.
- Pixley SK, Cotman CW (1985) Laminin supports short term survival of rat septal neurons in low-density serum-free cultures. *J Neurosci Res* 15:1–17.
- Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC, Cleary P (1988) The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. *Science* 239:586–592.
- Pulliam L, Herndier BG, Tang NM, McGrath MS (1991) Human immunodeficiency virus-infected macrophages produce soluble factors that cause histological and neurochemical alterations in cultured human brains. *J Clin Invest* 87:503–512.
- Report of a working group of the American Academy of Neurology AIDS Task Force (1991) Nomenclature and research case definitions for neurologic manifestations of human immunodeficiency virus-type 1 (HIV-1) infection. *Neurology* 41:778–785.
- Reynolds IJ (1990a) Arcaine uncovers dual interaction of polyamines with the *N*-methyl-D-aspartate receptor. *J Pharmacol Exp Ther* 255:1001–1007.
- Reynolds IJ (1990b) Arcaine is a competitive antagonist of the polyamine site on the NMDA receptor. *Eur J Pharmacol* 177:215–216.
- Reynolds IJ, Miller RJ (1988a) [3H] MK801 binding to the NMDA receptor/ionophore complex is regulated by divalent cations: evidence for multiple regulatory sites. *Eur J Pharmacol* 151:103–112.
- Reynolds IJ, Miller RJ (1988b) Multiple sites for the regulation of the *N*-methyl-D-aspartate receptor. *Mol Pharmacol* 33:581–584.
- Reynolds IJ, Rush EA, Aizenman E (1990) Reduction of NMDA receptors with dithiothreitol increases [3H] MK801 binding and NMDA-induced Ca^{2+} fluxes. *Br J Pharmacol* 101:178–182.
- Rosenberg PA, Aizenman E (1989) Hundred-fold increase in neuronal vulnerability to glutamate toxicity in astrocyte poor cultures of rat cerebral cortex. *Neurosci Lett* 103:162–168.
- Westbrook GL, Mayer ML (1987) Micromolar concentrations of Zn^{2+} antagonize NMDA and GABA responses of hippocampal neurons. *Nature* 328:640–643.
- Wiley CA, Schrier RD, Nelson JA, Lampert PW, Oldstone MBA (1986) Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. *Proc Natl Acad Sci USA* 83:7089–7093.
- Wiley CA, Masliah E, Morey M, Lemere C, DeTeresa R, Grafe M, Hansen L, Terry R (1991) Neocortical damage during HIV infection. *Ann Neurol* 29:651–657.