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24. The cell-free microtiter plate assay of O_2^- production was performed with PMN membranes and cytosol as described (*2*) except that the system was activated with arachidonic acid (40 μM) augmented with GTP-γ-S (5 μM). Studies on reconstitution of activity were done with the *E. coli*–derived rNCF-47k extract (*7*). Preliminary studies indicated that 0.5 μl of this rNCF-47k extract only slightly inhibited O_2^- production when added to normal PMN membranes and cytosol, while consistently augmenting O_2^- production in assays that contained NCF-47k–deficient PMN cytosol. This amount of extract had minimal background ferricytochrome C–reducing activity when added to either control PMN cytosol or membranes in the cell-free assay.
25. We thank B. D. Volpp, W. M. Nauseef, and R. A. Clark for their generous gift of B-1 antibody and for allowing us to use this antibody to clone NCF-47k; P. M. Murphy for providing the mRNA used to construct the expression library and for many helpful discussions; and D. Rotrosen for suggesting a comparison of NCF-47 and GAP protein sequence.

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In Vivo Activity Against HIV and Favorable Toxicity Profile of 2′,3′-Dideoxyinosine

ROBERT YARCHOAN, HIROAKI MITSUYA, ROSE V. THOMAS, JAMES M. PLUDA, NEIL R. HARTMAN, CARLO-FEDERICO PERNO, KATHY S. MARCZYK, JEAN-PIERRE ALLAIN, DAVID G. JOHNS, SAMUEL BRODER

The purine analog 2′,3′-dideoxyinosine (ddI), which has anti-retroviral activity in vitro was administered for up to 42 weeks to 26 patients with acquired immunodeficiency syndrome (AIDS) or severe AIDS-related complex (ARC). Ten of these individuals were AZT-intolerant. Eight dose regimens were studied. The drug was orally bioavailable and penetrated into the cerebrospinal fluid (CSF). Comparatively little evidence of an effect against human immunodeficiency virus (HIV) was seen at the lowest four doses. However, patients in the four highest dose groups (ddI at 1.6 milligrams per kilogram intravenously and then ≥3.2 milligrams per kilogram orally at least every 12 hours or higher) had increases in their circulating $CD4^+$ T cells ($P < 0.0005$), increased CD4/CD8 T cell ratios ($P < 0.01$), and, where evaluable, more than an 80% decrease in serum HIV p24 antigen ($P < 0.05$). The patients also had evidence of improved immunologic function, had reduced viremic symptomatology, and gained a mean of 1.6 kilogram with these comparatively infrequent dosing schedules (every 8 or 12 hours). The most notable adverse effects directly attributable to ddI administration at the doses used in this study included increases in serum uric acid (due to hypoxanthine release) and mild headaches and insomnia. These results suggest that serious short-term toxicity at therapeutic doses is not an inherent feature in the profile of agents with clinical anti-HIV activity. Further controlled studies to define the safety and efficacy of this agent may be worth considering.

SEVERAL DRUGS HAVE NOW BEEN shown to have clinical anti-retroviral activity against HIV (*1–4*), and one of these drugs, 3′-azido-2′,3′-dideoxythymidine (called zidovudine, or AZT), has been demonstrated to reduce the morbidity and mortality of patients with AIDS or AIDS-related complex (*2*). Widespread use of AZT, the only anti-retroviral drug currently approved for the treatment of severe HIV infection, is likely to be one factor above and beyond prophylaxis against *Pneumocystis carinii* pneumonia that has contributed to the increased survival of AIDS patients (both gay men and intravenous drug users) diagnosed since the end of 1986 (*5*). However, AZT and each of the other agents shown to have activity against HIV in vivo may also cause substantial toxicity in some patients with severe HIV infection (*1, 3, 4, 6*). In particular, 40 to 80% of AIDS patients do not tolerate therapeutic doses of AZT for 26 weeks because of bone marrow suppression or other toxicities (*6*). Moreover, the improvements induced by AZT are only transient in many patients with advanced AIDS (*1, 2, 7*), and Larder *et al.* have recently reported that isolates of HIV from patients receiving long-term AZT therapy may have reduced sensitivity to this drug (*8*). For these reasons, improved drugs and drug combinations are needed for AIDS and its related diseases.

2′,3′-Dideoxyinosine (ddI) (Fig. 1) is a purine dideoxynucleoside with potent activity against HIV in vitro in T cells (*9*) and monocytes (*10*). It is closely related to 2′,3′-dideoxyadenosine (ddA), a compound first synthesized by Robins and Robins in 1964 (*11*). [Indeed ddA is rapidly converted to ddI by the ubiquitous enzyme adenosine deaminase (*12*), so these drugs can for many purposes be considered alternate forms.] In human cells, ddI is metabolized to its active moiety, 2′,3′-dideoxyadenosine-5′-triphosphate (ddA-TP) (*13*). It is thought that ddA-TP inhibits HIV DNA polymerase (reverse transcriptase) activity preferentially, and thereby suppresses HIV infection by blocking the synthesis of a DNA copy of the viral genome. Its mechanism of action is thought to be chain termination, competitive inhibition of reverse transcriptase, or both (*14*). However, while ddI is a potent anti-retroviral agent, it is not a broad spectrum antiviral drug. Unlike the triphosphates of AZT or 2′,3′-dideoxycytidine (ddC), ddA-TP has a long half-life (over 12 hours) in cells exposed to ddI (*15*). Mitsuya and Broder found that ddI has a relatively high therapeutic index in vitro as compared to other dideoxynucleosides (*9*), and it has relatively little in vitro toxicity for human marrow progenitor cells (*16*). These observations suggested that ddI was worth testing in patients with HIV infection.

A total of 26 patients (25 male and 1 female) with HIV infection, aged 23 to 51, were entered into an initial clinical trial of ddI (*17*). Ten patients had AIDS and 16 had ARC (*18*). All had antibodies to HIV and less than 300 $CD4^+$ T cells per cubic millimeter (median 60; range 6 to 266). Ten of the patients were AZT-intolerant by virtue of having developed nausea, malaise, anemia, or headaches (*19*). All but one patient had received no AZT or anti-HIV therapy during the preceding 4 weeks. Prophylaxis for *Pneumocystis carinii* pneumonia was permitted during this study (*20*). Patients were given ddI intravenously for 14 days at doses

R. Yarchoan, H. Mitsuya, R. V. Thomas, J. M. Pluda, C.-F. Perno, K. Marczyk, S. Broder, Clinical Oncology Program, National Cancer Institute, Bethesda, MD 20892.
N. R. Hartman and D. G. Johns, Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD 20892.
J.-P. Allain, Abbott Laboratories, North Chicago, IL 60064.

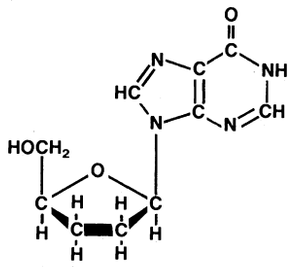

Fig. 1. Structure of 2',3'-dideoxyinosine (ddI).

ranging from 0.2 mg/kg every 12 hours (dose A) to 3.2 mg/kg every 8 hours (dose H) (Fig. 2). Patients then received ddI orally at twice the intravenous dose (but at the same dosing schedule). As ddI is acid labile (forming 2',3'-dideoxyribose plus hypoxanthine, a precursor of uric acid), oral doses were administered on an empty stomach after ingestion of antacids. Failure to take this into account would render the drug inactive by the oral route.

The peak ddI concentration (*21*) after intravenous administration of ddI over 90 min was roughly proportional to the administered dose and ranged from 0.5 μM in patients receiving 0.2 mg/kg to 10 μM in patients receiving 3.2 mg/kg. The average plasma half-life was 35 min. The oral bioavailability of liquid ddI in fasting patients to whom drug was administered 2 min after ingesting 30 cm^3 of antacids (for example, magnesium hydroxide) averaged 35%. Finally, the CSF/plasma ratio averaged 0.19 at 1 hour after the completion of an intravenous infusion of ddI.

All but three patients completed the first 10 weeks of dosing as planned (*22*). Overall, there was no clear trend in the $CD4^+$ T cells in the patients on the lowest dose (A), and in patients receiving the next three doses (B to D), there were comparatively small or transient increases (Fig. 2). However, starting with dose group E (ddI at 1.6 mg/kg intravenously every 12 hours), every patient had an increase in his or her circulating $CD4^+$ cells during the first 6 weeks on ddI (Fig. 2); the mean number of $CD4^+$ cells in these patients (receiving doses E, F, G, or H) increased from 144 ± 25 cells per cubic millimeter (mean ± SEM) at entry to 222 ± 37 cells per cubic millimeter at week 6 ($P < 0.0005$ (*23*). The mean $CD4^+$ cells in these patients remained essentially unchanged between weeks 6 and 10, and when most recently measured (at up to week 28) was still substantially higher (221 ± 43 cells per cubic millimeter) than at entry.

Along with the increase in $CD4^+$ T cells, patients in dose groups E to H had an increase in their CD4/CD8 T cell ratios from 0.17 ×/÷ 1.26 (geometric mean ×/÷SEM) at entry to 0.23 ×/÷ 1.28 at week 6 ($P < 0.01$) (*16*); an increase in their total lymphocytes from 1248 ± 74 cells per cubic millimeter at entry to 1566 ± 115 cells per cubic millimeter at week 6 ($P < 0.0005$); and an increase in their $CD8^+$ T cells from 666 ± 80 cells per cubic millimeter at entry to 823 ± 114 at week 6 ($P < 0.05$). These results are consistent with the restoration of T cell immune components after administration of ddI at concentrations sufficient to attain an anti-retroviral effect (*9*). The increase in both $CD4^+$ and $CD8^+$ T cells is noteworthy. In addition, the patients had evidence of an increase in their immune function. Seventeen patients were anergic before therapy (*1*), and five of these patients (four in dose groups E to H) developed a delayed-type cutaneous hypersensitivity response to at least one test antigen when retested after 6 weeks of therapy. In addition, of the nine patients tested in dose groups E to H, five had a ≥3× increase in their proliferative response to pokeweed mitogen, two had a ≥3× increased proliferation to tetanus toxoid, and two had a ≥3× increase in their proliferation to diphtheria toxoid (*24*).

As a measure of the effect of ddI on the HIV viral load in vivo, we periodically assessed serum HIV p24 antigenemia (*7*). The patients on the lower doses either had no change (dose A) or had moderate or transient decreases in this parameter (doses B to D) (*25*). In contrast, each of the six patients on doses E to H who had detectable p24 antigen at entry had a decline after administration of ddI ($P < 0.05$ at week 6) (Fig. 3); in five of these patients, the serum p24 antigen became undetectable and has remained so for up to at least 28 weeks (Fig. 3) (*25*). No patient at any dose group who was antigen-negative at entry became p24 antigenemic during therapy.

Fourteen of the patients reported increased energy, reduced fatigue, or decreased sleep requirements upon being given ddI. The one patient who had HIV-associated arthralgias reported improvement in this symptom, five patients reported an increase in appetite, and the patients overall gained an average of 1.6 kg by week 10. Six patients developed opportunistic infections (*20*) and one started on dose A has died. However, all but one of the infections developed in the 13 patients on the lower four doses (all but one of these in patients in dose groups A and B).

Overall, ddI was well tolerated for up to 42 weeks at the doses presented here. Most

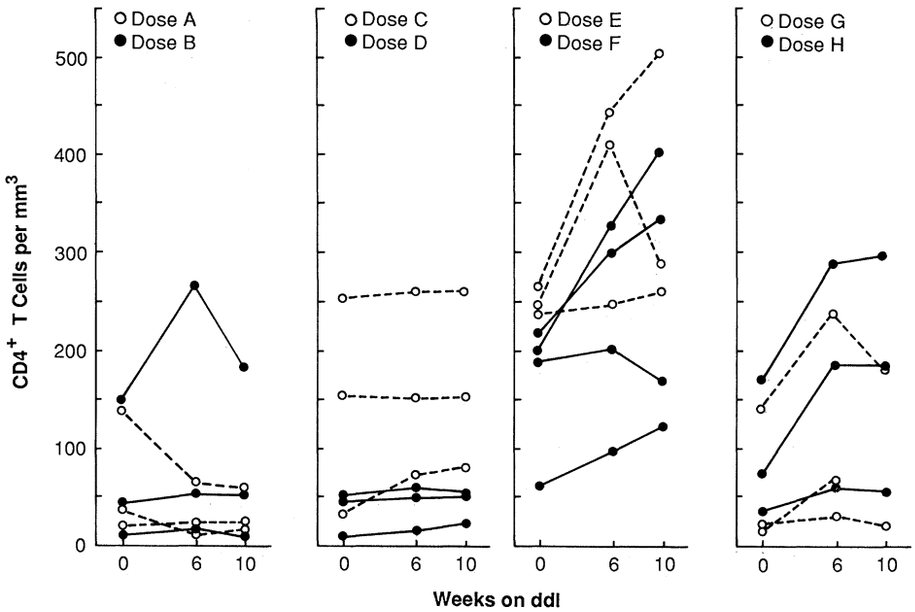

Fig. 2. Absolute numbers of circulating $CD4^+$ T lymphocytes in patients receiving ddI. During the first 2 weeks, the patients received ddI intravenously over 90 min according to the following dose schedules: dose A, 0.2 mg/kg every 12 hours; dose B, 0.4 mg/kg every 12 hours; dose C, 0.8 mg/kg every 12 hours; dose D, 0.8 mg/kg every 8 hours; dose E, 1.6 mg/kg every 12 hours; dose F, 1.6 mg/kg every 8 hours; dose G, 3.2 mg/kg every 12 hours; dose H, 3.2 mg/kg every 8 hours. After 2 weeks, the patients were given ddI orally at twice the intravenous dose. The number of $CD4^+$ lymphocytes was periodically determined by fluorescence-activated cell sorting. Each line represents the $CD4^+$ lymphocytes in a single patient. Where two pretreatment values were obtained within 2 weeks of starting therapy, these values were averaged for the entry value. One patient in dose group F (patient 18) could not be hospitalized for the course of intravenous therapy, and his results are for oral ddI only. The statistical significance of the increases at week 6 as compared to baseline for the patients in dose groups E to H is $P < 0.0005$. The equivalent significance calculated for all patients is also $P < 0.0005$. The increases in the number of $CD4^+$ T cells were also statistically significant (both for all patients and those in groups E to H) at the other time points examined (weeks 2 and 10).

patients in the highest two dose groups had increases of 1 to 3 mg/dl in their serum uric acid, which was almost certainly from ddI catabolism (through a hypoxanthine intermediate) (*13*). These changes were not clinically significant in our study. We also observed transient increases in serum triglycerides of uncertain clinical significance. Thirteen of the patients, particularly those who had previously developed constitutional symptoms on AZT, complained of mild headaches, restlessness, or insomnia on ddI. These symptoms were not dose-related and usually subsided by week 5. Other sporadic adverse effects that may have been caused either by ddI or alternatively, from the underlying HIV infection included seizures (two patients), neutropenia (two patients, one of whom was also on a folate inhibitor), increases in serum amylase (two patients, one of whom had pancreatitis), recurrence of preexisting hepatitis (one patient), dysesthesia of the feet (two patients), and a transient morbilliform skin rash (one patient). We did not observe a clear dose-related pattern to any of these, and do not believe that we achieved dose-limiting toxicity at the four doses (E to H) at which we observed consistent clinical activity. This represents a defined threefold dose range for activity without substantial toxicity. In particular, we did not observe macrocytic anemia (the hallmark toxicity of AZT).

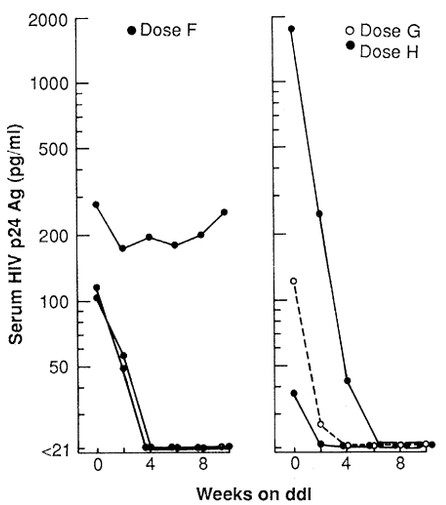

Fig. 3. Serum HIV p24 antigen, measured by an antigen-capture assay (*20*), in patients in dose groups E to H. All patients in these groups who had detectable p24 antigen at some point during therapy are shown (no patient in group E had detectable p24). In patients who had more than one determination during the 2 weeks prior to entry, the mean of these determinations is taken as the week 0 value. The statistical significance of the decreases in serum HIV p24 antigen at week 6 compared to week 0 in these six patients was $P < 0.05$. The decreases compared to baseline were also statistically significant at each of the other time points examined (weeks 2, 4, 8, and 10). The one patient in dose group F (patient 18) in whom p24 did not become undetectable could not be hospitalized for the course of intravenous therapy and the results for this patient are for oral dosing only. Also, this patient was taking ddI with meals and may have had lower bioavailability.

The results of this pilot study demonstrate that ddI can induce an increase in circulating $CD4^+$ T cells and total lymphocytes, a decrease in serum p24 antigen, and improvement in immunologic function when administered to patients with AIDS or severe ARC. The occurrence of these changes even with twice daily dosing is most likely caused by the long intracellular half-life of the active moiety of this compound, ddA-TP (*15*). The results of this study suggest that further research on the biochemical pharmacology of appropriate purine dideoxynucleosides may be useful in designing drugs with a favorable therapeutic index.

While the present Phase I study cannot be taken to demonstrate an effect on morbidity or mortality per se, the experience with other anti-HIV agents suggests that increases in the number of $CD4^+$ T cells and declines in serum p24 antigen as observed in the current report are predictive of prolonged survival and reduced incidence of opportunistic infections. Patients with less than 200 $CD4^+$ cells per cubic millimeter are at particular risk of developing opportunistic infections (*26*), and the average increase observed in this study (from <150 $CD4^+$ cells to >200 $CD4^+$ cells) might be expected to confer reduced risk of such infections. Also, in a related pilot study focused on patients with AIDS dementia, we have recently observed improvement in HIV-associated cognitive dysfunction in two patients receiving ddI (*27*).

Although patients have received ddI only for 42 weeks and we did observe increases in uric acid and occasional other adverse effects that theoretically may be related to the drug, the results overall suggest that ddI can induce clinical improvement at doses that cause minimal short-term toxicity. [Also, five additional patients who previously had received ddA, a precursor form of ddI, in the National Cancer Institute were subsequently switched to ddI, and these five patients have now tolerated ddA or ddI for more than 1 year (*25*)]. In particular, we did not observe macrocytic bone marrow suppression, the dose-limiting toxicity of AZT. Thus, the results of this study may contradict the notion that a clinical anti-retroviral effect can be attained only in the face of substantial toxicity. Nevertheless, we stress that the results here refer only to short-term administration of up to 19 mg/kg/day of ddI to a limited number of patients. One of the purposes of Phase I studies is to determine the maximum tolerated dose (dose-limiting toxicity) of a drug, and it would be quite unusual if a toxic dose could not be defined with this agent with further increases or very long term use (*28*). Indeed, we are still continuing our dose escalations, and at substantially higher doses than those reported here (or that we would recommend for larger trials of efficacy), have observed dysesthesia of the feet in two patients. Whether this or a sporadic pancreatic defect will prove to be the dose-limiting toxicity of ddI will require further studies.

Some AZT-resistant isolates of HIV from patients on long-term AZT therapy have been found to preserve their sensitivity to other dideoxynucleosides such as ddC and ddI in vitro (*8, 29*). In this regard, it will be of interest to determine whether patients with AZT-resistant strains of HIV respond to ddI in vivo or whether combination regimens utilizing two or more such drugs may reduce or delay the development of resistance. Also, as some AZT-intolerant patients can take ddI without difficulty, this subset of patients might be suitable for future studies of ddI. Additional trials of ddI in patients with severe HIV infection are now under way in several medical centers (*30*). Ultimately, large-scale controlled studies either comparing ddI (perhaps doses E or F) with AZT or comparing different doses of ddI in certain patients will probably be needed to determine its effect on survival in patients with AIDS or ARC, its long-term toxicity, and its particular role in the treatment of patients who cannot tolerate or who have developed viral resistance to AZT. Until these further studies are conducted, conclusions about the long-term safety and efficacy of this agent, and its appropriate role in the therapy of AIDS in comparison to other treatments, must be deferred.

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17. The protocol for this trial was approved by the Institutional Review Board of the National Cancer Institute. All subjects gave informed consent to participate in the study prior to entry. The ddI for this study was provided by the Developmental Therapeutics Program of the National Cancer Institute and by Bristol-Myers Co.
18. Of the ten AIDS patients, five had had *Pneumocystis carinii* pneumonia, one had had esophageal candidiasis, three had Kaposi's sarcoma, and one had wasting syndrome. Each of the ARC patients had oral candidiasis.
19. We specifically recruited patients who had received AZT for 4 months or less because of the concern that patients with end-stage AIDS who had been on AZT for a longer time might not have been able to manifest immunologic improvements upon being administered another anti-retroviral agent. As a result, a higher than usual percentage of AZT-intolerant patients had nausea, vomiting, or other constitutional symptoms as their principal AZT toxicity. Of the ten AZT-intolerant patients, two had AIDS and eight had ARC.
20. Prophylaxis against *P. carinii* pneumonia with trimethoprim-sulfamethoxazole was permitted throughout the protocol; 6 months into the study, prophylaxis with aerosolized pentamidine was also permitted. In dose groups A and B, one patient received trimethoprim-sulfamethoxazole throughout and one patient started receiving aerosolized pentamidine 4 months after entry; in dose groups C and D, two patients started receiving aerosolized pentamidine 2 months after entry and one patient started receiving trimethoprim-sulfamethoxazole 6 months after entry; in dose groups E and F, one patient received aerosolized pentamidine throughout; and in dose groups G and H, three patients received aerosolized pentamidine throughout.
21. The concentration of ddI in plasma samples was measured by high-performance liquid chromatography (N. R. Hartman, J. A. Kelly, D. G. Johns, unpublished data).
22. The three patients who did not complete the initial 10 weeks as planned were as follows. Patient 5 was diagnosed as having *Cryptococcal* meningitis after 1 week of therapy and was taken off ddI at that time; he was the patient not considered evaluable. Patient 18 could not undergo hospitalization because of an unexpected nonmedical problem and received only oral ddI. Patient 22 was temporarily taken off ddI at week 5, after a seizure; he subsequently resumed ddI therapy after initiating anti-seizure medication.
23. As specified in the original protocol, the statistical significance of the changes from baseline were assessed at week 6 with the two-sided Wilcoxon signed rank test for paired values. For serum HIV p24 antigen and CD4/CD8 T cell ratios, logarithmically transformed values were used.
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30. The ongoing trials of ddI are as follows: R. Dolin, F. Valentine and co-workers at the University of Rochester and New York University, co-sponsored by Bristol-Myers Co. and the NIAID; H. Liebman and co-workers at Boston University, sponsored by Bristol Myers Co.; J. Groopman and co-workers at The New England Deaconess Hospital, sponsored by Bristol-Myers Co.; P. Pizzo and co-workers in children at the National Cancer Institute, sponsored by the Cancer Therapy Evaluation Program. At the V International Conference on AIDS, investigators from the first two of these studies reported preliminary data showing a similar toxicity prolife of ddI at doses comparable to those reported here (J. Lambert *et al.*, *Abstracts of the V International Conference on AIDS, Montreal, June 4–9* (1989), p. 563; T. Cooley *et al.*, *ibid.*, p. 336.)
31. We thank C. McLaren, S. Steinberg, D. Venzon, W. W. Chamberlain, C. K. Grieshaber, K. P. Flora, J. Groopman, M. Bassilar, M. Leuther, J. Stewart, I. Naily, J. Tomaszewski; the Pharmacy staff of the NIH Clinical Center and the medical and nursing staffs of the Medicine Branch of the NCI for their help.

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Brain Region and Gene Specificity of Neuropeptide Gene Expression in Cultured Astrocytes

HISAHARU SHINODA, ANN M. MARINI, CRISTINA COSI, JOAN P. SCHWARTZ*

Astrocytes have many neuronal characteristics, such as neurotransmitter receptors, ion channels, and neurotransmitter uptake systems. Cultured astrocytes were shown to express certain neuropeptide genes, with specificity for both the gene expressed and the brain region from which the cells were prepared. Somatostatin messenger RNA and peptides were detected only in cerbellar astrocytes, whereas proenkephalin messenger RNA and enkephalin peptides were present in astrocytes of cortex, cerebellum, and striatum. Cholecystokinin was not expressed in any of the cells. These results support the hypothesis that peptides synthesized in astrocytes may play a role in the development of the central nervous system.

ASTROCYTES CARRY OUT VARIOUS functions originally thought to be neuronal in nature; these include maintenance of ionic balance, uptake and metabolism of certain transmitters such as excitatory amino acids, and the synthesis and secretion of a number of trophic agents. In addition, astrocytes contain functional neurotransmitter receptors and ion channels (*1*). We present evidence that neuropeptide genes not only are expressed in astrocytes but are expressed in gene- and brain region–specific ways. Furthermore, the proenkephalin gene is regulated by cyclic adenosine monophosphate (cAMP) in astrocytes just as it is in neurons. Thus astrocytes are capable of yet another set of neuronal functions.

Astrocytes were prepared from the cortex, cerebellum, and striatum of 3-day-old rat pups by a modification of the McCarthy-DeVellis technique (*2*). Cultures were 95 to 98% astrocytes (positive for glial fibrillary acidic protein by immunohistochemistry), with less than 1% contamination by microglia [antibody to Mac-I (*3*)] or oligodendrocytes [antibody to galactocerebroside (*4*)]. Analyses of mRNA and peptides were carried out 3 to 4 weeks after the cultures were prepared. RNA was isolated and analyzed by RNA blot or slot blot as previously described (*5*). Methionine enkephalin (met-enkephalin) and somatostatin were extracted and analyzed by specific radioimmunoassays (RIAs) (*6*).

RNA blot analysis of total or polyadenylated [poly(A)$^+$] RNA extracted from cortical, cerbellar, and striatal astrocytes demonstrated that somatostatin mRNA is present in cerebellar astrocytes but undetectable in the other two cultures (Fig. 1). Proenkephalin mRNA is present in approximately equal amounts in astrocytes from all three regions, in agreement with previous results (*7*). Both of these mRNAs are the size expected for the authentic brain mRNA; that is, 670 bases for somatostatin (*8*) and 1.4 kb for proenkephalin (*9*). In contrast, cholecystokinin mRNA could not be detected in either cortical or cerebellar astrocyte cultures, although it is readily detected in cortex of rat brain. Table 1 expresses the results of several RNA blots quantitatively.

These data demonstrated that astrocytes prepared from neonatal rat brain could express neuropeptide genes with a specificity for both the gene expressed and the brain region from which the astrocytes were derived. We then asked whether only the mRNA was synthesized or whether astrocytes could in fact translate the mRNA into precursor and process the precursor to the free bioactive peptides produced by neurons. Because proenkephalin gene transcription is stimulated by cAMP, via receptors

Clinical Neuroscience Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

*To whom correspondence should be addressed.