Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 7297–7301, August 1995
Biochemistry

A versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*: Polyethylenimine

OTMANE BOUSSIF*, FRANK LEZOUALC'H†, MARIA ANTONIETTA ZANTA*, MOJGAN DJAVAHERI MERGNY‡, DANIEL SCHERMAN‡, BARBARA DEMENEIX†, AND JEAN-PAUL BEHR*

*Laboratoire de Chimie Génétique, Unité de Recherche Associée 1386 du Centre National de la Recherche Scientifique, Faculté de Pharmacie, F-67401 Illkirch, France; †Unité de Recherche Associée 90 du Centre National de la Recherche Scientifique, Museum National d'Histoire Naturelle, 75231 Paris, France; and ‡Unité Mixte de Recherche 133, Centre National de la Recherche Scientifique/Rhône-Poulenc Rorer, B.P. 14, 94403 Vitry sur Seine, France

Communicated by Jean-Marie P. Lehn, Université Louis Pasteur, Strasbourg, France, April 12, 1995 (received for review February 25, 1995)

ABSTRACT **Several polycations possessing substantial buffering capacity below physiological pH, such as lipopolyamines and polyamidoamine polymers, are efficient transfection agents *per se*—i.e., without the addition of cell targeting or membrane-disruption agents. This observation led us to test the cationic polymer polyethylenimine (PEI) for its gene-delivery potential. Indeed, every third atom of PEI is a protonable amino nitrogen atom, which makes the polymeric network an effective "proton sponge" at virtually any pH. Luciferase reporter gene transfer with this polycation into a variety of cell lines and primary cells gave results comparable to, or even better than, lipopolyamines. Cytotoxicity was low and seen only at concentrations well above those required for optimal transfection. Delivery of oligonucleotides into embryonic neurons was followed by using a fluorescent probe. Virtually all neurons showed nuclear labeling, with no toxic effects. The optimal PEI cation/anion balance for *in vitro* transfection is only slightly on the cationic side, which is advantageous for *in vivo* delivery. Indeed, intracerebral luciferase gene transfer into newborn mice gave results comparable (for a given amount of DNA) to the *in vitro* transfection of primary rat brain endothelial cells or chicken embryonic neurons. Together, these properties make PEI a promising vector for gene therapy and an outstanding core for the design of more sophisticated devices. Our hypothesis is that its efficiency relies on extensive lysosome buffering that protects DNA from nuclease degradation, and consequent lysosomal swelling and rupture that provide an escape mechanism for the PEI/DNA particles.**

Nonviral gene-delivery techniques remain several orders of magnitude behind viral vectors when compared on the basis of the mean number of gene copies needed to transfect a cell. Despite this limitation, plasmid-mediated transfection has the major advantage that it raises none of the concerns of biological vectors for human therapy. Thus, much effort is presently devoted to improving nonviral techniques (1). Indeed, the advent of gene therapy has provided the impetus for improving, by appropriate chemical design, the efficiency of classical transfection agents such as cationic polymers (DEAE-dextran, Polybrene, polylysine) or inorganic aggregates (e.g., calcium phosphate). Various other polycationic cores have been developed, whether macromolecules (for review, see refs. 2–4), amphiphilic aggregates (for review, see ref. 5), or mixtures of both (6, 7), all of which ionically condense plasmid DNA and bind to the cell surface. Additional viral-like molecular properties have been added to these particles (2–9) to promote receptor-mediated endocytosis, fusogenicity, and karyophily.

Among the cationic cores described so far, two are constitutively efficient gene-delivery agents without any extra virus-derived function or lysosomotropic additive. Polyamidoamine cascade polymers (4) and lipopolyamines (5, 10–12), although quite different in chemical structures, share another feature besides their efficiency: both contain residues still protonable at physiological pH. This property may permit endosome buffering and thus prevent DNA from lysosomal degradation. With this in mind, we looked for another compound that had similar DNA-condensing and pH-buffering properties so as to test its transfection potential.

Polyethylenimine (PEI) is the organic macromolecule with the highest cationic-charge-density potential. Every third atom is an amino nitrogen that can be protonated. Acid-catalyzed polymerization of aziridine produces a highly branched network (13) that can ensnare DNA, and, owing to the close neighborhood of the many linker amino groups, PEI retains a substantial buffering capacity at virtually any pH. Our results suggest that this simple molecular property is related to the efficiency of the complex multistage process of transfection. Indeed, we show PEI to be a highly efficient vector for delivering oligonucleotides and plasmids both *in vitro* and *in vivo*.

MATERIALS AND METHODS

Polycations, Plasmids, and Oligodeoxyribonucleotide. PEI 800 kDa (Fluka) and PEI 50 kDa (Sigma) were used as 10 mM monomer aqueous stock solutions [9 mg of the 50% (wt/vol) commercial solution diluted in 10 ml of water]. The solutions were neutralized with HCl and filtered (Millipore, 0.2 μm). Transfectam (ref. 10; Promega) was used as a 2 mM ethanolic solution. Plasmids, pGL2-Luc (Promega), pCMV-Luc (R. Whalen, Institut Pasteur, Paris), and pT3RE-Luc (C. K. Glass, Molecular Medicine, La Jolla, CA) were propagated as described (14) and purified by PEG precipitation (14) or chromatography (Qiagen, Chatsworth, CA). The 3'-rhodamine-labeled antisense oligonucleotide (GCTGGGCTTCTGTTC-CAT, Eurogentec, Belgium) against the translation start region of the chicken α thyroid hormone receptor was purified on SDS/PAGE before use.

Cell Culture. 3T3 and HepG2 cell lines were cultured as described (9). COS-7 (M. Goeldner, Faculté de Pharmacie, Strasbourg, France), HeLa (A. Hall, Medical Research Council, London), and MRC-5 (Transgene, Strasbourg, France) cells were cultured in 75-cm^2 flasks (Costar) in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% fetal calf serum (GIBCO), streptomycin at 100 μg/ml (GIBCO), penicillin at 100 international units/ml (GIBCO), and glutamine at 0.286 g/ml (Lancaster) at 37°C in a humidified 5% CO_2-containing atmosphere. K-562 cells (O. Feugeas, Institut d'Hematologie, Strasbourg, France) were similarly cultured using RPMI 1640 medium (GIBCO) instead of DMEM. Primary brain capillary endothelial cells were prepared as described (15) and cultured for 6 days before

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.

Abbreviation: PEI, polyethylenimine.

transfection. Embryonic hypothalamic neurons were obtained from chicken embryos at day 6 of incubation (16).

Transfection Procedure. Cells from cell lines were seeded at 50,000 cells per well in 24-well dishes (Falcon) 18 hr before transfection. Immediately before transfection, cells were rinsed and supplemented with fresh serum-free culture medium (1 ml). The plasmid DNA (2 μg) and the desired amount of polymer solution (1 μg of DNA is 3 nmol of phosphate, and 1 μl of PEI stock solution contains 10 nmol of amine nitrogen) were each diluted into 50 μl of 150 mM NaCl and vortexed. After $\approx$10 min, the two solutions were mixed, and the resulting solution was vortexed. After 10 more minutes, the transfection mixture was added to the cells. The optimized Transfectam procedure has been described (17). After a 3- to 4-hr incubation, the medium was supplemented with 10% fetal calf serum. Luciferase gene expression was monitored 24 hr later by using a commercial kit (Promega) and photon counting (Biolumat LB 9500, Berthold, Nashua, NH). Each transfection experiment was done in triplicate and is expressed as mean light units per mg of cell protein [bicinchoninic acid (BCA) test, Pierce] $\pm$ SD. Each experiment was repeated several times; absolute values varied sometimes within an order of magnitude, depending on plasmid batch and the history of the cells, whereas relative values stayed within a factor of 2.

Oligonucleotide Uptake by Chicken Embryonic Neurons. Glass coverslips in four-well culture dishes (Costar) were treated for 30 min with gelatin (0.25 mg·ml^{-1}) and overnight with poly(D-lysine) (70 kDa, 10 mg·ml^{-1} in 0.15 M sodium borate buffer, pH 8.0). After being rinsed twice with 0.1 M phosphate-buffered saline (PBS) and twice with distilled water, dishes were coated with DMEM/Ham F-12, 1:1, supplemented with 10% fetal calf serum for 2–3 hr in a humid atmosphere of 93% air/7% CO_2. Cells were plated at 4×10^5 cells per well. The rhodamine-conjugated oligonucleotide (25 μM) was incubated with PEI (9 equivalents; see above) in 150 mM NaCl for 10 min. The mixture was added to the cells at a final concentration of 1.25 μM oligonucleotide and incubated for 2 hr. Cells were rinsed and fixed in 4% (vol/vol) formaldehyde/PBS for 30 min. After being rinsed twice with 0.1 M PBS and twice with distilled water, coverslips were mounted in Moviol (Hoechst) and visualized by fluorescence and Nomarski optics.

Intracerebral Gene Transfer in Newborn Mice. Neonatal OF1 mice of either sex were obtained from females bred in our colony or from commercial sources (Iffa Credo). These newborn mice were used for injections on postnatal days 2 and 3. Before intracranial injections, the mice were anesthetized by hypothermia on ice. The mouse head was held by hand, and a small incision was made with the tip of iris scissors through the skin overlying the sagittal suture to expose the skull. A small hole was made through the skull $\approx$1.5 mm lateral to the sagittal suture. A glass micropipette attached to a micromanipulator (Narishige, Tokyo) was lowered $\approx$1.5–2.5 mm through the incision and, with a microsyringe (Narishige), 2 μl of a 5% (wt/vol) glucose solution containing 2 μg of plasmid complexed or not complexed with PEI was injected bilaterally into the vicinity of the striatum. The solution was injected over 1–2 min, and the pipette was left in place for another 1–2 min before removal to limit back-diffusion from the site of release. After injection mice were kept warm until active and there were no signs of respiratory distress; then they were returned to the mother. After 24 hr, the injected mice were anesthetized and decapitated; the cerebral hemispheres were removed; the cerebellum and olfactory bulbs were discarded. The cerebral hemispheres were used for luciferase-activity analysis (luciferase assay kit; Promega). Briefly, separate hemispheres were homogenized in 200 μl of ice-cold lysis buffer, which was then centrifuged. Next, 20 μl of the supernatant was mixed with 100 μl of luciferase reagent, and the light emitted over 10 sec was measured in a single-well luminometer (ILA911, MGM Instruments, Hamden, CT) calibrated with a tritium standard. Results are expressed per group (six to nine animals). Background levels for empty tubes were substracted.

RESULTS

Variation of Transfection Efficiency and Cytotoxicity as a Function of Mean Ionic Charge of the Complexes. Polycation-mediated gene transfer is thought to involve DNA aggregation and binding of the resulting particles to anionic residues on the plasma membrane (5). To be efficient the complexes must bear a net positive charge. The fraction of nitrogen atoms that are protonated at neutral pH was unknown, so a wide range of PEI/DNA ratios (calculated on the basis of PEI nitrogen per DNA phosphate and expressed as PEI/DNA equivalents) had to be screened. Transfection results with the 3T3 murine fibroblast cell line are shown in Fig. 1. The luciferase gene driven by a relatively weak simian virus 40 promoter was used as the reporter system (pGL2Luc) to avoid saturating the cell expression machinery while retaining the $\approx$8 orders-of-magnitude dynamic range of this photon-counting enzyme detection. Luciferase expression after 24 hr was highest in cells transfected with complexes containing $\approx$10 PEI nitrogens per DNA phosphate. Lower ratios were less efficient due to reduced cell-surface binding, whereas very high ratios (>90) caused cytopathic effects, revealed by decreased cell number and morphological changes (also see below). The efficacy/toxicity balance of cationic polymers, such as polylysine, is known to increase with their molecular weights. Because both 800- and 50-kDa PEIs are available, we compared their transfection efficiencies on various cell types and found them to be within a factor of 4 (data not shown). Subsequent experiments were done with the higher-molecular-mass polymer.

In the next set of experiments, the PEI/DNA ratio was examined in detail. Fig. 2 shows that when working with between 6 and 20 nitrogens per phosphate, transfection levels are within 1 order of magnitude and display a broad maximum centered around 9. A roughly similar conclusion was reached with HeLa cells (data not shown), and this ratio was kept for subsequent experiments. Cytotoxicity was quantitated by the thiazolyl blue-tetrazolium reduction assay (Fig. 2 *Inset*). In the optimal transfection conditions, no reduction in cell activity was seen. Even increasing the PEI/DNA ratio to 18 did not significantly affect cellular metabolism.

Influence of Condensation Conditions on Transfection. Optical and electron microscopy show that 0.1- to 1-μm multimolecular PEI/DNA particles form in physiological con-

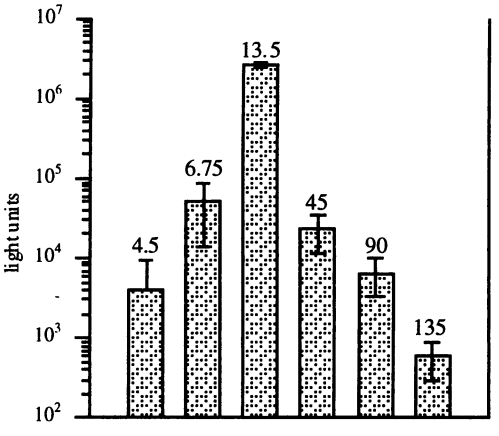

FIG. 1. Luciferase reporter gene expression is a function of the PEI nitrogen/DNA phosphate ratio. Murine 3T3 fibroblasts were transfected with pGL2-Luc (2 μg per well) and 4.5–135 equivalents of PEI 800-kDa nitrogen per DNA phosphate (see text for methods). Data are presented as means $\pm$ SEMs. Light units were normalized to 1 mg of cell protein.

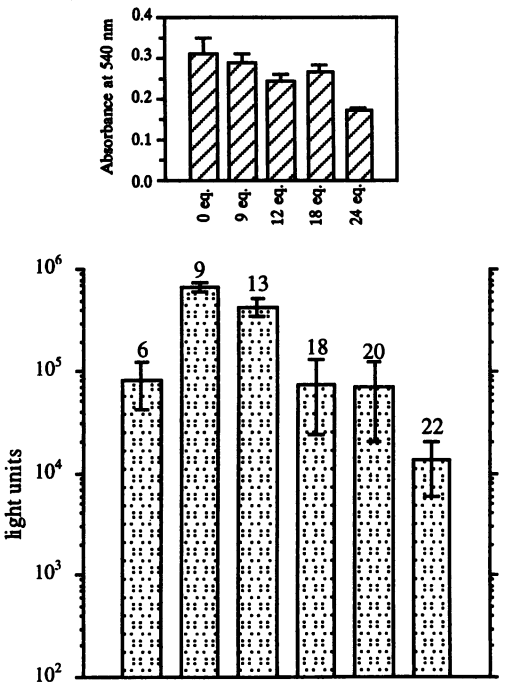

FIG. 2. Transfection efficiency is maximal at ≈9–13.5 equivalents of PEI nitrogen per DNA phosphate and does not affect cell metabolism. 3T3 cells were transfected with various ratios of PEI/plasmid pGL2-Luc (see text for methods). Data are presented as means ± SEMs. Light units were normalized to 1 mg of cell protein. Cytotoxicity was followed by using a thiazolyl blue-tetrazolium assay (18). Cells were incubated with thiazolyl blue (0.05 mg/ml; 90 min) and washed three times with PBS. After 10-min incubation in 1 ml of 10% SDS, the OD at 540 nm was read.

ditions. DNA condensation by polycations is a function of the nature and concentration of all ions present. We therefore varied pH, volume, and NaCl concentration of the compaction medium to narrow the size distribution. The total cationic charge of PEI increases with acidity, so transfection efficiencies were tested by using particles preformed at pH 7, 6, or 5 before coming in contact with the cells at pH 7.3 (Fig. 3). A nonsignificant trend to increased luciferase expression was seen in acidic conditions, and in a separate experiment decreasing pH to 2 did not further improve transfection. Modifying compaction solution volume (100 μl to 10 μl or 1 ml) had no effect. However, the order of adding reagents did influence

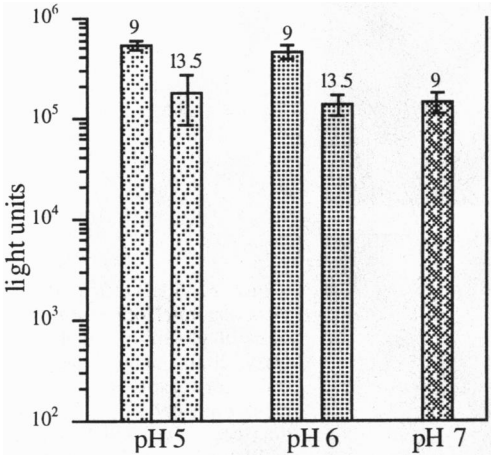

FIG. 3. Effect of compaction medium pH on PEI-dependent transfection efficiency. 3T3 cells were transfected with pGL2-Luc complexed with PEI at different pHs. Data are presented as means ± SEMs. Light units were normalized to 1 mg of cell protein.

properties of the resulting particles: adding the cationic polymer solution (0.5 ml) dropwise to the plasmid solution (0.5 ml) was 10-fold more efficient than adding plasmid to polymer. Polymer counterion condensation is a cooperative process that can be modulated and even inhibited in high salt. Plasmid/polylysine condensation was shown recently to lead to much smaller particles (hence more efficient vectors) in ≈1 M NaCl (19). We therefore condensed DNA with PEI in 0–5 M NaCl before dilution in culture medium (the volume of the condensing medium was adjusted so that the ionic strength of the cell culture medium varied <10%). A slight (3-fold) decrease in luciferase expression at high NaCl concentration resulted. Unlike polylysine, PEI is branched and has a much higher degree of polymerization, which may shift the NaCl concentration at which morphological change occurs to unattainable values.

PEI-Mediated Transfection Is Particularly Efficient. The dose dependency of transfection at optimal PEI/DNA ratio is shown in Fig. 4. The strong cytomegalovirus (CMV) promoter/enhancer led to much higher luciferase expression levels than those obtained with the simian virus 40 promoter. Levels were roughly proportional to the amount of DNA used; however, a pronounced nonlinear decrease of luciferase expression was observed at the lower DNA edge. This result has frequently been observed, whether transfecting with cationic lipids (17), cationic polymers (4), or by electroporation (20). Lipopolyamines are among the most efficient cationic lipid vehicles for carrying genes into eukaryotic cells *in vitro* (5, 11). So we compared PEI to the commercially available lipopolyamine Transfectam using optimized conditions (i.e., at PEI nitrogen/DNA base of 9 and Transfectam/DNA ionic charge ratio of 6). As seen from Fig. 4, the cationic polymer compares very favorably with the lipopolyamine and is 10^4-fold times more efficient than polylysine.

PEI/DNA Complexes Successfully Transfer Genes to Many Eukaryotic Cell Types. Because reporter gene expression depends, to a large extent, on promoter and cell type, several cell lines and primary cells were transfected with PEI and with Transfectam (Fig. 5). The results confirm our initial finding about the efficacy of PEI. The cationic polymer and lipid have quite similar properties; no systematic trend occurred in favor of either. However, Transfectam-mediated gene delivery is not depressed by serum-containing medium (17), whereas the situation seems more variable for PEI: efficiency changes of

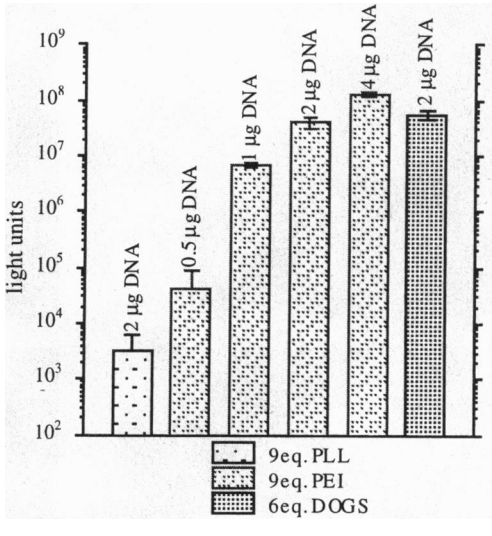

FIG. 4. Dose–response of luciferase activity versus the amount of pCMV-Luc plasmid used for transfection, including a comparison of PEI to polylysine and Transfectam. 3T3 cells were transfected with increased amounts of pCMV-Luc (0.5–4 μg) complexed to PEI 800 K. Data are presented as means ± SEMs. Light units were normalized to 1 mg of cell protein. PLL, polylysine; DOGS, Transfectam.

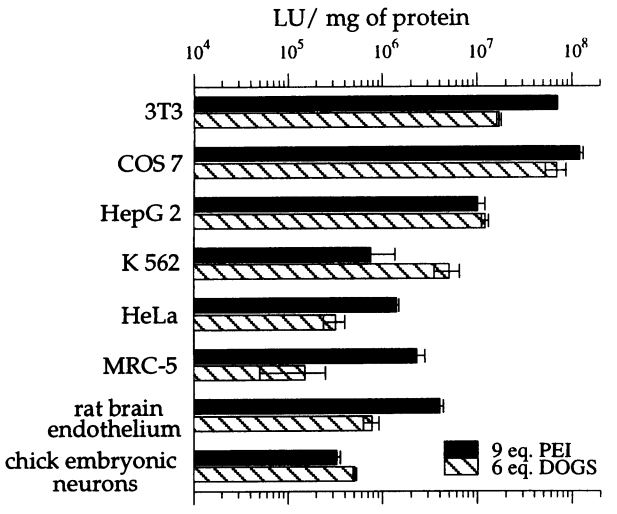

FIG. 5. Comparison of PEI- and Transfectam-mediated transfection of mammalian and avian cells. Origins of cell lines used are as follows: 3T3, murine fibroblasts; COS-7, monkey kidney; HepG2, human hepatoma; K-562, human leukemia; HeLa, human epithelial carcinoma; MRC-5, human lung epithelium. The two primary cultures are as indicated. All cells were transfected with 2 μg of plasmid complexed to PEI. Data are presented as means ± SEMs. Light units (LU) were normalized to 1 mg of cell protein. DOGS, Transfectam.

0.05–2 have been seen in 10% serum, depending on the complex-formation conditions and cell line; this point needs further investigation. Successful transfection of fragile primary embryonic neurons, as well as the response of transgene expression to physiological concentrations of thyroid hormone (a 2-fold increase in 1 nM hormone, data not shown), is evidence that the synthetic vectors do not severely perturb cellular metabolism.

PEI Carries Oligonucleotides to Cell Nucleus. Short single-stranded pieces of DNA do not generally require a carrier to enter a cell through fluid-phase endocytosis (21). However lysosomal degradation remains a problem. Polycations have been shown to help oligonucleotides reach their cytoplasmic or nuclear target (21–25). We therefore tried PEI as an oligonucleotide carrier. In the control experiment, chicken hypothalamic neurons were incubated with the rhodamine-labeled 18-mer oligonucleotide (10 μM) for 2 hr, then rinsed, and fixed. Fluorescence microscopy did not reveal any remaining cell-associated oligonucleotide or nucleoside (data not shown), which may be a consequence of extensive nuclease-mediated degradation followed by fluorescent nucleoside leakage (21, 26). In sharp contrast, PEI-mediated transport of as little as 1.25 μM oligonucleotide resulted in the appearance of almost 100% bright fluorescent nuclei (Fig. 6). As the neuronal culture was essentially postmitotic, this experiment shows either that some of the particles cross the nuclear membrane or that they disassemble in the cytoplasm where free oligonucleotides display nuclear tropism (21).

***In Vivo* Gene Transfer.** The weakness of nonviral gene carriers is particularly restrictive *in vivo*, although some limited success has been obtained with cationic amphiphiles (5) or targeted polylysine complexes (3). PEI transfection is orders of magnitude higher than that of polylysine *in vitro*, so we considered PEI a good candidate for nontargeted gene delivery *in vivo*. To this end, the luciferase-encoding plasmid was complexed with various amounts of PEI and injected into the brains of newborn mice. Using the optimal *in vitro* PEI/DNA ratio, high luciferase activity was detected in the brain extracts 24 hr after injection (Fig. 7). It should be emphasized that the luciferase levels obtained *in vivo*, $\approx 10^6$ light units per μg of DNA (the limiting factor being amount of DNA rather than number of cells), were as high as those obtained by transfection of primary brain cells in the less stringent *in vitro* conditions (Fig. 5).

DISCUSSION

The ultimate goal of designing synthetic gene-delivery vehicles is to build multimolecular DNA/vector assemblies that are inert and efficient enough to be used in human therapy. Polyethylenimine should fulfill both requirements. (*i*) It is presumably not toxic. PEI has been used widely in the environment for >30 yr. Applications as varied as water purification, mineral extraction, shampoos, etc. have established it as innocuous. No sign of acute or chronic toxicity has been reported, even when introduced into animals [$LD_{50} > 4$ g/kg when given by oral or s.c. routes in rats and rabbits (27)]. Our cytotoxicity data with PEI/DNA complexes in cell cultures are

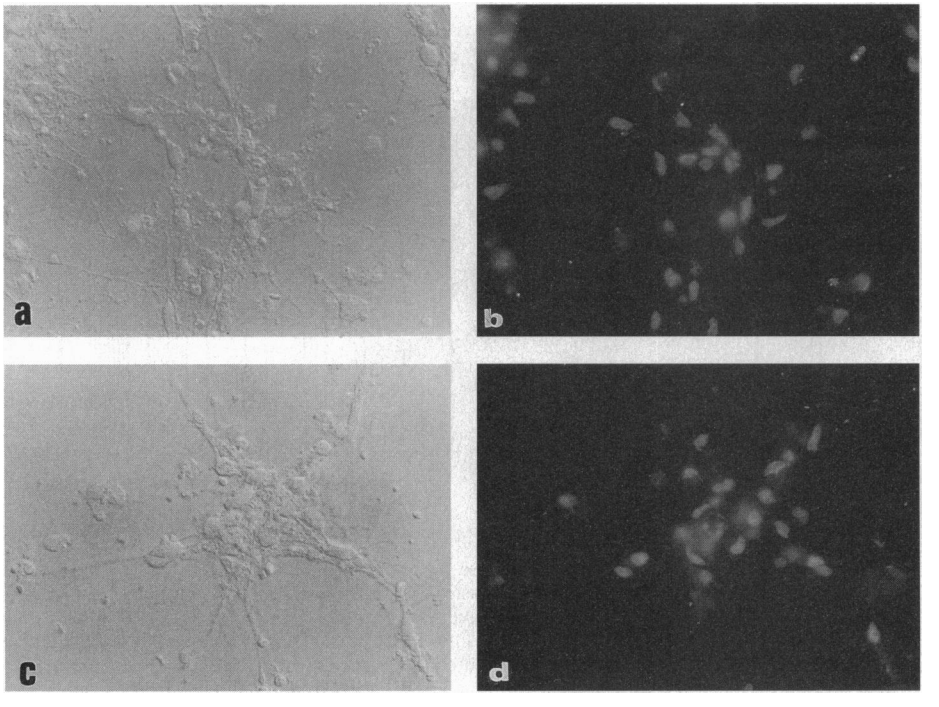

FIG. 6. PEI efficiently transfects oligonucleotides into the nucleus of postmitotic neurons. Primary cultures of hypothalamic neurons were exposed to 1.25 μM rhodamine-labeled oligonucleotides complexed with 9 equivalents of PEI, fixed, and visualized with Nomarski optics (*a, c*) or appropriate filters for fluorescence (*b, d*). Two typical fields are shown. (×75.)

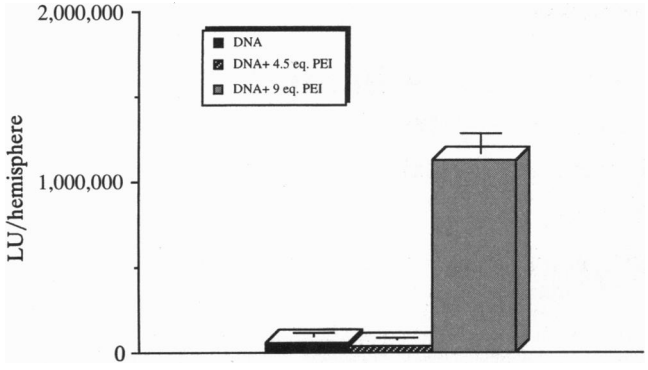

FIG. 7. *In vivo* transfection into the brains of newborn mice using PEI-complexed DNA. Injections of 2 μg of CMV-Luc with different ratios of PEI were made into the striata of anesthetized newborn mice. Mice were sacrificed 24 hr later, and luciferase activity in the homogenized brains were measured. Data are presented as means ± SEMs. LU, light unit.

somewhat less clear-cut: no perturbation of cell metabolism could be detected with up to twice the optimal concentration required for gene delivery, but higher amounts of complexes did result in toxicity. Thus, there is an apparent discrepancy between the innocuous nature of PEI as a molecule in the whole animal and the toxic cellular response to the entry of large numbers of PEI/DNA complexes through endocytosis. This toxicity might be a consequence of the endo/lysosomal enzyme release into the cytoplasm, consecutive to feeding a cell with many membrane-disrupting particles. This result is a drawback to high gene-transfer levels, which must be intrinsic to any carrier, whether synthetic or viral. (*ii*) PEI is highly efficient, clearly being one of the most efficient synthetic polynucleotide delivery systems. Indeed, a comparison using the luciferase reporter gene showed PEI to give transfection levels of the same order as lipopolyamines for various cell lines and primary cells *in vitro*. *In vivo* transfection is a more stringent test. Yet direct injection of PEI/luciferase DNA into the newborn mouse brain shows that this efficiency is maintained *in vivo*. The $\approx 10^6$ relative light units/μg of DNA luciferase expression levels found are 10^4-fold over background and comparable to *in vitro* transfection of primary cells of similar origin.

The choice of PEI among chemicals from a catalogue (as opposed to the design and synthesis of a new molecule) was based on our hypothesis that a causal relationship existed between the protonation reservoir of a molecule below neutrality and its transfection efficiency. This relationship may be the base for the design of new synthetic vectors, although the cellular mechanism underlying their actions has yet to be analyzed. Endosome buffering may protect DNA from lysosomal nucleases, but it may also perturb either the trafficking of endosomes or their osmolarity. For instance, massive vesicular ATPase-driven proton accumulation (28) followed by passive chloride influx into endosomes buffered with PEI should cause osmotic swelling and subsequent endosome disruption. The same mechanism holds for oligonucleotide delivery. Yet here the particles are smaller because the polyanion is no longer a macromolecule; this would explain the extensive nuclear accumulation we observed, a tropism important for both antisense and antigene therapeutical purposes (29). The oligonucleotide probably remains undegraded, as otherwise nucleoside leakage would have abolished the intracellular signal (21). Indeed, results obtained with lipidic proton sponges show complexed oligonucleotides to be protected (25).

The ionization properties of PEI have only recently been reported (30). According to the protonation vs. pH profile, every sixth nitrogen atom is protonated under physiological conditions. This protonation gives a mean cationic charge excess of ≈ 1.5 for the optimal PEI/DNA ratio (i.e., nine nitrogen atoms per phosphate). Low cationic charge density should be favorable for *in vivo* experiments (9), and this may explain why our *in vivo* experiments gave results close to those obtained under *in vitro* conditions. Moreover, given the pH difference between the extracellular space and lysosomes, when PEI moves from one to the other, the estimated fraction of protonated nitrogens will increase from 15% to 45%. This change means that in the lysosome every third nitrogen is participating in the buffering process. Furthermore, because the monomeric unit has such low molecular mass (43 Da), the whole molecule will behave as an extensive "proton sponge." PEI is a highly branched network polymer (25% cornerstone nitrogen atoms) that catches the unidimentional DNA polymer more efficiently than would a linear polycation, such as polylysine. Indeed our preliminary results using a linear analog of PEI confirm this interpretation. Taken together, these properties make polyethylenimine an outstanding core for the design of more sophisticated carriers for gene therapy.

We thank Corinne Benoist for help with the *in vivo* transfection assays. This work was supported by the Centre National de la Recherche Scientifique, the Programme Bioavenir, the Association Française contre les Myopathies, the Association Française de Lutte contre la Mucoviscidose, and the Association pour la Recherche contre le Cancer.

1. Behr, J. P. (1993) *Acc. Chem. Res.* **26,** 274–278.
2. Cotten, M. & Wagner, E. (1993) *Curr. Opin. Biotechnol.* **4,** 705–710.
3. Frese, J., Wu, C. H. & Wu, G. Y. (1994) *Adv. Drug Delivery Rev.* **14,** 137–152.
4. Haensler, J. & Szoka, F. C. (1993) *Bioconjugate Chem.* **4,** 372–379.
5. Behr, J. P. (1994) *Bioconjugate Chem.* **5,** 382–389.
6. Legendre, J. Y. & Szoka, F. C. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 893–897.
7. Mack, K. D., Walzem, R. & Zeldis, J. B. (1994) *Am. J. Med. Sci.* **307,** 138–143.
8. Plank, C., Oberhauser, B., Mechtler, K., Koch, C. & Wagner, E. (1994) *J. Biol. Chem.* **269,** 12918–12924.
9. Remy, J. S., Kichler, A., Mordvinov, V., Schuber, F. & Behr, J. P. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 1744–1748.
10. Behr, J. P., Demeneix, B., Loeffler, J. P. & Perez-Mutul, J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6982–6986.
11. Hawley-Nelson, P., Ciccarone, V., Gebeyehu, G., Jessee, J. & Felgner, P. L. (1993) *Focus* **15,** 73–79.
12. Remy, J. S., Sirlin, C., Vierling, P. & Behr, J. P. (1994) *Bioconjugate Chem.* **5,** 647–654.
13. Klotz, I. M., Royer, G. P. & Sloniewsky, A. R. (1969) *Biochemistry* **8,** 4752–4756.
14. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1986) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
15. Hughes, C. C. W. & Lantos, P. L. (1986) *Neurosci. Lett.* **68,** 100–106.
16. Lezoualc'h, F., Hassan, A. H. S., Giraud, P., Loeffler, J. P., Lee, S. L. & Demeneix, B. A. (1992) *Mol. Endocrinol.* **6,** 1797–1804.
17. Barthel, F., Remy, J. S., Loeffler, J. P. & Behr, J. P. (1993) *DNA Cell Biol.* **12,** 553–560.
18. Mosmann, T. R. (1983) *J. Immunol. Methods* **65,** 55–63.
19. Perales, J. C., Ferkol, T., Beegen, H., Ratnoff, O. D. & Hanson, R. W. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4086–4090.
20. Showe, M. K., Williams, D. L. & Showe, L. C. (1992) *Nucleic Acids Res.* **20,** 3153–3157.
21. Clarenc, J. P., Degols, G., Leonetti, J. P., Milhaud, P. & Lebleu, B. (1993) *Anti-Cancer Drug Des.* **8,** 81–94.
22. Bennett, C. F., Chiang, M. Y., Chan, H., Shoemaker, J. E. & Mirabelli, C. K. (1992) *Mol. Pharmacol.* **41,** 1023–1033.
23. Capaccioli, S., Dipasquale, G., Mini, E., Mazzei, T. & Quattrone, A. (1993) *Biochem. Biophys. Res. Commun.* **197,** 818–825.
24. Nestle, F. O., Mitra, R. S., Bennett, C. F., Chan, H. & Nickoloff, B. J. (1994) *J. Invest. Dermatol.* **103,** 569–575.
25. Lezoualc'h, F., Seugnet, I., Monnier, A. L., Ghysdael, J., Behr, J. P. & Demeneix, B. A. (1995) *J. Biol. Chem.*, in press.
26. Fisher, T. L., Terhorst, T., Cao, X. D. & Wagner, R. W. (1993) *Nucleic Acids Res.* **21,** 3857–3865.
27. Bikales, M. & Overberger, M. (1985) *Encyclopedia of Polymer Science and Engineering* (Wiley, New York), Vol. 1.
28. Nelson, N. (1991) *Trends Pharmacol. Sci.* **12,** 71–75.
29. Hélène, C. & Toulmé, J. J. (1990) *Biochim. Biophys. Acta* **1049,** 99–125.
30. Suh, J., Paik, H. J. & Hwang, B. K. (1994) *Bioorg. Chem.* **22,** 318–327.