opment of B lymphoma in transplant patients after prolonged treatment with CsA may be causally related to the inhibitory action of CsA on the synthesis of γ-IFN and other immunoregulatory lymphokines produced by T cells.

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References and Notes

1. J. F. Borel, C. Feurer, C. Magnée, H. Stahelin, *Immunology* **32**, 1017 (1977).
2. R. Y. Calne, D. J. G. White, D-B. Evans, *Br. Med. J.* **282**, 934 (1981); R. Calne, B. White, S. Thirzu, *Lancet* **1978-II**, 1323 (1978); T. E. Starzl, G. B. G. Klintman, K. A. Porter, S. Iwatsuki, G. P. J. Schroter, *N. Engl. J. Med.* **305**, 266 (1981).
3. S. Marker, N. Ascher, J. Kaliss, *Surgery* **85**, 430 (1979); S. Thiru, R. Y. Calne, J. Nagington, *Transplant Proc.* **13**, 359 (1981).
4. D. Crawford, J. Thomas, G. Janassy, *Lancet* **1980-I**, 1355 (1980); D. Crawford, J. Edwards, P. Sweny, *Int. J. Cancer* **28**, 705 (1981).
5. D. Bunjes, C. Mordt, M. Röllinghoff, *Eur. J. Immunol.* **11**, 657 (1981); E. Larsson, *J. Immunol.* **124**, 2828 (1980); A. D. Hess, J. P. Tutschka, Z. Pu, G. W. Santos, *ibid.* **128**, 360 (1982).
6. R. Palacios and G. Möller, *Nature (London)* **290**, 792 (1981); B. Ryffel, U. Gotz, B. Heuberger, *J. Immunol.* **129**, 1978 (1982).
7. N. H. Yeh, Y. K. Yip, G. H. Reem, paper presented at the Third International Congress for Interferon Research (1982).
8. G. Reem, L. A. Cook, D. M. Henriksen, J. Vilček, *Infect. Immun.* **37**, 216 (1982).
9. L. B. Epstein, *Tex. Rep. Biol. Med.* **35**, 42 (1977).
10. G. H. Reem, L. A. Cook, J. Vilček, paper presented at the Third International Congress for Interferon Research (1982).
11. S. Britton and R. Palacios, *Immunol. Rev.* **65**, 6 (1982).
12. J. Abb, H. Abb, S. Deinhardt, *Antiviral Res.* **2**, 361 (1982).
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Redox Delivery System for Brain-Specific, Sustained Release of Dopamine

Abstract. *Dopamine was transformed into a redox chemical system for delivery to the brain. The lipoidal form allowed penetration of the blood-brain barrier. Oxidative and hydrolytic processes then transformed the delivery form into a quaternary ammonium precursor of dopamine. The quaternary ammonium precursor was rapidly eliminated from the general circulation, whereas that formed in the brain was locked in, thereby providing a significant and sustained brain-specific dopaminergic activity.*

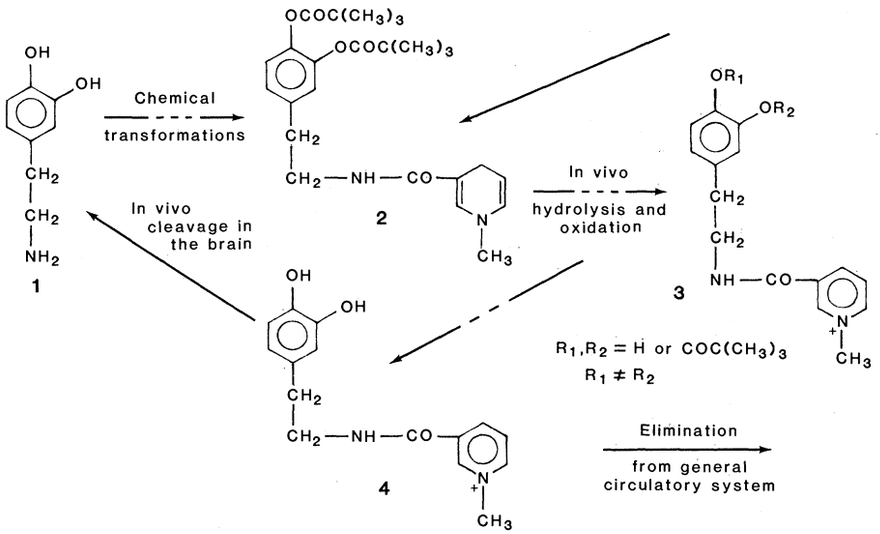
Dopamine (**1**) and related catecholamines do not cross the blood-brain barrier (*1*). Brain delivery of dopamine is critical in the treatment of parkinsonism (*2*), but L-dopa, the best known treatment for this disease, can have undesirable side effects (*3*). Dopamine agonists, used in the treatment of hyperprolactinemia associated with pituitary adenomas or amenorrhea (*4*), also have unwanted side effects. Effective delivery of dopamine itself to the brain would be the most desired way to treat both of these diseases, particularly if a sustained and controlled delivery of dopamine could be provided.

We demonstrated earlier that a redox system of drug delivery based on an interconvertible dihydropyridine ⇆ pyridinium salt carrier (*5, 6*) can deliver some simple amines, such as phenylethylamine, to the brain in a specific and sustained manner. The drug, when combined with the lipoidal, dihydropyridine carrier, crosses the blood-brain barrier. The carrier is then oxidatively transformed into a charged form, which has the effect of locking in the brain this new carrier-drug combination. The drug is then released in a sustained manner from the charged form. Dopamine, a more complicated molecule than phenylethylamine, requires a more complex delivery system. We now report successful site-specific and sustained delivery of dopamine to the brain, as demonstrated by the presence in the brain of the designed precursor and by the significant and prolonged inhibition of prolactin secretion in vivo.

The chemical delivery system for dopamine is structure **2**, where the amino function of dopamine is connected to the dihydropyridine carrier while the catechol function is protected as the corresponding dipivalyl ester. The brain-specific delivery of dopamine requires a succession of processes, including oxidation of the dihydropyridine ring to the corresponding pyridinium salt (for example, structure **3**), which provides the basis for the locking-in of the molecule in the brain, hydrolysis of the pivalyl esters (**4**) possibly via the 3- and 4-monopivalyl esters, and the release of dopamine (**1**) from **4**, which can be either by hydrolysis or by a reductive process. The possibility of a reductive release of dopamine was suggested by a model for a presynaptic terminal (*7*).

To evaluate this dopamine delivery system, we administered compound **2** intravenously to rats and evaluated the concentrations of the dopamine precursor **4** in the brain and plasma along with concentrations of dopamine in the brain.

After a single intravenous injection of the delivery system represented by structure **2**, compound **4** was effectively locked in the brain and quickly eliminated from the rest of the body (Fig. 1). No 3-*O*-methylated derivative of **4** could be detected in the brain, indicating that these amides are not good substrates for catechol *O*-methyl transferase (COMT). No dramatic increase was observed,



The brain-specific delivery of dopamine (**1**). Compound **2** is the chemical delivery system and **4** is the precursor locked in the brain and eliminated quickly from the rest of the body. Structure **3** depicts the intermediates formed during the stepwise hydrolysis and oxidation processes.

however, in the dopamine concentration in the brain. This was not unexpected since the facile metabolism and elimination of the dopamine formed should not allow its buildup. In fact, the sustained release and metabolism of the dopamine associated with significant pharmacological activity is the goal of this delivery system.

The biological system we used to evaluate dopamine activity in the brain after administration of **2** was prolactin release from the anterior pituitary. Dopamine and its agonists decrease prolactin secretion after they bind to the stereospecific receptors located on lactotrophs in the anterior pituitary (*8–10*). This suppressive effect of dopaminergic drugs shows a dose-dependent relationship following subcutaneous (*8, 9*), intraperitoneal (*10*), or intravenous (*11–13*) administration. That this effect of dopamine is exerted directly on the anterior pituitary is indicated by the observation of suppressed prolactin secretion after treatment with dopaminergic drugs in animals with massive lesions of the medial basal hypothalamus (*13*) or ectopically implanted pituitaries (*14*). In addition, dopamine and its agonists are potent inhibitors of prolactin secretion from the anterior pituitary in vitro (*15*).

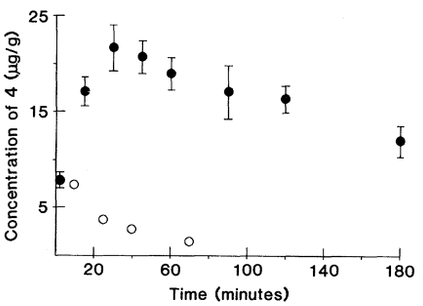


Fig. 1. Concentrations of **4** in brain (●) and in blood (○) after administration of **2**. Rats weighing 150 ± 10 g were given intrajugular injections of a freshly prepared solution of **2** (10 percent solution in dimethyl sulfoxide injected at 24 μl/min with a calibrated infusion pump) at a dose level of 50 mg/kg. After appropriate time periods, 1 ml of blood was withdrawn from the heart, the animal was perfused with 20 ml of saline and decapitated, and the brain was removed. The weighed brain was homogenized with 0.5 ml of water; 3 ml of acetonitrile was added; and the mixture was homogenized, centrifuged, filtered, and analyzed for **4** with high-performance liquid chromatography (HPLC). The blood samples were homogenized with 3 ml of acetonitrile and analyzed for **4** as above. A reverse-phase μBondapak C_{18} column was used for the HPLC; the mobile phase was acetonitrilephosphate buffer. Points represent an average of six animals. Quantitation was done by use of a recovery standard curve obtained by introducing a known amount of **4** in brain homogenate or blood and treated as above.

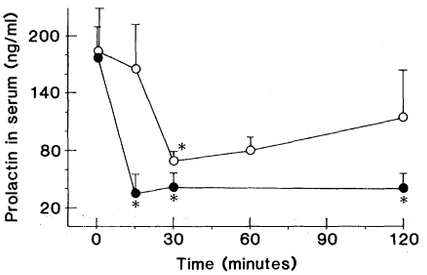


Fig. 2. Effects of compounds **2** (●) and **4** (○), administered intravenously at 1 mg/kg, on serum prolactin in rats. Adult male rats (Charles River; CD-1, 200–225 g) after being acclimatized for at least 1 week, received a single subcutaneous implant of a Silastic tube (interior diameter, 1.57 mm; overall size, 5 by 3.15 mm) packed with crystalline 17-β-estradiol. Two days later, the rats were given intravenous injections (jugular vein) of compound **4** (1 mg/kg) in saline or compound **2** (equivalent dose) in 10 percent ethanol in saline. Rats were decapitated in groups of six at various time intervals; trunk blood was collected and allowed to clot for 2 hours; and the serum was separated. Each serum sample was analyzed in duplicate by the double-antibody radioimmunoassay procedure described by the National Pituitary Agency Hormone Distribution Program. Serum prolactin concentrations are expressed in terms of the PRL-RP-2 reference preparation provided. The intra-assay coefficient of variation for ten replicate samples of pooled serum obtained from male rats was 13.8 percent. The effects of compounds **2** and **4** on serum prolactin concentrations were evaluated by one-way analysis of variance and the Student-Newman-Keuls test. The asterisk indicates a probability level of less than .05.

Intravenous administration of **2** to rats caused a 79 percent reduction in serum prolactin concentration, which was maintained for 120 minutes (Fig. 2). When the quaternary precursor **4** was given, no effect was observed at 15 minutes, but a 67 percent reduction was seen at 30 minutes. Prolactin then increased progressively to levels that were not significantly different from those in control animals receiving the vehicle only. An additional observation was that animals did not exhibit any stereotypic behavior characteristic of intense dopaminergic stimulation. Apomorphine at a dose of 1 mg/kg induces stereotypy (*8*). Thus, our inability to document large increases of dopamine in the brain after the administration of **4**, is consistent with the slow and persistent release of dopamine by this delivery system.

The rapid onset and prolonged inhibitory effect of **2** on prolactin secretion is consistent with the time course of appearance of **4** in the brain after administration of **2**. The trapping of **4** in the brain subsequent to intravenous injection of **2** appears to provide a constant source of a dopaminergic agent, either of **4** or of **1** formed from **4**. Since the median eminence of the brain does not contain a blood-brain barrier (*16*), **4** or **1** could be continuously delivered to the anterior pituitary via the hypophyseal portal system. Metabolism of **4** to dopamine in the hypothalamus may also take place. When **4** was administered peripherally, it showed prolactin-inhibiting activity, but for a much shorter time. The prolactin-

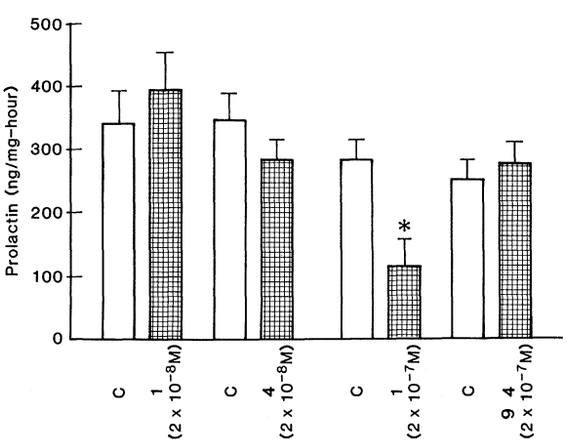


Fig. 3. Relative in vitro prolactin inhibitory activity of compound **4**, compared to dopamine (compound **1**) and the control (C). Adult female rats (Charles River) were decapitated, and their pituitary glands were quickly removed from the cranium. The anterior pituitary from each animal was divided into two equal parts and placed into incubation medium (essential medium, Gibco) at 37°C under continuous aeration. After 1 hour of incubation, the medium was replaced with fresh medium, containing either **1** or **4** ($2 \times 10^{-8}M$) or the control ($10^{-4}M$ ascorbic acid). In all cases, one-half of the anterior pituitary received the test drug; the other half, the ascorbate control. After 1 hour, samples were taken from the media and the rest was discarded. Fresh medium containing $2 \times 10^{-7}M$ compound **1** or **4**, was then added. One hour later, the samples were taken. After the 3-hour incubation period, the anterior pituitary halves were weighed. The samples were diluted 1:50 with buffered saline and assayed in triplicate (see Fig. 2). The data are given as nanograms of prolactin released per milligram of weight per hour. A paired Student's *t*-test was used to evaluate the significance of the inhibitory effect of the test drugs on prolactin secretion. Asterisks indicate $P < .05$. The control half and the drug-treated half of the anterior pituitary were used in each paired comparison.

suppressive effect of **2** was similar, from 15 to 120 minutes (the longest time tested) after administration, whereas that of **4** showed a time course similar to other dopaminergic agents such as apomorphine. Apomorphine given at a similar dose level (1 mg per kilogram of body weight, intravenously) to estrogen-primed female rats reduced serum prolactin by 50 percent at 30 minutes but was without effect at 60 minutes (*11*). Thus, the brief activities of apomorphine and of **4** seem to be due to their rapid clearance from the body. In the case of **4**, however, dopamine formed at the anterior pituitary could still be responsible for the transient reduction in prolactin secretion. To evaluate this possibility, we compared the activity of **4** in vitro with that of dopamine (**1**) (Fig. 3). If it is active at all, compound **4** is considerably less active than **1**. The lack of activity by **4** at 2×10^{-7} *M* also indicates that metabolism to **1** does not take place to any significant extent in the media used for testing in vitro.

Thus, we have succeeded in producing significant and sustained brain-specific dopaminergic activity by a mechanism of sustained local release of dopamine from the locked-in delivery form. This delivery system may be useful in the treatment of parkinsonism and hyperprolactinemia, and similar methods could be used for the selective delivery of other neurotransmitters to the brain.

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References and Notes

1. B. E. Roos and G. Steg, *Life Sci.* **3**, 351 (1964).
2. H. Ehringer and O. Hornykiewicz, *Klin. Wochenschr.* **38**, 1236 (1960).
3. A. Barbeau, *Trends Pharmacol. Sci.* **2**, 297 (1981).
4. R. F. Spark and G. Dickenstein, *Ann. Int. Med.* **90**, 949 (1979).
5. N. Bodor, H. H. Farag, M. E. Brewster, *Science* **214**, 1370 (1981).
6. N. Bodor and H. H. Farag, *J. Med. Chem.* **26**, 313 (1983).
7. L. L. Miller, A. N. K. Lau, E. K. Miller, *J. Am. Chem. Soc.* **104**, 5242 (1982).
8. G. P. Mueller, J. W. Simpkins, J. Meites, K. E. Moore, *Neuroendocrinology* **20**, 121 (1976).
9. W. E. Wuttke, E. Cassell, J. Meites, *Endocrinology* **88**, 737 (1971).
10. J. A. Clemens, E. B. Smalstig, C. J. Shaar, *Acta Endocrinol.* **79**, 230 (1975).
11. D. M. Lawson and R. R. Gala, *Endocrinology* **96**, 313 (1975).
12. D. Durand, J. B. Martin, P. Brazeau, *ibid.* **100**, 722 (1977).
13. C. Y. Cheung and R. I. Weiner, *ibid.* **99**, 914 (1976).
14. G. H. Zeilmaker and R. A. Carlsen, *Acta Endocrinol.* **41**, 321 (1962).
15. R. M. MacLeod, in *Frontiers in Neuroendocrinology*, L. Martini and W. F. Ganong, Eds. (Raven, New York, 1976), vol. 4, pp. 169–194.
16. J. Dobbins, *Physiol. Rev.* **41**, 130 (1964).
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Transmission of Integrated Sea Urchin Histone Genes by Nuclear Transplantation in *Xenopus laevis*

Abstract. *Sea urchin histone genes contained in a recombinant plasmid pSp102 were microinjected into the cytoplasm of fertilized eggs of* Xenopus laevis. *By the late blastula stage, plasmid DNA sequences were detected comigrating with the high molecular weight cellular DNA (greater than 48 kilobases). Analysis of the DNA from injected embryos digested with various restriction endonuclease demonstrated that the injected DNA was integrated into the frog genome. Clones of embryos containing the pSp102 DNA sequences were produced by means of nuclear transplantation. Individuals of the same clone contain the pSp102 sequences integrated into similar chromosomal locations. These sites vary between different clones.*

Recently the developmental and tissue specific expression of individual cloned genes has been examined by microinjection into the fertilized eggs of amphibians, mammals, and insects (*1–11*) [for review, see (*12–14*)]. During early developmental stages in the frog the injected circular DNA sequences migrate on agarose gels both as the circular plasmid DNA and as high molecular weight cellular DNA, and appear to replicate (*1–3*). In most cases, after the gastrula stage of development, the circular plasmid sequences are no longer detected (*1–3*). In the mouse and fruit fly the injected genes are integrated and the integrated DNA sequences are transmitted in the germ line to the next generation (*4, 7, 8, 10, 11*). Injected DNA sequences are expressed during early development (*1–3*) and in adult tissues (*4–10*) but not in a predictable manner.

The aberrant expression of injected DNA's may be due to expression of nonintegrated sequences during early development or the integration of exogenous genes into various locations in the host chromosome. Therefore, even though the injected genes contain the DNA sequences necessary for transcription (that is, CCAAT and TATA; C, cytosine; A, adenine; T, thymine) located at their 5′ end, their developmental or tissue specific expression may be affected by the absence of other sequences that normally surround the gene in the chromosome.

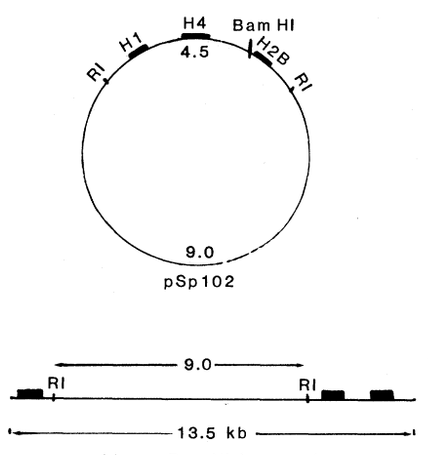

Fig. 1. Diagram of Bam HI and Eco RI restriction sites in plasmid pSp102. Treatment of plasmid DNA with Bam HI produces a 13.5-kb linear DNA fragment. There are no Hind III sites in the plasmid.

We now report that sea urchin histone genes are integrated into the frog genome after microinjection into the cytoplasm of fertilized eggs of *Xenopus laevis*, and that they can be transmitted to clones of frogs by nuclear transplantation. This procedure permits the analysis of developmental and tissue-specific expression of injected genes in clones of frogs in which the gene is integrated into a specific chromosomal location, thus enabling us to assess the effect of chromosomal location on the expression of injected genes.

Copies (10^4 to 10^6) of pSp102 (*15*) containing the sea urchin histone genes H1, H4, and H2B in Col El were injected into the cytoplasm of each egg within 1 hour after the eggs were artificially inseminated. High molecular weight DNA was extracted from embryos and larvae with phenol, chloroform, and isoamyl alcohol. The DNA was separated by electrophoresis on 0.7 percent agarose gels and transferred to nitrocellulose filters (*16*). The blots were hybridized with a nick-translated probe prepared from pSp102 (*17, 18*). The pSp102 DNA sequences were detectable in 90 percent of the injected blastulas (*3*). During early cleavage stages most of the DNA comigrated with circular plasmid DNA molecules (that is, either form I, supercoiled, or form I^r, relaxed, and form II). By the late blastula stage a portion of the exogenous DNA comigrated with the high molecular weight cellular DNA in approximately 20 to 30 percent of the injected embryos. After the blastula stage, there was a gradual loss of the circular molecules until few, if any, circular DNA molecules were detectable by the late tail bud stage [stages 29 and 30 (*19*)] (*1–3*).

Integration of exogenous DNA sequences was shown by digestion of DNA