Controlled Release of Dopamine from a Polymeric Brain Implant: In Vivo Characterization

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Intracerebral microdialysis was used to evaluate the long-term in vivo release of dopamine from ethylene-vinyl acetate (EVAc)-dopamine copolymer matrix discs for up to 65 days following striatal implantation. Dopamine release occurred through a single cavity present on one side of the disc, which was otherwise fully coated with an additional, impermeable layer of EVAc. At 20 days following implantation of the device, extracellular concentrations of dopamine within the striatum reached micromolar levels, over 200-fold greater than control values. Release of dopamine was shown to be stable and maintained for the 2-month duration of the experiment. Histological examination confirmed the biocompatible nature of the implant. There are potential applications of this technology to the treatment of Parkinson's disease and other neurological and psychiatric disorders.

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A number of neurological and psychiatric conditions can be treated pharmacologically. Perhaps the best example of such a condition is Parkinson's disease, in which there is a striking depletion of striatal dopamine levels [1, 2]. Current therapies hinge on the use of the dopamine biosynthetic precursor L-dopa (dihydroxyphenylalanine) to restore dopaminergic neurotransmission of this system [3–6]. Initial response to orally administered L-dopa is dramatic; however, response fluctuations and eventual refractoriness to L-dopa limit its therapeutic efficacy [7–12]. Studies using continuous intravenous infusion have demonstrated a reduction in these fluctuations, as have manipulations of oral dose and diet, suggesting that steady delivery of L-dopa or dopamine to the brain is desirable for optimal and perhaps long-term clinical response [13–21].

The need for improved therapy has resulted in suggestions of alternative treatments. Of these, tissue transplants (of either adrenal or fetal neural origin) have been studied in both animals and humans [22–27]. Although preliminary data indicate some beneficial response, this approach is beset by a number of problems. The mechanism responsible for the observed therapeutic effects remains unclear, as does the long-term efficacy of these transplants. Furthermore, the ethical issues surrounding fetal tissue transplants are a concern [27–32].

We have developed an alternative potential therapeutic modality based on the localized delivery of dopamine to the corpus striatum from a polymeric brain implant. Characterization of this release was performed using the newly developed technique of intracerebral microdialysis [33–35]. Postmortem tissue analysis confirmed the biocompatible nature of these implants and demonstrated maintenance of normal tissue architecture.

Materials and Methods

Ethylene-vinyl acetate (EVAc)-dopamine copolymer matrix devices were prepared by solvent casting as previously described [36, 37]. Based on previous in vitro data [38], an optimal matrix design for prolonged, linear release of dopamine was obtained by 30% loading in the presence of a full coating and a single cavity.

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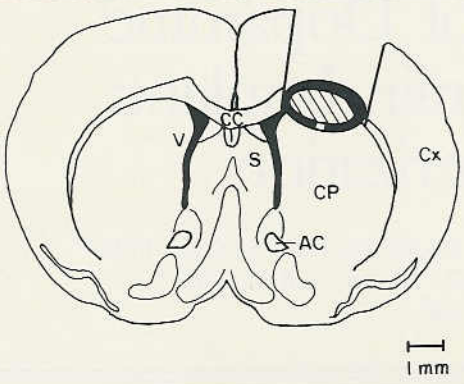

Fig 1. Coronal schematic diagram of placement of the polymer implant. Cortex (Cx) was aspirated as described in the text, and the implant was placed overlying the caudate nucleus (CP). V = ventricle, CC = corpus callosum, AC = anterior commissure, S = septum.

Male Sprague-Dawley rats (180 to 220 gm) were anesthetized with pentobarbitol (60 mg/kg IP) and placed in a Kopf stereotaxic frame. The skull was exposed and a circular hole drilled over the right striatum (anteroposterior [AP]: + 0.4 mm relative to Bregma; lateral [L]: 2.6 mm) [39]. Neocortex overlying this part of the striatum was carefully aspirated using mild suction down to the dorsal aspect of the corpus striatum (approximately 3 mm ventral to dura). Either a control (EVAc only, n = 4) or a dopamine-containing (n = 12) polymer device was implanted into each rat with the pore facing the striatum (Fig 1).

In Vivo Dialysis

In vivo release of dopamine and its major acidic metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), was measured by in vivo dialysis [33–35] on the third, tenth, twentieth, forty-fifth, and sixty-fifth days following implantation of the polymeric devices. Rats were anesthetized with pentobarbital (60 mg/kg) and placed in a Kopf stereotaxic frame. The skull was exposed and a Carnegie Medicin (Solna, Sweden) dialysis probe (membrane length, 4 mm; outer diameter, 0.5 mm; cut off, 5,000 Dalton molecular weight) was implanted into the striatum (AP: +2.1 mm relative to Bregma; L: 1.9 mm; dorsoventral: −7.0 mm) [39]. Probes were perfused with an artificial cerebrospinal fluid (Na+, 147 mM; K+, 3.5 mM; Ca++, 1.0 mM; Mg++, 1.2 mM; Cl−, 129 mM; phosphate, 1 mM; HCO_3-, 25 mM; pH, 7.4) at a flow rate of 1.5 μL/min using a Harvard infusion pump. Fifteen-minute samples (22.5 μL) were collected into 5 μL of 0.5 M perchloric acid. The probe was calibrated by measuring the recovery of standards of known concentration. Dialysates were immediately assayed by direct injection onto a highly sensitive reverse-phase, isocratic high-performance liquid chromatography (HPLC) system (3 μm, C18 column) with an ESA 5100 coulometric detector (ESA, Bedford, MA) [40]. Chromatograms were completed within 12 minutes. After an initial period (approximately 90 minutes following implantation of the probe) of "injury release," during which dopamine concentrations are elevated secondary to neuronal damage [41], stable levels of dopamine were measured for a minimum of four 15-minute periods.

In 4 rats, levels of dopamine, HVA, and DOPAC were monitored for 7 hours following implantation of the probe at day 45 (45 days after polymer implantation). Microdialysis was also performed on days 3 and 20 after implantation on the contralateral (left) side of 4 rats that had received dopamine-polymer implants. Levels of dopamine in extracellular fluid were also measured in the right striatum of normal untreated rats (n = 5) weighing between 200 and 400 gm, thus covering the timespan of this experiment.

Histology

Anatomical examination of the brains from the rats with a dopamine-polymer implant was performed at 70 days following implantation of the device. Animals were deeply anesthetized and transcardially perfused with 4% paraformaldehyde in 0.1 M sodium phosphate buffer. Frozen sections were subsequently cut at 40-μm intervals through the striatum and the tissue stained with neutral red for Nissl substance.

Results

Long-Term Release In Vivo

Dopamine concentrations in the striatal extracellular fluid in the rats that received a control implant and the normal rats were stable over the course of these experiments: 22 ± 5 nM and 29 ± 5 nM, respectively. In contrast, extracellular concentrations of dopamine reached unprecedented elevations of as much as 7.2 μM at days 10 through 65 after implantation of the dopamine-polymer device in the ipsilateral corpus striatum of the experimental rats (Fig 2). Stable levels were reached at 20 days after implantation and were maintained throughout the length of the experiment (day 65). The stability of dopamine release was further demonstrated with a prolonged dialysis experiment on 2 rats which was performed at 45 days after polymer implantation; stable release of dopamine over a period of 5 hours was observed (Fig 3). Levels of dopamine (26 ± 4 nM) in the contralateral, left side in rats with dopamine-polymer implants showed no difference from levels in the right striatum of rats with the control polymer implant or normal rats (data not shown). In the animals with the dopamine-polymer implant, in which stable levels of DOPAC and HVA were measured, DOPAC concentrations were 15.40 ± 2.90 μM and HVA levels were 1.79 ± 0.40 μM (n = 5, days 20, 45, and 65 after implantation). In contrast, control levels of DOPAC and HVA in the striatum were 8.05 ± 0.57 μM and 4.50 ± 0.25 μM, respectively. Thus, the ratio of DOPAC to HVA was elevated from 1.8:1 in normal rats to 8.6:1 in the rats with the dopamine-polymer implant. The rats survived and their behavior appeared normal for the duration of the study.

Histology

The dopamine-polymer devices were located in the rostral striatum and extended caudally to the rostral

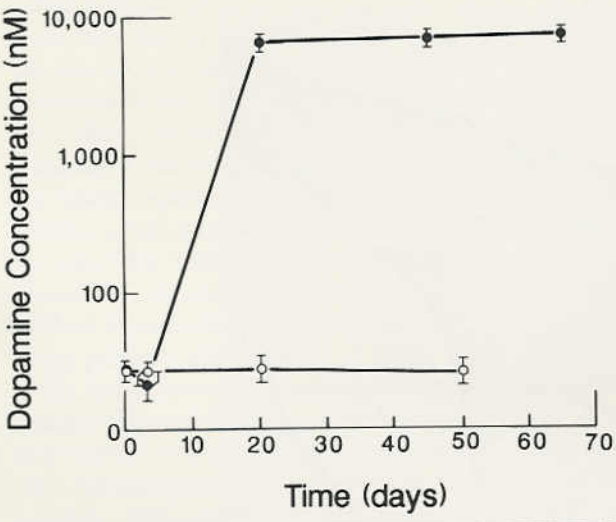

Fig 2. Time course of in vivo dopamine release. Concentrations of dopamine in extracellular fluid were estimated using intrastriatal microdialysis. Each value represents the mean (± SEM) of a minimum of four measurements. A total of 12 rats (dark circles) *received a dopamine-polymer device (30% loading, fully coated except for one cavity), and 4 rats* (empty circles) *received a control polymer device. Animals were randomly selected for dialysis at the indicated time points.*

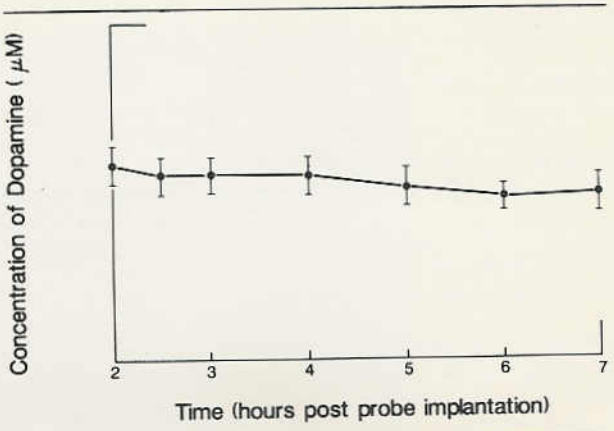

Fig 3. In vivo dopamine release over the course of several hours. Concentrations of dopamine in extracellular fluid (mean ± SEM) were measured using intrastriatal microdialysis in 4 rats. Samples were collected over a 5-hour time period 45 days after implantation of a dopamine-polymer device (30% loading, fully coated except for one cavity).

pole of the globus pallidus. Medially, the device encroached on the lateral ventricular wall but did not broach the ventricular ependyma. Caudally, continuous cortical tissue could be seen overlying the position of the implant, suggesting some expansion of the volume of the implant matrix over the 70-day survival period (Fig 4).

Immediately ventral to the device, a zone of moderate gliosis extending for approximately 250 μm was seen. However, within 500 μm of the implant-striatal interface, striatal morphology was essentially normal (Fig 5).

Discussion

The extracellular fluid levels of dopamine obtained by microdialysis in the rats implanted with a dopamine copolymer matrix were several orders of magnitude greater than the levels in rats with the control implant or in normal rats. These levels far exceed concentrations achieved by such manipulations as *d*-amphetamine administration or potassium-induced depolarization [41, 42]. These elevated dopamine concentrations were sustained from day 20 to at least day 65 after implantation (at which time the experiment was terminated). The DOPAC-to-HVA ratio in the dialysates of the rats with the polymer implants was markedly elevated. The predominance of DOPAC as the major metabolite when the extracellular fluid is flooded with dopamine suggests that the monoamine oxidase capac-

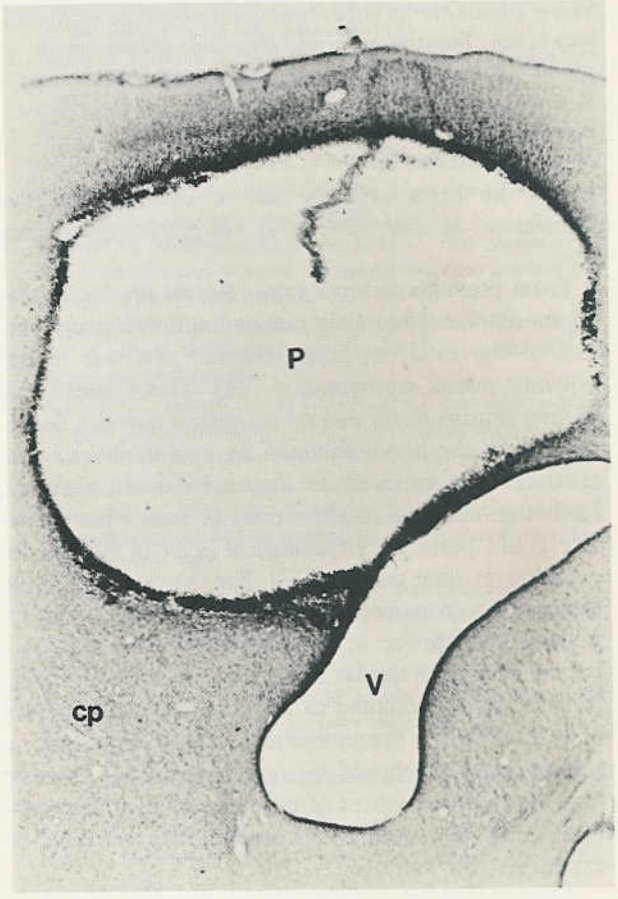

Fig 4. Placement of the polymer implant (P) overlying the posterior striatum (cp). This level represents the caudal end of the implant, where it can be seen to be covered by overlying cortex. The ventricle (V) was not penetrated. (×50 before 29% reduction.)

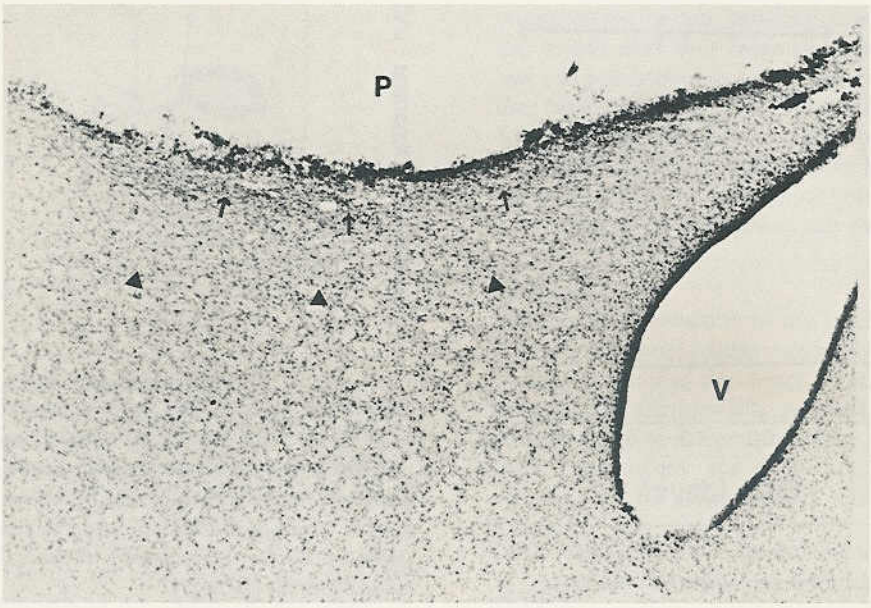

Fig 5. An area of gliosis (small arrows) *immediately adjacent to the implant, extending up to 250 μm, is visible. In addition, a zone of hypocellularity* (arrowheads) *underlying the gliotic area may be seen. The striatum beyond these areas appears grossly normal. (×100 before 30% reduction.) P = polymer implant, V = ventricle.*

ity of the brain exceeds that of catechol-O-methyl transferase, at least under this nonphysiological situation.

From previous in vitro experimental results, by day 65 the implants had only released approximately one-half of the total dopamine content that was in this polymer matrix configuration [38]. This suggests that in vivo release could extend to several months. Moreover, since the levels obtained far exceed desired concentrations of extracellular dopamine in the striatum, further geometrical modifications of such a matrix device could permit a physiological level of release for even longer time periods [37]. For the potential application to the treatment of Parkinson's disease, this approach could prove to be particularly desirable, as a polymeric device similar in size to the one used in this study could be designed to release a sufficient amount of dopamine for symptomatic control for as long as several years. It should be noted, however, that the long-term consequences of exposure to very high concentrations of dopamine are not known and must be experimentally assessed.

Recent advances in slow-release technology include the use of biodegradable polymers, such as polyanhydrides, which obviate the need for removal of residual polymer if subsequent implants are desired [43]. An additional level of control could be obtained by the implantation of miniature magnets within the polymer device, permitting modulation of release by an external electromagnetic field [44]. Further refinement of this technology may also lead to the use of small spherical polymer matrices (microspheres), which can be injected directly into desired brain regions stereotaxically [45].

Perhaps the major advantage of this technology relates to the stability of the prolonged release. Numerous studies have shown that fluctuations in plasma (and presumably brain) levels of L-dopa resulting from oral therapy are in part responsible for fluctuations in clinical response, including the well-established "on-off" effect [7–12]. In fact, oral "slow-release" formulations of L-dopa, which extend the half-life of plasma L-dopa levels by several hours, and intravenous therapy have been shown to improve fluctuations of symptoms [13–21]. The oral "slow-release" formulations still require repeated dosing and the variations in plasma levels remain significant [13]. The disadvantages of intravenous infusion are well known: patients must be hospitalized and attached to an intravenous line or a reservoir, there is potential for infection, and it is costly. Additional advantages of polymer implants include relative cost-effectiveness, as little drug is wasted because of low efficiency of gastrointestinal absorption [46], and the elimination of patient compliance problems. Given the large number of parkinsonian patients with memory deficits, compliance with a strict oral drug regimen is a particular concern [47–51].

Recently, tissue transplantation into the brain has gained much attention as a treatment for Parkinson's disease, and a number of clinical trials have been initiated [24, 25, 29, 32]. However, there are problems associated with such tissue transplants. The mode of

action remains unclear, the long-term efficacy has not been established, and the ethical questions raised by the use of fetal tissue transplants are likely to be controversial, at best [27–32]. Implantable polymer matrices offer a number of advantages over such tissue transplants. Moreover, since it has been proposed that the tissue transplants may function by the release of a trophic agent [30], this agent, once purified, could also be imbedded within such a matrix device system. Alternatively, either dopamine agonists or monoamine oxidase B inhibitors could be delivered to the striatum using polymer technology.

To define more clearly such potential clinical applications of controlled release technology, we have begun studies investigating the behavioral correlates of the dopamine-polymer implants in dopamine-deficit animals. Although this technology has clear implications for potential application to patients with Parkinson's disease, we believe it could also be used in the treatment of other neurological or psychiatric disorders in which sustained or controlled delivery of a drug into the brain might be beneficial.

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