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

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A biocompatible polymeric matrix system for the long-term controlled release of dopamine has been developed. Solid particles of this bioactive agent were encapsulated in ethylene–vinyl acetate copolymer (EVAc). Following immersion in an aqueous buffer solution, the release rate of dopamine from the polymer matrix was found to depend on the initial concentration of dopamine in the polymer. After coating the matrix devices with an additional impermeable layer of EVAc, constant rates of release were obtained by creating a cavity in this impermeable layer. The observed experiments are consistent with a diffusion-limited model of dopamine release; all the *in vitro* experimental results were therefore correlated by the effective diffusion coefficient of dopamine through the porous polymer network. These results are discussed in terms of potential design modifications to achieve desired release characteristics for a variety of neuroactive substances, including neurotransmitters or their precursors.

INTRODUCTION

A major obstacle in the treatment of many neurological disorders is the inability to deliver drugs into the brain, particularly within discrete regions, at a controlled or constant rate. The primary cause for this problem is the presence of a blood–brain barrier which precludes the entry of many plasma-borne substances into the brain (24). Even if such a substance were to bypass this barrier, it would distribute itself throughout the brain in a relatively anatomically undefined manner, with levels particularly dependent on fluctuations in plasma concentrations (23). Thus, several methods have been developed in an attempt to circumvent some or all of these problems, including osmotic disruption of the blood–brain barrier (21, 22), intracerebral delivery using infusion pumps (10, 11), and the implantation of adrenal or fetal neural tissue which can release neurotransmitters or trophic factors (1, 16–18). Each of these methods has real or potential deficiencies, including, respectively, severe toxic side effects; size, safety, and reliability problems; and serious ethical and cost concerns (12, 19, 21, 22). Furthermore, although clinical trials of adrenal and fetal tissue implants in parkinsonian patients have already begun, the mechanism and long-term efficacy of tissue transplants within the nervous system remain unclear (3, 28).

We have attempted to overcome these difficulties by developing a biocompatible polymeric matrix system which permits the sustained and localized release of neuroactive substances directly into the brain. Polymeric matrix devices are more reliable and less dangerous than alternative therapeutic modalities. Furthermore, these devices appear to raise fewer ethical questions than fetal or other tissue brain transplants. Preliminary reports have shown that peripheral polymer implants can release proteins, such as insulin, at a constant rate for at least 100 days *in vivo* (5, 6). Thus, it seemed possible that similar biocompatible polymeric systems could release neurally active substances, such as catecholamines, directly into the brain for extended periods of time. The present study demonstrates the *in vitro* release of dopamine from various ethylene–vinyl acetate copolymer matrix devices for extended periods of time. By selection of an optimal loading and geometric configuration, linear and sustained release could be achieved.

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MATERIALS AND METHODS

In Vitro Studies

Ethylene–vinyl acetate (Elvax 40P, DuPont, Wilmington, DE) was washed as previously described (25), and slabs containing 0, 30, 40, and 50% dopamine (Sigma, St. Louis, MO) by weight, formed by solvent casting, were punched out to form round discs 4.0 mm in diameter and 1.0 mm in depth (final weight, 16 ± 1 mg) (25). These discs were then coated by an additional layer of ethylene–vinyl acetate copolymer either (i) completely; (ii) completely with one cavity present; (iii) completely with two cavities present, one on each surface; (iv) partially with only one surface coated; or (v) not coated at all. In order to coat the matrices with an impermeable layer of EVAc, the matrix was first impaled on a 30-gauge syringe needle. The matrix was immersed in liquid nitrogen for 10 to 15 s and then immersed in a 20% (w/v) solution of EVAc in methylene chloride. The coated matrix was held under house vacuum for several hours and the procedure was repeated. When the needle was removed from the twice-coated matrix, a pinhole cavity remained in the otherwise impermeable coating. By measuring the size of the resulting polymeric device, the thickness of this impermeable coating was calculated to be approximately 500–700 μm. For coating one side of the matrix, the procedure was identical except that the matrix was not fully immersed in the polymer solution. Fully coated polymer matrices were produced by (i) pouring a thin film of 10% (w/v) EVAc/methylene chloride into a level mold on dry ice, (ii) waiting 10 to 15 min for this bottom layer to freeze completely, (iii) placing EVAc/dopamine matrices on top of this pure EVAc layer, and (iv) carefully pouring a second film of 10% EVAc/methylene chloride into the dry ice temperature mold. The mass of EVAc in each layer was adjusted to obtain a 500- to 700-μm-thick coating on each face of the matrix. This sandwich matrix was evaporated as previously described (2 days at −20°C and 2 days at room temperature and house vacuum) and fully coated devices were obtained by cutting around the encapsulated matrix.

Quadruplicates of these devices were then separately incubated in vials containing 5 ml of a 150 m*M* NaCl, 0.2% EDTA (as antioxidant) solution, and maintained on an Orbitron oscillating platform (Boekel Industries, PA) in a 37°C oven. *In vitro* release was monitored by spectrophotometric analysis (at 280 nm) of the bathing solution, which was replaced each time a measurement was taken (13). Quantification of dopamine levels was based on comparison to a standard solution curve. The identity of the compound released at various time points was confirmed as >99.9% dopamine by use of high-performance liquid chromatography (HPLC) analysis. This system (8) consisted of an Allex 100A pump; a 20-μl sam-

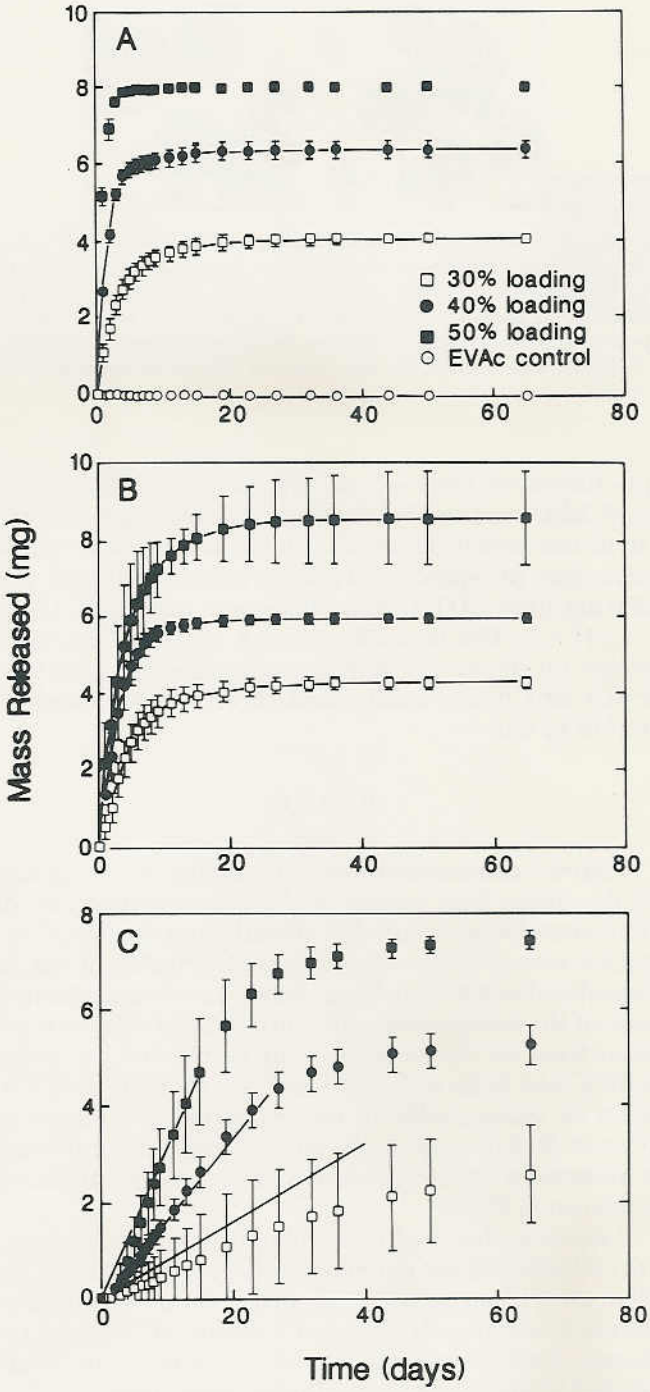

FIG. 1. Release of dopamine from dopamine/ethylene–vinyl acetate copolymer matrices. Each experimental point represents the mean (± standard deviation) cumulative mass of dopamine released for four sample matrices. Each dopamine/polymer matrix initially contained 0% (empty circle), 30% (empty square), 40% (filled circle), or 50% (filled square) by weight dopamine. The cumulative mass of dopamine released is shown for matrices with different geometries: (A) a simple disc, (B) a disc with one impermeable face, and (C) a disc with a completely impermeable coating except for a single cavity in one face. Solid lines in the bottom panel demonstrate the linear release predicted by a model of diffusion of dopamine through the prescribed geometry.

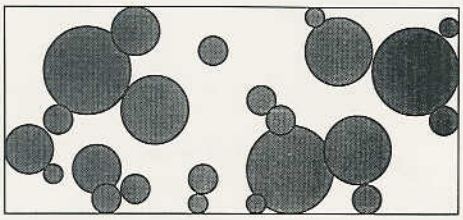

FIG. 2. Schematic cross section of the dopamine/copolymer devices showing the solid particles (dark) of dopamine encapsulated in a continuous polymer phase. Although the polymer phase is impermeable to the encapsulated molecules, release occurs as water enters the pore space, dissolving the solid particles. Molecules counter diffuse out of the polymer through the pore network created by dissolution (29, 30, 31).

ple Rheodyne loop; a 3-μm HR-80 column (ESA, Bedford, MA); and an ESA 5100A coulometric detector with an in-line conditioning cell. The mobile phase consisted of sodium phosphate, 0.6 g/liter; heptane sulfonic acid, 350 mg/liter; EDTA, 80 mg/liter; and methanol, 5% (v/v); *p*H 4.2. The flow rate was 1.8 ml/min. Chromatograms for dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were completed within 12 min.

RESULTS

Figure 1 demonstrates the cumulative *in vitro* release of dopamine from a variety of polymer configurations. This cumulative release was directly proportional to the square root of time, suggesting that diffusion of the encapsulated solute from the polymer was the rate-limiting step in the release process (7, 30). Since release of dopamine from the device was totally eliminated by coating with a thin layer of EVAc (data not shown), the EVAc must be impermeable to the dopamine. Therefore, release of dopamine from the polymer must occur through a network of interconnected, aqueous pores (29, 30, 32) as shown in Fig. 2.

Assuming that dopamine solubility in aqueous buffer (C_s) is high, that the diameter of the internal pores is less than the thickness of the device (L), and that release occurs predominantly in one dimension through the two largest faces of the slab, the release process can be described by the continuum diffusion equation

$$\frac{\partial C}{\partial t} = D_{\text{eff}} \frac{\partial^2 C}{\partial x^2}, \qquad [1]$$

where C is the concentration of dopamine in the matrix at Position x and Time t, and D_{eff} is the effective diffusion coefficient of dopamine through the pore space. Appropriate boundary and initial conditions for this formulation are

$$C = C_0 \quad \text{at } t = 0 \quad \text{for } 0 < x < L$$
$$C = 0 \quad \text{at } x = 0, L \quad \text{for } t > 0. \qquad [2]$$

The complete solution to this equation—giving C as a function of x and t—for the stated boundary conditions is available (7). The mass of solute released at any time is found by integrating the expression for C to find the mass of solute remaining in the slab. For short times, when more than 40% of the encapsulated solute remains in the slab, the mass of solute released, M_t, is proportional to the square root of time,

$$M_t = 4M_0 \sqrt{\frac{D_{\text{eff}} t}{L^2 \pi}}, \qquad [3]$$

where M_0 is the mass of solute initially present in the matrix.

Comparison of the release profiles shown in Fig. 1A with Eq. [3] yields an experimental value for the effective diffusion coefficient. The experimentally determined effective diffusion coefficients for dopamine in the polymer pore space depend on the mass of dopamine initially incorporated within the matrix, as shown in Table 1. This is consistent with previously observed results for the release of other bioactive agents from EVAc slabs (2, 20, 29).

Although the absolute value of mass released changed, the same dependence of release on the square root of time was observed when the devices were coated with an impermeable polymer layer on one face (Fig. 1B). This is the expected behavior for release from a slab with one impermeable boundary (7). Effective diffusion coefficients for this configuration are consistent with the results provided in Table 1.

With a complete EVAc coating over the matrix, no solute was released over the 65-day period (results not shown). When a single cavity was introduced into the impermeable coating, release was linear with time (Fig. 1C). Release rates of 0.06, 0.17, and 0.30 mg/day were obtained from matrices of 30, 40, and 50% loading, respectively. For times when less than ~60% of the initially encapsulated solute has been released, this configuration should behave as a coated hemispherical vehi-

TABLE 1

Dependence of Diffusion Coefficients on Initial Dopamine Concentrations

Initial concentration of dopamine (mass %)	D_{eff} (cm^2/s)
30	2.5×10^{-9}
40	5.1×10^{-9}
50	9.5×10^{-9}