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Preparation and in vivo effect of microencapsulated cholinotoxin

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Summary

Long-term controlled release of a neurotoxin was achieved in vivo by microencapsulating the cholinotoxin in a poly(lactic acid) matrix. A dose of 0.59 nmol of the toxin (in 20 mg of microcapsules) produced a significant reduction in choline acetyltransferase (ChAT) activity in the cortex without affecting other brain structures.

Introduction

A variety of neurotoxic compounds have been used to study the neurochemical, morphological and behavioral sequelae of the neurotransmitter (e.g. cholinergic, dopaminergic) deficiencies occurring in some neurodegenerative disorders (Coyle, 1985; Smith, 1988). Based upon findings of extensive cell loss in the Nucleus basalis of Meynert (NBM) in Alzheimer's disease patients relative to normal controls (Whitehouse et al., 1983), investigators began to produce lesions in the corresponding nuclei of the rodent and non-human primates. Lesions have been produced with several different neurotoxins (ibotenic, kainic, and quisqualic acids, ethylcholine aziridinium ion (AF64A) and various hemicholinium analogs) leading to anterograde and retrograde neuronal degenerations (Coyle and Schwarz, 1983; Coyle, 1985; Fischer and Hanin, 1986; Maysinger et al., 1986; Tagari et al., 1986). The specificity of the most widely used cholinotoxin AF64A has been questioned as a result of controversial findings by several laboratories (Levy et al., 1984; Potter et al., 1985; Colhoun et al., 1986; Eva et al., 1987). Discrepancies between studies demonstrating the cholinergic specificity of AF64A and those showing nonselective damage may be accounted for on the basis of differing rates of toxin injection and variations in local concentrations of the toxin produced at the site of injection.

Administration of neurotoxic compounds leading to specific/nonspecific central nervous system lesions was mostly achieved by a single injection of drug into the lateral ventricle or into a particular brain structure (e.g. cortex, striatum, hippocampus). This often caused local tissue irritation and necrosis. So far, the only way of achieving chronic administration of these toxins into the central nervous system was by the use of osmotic minipumps and permanently installed stainless-

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steel cannulae. However, this has some serious limitations. It has recently been shown that a microencapsulated substance can provide a convenient means of chronic administration of a drug (Maysinger et al., 1989).

In an attempt to achieve the chronic delivery of neurotoxins circumventing these limitations, we have prepared microcapsules containing a model cholinergic toxin. It seemed reasonable to assume that neurotoxin microencapsulated in a biodegradable material, poly (lactic acid), could release a toxin over an extended period of time in a manner more physiological than that provided by minipumps, thus more efficiently mimicking the conditions under which neuronal degeneration may occur.

Materials and Methods

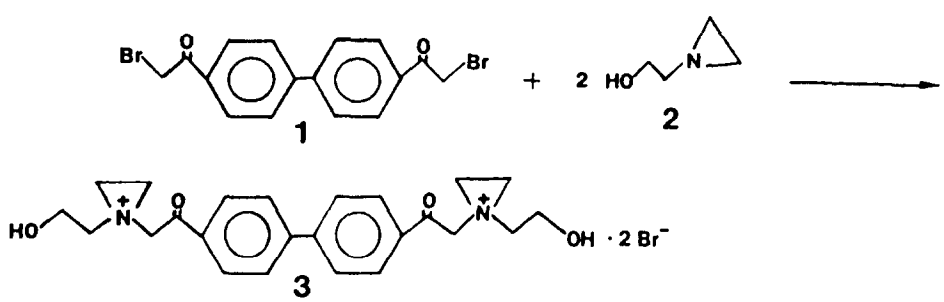
Compounds

α,α'-Dibromo-4,4′-biacetophenone (compound (**1**), scheme 1) was prepared by the method of Long and Schueler (1954). 1-(2-Hydroxyethyl-aziridine) (**2**) was synthesized from ethylene oxide and ethylenimine according to the method of Funke and Benoit (1953). Ethylenimine was prepared from ethanolamine (Wenker, 1935).

α,α'-Bis(1-hydroxyethylaziridinium)-4,4′-biacetophenone dibromide (**3**) was prepared from (**2**) (0.78 g, 0.01 mol) which was added with vigorous stirring to a solution of 1.98 g (0.005 mol) of (**1**) in 60 ml of hot 1,4-dioxane. The cooled reaction mixture was stirred for 1 h at room temperature. The precipitate was filtered, washed with dry ether and dried in vacuo to yield 0.42 g (15%) of (**3**). The mother liquor was concentrated under reduced pressure. After the addition of 50 ml dry ether, precipitation of the product (**3**) occurred (1.55 g, 54%). m.p. 170–172° C. UV spectra were taken on a Pye Unicam Sp 8-100 spectrophotometer, and IR spectra on a Perkin-Elmer 457 spectrophotometer. UV (MeOh): λ_{max} 294 nm. IR (KBr): 3380, 2940, 1680, 1605, 1450, 1400, 1230, 1115, 1070, 910, 825 cm^{-1}. Analysis for $C_{24}H_{30}Br_2N_2O_4$ (570.33); calculated: C, 50.54; H, 5.30; N, 4.91; found: C, 50.43; H, 5.59; N, 4.79.

Microencapsulation of the toxin (3)

Poly(+)lactide (Southern Research Institute, Birmingham, AL) (0.5 g) was dissolved in 10 ml dichloromethane and 95.2 mg of toxin (**3**) was added. This mixture was then gradually poured into a 0.3% aqueous methylcellulose solution (50 ml). The final mixture was stirred (700 rpm) at room temperature until the dichloromethane completely evaporated. The resulting microencapsulated toxin was decanted, washed several times with water and filtered. The product was dried at ambient temperature for 24 h and then stored in a desiccator. Scanning electron microscopy (SEM) was carried out by applying the gold technique (Joel JSM T-200 at Pulp and Paper Co., Pointe-Claire, Canada). The content of neurotoxin (**3**) in the microcapsules was determined spectrophotometrically (Pye Unicam SP 8-100): 10 mg of microencapsulated toxin was extracted with 5 ml MeOH overnight. The suspension was filtered and the concentration of toxin (**3**) was determined by measuring the absorbance at 294 nm.



Scheme 1. Structures of compounds (**1**)–(**3**).

Administration of microencapsulated toxin (3) and determination of enzymatic activities in the rat brain

Male Wistar rats aged 2 months and weighing 200–250 g were used. The bone flap (to expose the dura mater) was removed under Equithesin anaesthesia (1 ml/300 g body wt). The dura mater was gently nicked in three places and 1, 5 or 20 mg of microencapsulated toxin (**3**) incorporated into a thin gelatin film ($10 \times 0.9 \times 0.1$ cm) was layered over the exposed brain surface. Sham-operated animals had the bone flap removed and a gelatin film containing microcapsules without neurotoxin was placed instead of the bone flap. 30 days followed administration, animals were killed by decapitation, and their brain tissues were quickly dissected on ice and extracted for determinations of enzymatic activities. Choline acetyltransferase (ChAT) activity was assayed according to Fonnum (1975), glutamic acid decarboxylase (GAD) activity was determined as described by Atterwill et al. (1981), and proteins were determined by the method of Bradford (1976).

Results and Discussion

Fig. 1 shows scanning electron micrographs of microcapsules containing toxin (**3**). They are spherical in shape, have few small pores and do not vary very much in size. According to the spectrophotometric analysis the content of toxin (**3**) in this preparation was 1.57%.

Three different doses of neurotoxin (**3**) corre-

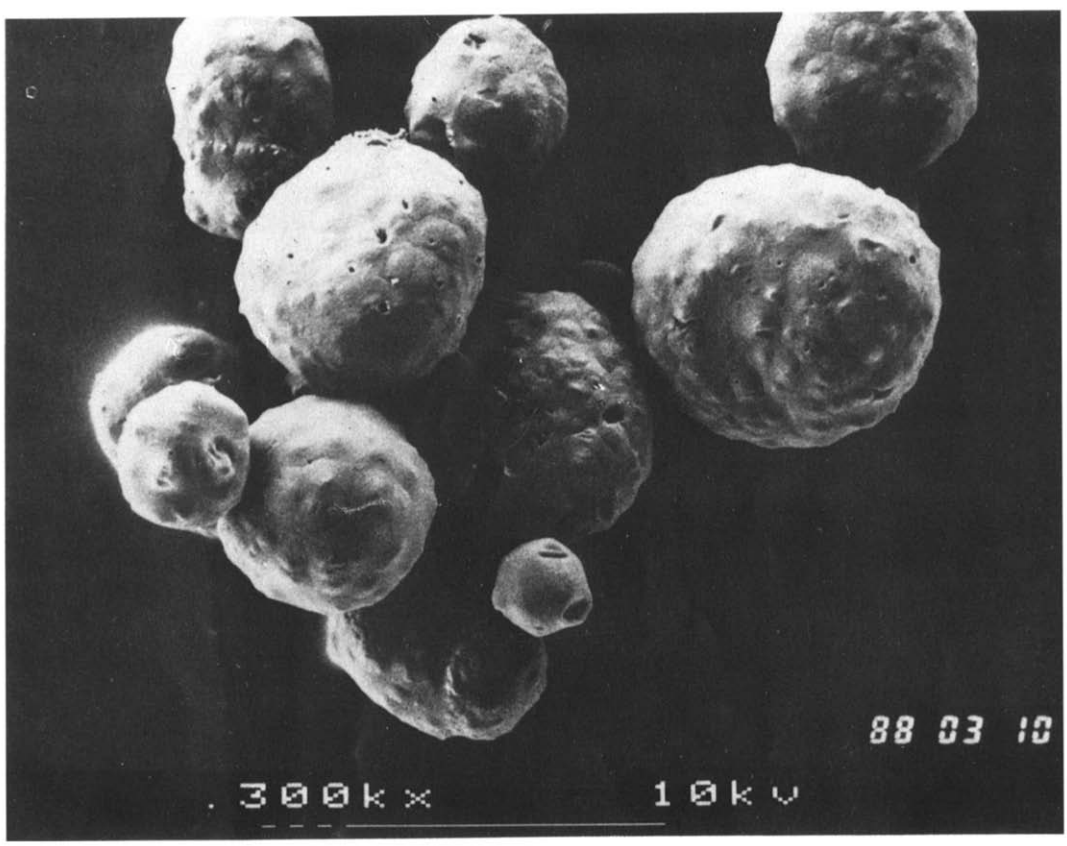


Fig. 1. Scanning electron micrographs of PLA-microcapsules containing neurotoxin (3).

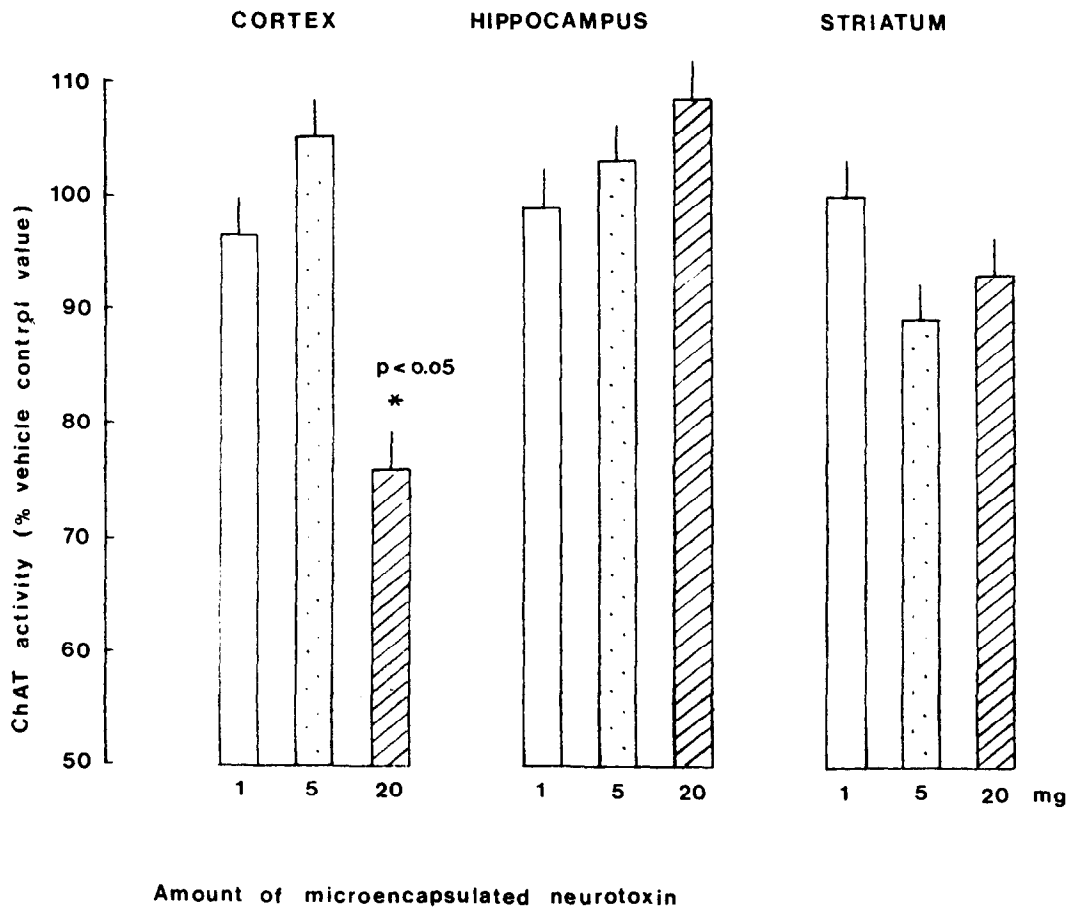


Fig. 2. Choline acetyltransferase activity vs. amount of microcapsules containing neurotoxin (3). Toxin content in microcapsules: 1.67%.

sponding to 1, 5 and 20 mg of the microencapsulated product were administered to rats. Sham-operated animals served as controls. Gelatin did not have any effect on the measured ChAT or GAD enzymatic activities and maintained the microcapsules localized in place of the removed bone flap.

Low doses of toxin (3) contained in 1 and 5 mg of PLA-microcapsules were ineffective. Only relatively large dose of toxin (3) (0.3 mg, 0.59 nmol) produced a significant, albeit small, reduction in ChAT enzymatic activity in the cortex without affecting other brain structures (Fig. 2). GAD activity (expressed in nmol/mg protein per h) remained unchanged in all brain areas examined: hippocampus, 210 ± 10.2 ($n = 15$); striatum, 284.5 ± 17.5 ($n = 15$); cortex, 191.1 ± 10.8 ($n = 15$).

This study demonstrates that long-term controlled release of neurotoxins can be achieved in vivo by microencapsulating the cholinotoxin in a poly(lactic acid) matrix. PLA is known to be well tolerated by the host tissue. However, both biodegradable matrix material and the model neurotoxic compound presented in this report can be substituted with other materials. Microparticles containing colchicine, ibotenic, kainic or quisqualic acid as well as AF64A could provide a chronic supply of these agents to various brain areas. Administration of microencapsulated toxins could be a viable alternative to other existing modalities

for their administration into the central nervous system, avoiding both local tissue necrosis and other nonspecific effects.

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