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ELECTRICALLY STIMULATED RELEASE OF NEUROTRANSMITTER FROM A CONDUCTING POLYMER THIN FILM ON THE MODEL OF A SYNAPSE

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A microtip of polypyrrole film (50 μm in diameter) was devised on the model of a presynaptic membrane. The microtip incorporated neurotransmitter such as glutamic acid at an electrochemically oxidized state. The incorporated glutamic acid was released from the film on call by electric pulse stimulation.

The extent and orientation of nerve terminal branching results in synapses of various configurations. The nerve ending, presynaptic membrane, is separated from the postsynaptic membrane by a gap of 20-30 nm. Neurotransmitters such as acetylcholine are packed in quanta of less than 10^4 molecules contained in vesicles in the presynaptic membrane. Electric pulse (impulse) generates at the nerve ending, transfers through the axon and reaches the presynaptic membrane to release the packed neurotransmitter. The presynaptic membrane thus transduces the electric into the chemical information.

This communication concerns itself with a novel information transducing membrane modeled on the presynaptic membrane which releases a chemical messenger on call. Miller and his coworkers covalently immobilized dopamine to a polymer coated electrode, and released bound dopamine from the electrode by the electrochemical reductive cleavage of the linkage.[1] We have developed a completely different type of electrically stimulated release of neurotransmitter, which is characterized by the use of a conducting polymer thin film. The polymer film employed is made by electrochemical polymeri-

zation of pyrrole onto a Pt electrode. We should emphasize that the purpose of the study is to demonstrate the concept of making a device for electrically stimulated release of neurotransmitters based on electrochemical principles.

Polypyrrole thin films were prepared on a Pt plate (2 cm^2) by the electrochemical oxidative polymerization of pyrrole at controlled potential of 1.0 V vs. Ag/AgCl.[2] The electrolyte solution contained 0.1 mol dm^{-3} pyrrole and 0.1 mol dm^{-3} tetra-n-butylammonium perchlorate in acetonitrile. The polymer film was grown up to about 500 molecular layers in thickness.[3] The electrochemical incorporation and release of anionic neurotransmitters such as glutamic acid were carried out with the polymer-coated Pt electrode in a borate buffer solution (pH 9.0).[4] Potential of the electrode was controlled refering to a Ag/AgCl electrode with a potentiostat and a function generator (EG&G Princeton Applied Research Model 363 and 175). A Pt plate was used as a counter electrode. Pulsed current was recorded through a signal recorder (EG&G Princeton Applied Research Model 4102). Neurotransmitter released into the electrolyte solution was determined by the fluorescent method using fluorescamine.[5]

Figure 1 shows the cyclic voltammogram of the polypyrrole film deposited on the Pt electrode in a borate buffer solution (pH 9.0) containing 10 mmol dm^{-3} glutamic acid. Polypyrrole was oxidized in the potential range above -0.2 V, which was accompanied by the incorporation of glutamic acid and borate into the membrane matrix to compensate the net charge. The reverse scanning resulted in the reduction of the oxidized polypyrrole into the neutral state, which was followed by the release of the incorporated

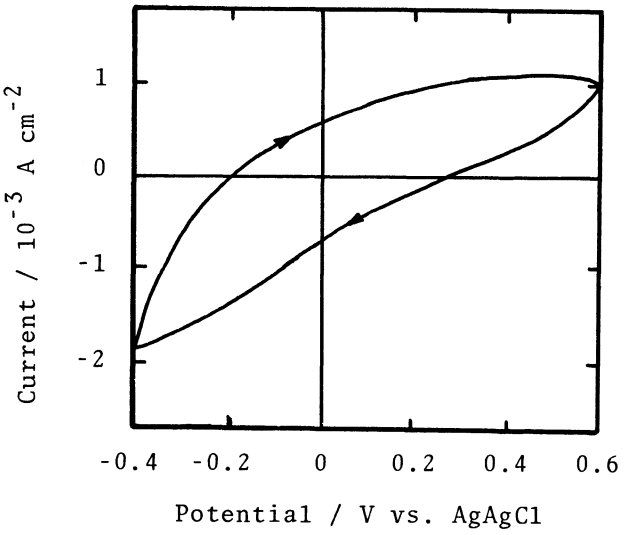

Fig. 1. Cyclic voltammogram of a polypyrrole film in 0.2 mol dm^{-3} borate buffer solution (pH 9.0) containing 10 mmol dm^{-3} glutamic acid. Potential was scanned at a rate of 100 mV s^{-1}.

glutamic acid and borate.

The potential of the polymer coated Pt was controlled at 0.15 V to incorporate glutamic acid into the membrane. The time course of anodic current is shown in Fig. 2(a). Electrochemical incorporation was continued for 3 min. The polymer-coated Pt was thoroughly rinsed with a glutamic acid-free borate buffer solution (pH 9.0) and then immersed in the same solution. The potential was set at 0.15 V again. Almost no release of the incorporated glutamic acid occurred at this potential. Subsequently the potential was steped to -0.40 V to release the incorporated glutamic acid out of the polypyrrole membrane. The time course of cathodic current, which is attributed to the reduction of polypyrrole and the release of the incorporated glutamic acid and borate, is presented in Fig. 2(b). Charge of the electrochemical oxidation and reduction, which were shown in Figs. 2(a) and 2(b) respectively, were almost same. The borate buffer solution was assayed for glutamic acid by the fluorescent method using fluorescamine before and after the electrochemical release. It indicated that 2.5×10^{-9} mol of glutamic acid was released from the polypyrrole membrane. The amount of released glutamic acid depended on the conditions of the incorporation. From these results, we conclude that glutamic acid can be incorporated into the polypyrrole membrane and be released from it by the electrochemical redox reaction of polypyrrole, and that our conceptual de-

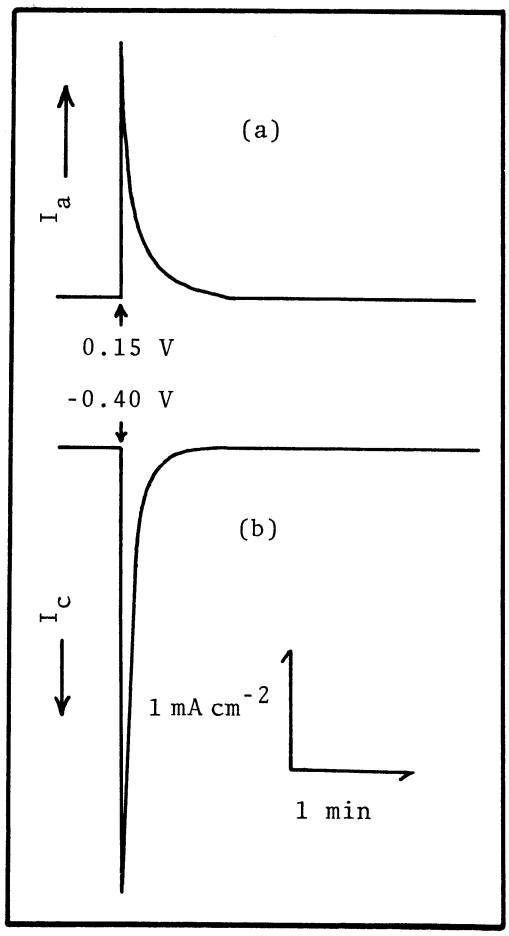

Fig. 2. Electrochemical incorporation and release of glutamic acid at a polypyrrole film. (a) Incorporation at a controlled potential of 0.15 V in 0.2 mol dm^{-3} borate buffer solution (pH 9.0) containing 10 mmol dm^{-3} glutamic acid. (b) Release at a controlled petential of -0.40 V in 0.2 mol dm^{-3} borate buffer solution (pH 9.0).

vice modeled on a presynaptic membrane may be accomplished using polypyrrole.

A microtip of polypyrrole membrane was made by the electrochemical polymerization of pyrrole in acetonitrile as described above onto a Pt fiber (50 μm in diameter) with Teflon shroud. Glutamic acid was incorporated into the microtip in the same method. After rinse, the microtip was immersed in a glutamic acid-free borate buffer solution (pH 9.0) and set at the potential of 0.15 V to hold the incorporated glutamic acid. The device was then subject to a -350 mV voltage pulse with 1 ms width. Figure 3 shows the time course of the current derived from the device. It indicates that glutamic acid is released from the microtip of polypyrrole membrane in response to electric stimulation with 1 ms width.

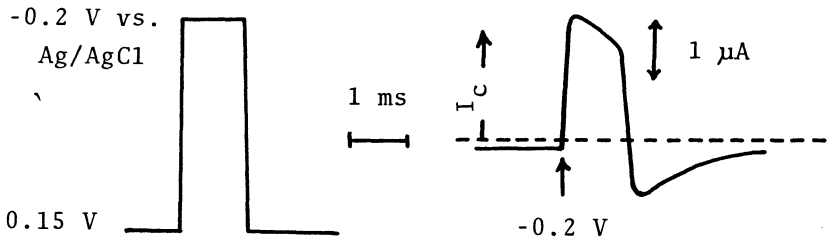

Fig. 3. Electrically stimulated release of glutamic acid from a polypyrrole microtip in 0.2 mol dm^{-3} borate buffer solution (pH 9.0).

References

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2) A. F. Diaz, Chem. Scripta, 17, 145 (1981).
3) The film thickness was estimated from the charge passed during electrochemical polymerization on the assumption that 1.8×10^{-4} C cm^{-2} was required to form a monolayer of polypyrrole.
4) A pH 9.0 of borate buffer solution was used as a standard solution in all measurements, because glutamic acid might dissociate to give negative charge.
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