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BRIEF COMMUNICATION

A New Method For Drug Application Using Electrolysis of Water

TOMOYA YAMAMOTO, YUTAKA OOMURA,*[1] SEIJI NEMOTO
ICHIRO FUJITA AND HITOO NISHINO

Department of Biological Control System, National Institute for Physiological Sciences
Okazaki 444
*and *Department of Physiology, Faculty of Medicine*
Kyusyu University, Fukuoka 812, Japan

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YAMAMOTO, T., Y. OOMURA, S. NEMOTO, I. FUJITA AND H. NISHINO. *A new method for drug application using electrolysis of water.* BRAIN RES BULL **15**(5) 527–528, 1985.—A micropipette for drug application using the electrolysis of water is described. The drug solution is applied from the tip of a micropipette by the pressure of the hydrogen and oxygen gas that are produced by electrolysis of water in the micropipette. The amount of the drug applied is proportioned to the applied current and time of electrolysis. This method is simple, reliable and effective for the application of any kind of drug.

Pressure injection Electrolysis Micropipette Neuronal activity Multi-barrel electrode

IN recent years, there has been increasing interest in the study of effects of drug applications *in vivo* and *in vitro*. For this purpose, electrophoresis [1,3] and pressure injection [2,4] have been used. These methods, however, require complicated equipment and special care to avoid electrical and mechanical artifacts. The present method was designed to overcome these difficulties by using the electrolysis of water to create pressure in a micropipette.

ELECTRODE ASSEMBLY

The micropipette was made from 3 mm diameter cored glass capillary with a tip diameter of less than 1 μm (d.c. resistance, approximately 25 MΩ if filled with 3 M KCl, Fig. 1). The drug solution, silicone oil (SRX310, Traysilicone Co., Tokyo) for isolation and electrical insulation, and 0.1% NaCl solution were placed in layers in the micropipette. A pair of twisted enamel-coated Ag wires with exposed Ag-AgCl ends was situated in the NaCl solution. The top end of the micropipette was sealed with polycyanoacrylate glue (ARONALPHA, Toagoseikagakukogyo Co., Tokyo) and dental acrylic. A direct current (250 μA) was applied for the electrolysis. The drug solution was ejected from the tip of the micropipette by the pressure of the hydrogen and oxygen that were produced by electrolysis of the water in proportion to the electrical current and the time applied.

MEASUREMENT OF AMOUNT APPLIED

The amount of solution ejected was measured by colorimetry using a dye (Coomassie Brilliant Blue R-250, $CH_{45}H_{44}N_3NaO_7S_2$, MW=825.96, Nakarai Chemical Co., Kyoto). The dye solution (0.1%) was directly ejected from the micropipette into the colorimeter cell which contained 3 ml distilled water. The tip diameter of the micropipette was 10 μm in order to measure rather large amounts of ejecta (several μl). The ejected amount was calculated from the absorbance at 550 nm. Figure 2A shows the relationship between the amount of the dye solution ejected and the time of current applied. The amount of solution ejected from the micropipette was theoretically estimated using the following equation.

$$V = i \times t \times 1/F \times 0.75 \times R \times T \times 1/(P_a + P_t - P_w) \quad \text{(eq.1)}$$

where V=ejected amount in liters, i=applied current in A, t=time of current in sec, F=Faraday constant, R=gas constant, T=absolute temperature, P_a=atmospheric pressure in atm, P_t=tissue pressure in atm and P_2=water vapor pressure in atm.

The measured amount of the dye solution ejected was 1.71×10^{-1} μl for 1 mA applied for 1 sec. This value agreed with the theoretical value of 1.93×10^{-1} μl (295°K, P_a=1 atm, P_t=0 atm and P_2=0.027 atm). The calculated efficiency of the ejection was 87%. There was no significant difference in efficiency among micropipettes of 3, 10 and 30 μ in tip diameter (*t*-test, Fig. 2B).

APPLICATION TO PHYSIOLOGICAL EXPERIMENTATION

This method can be combined with the multi-barrel tech-

[1]Requests for reprints should be addressed to Y. Oomura.

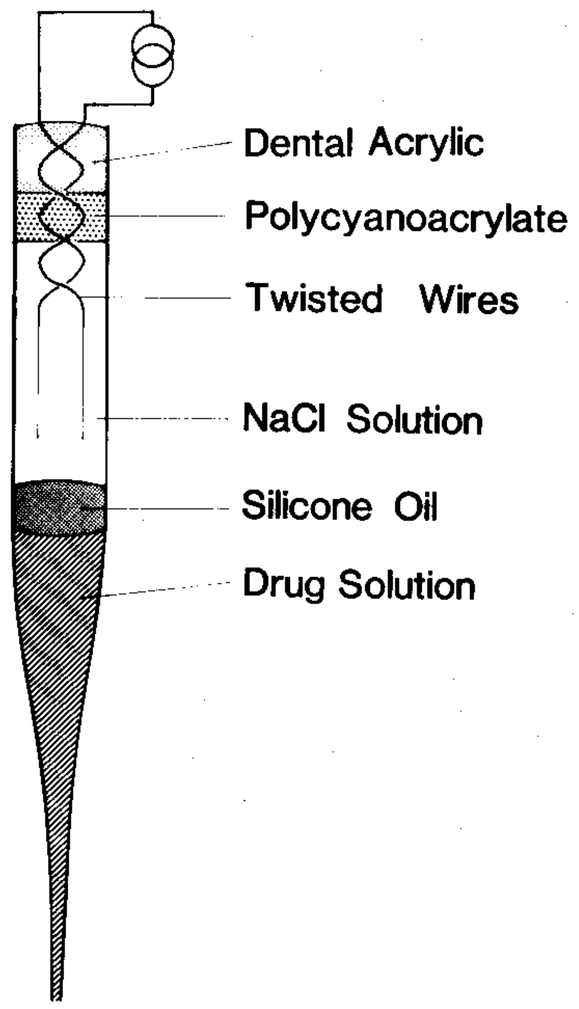

FIG. 1. Micropipette assemply. Drug solution, silicone oil and NaCl solution were placed in layers without bubbles. Chlorided ends of twisted, insulated Ag wires were situated in NaCl solution. Top end of micropipette was sealed with polycyanoacrylate glue and dental acrylic. A direct current (250 μA) was applied to twisted wires to produce mainly hydrogen gas and oxygen gas in NaCl solution. The drug solution was ejected from the tip by the gas pressure.

nique. Figure 2C shows the activity change of a single neuron in the cerebral cortex of a rat (adult Wistar rat weighing 220 g under ketamine anesthesia, 150 mg/kg) in response to 0.1 M sodium L-glutamate (PH=8) application using the method described here. The electrode assembly was a nine-barrel pipette with a carbon fiber (7 μm in diameter) in the central recording channel (5 MΩ at 60 Hz). One of the surrounding barrels, (individual tip diameters, less than 1 μ), filled with 0.1 M sodium L-glutamate, was used as an application channel. A clear dose-dependent relationship between duration of current (250 μA) application and the increase in single neuron activity was observed.

DISCUSSION

The electrophoresis method has been used to study the effects of drug applications on the activity of single neurons. This method, however, requires careful observation to avoid the current effect [1,3]. Although pressure injection became a popular alternative to electrophoresis for drug application, this method requires special equipment and sometimes suffers from inaccuracy of the ejected amount. In conventional

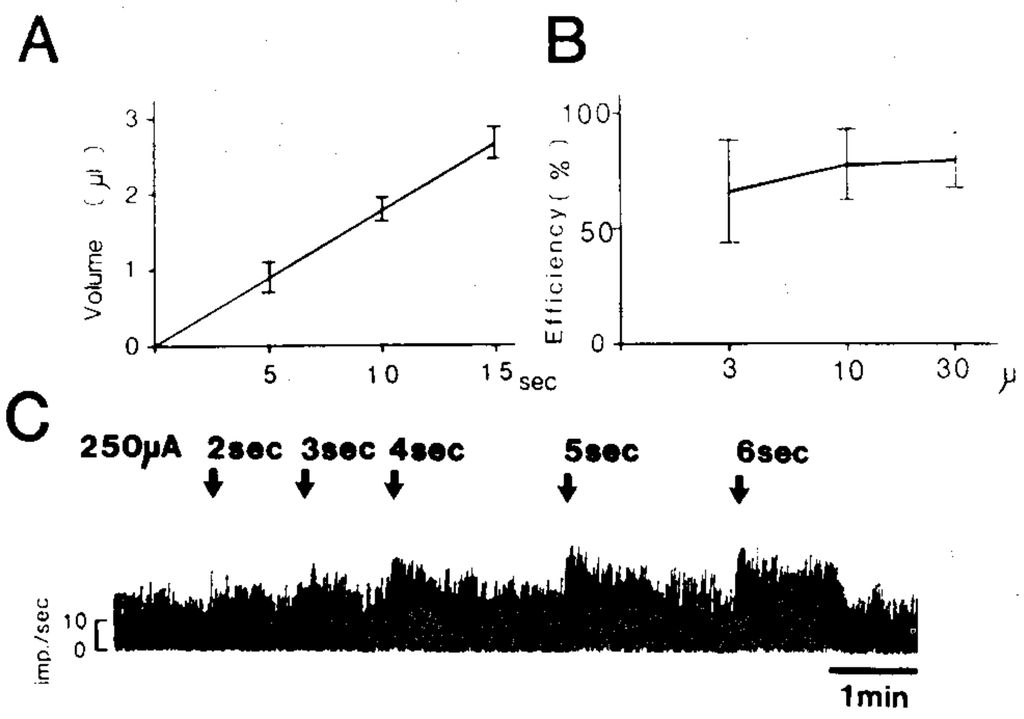

FIG. 2. A: Relationship between amount of dye ejected in μl and time of current (1 mA) applied in sec. Micropipette was kept in the colorimeter cell for 5 min after current application. Data from 5 trials. Average and standard deviation (I). B: Efficiency of ejection against the theoretical value and tip diameter. Data from 5 micropipette for each diameter at 0.5 mA for 1 sec. Average and standard deviation (I). C: Activity of single neuron in anesthetized rat cerebral cortex during 0.1 M sodium L-glutamate (PH=8) application by the present method. Activity increased in a dose-dependent manner with increasing time of current (250 μA) applied in sec.

method, the amount of ejection, controlled by the duration of pressure application, has been easily affected by the micropipette properties. Furthermore, comparatively large dead space deriving from long tubing was easily affected by the mechanical condition such as pressure, vibration and bending. The present method is simple, reliable and effective for the application of virtually any kind of drugs without electrical and mechanical artifacts. Theoretically, the ejection from the tip continues until the inner pressure of the micropipette attains equilibrium state with the circumstantial pressure. And the ejected solution is replaced with gas produced by electrolysis of the water. So, the amount of drug applied was accurately in proportion to the current and time, and agreed with the theoretical value irrespective of micropipette properties. This method can be used to study the effects of drug application on the activity of single neurons when it is combined with the multi-barrel electrode technique.

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REFERENCES

1. Aou, S., Y. Oomura and H. Nishino. Influence of acetylcholine on neuronal activity in monkey orbitofrontal cortex during bar press feeding task. *Brain Res* **275:** 178–182, 1983.
2. McCaman, R. E., D. G. McKenna and J. Ono. A pressure system for intracellular ejections of picoliter volumes. *Brain Res* **136:** 141–147, 1977.
3. Oomura, Y. Recent techniques and investigations on the influence of noradrenaline on feeding and behavior. In: *Methods in Biogenic Amine Research*, edited by S. Parpez, T. Nagatsu, I. Nagatsu and H. Parvez. Amsterdam: Elsevier Science Publishers, 1983, pp. 953–979.
4. Sakai, M., B. E. Swartz and C. D. Woody. Controlled micro release of pharmacological agents: Measurements of volume ejected *in vitro* through fine tipped glass microelectrodes by pressure. *Neuropharmacology* **18:** 209–213, 1979.