

## LITERATURE CITED

- (1) Ashworth, M. "Titrimetric Organic Analysis, Part 2"; Wiley: New York, 1965; p 329.
- (2) Siggia, S. "Quantitative Organic Analysis via Functional Groups", 3rd ed.; Wiley: New York, 1963; p 526.
- (3) Wolff, G.; Nurnberg, H. W. *Fresenius' Z. Anal. Chem.* **1965**, *216*, 169.
- (4) Bottei, R. S.; Furman, N. H. *Anal. Chem.* **1955**, *27*, 1182.
- (5) Tiwari, R. D.; Sharma, J. P. *Anal. Chem.* **1963**, *35*, 1307.
- (6) Tandon, J. P. *Fresenius' Z. Anal. Chem.* **1954**, *167*, 184.
- (7) Aikens, D. A.; Carlita, S. C., Sr. M. *Anal. Chem.* **1965**, *37*, 459.
- (8) Bourg, P.; Astrue, M.; Bonastre, J. *Analisis* **1975**, *3*, 252.
- (9) Sheytanov, C.; Neshkova, M. *Anal. Chim. Acta* **1970**, *52*, 455.
- (10) Bard, A. J.; Petropoulos, A. G. *Anal. Chim. Acta* **1962**, *27*, 44.
- (11) Lindbeck, M. R.; Freund, H. *Anal. Chim. Acta* **1966**, *35*, 74.
- (12) Bergman, I.; James, J. C. *Trans. Faraday Soc.* **1954**, *50*, 60.
- (13) Tomilov, A. P.; Mairanovskii, S. G.; Fioshin, M. Y.; Smirnov, V. A. "Electrochemistry of Organic Compounds", English Translation; Halsted Press: New York, 1972; p 248.
- (14) Reilley, C. N.; Cooke, W. D.; Furman, N. H. *Anal. Chem.* **1959**, *23*, 1223.

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## Normal Pulse Polarography with Carbon Fiber Electrodes for in Vitro and in Vivo Determination of Catecholamines

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**An electrochemical microsystem using three electrodes is described. The active surface of the working electrode is a carbon fiber of 8- $\mu$ m o.d. In normal pulse polarography, this electrode presents, in vitro, good sensitivity and selectivity, and a wide potential range. Using pulse amperometry, these electrodes can be used in vivo to study the release of dopamine in the rat neostriatum. The specificity and the stability of electrochemical response in acute or chronic preparations are discussed.**

Previous studies have shown that electrochemistry permits the in vitro determination of the catecholamines (dopamine (DA), norepinephrine (NE) and serotonin (5-HT) (1-6), and is also a possible "tool" for their in vivo determination. The concentrations of DA and its metabolite homovanillic acid (HVA), have been determined in the neostriatum and cerebrospinal fluid (CSF) (7-12) and that of 5-HT and its metabolite, 5-hydroxyindole acetic acid (5-HIAA) in the hippocampus and the CSF (13-16).

This paper describes a new type of working electrode with a carbon fiber (o.d. 8  $\mu$ m) for its active surface, and its electrochemical characteristics measured in vitro (potential range, sensitivity, resolution). The improved characteristics of this electrode in vivo are also discussed (17).

### EXPERIMENTAL

**Preparation of Electrodes.** The working electrodes are prepared with carbon fibers (8- $\mu$ m o.d., Le Carbone Lorraine, ref. Agt 10,000), graphite powder (1- $\mu$ m particles, Ultra Carbon, ref. UCP 1 M), polyester resin (Escil, ref. Sody 33), glass tubes (1.5-mm o.d.; 1.05-mm i.d., Clark Electromedical Instruments, ref. 26 C 150 T-4), and a "pipet puller" (David Kopf Instruments, ref. 700 C).

A dried glass tube is drawn using the pipet puller to obtain a tip diameter of few micrometers (Figure 1 A). A carbon fiber (length 20 to 40 mm) is threaded into the capillary (length 10 to 30 mm) until it is blocked by the fringed tip (Figure 1 B). The glass capillary is cut at the level where the fiber is blocked, thus enabling the fiber to be pushed a few millimeters through the

capillary. This method minimizes the interstitial space between the capillary and the carbon fiber. A microscope facilitates preparation of the electrode.

The capillary is inverted into the mixture, prepared with the graphite powder and the polyester resin (0.3 g of graphite powder for 350  $\mu$ L of resin) in order to fill 4-5 mm of the body with the paste (Figure 1C). An appropriate plunger was used to force the paste into the tip of the capillary (Figure 1D). At the tip of the capillary this resin is separated from the graphite powder, which ensures insulation from the electrode's inactive part (Figure 1E). Humidity should be avoided because it modifies the polymerization, and as well as the electrochemical characteristics. The other resins studied are too fluid (Ciba-Geigy, ref. Araldite E and HY 842) and produce drops of resin at the tip of the capillary or polymerize too fast and inhibit the good separation between the resin and the graphite powder at the tip of capillary.

A contact wire is then pushed as far as possible into the barrel filled with the paste. The electrodes are dried at least 24 h before use. Immediately before use in vitro or in vivo, the electrodes are cut to a length of 0.5 mm (Figure 2) (then, they have a resistance in the range of k $\Omega$ 's).

With the above preparation scheme, 7 to 8 electrodes out of 10 have the same electrochemical characteristics.

The reference electrode is constituted in vitro by a standard saturated calomel electrode (SCE) (Tacussel, ref. C 6), and in vivo by a micro Ag/AgCl electrode (a silver wire (o.d. 0.2 mm)) is coated with AgCl and inserted into a glass micropipet filled with a gelatin-salt solution (1 g of gelatin in 9 mL of 3 M NaCl).

A platinum wire (0.5-mm o.d.) is used in vitro and in vivo as the auxiliary electrode.

**Reagents.** All solutions are prepared with pyrodistilled water. Buffer solutions are prepared from analyzed reagent grade chemicals without further purification. Unless otherwise noted, the supporting electrolyte was a phosphate buffered saline solution (PBS pH 7.4). The following reagents were obtained from Sigma Chemical Company: dopamine hydrochloride, norepinephrine hydrochloride, 5-hydroxytryptamine hydrochloride, 5-hydroxyindoleacetic acid, 3,4-dihydroxyphenylalanine (DOPA), 4-hydroxy-3-methoxy-phenylacetic acid (HVA), 4-hydroxy-3-methoxymandelic acid (VMA), normetanephrine (NME), 3-methoxytyramine (MT) hydrochloride, epinephrine (E), ascorbic acid (AA). All solutions were made up just prior to use, and tested at 25  $^{\circ}$ C.

**Pulse Voltammetric Techniques.** The voltammetric measurements are made with a Tacussel model PRG 5 electrochemical system. Normal pulse polarography (NPP) is more satisfactory than direct current polarography, since the charging

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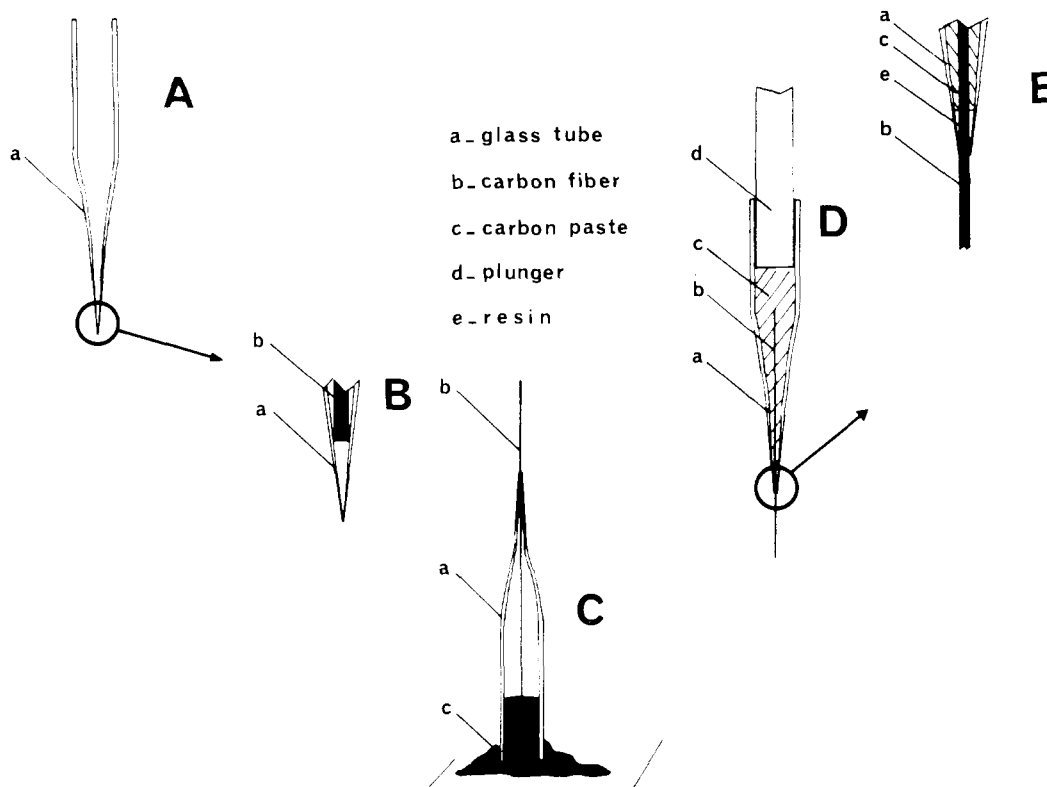


Figure 1. Different steps of preparation of carbon fiber electrodes (see text for details)

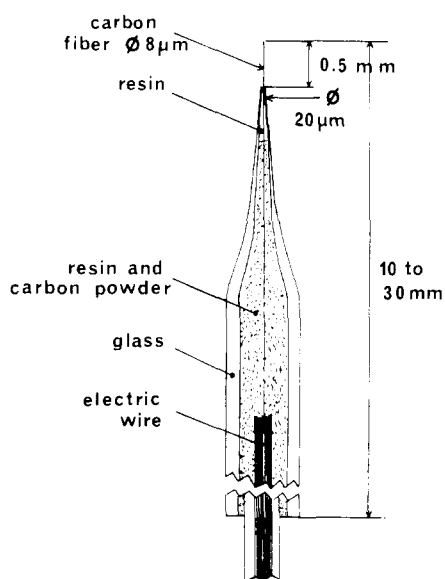


Figure 2. Scheme of standard carbon fiber electrode

current is minimized. The reactions at the electrode are also simpler because the potential returns to its initial value between each pulse (so the oxidized compound on the electrode surface is again reduced (18)). The analytical utility of the pulse technique with solid electrodes, especially for oxidation, has been recently developed by Soderhjelm (19).

In our study, the optimum parameters *in vitro* were  $-0.1$  V/SCE as initial potential ( $E_i$ ), a anodic sweep rate of  $10$  mV/s, a length of  $1$  s for the pulse cycle ( $T$ ), a duration of  $88$  ms for each pulse (including  $8$  ms of current measure).

*In vivo*, the "pulse amperometry" (every  $5$  s, a pulse of  $88$  ms brought the potential of the working electrode from  $0$  to  $0.5$  V vs. SCE) permits the recording of the variation of the oxidation current.

## RESULTS AND DISCUSSION

**Potential Range and Residual Current.** The experimental NPP current curves for PBS are shown in Figure 3.

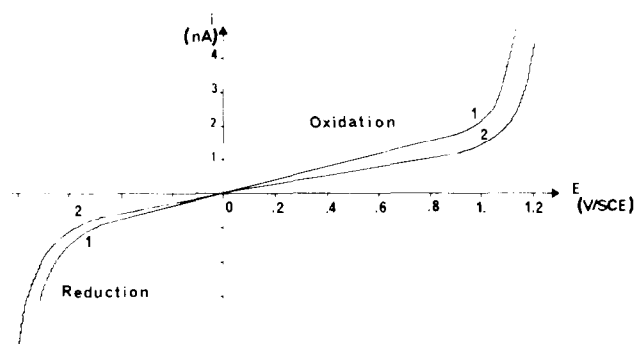


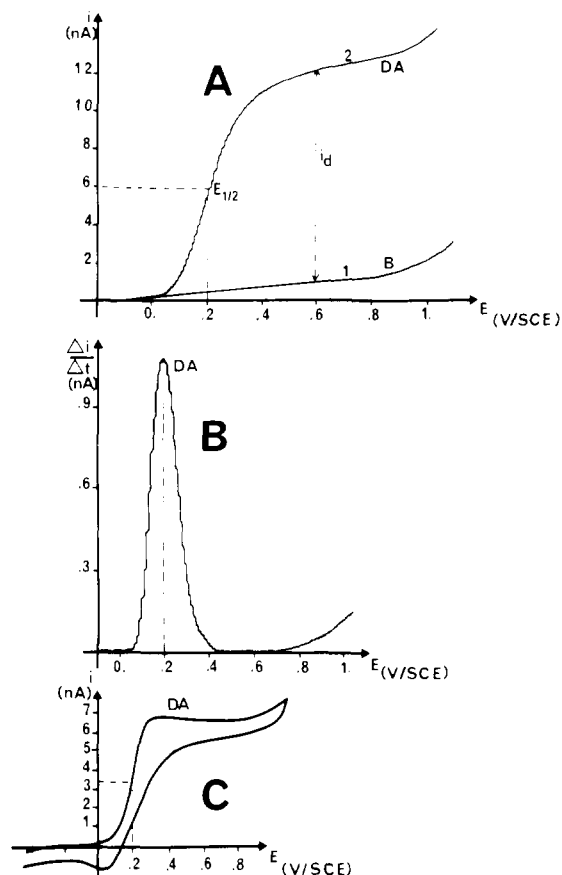
Figure 3. Experimental NPP (normal pulse polarography) current-potential curve for phosphate buffered saline (PBS). NPP parameters:  $T = 1$  s;  $t_p = 88$  ms;  $V = 10$  mV/s. Curve 1: first sweep. Curve 2: second sweep (and following ones)

The potential range of a carbon fiber electrode ( $-0.8$  V;  $+1.2$  V vs. SCE) is comparable to the carbon paste electrode but is more satisfactory than the treated platinum electrode (20, 21).

The residual current is smaller for the second sweep and following sweeps due to a electrochemical cleaning or conditioning of the surface. This residual current is between  $0.5$  and  $1$  nA (at  $+0.6$  V vs. SCE) for standard electrodes ( $0.5$  mm length of fiber).

**Oxidation of DA.** The oxidation pathways of catecholamines in "physiological" solutions have already been studied (22, 23): DA is oxidized to dopamine-*o*-quinone (DOQ) which can be intracyclized and again oxidized. But the rates of these secondary reactions for DA are such ( $t_{1/2}$  for second stage:  $2.7$  s) that they can be observed in cyclic voltammetry at very slow sweep rates and after several successive sweeps. On the other hand in the NPP each pulse is applied for a comparatively short time ( $88$  ms) and the reduction of DOQ back to DA occurs before the intracyclization.

The oxidation wave of DA/DOQ is shown by Figure 4A. The half wave potential ( $E_{1/2}$ ) is  $+0.2$  V vs. SCE; this result is verified by normal pulse polarography with "differential



**Figure 4.** Oxidation of dopamine (DA). All current-potential curves are plotted analogously to Figure 3, with oxidation current above the zero current line. (A) NPP (same parameters as in Figure 3). Curve 1: blank or PBS. Curve 2: DA  $3 \times 10^{-5}$  M. (B) NPP with "differential" detection of the current (same parameters as in Figure 3) for DA  $3 \times 10^{-5}$  M. (C) Cyclic voltammetry ( $V = 20$  V/min) for DA  $4 \times 10^{-5}$  M.

detection" of the current (Figure 4 B), and by cyclic voltammetry (Figure 4C).

The mathematical treatment of waves obtained in NPP showed that the oxidation is not completely reversible. The irreversibility of this system can be observed in NPP with "differential detection" of the current since the peak half width is three times higher than expected for a reversible system with a 2-electron oxidation. Furthermore, in cyclic voltammetry, the reduction peak is very small in relation to the oxidation peak and the gap between these peaks is larger than the theoretical value.

The irreversibility of this system explains the discrepancies between the experimental and theoretical values for plots of  $E_{1/2}$  vs. pH. According to Lewis (24), DA is a weak diprotic acid with  $K_{a1} \approx 10^{-9}$  mol L $^{-1}$  and  $K_{a2} \approx 10^{-12}$  mol L $^{-1}$ , therefore a standard treatment of  $E_{1/2}$  vs. pH for a reversible system should have a slope for DA of 0.06 V between pHs less than 6 (when  $H^+ \gg K_{a1}$ ) and a shift of 0.03 V per pH at about pH 7–8 (25). The experimental slope between pH 2 and pH 8 (0.076 V per pH) is greater than the theoretical value in the low pH range, also there is no indication of discontinuity at about pH 7–8 as is seen for the theoretical curve. This has also been observed for the carbon paste electrode (6).

**Sensitivity.** The diffusion current is proportional to the concentration between  $2.10^{-6}$  and  $10^{-3}$  M, and to the fiber length (or the area of the electrode) between 0.1 to 2 mm. Then the experimental equation (Equation 1) is in agreement with the theoretical expression for the current in NPP (26).

$$i_d = kC \cdot L = KCA \quad (1)$$

$k = K\pi d = 0.75$  nA  $\mu\text{M}^{-1}$  mm $^{-1}$ ;  $d$  = fiber diameter (mm);  $C$

**Table I.** Half-Wave Potentials of Neurotransmitters and Their Derivatives (Values Determined in NPP)

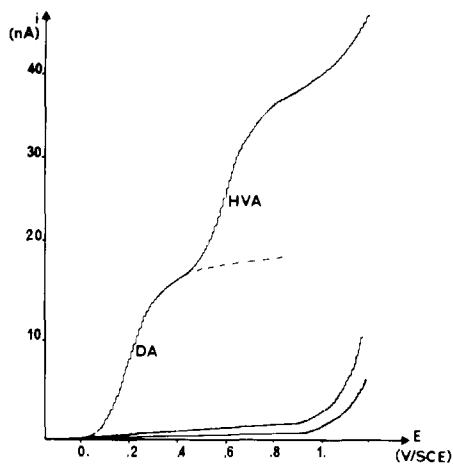
reagents	symbols	$E_{1/2}$ , mV/ SCE
dopa	<chem>Oc1ccc(cc1)C(C(=O)O)N</chem>	450
methoxytyramine	<chem>COc1ccc(cc1)CN</chem>	460
dopamine	<chem>Oc1ccc(cc1)CN</chem>	200
homovanillic acid	<chem>COc1ccc(cc1)C(=O)O</chem>	530
normetanephrine	<chem>COc1ccc(cc1)C(O)N</chem>	500
norepinephrine	<chem>Oc1ccc(cc1)C(O)N</chem>	240
vanilmandelic acid	<chem>COc1ccc(cc1)C(O)C(=O)O</chem>	570
epinephrine	<chem>Oc1ccc(cc1)C(O)CN(C)C</chem>	380
serotonin	<chem>Oc1ccc2c(c1)c(c[nH]2)CCN</chem>	340
5-hydroxy-indole 3-acetic acid	<chem>Oc1ccc2c(c1)c(c[nH]2)CC(=O)O</chem>	500

= initial concentration of oxidized specie ( $\mu\text{M}$ );  $L$  = fiber length (mm);  $A \approx \pi dL$  (mm $^2$ );  $i_d$  = diffusion current (nA).

In spite of the small surface of carbon fiber electrode ( $1.2 \times 10^{-4}$  cm $^2$ ) the detection limit is  $10^{-6}$  M which is very promising when compared to the carbon paste electrode (20).

**Oxidation of Neurotransmitters and Their Metabolites.** The broad potential range of this electrode in NPP allows a study of the oxidation of many compounds in the CNS such as the catecholamines and their metabolites, serotonin and its metabolite. Several other neurotransmitters cannot be oxidized: acetylcholine,  $\gamma$ -aminobutyric acid, and most of the amino acids.

Table I shows the anodic half wave potentials of the principal compounds. The direct electrochemical determination of mixtures of DA and NE is very difficult. The methoxy and nonmethoxy derivatives are well differentiated as shown in Figure 5 for DA and its principal metabolite (HVA). All of these compounds are better differentiated by the use of differential pulse polarography (DPP) or differential pulse cyclic voltammetry (10). The anodic half wave potential for AA ( $E_{1/2} = +0.45$  V vs. SCE) is shifted to more positive potentials than those for the catecholamines. This minimizes the overlap of DA by AA seen in vivo with the carbon paste electrode (7, 11). The transposition of these results in vitro to in vivo measurements in CSF is relatively easy (8), but this is not the case for the central nervous system (CNS) where the elements in the vicinity of the electrode are not well defined (27). Although brain tissue is highly conductive, this "solution" at the same time serves as supporting electrolyte,



**Figure 5.** Oxidation of dopamine (DA) and its metabolite, homovanillic acid (HVA). The current-voltage curve is plotted analogously to Figure 3. The NPP parameters are the same as in Figure 3 DA and HVA  $5 \times 10^{-5}$  M

the anodic half wave potentials are shifted to more positive potential (11). The principal oxidized compounds of the brain structure in the vicinity of the electrode are easily determined by differential pulse cyclic voltammetry.

As we have already shown in a previous publication (17), the following arguments suggest that the implantation of carbon fiber electrode in the neostriatum of a rat permits to measure in vivo the extracellular concentration of DA: (a) Injection of amphetamines or the death of the animal, which give an increase in the release of DA, produce an increase in the oxidation current having characteristics (amplitude and duration) similar to those already seen in other experimental approaches (28–30). (b) Moreover injection of methyl-*p*-tyrosine ( $\alpha$ -MTP) which causes an inhibition of catecholamine synthesis (31, 32), produces a decrease in the oxidation current. (c) Finally, the effect of amphetamines is eliminated after selective degeneration of the striatal dopaminergic terminals by injection of 6-hydroxydopamine (6-OHDA) into the substantia nigra (33) or after inhibiting the synthesis of the transmitter by  $\alpha$ -MPT (34).

Having established the specificity of this technique, it was very possible to monitor the release of DA for several days in unanaesthetized and free moving rats (chronic preparation). The stability of the carbon fiber electrode was demonstrated by injection of amphetamines (2 mg/kg i.p.) which produces a similar increase at the beginning (mean  $\pm$  SEM = 22%  $\pm$  5% (2nd day)) and the end of each experiment (means  $\pm$  SEM

= 21%  $\pm$  7% (30th day, 3 animals/7).

## LITERATURE CITED

- (1) Adams, Ralph N.; Hawley, M. Dave; Feldberg, Stephen W. *J. Physiol. (Paris)* **1967**, *71*, 851–855.
- (2) Adams, Ralph N.; *J. Pharmacol. Sci.* **1969**, *58*, 1171–1184.
- (3) Adams, Ralph N.; Murril, E.; McCreery, Richard L.; Blank, C. L.; Karolczak, M. *Eur. J. Pharmacol.* **1972**, *17*, 287–292.
- (4) Papouchado, L.; Petrie, G.; Adams, Ralph N. *J. Electroanal. Chem.* **1972**, *38*, 389–395.
- (5) Petek, M.; Bruchenstein, Stanley; Feinberg, B.; Adams, Ralph N. *J. Electroanal. Chem.* **1973**, *42*, 397–401.
- (6) Sternson, Arlene W.; McCreery, Richard L.; Feinberg, B.; Adams, Ralph N. *J. Electroanal. Chem.* **1973**, *46*, 313–321.
- (7) Adams, Ralph N. *Anal. Chem.* **1976**, *48*, 1126A–1137A.
- (8) Kissinger, Peter T.; Hart, Jonathan B.; Adams, Ralph N. *Brain Res.* **1973**, *55*, 209–213.
- (9) Lane, Ross F.; Hubbard, Arthur T.; Fukurraga, Ken; Blanchard, Robert J. *Brain Res.* **1976**, *114*, 346–352.
- (10) Lane, Ross F.; Hubbard, Arthur T.; Blaka, Charles D. *Bioelectrochem. Bioenergetics* **1978**, *5*, in press.
- (11) McCreery, Richard L.; Dreiling, Roger; Adams, Ralph N. *Brain Res.* **1974**, *73*, 23–33.
- (12) Wightman, R. Mark; Strobe, Elaine; Plotsky, Paul M.; Adams, Ralph N. *Brain Res.* **1978**, *159*, 55–68.
- (13) Adams, Ralph N. *Neurosciences* **1978**, in press.
- (14) Conti, James C.; Strobe, Elaine; Adams, Ralph N.; Mardsen, Charles A. *Life Sci.* **1978**, in press.
- (15) Wightman, R. Mark; Strobe, Elaine; Plotsky, Paul M.; Adams, Ralph N. *Nature (London)*, **1976**, *262*, 145–146.
- (16) Mardsen, Charles A.; Conti, James C.; Strobe, Elaine; Curzon, G.; Adams, Ralph N. *Brain Res.* **1978**, in press.
- (17) Gonon, François; Cespuccio, Raymond; Ponchon, Jean-Luc; Buda, Michel; Jouvot, Michel; Adams, Ralph N.; Pujol, Jean-François C. *R. Hebd. Seances Acad. Sci., Ser. D* **1978**, *286*, 1203–1206.
- (18) Christie, Joseph H.; Osteryoung, Robert A. *J. Electroanal. Chem.* **1974**, *49*, 301–311.
- (19) Soderhjelm, Petter J. *Electroanal. Chem.* **1976**, *71*, 109–115.
- (20) Adams, Ralph N. "Electrochemistry at solid electrodes"; Marcel Dekker: New York, 1969.
- (21) Lane, Ross F.; Hubbard, Arthur T. *Anal. Chem.* **1976**, *48*, 1287–1293.
- (22) Hawley, M. D.; Tatawawadi, S. V.; Pierkarski, S.; Adams, Ralph N. *J. Am. Chem. Soc.* **1967**, *89*, 447–450.
- (23) Brun, André; Rosset, R. J. *Electroanal. Chem.* **1974**, *49*, 287–300.
- (24) Lewis, G. P. *Brit. J. Pharmacol.* **1954**, *9*, 488–493.
- (25) Elving, P. J. *Pure Appl. Chem.* **1963**, *7*, 432–437.
- (26) Parry, E. P.; Osteryoung, Robert A. *Anal. Chem.* **1965**, *37*, 1634–1637.
- (27) Clark, L. C.; Sachs, G. *Ann. N. Y. Acad. Sci.* **1968**, *148*, 133.
- (28) Besson, Marie-Jo; Cheramy, André; Feltz, Paul; Glowinski, Jacques *Brain Res.* **1971**, *32*, 407–424.
- (29) Chiueh, C.; Moore, K. E. *J. Pharmacol. Exp. Ther.* **1975**, *192*, 642–653.
- (30) Nieoullon, André; Cheramy, André; Glowinski, Jacques J. *Neurochem.* **1977**, *28*, 819–828.
- (31) Besson, Marie-Jo; Cheramy, André; Gauchy, E.; Glowinski, Jacques *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1973**, *278*, 101–105.
- (32) Javoy, France; Glowinski, Jacques J. *Neurochem.* **1971**, *18*, 1305–1311.
- (33) Javoy, France; Sotelo, Constantino; Herbert, A.; Agid, Yves *Brain Res.* **1976**, *102*, 201–215.
- (34) Papeschi, R. *Psychopharmacologia* **1975**, *45*, 21–28.

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