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In Vivo Voltammetric Recording With Nafion-Coated Carbon Paste Electrodes: Additional Evidence That Ascorbic Acid Release is Monitored

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MUELLER, K. In vivo *voltammetric recording with nafion-coated carbon paste electrodes: Additional evidence that ascorbic acid release is monitored.* PHARMACOL BIOCHEM BEHAV **25**(2)325–328, 1986.—The response of nafion-coated carbon paste electrodes was studied *in vitro* and *in vivo* with linear sweep semidifferential voltammetry. *In vitro* nafion-coated electrodes were insensitive to ascorbic acid (AA) but were equally sensitive to dopamine (DA) as were the uncoated electrodes. *In vivo* (anterior caudate) nafion-coated electrodes recorded small, poorly defined peaks. However, nafion-coated electrodes were equally responsive to microinfusion of DA as observed with the uncoated electrodes. Nafion-coated electrodes were insensitive to micro-infusion of AA while uncoated electrodes showed a large response to AA. These data suggest that endogenous DA levels are below the sensitivity of carbon paste electrodes in caudate and that the endogenous peaks recorded with uncoated carbon paste electrodes reflect AA.

Linear sweep voltammetry Nafion Carbon paste electrodes Ascorbic acid

VOLTAMMETRIC recording *in vivo* offers the exciting possibility of monitoring dopamine (DA) and serotonin (5HT) release in multiple brain areas of behaving organisms. Many aspects of brain-behavior relationships could therefore be easily studied with voltammetry. However, a consistent problem with voltammetry has been the difficulty of distinguishing between DA release and ascorbic acid (AA) release [7]. This paper presents additional data which support the contention that carbon paste voltammetric electrodes monitor AA release rather than DA release.

Linear sweep voltammetry with carbon paste electrodes has often been used to monitor DA release [2, 9, 10, 12]. However, the ability of this technique to distinguish between AA release and DA release has been very controversial. To complicate matters, extracellular AA levels are often increased by the same stimuli that increase DA release; for example, amphetamine increases release of both DA and AA [14]. Some investigators have attempted to solve the problem by monitoring subtle changes in the position of the signal or by using microprocessor based data analysis [9, 10, 12]. Various modifications of the working electrode have also been proposed to enhance selectivity of the electrodes [6]. One approach is to coat the electrodes with nafion, a polymer which is impermeable to anions [5]. The following research compared the response of nafion-coated carbon paste electrodes to uncoated electrodes. The data suggest that carbon paste electrodes are completely insensitive to levels of DA and the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) found in the extracellular space of anterior caudate, a DA rich area of brain.

METHOD

Electrochemical Recording

Carbon paste working electrodes were fabricated from teflon coated silver wire (Medwire, o.d. 0.013) and a silicone oil based carbon paste as previously described [8]. Some working electrodes were coated with 5% nafion (C. G. Processing, Rockland, DE). The efficiency of coating varied greatly; to obtain consistent results, electrodes were dipped in nafion 10 times with at least a 2 minute interval between dips (this procedure ensured a dramatic reduction of the AA signal *in vitro*). A silver-silver chloride reference electrode was constructed by placing an anodized silver wire in a disposable pipette tip containing 3 M NaCl. A silver wire (attached to a skull screw *in vivo*) served as an auxiliary electrode.

A DCV5 voltammetry controller (BAS) was used to perform linear sweep (−100 to +500 mV at 10 mV per second) semidifferential voltammetry. Electrodes were scanned every 12 minutes. For *in vivo* studies the DCV5 was interfaced with an Apple II+ microcomputer for remote operation. For *in vitro* studies chemicals were prepared in a phosphate buffer 0.1 M pH 7.4. Electrodes were tested in a series of solutions; some electrodes were then coated with nafion and the series was repeated.

Surgery and In Vivo *Testing*

Four working electrodes were implanted in the anterior caudate of male Wistar rats with two on each side of the

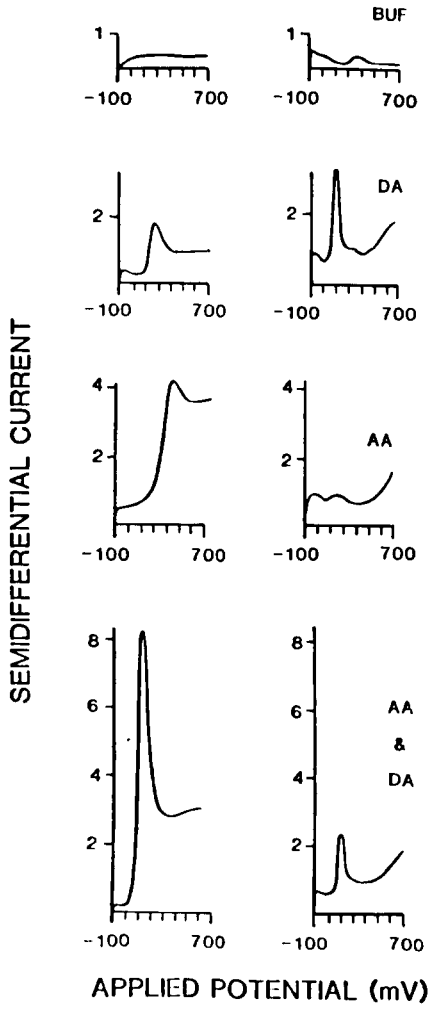

FIG. 1. Representative *in vitro* response of a carbon paste electrode before (left) and after (right) coating with nafion. (Uncoated electrodes which were taken through the series twice showed similar responses on both the first and second series.)

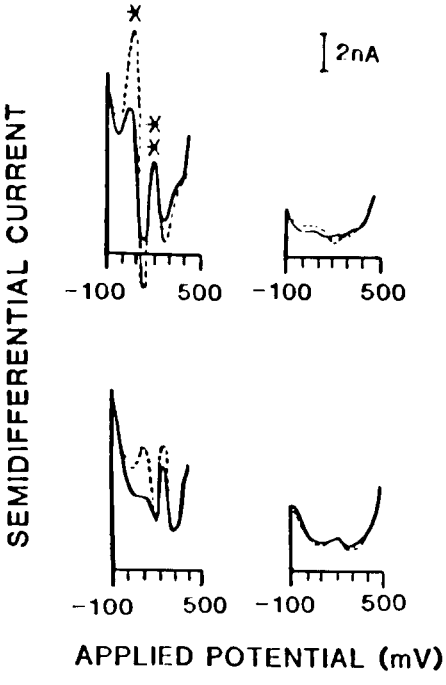

FIG. 2. Representative *in vivo* (anterior caudate) scans from two nafion-coated electrodes (right) and the uncoated electrodes implanted adjacent to them (left). The solid lines are baseline scans; the dashed lines are after 4 mg/kg amphetamine (subcutaneously). The large "signal" on the far left is charging current and is not considered a peak. Peak 1 is identified by a single asterisk; peak 2 identified by a double asterisk.

brain (3.0 mm anterior to bregma, 4.8 mm below the cortex, 2.8 mm lateral [13]). Usually, one working electrode on each side of the brain was nafion-coated and the other was uncoated. Occasionally a guide cannula (Plastic Products) was implanted unilaterally to allow micro-infusion of substances into the brain and onto the surface of two electrodes. Surgery was performed under Nembutal (50 mg/kg) anesthesia; 48 hours were always allowed for recovery. Placement of electrodes was always verified histologically after completion of testing.

During each testing session recording was conducted for two hours to ensure a stable baseline before infusion or injection. Chemicals were infused in phosphate buffer 0.1 M pH 7.4 at a speed of 0.5 μl per minute. The total infusion volume was always 1.0 μl. All *in vivo* experiments were conducted in conscious behaving animals.

RESULTS

Representative scans from the *in vitro* studies are shown in Fig. 1. After being coated with nafion the electrodes often exhibited an enhanced response to 0.1 mM DA(uncoated electrodes which were taken through the series twice showed very similar responses in both the first and second series). Coating the electrode with nafion dramatically reduced the signal in 0.5 mM AA and in a solution of both DA and AA.

Representative scans from the *in vivo* studies are shown in Fig. 2. The coated electrodes did not record well defined peaks while the uncoated electrodes adjacent to them virtually always recorded at least two distinct peaks. When six rats were injected with 4 mg/kg amphetamine the uncoated electrodes always showed a dramatic increase in both peaks 1 and 2 after amphetamine; the coated electrodes were apparently unaffected by any neurochemical changes produced by the amphetamine. In general the nafion coated electrodes had a much smaller background current than the uncoated electrodes.

To confirm that the coated electrodes were still responsive to DA *in vivo*, DA was microinfused onto the electrodes *in situ* in seven rats. Figure 3 shows the response to microinfusion of DA of an uncoated electrode and the coated electrode implanted adjacent to it in two different animals. The dramatic peak confirms that the nafion-coated electrode is, in fact, responsive to DA *in vivo* and indicates that the nafion coated electrode is just as sensitive to DA as the uncoated electrode.

Figure 4 shows the response of adjacent coated and uncoated electrodes to microinfusion of DOPAC (3.3 μg) and AA (3.3 μg). As expected the uncoated electrodes record a dramatic increase in peak 1 while the coated electrodes are

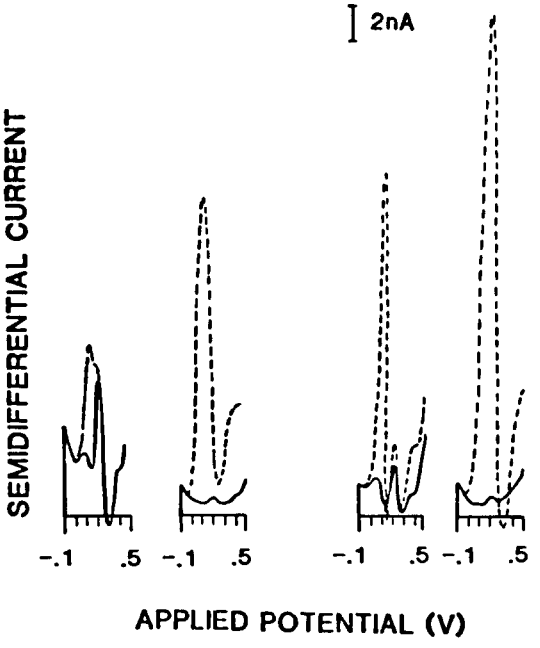

FIG. 3. Response *in vivo* of two nafion-coated electrodes (right) and the uncoated electrode implanted immediately adjacent to it (left) to microinfusion of DA. The solid lines are baseline scans; the dashed lines are after infusion of DA. The two scans to the far left show the response to 3.0 μg of DA; the 2 scans on the right show the response to 4.5 μg of DA.

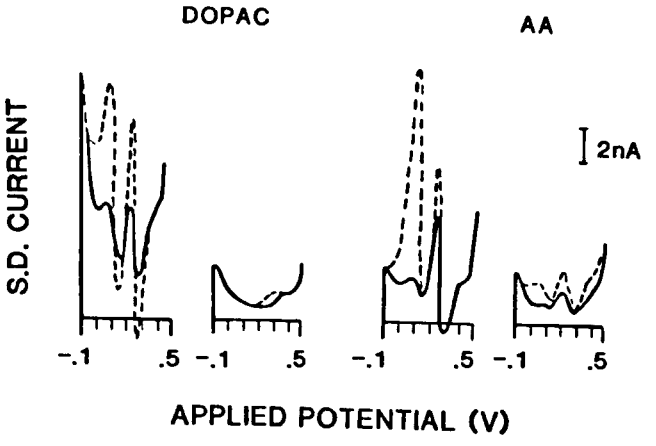

FIG. 4. Response *in vivo* of a nafion-coated electrode (right) and the uncoated electrode implanted adjacent to it (left) to microinfusion of 3.3 μg DOPAC and 3.3 μg AA. The solid lines are baseline scans; the dashed lines are after infusion. Semidifferential (SD) current is shown.

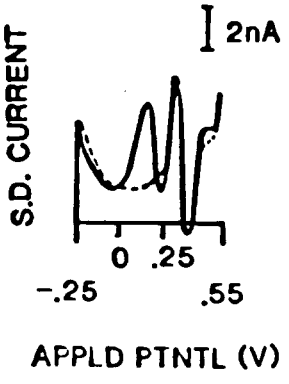

FIG. 5. Response of an uncoated electrode to microfusion of 0.01 unit of ascorbate oxidase. The solid line is baseline current; the dashed line is after infusion. Semidifferential (S.D.) current is shown.

either unaffected or record only a slight increase in signal. Thus, the nafion coated electrodes are insensitive to both DOPAC and AA *in vivo* as well and *in vitro*.

These data strongly suggest that peak 1 recorded by uncoated carbon paste electrodes from anterior caudate is due to the oxidation of an acidic substance—either AA or DOPAC. Microinfusion of 0.01 unit ascorbate oxidase (Sigma; 1700 units per mg protein) eliminates peak 1 within 30 minutes as shown in Fig. 5. Since ascorbate oxidase does not interfere with the DOPAC signal *in vitro* (data not shown) the data suggest that peak 1 is due to oxidation of AA rather than DOPAC.

Although nafion-coated electrodes were successful in eliminating the AA signal in the short term, caution is advised when using these electrodes over a period of days. As shown in Fig. 6, the response profile of the electrodes changed after about 10 days perhaps because the nafion does not adhere to the oil based paste over the long term.

DISCUSSION

Coating electrodes with nafion was suggested to enhance the selectivity of voltammetric electrodes by preventing AA from gaining access to the electrode [5]. The data obtained from *in vitro* recordings confirmed that nafion-coated carbon paste electrodes were insensitive to AA without a loss in sensitivity to DA. But when implanted in anterior caudate, the nafion coated electrodes did not record well defined peaks. Because the nafion-coated electrodes were just as sensitive to microinfusion of DA as the uncoated carbon paste electrodes, the failure of nafion coated electrodes to record a prominent signal would seem to be because the

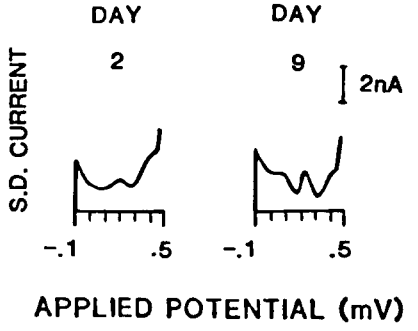

FIG. 6. Response *in vivo* of the same nafion-coated electrode on day 2 and day 9 (after implantation). Semidifferential (S.D.) current is shown.

species responsible for the signal is either AA or DOPAC. Since the signal can be eliminated by microinfusion of ascorbate oxidase, one must conclude that peak 1 recorded by uncoated carbon paste electrodes reflects the oxidation of AA not DOPAC.

The elimination of peak 1 by ascorbate oxidase has been reported previously [1] and is strong evidence that carbon paste electrodes are recording AA rather than DOPAC. One might counter that AA is somehow necessary for proper neuronal function and that in its absence DA is not released. However, the lack of effect of ascorbate oxidase on peak 2 argues against this hypothesis; peak 2 is apparently sensitive to changes in neuronal activity since it is affected by various psychoactive drugs [8,11].

Although coating electrodes with nafion successfully eliminated the AA signal, it also eliminated peak 2 recorded by carbon paste electrodes. Thus, one might suspect that peak 2 reflects the oxidation of some acidic substance. Indeed several recent reports indicate that peak 2 reflects the oxidation of uric acid, particularly when carbon paste electrodes are used [3, 8, 11]. Peak 2 is dramatically reduced by allopurinol (which inhibits the synthesis of uric acid) and peak 2 is eliminated by microinfusion of uricase. Thus, the failure of nafion-coated electrodes to record a prominent peak 2 is consistent with previous research on peak 2.

Amphetamine produces a dramatic increase of extracellular AA in striatum which correlates with the electrochemical response to amphetamine [9]. One might suggest that the electrochemical response to amphetamine and to other drugs as well may reflect AA changes rather than catechol changes when carbon paste electrodes are used.

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