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“Negative Electrocatalysis”-Based Specific Analysis of Dopamine at Basal Plane HOPG in the Presence of Structurally Related Catecholamines

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Abstract.

Oxidations of dopamine and structurally related catecholamines were shown to proceed with the essentially different overpotentials at basal plane HOPG electrodes as compared to glassy carbon electrodes, consistent with the concept of “negative electrocatalysis”. Electrode reactions of norepinephrine, epinephrine, and catechol slowed down on HOPG to a higher extent than oxidation of dopamine. Larger overpotentials for norepinephrine, epinephrine, and catechol oxidations allowed analytical discrimination and specific 0.05 to 4 μM detection of dopamine in an artificial cerebrospinal fluid in the presence of 0.5 M ascorbic acid and excessive amounts of norepinephrine, epinephrine and catechol. The results offer a new simple and powerful strategy for designing electrodes for specific analysis of dopamine in biological systems.

Keywords. Dopamine; Norepinephrine; Catechol; Basal plane, Highly oriented pyrolytic graphite (HOPG), Negative electrocatalysis.

1. Introduction

Detection of dopamine in the presence of structurally-related catecholamine neurotransmitters represents one of the largest challenges in neuroscience and neuropathology research [1]. Specific electroanalysis of co-existing catecholamines is strongly interfered by their very close redox potentials, predetermined by the common to all of them catechol group (**Figure 1**) [2]. Numerous modified-electrode approaches for the electrocatalytic discrimination of dopamine oxidation from those of ascorbic and uric acids have been suggested [3, 4], with hitherto no success in specific detection of dopamine over other catecholamines. Strategies for specific bioelectroanalytical recognition of dopamine, based on the aptamer electrodes [5, 6] and laccase microreactors [7] for *in vitro* detection of dopamine in serum and brain microdialysates, are not yet applicable for *in vivo* analysis. Tyrosinase electrodes used for analysis of dopamine in the brain showed a good performance, but their general O₂-and catechol-dependence may interfere with the assay robustness [8].

Figure 1. Electrochemical transformation of dopamine (DA), norepinephrine (NE), epinephrine (E), as discussed in [9-11].

Recently, Compton and coworkers introduced the concept of “negative electrocatalysis” as a valuable analytical alternative to the routinely used “electrocatalysis” approach, which might enable beneficial voltammetric discrimination between an analyte and interferents [12]. In contrast to the frequently experimentally targeted electrocatalytic reduction of the reaction overpotential for the analyzed species enabling better discrimination of the analyte over interferents whose electrode kinetics remains unchanged, in “negative electrocatalysis” not acceleration but slowing down of the reaction

of interest should allow such analytical discrimination. Unusual surface properties and electrochemical reactivity of basal plane highly ordered pyrolytic graphite (HOPG), composed of stacked, nearly atomically smooth hexagonal sp^2 carbon planes [13] at which electrochemical reactions generally slow down both kinetically and thermodynamically [14, 15], as well as a very special electrochemical behavior of dopamine at such and similar surfaces [12, 16] forced us to study HOPG electrodes as a potential platform for the “negative electrocatalysis” – based discrimination between dopamine and other catecholamines. Since dopamine oxidation at HOPG proceeds at potentials higher than at other carbon electrodes [16], we suggested that electrochemical oxidations of structurally related catecholamines could also proceed at increased overpotentials, different from those of dopamine, and that could allow specific detection of dopamine with no interference from other catecholamine neurotransmitters.

Here, we show that electrochemical oxidations of dopamine and such related catecholamines as norepinephrine, epinephrine and catechol slows down on the basal plane HOPG electrode compared to glassy carbon, and it allows electrochemical discrimination and specific detection of dopamine at 5-6-fold excessive concentrations of related species, also in the presence of ascorbic acid.

2. Experimental

All reagents were from Sigma-Aldrich (Germany) and used as received; solutions were prepared using ultrapure Milli-Q (MQ, 18.2 M Ω cm at 25 °C) water (Millipore, Bedford, USA). Catecholamine solutions were freshly prepared either in 20 mM phosphate buffer solution containing 0.15 M NaCl (PBS) or in an artificial cerebrospinal fluid (CSF: 108 mM NaCl, 2.5 mM KCl, 8.2 mM MgCl₂, 2 mM CaCl₂, 4 mM NaHCO₃, 1 mM Na₂HPO₄, 10 mM glucose), both pH 7.4, on the day of the experiments and kept in the dark when not in use. Glassy carbon (GC) electrodes ($\varnothing$ 3 mm) were from CH Instruments Inc. and basal plane HOPG (ZYH grade) was from Bruker (both USA).

HOPG electrodes were prepared 15-20 min before electrochemical measurements, by peeling off a top layer of HOPG by a single-sided Scotch tape, which was then placed, with its HOPG side facing solution, into the homemade Teflon holder with a 3 mm diameter window. GC electrodes were polished in 0.1 μm alumina slurry and ultrasonicated in MQ water for 10 min afterwards. Electrochemical measurements were performed at 23 ± 1 °C in a three-electrode cell connected to a potentiostat Autolab PGSTAT 302N (Eco Chemie B.V., the Netherlands) equipped with NOVA 1.10 software. An Ag/AgCl (3 M KCl) and a Pt wire were the reference and counter electrodes, correspondingly. Reproducibility of the results was verified by measurements with at least three independently prepared electrodes, and the average values were reported.

3. Results and Discussion

Compared to the routinely used GC electrodes, exhibiting a reversible electrochemistry of dopamine transformation [13], with a formal potential of 151 ± 3 mV at the studied pH (an oxidation wave starting from 70 mV for a 50 mV s^{-1} potential scan rate), voltammetric oxidation of dopamine on the HOPG electrode proceeded with an overpotential of 126 ± 10 mV vs. the process at GC as evaluated from the half-wave potential differences, oxidation starting at 146 ± 3 mV (**Figure 2A, Table 1**). Thus, the reaction of dopamine oxidation slowed down on HOPG compared to GC, on the latter being fast and reversible, as earlier discussed, due to the presence of essentially large number of surface oxygen functionalities [13, 17]. Therewith, oxidations of both norepinephrine and catechol at HOPG proceeded at potentials higher than those of dopamine (**Table 1**), with essential overpotentials versus their oxidations on GC, 213 ± 4 and 178 ± 3 mV for norepinephrine and catechol oxidations, respectively (as determined from the half-wave potential difference, **Figure 2B,C, Table 1**).

Figure 2. Representative CVs (1st scans) recorded with (1,1') GC and (2,2') HOPG electrodes in (1',2') blank CSF and (1,2) in the presence of 10 μ M (A,D) dopamine, (B,E) norepinephrine and (C,F) catechol.. The potential scan rate was (A-C) 0.05 and (D-F) 10 V s⁻¹.

At low scan rates, single, well-separated waves of dopamine and other catecholamine (with this term we will refer also to catechol) oxidations on HOPG could be followed in cyclic voltammograms (CVs) (**Figure 2A-C**), (**Figure 2D-F**). Therewith, at high scan rates, the overpotentials for catecholamine oxidations at HOPG (relative their oxidations at GC) decreased and the redox couples' formal potentials became closer to those observed on GC electrodes (**Table 1**).

The obtained results gave rise to two questions: 1). Why electrochemical oxidations of catecholamines slow down on basal plane HOPG, namely, if the phenomenon is truly related to the concept of “negative electrocatalysis”, and 2). What is the reason of such a different electrochemical

behavior of dopamine and structurally related catecholamines, compounds having the very same redox centers, on the basal plane HOPG electrodes?

Table 1. The formal potentials (E^0) and anodic peak half-wave potentials ($E_{pa\ 1/2}$) for dopamine (DA), norepinephrine (NE) and catechol (CA) transformations in CSF, pH 7.4						
	Basal plane HOPG			GC		
	DA	NE	CA	DA	NE	CA
E^0 , mV ^(a)	n.d.	n.d.	n.d.	151±3	188±2	158±1
$E_{pa\ 1/2}$, mV ^(a)	246±7	375±6	328±3	132±3	167±2	151±1
E^0 , mV ^(b)	229±9	247±5	229±7	174±1	198±1	186±3
Evaluated from CVs at ^(a) 0.05 and ^(b) 10 V s ⁻¹ . n.d.: not detectable due to the irreversibility of the process (only anodic process could be followed)						

Since electrochemical oxidations of catecholamines proceeded with the essential overpotentials and showed significantly different patterns on basal plane HOPG (and not on GC) surfaces, then the observed differences in electrochemical oxidations were assumed to be connected with a different surface reactivity of the HOPG electrodes relative studied compounds. First, we explored if our results were consistent with the concept of the basal plane HOPG reactivity proposed by Compton and coworkers [18]. Within this model, the heterogeneous surface of basal plane HOPG is approximated by basal-plane surface regions of low (referred to as “negative”) electrocatalytic reactivity decorated by edge-plane areas where an electrode reaction can proceed at essentially higher electron transfer (ET) rates [18]. The voltammograms recorded should then reflect the ET heterogeneity of such surfaces and represent a balance of reactions that can occur at either of these surface areas.

Consistent with the model, at low scan rates, slow irreversible oxidation of dopamine, norepinephrine and catechol proceeded predominantly at the basal-plane regions dominating the heterogeneous HOPG surface, with a low contribution from the edge-plane regions to the overall surface reactivity (**Figure 2A-C**). That allowed the beneficial “negative electrocatalysis” discrimination between dopamine and other catecholamines. At high scan rates, voltammetric signals appeared to be strongly

affected by the composition of the working solution. At HOPG, both in CSF and PBS, irreversible oxidation waves observed in low-scan rate CVs transformed into CVs characteristic of a quasi-reversible redox-couple at scan rates exceeding $1\text{--}2\text{ V s}^{-1}$ (**Figure 2A-C** versus **2D-F** and **Figure 3A**). Only in CVs recorded in PBS the contribution from “fast” edge-plane regions started to shape CVs and the oxidation wave shifted to less positive potentials as compared to the oxidation wave in CSF (**Figure 3A**) by this masking and even almost annihilating the “negative electrocatalysis” – based oxidation potential difference between dopamine and other catecholamines. Coexistence of two oxidation processes, simultaneously proceeding at “slow” and “fast” HOPG regions, could be also followed through the decreased peak separation and peak broadening/its eventual splitting into two peaks (exhibited as appearance of a hump at 0.7 V , in the anodic wave, **Figure 3A**), correlating with the fast-scan-rate triggered peak splitting predicted for heterogeneous surfaces of different reactivity [18]. Those effects were not observed at energetically uniform GC (**Figure 3B**) and support the assumption that at lower scan rates oxidations occurred predominately at basal plane regions of HOPG and thus could be related to the “negative electrocatalysis” phenomenon.

Figure 3. Representative CVs (1st scans) recorded with (A) HOPG and (B) GC electrodes in solutions of $30\text{ }\mu\text{M}$ dopamine in (1) PBS and (2) CSF, both pH 7.4, at a scan rate of 10 Vs^{-1} .

It is important to stress that any peak splitting was absent in CSF, while the phenomenon of “negative electrocatalysis” did not totally disappear with increasing scan rate, but was minimized. As a matter

of fact, at high scan rates electrochemical oxidation of dopamine in PBS followed the mixed diffusion/surface-confined ET mechanism, while in CSF it was purely diffusion limited (based on analysis of scan rate and square-root scan rate dependences of the oxidation peak currents). Thus, the composition of a buffer solution, in particular, the presence of organic matter [15], can have a strong effect on the electrochemical properties/reactivity of basal plane HOPG, consistent with previous reports on varying surface and electrochemical properties of freshly cleaved and exposed to air/air-aged basal plane HOPG surfaces [14-16], exhibiting a whole spectra of electrochemical reversibility/irreversibility in reactions with solution-present redox species.

Another question was the reason for the different electrochemical behavior of dopamine and other catecholamines on basal plane HOPG surfaces. Since the redox transformation of catecholamines is not just a two electron transfer reaction but is proton transfer-coupled (**Figure 1**), then the proton transfer step can be implicated and contribute in a variety ways to the kinetics of catecholamine oxidation, either by affecting the overall rate of oxidation of species differently interacting with HOPG or by introducing local pH changes at the electrode surface [19]. Our recent studies on electrodeposition of IrO_x nanoparticles on basal plane HOPG show that the working solution pH strongly affects surface properties of HOPG, including its wettability and ability to accommodate nanoparticles (unpublished data). Similarly, a local decrease in the effective pH at the HOPG surface that can occur during catecholamine oxidations and be different for dopamine and related catecholamines, may be responsible for observed differences in kinetics and actually thermodynamics of the electrode reactions at HOPG.

Thus, a more pronounced “negative electrocatalysis” [12] observed for norepinephrine (very similar data for epinephrine were obtained) and catechol oxidations on HOPG as compared to dopamine allowed discrimination between dopamine and those catecholamine oxidation signals (**Figure 4A**). It was used for specific analysis of dopamine in CSF at its concentrations corresponding to *in vivo*

conditions (within the 10 nM (basal levels) to 4-6 μ M range) [20, 21], in the presence of norepinephrine, epinephrine, catechol and ascorbic acid (on GC those processes overlapped).

As can be seen from differential pulse voltammograms (DPVs), signals from dopamine and epinephrine/catechol were well-separated (**Figure 4A**), the latter having very small interference with the dopamine oxidation process. Norepinephrine had eventually no effect on the dopamine oxidation currents at the DPV half-wave potential of around 0.1 V, though at comparable concentration levels (**Figure 4A, inset**). In the presence of the physiologically relevant level of ascorbic acid, which oxidation proceeds at lower potentials, the oxidation peaks of dopamine and ascorbic acid were also well-separated, and once the ascorbic acid concentration was kept constant, it did not affect analysis of dopamine (**Figure 4B**).

Figure 4. (A) Representative DPVs, corrected for background signals, recorded with the HOPG electrodes in 10 μ M solutions of (1) dopamine, (2) epinephrine, (3) catechol and a mixture of (4) 10 μ M dopamine, 10 μ M norepinephrine and 500 μ M ascorbic acid in CSF; Inset: DPV signals from 10 μ M (1) dopamine in CSF spiked with (2) 10 μ M norepinephrine and (3) 20 μ M norepinephrine and normalized for the dopamine peak current signal ($I_{\max}$) recorded in (1). Modulation amplitude: 50 mV, modulation time: 50 ms, step potential: 5 mV, apparent scan rate: 10 mV s⁻¹. (B) Amperometric current responses recorded at 0.1 V with the HOPG electrodes in CSF upon successive additions of (1) dopamine, (2) epinephrine, (3) norepinephrine and (4) catechol, in the presence of 500 μ M ascorbic acid. Inset: data for the GC electrodes, all the rest conditions as in the main figure. (C) Representative CVs (1st scans) recorded with the HOPG electrodes in solutions of 10 μ M (1) dopamine, (2) norepinephrine, (3) epinephrine and (4) catechol in CSF, potential scan rate: 0.05 V s⁻¹.

Chronoamperometric (CA) detection of dopamine was performed at the HOPG electrodes at 0.1 V.

The electrode response to increasing concentrations of dopamine was linear within the 0.05 to 4 μ M

concentration range, and the limit of dopamine detection was 40 ± 5 nM (**Figure 4B**). CA responses of norepinephrine at this potential were significantly lower, which allowed detection of dopamine over an almost four-fold excess of norepinephrine. Importantly, epinephrine and catechol, also demonstrating slow kinetics of oxidation at HOPG at potentials approaching that of norepinephrine, were even less interfering with dopamine detection (**Figure 3B**). Such signal discrimination was not possible at GC ((**Figure 3B, inset**)). Those results were obtained in the presence of 0.5 M ascorbic acid (roughly correlating with the ascorbic acid levels in the brain). It should be emphasized that the detection potential could be further decreased to improve discrimination between dopamine and other catecholamines (**Figure 4C**), though, it would result in lower sensitivity of analysis. Thus, the “negative electrocatalysis” concept appeared to be most productive for electrochemical discrimination of dopamine oxidation from other catecholamine oxidations, under conditions close to physiological ones. This approach is promising for further design of electrodes with surface ET properties useful for specific detection of dopamine in mixtures of structurally related compounds, either directly by CA (**Figure 3B**) or by electrochemical recycling at microarray electrodes [22, 23]. Due to the “negative-electrocatalysis” features of the heterogeneous basal plane HOPG surface becoming less pronounced at high potential scan rates fast scan voltammetry currently seems to be less suitable for practical “negative-electrocatalysis”-based analytical applications.

4. Conclusions

We showed that oxidations of dopamine and norepinephrine (as well as epinephrine and catechol) slowed down at basal plane HOPG electrodes and proceeded at potentials that allowed analytical discrimination between dopamine and structurally related catecholamines as well as dopamine detection in CFS in the presence of ascorbic acid and norepinephrine, epinephrine and catechol. The established phenomenon illustrates the concept of “negative electrocatalysis” and offers

technologically advanced, simple and powerful tools for designing electrodes for specific analysis of dopamine in mixtures, including the development of microelectrodes for *in-vivo* analysis.

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