Adsorption of Catecholamines on a Gold Electrode Surface Studied by Specular Reflection Measurement

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The adsorption of catecholamines including norepinephrine, epinephrine, isoproterenol, dopamine, dopa and homocatechol on a gold electrode surface was studied in phosphate buffer of pH 6.9 by specular reflection measurement. Norepinephrine is oxidized at potentials more positive than +0.1 V vs. saturated calomel electrode (SCE) and adsorbed at the potential range from -0.7 to -0.05 V. Its antagonists, phenoxybenzamine, tolazoline, ergotamine, oxprenolol and propranolol, are also adsorbed on the electrode, more strongly than norepinephrine. The adsorption behavior of catecholamines on the electrode surface may reflect electrostatic interactions between adrenergic transmitter substances and their receptors.

Keywords — catecholamine; adsorption; gold electrode; specular reflectivity; adrenergic receptor; electrode-solution interface

Catecholamines, including norepinephrine, dopamine and epinephrine, are of considerable biological and pharmacological importance owing to their involvement in chemical neurotransmission processes and their usefulness as drugs for treating various clinical disorders such as hypertension, shock, cardiac failure and arrhythmia, asthma, allergy and anaphylaxis.

Norepinephrine is the transmitter of most sympathetic postganglionic fibers and certain tracts in the central nervous system. Dopamine is the predominant transmitter of the mammalian extrapyramidal system and several mesocortical and mesolimbic neuronal pathways. In an adrenergic neurotransmission system, neurotransmitter substances such as norepinephrine and dopamine travel by convective or diffusive mass transport in the synaptic gap and reach receptor sites on the postsynaptic membrane. Interaction between transmitter substances and receptors results in the generation of new electrical signals to cause neurotransmission.

Hirono et al. calculated the electrostatic potential at the molecular surface of a dopamine targeting dopamine receptor, using Cartesian coordinates and Mulliken net atomic charges obtained by the semiempirical molecular orbital method. The electrostatic potential image indicated that a small absolute potential region (-2 --2 kcal/mol) exists perpendicular to the catechol ring plane, on one side of which there is a positive potential region...
The electrostatic potential of most of the molecular surface was within ±6 kcal/mol. Thus, the dopamine molecule may be capable of interacting electrostatically with surfaces charged within ±0.3 eV. In our preliminary experiment on the adsorption of catecholamines, the electroosorption of dopamine seemed to occur in the potential region within about ±300 mV vs. point of zero charge (pzc), which is in fair agreement with the above charge. This implies that the adsorption behavior of transmitters on an electrode surface may reflect the electrostatic interactions between transmitters and the receptor surface.

Therefore, we carried out a study on the adsorption of adrenergic transmitters and related substances onto a gold electrode surface as a model of a certain type of interactions between transmitter substances and receptors. Since an electrode surface may be regarded as a very simplified biosurface charged negatively or positively and in contact with an electrolyte solution, an understanding of the surface behavior of these substances should help to elucidate the electrostatic interactions involved in their neurochemical behavior at receptor sites. Specular reflection was used to detect trace amounts of adsorbed species on electrode surfaces with high sensitivity.

Experimental

Reagents—L-Epinephrine, DL-isoproterenol hydrochloride, ergotamine tartrate, oxprenolol hydrochloride and tolazoline hydrochloride were obtained from Sigma Chemical Company. L-Norepinephrine and phenoxybenzamine hydrochloride were from Tokyo Kasei Industries Ltd. and dopamine hydrochloride from Wako Pure Chemical Industries Ltd. L-Dopa and propranolol hydrochloride were purchased from Nakarai Chemicals Ltd. All reagents were used without further purification.

Phosphate solutions (0.067 M, pH 6.9 and pH 9.3) served as supporting electrolytes and were prepared from phosphate salts (NaH₂PO₄, K₂HPO₄, Na₃PO₄) and freshly purified water by using a Barnsted D-27942 three-module system.

Instruments—The apparatus for specular reflectivity measurements, coupled with a Hokuto Denko HA-101 potentiostat and Hokuto Denko HB-107A function generator, was the same as that in the previous papers.3,4) Electrodes—A gold plate (99.99% pure, 25 × 19 mm) served as the working electrode. It was polished with a 0.3 μm α-alumina slurry on a nylon cloth (Buehler Ltd.) and thoroughly rinsed with freshly purified water. A saturated calomel electrode (SCE) and gold plate were used as the reference and counter electrodes, respectively.

Procedures—The procedures used to obtain reflectivity (R/R₀)-potential (E) and R/R₀-time (t) curves were the same as described in the previous paper.3) All measurements were carried out using a light beam at a wavelength of 500 nm and perpendicular polarization at an incident angle of 15°. Before making an actual measurement, a sufficient amount of pure argon gas was bubbled through the electrolyte solution to expel dissolved oxygen.

Results and Discussion

Current–Potential and Reflectivity–Potential Curves

On a current (i)-E curve of norepinephrine in a phosphate buffer solution (pH 6.9) at the potential range from -0.8 to 0.5 V, an anodic peak appeared at +0.2 V. This was due to a two-electron oxidation of norepinephrine followed by the chemical reactions shown below.5)

In these reactions, some products such as noradrenochrome absorb light near 500 nm; this is undesirable for specular reflection measurement. Consequently, the potential examined was restricted to the range from -0.8 to -0.05 V. The i-E curve of norepinephrine in this potential range is given in the upper half of Fig. 1 and shows no redox wave.

The R/R₀-E curves measured simultaneously with the i-E curves are shown in the lower half of Fig. 1. The R/R₀-E curve of gold in a phosphate buffer solution (pH 6.9) had two approximately linear portions with different slopes intersecting at about -0.25 V (see curve c). This potential value appeared to be the pzc for gold in the solution.

The addition of norepinephrine to the solution caused no change in the R/R₀ value at potentials between -0.8 and -0.7 V, but a marked decrease in R/R₀ was observed in

(2 kcal/mol) and on the other side, a nitrogen atom lone pair (< -2 kcal/mol). The electrostatic potential of most of the molecular surface was within ±6 kcal/mol. Thus, the dopamine molecule may be capable of interacting electrostatically with surfaces charged within ±0.3 eV. In our preliminary experiment on the adsorption of catecholamines, the electroosorption of dopamine seemed to occur in the potential region within about ±300 mV vs. point of zero charge (pzc), which is in fair agreement with the above charge. This implies that the adsorption behavior of transmitters on an electrode surface may reflect the electrostatic interactions between transmitters and the receptor surface.

Therefore, we carried out a study on the adsorption of adrenergic transmitters and related substances onto a gold electrode surface as a model of a certain type of interactions between transmitter substances and receptors. Since an electrode surface may be regarded as a very simplified biosurface charged negatively or positively and in contact with an electrolyte solution, an understanding of the surface behavior of these substances should help to elucidate the electrostatic interactions involved in their neurochemical behavior at receptor sites. Specular reflection was used to detect trace amounts of adsorbed species on electrode surfaces with high sensitivity.
potential regions more positive than about $-0.7$ V (Fig. 1, curves d–g). The reflectivity change was enhanced by increasing the norepinephrine concentration but tended to saturate, reaching a limiting value at ca. $1 \times 10^{-4}$ M. These observations suggest that adsorption of norepinephrine takes place on the gold electrode surface, since a decrease in reflectivity was often found to result from adsorption of organic compounds, and it was explained in terms of the following linear approximation equation.\(^7\)

\[
(AR/R_0)_{\perp, \theta=1} = \frac{8\pi n_s \cos \phi}{\lambda} \frac{\varepsilon_0^2(n_m^2 - \varepsilon_m^2) + \varepsilon_0^2\varepsilon_m^2(n_m^2 - \varepsilon_m^2)}{(n_m^2 - \varepsilon_m^2)^2 + \varepsilon_m^2}
\]

where $AR/R_0$ denotes the reflectivity change at monolayer coverage ($\theta = 1$) observed by the perpendicularly polarized light ($\perp$), $\phi$ is the incidence angle, $\lambda$ the wavelength, $n_s$ the refractive index of the bulk electrolyte solution, and $\varepsilon'$ and $\varepsilon''$ are the real and imaginary parts of the complex dielectric constants, respectively. The subscripts ad and M represent the adsorbed layer and metal surface, respectively. When an optically transparent layer of an organic compound is formed on the substrate in water, $\varepsilon''_{ad} \approx 0$ and $\varepsilon''_{ad} > n_s^2$, and therefore, $AR/R_0$ is always negative.

**Potential and Concentration Dependence of Adsorption**

The relationship between reflectivity change $AR/R_0$ and surface coverage $\theta$ of an
electrode by an adsorbate was derived as follows,

\[(\Delta R/R_0)_{t=0} = \theta \Delta R/R_0 \quad (\theta = 0, 1)\]  \hspace{1cm} (2)

provided that the thickness of the monolayer, \(d\), and the optical constants of the adsorbed layer and metal surface are not altered during the adsorption process.\(^7\)

Measurements for other catecholamines such as epinephrine, dopamine, dopa, isoproterenol and homocatechol, were also carried out. All compounds were found to be adsorbed on the gold electrode surface.

The \(\Delta R/R_0\) values due to adsorption of the catecholamines were obtained by the potential-step method using solutions of different catecholamine concentrations. The potential was first set at \(-0.8\) V, where no adsorption occurs, and then stepped to more positive potentials, and the \(R/R_0\) vs. \(t\) curves were recorded until the \(R/R_0\) no longer decreased. The same procedure was also applied to the supporting electrolyte solution. Reflectivity changes at equilibrium in the presence and absence of catecholamines at the same potential were obtained. By subtracting the latter from the former at the same potential, the net change in \(R/R_0\) due to the adsorption, denoted as \(\Delta R/R_0\), was obtained. Each curve in Fig. 2 has a maximum \(|\Delta R/R_0|\) at a potential near the pzc and \(|\Delta R/R_0|\) decreases with deviation from the potential, suggesting that each catecholamine behaves as a neutral organic compound in the adsorption. However, the potentials of the \(|\Delta R/R_0|\) maxima were actually somewhat more positive than the pzc of gold in the supporting electrolyte. This can be ascribed to the contact of a negatively charged part, perhaps the catechol moiety, of an adsorbed molecule with the gold electrode surface.

The smooth \(|\Delta R/R_0| - E\) curves of norepinephrine suggest that no orientational change takes place, at least in the potential region from \(-0.8\) to \(-0.2\) V. Supposing that the limiting value of \(|\Delta R/R_0| = 0.78\%\) in Fig. 2, A corresponds to the reflectivity change at monolayer coverage of norepinephrine, \(\theta\) values can be obtained by the use of Eq. 2. In Fig. 3, \(\theta\) values are plotted against norepinephrine concentrations, and Langmuir-like adsorption isotherms were obtained. They were analyzed by using Eq. 3, initially derived by Bockris and Swinkles\(^9\).

Fig. 2. Potential Dependence of Reflectivity Change Obtained for (A) Norepinephrine, (B) Epinephrine, (C) Isoproterenol, (D) Dopamine hydrochloride, (E) Dopa and (F) Homocatechol

Concentration: A (○) \(3.0 \times 10^{-8}\), (△) \(8.0 \times 10^{-8}\), (□) \(3.0 \times 10^{-6}\), (●) \(1.5 \times 10^{-4}\); B (○) \(3.0 \times 10^{-6}\), (△) \(8.0 \times 10^{-8}\), (□) \(3.0 \times 10^{-5}\), (●) \(1.5 \times 10^{-4}\); C (○) \(2.0 \times 10^{-6}\), (△) \(7.0 \times 10^{-6}\), (□) \(2.6 \times 10^{-5}\), (●) \(1.4 \times 10^{-4}\); D (○) \(3.0 \times 10^{-6}\), (△) \(2.0 \times 10^{-5}\), (□) \(6.5 \times 10^{-6}\); E (○) \(1.5 \times 10^{-4}\), (△) \(2.7 \times 10^{-3}\), (□) \(1.4 \times 10^{-4}\), (●) \(2.3 \times 10^{-4}\); F (○) \(5.1 \times 10^{-6}\), (△) \(3.5 \times 10^{-3}\), (□) \(1.5 \times 10^{-4}\), (●) \(3.7 \times 10^{-4}\) M.
for the adsorption of organic molecules in solution, and applied to certain cases by Dahms and Green.\textsuperscript{10)}

\[
\theta/(1 - \theta)^p = cK
\]  

(3)

where \( p \) is the number of water molecules replaced by one molecule of the adsorbed species, and \( c \), the concentration of organic molecule, used in place of activity in a dilute solution. The constant \( K \) is the adsorption coefficient. For norepinephrine adsorption at potentials ranging from \(-0.8\) to \(-0.2\) V, the plot of \( \log(\theta/c) \) vs. \( \log(1 - \theta) \) gave a straight line, indicating that Eq. 3 holds for the adsorption isotherm of norepinephrine. Similar adsorption isotherms were also obtained for other catecholamines, but not dopa.

From these results, it can be noted that most of the catecholamines exhibit a rather simple pattern of adsorption behavior as a neutral molecule with no orientational change on the electrode surface.

**Adsorption of Adrenergic Receptor-Blocking Drugs**

To determine if the surface behavior of neurotransmitters, such as norepinephrine and epinephrine, would shed some light on their interactions with receptor sites, we investigated the adsorption behavior of adrenergic receptor-blocking drugs,\textsuperscript{11)} which offset the action of catecholamines by occupying receptors.

The following five adrenergic receptor-blocking drugs were examined: as \( \alpha \)-blockers, phenoxybenzamine, tolazoline and ergotamine; as \( \beta \)-blockers, oxprenolol and propranolol. None of these compounds participated in redox reactions in the potential range from \(-0.8\) to \(0\) V in the phosphate buffer solution (pH 6.9). All, except phenoxybenzamine, gave a reflectivity decrease in the \( |\Delta R/R_0| - E \) curves obtained in the solution throughout the potential range examined. It is clear that tolazoline, ergotamine, oxprenolol and propranolol are adsorbed at all potentials examined.

Because of hydrogen evolution on a gold electrode, observation in the negative potential region is limited up to \(-0.8\) V at pH 6.9. To obtain the \( |\Delta R/R_0| - E \) curves for the above drugs, phosphate solution of pH 9.3 was used and the data thus obtained are shown in Fig. 4. In Fig. 4, B and C, the \( |\Delta R/R_0| - E \) curves for tolazoline and ergotamine (\( \alpha \)-blocker) are bell-shaped with maxima at \( \approx 0.4\) V (i.e., the pzc of gold in the phosphate solution of pH 9.3). Figure 4, D and E, shows the \( |\Delta R/R_0| - E \) curves of oxprenolol and propranolol (\( \beta \)-blockers). These drugs may be adsorbed on the electrode surface even from a fairly dilute solution (ca. \( 10^{-5} \) M), but their adsorption isotherms did not obey the Langmuir-like adsorption described by Eq. 3. This probably is due to orientational changes depending on potential and/or mutual interactions of the adsorbed molecules.

The adsorption behavior of these antagonists was compared qualitatively with that of norepinephrine. It can be seen from their adsorption dependence on concentration and electrode potential that the adsorptivity of the antagonists is stronger than that of

![Fig. 3. Concentration Dependence of the Reflectivity Change for Norepinephrine at a, -0.3 V; b, -0.4 V; c, -0.5 V.](image-url)
norepinephrine, especially in the potential region more negative than $-0.3 \text{ V}$. That is, the antagonist drugs are readily adsorbed on a negatively charged electrode surface.

**Electrochemical Approach to Adrenergic Chemical Neurotransmission Processes**

In chemical neurotransmission, a neurotransmitter present in the synaptic gap may initially be attracted to the post synaptic membrane through electrostatic interactions and then bind to the receptor site in a particular orientation. Pilla\(^{12}\) pointed out the similarities of electrical behavior between a biomembrane surface/biological fluid interface and an electrode/electrolyte solution interface. Although biomembranes have relatively complex structure, they are charged because of the presence of polar groups such as phospholipid head, and they are in contact with biological fluid which contains electrolytes. Furthermore, the biomembrane surface is regarded as being directly covered in the first sphere with a structured water layer whose properties are different from those of bulk water. Such features resemble those of an electrical double layer of electrode/solution interface.\(^{13}\) Thus, the adsorption behavior of neurotransmitters at the electrode/solution interface is expected to reflect the electrostatic interactions between a neurotransmitter and its receptor site.

Based on the adsorption isotherm given by Eq. 3, the value of $p$ for norepinephrine was obtained to be $4.1 \pm 0.4$ from the slope of $\log (\theta/c)$ vs. $\log (1-\theta)$. The area occupied by one adsorbed norepinephrine molecule can be estimated from $p$ by comparison with the area of an adsorbed water molecule, using the CPK molecular model. Assuming a water molecule to occupy an area of $0.09 \text{ nm}^2$, the area occupied by norepinephrine was calculated to be $0.37 \text{ nm}^2$. Comparing this value with the surface area of the norepinephrine molecule based on the crystal structure,\(^{14}\) the most probable orientation of adsorbed norepinephrine is that the...
catechol moiety and hydroxy groups at the $\beta$-position may be in contact with the electrode surface in a flat orientation.

The activity of norepinephrine is due to its complexation with adrenergic receptors such as $\alpha$-adrenergic and $\beta$-adrenergic receptors. From QSAR experiments, the following model of drug-receptor interaction is proposed. Phenolic hydroxy groups assist in the fixation to the $\beta$-adrenergic-receptor site through electrostatic forces. The aromatic ring, attaching itself to the receptor by van der Waals’ forces, is also essential for $\beta$-adrenergic activity. The alcoholic hydroxy group allows other electrostatic bonding with the receptor. The presence of an amino group is essential, especially for $\alpha$-adrenergic activity, owing to its interaction in cationic form with the receptor’s anionic phosphate groups. Comparing the possible orientation of norepinephrine at the electrode surface with that at the receptor based on the drug-receptor model, interactions between norepinephrine and the electrode surface may reflect mainly drug-receptor interactions on the $\beta$-receptor.

A study of the electrosorption behavior of norepinephrine and adrenergic blocking drugs may provide basic information concerning the competitive inhibitory effects of antagonists on the interactions of the agonist with solvated and charged surfaces.

References and Notes

8) The magnitude of net reflectivity change is given in terms of $|\Delta R/R_0|$ in the present paper, since all the $\Delta R/R_0$ values experimentally obtained are negative.