Ion-Transfer Voltammetry of Drugs at the Nitrobenzene/Water Interface

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Ion-transfer voltammetry of calcium antagonists, propranolol, and acetylcholine were carried out by current-scan polarography with an ascending water electrode. The pH effect of the aqueous phase on the half-wave potential of the ion-transfer voltammogram was examined for each drug. For nifedipine, flunarizine and flunarizine, the half-wave potential became more positive by 0.059 V per unit pH over the pH range (pH 4-8). For propranolol, the half-wave potential became more positive by 0.059 V per unit pH at pH values above 6.8, whereas at pH values below 6.8, this potential remained constant. The half-wave potential of acetylcholine was independent of pH at pH 5-8.5. These results could be explained by the $E_{1/2}$ equations of for ion transfer of drugs based on the ionization of drugs in the aqueous phase and distribution of neutral drugs between the nitrobenzene and aqueous phases.

Keywords Ion-transfer voltammetry, drug, calcium antagonists, propranolol, acetylcholine

The interfaces between the lipid and aqueous phases in a biological system play an important role in drug transport across a biomembrane and the occurrence of pharmaceutical activity of drugs.¹ An immiscible oil/water interface is the simplest model of such an interface. Ion-transfer voltammetry serves as a method for clarifying drug behavior at the polarized oil/water interface and thus is of interest from pharmaceutical as well as electroanalytical viewpoints. This method provides thermodynamic parameters such as the free energy of ion transfer and the partition coefficient. Ion-transfer voltammetry at the polarized oil/water interface was established by Gavach *et al.*,² and has been used to examine the ion transfer of various types of drugs.³

Most of these drugs are weak electrolytes. Recently,⁴ ion-transfer voltammetry of weak bases was examined using scopolamine and lidocaine. Half-wave potential ($E_{1/2}$) equations were derived on the basis of the ionization of drugs in the aqueous phase and the distribution of neutral drugs between the nitrobenzene and aqueous phases. Thus, ion-transfer voltammetry of other cationic drugs is a further point of interest.

In this study, ion-transfer voltammetry of calcium antagonists, propranolol, and acetylcholine were conducted at the nitrobenzene/water interface and the pH dependence of $E_{1/2}$ is discussed. The calcium antagonists in this study and propranolol

are amines, and acetylcholine is a quaternary ammonium ion. The pH dependence of $E_{1/2}$ will be explained by the equation of $E_{1/2}$ for ion transfer of drugs based on the ionization of the drugs in the aqueous phase and distribution of the neutral drugs between the nitrobenzene and aqueous phases.

Experimental

Apparatus

Current-scan polarography at an ascending water electrode⁵ was applied to the measurement of the ion-transfer voltammograms. A drop of aqueous solution was introduced into a nitrobenzene solution from the tip of a Teflon capillary (0.5 mm inner diameter and 0.75 mm length). A capillary (0.5 mm inner diameter and 2 m length) was inserted and the reservoir height was fixed at a constant height so that the flow rate of the aqueous solution could be maintained at a fixed value. A four-electrode potentiostat/galvanostat (Huso Electrochemical System, HECS-310B) was combined with a laboratory-made instrument for compensating the iR-drop due to solution resistance. Reference electrodes in the nitrobenzene and aqueous phases were Ag/AgCl electrodes, and auxiliary electrodes in both phases were platinum electrodes.

The electrolytic cell for ion-transfer voltammetry was

Ag/AgCl	0.01 M TPenACl + 0.09 M LiCl (W1)	0.1 M TPenA TPhB (+drug) (NB)	0.1 M LiCl + buffer (0.1 M) (+drug) (W2)	0.1 M LiCl (W3)	Ag/AgCl
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where TPenACl is tetrapentylammonium chloride, and TPenATPhB is tetrapentylammonium tetraphenylborate. Each drug was added to aqueous phase W2 containing a pH buffer solution or phase NB.

Reagents

TPenATPhB was prepared by mixing an ethanol solution of tetrapentylammonium iodide with an ethanol solution of sodium tetraphenylborate and purified by recrystallization from an acetone-ethanol mixture. Nitrobenzene (analytical grade

Fig. 1 Voltammograms in the presence of 0.2 mM nicardipine in NB at various pH. Scan rate: 0.2 mA/s.

Fig. 2 Plots of the half-wave potential of the voltammogram in the presence of nicardipine (●), barnidipine (○), and benidipine (□) vs. pH.

from Wako Pure Chemicals) was washed with water three times just before use. Water used in this study was distilled and then purified with a Nano Pure II filtering system (Barnstead Co.). The other chemicals were obtained commercially and were used without further purification.

Results and Discussion

Calcium antagonists

Nicardipine, barnidipine and benidipine were used as calcium antagonists. Each drug in the neutral form was added to NB because of its low solubility in water. Figure 1 shows the voltammograms obtained in the presence of 0.2 mM (solid line) and 0 M (dotted line) nicardipine in W2 at pH 4-8. In the voltammograms, the anodic current (positive current) corresponds to the transfer of cations from W2 to NB or anions from NB to W2. The final anodic and cathodic current rises correspond to the transfer of supporting electrolyte ions in the aqueous and/or nitrobenzene phases. At each pH, an anodic wave was observed in the potential window when nicardipine was present in NB. The limiting current (I_d) increased linearly with nicardipine concentration added to NB. Figure 2 shows plots of $E_{1/2}$ of nicardipine, barnidipine, and benidipine versus pH. $E_{1/2}$ becomes more positive by 0.059 V per unit pH over the pH range. I_d was independent of pH. Essentially the same behavior was obtained using barnidipine and benidipine. Though nifedipine and isradipine have a main structure similar to nicardipine, no voltammetric wave corresponding to the drugs was seen. The chemical structures of the calcium antagonists in this study are shown in Fig. 3. Nicardipine, barnidipine, and benidipine are tertiary amines and are weak bases. They are ionized by accepting protons in the aqueous and nitrobenzene phases. Protons or ionized drugs can be transferred at the interface, thus showing a voltammetric wave. Nifedipine and isradipine have no ability to accept protons at the pH in this study and remain in the neutral forms. Thus no voltammetric wave was seen.

Fig. 3 Chemical structures of calcium antagonists: (a) nicardipine, (b) barnidipine, (c) benidipine.

Fig. 4 Voltammograms in the presence of 0.2 mM propranolol in W2 at various pH. Scan rate: 0.2 mA/s.

Fig. 5 Plots of the half-wave potential of the voltammogram in the presence of propranolol vs. pH.

Propranolol

Fig. 4 shows the ion-transfer voltammograms in the presence of 0.2 mM propranolol hydrochloride in W2 at pH 5-8.5. Each voltammogram showed a reversible anodic wave. I_d increased with propranolol concentration and was independent of pH. $E_{1/2}$ becomes more positive by 0.059 V per unit pH at pH values above 6 (Fig. 5), whereas below pH 6, $E_{1/2}$ remained constant.

Acetylcholine

The ion-transfer voltammograms in the presence of 0.2 mM acetylcholine chloride in W2 are shown

Fig. 6 Voltammograms in the presence of 0.2 mM acetylcholine in W2 at various pH. Scan rate: 0.2 mA/s.

Fig. 7 Plots of the half-wave potential of the voltammogram in the presence of acetylcholine vs. pH.

in Fig. 6. I_d increased with acetylcholine concentration and was independent of pH. A reversible anodic wave was seen in each voltammogram. As shown in Fig. 7, $E_{1/2}$ was independent of pH at pH 5-8.5. $E_{1/2}$ obtained was 0.041 V vs. TPhE, a value essentially the same as that in the literature.⁶⁻⁸

pH dependence of half-wave potential

Three types of pH vs. $E_{1/2}$ plots were obtained.

Propranolol: having two regions; one where $E_{1/2}$ increases with pH and the other

where $E_{1/2}$ is independent of pH (Type I)

Nicardipine, barnidipine, and benidipine:

$E_{1/2}$ increases with pH (Type II)

Acetylcholine: $E_{1/2}$ is independent of pH (Type III)

Since propranolol, nicardipine, barnidipine, and benidipine are amines, the ionization of the drugs in the aqueous phase and the distribution of the neutral drugs between the nitrobenzene and aqueous phases should participate in the ion transfer process. For deriving the $E_{1/2}$ equations for ion transfer of a drug, the following conditions were assumed:

(1) The dissociation of a drug in the nitrobenzene phase and ion pair formation with supporting electrolyte ions are negligible.

(2) The aqueous phase is well buffered so that protons at the interface are readily available in abundance relative to ion transfer.

(3) All equilibrium reaction rates participating in the ion transfer are much higher than that of the ion transfer.

Thus, the $E_{1/2}$ equations⁴ for ion transfer of drugs are given as Eqns. 1 and 2 :

Case I:

$$(K_a / [H^+]) K_D (D_B^0 / D_B^w)^{1/2} \ll 1$$

(that is, $pH < pK_a - \log K_D - \log(D_B^0 / D_B^w)$)

$$E_{1/2} = E^0 + (RT/2zF) \ln (D_{BH^+}^w / D_{BH^+}^0) \quad (1)$$

Case II:

$$(K_a / [H^+]) K_D (D_B^0 / D_B^w)^{1/2} \gg 1$$

(that is, $pH > pK_a - \log K_D - \log(D_B^0 / D_B^w)$)

$$E_{1/2} = E^0 + (RT/2zF) \ln (D_{BH^+}^w / D_{BH^+}^0) + (RT/zF) \ln K_a + (RT/zF) \ln K_D - (RT/zF) \ln [H^+] \quad (2)$$

where R is the gas constant, T absolute temperature, z ion charge, F Faraday's constant, K_a dissociation constant in the aqueous phase, and K_D partition coefficient. D is the diffusion constant whose superscripts w and o stand for the aqueous and organic phases, respectively, and subscripts B and BH^+ , the molecular and ion forms of a drug, respectively. E^0 is the standard potential of ion transfer defined by $\Delta G^0 = -zFE^0$, with ΔG^0 the standard Gibbs energy of ion transfer. Accordingly, $E_{1/2}$ of the drugs should become more positive by 0.059 V per unit pH at $pH > pK_a - \log K_D - \log(D_B^0 / D_B^w)$, whereas at $pH < pK_a - \log K_D - \log(D_B^0 / D_B^w)$, $E_{1/2}$ should remain constant. From Eqn. 2, the $E_{1/2}$ value obtained from a voltammogram at $pH < pK_a - \log K_D - \log(D_B^0 / D_B^w)$ can be thought of as $E_{1/2}$ of the drug ion itself, though the anodic waves in the voltammograms in Figs. 1 and 4 may be due to the proton transfer facilitated by neutral propranolol in NB.

The pK_a of propranolol was obtained from the literature⁹ and $\log(D_{BH^+}^0 / D_{BH^+}^w)$ was determined from the peak height ratio of anodic and cathodic

waves in the cyclic voltammogram. $\log K_D$ was determined by potentiometric titration. Thus, $pK_a - \log K_D - \log(D_B^0 / D_B^w)$ is estimated as 6.8. That is, $E_{1/2}$ of the drugs should become more positive by 0.059 V per unit pH at $pH > 6.8$, whereas at $pH < 6.8$, $E_{1/2}$ should remain constant. This is very consistent with a pH vs. $E_{1/2}$ plot in Fig. 2, and the $E_{1/2}$ equations could be applied to propranolol, as well as scopolamine and lidocaine.⁴ This kind of drug shows a pH vs. $E_{1/2}$ plot of Type I.

For nicardipine, barnidipine and benidipine, the pH dependence of $E_{1/2}$ according to Eqn. 1 was seen over the pH range. A pH-independent region may possibly be present at a lower pH. At this time, the anodic wave must be shown at a more negative potential, which is far out of the potential window. Thus, the pH-independent region could not be seen. This may be due to the large pK_D values of the calcium antagonists. This type of drug shows a pH vs. $E_{1/2}$ plot of Type II.

Acetylcholine is a quaternary ammonium ion and thus, the $E_{1/2}$ value obtained was that of the drug ion itself without regard to Eqns. 1 and 2. A plot of Type III is shown by this kind of drug.

The pH vs. $E_{1/2}$ plots of calcium antagonists, propranolol, and acetylcholine were successfully explained by the $E_{1/2}$ equations for ion transfer of drugs based on the ionization of the drugs in the aqueous phase and the distribution of the neutral drugs between the nitrobenzene and aqueous phases. Since ion-transfer voltammetry provides thermodynamic parameters such as the free energy of ion transfer and the partition coefficient of drugs, this method is a promising one for clarifying drug behavior at biomembranes such as adsorption and transport of a drug through a biomembrane.

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