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Membrane transport of neurotransmitter acetylcholine and related compounds across a phospholipid bilayer by a calix[6]arene ester

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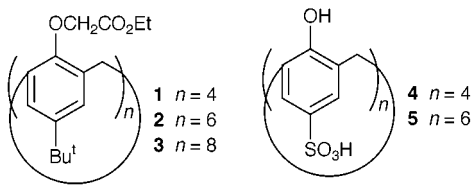
A calix[6]arene ester 2 acts as a carrier for neurotransmitters such as acetylcholine, carbachol and choline in a phospholipid bilayer membrane.

Artificial acetylcholine receptors have been of great interest for the development of acetylcholine detection techniques[1,2] and for the understanding of biological recognition[3] of acetylcholine (ACh). Several macrocyclic compounds[1–6] have been reported as artificial acetylcholine receptors which recognize the quaternary ammonium moiety of acetylcholine. Recently, it was reported that sulfonated calix[4]- and calix[6]-arenes have binding abilities toward acetylcholine and related choline derivatives in water.[2,6] To the best of our knowledge, however, there has been no report of membrane transport of acetylcholine mediated by synthetic ionophores for the detection and separation of acetylcholine. We now report that a calix[6]arene ester **2** can act as a selective acetylcholine carrier in a phospholipid bilayer membrane.

Calixarene esters are known to have selective ion complexing abilities, and also have ion transporting abilities toward alkali-metal ions.[7–10] For example, calix[4]-, calix[5]- and calix[6]-arene esters act as selective Na^+ ,[7–10] K^+ [10] and Cs^+ carriers[7,10] in bilayer membranes as well as in bulky liquid membranes. Although much information has been accumulated on the metal ion transport properties of calixarene esters, molecular ion transport properties of the calixarenes are known to a lesser extent.[11] Here we have focused our attention on the use of calixarene esters as quaternary ammonium ion carriers, and examined their membrane transport abilities for neurotransmitters such as acetylcholine, carbachol (carbamylcholine) and choline in a phospholipid bilayer system.



Five calixarene derivatives **1**–**5** were tested for their neurotransmitter transport abilities. Compounds **1**–**3** were prepared according to the literature method.[7] The sulfonated calixarenes **4** and **5** were purchased from Acros Organics. Membrane transport of neurotransmitters by calixarene derivatives across planar phospholipid bilayers was investigated by a voltage clamp method.[10,12] Planar phospholipid bilayers[12,13] (soybean phospholipids) were prepared at an aperture (diameter, 0.2 mm) in a Teflon film (thickness, 12.5 μm) which separated two Teflon chambers† (*cis* and *trans* chamber; volume, 1.7 ml). Calixarenes were added as 100 μM DMSO solutions to the *cis* chamber after the formation of the bilayers.

Fig. 1(*a*) shows the generation of membrane currents upon the addition of calix[6]arene ester **2** to the *cis* chamber, where both chambers were filled with 100 mM acetylcholine chloride solutions (pH 7.2, 25 mM HEPES-TRIS buffer). Membrane currents resulting from the transport of acetylcholine cations (ACh^+) were measured at an external voltage at 100 mV. In the absence of **2**, membrane conductance was *ca*. 200 GΩ: the control level of the membrane current was 0.5 pA at 100 mV. When a microliter aliquot (20 μl) of the DMSO solution of **2** was added to the chamber, the membrane conductance immediately increased. A control experiment was performed where only neat DMSO was added to the *cis* chamber. The addition of 200 μl of DMSO did not change the membrane conductance. To confirm the transport of ACh^+ across the phospholipid bilayer, the membrane conductance was measured under LiCl (100 mM)/LiCl (100 mM) ionic conditions. The addition of **2** (20 μl) to the chamber filled with the LiCl solution did not lead to the generation of membrane current, suggesting that the membrane current observed in Fig. 1(*a*) is due to the transport of ACh^+ cations, not the transport of Cl^- anions. Fig. 1(*b*) shows the concentration dependence of calix[*n*]arene esters *versus* the membrane currents. With increasing concentration of **2**, the membrane currents significantly increased. In contrast, the addition of **1** and **3** did not increase the membrane conductance for concentrations of calixarenes up to 10 μM. Furthermore, we tested water-soluble sulfonated calix[4]- and calix[6]-arene, which are known to form 1:1 acetylcholine complexes[6] in water. The addition (100 μl) of these calixarenes, however, did not increase the membrane conductance. Except for **2**, we could not detect ion transport for acetylcholine in the bilayer membrane.

The evidence for the complexation of acetylcholine with **2** was confirmed by the ^1H NMR measurements in $CDCl_3$. Fig. 2 shows the changes in ^1H NMR spectrum of acetylcholine in the

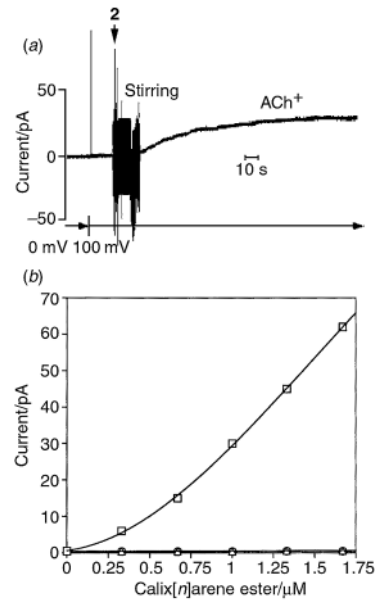


Fig. 1 (*a*) Generation of membrane currents (ACh^+) at 100 mV upon the addition of 20 μl of **2** (100 μM DMSO solution); (*b*) the membrane currents (ACh^+) *versus* the concentration of calix[*n*]arene ester in the *cis* chamber: (○) **1**, (□) **2** and (△) **3**.

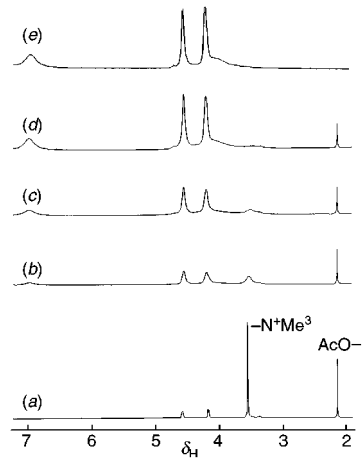


Fig. 2 Changes in ^{1}H NMR spectra ($CDCl_3$) of acetylcholine in the presence of **2**: [**2**]/[ACh] = (*a*) 0, (*b*) 0.5, (*c*) 1 and (*d*) 2, [ACh] = 14 mM. For comparison, the ^{1}H NMR spectrum of **2** is shown in (*e*).

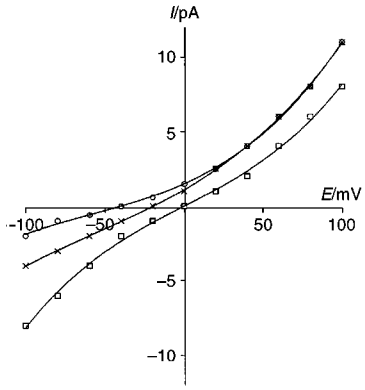


Fig. 3 The current–voltage (*I-E*) curves for different ionic conditions (pH = 7.2, 25 mM HEPES–TRIS buffer). All measurements were carried out at 25 °C. (□) ACh^+(*cis*)/ACh^+(*trans*), (×) $Carbachol^+/ACh^+$ and (○) $Choline^+/ACh^+$.

presence of **2**. Upon addition of **2**, the N^+Me_3 protons (δ 3.54) in acetylcholine moved to lower field ($\Delta\delta$ −0.06 ppm at [**2**]/[ACh] = 2) and the signal became very broad upon increasing the ratio of [**2**]/[ACh]. On the other hand, the chemical shift of the CH_3CO protons in acetylcholine was not affected by the addition of **2**: the line broadening of the CH_3CO singnal was much less than that of the N^+ Me_3 signal. The shift of the N^+ Me_3 protons to lower magnetic field suggests that the binding site of acetylcholine toward **2** is the quaternary ammonium moiety, not the acetyl moiety.[2] The line broadening of both the N^+Me_3 and CH_3CO protons in the presence of **2** may be explained by a fast exchange between the complexed and free acetylcholines on the NMR time scale: the resolved signals resulting from the two acetylcholine species were not observed at [**2**]/[ACh] < 1.

To evaluate the transport selectivity of **2** for acetylcholine and related compounds (carbachol and choline), we examined current–voltage (*I-E*) relations. Fig. 3 shows the *I-E* curves for three sets of ionic conditions (100 mM Cl^- salts). In the case of ACh^+/ACh^+ ionic conditions, a non-linear *I-E* curve which did not obey Ohm's law was obtained. This voltage-dependent *I-E* curve suggests that the transport of acetylcholine proceeds *via* a carrier mechanism.[14,15] In fact, a similar voltage-dependent *I-E* curve for a ACh^+/ACh^+ system has also been observed in the case of calix[*n*]arene ester-mediated carrier transport of alkali-metal ions across phospholipid bilayer membranes.[8,10] To determine ion permeability ratios, we exchanged the acetylcholine solution in the *cis* chamber with other neurotransmitter solutions and then measured *I-E* curves. It should be noted that in the X^+ (*cis*)/ACh^+ (*trans*) ionic conditions, positive currents of *ca*. 2 pA were generated at 0 V. This implies that acetylcholine cations are transported by **2** from the *trans* to the *cis* chamber in the absence of external voltages. From the *I-E* curves in the X^+/ACh^+ ionic conditions, the reversal potentials were determined as follows: −18 mV for $Carbachol^+/ACh^+$ and −42 mV for $Choline^+/ACh^+$. According to the Goldman–Hodgkin–Katz equation,[15] the reversal potentials ($\Delta\phi$) in XCl (*cis*)/YCl (*trans*) systems is related to the ion permeability (*P*), as shown in eqn. (1), where *R* and *F* express the gas constant and

$$\Delta\phi = \frac{RT}{F}\ln\frac{P_X[X]_{cis} + P_{Cl}[Cl]_{trans}}{P_Y[X]_{trans} + P_{Cl}[Cl]_{cis}} \quad (1)$$

the Faraday constant, respectively. Since **2** does not transport choline anions across the bilayer membranes (P_{Cl} = 0), the cation permeability ratio (P_X/P_Y) can be easily calculated as follows: 0.48 for $P_{Carbachol^+}/P_{ACh^+}$ and 0.18 for $P_{Choline^+}/P_{ACh^+}$. The permeability of acetylcholine across the planar bilayer membrane is larger than that of carbachol and choline by a factor of 2.1 and 5.6, respectively. This result shows that the calix[6]arene ester **2** acts as a selective acetylcholine carrier in a phospholipid bilayer membrane. The lower transport selectivity of choline and carbachol toward **2** may be due to the higher hydrophilicities of those compounds, which depress the stability of the complex in the water–lipid interface.

In conclusion, we have demonstrated that a calix[6]arene ester **2** can transport neurotransmitters, especially acetylcholine, across a phospholipid bilayer. For the other calix[4]- and calix[8]-arene esters (**1** and **3**) and sulfonated calixarenes (**4** and **5**), we could not determine the neurotransmitter transport activity in the bilayer membrane. To the best of our knowledge, this is the first report of an artificial acetylcholine carrier which is active in a phospholipid bilayer membrane. We believe that the calix[6]arene ester **2** has potential for use as an acetylcholine carrier in biological membrane systems.

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Notes and references

† The side to which the compounds were added was defined as the '*cis*' chamber and the opposite side was the '*trans*' chamber. The external voltage was fed to the *trans* chamber *via* an Ag/AgCl electrode and the *cis* chamber was grounded *via* an Ag/AgCl electrode.

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