DIPOLE MOMENT OF ACETYLCHOLINE
AND ITS RELEVANCE TO
THE CHEMICAL SYNAPTIC TRANSMISSION

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ABSTRACT The dipole moment of acetylcholine (AcCh) has been measured in chloroform and a value of 8.49 D was obtained. Such a value actually represents the total dipole moment of the ion pair $(AcCh)^+(Cl)^-$. The dipole moment of the $(AcCh)^+$ cation alone turned out to be 2.65 D whereas its theoretical value obtained after a vectorial calculation was 1.65 D. The discrepancy was related to an interaction between AcCh and the solvent. The meaning of the measured value is discussed on the basis of a recent theory of chemical synaptic transmission based on the assumption of a much higher dipole moment value for the AcCh molecule.

INTRODUCTION

The mechanism of chemical synaptic transmission has been discussed in recent times by Wei (1968, 1969 *a*, *b*), who proposed a general theory, or, better, a physical model encompassing the main events of the phenomenon of chemical synaptic transmission. The model is based upon the predicted dipolar characteristics of AcCh, which would be responsible for the formation of an electric field formed by a multilayer array of AcCh dipoles within the synaptic cleft. The formation of such a field would allow ions to overcome the junction barrier of the postsynaptic membrane and then originate the bioelectrical change which is the basis of chemical transmission.

Besides the value assumed for the dipole moment of AcCh, Wei's model raises several questions related to the physical characteristics of the synaptic cleft, which has been assumed to contain water but more likely has the features of a hydrophobic "milieu" (Galzigna, 1970), and the ion-ion interactions.

This paper reports the measurement of the dipole moment of AcCh and attempts to discuss the chemical synaptic transmission phenomenon from the standpoint of a sound experimentation.

The actual value of the dipole moment of AcCh in solution is central to any theory on the chemical transmission, and we carried out our measurements using AcCh as a chloride salt. In fact it is unlikely that it exists "in vivo" as a free cation and it is known that AcCh acts at the synaptic junction together with a number of anions including chloride.

DIPOLE MOMENT MEASUREMENTS

A method was first set up for measuring the dipole moment of molecules dissolved in both polar and nonpolar solvents. Such a method is badly needed since the dipole moment values of a large number of compounds which can act as central transmitters have to be measured in various solvents, which might reflect the physicochemical features of the synaptic cleft.

Starting from the basic equation of polarization (P)

$$P = (\epsilon - [1/4]\pi)E, \tag{1}$$

where ϵ is the permittivity of the medium and E is the applied electric field, according to Onsager (1936) and Frohlich (1950), we write:

$$P = \sum_j N_j[\alpha_j(E_i)_j + (\mu_j^2/3KT)(E_r)_j], \tag{2}$$

$$\epsilon = 1 + \sum_j 4\pi N_j/E[\alpha_j(E_i)_j + (\mu_j^2/3KT)(E_r)_j]. \tag{3}$$

In these equations α_j is the induced polarization of a molecule j in a uniform electric field E_r called the director field, $(E_i)_j$ represents the value of the internal field of a molecule j, and N_j is the number of molecules per unit volume.

For a two-component mixture an expression can be worked out which gives the dipole moment of the solute (μ_2) as a function of the molar dielectric increment:

$$\delta = \Delta\epsilon/C_2, \tag{4}$$

where $\Delta\epsilon$ is the measured dielectric increment and C_2 is the concentration of solute expressed in moles per liter.

By introducing the values for the internal field (E_i) and for the director field (E_r) given by Bottcher (1952), and the value of the molar refraction of solute (R_2), if C_2 tends toward zero, the dipole moment of solute is

$$\mu_2 = [(9KT/4\pi\mathfrak{N})(1 - [R_2C/V_2])([\delta/A] + [B/A] - R_2)]^{1/2}, \tag{5}$$

where

$$A = 9\epsilon_1/1000(1 - [R_2C/V_2])(2\epsilon_1 + 1),$$

$$B = (\epsilon_1 - 1)V_2/1000,$$

$$C = (2\epsilon_i - 2)/(2\epsilon_1 + 1),$$

$$\mathfrak{N} = \text{Avogadro's number.}$$

Equation 5 was used for the calculation of the dipole moment of AcCh. In our experiments chloroform was used as a solvent because of the negative dielectric increment observed for AcCh dissolved in polar solvents.

The theoretical value of the dipole moment of AcCh was calculated vectorially on the basis of the structural parameters given by Canepa et al. (1966). The dipole moment values reported by Smyth (1955) for each single bond were also utilized for a structure such as the one represented in Fig. 1. For simplicity, the vector calculation was done in two dimensions since the ester group is planar, the conformation of the molecule depends mainly on the values of the torsion angles of the portion C_5-O_1-C_6-O_2-C_7 (Chothia, 1970), and the spheric symmetry around the N atom implies a resulting dipole moment of zero for the portion $(C_1C_2C_3)N$-C_4.

We obtained therefore:

$$\mu(\overleftarrow{C_5O_1}) = 1.14D = \mu_1,$$

$$\mu(\overleftarrow{C_6O_1}) = 0.74D = \mu_2,$$

$$\mu(\overrightarrow{C_6C_7}) = 0.40D = \mu_3,$$

$$\mu(\overleftarrow{C_6O_2}) = 2.40D = \mu_4,$$

$$\mu_x = \mu_1 - \mu_2 \cos 65 - \mu_3 \cos 5 - \mu_4 \cos 58 = -0.845D,$$

$$\mu_y = -\mu_2 \cos 25 + \mu_4 \cos 31 + \mu_3 \cos 85 = 1.42D,$$

$$\mu_{th} = 1.65D,$$

and the angle $(\overrightarrow{\mu_{th}}, O_1C_5)$ is 120°.

The use of chloroform as a solvent ($\epsilon = 4.84$) and AcCh as a chloride salt must result in a close association of the two charged ions in the low dielectric so that the actual particle we are dealing with is the couple $(AcCh)^+(Cl)^-$. Under these conditions, according to the electrostatic laws, the permanent and induced dipole
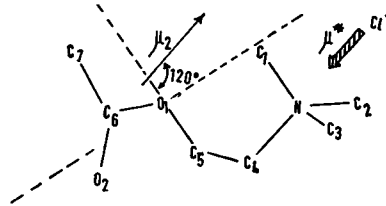

FIGURE 1 Structure of acetylcholine with dipole orientation.

moment of the AcCh molecule turns out to be parallel and opposite to the principal dipole moment of the ion pair (μ^*).

A detailed theory on the measurement of the dipole moment of ion pairs has been developed by Debye (1929) and Bauge and Smith (1964), starting from the basic equation:

$$\mu^* = e \times r, \tag{6}$$

where e is the electrical charge and r the distance between ions. If we consider $(AcCh)^+$ and $(Cl)^-$ in close contact we have:

$$r(Cl)^- = 1.72A,$$

$$r[N^+(CH_3)_4] = 2.16A,$$

$$r = r(Cl)^- + r[N^+(CH_3)_4] = 3.88A.$$

By assuming that the polarizability of the ion pair is the sum of the two polarizabilities, and that no solvent molecule is placed between the two ions, we obtain the dipole moment of the cation $(AcCh)^+$ alone in chloroform. In fact:

$$\alpha_1 (\text{polarizability of } Cl^-) = 3.012 \times 10^{-24} \text{ cm}^3,$$

$$\alpha_2 (\text{polarizability of } AcCh^+) = 14.943 \times 10^{-24} \text{ cm}^3.$$

If $\epsilon = 1$, i.e. a vacuum exists between the two ions, we can express the value of μ by Debye's formulation (1929) as:

$$\mu = \mu^* - (\mu_1 + \mu_1{}') - (\mu_2 + \mu_2{}'), \tag{7}$$

$$\mu = er - (\mu_1 + \mu_2) - [er^4(\alpha_1 + \alpha_2)/\epsilon + 2r^3(\alpha_1\mu_2 + \alpha_2\mu_1)/\epsilon]/r^6$$

$$- 4\alpha_1\alpha_2/\epsilon^2 + [4\alpha_1\alpha_2 er/\epsilon^2 + 4\alpha_1\alpha_2(\mu_1 + \mu_2)/\epsilon^2]/r^6$$

$$- 4\alpha_1\alpha_2/\epsilon^2, \tag{8}$$

Since the dipole moment of $(Cl)^-$ is equal to zero ($\mu_1 = 0$) equation 8 becomes:

$$\mu = er - \mu_2 - [er^6(\alpha_1 + \alpha_2)/\epsilon + 2r^3\alpha_1\mu_2/\epsilon + 4\,er\alpha_1\alpha_2/\epsilon^2 \tag{9}$$

$$+ 4\alpha_1\alpha_2\mu_2/\epsilon^2]/r^6 - 4\alpha_1\alpha_2/\epsilon^2.$$

This equation was used to calculate the value of the dipole moment of the $(AcCh)^+$ cation alone from the measured dipole moment of the ion pair in chloroform and from the value of the other parameters given above.

The ion pair is to be considered quite rigid in chloroform, since it has been seen from NMR (nuclear magnetic resonance) spectrograms of AcCh chloride in CDCl$_3$

that the Cl⁻ ion induces some perturbations in the proton signal of the CH_2 groups of AcCh (J. Parello. Personal communication).

EXPERIMENTAL

The AcCh used throughout this study was the chloride salt purchased from Fluka A.G. (Buchs, Switzerland). Solvents (Merck, Darmstadt W. Germany) were redistilled and dehydrated on calcium chloride after repeated washings with sulfuric acid 10%, sodium hydroxide 20%, and doubly distilled water. AcCh·Cl was desiccated at 100°C for 4 days and its solutions were prepared by weighing the compound in the absence of air, to prevent hydration.

The dielectric increment was measured with a resonant circuit system by using a Rhode-Schwarz RCL circuit fed by a variable frequency oscillator (10–100 MHz). The scheme of the circuit is given in Fig. 2. X is the cell where the solutions at different concentration were used as dielectrics after a calibration was made with solvent alone.

The details of the apparatus and a thorough description of the method have been given elsewhere (Maurel, 1969).

The molar volume was measured with a Jaulmes pycnometer at 20°C, the molar refraction with a Pulfrich-Abbe refractometer equipped with a sodium lamp, and the conductivity with a conductivity meter (Radiometer Co., Copenhagen, Denmark) attached to a PP1042 cell.

The dielectric increment value was calculated from the slope of the straight line obtained by plotting $\Delta\epsilon$ vs. the concentration of the compound.

The dipole moment values, measured or calculated as described, are summarized in Table I. The frequency used in the circuit was 10 MHz.

Equation 5 was used for the calculation of the dipole moment of AcCh·Cl after introducing in it values obtained for R_2 (45.31 cm³), ϵ (4.84), and V_2 (142.17 cm³).

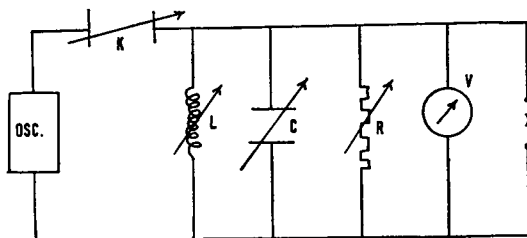The actual dipole moment for the (AcCh)⁺ cation differs somewhat from the expected

FIGURE 2 Scheme of the RCL-circuit.

TABLE I

Compound	Solvent	Dipole moment	δ
		D	(M^{-1})
AcCh·Cl	water-dioxane (1:1)	—	−221
AcCh·Cl	chloroform	8.5 ±0.4	10.8 ±0.05
AcCh⁺	chloroform	2.6 ±0.2	—
AcCh⁺	theoretical	1.65	—

value (1.65 D), but an association effect must be considered, since it has been shown that chloroform is able to interact with alkylammonium compounds (Galzigna, 1969; Galzigna and Mery, 1971).

The dipole moment of chloroform is 1 D so that we can calculate for the AcCh-chloroform complex a theoretical dipole moment value of about 2.65 D, which is quite similar to the value obtained for the (AcCh)$^+$ cation.

The value of 8.5 D for AcCh·Cl in chloroform is of the same order of the one obtained by Bauge and Smith (1946) for tetrabutylammonium iodide in benzene (12 D).

DISCUSSION

A strong discrepancy exists between the dipole moment value for AcCh assumed by Wei (1968), i.e., $\mu = 48$ D, and both our theoretical and measured values.

The value calculated by Wei was based, however, on two assumptions: (*a*) that two unit charges are localized on the N$^+$ atom and on the O$_2$ oxygen atom respectively; and (*b*) that the whole length of the molecule is 10 A.

We have considered that only a *partial* negative charge is localized on the oxygen, and that the whole length of the molecule varies from 6 to 5 A taking the *trans* and *cis* forms respectively (Canepa et al., 1966).

Despite wrong assumption as to an AcCh dipole moment value, some of Wei's ideas have received experimental support (Segal, 1968; Cohen et al., 1968; Fraser and Frey, 1968), and we wish to consider the apparently meaningful expression for the intensity of the electric field across the synapse:

$$F = np/2\epsilon R. \tag{10}$$

We consider that besides p (dipole moment of AcCh), the values assumed for n (dipole density), R (dimensions of the synapse) and ϵ (permittivity of the cleft) should also be modified. In fact ϵ and R must be lower than the proposed ones if the presence of synaptic structures within the cleft is to be taken into consideration (Bloom, 1969; Gray and Guillery, 1966; Gray, 1964; De Robertis, 1964; Hama, 1963; Whittaker, 1970). The presence of organic connections bridging the synaptic gap introduces a new parameter related to the modification of the above values and to the possibility of an anchoring of the dipoles to structures other than the postsynaptic membrane. Moreover, we should consider the presence of anions in the intersynaptic space, and the favorable electrostatic association of ion pairs due to the exchange cation-to-water and anion-to-water bonds. Our previous measurements of the conductivity of brain homogenates (Galzigna, 1970) showed an average conductivity of 5 microsiemens, which is closer to the value found for a 3×10^{-2} M solution of AcCh in chloroform (10 microsiemens) than the value found for the same concentration of AcCh in water (1.57 millisiemens). Physiological measurements have indicated that the synaptic gap, e.g. in the squid giant axon, is a pathway of low conductivity, since large changes in presynaptic mem-

brane potential induced by injecting current did not produce measurable electric leakage to postsynaptic axon (Hagiwara and Tasaki, 1958).

From the above considerations we think it likely that the actual dipoles acting in the cleft are ion-ion pairs such as $(AcCh)^+(Cl)^-$. In this case, the only configuration of the AcCh molecule vectorially compatible with the orientation of the moment is the *cis* one. In fact for symmetry reasons the Cl^- ion should interact with the N^+ along the $N-C_4$ axis and the $(AcCh)^+$ cation must assume a configuration such that its dipole moment is parallel to the same axis.

It is of interest that the *cis* form has been associated with the nicotinic activity and the *trans* form with the muscarinic activity of AcCh (Armstrong et al., 1968). The change from one configuration to the other must be associated with an important change in the dipole moment of the molecule. The transition from an ion pair to a cation $(AcCh)^+$ alone might play a paramount role in such a dipole moment change. The effect of other molecules (e.g. other neurotransmitters) coexisting with AcCh (Wise and Ruelius, 1968), and a number of metal ions such as Cu, Zn, and Fe (Colburn and Maas, 1965) found present in the synaptic structures must also be considered. These molecules might participate in the electric field formation by drastically influencing the conductivity of the intersynaptic space. The same function might be associated with a nonrigid behaviour of the synapse.

We think that the divalent behaviour of AcCh at the excitatory and inhibitory synapses must be related to its conformational plasticity, which allows it to assume two different configurations, nicotinic and muscarinic. The configuration of the transmitter molecule, its degree of hydration (Horne and Young, 1968), the presence of other molecules, and the physical characteristics of the synaptic cleft, which are all related and are modulable parameters, should contribute together to render one synapse either excitatory of inhibitory.

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