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Inelastic Electron Tunneling Spectroscopic Study of Interaction of Acetylcholine and β-Methyl Acetylcholine with Alumina Surface

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SUMMARY

The conformation of acetylcholine (Ach) and its muscarinic analogue β-methyl acetylcholine (β-MeAch) on an alumina surface was analyzed by inelastic electron tunneling spectroscopy (IETS). This method detects vibrational modes of organic molecules that are active in both Raman (R) and IR spectroscopies. By using previously recorded and interpreted R and IR spectra of Ach and β-MeAch in solid-state and aqueous solutions we studied the perturbations due to adsorption. The results were used to interpret the interaction of both molecules with the alumina surface, and a comparison to that with receptors or with acetylcholinesterase was attempted. In the case of nonhydrolytic interaction, the positive trimethylammonium groups of both molecules seemed to be attracted by the negative oxygen ions of the surface. There was evidence that the O—C—C—N skeleton of Ach changed its conformation in aqueous solution and adopted the solid-state conformation, which is very similar to that of β-MeAch. This conformation once established, Ach appeared to interact with the alumina surface in the same way as did β-MeAch: both tunneling spectra were very similar. There was also evidence that in the acetyl part of both molecules the C=O double bond was broken and that the oxygen atom coordinated with an Al^+ cation. The acetyl skeleton did not show important conformational changes for either molecule. In the case of hydrolytic interaction of Ach or β-MeAch, the products of the hydrolysis, acetate ion and choline—the latter also adsorbed in ionic form—were found on the alumina surface. In both cases the conformation of the lateral groups bonded to the choline and acetyl skeletons was also analyzed.

INTRODUCTION

Ach[1] is a synaptic transmitter that interacts with two distinct receptors of the postsynaptic membrane, the nicotinic and muscarinic receptors, as well as with Ach*E* (1). To understand the stereochemistry of these interactions, information on molecular structure is necessary. Many efforts have been made to elucidate the conformation of Ach and related compounds. Structural details in the solid state have been obtained by X-ray diffraction studies (2-8), and a large number of quantum mechanical calculations have been performed (9). NMR spectroscopy has provided interesting information about aqueous solutions (10-14). However, the complexity of the problem prevented achieving a complete determination of the structure and of the conformational possibilities of these molecules; therefore, methods that allow simultaneous observation in the solid state and in aqueous solution are quite desirable. R spectrometry complemented by IR spectrometry has been used to study the vibrational properties of Ach (15) as well as those of some of its analogues, namely a nicotinic analogue, nicotine (16), and two muscarinic analogues, muscarine and β-MeAch (17). Their conformational changes when passing from the solid state to an aqueous solution have been discussed in detail. Nevertheless the problem of the conformational possibilities of Ach and its analogues when attached to the receptors of the postsynaptic membrane remains unsolved. It has been the subject of many hypotheses, but experimental work is lacking.

We report here, as an extension of our earlier work (18), a detailed interpretation of the IET spectra of Ach and β-MeAch, obtained by adsorption on an alumina surface (Al_2O_3).

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[1] The abbreviations used are: Ach, acetylcholine; Ach*E*, acetylcholinesterase; R, Raman spectra; β-MeAch, β-methyl acetylcholine; IETS, inelastic electron tunneling spectroscopy.

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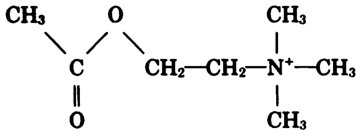
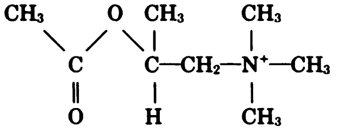


Ach



β-MeAch

Despite the fact that the alumina surface is not exactly a postsynaptic membrane, it does possess complementary adsorption sites ($Al^{+}—O^{-}$ acid-base pairs) which may provide a model for the complementary sites believed to exist in Ach receptors and Ach*E* (19). The interaction of Ach and β-MeAch with a surface being determined especially by their conformational possibilities, we believe that our results could be relevant to the situation at the membrane level.

The IETS method is particularly well adapted for adsorption studies and can detect vibrational modes of organic molecules that are active in both R and IR spectra in the range of 300–4000 cm^{-1} without significant interference from the vibrational modes of the adsorbate. The details of the spectra can be interpreted in terms of molecular conformation and of perturbations due to adsorption. The method has a resolution of about 0.5 meV (4 cm^{-1}) in the present case, and a very high sensitivity (down to 1/30 monolayer) (20). Tunneling spectra have been obtained for relatively small molecules as well as for large species such as amino acids and proteins and other biological materials (20).

In this study we show that IETS provides important information about the interaction of Ach and β-MeAch with an alumina surface. Ach is a flexible molecule. Its unsubstituted O—C—C—N chain is capable of relatively unhindered rotation around the O—C and C—C bonds, whereas the β-methyl substitution in β-MeAch limits this possibility. Thus, in transition from the solid state to an aqueous solution, the Ach conformation changes considerably (15) but only slight differences have been observed in the case of β-MeAch (17). It has been proposed that the conformation of β-MeAch in solution is already closer to the structure fitting the receptor molecule, whereas Ach takes its "active" form only in the neighborhood of the receptor (17). The present analysis has yielded experimental results on the behavior of the molecules in the vicinity of an alumina surface in terms of molecular configuration in the absorbed state and illustrates analogies with adsorption on a receptor site.

EXPERIMENTAL PROCEDURES

The principle and some applications of tunneling spectroscopy have been reviewed recently (20). The molecules to be studied were adsorbed on the oxide surface of a metal-oxide-metal tunnel junction. When a bias is applied to the junctions, the electrons that tunnel through the oxide layer (about 20 Å thick) may excite vibrational transitions in the molecules. When the bias becomes sufficient for that to occur ($eV = \hbar\omega$, where eV is the bias and $\hbar\omega$ is the energy of the transition), the conductance of the junction varies slightly. This is rendered more visible in the derivative of the junction conductance versus voltage: a transition is then marked by a peak, hence spectra such as those shown in Fig. 1. The spectra are measured at liquid helium temperature to avoid thermal broadening. Both IR-active and R-active modes as well as inactive modes are observed by IETS. Peak positions coincide if a slight shift due to the dipole image in the counter electrode is taken into account. This shift is of the order of a few tenths of 1% (21, 22). On the other hand, the relative intensities of the peaks are quite different from those observed in the R and IR spectra. They are not well understood, although it appears that orientation [modes with the dipole derivatives parallel to the plane of the junction are strongly attenuated (20)] and proximity to the counter electrode (21) play important roles. Great care must therefore be taken in interpreting intensities in tunneling spectra.

In the present study, aluminum-oxide-lead junctions were used. A film of aluminum (2000 Å) was deposited on a glass substrate and oxidized in a plasma of oxygen. The oxide layer was roughly 15 Å thick. The slide was then removed from the evaporator for treatment with an aqueous solution of Ach or β-MeAch. This was done under two different conditions.

Condition A. A 10^{-4} M aqueous solution of AchI or β-MeAchBr was deposited onto the alumina surface and left for 2 min. The excess solution was then blown off with a jet of argon.

Condition B. The molecules were deposited as indicated above, but the sample was rinsed with distilled water and then dried with argon.

The samples were returned to the bell jar, which was evacuated to 10^{-6} torr, and a lead counter electrode was deposited. The whole process took place at room temperature.

AchI and β-MeAchBr were obtained from Sigma Chemical Company (St. Louis, Mo.) and were used without further purification.

RESULTS

Tunneling Spectra of Ach and β-MeAch Recorded under Experimental Condition A

In Fig. 1 are shown the tunneling spectra of an undoped Al-Al_2O_3-Pb junction (*a*) and the same junction doped with AchI (*b*) and β-MeAchBr (*c*) under Condition A. Table 1 lists the peak positions and the apparent peak intensities. For purposes of comparison we have added the corresponding positions and assignments of vibrational R and IR modes of AchI and β-MeAchBr in the solid state and in aqueous solutions, studied previously (15, 17).

An important feature which characterized the interaction of Ach and β-MeAch with the alumina surface was the similarity of their tunneling spectra (Fig. 1, *b* and *c*). We present the analyses of these spectra successively for the two parts of the molecules: the choline part and the acetyl part. In each one we distinguish between the skeleton and lateral group vibrations.

Choline part.

Choline skeleton (O—C—C—N). Most significant for this skeleton was the strong bending vibration at 443 cm^{-1}, which appeared in the tunneling spectra of both Ach and β-MeAch. This vibration was also very strong in the aqueous solution and solid-state R spectra of β-MeAch at 450 cm^{-1} and 455 cm^{-1}, respectively. The solid-state R spectrum of AchI showed the vibration at 450 cm^{-1}, but it was barely visible in the aqueous-solution R spectrum of AchI (Table 1). Thus the O—C—C—N skeleton of the choline part of β-MeAch retained its aqueous-solution and solid-state conformation in the vicinity of the alumina surface. In contrast, the O—C—C—N skeleton of Ach on the surface adopted its solid-state conformation, which is similar to that of the β-MeAchBr skeleton (17) (Table 1). This was confirmed by the presence of two other choline skeleton vibrations (shoulders) at 484 and 548 cm^{-1} in the tunneling spectrum of β-MeAch and of one shoulder at 532 cm^{-1} in the spectrum of Ach. We found the corresponding R and IR vibrations at 502 cm^{-1} and 548 cm^{-1} for solid-state β-MeAchBr at 485 cm^{-1} and 535 cm^{-1} for AchI. The R spectra revealed another vibration at 435 cm^{-1} for β-MeAch and at 422 cm^{-1} for Ach which was not visible in the tunneling spectra of the same molecules. It may have been concealed by the slope of the strong peak at 443 cm^{-1}.

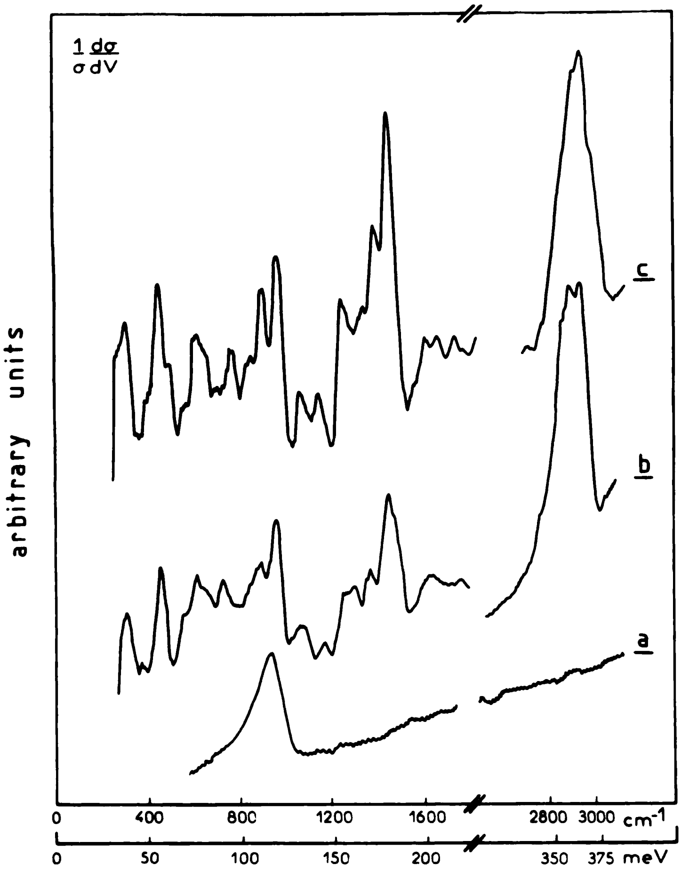


FIG. 1. *Tunneling spectra of Al-Al$_2$O$_3$-Pb junctions under Condition A (see text)*

a, Undoped; *b*, doped with AchI; *c*, doped with β-MeAchBr. Spectra were recorded at 4.2 K with a 2-mV rms modulation.

Lateral groups bonded to the choline skeleton. Three methyl groups were bonded to the positive nitrogen atom in both molecules. They were characterized by two stretching vibrations: the symmetrical $\nu_s(CH_3)N$, and the asymmetrical $\nu_a(CH_3)N$. In the solid-state R spectra we found them at about 2930 cm^{-1} and 3000 cm^{-1}, respectively (15) (Table 1). In tunneling spectra $\nu_s(CH_3)N$ appeared as a shoulder at 2911 cm^{-1} for both Ach and β-MeAch. Although $\nu_a(CH_3)N$ was very strong for Ach at 3006 cm^{-1}, it appeared as a shoulder for β-MeAch.

The methyl groups were also characterized by two bending vibrations. The in-plane bending vibration, $\delta(CH_3)N$ rocking, was situated in the solid-state AchI and β-MeAchBr R and IR spectra at 913 cm^{-1} and 912 cm^{-1}, respectively. It disappeared completely in tunneling spectra. The out-of-plane bending vibrations, $\delta(CH_3)N$ twisting or wagging, found at about 1404 cm^{-1} in the solid-state Ach and β-MeAch R and IR spectra, were probably included in the wide and strong peak at about 1440 cm^{-1}. Equally invisible in the tunneling spectra of β-MeAch was the asymmetrical stretching vibration of the β-methyl group, whereas it appeared strong in the solid-state R spectrum at 2967 cm^{-1} (Table 1).

We also include here the stretching vibrations of the C—N bonds. The ν_s(C—N) mode at 895 cm^{-1} for β-MeAch was strong in intensity. We found it at 871 cm^{-1} for Ach, medium in intensity. The frequencies of the ν_a(C—N) vibrations were as follows: 1315 cm^{-1}, weak for β-MeAch; 1330 cm^{-1}, strong for Ach. The latter was displaced by 26 cm^{-1} from the corresponding R and IR solid-state vibrations.

These observations indicate that on the alumina surface the conformation of the methyl groups bonded to the positive nitrogen atom were different for Ach and β-MeAch. These conformations were also different from the conformations of the same groups in the solid-state and aqueous-solution Ach and β-MeAch.

The methylene groups (CH_2) of the choline part of the molecules could be characterized by two vibrations in the solid-state and aqueous-solution spectra which were not mixed with other vibrations, namely $\nu_a(CH_2)$ at about 2946 cm^{-1}, and $\delta(CH_2)$ rocking at 759 cm^{-1} for β-MeAch and 720 cm^{-1} for Ach (Table 1). The tunneling spectra indicated that these groups preserved their respective conformations on the alumina surface.

The choline and acetyl part of both Ach and β-MeAch are bound together by a C—O—C bond. In the IR and R spectra (Table 1) we found the symmetrical stretching vibration, ν_s(C—O), at 952 cm^{-1} (Ach) and 973 cm^{-1} (β-MeAch) for aqueous solutions (Table 1). In the tunneling spectra of both molecules the same vibration, ν_s(C—O), was mixed with the ν(Al—O) mode. The asymmetrical vibration, ν_a(C—O), was found at 1242 cm^{-1} for Ach as a wide peak. In the β-MeAch spectrum the ν_a(C—O) was situated at 1234 cm^{-1}. The solid-state IR spectrum showed it at 1234 cm^{-1}, and the aqueous-solution IR spectrum at 1249 cm^{-1}. Thus, at least for β-MeAch, we could conclude that the C—O—C bond did not change its conformation on the alumina surface.

Acetyl part.

Acetyl skeleton (C—C—O). The tunneling spectrum of the acetyl skeleton was also similar for both molecules

Peak positions and intensities in IR, R, and tunneling spectra (Condition A) for AchI and β-MeAchBr

The abbreviations used are: vw, very weak; w, weak; m, medium; s, strong; vs, very strong; sh, shoulder.

β-MeAchBr				Tunneling spectra				AchI				Assignment
Aqueous solution		Solid state		β-MeAchBr		AchI		Solid state		Aqueous solution		
R	IR	R	IR	cm^{-1}	meV	cm^{-1}	meV	R	IR	R	IR	
420 vw		435 m						422 m	423 w	418 vw		
450 vw		455 vs	454 vw	443 s	55 s	443 s	55 s	450 vs	453 m	450 vw		δ choline skeleton
		502 m	468 vw	484 sh	60 sh			485 m	482m			
			501 m									O—C—C—N
544 m		548 m	546 w	548 sh	68 sh	532 sh	66 sh	535 m	532 vw	525 vw		
		598 w	599 m	605 s	75 s	587 s	74 s	608 w	607 m			
610 w												δ acetyl skeleton
646 s		638 vs	636 m	653 sh	81 sh	645 sh	80 sh	644 vs	644 m	645 vs		C—C—O
834 s		828 vs	827 w	839 w	104 w			828 vs	827 m	834 s		
762 vs		759 vvs		750 m	94 m	710 m	88 m	720 vs		720 vs		δCH_2r
895 s	891 m	892 s	893 s	895 s	111 s	871 m	108 m	863 m	864 m	875 s	874 m	$\nu_sC—N$
909 sh	907 m	913 sh(w)	912 m					913 m	912 m	960 sh	960 s	$\delta(CH_3)Nr$
952 sh	945 s	940 sh(m)	937 s									$\nu C—C$
964 s	962 sh	958 sh(s)	957 m	960 s	119 s	944 vs	117 vs	953 m	955 vs	952 vs	951 s	$\nu_sC—O + \nu(Al—O)$
975 sh	973 s	976 vs	973 s									
1025 vw	1020 m	1020 w	1016 s									
			1037 m									
1070 w	1065 s	1065 m	1063 s	1048 s	130 s	1048 m	130 m	1055 vw	1052 vs	1055 w	1055 s	
			1073 m						1072 vs		1089 m	$\nu C—C + rCH_3$
1100 w	1127 s	1098 w	1102 w	1113 m	138 m							
1131 w	1134 sh	1134 w	1128 m	1120 m	139 m	1145 m	142 m	1139 vw	1136 m	1143 vw	1135 w	
		1150 sh	1141 m									
1225 w		1225 w	1222 sh									
1245 w	1249 vs	1245 sh	1234 vs	1234 m	153 m	1242 m[a]	154 m[a]	1221 w	1223 vs	1240 vw	1248 vs	$\nu_aC—O$
1280 vw		1280 m						1277 w		1271 vw		δCH_2t *ou* *w*
1329 w	1321 vw	1327 s	1321 w	1315 w	163 w	1330 s	165 s	1304 w	1303 vs			$\nu_aC—N$
1362 w	1379 m	1359 s	1355 sh			1354 m	167 m	1362 vw	1366 vs			$\delta CH_2w + \delta_s(CH_3)C$
1382 sh			1365 s	1379 m	171 m				1381 vs			
	1403 vw	1404 s	1398 w					1409 s	1410 m			$\delta(CH_3)Nt$ *ou* *w*
1428 sh	1421 w	1420 sh	1417 sh							1420 sh	1424 sh	
			1426 m									$\delta CH_2 + \delta CH_3$
		1442 sh	1440 vw	1442 s	178 s	1434 s	177 s	1445 s	1448 vw			
1450 s		1454 s	1455 vw					1459 sh		1450 vs	1445 sh	
1470 sh	1477 s	1477 sh	1475 m					1475 sh	1477 vs	1475 sh	1478 s	δCH_2
	1487 s		1487 s								1487 s	
1725 m	1728 vs	1730 s	1732 vs					1740 m	1737 vs	1735 m	1734 vs	$\nu C═O$
2893 vw		2887 sh	2884 w					2867 vw				ν_sCH_2
		2935 s		2911 sh[b]	367 sh[b]	2911 sh	361 sh	2920 m				$\nu_s(CH_3)N$
2948 s		2946 s	2947 w	2952 vs	366 vs	2944 vs(sh)	365 vs(sh)	2946 s		2945 s		ν_aCH_2
		2967 s	2968 m									ν_aCH_3(β-MeAch)
2980 sh		2983 s	2982 w					2978 s	2975 m	2978 sh	2973 vw	$\nu_a(CH_3)C$
										2989 vs		
2988 s		2996 sh	2997 s									
		3002 vs	3000 s	3006 sh	372 sh	3006 vs	373 vs	3005 vs	3002 m			
			3008 s					3011 vs				$\nu_a(CH_3)N$
3045 m		3021 sh	3019 sh					3030 vw	3029 w	3045 w	3040 vw	
		3035 vw										
		3054 vw	3050 vw									

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[a] Broad.

[b] Badlv resolved.

and was characterized by strong vibration at 587 cm^{-1} for Ach and at 605 cm^{-1} for β-MeAch. It was accompanied by a shoulder at 645 cm^{-1} in the spectrum of Ach and at 653 cm^{-1} in that of β-MeAch. The latter had another weak vibration at 839 cm^{-1} which was not visible for Ach, but possibly it was hidden by the noise.

All of these vibrations corresponded well to the acetyl skeleton vibrations in the solid-state and aqueous-solution R and IR spectra of Ach and β-MeAch (Table 1). Thus this skeleton did not present radical modifications on the alumina surface.

Lateral groups bonded to the acetyl skeleton. Two groups belong here: C—CH_3 and C═O.

The $\upsilon_a(CH_3)C$ of the methyl group bonded to the carbon atom was identified in the R and IR spectra of both molecules at about 2980 cm^{-1} (Table 1). However, it could not be distinguished in their tunneling spectra, probably being masked by the neighboring C—H vibrations; this was an indication of its low intensity on the alumina surface.

The stretching vibration of the C═O group (υC═O) appeared very strong, especially, in the solid-state and aqueous-solution IR spectra, at 1740 cm^{-1} (Ach) and at 1730 cm^{-1} (β-MeAch). This vibration was absent in both tunneling spectra (Table 1), which showed unambiguously that the double bond was broken.

Tunneling Spectra of Ach and β-MeAch Recorded under Experimental Condition B

Figure 2 shows the tunneling spectra of AchI (*a*) and β-MeAchBr (*b*) on the alumina surface under Condition B, and the acetate ions formed at the junction treated with acetic acid (*c*) and ChI (*d*). Table 2 lists the peak positions and apparent peak intensities in the tunneling spectra recorded under Condition B. It is completed with the R and IR vibrations of AchI in aqueous solution, before and after non-enzymatic hydrolysis; the aqueous solution vibrations of ChI, studied previously (15, 17); and the tunneling vibrational modes of acetic acid and ChI.

Under Condition B, after the deposition of Ach and β-MeAch, the surface was rinsed with distilled water. The tunneling spectra were similar for both molecules but they were very different from the spectra obtained under Condition A.

Significant differences appeared in the choline and acetyl skeleton vibrations. Thus the principal vibration of the choline skeleton (O—C—C—N) at 443 cm^{-1} (Table 1) was now split, widened, and shifted toward lower frequencies: 411 cm^{-1} and 427 cm^{-1} for Ach, and 403 cm^{-1} and 427 cm^{-1} for β-MeAch (Table 2). It was the only vibration which characterized the choline skeleton of both molecules under these conditions.

In the acetyl skeleton (C—C—O) the strong vibration at 587 cm^{-1} for Ach and at 605 cm^{-1} for β-MeAch (Table 1) was now shifted to 621 cm^{-1} and 613 cm^{-1}, respectively. The weak vibration at 645 cm^{-1} and 653 cm^{-1} was missing, whereas a very intense peak appeared at 685 cm^{-1} (Ach) and 677 cm^{-1} (β-MeAch).

These skeleton modifications, together with the appearance of a predominant peak at 1596 cm^{-1} and the disappearance of the strong vibration υ_a(C—O) at about 1220 cm^{-1} (Fig. 2; Table 2), provided strong evidence that a reaction had taken place on the alumina surface leading to structural changes in the adsorbed molecules with formation of a new species.

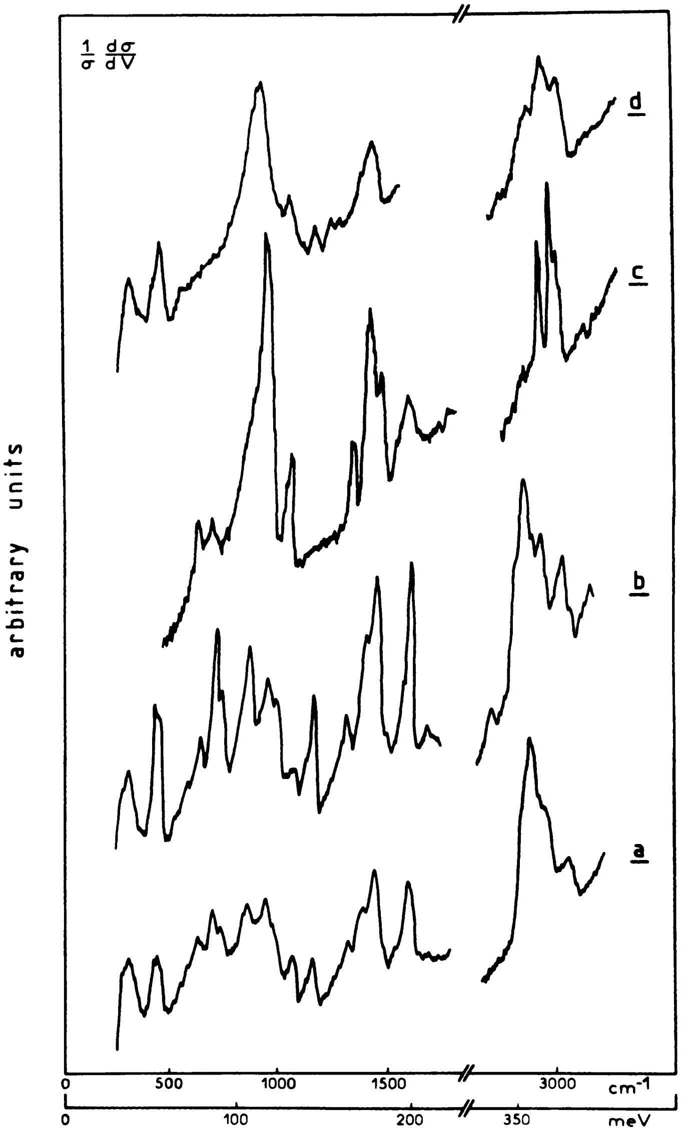


FIG. 2. *Tunneling spectra of Al-Al_2O-Pb junctions under Condition B (see text)*

a, Doped with AchI; *b*, doped with β-MeAchBr; *c*, doped with acetic acid; *d*, doped with ChI. Spectra were recorded at 4.2 K with a 2-mv rms modulation.

Some spectral features listed in Table 2 enable us to clarify the phenomenon. Thus a survey of the R and IR spectra of Ach after hydrolysis (Table 2) revealed that they possessed three very strong peaks which are characteristic of the $\upsilon_s COO^-$, $\upsilon_a COO^-$, and υ(C—C) modes of the acetate ion formed as a final product of the hydrolysis, together with choline (23). The $\upsilon_a COO^-$ vibration appeared at 1566 cm^{-1} (seen only in IR spectra), the $\upsilon_s COO^-$ appeared at 1420 cm^{-1} (R) and 1413 cm^{-1} (IR), and the υ(C—C) was found in the R spectrum at 930 cm^{-1}. The formation of the acetate ion after hydrolysis was accompanied by the disappearance of the very strong υC═O vibration at 1734 cm^{-1} and υ_a(C—O) at 1251 cm^{-1}.

TABLE 2

Peak positions and intensities in IR, R, and tunneling spectra (Condition B) for AchI, β-MeAchBr, CH_3COOH, and ChI

The abbreviations used are: vw, very weak; w, weak; m, medium; s, strong; vs, very strong; sh, shoulder.

IR and R Spectra								Tunneling spectra								Assignment
AchI: aqueous solution				ChI				AchI		β-MeAch		CH_3COOH		Choline		
Before hydrolysis		After hydrolysis		Aqueous solution		Solid state		cm^{-1}	meV	cm^{-1}	meV	cm^{-1}	meV	cm^{-1}	meV	
R	IR	R	IR	R	IR	R	IR									
418 vw				419 vw		420 m	410 vw	411 vs	51 vs	403 vs	50 vs					
450 vw						449 s	450 w	427 vs	53 vs	427 vs	53 vs			440 s	55 s	δ choline skeleton (O—C—C—N)
				477 vw		468 sh	468 w									
525 vw				526 vw		531 m	531 w									
								621 m	77 m	613 m	76 m	613 w	76 w			δ acetyl skeleton (O—C—O)
645 s		645 vvw						685 s	85 s	677 s	84 s	677 w	84 w			
834 s																
720 vs		709 vs		709 vs		713 vs		726 sh	90 sh	718 sh	89 sh					$\delta CH_2 r$
875 s	874 m	864 m		864 m		859 s	860 m	847 s	105 s	847 s	105 s					ν_sC—N
		930 vs						935 m	116 m	935 m	116 m	935 s	116 s	935 vs	116 vs	νC—C acetate + νAl—O
960 sh	960 s			879 s	880 vw	896 s	895 m	976 sh	121 sh	976 sh	121 sh					$\delta(CH_3)N r$
952 s	951 s	953 s		953 vs		956 vs	956 vs									ν_sC—O
												1032 sh	128 sh			
1055 w	1055 s			1052 m	1052 m	1054 m	1053 m	1056 m	131 m	1056 m	131 m	1048 s	130 s	1056 w	131 w	
	1089 m				1085 m	1079 w	1079 s									νC—C + $\delta CH_3 r$
1143 vw	1135 w			1136 m	1132 m	1133 w	1129 m	1145 s	143 s	1145 s	143 s			1173 w	146 w	
	1251 vs					1231 vs	1231 vw									ν_aC—O
						1334 m		1306 m	162 m	1306 m	162 m					ν_aC—N
1346 m		1346 m					1367 sh							1371 sh	170 sh	δCH_2 *w ou t*
												1339 s	166 s			$\delta(CH_3)C$
								1387 sh	172 sh	1387 sh	172 sh	1411 vs	175 vs			
		1420 s	1413 vs					1429 vs	177 vs	1435 vs	178 vs	1468 s	182 s	1444 s	179 s	$\nu_s COO^-$
1450 vs	1445 sh	1450 vs		1446 vs		1454 sh	1454 sh									$\delta_a CH_3$
						1458 s										
1475 sh	1477 s	1475 sh	1477 s	1471 sh	1477 w		1477 m									δCH_2
	1488 sh		1488 sh													
			1566 vs					1596 vs	198 vs	1596 vs	198 vs	1597 s	198 s	1605 w	199 w	$\nu_a COO^-$
1735 m	1734 vs															νC=O
						2885 m	2881 w	2879 vs	357 vs	2879 vs	357 vs	2839 sh	352 sh	2855 sh	354 sh	
												2911 s	361 s			$\nu_s CH_2 + \nu_s(CH_3)C$
2945 s		2941 s		2941 m		2930 s	2925 vw							2935 s	364 s	$\nu_s(CH_3)N$
2978 sh	2973 vw			2984 vs		2954 s	2948 m	2952 vs	366 sh	2944 vs	365 vs	2964 vs	368 vs			$\nu_a CH_2 + \nu_a(CH_3)C$
2989 vs		2989 vs										2992 s	371 s			
						3008 vs	3006 m									
						3012 vs								3008 s	373 s	$\nu_a(CH_3)N$
						3027 s	3023 m									
3045 w		3045 w		3045 w		3045 w		3048 m	378 m	3056 m	379 m					

All of the features discussed above were found in the tunneling spectra (Table 2). We conclude therefore that under these experimental conditons the alumina surface acts as a catalyst for the hydrolysis of Ach and β-MeAch, as does a basic aqueous medium with pH greater than 9 (23) or as does Ach*E* on the postsynaptic membrane. Further confirmation of this conclusion has been obtained by investigation of the tunneling spectrum of acetic acid chemically adsorbed on alumina (24) as well as the spectrum of choline alone recorded in this study (Fig. 2, *c* and *d*; Table 2).

When the COOH group of acetic acid reacts with the alumina surface, a COO^- ion is formed and the extra electron on the oxygen atom participates in the coordinate bonding to an aluminum atom in the substrate. The features seen in the spectrum of the acetate ion (Fig. 2, *c*) were present in the Ach and β-MeAch spectra (Fig. 2, *a* and *b*), but the $\nu_a COO^-$ and δ(C—C—O) modes were relatively more intense.

As far as the choline tunneling spectrum is concerned (Fig. 2, *d*), it was of interest to find the same features as those in the spectrum of adsorbed acetate ions: two peaks, situated at 1444 cm^{-1} and at 1605 cm^{-1}, which corresponded to $\nu_s COO^-$ and $\nu_a COO^-$. The liberated choline also appeared to adsorb in ionic form on the alumina surface. Thus the large intensities for the $\nu_a COO^-$ modes in the tunneling spectra of Ach and β-MeAch as compared with the spectrum of acetate ions could be explained by the superposition of vibrations of the νCOO^- groups coming from the acetyl and choline parts of the hydrolyzed and ionized molecules.

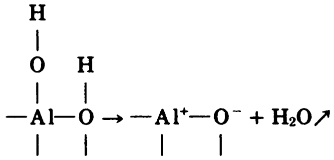
The presence of both products of hydrolysis on the alumina surface considerably complicated interpretation of the lateral vibrations. Nevertheless some comments can be made about the methyl and methylene groups. The $\nu_s(CH_3)N$ vibration, which previously was present at 2911 cm^{-1} for Ach and β-MeAch, was missing here. The $\nu_a(CH_3)N$ vibration, which was present at 3006 cm^{-1} for Ach and β-MeAch, was now shifted to 3048 cm^{-1} for Ach and 3056 cm^{-1} for β-MeAch and corresponded well to the respective frequencies of the aqueous-solution Ach and β-MeAch spectra. The $\delta(CH_3)N$ rocking vibration, which was missing from the tunneling spectra in Table 1, appeared here as a shoulder at 976 cm^{-1} for both molecules (Table 2). The stretching ν_s(C—N) vibrations, which appeared at 871 cm^{-1} (Ach) and 895 cm^{-1} (β-MeAch), as well as the ν_a(C—N) vibration at 1330 cm^{-1} and 1315 cm^{-1}, respectively (Table 1), were also modified. The ν_s(C—N) was now at 847 cm^{-1} and ν_a(C—N) at 1306 cm^{-1} for both molecules (Table 2). Thus the conformation of the methyl groups bonded to the nitrogen atom seemed to change greatly as compared with that of the choline part of nonhydrolyzed Ach and β-MeAch, and became almost identical for both molecules.

As in the case of experimental Condition A, the methylene groups can be characterized by the nonmixed vibrations, $\nu_a CH_2$ and $\delta CH_2 r$. The $\nu_a CH_2$ mode remained unchanged at 2952 cm^{-1} for Ach and at 2944 cm^{-1} for β-MeAch. The $\delta CH_2 r$, found at 710 cm^{-1} for Ach and at 750 cm^{-1} for β-MeAch (Table 1), was now located at 726 cm^{-1} and at 718 cm^{-1}, respectively (Table 2). Thus the conformation of the methylene groups did not show significant modifications except that here again the difference between Ach and β-MeAch seemed to be suppressed.

DISCUSSION

Careful attention must be given to defining the nature of the alumina in tunneling junctions. γAl_2O_3 has been widely studied by IR spectrometry but nearly always in its dehydrated state after prolonged evacuation above 500°.

The alumina of tunneling junctions has not been subjected to such treatment. It is formed under a residual water vapor pressure of the order of 10^{-7}–10^{-6} torr, and the presence of hydroxyl groups on its surface is proven by the OH stretching vibrations present in all spectra. Nevertheless, the fact that tunneling alumina is exposed to a pressure of 10^{-6} torr probably causes a partial dehydroxylation of the surface. Weak acid-base pairs then appear (27). We can schematize the reaction as follows:



$$\begin{array}{c} H \\ | \\ O \quad H \\ | \quad\; | \\ —Al—O \end{array} \rightarrow \begin{array}{c} \\ \\ \\ \\ —Al^+—O^- \end{array} + H_2O\nearrow$$

These sites have been shown to be present on the surface of the alumina of tunnel junctions (24).

Ammonia and pyridine adsorb with difficulty on tunnel junctions (pyridine can be held by prior physical adsorption at 77 K and, in this case, the presence of Lewis acid sites is indicated). On the other hand, acids (in particular, carboxylic acids) adsorb very well. This adsorption takes place via two different mechanisms, depending on the acid coverage (24). The acid decomposes, its proton remaining on the surface in the first stage, whereas at higher coverage the proton combines with an —OH group to desorb as water.

In the following discussion we use this information first to describe the interaction of the molecules with the alumina surface in the nonhydrolyzed state (Condition A) and then to suggest a mechanism of hydrolysis under Condition B.

Nonhydrolytic Interaction of Ach and β-MeAch with the Alumina Surface (Condition A)

There are two active groups in both Ach and β-MeAch: the trimethylammonium group of the choline part, which carries a full positive charge, and the ester group of the acetyl part, which is strongly polarized. It has been suggested (25) that on the active surface of the receptor there are two corresponding and complementary groups, anionic and esteratic. The negative charge of the anionic site in the receptor molecule which interacts with the N^+ of Ach has been associated with a small group, perhaps an oxygen atom. From electron density calculations in the Ach molecule (9), a dipole-dipole interaction between the C═O group of the acetyl part of Ach and an unknown charge in the electrophilic portion of the Ach receptor has been suggested (25), but other interactions are possible.

On the alumina surface the two corresponding and complementary active centers which can interact with Ach and β-MeAch could be the Al^+ and O^- ions. Thus we could explain the interactions of both molecules in the following way.

Choline part. The positive trimethylammonium group is attracted by the negatively charged oxygen atom of the surface. The interaction does not seem to influence the conformation of the choline skeleton of β-MeAch, which remains close to that of its solid state and aqueous solution (Table 1). This is probably due to the great distance between the two opposed charges forming an ionic bond, as suggested by Nachmansohn (19). If it is assumed that the unbonded oxygen radius is 1.5 Å and the radius of the trimethylammonium is 3.5 Å, the closest approach of the two charges can be estimated at 5 Å.

When the flexible Ach molecule approaches the alumina surface, there is evidence that the conformation of the choline skeleton changes by a rotation around the O_1—C_5 bond (15) and adopts the solid-state conformation which is very similar to that of the β-MeAch skeleton (Table 1). This conformation once established, Ach seems to interact with the alumina surface in the same way as does β-MeAch: both tunneling spectra were very similar (Fig. 1, *a* and *b*; Table 1). This similarity applies especially to the conformation of the choline and acetyl skeletons. However, there are slight differences in the conformations of the three methyl groups bonded to N^+. Some differences were noted when we compared the tunneling spectrum of Ach with that of β-MeAch; they are not surprising because of the differing solid-state conformations of these groups in both molecules (17). Other differences were found in comparing the tunneling spectrum of each molecule with its solid-state R and IR spectra; we cannot give a satisfactory explanation for these differences. In contrast, the methylene groups (CH_2) do not indicate any conformational changes on the alumina surface.

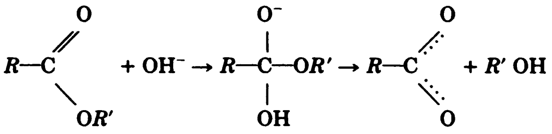
Acetyl part. Tunneling spectroscopic studies have been conducted on the interaction with the alumina surface of various molecules with C═O bonds (26). Carboxylates have been found to form on the surface.

Our tunneling spectra indicate (Fig. 1; Table 1) that the acetyl part of both molecules interacts with positive Al^+ sites on the alumina surface via the strongly polarized oxygen atom of the C═O group. This is indicated by the absence of the characteristic vibration $\upsilon(C{=}O)$, expected at about 1740 cm^{-1}, in the tunneling spectra. However, it is clear that the carboxylate is not formed because we did not find the typical peak for the $\nu_a COO^-$ vibration at about 200 meV (1600 cm^{-1}). Therefore, we propose that in the course of the interaction the C═O double bond is broken and that the O is coordinately bonded to an Al^+ cation.

Hydrolytic Interaction of Ach and β-MeAch with the Alumina Surface

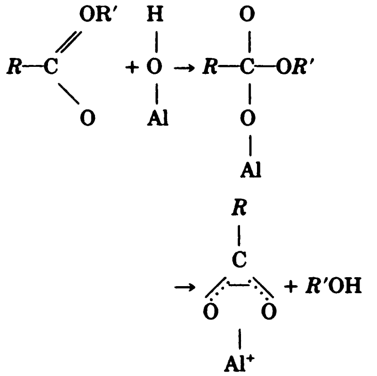
In both series of experiments (Conditons A and B), aqueous solutions of Ach and β-MeAch were deposited on the alumina surface, but hydrolysis occurred only when the samples were rinsed with distilled water. It is known from previous experiments that enzymatic hydrolysis is maximal at a pH between 7.8 and 8.5 (19) and that non-enzymatic hydrolysis requires a more basic environment [above pH 10 (23)].

According to the classical scheme (28), hydrolysis of the esters takes place as follows:



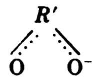
where $R = CH_3$ and $R' = CH_2CH_2N(CH_3)_3$ in the case of Ach.

Assuming that OH^- is an alumina surface radical, we can write the reaction:


(1)

(2)

The choline ($R'OH$) would then react with the alumina surface, as alcohols are known to do (29), to form a carboxylate ion:



Configuration 1 would have a spectrum close to the one that we observed under Condition A, the molecules being only slightly affected, apart from the disappearance of the double bond C═O. Configuration 2 would correspond to the case where the molecule is indeed hydrolyzed, as under Condition B.

It is not clear why a rinse in distilled water should provoke the change from Configuration 1 to Configuration 2. It is possible that the molecules diffuse rapidly toward the alumina surface under Condition A, forming a local concentration much higher than that of the doping solution. Rinsing the samples could lower this concentration, displacing the equilibrium of the reaction: Configuration 1 → Configuration 2. This suggestion is highly speculative in the absence of precise knowledge of the molecular interactions near and on the alumina surface.

CONCLUSIONS

The results presented in this paper show that IETS can be used to obtain information about the interaction of Ach and its analogue, β-MeAch, with an alumina surface which can be useful in understanding the "active conformation" of Ach at the postsynaptic membrane surface as well as its hydrolysis by Ach*E*. Thus, in the course of nonhydrolytic interaction of Ach and β-MeAch with the alumina surface, the positive trimethylammonium groups of both molecules seem to be attracted by the negative oxygen atoms of the surface. This interaction does not influence the choline skeleton of β-MeAch; its conformation remains close to that of solid-state and aqueous-solution β-MeAch. In contrast, in Ach the choline skeleton assumes its solid-state conformation, similar to that of β-MeAch.

There is evidence that in the acetyl part of the molecules the C=O double bond is broken and that the oxygen atom coordinates with an Al^+ cation. The acetyl skeleton itself does not show important conformational changes for either molecule.

Among the lateral groups bonded to the two skeletons, the methylene groups do not indicate any conformational changes on the alumina surface, but the methyl groups bonded to the positive nitrogen atom exhibit some differences between Ach and β-MeAch as well as between tunneling spectra and solid-state R spectra for each molecule.

Under Condition B, alumina catalyzes the hydrolysis of both molecules, as does AchE on the postsynaptic membrane or as does a basic aqueous medium with a pH greater than 9. Products of hydrolysis were found on the alumina surface: acetate ion and choline, the latter also adsorbed in ionic form.

The following observations have been made concerning the lateral methyl and methylene groups of the separated choline: (a) The conformation of the methyl groups bonded to the positive nitrogen atom becomes almost identical for both molecules and is similar to the aqueous-solution conformation; it differs greatly from the conformation of nonhydrolyzed Ach and β-MeAch. (b) The conformation of the methylene groups does not show significant modifications; here also the differences between Ach and β-MeAch seem to be suppressed.

A tentative hypothesis is given to explain this hydrolysis.

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