The rate of action of cyclic ammonium and sulfonium analogues of acetylcholine[1]

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Summary. The rate of action of cyclic acetylcholine analogues in the 4-acetoxypiperidine and 4-acetoxythiacyclohexane series has been determined by using the isolated left guinea-pig atrium. The kinetic data obtained has been correlated with the experimental ED_{50}-value on the muscarinic receptor.

Previous work in our laboratories[3-6] has shown that compounds **I–IV** interact with the muscarinic receptor in the same manner as acetylcholine. These cyclic analogues activate the muscarinic receptor directly and specifically. Their nicotinic activity is only weak. **I–IV** are substrates for acetylcholinesterase.

It was found that stereochemical and thermodynamic parameters are responsible for great differences in the muscarinic activity of **I–IV**. The stereochemical demands, which are responsible for a high muscarinic potency of compounds like **I–IV**, can be summarized as follows: 1. there should be only 1 methyl group on the heteroatom (in the axial orientation), 2. the acetoxy group should be equatorial, and 3. the energy barrier between the recognition and the active conformation should be low. Only compound **III** fulfils all these conditions, and therefore only **III** shows a high muscarinic activity. The active conformation of **III** is **III**-cis-e in the figure (=29% population in D_2O solution).

Earlier investigations[7-9] have shown that in principle it does seem possible to study drug-receptor kinetics by means of analyzing time-effect curves. It was found[8,9] that the interaction of muscarinic drugs with their receptor seems to be the rate-limiting step. Therefore, the estimation of the association and the dissociation rate constants of the drug receptor complexes, and the correlation of structural and thermodynamic parameters of the drugs not with an overall equilibrium constant but with the single rate constants, should be of value in a better understanding of structure and conformation activity relationships. Thus, we have determined the kinetic parameters of **I–IV** on the muscarinic receptor of isolated electrically driven guinea-pig atria.

Methods. The experiments were performed on isolated left atria of guinea-pigs electrically driven with a frequency of 2 Hz and 3 msec duration by means of rectangular impulses of 4–6 V. The preparations were suspended in an organ bath under a tension of 0.5 g containing Tyrode solution at 27 °C. The incubation medium was vigorously gassed with a mixture of 95% O_2 and 5% CO_2 and contained 7×10^{-5} g/ml hexamethonium. The acetylcholinesterase was blocked by pretreatment of the organs with 5×10^{-5} M Di-isopropyl fluorophosphate. The contractions were measured isometrically. The drugs were dissolved in 0.9% NaCl solution and the negative inotropic responses evoked by separate additions of the agonists were recorded until equilibrium was reached. After each test the agonist was completely washed out, and the contractions were allowed to recover to the basal amplitude before any further drug additions were made. 5 different concentrations were chosen which produced negative inotropic effects between 20 and 85% reduction of the contractile force. 4 runs were made for each drug concentration.

To obtain the kinetic data, the following equations were used[7-9]:

$$[D]+[R] \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} [DR]; \quad (1)$$

$$\frac{d[DR]}{dt} = k_{12}[D][R]-k_{21}[DR]; \quad (2)$$

$$t_{1/2}=\frac{\ln 2}{k_{12}[D]+k_{21}}; \quad (3)$$

$$\frac{\ln 2}{t_{1/2}} = k_{12}[D]+k_{21}; \quad (4)$$

k_{12} = the association rate constant; k_{21} = the dissociation rate constant; [D] = concentration of free drug molecules; [R] = the concentration of free receptors; [DR] = the concentration of drug-receptor complex; $t_{1/2}$ = the half-life of the generation of the effect.

The time course of all the effects was determined by measuring the contraction amplitude just before and frequently after the onset of the drug effect. The responses determined were expressed as fractional effects. These were plotted against time on a semilogarithmic scale. In all cases, straight lines were obtained[8,9]. That means that the time course of the drug effects was an exponential function and could be described by rate constants and half-life values according to the equations (1)-(4). $\ln 2/t_{1/2}$ and [D] values were fitted by a least-squares technique to equation (4). The slopes of these linearized functions (4) give k_{12}, the y intercepts k_{21}, and the x intercepts $-K$, the dissociation constant of the drug-receptor complex.

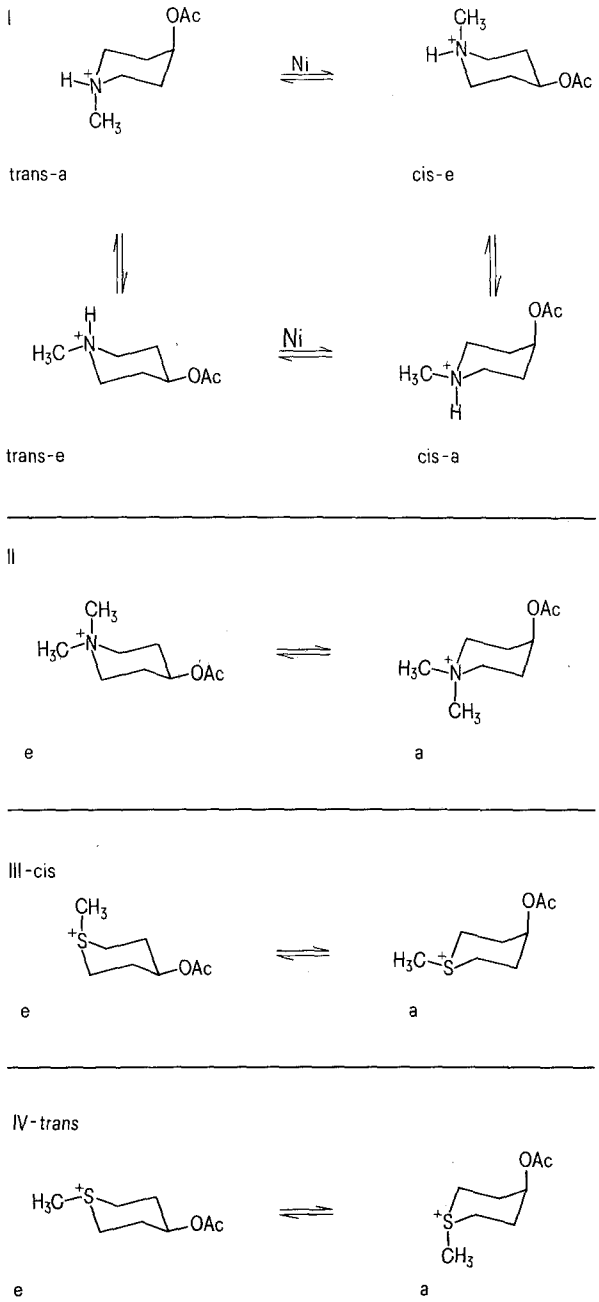
Results and discussion. The dissociation constants K of all the compounds under investigation obtained by kinetic analysis are in good agreement with the ED_{50}-values determined from dose-response curves via a logit transformation and based upon equilibrium values. These agreements show that there are no differences in the intrinsic activities of the compounds **I–IV** and acetylcholine. However, it is impossible to determine intrinsic activities from dose-response curves using the negative inotropic response of the cardiac muscles. But the similarity of the intrinsic activities of **I–IV** and acetylcholine was found when using a smooth muscle preparation (isolated ileum of guinea-pig)[4].

The order of the association rate constants k_{12} of **I–IV** and acetylcholine runs inversely proportional to the respective ED_{50}-values. Assuming that the response in time is a real reflection of the degree of receptor occupation, these findings lead to the conclusion that the affinity of the drugs is predominantly governed by the rate of association. The lower association rate constant of compounds **I**, **II** and **IV** may result from the imposition of severe structural and geometric restrictions upon their binding to the receptor. We have suggested that the correct active conformation of compounds like **I–IV** must have only 1 axial methyl group on the heteroatom and the acetoxy group in the equatorial position as depicted in the figure for **I**-cis-e and **III**-cis-e. The transformation of the trans sulfonium compound **IV** from any possible conformation into this active conformation is not possible. This may be the reason for the low association rate constant of **IV**. The low association rate constant of **II** may be seen as a result of steric hindrance due to the additional 2nd nitrogen methyl group of the compound. As one can see in the figure, the ammonium compound **I** can exist in the correct active conformation (**I**-cis-e), but ^1H NMR-data and MO calculations have shown

Pharmacological and kinetic data for compounds **I–IV** and acetylcholine (Ach) on the muscarinic receptor of guinea-pig atria, means ± SEM

	$ED_{50} \times 10^{-8}$ M*	$K \times 10^{-8}$ M	$k_{12} \times 10^3$ ($L \times mole^{-1} \times sec^{-1}$)	k_{21} (sec^{-1})	$t_{1/2}$** (sec)
Ach	3.08± 0.38	2.47± 0.88	506 ±54	0.012±0.004	19.9±3.6
I	563 ±60	616 ± 43	1.79± 0.20	0.011±0.001	31.1±1.2
II	690 ±50	880 ±114	1.99± 0.11	0.017±0.002	23.8±0.4
III	1.60± 0.10	2.39± 0.29	468 ±44	0.012±0.002	17.5±3.4
IV	146 ±23	221 ± 73	8.23± 1.19	0.017±0.003	14.3±1.2

*These ED_{50}-values were calculated with data from semi-logarithmic dose-response curves[4] by means of the logit transformation, based on the same model of drug activity as used to calculate the kinetic parameters[10]. **Estimated for the concentration of ED_{50}.



Structures and conformation equilibria of **I–IV**. Ni = nitrogen inversion in the free amines conformers. e = equatorial acetoxy group, a = axial acetoxy group.

that this conformation is energetically unfavourable and of low density population (about 0.3%)[4]. This may explain the low association rate constant of **I**.

The dissociation rate constants k_{21}, reflecting the probability of the dissociation of a drug molecule from the receptor site, are in the same range as recently published for the muscarinic antagonist atropine ($k_{21} = 0.011$[11]). The values for $t_{1/2}$ of the negative inotropic responses show some parallelism to the k_{21}-values. Therefore, the rate of dissociation seems to be an important factor in limiting the generation of the effects.

It has been published that the final equilibrium of the negative inotropic response will be reached more rapidly by quaternary than by tertiary muscarinic agents[9]. The relative high value for $t_{1/2}$ of the tertiary compound **I** (31 sec) confirms these results. The fact that tertiary amines, like **I**, are accumulated by heart muscle cells[12,13], could be of influence on the kinetics of the drug-receptor interaction, because the lipophilic unprotonated molecules of **I** in the equilibrium at the pH of the bath fluid (7.20) will disappear from the biophase similarly due to enzymatic hydrolysis of acetylcholine by acetylcholinesterase. Published experiments of Lüllmann and Ziegler[12] show that the rates of cellular uptake of tertiary radio-labelled cholinergic drugs by electrically driven atria are very low in comparison with the rates of the mechanical responses. Therefore, any interference of such an uptake process with the interaction of **I** with the muscarinic receptor in the atria seems highly improbable.

1 Structure and Conformation Activity Relationships of Cyclic Acetylcholine Analogues. **VI**.
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