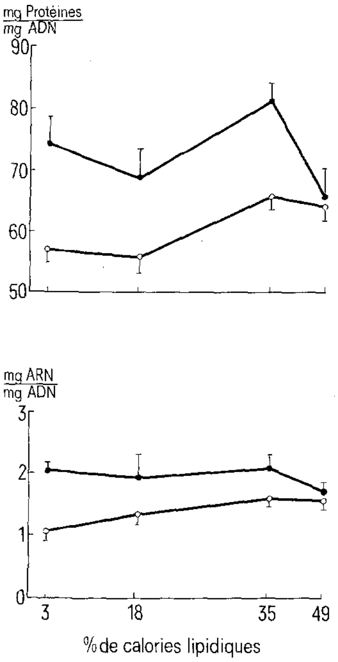
tre part une augmentation de la teneur en eau du tissu. Chez les animaux intoxiqués, la teneur en protéines, exprimée en g/100 g de poids corporel, et la teneur en ARN du tissu, augmentent alors que le taux d'ADN est moins élevé, bien que le poids frais du foie soit plus important. De ce fait, les rapports protéines/ADN et ARN/ADN sont toujours plus élevés chez les traités que chez les témoins (voir figure). Ceci signifie donc que les cellules sont moins nombreuses mais plus développées[15,16]. L'augmentation du rapport protéines/ADN n'est pas suffisante pour expliquer à elle seule, le taux d'hydratation plus élevé du foie des animaux traités. En effet, bien que la teneur relative en protéines (exprimée pour le foie total/100 g de poids corporel), soit plus élevée chez les traités que chez les témoins, elle n'est cependant pas suffisante pour expliquer l'augmentation de la teneur en H_2O qui accompagne la synthèse des protéines comme cela a été observé récemment[17]. Malgré le taux élevé de lipides de la ration, on n'observe pas une stéatose au niveau du foie, même en présence d'oxythioquinox. Le taux de calories lipidiques ne semble pas avoir d'effet significatif ni sur la teneur en eau ni sur les différents constituants du foie. Ceci est sans doute du au maintien du rapport calories totales/poids des protéines en g, du régime à une valeur constante quelque soit le taux de calories lipidiques du régime. Les effets du taux de lipides du régime et ceux de l'oxythioquinox semblent être antagonistes pour la plupart des paramètres étudiés et l'intéraction est toujours significative. Enfin il est important de souligner que cette augmentation du poids du foie n'est pas du type adaptatif car elle ne s'accompagne pas d'une augmentation de l'activité des microsomes hépatiques mais au contraire d'une diminution de cette activité[5].



Protéines/ADN et ARN/ADN du foie en fonction du pourcentage de calories lipidiques du régime: ○ Témoin, ● oxythioquinox.

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Offset rate of action of muscarinic antagonists depends on their structural flexibility

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Summary. Time course measurements of the action of muscarinic antagonists were performed in the spontaneously beating carp atrium. Several high affinity drugs, which embody the quinuclidine structure were examined. The structural flexibility of these molecules was reflected in the dissociation of the drugs from the muscarinic receptor. The dissociation of rigid drugs was very much prolonged as compared to flexible drugs of the same affinity.

It has been proposed[2,3] that the molecular flexibility of drugs finds expression in the kinetic parameters of drug-receptor interaction. It has also been stated that the offset rates in such interactions are a priori very uncertain[4], whilst onset rates are mainly diffusion-controlled[5].

We propose that the offset rates of muscarinic antagonists can be anticipated from the extent of flexibility allowed in the framework of the respective molecules. A rigid drug dissociates from the receptor at a slower rate. The muscarinic pharmacophore consists of 3 'active' sites which, when bound to the corresponding sites on the receptor, will necessarily impose almost equal rigidity on all such antagonists, irrespective of structural flexibility of the isolated molecule. In view of this, flexibility (or rigidity) will be of consequence only when the drug molecule is in a state of partial binding to the receptor as might occur during associative or dissociative processes.

Prolonged time course measurements in isolated organs obtained from homeotherms[6,7] are not practical. A more suitable preparation is the spontaneously beating carp

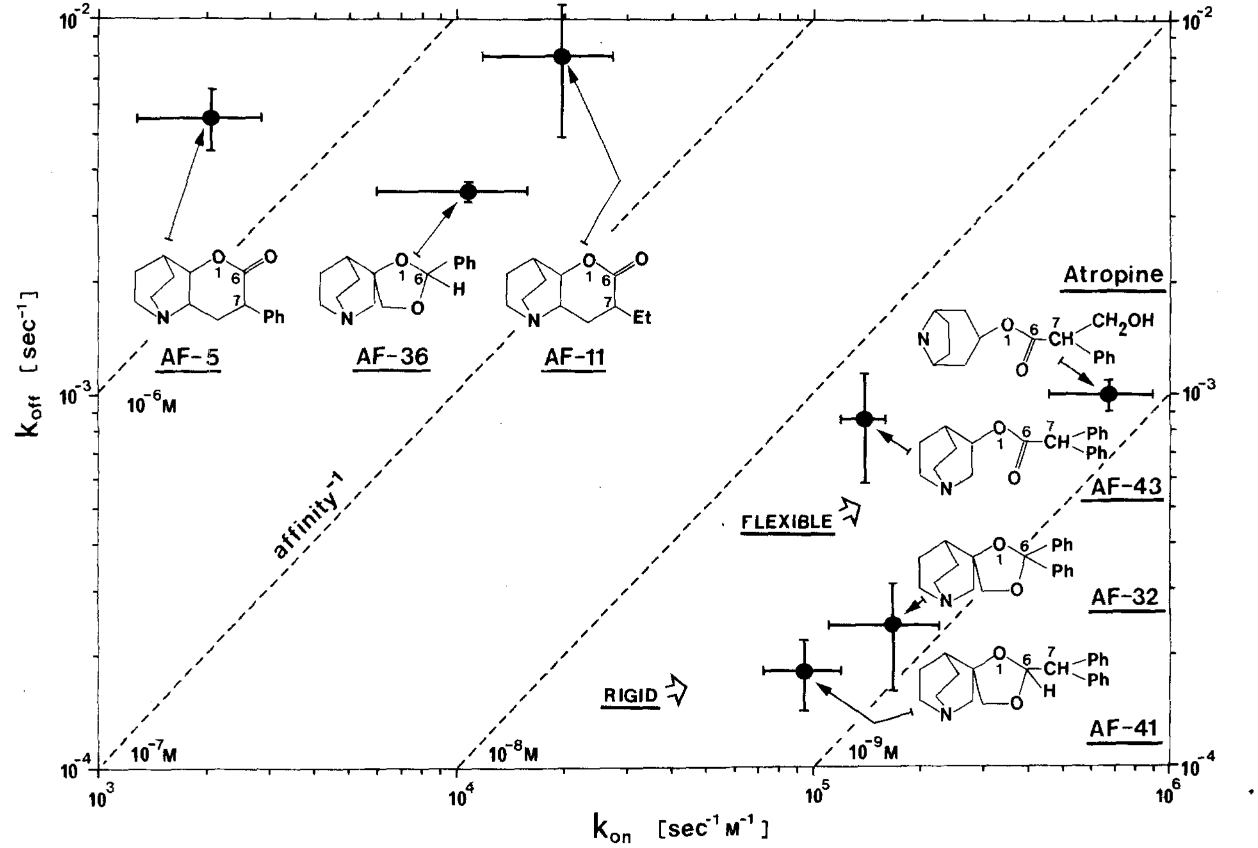

Fig. 1. Structure and kinetic constants of muscarinic antagonists. The dashed diagonals are equal-affinity lines, where affinity^{-1} is defined as k_{off}/k_{on}. Elimination of 1 hydrophobic group reduces the affinity by 2 log units. Among the high affinity drugs, rigid drugs dissociate from the receptor-drug complex at a slower rate than corresponding flexible drugs.

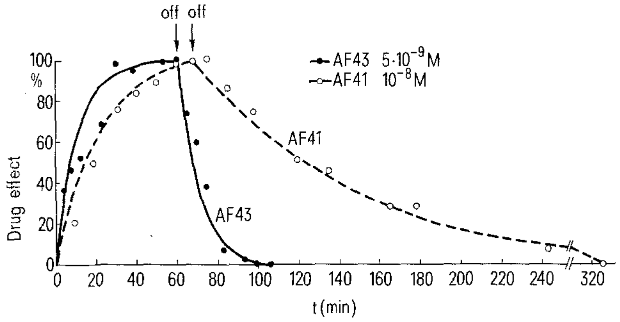

Fig. 2. The time course of antagonism of the structurally related AF-41 and AF-43. Each point represents the relative reduction of the negative inotropic effect of ACh as a function of time; 0% indicates no effect of the antagonist and 100% indicates its effect at equilibrium. k_{off} is derived from the time constant τ of the declining phase by applying the relation $\tau = k_{off}^{-1}$; k_{on} is derived from the time constant of the rising phase, τ_1 where $\tau_1 = [k_{on}[D] + k_{off}]^{-1}$, and [D] = drug concentration. In the present case, AF-41 (rigid) dissociated with $\tau = 83$ min, whereas AF-43 (flexible) did so with $\tau = 11$ min.

atrium, which is removed from live fish *(Cyprinus carpi)* immediately before the experiment. Unlike the guinea-pig atrium, this preparation exhibits a remarkably steady base line and will deliver about 30,000 spontaneous contractions at room temperature over a period of 10 h. Isolated atrial muscle bands were suspended in a 5 ml organ bath and bathed with carp-Ringer solution[8] at room temperature (mM, NaCl 120; KCl 2.7; $CaCl_2$ 2.9; $MgCl_2$ 1.0; $NaHCO_3$ 10). Spontaneous contractions were recorded via an isometric strain gauge. Addition of acetylcholine (ACh) to the bath elicited a negative inotropic effect which reached peak effect in about 10 sec. Recovery after wash out of ACh was complete in about 25 sec. Responses were reproducible with no signs of desensitization over a period of many hours.

The effect of ACh was attenuated or completely blocked by atropine and other antagonists which embody the muscarinic pharmacophore in a framework of varying rigidity[9-12]. The structures of these are given in figure 1. The preparation was bathed in a solution containing one of the antagonists at a given concentration, and the onset of its effect was progressively followed by challenging the preparation with an occasional test dose of ACh. When a steady response had been reached, the preparation was washed with antagonist-free Ringer solution and occasionally challenged with ACh, until complete recovery to the control negative inotropic effect of ACh was reached. For each drug, the following parameters were independently derived: The dissociation constant (K_i), from the parallel shift in the steady-state dose-response curve of ACh; the kinetic constants, k_{on} and k_{off} from the onset and offset rates of drug action[13]. A digital computer (CDC 3600) with a least square routine was used to calculate these constants.

The experimental derivation of onset and offset rates is shown in figure 2 for 2 structurally related drugs. The onset time constant $(K_{on} \cdot D + K_{off})^{-1}$ was obviously concentration (D) dependent, whereas the offset time constant $(K_{off})^{-1}$ was concentration independent. The dissociation of AF-41 (rigid) was very much prolonged as compared to AF-43 (flexible). All experimental results are summarized in figure 1. The drugs tested may be classified into high- and low-affinity groups, depending on the number of hydrophobic moieties on each drug molecule. The high affinity drugs differ significantly from each other in their rate constants, especially in offset rates.

From CPK space-filling models we found that AF-41 is a fairly rigid molecule with highly restricted rotation around the C_6-C_7 and C_7-phenyl bonds. Since AF-41 is also a potent muscarinic antagonist, we infer that its structure reflects an image of the most important steric and electronic elements of the binding site. In the case of the more flexible molecules, AF-43 and atropine, proper positioning on the receptor requires that these drugs assume an 'active' conformation whereby the muscarinic pharmacophore, consisting of the triangle N, O_1 and C_7[3,14,15] be preserved (figure 3). Indeed, it could be shown with the help of space-filling models that all the drugs considered in this report, rigid or flexible, of low or high affinity, are capable of displaying the 'active' pharmacophore within tolerable limits.

The contribution of structural flexibility in these molecules becomes appreciable when binding to the receptor is *partial*, e.g., as in the case where binding is at N and O_1 only, without participation of C_7. In this case, it may be shown that any structural flexibility in the drug molecule will be operative only beyond C_6, e.g. at the C_6-C_7 bond. This is where the relatively flexible atropine and AF-43 differ from the rigid AF-41 and AF-32. In AF-41, steric hindrance will prevent rotation around the C_6-C_7 bond even when the drug molecule is in a state of partial binding to the receptor.

We propose that variance in structural flexibility finds expression in the drug-receptor dissociative process. The rate-determining step in this process is controlled by an energy barrier which could reach a value of 20 kcal per mole[16] for k_{off} values in the range of 10^{-3} to 10^{-4} sec^{-1}. Since all our antagonists display the muscarinic pharmacophore in an unstrained conformation, we ascribe the energy barrier to a transient state which arises in the course of the dissociative process. This energy barrier is higher for the more rigid drugs, suggesting that their removal from the receptor imposes a greater transient strain on the drug-receptor complex[17]. We suggest that structural rigidity of drugs may afford a useful guide to their offset rates and, possibly, to the duration of drug action in vivo.

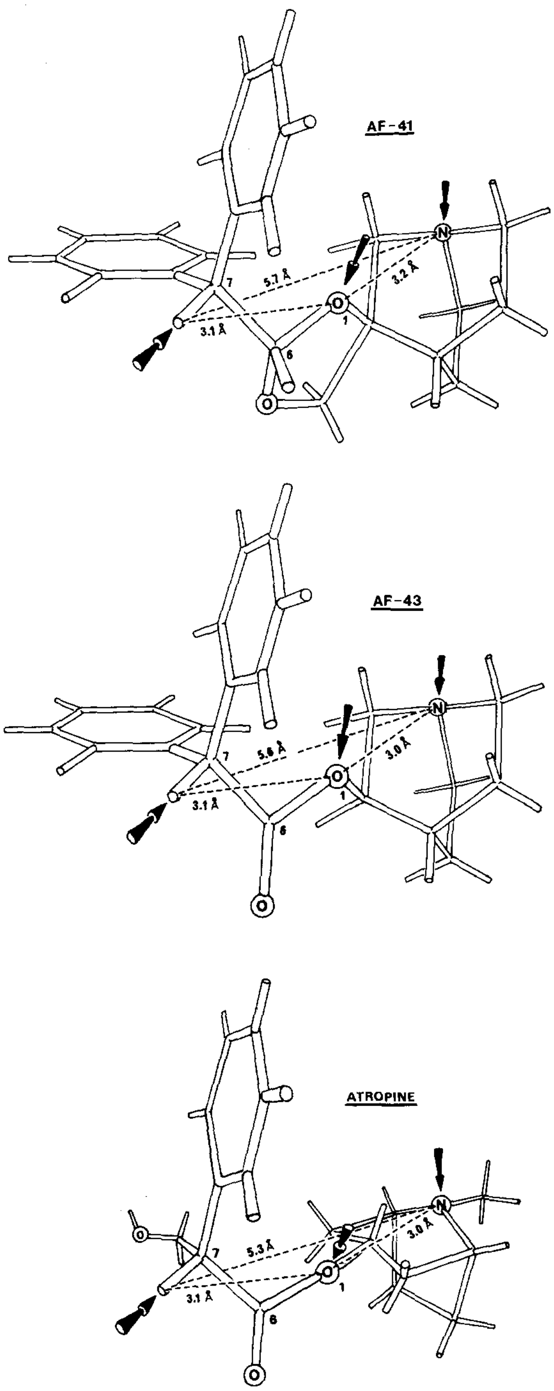

Fig.3. Unstrained conformations of atropine and AF-43 corresponding to the 'fixed' conformation of AF-41. The drawing is based of CPK space-filling models. The distances shown were measured from the centres of the Van der Waals surfaces of atoms constituting the muscarinic pharmacophore. Arrows show the orientations of the symmetry axes of the functional groups.

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