Br. J. Pharmac. (1985), **85**, 783–786

Affinity and efficacy correlate with chemical structure more than potency does in a series of pentatomic cyclic muscarinic agonists

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1 The efficacy and affinity of nine pentatomic cyclic muscarinic agonists were determined on the guinea-pig ileum, according to the method of Furchgott & Bursztyn (1967).

2 The efficacy and affinity of these agonists are affected differently by structural modifications.

3 Our results suggest that a strong dipole oriented in the same direction as that of the hydroxy group of muscarine, or the presence of a polarizable atom in the same position, are important for efficacy.

Introduction

In a series of pentatomic cyclic compounds related to muscarine (*1*) and dioxolane (*2*), *cis*-2-methyl-5-dimethylaminomethyl-1,3-oxathiolane methiodide (*3*) shows an outstanding muscarinic activity being more potent than acetylcholine, muscarine and dioxolane, and also than its own sulphoxide *4* (Pigini *et al.*, 1981).

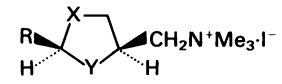
As far as substitution at the X position (Figure 1) is concerned, this pattern of potencies is not consistent with the chemical properties of the atoms or the groups of atoms substituting the oxygen of dioxolane. In fact, if the interaction at the receptor site were through a hydrogen bond (Beckett, 1967), *3* should be less potent than *2* since it is known that sulphur gives weaker hydrogen bonds than oxygen. On the other hand, should a dipole-dipole interaction be active at the same site (Gualtieri *et al.*, 1979), sulphoxide *4* should be more potent than *3*, since it carries a strong dipole orientated in the same direction as the hydroxy group of muscarine.

Structure-activity relationships (Angeli *et al.*, 1984; Gualtieri *et al.*, 1985) suggest that compound *3* may be behaving differently from other cyclopentatomic agonists. It is therefore necessary to determine how modifications of agonist structure affect affinity and efficacy.

Therefore using the method of Furchgott & Bursztyn (1967) we measured the dissociation constants (K_D) and the relative efficacies (e_r) of a number of selected agonists whose structures are shown in Figure 1. The validity of this method has been questioned (El-Fakahany & Richelson, 1981; Siegel & Triggle, 1982) but Ringdahl has recently shown (Ringdahl, 1984) that it gives results in excellent agreement with those obtained by other independent methods.

Methods

Male guinea-pigs weighing 200–300 g were killed by cervical dislocation. Segments of ileum 2–3 cm long were carefully removed and suspended in a 10 ml organ bath containing a solution of the following composition (mM): NaCl 137, $NaHCO_3$ 12, KCl 2.7, $MgSO_4$ 1, NaH_2PO_4 0.4, $CaCl_2$ 1.8 and glucose 5, which was kept at 37°C and aerated with O_2 containing 5% CO_2. Contractions were recorded isometrically at



X = CHOH	(*trans*)	Y = O	R = CH_3	(*1*) (±) muscarine
X = O		Y = O	R = CH_3	(*2*) (±) dioxolane
X = S		Y = O	R = CH_3	(*3*) (±) oxathiolane
X = SO	(*trans*)	Y = O	R = CH_3	(*4*)
X = SO_2		Y = O	R = CH_3	(*5*)
X = CO		Y = O	R = CH_3	(*6*) (±) muscarone
X = CHOH	(*trans*)	Y = CH_2	R = CH_3	(*7*) (±) deoxamuscarine
X = S		Y = O	R = H	(*8*)
X = SO	(*trans*)	Y = O	R = H	(*9*)

Figure 1 Pentatomic cyclic compounds related to muscarine and dioxolane used in these experiments.

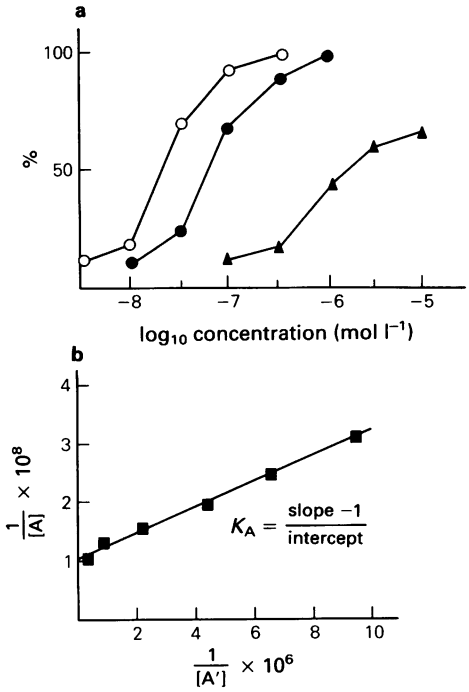


Figure 2 Dose-response curves for muscarone in the guinea-pig isolated ileum (a) and double-reciprocal plot of A versus A′ (b). In (a), (○) represent the muscarone dose-response curve before dibenamine treatment (control curve); (●) and (▲) represent the experimental dose-response curves of muscarone after two successive 20 min incubations with dibenamine at 10^{-5} M and 5×10^{-6} M, respectively. Values for A and A′ were obtained from the control dose-response curve and the plotted points (▲) after the second dibenamine incubation, respectively.

1 g tension using an electromechanical transducer connected with a Gemini II poligraph.

Dissociation constants and relative efficacies were determined according to the method of Furchgott & Bursztyn (1967) using cumulative additions of the agonist. After determination of the dose-response curve, the preparation was treated with an adequate amount of dibenamine (5–10 μM for 20 min) to occlude a fraction of the receptors. The tissue was then washed for 20 min and a new dose-response curve constructed on the dibenamine-treated tissue. Several equipotent doses of the agonist before (A) and after (A′) dibenamine treatment were determined graphically. 1/A was plotted vs. 1/A′ and the points were fitted to a straight line by linear regression analysis. The dissociation constant (K_D) was calculated from the slope of the regression line and the intercept on the ordinate scale. Results of a typical experiment are shown in Figure 2. The efficacy of the agonist under study (e_r) relative to that of muscarine was determined by the following equation:

$$e_r = \frac{e_x}{e_{mus}} = \frac{RA_{mus}}{RA_x} = \frac{\dfrac{ED_{50\,mus}}{K_{D\,mus} + ED_{50\,mus}}}{\dfrac{ED_{50\,x}}{K_{D\,x} + ED_{50\,x}}} \quad (1)$$

where RA_{mus} and RA_x are the percentages of the receptor to be occupied by muscarine and by the compound under study respectively to elicit 50% of the maximal response. Compounds *3*, *4*, *5*, *7*, *8*, *9*, were prepared as already described (Melchiorre *et al.*, 1975; Elferink & Salemink, 1975; Pigini *et al.*, 1981; Angeli *et al.*, 1984). (±)Muscarine (*1*) and dibenamine were purchased from Sigma Chemical Company. (±)-Dioxolane (*2*) and (±)-muscarone (*6*) were generously donated by Prof. P. Pratesi (University of Milan). Agonist stock solutions were prepared in double distilled water and stored for no more than three days. Dilutions were made just before the experiment began. Dibenamine was freshly prepared just before use.

Results

The results for agonists *1*–*9* are shown in Table 1 together with those for carbachol, which was used to check the reliability of our protocol; the dissociation constant found agrees with that reported by Ringdahl (1984).

Muscarine was used as reference compound in calculating e.p.m.r., relative affinity and efficacy of the investigated agonists. Muscarone (*6*) was the most potent compound ($ED_{50} = 1.13 \times 10^{-8}$) displaying the same efficacy as muscarine. Dioxolane (*2*) showed the highest affinity ($K_D = 1.34 \times 10^{-7}$) and the lowest relative efficacy (0.41). Finally, oxathiolane sulphoxide (*4*) displayed the highest relative efficacy (14) showing the largest fraction of spare receptors.

Discussion

The data confirm the findings of Furchgott & Bursztyn (1967), Stephenson (1956) and more recently of Ringdahl (1984), and Ringdahl & Jenden (1983) that efficacy and affinity respond to different structure-activity relationships, and furthermore stress the complex relationships between affinity, efficacy, potency and chemical structure.

In addition, these results give a fairly good explanation of the high potency of *3* as compared to dioxolane (*2*), muscarine (*1*) and the sulphoxide *4*. While the affinity of *3* is lower than that of *2* (as was expected

Table 1 Pharmacological parameters of selected pentatomic cyclic compounds on guinea-pig ileum[a]

Agonist	ED_{50} (± s.e.mean)	*e.p.m.r.*[b]	K_D (± s.e.mean)	*Relative*[b] *affinity*	K_D/ED_{50}	e_r^b (± s.e.)[c]	*% receptor occupied at* ED_{50}
1	$5.86 \pm 0.70 \times 10^{-8}$	1	$9.43 \pm 2.6 \times 10^{-7}$	1	16.1	1	5.9
2	$2.21 \pm 0.19 \times 10^{-8}$	0.4	$1.34 \pm 0.21 \times 10^{-7}$	7.04	6.06	0.41 ± 0.04	14.2
3	$2.03 \pm 0.66 \times 10^{-8}$	0.3	$2.80 \pm 0.55 \times 10^{-6}$	0.34	138	8.1 ± 2.3	0.72
4	$1.25 \pm 0.20 \times 10^{-7}$	2.1	$3.01 \pm 0.41 \times 10^{-5}$	0.031	241	14 ± 2.5	0.41
5	$2.64 \pm 1.0 \times 10^{-5}$	450	$4.04 \pm 1.5 \times 10^{-4}$	0.0023	15.3	0.95 ± 0.14	6.1
6	$1.13 \pm 0.16 \times 10^{-8}$	0.2	$1.90 \pm 0.46 \times 10^{-7}$	4.96	16.8	1.0 ± 0.06	5.6
7	$9.76 \pm 1.8 \times 10^{-7}$	16.7	$1.04 \pm 0.29 \times 10^{-5}$	0.091	10.7	0.68 ± 0.05	8.6
8	$7.23 \pm 1.2 \times 10^{-7}$	12.3	$3.72 \pm 0.74 \times 10^{-5}$	0.025	51.5	3.1 ± 0.38	1.9
9	$8.45 \pm 1.2 \times 10^{-6}$	144	$1.27 \pm 0.30 \times 10^{-4}$	0.0074	15.0	0.94 ± 0.06	6.2
CCh[d]	$2.11 \pm 0.44 \times 10^{-7}$	3.6	$1.07 \pm 0.33 \times 10^{-5}$	0.088	50.2	3.0 ± 0.35	1.9

[a] The number of observations varies between 8 and 12.
[b] Muscarine = 1 : e.p.m.r., equipotent molar ratio: e_r, relative efficacy.
[c] s.e. was calculated using the error propagation theory (Bevington, 1969) from equation (1).
[d] CCh = carbachol.

considering that sulphur gives weaker hydrogen bonds than oxygen), its efficacy is increased so that the potency of *3* is higher than that of *2*. Oxidation of *3* further increases efficacy but at the same time lowers affinity so that of *4* is less potent than *3*. It can be argued that a strong dipole in the direction corresponding to the hydroxy group of muscarine *1*, or an easily polarizable atom like sulphur, are crucial to efficacy. The importance of the direction of the dipole for efficacy is well documented by the fact that muscarone, which has a strong dipole and which is a potent muscarinic agonist, shows lower efficacy than *3*. The same holds true for sulphone *5* as compared to sulphoxide *4*. In both cases the dipole lies more or less in the plane of the pentatomic ring. From the other results shown in Table 1 it can be observed that removal of the ether oxygen of muscarine *1* to give deoxamuscarine *7* influences affinity and, to a much lesser extent, efficacy. Removal of the methyl groups from *3* and *4* gives intriguing results. Removal of the 2-methyl from oxathiolane sulphoxide *4* to give *9* decreases both efficacy and affinity, whilst removal of the 2-methyl group from oxathiolane *3* to give *8* decreases affinity to a similar extent, but has little effect on efficacy. These results might be rationalized by the binding of the 2-methyl moiety allowing the molecule to present the function in the X position in the right orientation with the receptor molecule. As a consequence, the methyl group is more important for the efficacy of compounds where the dipole has a definite direction as in *4*, than for compounds like *3* where the sulphur atom can be polarized in different directions. This is also in accordance with the early observation (Pratesi *et al.*, 1983) that binding of the methyl group and the oxygenated functions is highly cooperative, and stresses once again the crucial influence of the methyl group on binding (Triggle, 1976; Gualtieri *et al.*, 1979). Considering these results it seems possible to distinguish within the ligand molecule, functions that are mainly responsible for affinity and functions that are mainly responsible for efficacy. Besides the onium group, a carbonyl group or an oxygen in X position, a methyl group in position 2 and an oxygen in Y position contribute to the affinity of the molecules. On the other hand, a strong dipole or a polarizable atom in the X position are the features that provide the molecule with high efficacy.

At a molecular level our findings could mean that a strong dipole in the same direction as the hydroxy group of muscarine or an easily polarizable atom enhance the ability of the ligand to induce a productive conformational change in the receptor molecule.

One very interesting working hypothesis is that the dipole associated with the carbonyl bond of acetylcholine could be responsible for the activation of the receptor. According to Jensen (1984) the CH_3COOCH_2- portion of the acetylcholine molecule lies roughly in the same plane. Therefore, in order to be oriented in the same direction as the sulphoxide group of *4*, the carbonyl group of acetylcholine would have to deviate from the more stable planar conformation. This is possible in principle, since we do not know whether the active conformation of acetylcholine is actually the more stable one (Casy, 1975).

Finally, the results shown in Table 1 indicate that for most of the compounds examined, the variation in potency is due to a change in affinity rather than in efficacy. This makes comparison of the potencies still

reliable and useful in most cases. Nevertheless, when structure-activity relationships are not consistent, one should take both efficacy and affinity changes into consideration before suggesting different modes of binding or the existence of receptor subclasses (Kenakin, 1984).

This work was supported by the National Research Council of Italy, grant n. 83.00248.95 from "Progetto Finalizzato, Chimica Fine e Secondaria." We are grateful to Mrs Maria Scarlattini on leave from the Fisiologia Clinica of CNR, Pisa, for excellent technical assistance and to Dr Anna Cassinelli who participated in some of these experiments.

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(Received October 26, 1984.
Revised February 20, 1985.
Accepted March 21, 1985.)