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Selectivity of Partial Agonists Related to Oxotremorine Based on Differences in Muscarinic Receptor Reserve Between the Guinea Pig Ileum and Urinary Bladder

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SUMMARY

The muscarinic effects of analogs of oxotremorine were compared on strips of the guinea pig ileum and urinary bladder. In a series of eight analogs, full or nearly full contractile responses compared to carbachol were observed on the ileum. On the bladder, the analogs were full agonists, partial agonists, or competitive antagonists. Although EC_{50} values estimated on the bladder were 10- to 20-fold greater than those obtained on the ileum, the dissociation constant and relative efficacy of each agonist were similar in the two tissues, as were dissociation constants of competitive antagonists including pirenzepine. The ability to discriminate between responses in the ileum and bladder was related to intrinsic efficacy. Highly efficacious compounds such as carbachol and oxotremorine-M were full agonists in both tissues, although less potent on the bladder. Compounds having intermediate intrinsic efficacy, e.g., oxotremorine, were partial agonists on the bladder, whereas BM 5, having low intrinsic efficacy, was a competitive antagonist. These results suggest a mechanism, based on tissue differences in receptor reserve, by which selectivity may be achieved among muscarinic stimulants, even in the absence of distinct subtypes of muscarinic receptors.

In any given tissue, the pharmacological activity of an agonist is determined by essentially three factors: (i) the ability of the agonist to bind to specific receptors in the tissue, (ii) the efficacy of the agonist in producing a stimulus, and (iii) the ability of the tissue to translate the stimulus into a response (1–3). The two latter factors depend on the number of receptors in the tissue which, therefore, also is an important determinant of agonist activity. The ability to bind to the receptor, as reflected in the affinity of the agonist, is a purely drug-receptor-related property and, as such, is of value in classification of agonists and their receptors. Tissue differences in agonist affinity therefore imply receptor heterogeneity and thus potential for selective agonist effects. Undoubtedly, many agonists display selectivity at the receptor level but, in view of the multitude of factors involved in the generation of an agonist response, it may be unnecessarily confining to think of agonist selectivity solely in terms of receptor selectivity (4). There is growing evidence, for example, from studies with dopamine receptor agonists (5) and β_1-adrenoceptor agonists (6, 7), that the efficacy of an agonist may be more of a determinant of selectivity than its affinity. In a previous study of acetamides related to the muscarinic agent oxotremorine, we observed that their properties on the guinea pig urinary bladder ranged from that of a full agonist to those of partial agonists and competitive antagonists (8). This behavior contrasted with previous observations made on the guinea pig ileum, where the compounds were full agonists. Although a detailed comparison between the two tissues was not made, it appeared that the dissociation constants and relative efficacies of these acetamides at muscarinic receptors in the bladder agreed reasonably well with those estimated previously on the ileum. Desensitization and diffusional factors did not seem to be responsible for these striking differences in agonist activity, and we suggested that they were due primarily to a smaller muscarinic receptor reserve (a function of agonist efficacy) for the compounds in the bladder as compared to the ileum.

In this study we have extended these observations by comparing directly muscarinic activities, dissociation constants, and relative efficacies of some analogs of oxotremorine (Fig. 1), including three enantiomeric pairs, in strips of the guinea pig ileum and urinary bladder. The results confirm previous suggestions (8) that analogs of oxotremorine may exert selective actions in the guinea pig ileum and urinary bladder in spite of apparent homogeneity of muscarinic receptors in the two tissues with respect to such analogs. A preliminary account of part of this work has appeared (9).

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ABBREVIATION: PrBCM, propylbenzilylcholine mustard.

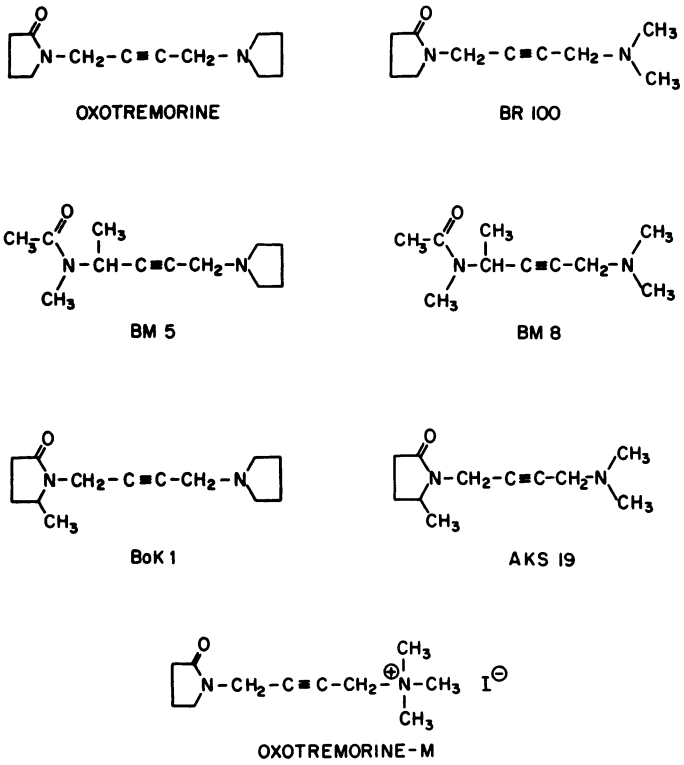

Fig. 1. Chemical structures of some analogs of oxotremorine.

Materials and Methods

Guinea pig ileum. Male Hartley guinea pigs (350–400 g body weight) were sacrificed by a blow to the head and bled. Segments of the ileum (about 2 cm long) were removed and suspended in a 10-ml organ bath containing Tyrode solution at 37° and continuously gassed with a 5% CO_2/95% O_2 mixture. The Tyrode solution had the following composition (mM): NaCl, 137; $NaHCO_3$, 12; KCl, 2.7; $MgSO_4$, 1.0; NaH_2PO_4, 0.4; $CaCl_2$, 1.8; and glucose, 5.0 (pH 7.4). It also contained hexamethonium (300 μM). Contractions were recorded isotonically at 1 g of tension, using an electromechanical displacement transducer and a potentiometric recorder. The ileal strips were allowed to equilibrate for 1 hr before drug addition. Concentration-response curves were constructed by the cumulative dose-response technique by increasing stepwise the concentration of agonist by a factor of 2.15. The interval between addition of drug doses was about 15 sec.

Dissociation constants (K_A) and relative efficacies of oxotremorine and carbachol at ileal muscarinic receptors were estimated by using a modification (10, 11) of the method of Furchgott and Bursztyn (12). After construction of control concentration-response curves, from which EC_{50} values were obtained, the ileum was treated with PrBCM to inactivate irreversibly a fraction of the receptors. The tissue was washed for 1 hr and concentration-response curves were then obtained in the PrBCM-treated tissue. A concentration of precyclized PrBCM that upon 15-min incubation reduced the maximum response to the agonists by about 20–60% was found suitable. Carbachol, for example, required a concentration of PrBCM of about 3 μM. Several equieffective concentrations of each agonist before [A] and after [A'] treatment with PrBCM were estimated graphically. A hyperbolic function (Eq. 1) was then fitted by nonlinear regression analysis to pairs of concentrations [A] and [A'] to give best estimates of K_A values and fraction (y) of receptors inactivated (10):

$$[A] = \frac{[A']}{[A'] + K_A/y} K_A \frac{1 - y}{y} \quad (1)$$

The efficacy of oxotremorine relative to that of carbachol and percentage receptor occupancy required for half-maximal response were computed from the EC_{50} and K_A values as described previously (12). The efficacies of oxotremorine-M, BR 100, and the enantiomers of BM 8 and AKS 19, previously determined relative to that of oxotremorine, were multiplied by 0.13 which is the efficacy of oxotremorine relative to that of carbachol as determined above. In this way, the efficacies of oxotremorine-M, BR 100, and the enantiomers of BM 8 and AKS 19 relative to that of carbachol were determined.

The dissociation constant of BM 5 was estimated by the method of Waud (13) by comparison of its concentration-response curve with that of carbachol. Several equieffective concentrations of BM 5 [P] and carbachol [A] were determined graphically. A hyperbolic function (Eq. 2) was then fitted to the data points by nonlinear regression analysis (10) to give the dissociation constant (K_P) of BM 5:

$$[A] = \frac{[P]}{[P] + K_P} K_A \frac{e_P}{e_A} \quad (2)$$

In Eq. 2, e_P/e_A is the efficacy of BM 5 relative to that of carbachol and K_A is the dissociation constant of carbachol. Since the latter was known from application of the method of Furchgott and Bursztyn (12) (above), e_P/e_A could be calculated (14). The accuracy of the method was greatly enhanced by using a K_A value of carbachol that had been determined on the same piece of tissue.

Guinea pig urinary bladder. Strips of the bladder, about 10 mm long and 1–2 mm thick, were cut out longitudinally and suspended under 1 g of tension at 37° in Krebs-Henseleit solution of the following composition (mM): NaCl, 117; $NaHCO_3$, 25; KCl, 5.4; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; $CaCl_2$, 2.5; and glucose, 10 (pH 7.4). The Krebs-Henseleit solution also contained hexamethonium (300 μM). It was gassed with a 5% CO_2/95% O_2 mixture. Contractions were recorded as described above. Agonist doses were added cumulatively at intervals of about 3 min (quaternary ammonium compounds) or 5 min (tertiary amines) by increasing the concentration by a factor of 3.16.

K_A values and relative efficacies of carbachol, oxotremorine-M, and BR 100 at muscarinic receptors in the urinary bladder were estimated by the method of Furchgott and Bursztyn (12) as described above. The concentration of PrBCM (0.3 μM) required to depress the maximum response to these agonists by 20–60% was about 10-fold lower than on the ileum.

Dissociation constants (K_P) of oxotremorine, (+)-BM 8, (−)-BM 8, (+)-AKS 19, and (−)-AKS 19 were determined by comparison of their concentration-response curves with that of carbachol as outlined above for BM 5 on the ileum. The curves were arranged in such a way that the concentration-response curve for oxotremorine and the enantiomers of BM 8 and AKS 19 each was preceded by a concentration-response curve to carbachol. The hyperbolic function (Eq. 3), derived from an equation of Mackay (15), rather than Eq. 2, was fitted by nonlinear regression analysis to pairs of equieffective concentrations of carbachol [A] and the compounds studied [P]:

$$[A] = \frac{[P]}{[P] + K_P \frac{e_A}{e_A - e_P}} K_A \frac{e_P}{e_A - e_P} \quad (3)$$

Since the dissociation constant of carbachol (K_A) was known (17.0 μM, obtained independently as described above), Eq. 3 provided estimates of the efficacy of the partial agonist relative to that of carbachol (e_P/e_A) and of the dissociation constant of the partial agonist (K_P).

Dissociation constants (K_B) of BM 5, (+)-BoK 1, (−)-BoK 1, and pirenzepine on the bladder and of pirenzepine on the ileum were estimated as described by Arunlakshana and Schild (16). Concentration-response curves to carbachol were recorded in the absence and presence of various concentrations of the compound studied. Antagonists were allowed to equilibrate with the tissue for 30 min. Dose ratios (ratio of the EC_{50} values of carbachol in the presence and absence of antagonist) were calculated for each antagonist concentration. K_B values were obtained from the relationship K_B = [antagonist]/(dose ratio − 1). The logarithm of (dose ratio − 1) was plotted against the negative logarithm of the molar concentration of antagonist. In the

TABLE 1

Parameters characterizing the muscarinic activity of some oxotremorine analogs in the isolated guinea pig ileum

Maximum contractile response (E_{max}) and efficacy are given relative to those of carbachol. Percentage receptor occupancy required for 50% response was calculated from the law of mass action as $100 \times EC_{50}/(K_A + EC_{50})$. K_A values, relative efficacies (recalculated as described under Materials and Methods), and percentage occupancy for oxotremorine-M, BR 100, (+)-BM 8, (−)-BM 8, and (+)-AKS 19 are from Refs. 20, 22, and 23. K_B values for (+)- and (−)-BoK 1 are from Ref. 20. Values are means ± standard errors. *n* equals number of preparations used.

Compound	n	EC_{50}	E_{max}	K_A	Relative efficacy	Percentage occupancy at EC_{50}
		μM		μM		
Oxotremorine-M	5	0.023 ± 0.004	1.01 ± 0.01	2.9 ± 0.1	0.95 ± 0.04	0.46
BR 100	5	0.19 ± 0.009	1.04 ± 0.02	22.9 ± 2.7	0.85 ± 0.09	0.55
Oxotremorine	6	0.035 ± 0.002	1.00 ± 0.02	0.93 ± 0.08	0.13 ± 0.02	3.6 ± 0.4
(+)-BM 8	5	0.39 ± 0.02	1.02 ± 0.01	6.4 ± 0.8	0.13 ± 0.01	3.8
(−)-BM 8	5	7.3 ± 0.6	1.01 ± 0.02	107 ± 15	0.10 ± 0.01	4.7
(+)-AKS 19	5	0.49 ± 0.03	1.02 ± 0.02	2.3 ± 0.4	0.038 ± 0.008	11.0
(−)-AKS 19	3	5.0 ± 0.8	1.01 ± 0.02	32.1 ± 5.9	0.042 ± 0.011	10.4 ± 3.1
BM 5	4	0.19 ± 0.03	0.83 ± 0.03[a]	0.24 ± 0.07[b]	0.013 ± 0.002	45.3 ± 4.2
(+)-BoK 1	4		0	0.051 ± 0.008[c]		
(−)-BoK 1	4		0	0.98 ± 0.10[c]		
Pirenzepine	5		0	0.15 ± 0.02[c,d]		
Carbachol	6	0.10 ± 0.01	1.00	15.4 ± 3.1	1.00	0.50 ± 0.05

[a] Significantly different from the E_{max} value of carbachol ($p = 0.005$).
[b] K_P value.
[c] K_B value.
[d] Barlow *et al.* (35) found a K_B value of 0.22 μM for pirenzepine.

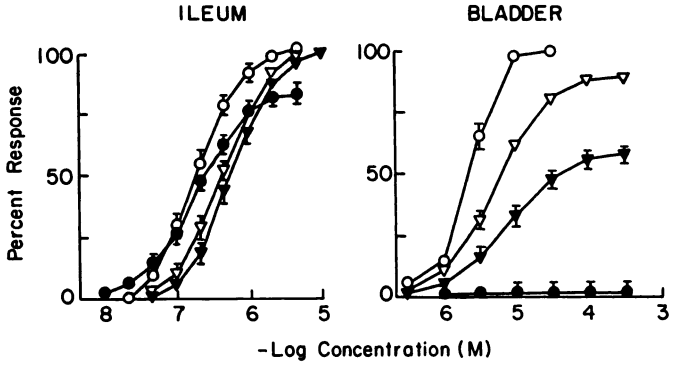

Fig. 2. Concentration-response curves of oxotremorine analogs in strips of the guinea pig ileum and urinary bladder. Responses to BR 100 (○), (+)-BM 8 (▽), (+)-AKS 19 (▼), and BM 5 (●) are expressed relative to the maximum response elicited by carbachol. *Vertical bars* show standard error. Number of preparations used is given in Tables 1 and 2.

case of competitive antagonism, a straight line having a slope of 1 should result (16).

Statistical analysis. Statistical significance ($p < 0.05$) of differences between means was determined by Student's *t* test. When groups to be compared had potentially different variance, the test described by Cochran and Cox (17) was employed.

Drugs. BR 100 and oxotremorine-M (18); (*R*)-(+)-BM 8 and (*S*)-(−)-BM 8 (19); (*R*)-(+)-AKS 19, (*S*)-(−)-AKS 19, (*R*)-(+)-BoK 1, and (*S*)-(−)-BoK 1 (20); and BM 5 (21) were prepared as described previously. Other drugs and their sources were: oxotremorine sesquioxalate (synthesized in this laboratory), PrBCM, *N*-2-chloroethyl-*N*-propyl-2-aminoethyl benzilate hydrochloride (generous gift of Dr. N. J. M. Birdsall, London), pirenzepine dihydrochloride (Boehringer Ingelheim Ltd.), carbamoylcholine chloride (Aldrich Chemical Company), and hexamethonium chloride (K & K Laboratories).

Results

Guinea pig ileum. With the exception of pirenzepine and (+)- and (−)-BoK 1 which were antagonists, the compounds studied produced full or nearly full contractile responses on the ileum as compared to carbachol (Table 1). Oxotremorine and oxotremorine-M were the most potent and (−)-AKS 19 the least potent compounds. The other analogs, which had quite similar potencies (Fig. 2), were only slightly less potent than carbachol. These results are in good agreement with those obtained previously (20–23). The K_A values of oxotremorine-M and BR 100 (22), (+)- and (−)-BM 8 (23), and (+)-AKS 19 (20) at ileal muscarinic receptors, given in Table 1, are quoted from previous studies in which efficacies were determined relative to oxotremorine. In order to allow comparison with the urinary bladder (Table 2), it was desirable to express efficacies relative to carbachol. Therefore, K_A values and relative efficacies of oxotremorine and carbachol were estimated by the method of Furchgott and Bursztyn (12). The results (Table 1) were in excellent agreement with previous estimates (11). The dissociation constant and relative efficacy of BM 5 were estimated by the method of Waud (13). The important assumption underlying this method is that the efficacy difference between the agonists compared is large, i.e., that the full agonist has a large receptor reserve (13). Carbachol clearly satisfied this criterion as it required only 0.5% receptor occupancy in the ileum for half-maximal response (Table 1).

In spite of the similar spasmogenic potency of the compounds in Fig. 2 (potency range 2.6-fold), their affinity for ileal muscarinic receptors varied over a range of 95-fold and their relative efficacies over a range of 69-fold (Table 1). Furthermore, within the whole series of compounds, there was no correlation between dissociation constants and relative efficacies. These results suggest that the structural requirements underlying affinity and efficacy are different and also clearly illustrate the importance of separating the response of stimulant drugs into affinity and efficacy components to acquire more detailed knowledge of drug-receptor interactions (11, 22, 23).

Guinea pig urinary bladder. Although the compounds investigated [except pirenzepine and (+)- and (−)-BoK 1] were full or nearly full agonists on the ileum, they were full agonists, partial agonists, or competitive antagonists on the bladder (Fig. 2). Furthermore, EC_{50} values obtained on the bladder (Table 2) were 10- to 20-fold greater than those estimated on the ileum. This difference was highly significant ($p < 0.005$) for all com-

TABLE 2

Parameters characterizing the muscarinic activity of some oxotremorine analogs in the isolated guinea pig urinary bladder

Maximum contractile response (E_{max}) and efficacy are given relative to those of carbachol. Percentage receptor occupancy required for 50% response was calculated from the law of mass action as $100 \times EC_{50}/(K_A + EC_{50})$. Values are means ± standard errors. *n* equals number of preparations used.

Compound	n	EC_{50}	E_{max}	K_A	Relative efficacy	Percentage occupancy at EC_{50}
		μM		μM		
Oxotremorine-M	6	0.37 ± 0.03	1.01 ± 0.01	4.3 ± 0.7	0.88 ± 0.12	8.4 ± 1.1
BR 100	4	2.3 ± 0.2	1.00 ± 0.02	35.0 ± 6.5	1.1 ± 0.08	6.5 ± 0.8
Oxotremorine	6	0.77 ± 0.17	0.80 ± 0.03[a]	1.4 ± 0.2[b]	0.14 ± 0.008	42.1 ± 5.1
(+)-BM 8	4	5.5 ± 0.7	0.89 ± 0.01[a]	11.7 ± 1.2[b]	0.21 ± 0.03	32.8 ± 5.1
(−)-BM 8	4	156 ± 18	0.88 ± 0.01[a]	262 ± 71[b]	0.15 ± 0.02	35.8 ± 6.3
(+)-AKS 19	4	8.1 ± 1.9	0.58 ± 0.03[a]	6.4 ± 0.9[b]	0.064 ± 0.008	53.7 ± 7.9
(−)-AKS 19	3	66.1 ± 5.5	0.56 ± 0.04[a]	52.8 ± 19.0[b]	0.056 ± 0.019	57.1 ± 11.3
BM 5	6		<0.05	0.14 ± 0.02[c]		
(+)-BoK 1	4		0	0.057 ± 0.002[c]		
(−)-BoK 1	3		0	1.4 ± 0.15[c]		
Pirenzepine	5		0	0.19 ± 0.02[c]		
Carbachol	9	0.84 ± 0.10	1.00	17.0 ± 2.1	1.00	6.1 ± 0.7

[a] Significantly different from the E_{max} value of carbachol ($p < 0.005$).
[b] K_P value.
[c] K_B value.

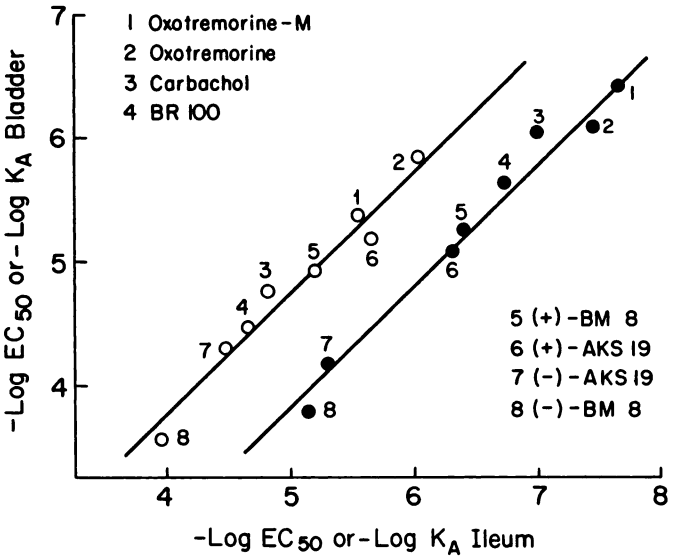

Fig. 3. Relationships between spasmogenic activity of oxotremorine analogs in the guinea pig ileum and urinary bladder and between their affinity for muscarinic receptors in the two tissues. −Log EC_{50} (pD_2) values (Tables 1 and 2) were used as measures of spasmogenic activity (●) and −log K_A (pK_A) values (Tables 1 and 2) as measures of affinity (○) except for oxotremorine and the enantiomers of BM 8 and AKS 19, in which case K_P values obtained on the bladder (Table 2) were employed. The regression lines are described by pD_2(bladder) = −1.28 + (1.02 ± 0.06) × pD_2(ileum) ($t_6 = 16.9$; $P = 3 \times 10^{-6}$) and pK_A(bladder) = −0.31 + (1.02 ± 0.08) × pK_A(ileum) ($t_6 = 13.2$; $P = 1 \times 10^{-5}$).

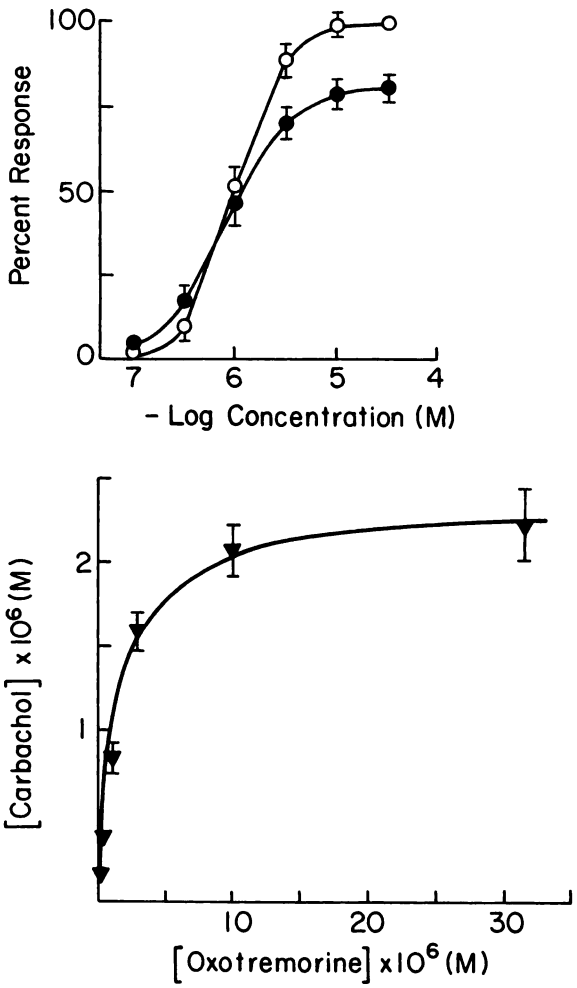

Fig. 4. Determination of the dissociation constant (K_P) of oxotremorine at muscarinic receptors in the guinea pig urinary bladder. Concentration-response curves of oxotremorine (●) and carbachol (○) (*top*) and plot of equieffective concentrations of the two agonists (*bottom*). The theoretical curve in the lower graph represents the best fit to Eq. 3 under Materials and Methods. *Vertical bars* show standard errors of six experiments.

pounds. The different composition of the physiological salt solutions used in the experiments on the ileum and bladder did not appear to contribute to the lower potency on the bladder. Thus, the EC_{50} values of carbachol and oxotremorine were nearly identical whether the bladder strips were suspended in Krebs-Henseleit or in Tyrode solution. A linear relationship was observed between negative logarithms of EC_{50} values (molar concentrations) in the two tissues (Fig. 3). BR 100 and oxotremorine-M were the only compounds that elicited a maximum response as compared to carbachol (Table 2).

Estimation of K_A values by the method of Furchgott and Bursztyn (12) revealed that carbachol, in order to produce a

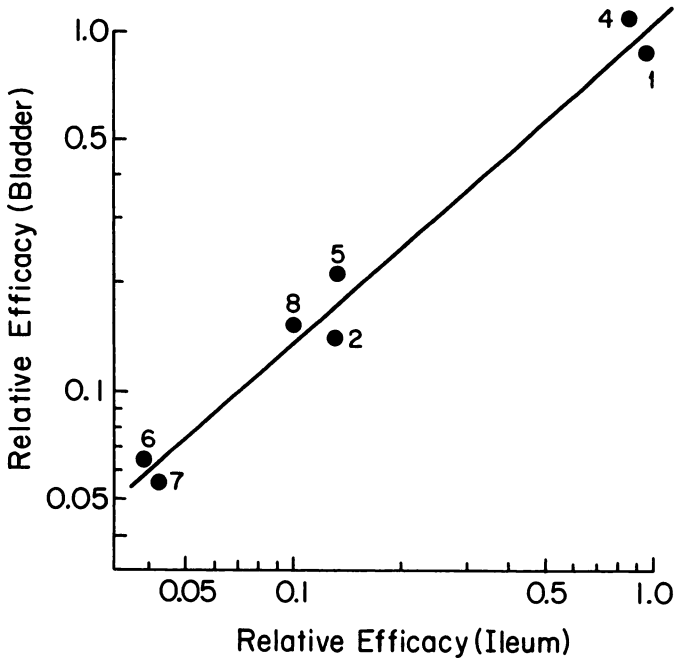

Fig. 5. Relationship between logarithms of relative efficacies (e_r) of some oxotremorine analogs at muscarinic receptors in the guinea pig ileum and urinary bladder. For explanation of symbols, see Fig. 3. Relative efficacies are from Tables 1 and 2. The regression line is described by: log e_r(bladder) = 0.034 + (0.89 + 0.06) × log e_r(ileum) (t_5 = 15.6; $P = 2 \times 10^{-5}$).

half-maximal response, required 10-fold higher receptor occupancy in the bladder compared to the ileum. In experiments where carbachol was employed to estimate dissociation constants of partial agonists, data analysis was therefore performed by the method of Mackay (15). This method, in contrast to the method of Waud (13), which was used to estimate K_P values of partial agonists on the ileum, does not rest on any assumptions about the magnitude of the receptor reserve for the full agonist, but requires prior knowledge of its K_A value. Application of this method to oxotremorine is illustrated in Fig. 4. As noted previously (8), BM 5 elicited a small contractile response (<15%) in some experiments, but this was not consistently observed. Its dissociation constant, like those of pirenzepine and (+)- and (−)-BoK 1, was estimated from antagonism of carbachol-induced contractions. All compounds gave linear Schild plots with slopes not significantly different from unity. Enantiomeric potency ratios of BM 8 and AKS 19 and enantiomeric affinity ratios of BoK 1, BM 8, and AKS 19 showed good agreement in the ileum and bladder. The enantiomers of BM 8 and AKS 19 did not differ in efficacy in either tissue.

Test of significance showed that, for all compounds except (+)-BM 8 and (+)-AKS 19, the dissociation constants at muscarinic receptors in the ileum and bladder were not significantly different. Regression of $-\log K_A$ values estimated on the ileum on $-\log K_A$ (or $-\log K_P$) values estimated on the bladder revealed a highly significant linear relationship between the two parameters (Fig. 3). The slope of the regression line was not significantly different from unity, but the line appeared to deviate slightly from the line of equivalence. There was no significant difference between relative efficacies estimated on the ileum and bladder for any of the compounds. The relationship between relative efficacies in the two tissues is shown graphically in Fig. 5. In contrast, the degree of receptor occupancy required by a particular agonist to produce a given level of response (e.g., 50% response) was smaller in the ileum than in the bladder, resulting in a receptor reserve for most of the compounds in the ileum. In the bladder, however, only highly efficacious agonists (BR 100, oxotremorine-M, and carbachol) displayed a receptor reserve. These differences in tissue receptor reserve may be utilized to activate responses selectively on the ileum or to block responses selectively on the bladder using partial agonists such as BM 5 (Fig. 2).

Discussion

The affinity of an agonist, defined as the reciprocal of the equilibrium dissociation constant of the agonist-receptor complex, should vary among tissues only if the receptors differ. The efficacy of an agonist determines the magnitude of the stimulus produced at any given level of receptor occupancy. In contrast to affinity, efficacy is not solely a characteristic of the agonist-receptor pair in question. It also depends, among other things, on receptor density which, for a given type of receptor, may vary from tissue to tissue. Therefore, the efficacy of an agonist may vary among tissues even if they contain identical receptors.

Although agonist efficacy, as defined above, is a tissue-dependent parameter, measurements of relative efficacies of agonists in a tissue provides a receptor-dependent parameter because tissue factors cancel (24). Methods of quantifying agonist efficacy require an accurate estimate of the dissociation constant of the agonist-receptor complex. The pharmacological methods used in the present study to determine dissociation constants of analogs of oxotremorine were shown previously to provide reliable and consistent estimates of agonist affinity at ileal muscarinic receptors (11, 22, 23). In spite of striking differences in agonist potency and relative maximal responses between the ileum and bladder (Fig. 2), no corresponding differences were observed between dissociation constants in the two tissues. Dissociation constants estimated on the bladder in general were slightly greater than those obtained on the ileum, although significantly so only for (+)-AKS 19 and (+)-BM 8. The dissociation constants of these two compounds were estimated on the ileum by the method of Furchgott and Bursztyn (12) and on the bladder by the method of Mackay (15). As noted previously (8), there is evidence that the latter method as applied here may underestimate the affinity of partial agonists at muscarinic receptors in the bladder, especially the affinity of tertiary amines. Collectively, these observations and the excellent agreement between K_B values of antagonists in the two tissues observed in this and in a previous study (8) suggest that muscarinic receptors in the guinea pig ileum and urinary bladder are pharmacologically similar. In agreement with these suggestions, the novel antimuscarinic agent pirenzepine, which has been claimed to distinguish between muscarinic receptors in other tissues (25–27), had identical affinity for receptors in the ileum and bladder (Tables 1 and 2). Furthermore, the relative efficacies of each agonist agreed, as would be expected if the receptors are similar. Radioligand binding studies also have indicated homogeneity of muscarinic receptors in the guinea pig ileum and bladder with respect to a series of antimuscarinic agents including pirenzepine and atropine (28, 29).

In contrast to these similarities, the degree of receptor occupancy required for half-maximal response was considerably

larger in the bladder than in the ileum. Muscarinic receptor occupancy therefore appears to be less efficiently translated to a contractile response in the bladder. Thus, the observed selectivity for stimulating responses in the ileum (or antagonizing responses in the bladder) seems to be derived from differences in tissue sensitivity resulting from a smaller effective receptor reserve in the bladder. The latter may be due to low receptor density or to less efficient coupling between receptor occupancy and contractile response (24). Although the pharmacological methods used in this study do not distinguish between these two possibilities, it may be worth noting that the density of muscarinic binding sites in the guinea pig urinary bladder is 5-fold lower than in the ileum (28, 30). The dependence of agonist activity upon the magnitude of the effective receptor reserve is clearly a function of agonist efficacy (24, 31). As shown here, responses to agonists of low efficacy were more affected by tissue differences in receptor reserve than were responses to agonists of high efficacy. Thus, in the guinea pig bladder, responses to agonists of low efficacy were depressed or even abolished, whereas more efficacious agonists still produced a full response.

Traditionally, drug selectivity is thought of in terms of receptor selectivity. For example, the selective antimuscarinic effects of pirenzepine (25, 26) appear to be due to its ability to differentiate between subpopulations of muscarinic receptors (27). However, attempts to find receptor-selective muscarinic agonists have met with only limited success. The present results suggest an alternate and virtually unexplored mechanism, based on tissue differences in receptor density and/or in the efficiency of the receptor-effector coupling, by which selectivity may be achieved among muscarinic stimulants, even in the absence of distinct subtypes of muscarinic receptors. They also show the importance of carefully evaluating both the affinity and efficacy components of agonist potency before ascribing apparently selective actions to receptor-selective properties of an agonist. Thus, the muscarinic agent McN-A-343 (32) has been classified as a selective M1-receptor agonist (26). Recent evidence suggests, however, that its selective actions at some sites may be equally well explained by a combination of low intrinsic efficacy and tissue differences in receptor reserve (33, 34).

Acknowledgments

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