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Binding Profiles of Oxomemazine to the Muscarinic Receptor Subtypes

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ABSTRACT

The binding properties of oxomemazine to muscarinic receptors using the ability of oxomemazine to inhibit [^{3}H]QNB binding in membrane fractions of rat cerebrum and guinea pig ventricle and ileum were investigated. [^{3}H]QNB bound to a single class of muscarinic receptors with a dissociation constant of approximately 60 pM in three tissue preparations. Pirenzepine and oxomemazine inhibited [^{3}H]QNB binding in cerebrum with a Hill coefficient lower than unity, and the inhibition data were best described by a two-site model. The relative densities of the high (M_1) and low (M_2) affinity sites for pirenzepine were 60 and 40%, with corresponding Ki values of 16 and 431 nM, and those (O_H and O_L) for oxomemazine 40 and 60%, with corresponding Ki values of 80 and 1350 nM. However, the inhibition data of both drugs vs [^{3}H]QNB in ventricle and ileum appeared to obey the law of mass-action (Hill coefficient close to 1). The apparent Ki values of pirenzepine were 850 and 250 nM, and those of oxomemazine 1460 and 570 nM in ventricle and ileum, respectively. Thus, oxomemazine like pirenzepine has high affinity for cerebrum, moderate affinity for ileum and low affinity for ventricle. These results suggest that oxomemazine could recognize the muscarinic receptor subtypes with a high affinity for the M_1 sites.

Key Words: Muscarinic receptor subtype, Oxomemazine, Pirenzepine, Receptor binding

INTRODUCTION

From a number of functional and ligand binding studies, cholinergic muscarinic receptors have been classified into three subtypes, namely M_1, M_2, and M_3. M_1 receptors with a high affinity for pirenzepine are present mainly in the cerebral cortex and sympathetic ganglia, while M_2 receptors with a low affinity for pirenzepine exist in the ileum and heart (Berrie *et al.*, 1983; Birdshall *et al.*, 1980; Clague *et al.*, 1985; Hammer *et al.*, 1980; Hammer and Giachetti, 1982; Hirschowitz *et al.*, 1984; Luthin and Wolfe, 1984; Wamsley *et al.*, 1984; Watson *et al.*, 1983). The M_2 receptors as defined with pirenzepine are further divided into a cardiac M_2 receptor having a high affinity for 11-(2-[(diethylamino)methyl]-1-piperidinyl acetyl)-5,11-dihydro-6H-pyrido(2,3-b) (1,4)-benzodiazepine-6-one (AF-DX 116), and a glandular M_3 receptor having a low affinity for this drug (Giachetti *et al.*, 1986; Hammer *et al.*, 1986). 4-Diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP), which is more selective towards a ileal M_3 receptor than a cardiac M_2 receptor, can also discriminate between cardiac and ileal muscarinic receptor subtypes (Doods *et al.*, 1987; Giraldo *et al.*, 1987; Lazareno and Roberts, 1989). Thus, the discovery of selective antagonists at these three subtypes

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has provided an important pharmacological basis to clarify subclassification of the muscarinic receptors.

Oxomemazine, a phenothiazine derivative, prevents the action of histamine on histamine H_1 receptors of target cells. Like many traditional antihistamines, this drug elicits antimuscarinic side effects such as dryness of the mouth, throat, and nasal airway, constipation, urinary retention, tachycardia, and blurred vision (Ambre *et al.*, 1991; Gilman *et al.*, 1990). The muscarinic receptor blocking potencies of H_1 receptor blockers vary (Lee *et al.*, 1988; 1990; 1993). Our previous reports (Lee *et al.*, 1990; 1993) indicated that oxomemazine was about 30 times less potent than promethazine in the muscarinic receptor blocking action. In addition to these results we found that, whereas most of traditional antihistamines lacked selectivity at above mentioned muscarinic receptor subtypes, oxomemazine showed the shallow inhibition curve in competition binding with [^{3}H]QNB to the rat cerebral microsome, indicating the presence of muscarinic receptor subtypes with different affinity for this drug (Lee *et al.*, 1990). However, no detailed study has been performed whether high and low affinity sites for oxomemazine relate to M_1 and M_2 receptors for pirenzepine.

The purpose of this study was to assess the binding affinity of cerebral (M_1), cardiac (M_2), and ileal (M_3) muscarinic receptors for pirenzepine and oxomemazine, in order to define oxomemazine-recognized heterogeneity in terms of the M_1, M_2 classification defined by pirenzepine.

MATERIALS AND METHODS

Materials

Atropine sulfate, 1,4-bis[2-(5-phenyloxazolyl)] benzene (POPOP), 2,5-diphenyloxazole (POP), pirenzepine dihydrochloride, and tris-(hydroxymethyl) aminomethane (Tris) were obtained from Sigma Chemical Co. (St. Louis, MO). [^{3}H]QNB (43 Ci/mmol) was from New England Nuclear (Boston, MA). Oxomemazine hydrochloride was a generous gift from Dr. K. W. Ha(National Institute of Safety Research, Korea). All other chemicals were of reagent grade purity.

Tissue preparations

Male rat (Sprague-Dawley, 200~250 g) cerebrum, and male guinea pig (Hartley, 400~450 g) heart and ileum were used. Rats and guinea pigs were killed by decapitation and a blow to the head, respectively. The pertinent organs were quickly removed and placed in ice-cold 10 mM Tris • Cl (pH 7.4, medium A). All subsequent operations were carried out at 2~4℃. Following an initial rinse, rat cerebrums were weighed, minced with scissors and homogenized four times (separated by 30 sec intervals) in 30 volumes of ice-cold medium A with a blender at full speed for 5 sec. The homogenate was centrifuged at 1,100×g for 5 min to remove unhomogenized materials. The supernatant was further centrifuged at 7,700 ×g for 20 min and centrifuged again at 55,000×g for 30 min. The final pellet was suspended in a small volume of medium A(2~3mg of protein/ml) using 5~6 strokes of a hand driven glass-teflon pestle.

Guinea pig ventricle was minced with scissors and homogenized twice in 5 volumes of medium which contained 10 mM $NaHCO_3$ and 5 mM NaN_3, pH 7.0, with a Polytron (PT-20) at one-half maximal speed for 15 sec. The homogenate was centrifuged for 20 min at 8,700×g and the pellet was suspended in 6 volumes of medium A using 4 passes of a glass-teflon motor driven homogenizer (80% of 140 volts). After centrifugation at 8,700×g for 20 min, the supernatant was centrifuged again at 35,000×g for 20 min to obtain pellet. This pellet was resuspended in a small glass homogenizer by 5 passes of a hand driven glass-teflon pestle in medium A to a final protein concentration of 3~4 mg/ml.

Ileum rinsed with cold medium A was minced with scissors and homogenized on ice in 10 volumes of medium A for 4×15 sec periods with 30 sec cooling between each burst. The homogenate was centrifuged at 3,600×g for 10 min at 4℃ and the pellet was discarded. The supernatant was centrifuged at 45,000×g for 20 min. The resulting pellet was suspended in an appropriate volume of medium A, using a hand driven glass-teflon homogenizer to give a final protein concentration of 10~15 mg/ml. All tissue preparations were either used immediately or stored in small aliquots at

−70℃ until use in the binding assay. Protein concentrations were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Binding studies

Tissue preparations were incubated in a final volume of 5 ml with [^{3}H]QNB in medium containing 50 mM Tris · Cl and 10 mM $MgCl_2$, pH 7.4, for 150 min at 37℃. Nonspecific binding was defined by the addition of 10 μM atropine to the incubation medium. The incubation was terminated by the filtering the suspension through a Whatman GF/B (2.5 cm) glass fiber filters under a vacuum. The filters were washed four times with 5 ml of ice-cold buffer. Filters were then dried for 3hr at room temperature and placed for 12hr in plastic scintillation vials with 8ml of scintillation cocktail (PPO: 6g, POPOP: 0.225g, Triton X-100: 500g, toluene: 1 ℓ). Radioactivity was measured in a liquid scintillation counter (Packard) at a counting efficiency of 45%.

Saturation studies were performed in the presence of various concentrations (10～800 pM) of [^{3}H]QNB. For inhibition experiments of [^{3}H]QNB binding, the preparations were incubated as indicated above in 100 pM or 300 pM [^{3}H]QNB and various concentrations of either pirenzepine or oxomemazine. All measurements were made in duplicate at least three independent assays.

Data analysis

Saturation isotherms were transformed according to the method of Scatchard(1949) and K_D value of [^{3}H]QNB were obtained by unweighted linear regression analysis of the transformed data. The Hill coefficient (nH) of [^{3}H]QNB binding was determined from the equation:

$$\log[Y/(1-Y)] = nH \cdot \log[F] - \log[K_D]$$

Where Y is the bound [^{3}H]QNB(B)/total binding sites (Bmax) and F the free (unbound) [^{3}H]QNB concentration. IC_{50} values, the concentration of unlabeled drug that inhibits 50% of specific [^{3}H]QNB binding and Hill coefficients of unlabeled drugs were calculated using linear regression analysis of log-logit plots of the inhibition data according to the following equation:

$$\log[I/(100 - I)] = nH \cdot \log[D] - \log[IC_{50}]$$

Where I is the percentage inhibition of [^{3}H]QNB binding and D the concentration of unlabeled drugs. IC_{50} values were transformed to Ki values for unlabeled drugs, using the method of Cheng and Prusoff(1973).

$$Ki = IC_{50}/(1 + F/K_D)$$

Where K_D is the dissociation constant for [^{3}H]QNB, IC_{50} is the concentration of unlabeled drug that inhibits 50% of specific [^{3}H]QNB binding, and F is [^{3}H]QNB concentration used for competition experiments.

Inhibition data were also analyzed by nonlinear least squares curve-fitting program LIGAND (Munson and Rodbard, 1980). The statistical difference between one and two-site models was assessed with the partial F test implemented in the LIGAND program. Data were expressed as mean ± standard error (S.E.M.) of the indicated number of experiments and analyzed using a two-tailed Student's *t* test. Statistical significance was considered at $p<0.05$.

RESULTS

Binding properties of [^{3}H]QNB to cerebrum, ventricle, and ileum

In the preliminary experiments performed under standard assay conditions (100 pM [^{3}H]QNB, 37℃), the specific binding of [^{3}H]QNB to each preparation reached equilibrium by 60 to 90 min without significant decrease up to 180 min and increased linearly with protein concentrations in a range of 0.05 to 1.0mg protein/tube (data not shown). The radioactivities by [^{3}H]QNB binding to 0.05 mg of cerebral membrane protein were similar to those to 0.2 mg and 0.5 mg of ventricular and ileal proteins, respectively. All binding assays in this study were therefore carried out at 37℃ for 150 min with 0.05 mg, 0.2 mg, or 0.5 mg of protein.

To determine the dissociation constant(K_D) for [^{3}H]QNB in three tissues, the specific binding of [^{3}H]QNB was measured as a function of the added [^{3}H]QNB concentration. The [^{3}H]QNB binding to all three tissue preparations demonstrated saturability with maximum specific binding at a concentration of about 500 pM. Scatchard

Table 1. The binding parameters of [^{3}H]QNB to rat cerebral microsome, guinea-pig ventricular microsome and ileal homogenate

	K_D(pM)	Bmax(fmol/mg)	nH
Cerebrum	63.6 ± 3.8	4238.4 ± 12.7	1.01 ± 0.02
Ventricle	65.3 ± 4.1	523.7 ± 22.5	0.97 ± 0.03
Ileum	56.7 ± 3.4	162.2 ± 15.3	1.03 ± 0.04

Each sample was incubated with various concentrations of [^{3}H]QNB for 150 min at 37℃ in a final volume of 5 ml and the reaction terminated by rapid filtration over glass fiber (GF/B) filters. Specific [^{3}H]QNB binding was calculated by subtracting the nonspecific binding determined in the presence of 10^{-5} M atropine. K_D and Bmax were determined by Scatchard analysis. Hill coefficient (nH) was calculated from Hill plot. Values are the mean ± S.E.M. of four experiments.

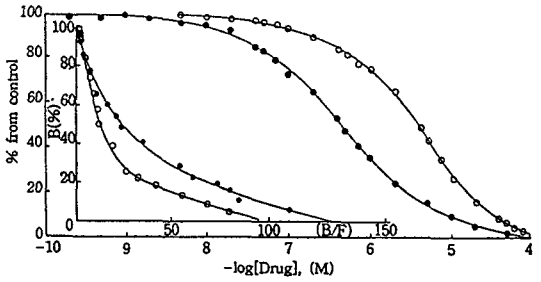

Fig. 1. Inhibition of [^{3}H]QNB binding by pirenzepine and oxomemazine to rat brain microsome. Microsomes were incubated with 300 pM [^{3}H]QNB and various concentrations of pirenzepine (●) or oxomemazine (○) for 150min at 37℃. Inset: Hofstee plot of the competition binding data. B represents the percentage inhibition of [^{3}H]QNB binding and F the free unlabeled drug concentration. Each point represents the mean of five separate determinations.

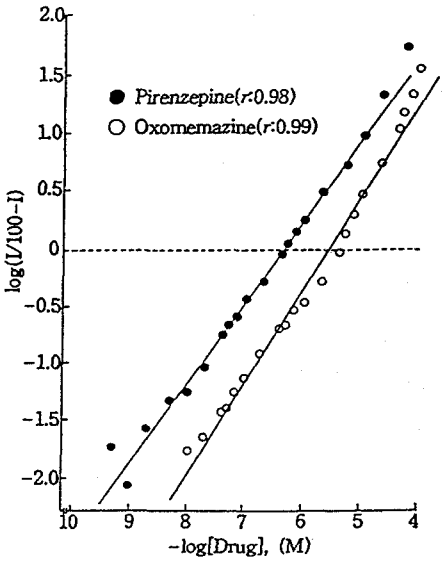

Fig. 2. Hill plot of the inhibition data in Fig. 1. I represents the percentage inhibition of [^{3}H]QNB binding at a given unlabeled drug concentration. Other legends are as described under Fig. 1 and " Method ".

and Hill plots of these binding data were linear and the Hill coefficients(nH) were not significantly different from unity, indicating that [^{3}H]QNB bound to a single populaton of muscarinic receptors with the apparent K_D value of about 60 pM in all three tissues (Table 1).

Pirenzepine inhibiton of [^{3}H]QNB binding in rat cerebrum

Inhibiton of [^{3}H]QNB binding by pirenzepine occured in a dose-dependent manner, and complete inhibition was obtained at a concentration of 100 μM(Fig. 1). The competition curve of pirenzepine vs [^{3}H]QNB was shallow and yielded a Hill coefficient of about 0.67 (Fig. 2). Hofstee plot of the inhibition data was also not linear (Fig. 1, inset), indicating the presence of the two receptor subclasses with different affinity for pirenzepine. The analysis of the inhibition curve with LIGAND program of Munson and Rodbard (1980) revealed that the data were fitted better according to a two-site than to a one-site model. The

Table 2. Ki values of pirenzepine and oxomemazine for the total sites (M_1+M_2, or O_H+O_L), the high and low affinity sites in rat cerebral microsome

	Pirenzepine			Oxomemazine		
	$M_1 + M_2$	M_1	M_2	$O_H + O_L$	O_H	O_L
nH	0.67 ±0.01			0.81 ± 0.01		
Ki(nM)	71.2±19.3	15.6±4.5	431.0 ± 37	490 ± 30.0	80.0 ± 10.0	1350 ± 120
%	100	61.2±1.8	38.8 ± 1.8	100	38.6 ± 2.03	61.4 ± 2.03

The non-line ar or Hofstee plot shown in Fig.1 was analysed according to a two-site model by LIGAND as described by Munson *et al*,.The M_1 and M_2 for pirenzepine, and O_H and O_L for oxomemazine represent high and low affinity sites for each drug, respectively. Hill coefficient (nH) from Hill plot in Fig. 2 and Ki value from $Ki = IC_{50}/(1 + F/K_D)$ were calculated. Values are the mean ± S.E.M. of four experiments.

IC_{50} values for high-and low-affinity sites (defined as M_1-and M_2-sites respectively) were 88.7 nM and 2.46 μM, respectively, with corresponding relative receptor densities of 61.2 and 38.8%. The respective Ki values of pirenzepine for M_1-and M_2-sites estimated by the equation of Cheng and Prusoff (1973) were 15.6 and 431 nM (Table 2).

Oxomemazine inhibition of [H^3]QNB binding in rat cerebrum

In order to evaluate whether oxomemazine is capable of discriminating the muscarinic receptor subtypes which coexist in our cerebral preparations, the effect of oxomemazine on [^{3}H]QNB binding was examined. Incubation of rat cerebral preparations with increasing concentrations of oxomemazine inhibited progressively the specific binding of 300 pM [^{3}H]QNB to muscarinic receptors (Fig. 1). However, oxomemazine was less potent than pirenzepine for the inhibition of [^{3}H]QNB binding. The competition binding curve of oxomemazine with [^{3}H]QNB was shallow with a Hill coefficient of 0.81. In addition to statistically significant difference of this Hill coefficient from unity, the Hofstee plot of the competition binding data showed a non-linear (Fig. 1, inset). Therefore, the binding data were analysed by a two-site model and summarized in Table 2. About 40% of the total receptor sites in cerebrum were of the high affinity sites (O_H) for oxomemazine with a Ki value of 0.08 μM, whereas the remaining 60% of the low affinity sites (O_L) with a Ki value of 1.35 μM.

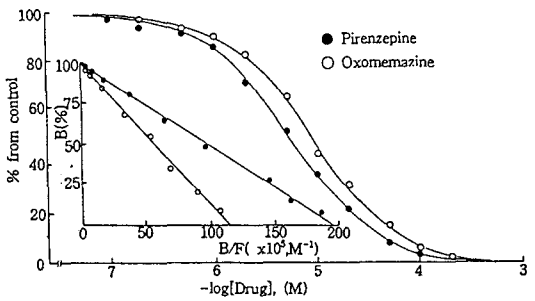

Fig. 3. Inhibition of [^{3}H]QNB binding by pirenzepine and oxomemazine to guinea pig ventricular microsome
Microsomes were incubated with 100 pM [^{3}H]QNB and various concentrations of pirenzepine (●) or oxomemazine (○) for 150 min at 37℃ in a final volume of 5ml.
Inset: Hofstee plot of the competition binding data. Each point represents the mean of three separate determinations.

Inhibition of [^{3}H]QNB binding by pirenzepine and oxomemazine in guinea pig ventricle

The binding parameters of pirenzepine and oxomemazine for the muscarinic receptors in ventricle, which contains almost exclusively the M_2 subtype (Watson *et al.*, 1983), were investigated to determine whether the M_1 and M_2 sites are the O_H and O_L sites, respectively. Pirenzepine and oxomemazine exhibited parallel inhibitory effects on [^{3}H]QNB binding in ventricular preparations

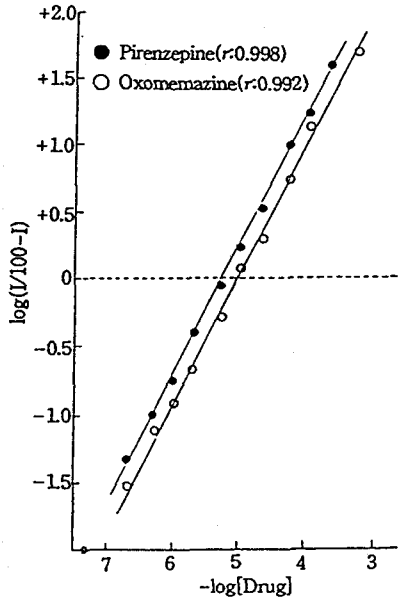

Fig. 4. Hill plot of the inhibition data in Fig. 3.
Legends are as described under Fig. 2 and Fig. 3.

with IC_{50} values of 2.14 μM for pirenzepine and 3.68 μM for oxomemazine, and fully displaced at a concentration of 100 μM (Fig. 3). The Hofstee plots of the inhibition data were linear (Fig. 3, inset). The Hill plots were also linear with the slopes of 0.95 and 1.04, which were not significantly different from 1 (Fig. 4). Analysis of the data from ventricle best fitted a one-site model, indicating that both drugs interacted with a single muscarinic binding site. The Ki values of pirenzepine and oxomemazine for this site were 0.85 and 1.46 μM, respectively, which were similar to those of these drugs for low affinity site (M_2,or O_L) in cerebrum (Table 3).

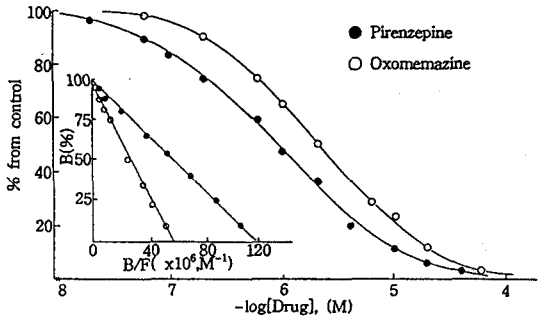

Fig. 5. Inhibition of [^{3}H]QNB binding by pirenzepine and oxomemazine to guinea pig ileal homogenate
Legends are as described under Fig. 3.

Inhibition of [^{3}H]QNB binding by pirenzepine and oxomemazine in guinea pig ileum

To further assess whether the O_H, or the O_L sites for oxomemazine are related to the M_3 sites, the binding nature of these drugs for the muscarinic receptors in guinea pig ileum was studied. Incubation of ileal preparations with increasing concentrations of pirenzepine or oxomemazine produced progressive inhibition of the [^{3}H]QNB (100 pM) binding to muscarinic receptors (Fig. 5). The IC_{50} values for pirenzepine and oxomemazine were 0.63 and 1.69 μM, respectively. Analysis of the inhibition curves of [^{3}H]QNB binding by both drugs was best fitted with binding to a single class of sites. This homogeneity of the binding sites was supported by a Hill coefficient close to unity (0.92 for pirenzepine and 0.99 for oxomemazine) (Table 3) and a linearity of Hofstee plots (Fig. 5, inset). Thus, in ileum both drugs bind to a homogeneous

Table 3. Ki value and Hill coefficient of pirenzepine and oxomemazine in guinea pig ventricle and ileum

	Pirenzepine		Oxomemazine	
	Ki(μM)	nH	Ki(μM)	nH
Ventricle	0.85 ± 0.05	0.95 ± 0.03	1.46 ± 0.16	1.04 ± 0.05
Ileum	0.25 ± 0.02	0.92 ± 0.05	0.67 ± 0.05	0.99 ± 0.03

Ki values are the equilibrium dissociation constants of drugs. nH = Hill coefficient. Data are presented as the mean ±S.E.M. of three experiments.

population of low affinity sites with the Ki values of 0.25 and 0.67 μM for pirenzepine and oxomemazine, respectively.

DISCUSSION

The present results demonstrate that oxomemazine recognizes the muscarinic receptor heterogeneity. The greater affinity of oxomemazine for the muscarinic receptors of cerebrum compared with those of heart and ileum is the distinguishing nature of this drug.

Muscarinic receptors have been shown to exhibit different affinities for pirenzepine in various tissues. Traditionally receptors with high and low affinity for pirenzepine are referred to as M_1 and M_2 (Goyal and Rattan; 1978, Hammer *et al.*, 1980). On the basis of binding affinity for pirenzepine, the M_2 sites could be further subdivided into intermediate (M_3, exocrine gland and ileum) and low (M_2, heart) affinity sites (Hammer *et al.*, 1980). This subclassification of the M_2 receptors into a cardiac M_2 subtype and a glandular M_3 subtype has been supported by the studies with a cardioselective muscarinic antagonist AF-DX 116 (Barlow and Shepherd, 1985; Doods *et al.*, 1987; Giraldo *et al.*, 1987; 1988) and a gland selective antagonist 4-DAMP (Barlow *et al.*, 1976; Barlow and Shepherd, 1985; Eglen and Whiting, 1986). In the present study, to determine the selectivity of oxomemazine for these three muscarinic receptor subtypes the binding characteristics of oxomemazine for cerebral, ventricular, and ileal muscarinic receptors were compared to those of pirenzepine.

The specific binding of [^{3}H]QNB to cerebral, ventricular, and ileal preparations was saturable, of high affinity and inhibited by specific muscarinic antagonist atropine. The Scatchard plots of [^{3}H]QNB saturation binding data were linear. These results are consistant with previous reports (Field *et al.*, 1978; Hammer *et al.*, 1980; Yamamura and Snyder, 1974), which were described that [^{3}H] QNB recognizes the muscarinic receptor subtypes with equal affinities. Therefore, we used [^{3}H]QNB as a suitable radioligand for labeling all three muscarinic receptor subtypes in cerebrum, ventricle and ileum.

In this study, the competition binding curves for pirenzepine in ventricle and ileum were compatible with an interaction at one-binding site ($nH \cong 1$) with a Ki value of 850 nM and 250 nM, respectively, whereas the interaction of pirenzepine with the cerebral receptors did not follow the law of mass-action ($nH = 0.67$), indicating the presence of a heterogeneous population of receptors ; approximately 60% of the total receptor sites were the high affinity (M_1) sites for pirenzepine with a Ki value of 16 nM, and the remaining (M_2) sites (40%) bound to pirenzepine with a Ki value of 430 nM, showing a lower affinity for this drug. Thus, in agreement with earlier studies (Hammer *et al.*, 1980; Hammer and Giachetti, 1982; Luthin and Wolfe, 1984; El-Fakahany *et al.*, 1986) pirenzepine recognized two classes of muscarinic receptors in cerebral preparations with about a 28-fold difference in the affinities for the two sites. Similarly to what was observed with pirenzepine, the oxomemazine inhibition curve of [^{3}H]QNB binding to cerebral preparations deviated from the law of mass-action, as indicated by a Hill coefficient significantly differing from one ($nH = 0.81$). Analysis of the inhibition data with a two-site model revealed that about 40% and 60% of the total receptor sites had a high affinity (O_H) and a low affinity (O_L) for oxomemazine, respectively, with corresponding Ki values of 80 nM and 1350 nM. These results demonstrate the ability of oxomemazine to recognize muscarinic receptor heterogeneity.

The results from cerebrum do not ascertain whether the subpopulations for oxomemazine correspond to the M_1 and M_2 subtypes defined by pirenzepine. The inverse relationship in the relative densities of the M_1 and M_2 sites (60 : 40) compared to the O_H and O_L sites (40 : 60) may reflect that the O_H sites correspond to the M_2 sites. In this case, oxomemazine, like AF-DX 116 and 4-DAMP which are selective for cardiac M_2 and ileal M_3 sites, respectively, should bind to heart or ileum with a high affinity. However, the present results obtained from the analysis of competition curve in ventricle and ileum indicated that oxomemazine bound to a single class of muscarinic receptors in both tissues with a Ki value of 1500 nM and 700 nM, respectively. These binding profiles clearly demonstrate that oxomemazine has a low affinity for the M_2 sites.

In our present study, oxomemazine showed a

high affinity for cerebral (M_1) sites and a low affinity for ventricular (M_2) sites. It has been known that the rat cerebral cortex contains a mixed population of the M_1 and M_3 sites (Doods *et al.*, 1987), and that drugs as 4-DAMP and dicyclomine possess high affinity for both M_1 and M_3 sites (Barlow *et al.*, 1976; Barlow and Shepherd, 1985; Eglen *et al.*, 1986; Kenny *et al.*, 1985; Nilvebrant and Spart, 1986). Accordingly, when a drug shows high affinity for cerebral sites and low affinity for cardiac sites this difference is not sufficient to designate this drug as M_1 selective. In the case of oxomemazine, however, the selectivity for the M_1 receptors was confirmed by the low affinity binding nature of this drug for the ileal (M_3) sites. Therefore, it is concluded that oxomemazine like pirenzepine does not discriminate between the binding sites (M_2) present in ventricle and those (M_3) in ileum (only 2~3-fold difference in affinity), but discriminates very effectively between the M_1 and M_2 sites (17~19-fold difference).

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=국문초록=

Oxomemazine의 Muscarinic Receptor Subtypes에 대한 결합성질

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Oxomemazine이 muscarinic receptor subtypes에 대하여 선택성을 가지는지에 관한 지견을 얻고자, 대뇌, 심실 및 회장 muscarinic receptor에 대한 oxomemazine의 결합성질을 조사,비교하였다. [^{3}H]QNB 포화결합실험 결과 세 조직의 muscarinic receptor는 [^{3}H]QNB에 대해서는 affinity가 약 60pM인 단일 receptor인 것으로 추정되었다. 대뇌에서 pirenzepine과 oxomemazine의 [^{3}H]QNB 결합억제에 대한 Hill coefficient는 각각 0.67 및 0.8로서 대뇌에는 이들 약물에 대하여 affinity가 서로 다른 두 종류의 muscarinic receptor subtypes가 존재하는 것으로 나타났으며, pirenzepine에 대한 high affinity(M_1)와 low affinity(M_2) receptor 및 oxomemazine에 대한 high affinity(O_H)와 low affinity (O_L) receptor의 분포비는 약 60:40 및 40:60이었고, M_1과 M_2 receptor에 대한 pirenzepine의 K_i치는 16 nM 및 431 nM, O_H와 O_L receptor에 대한 oxomemazine 의 K_i치는 80nM 및 1350 nM이었다. 그러나 심실과 회장에서 이들 약물의 [^{3}H] QNB 결합억제에 대한 Hill coefficient는 1에 가까웠다. 심실과 회장 muscarinic receptor에 대한 pirenzepine의 K_i치는 850 nM 및 250 nM, oxomemazine의 K_i치는 1460 nM 및 670 nM로서 대뇌에서 이들 약물의 low affinity receptor에 대한 K_i치에 가까웠다. 즉, muscarinic receptor에 대한 affinity면에서 oxomemazine은 pirenzepine과 같이 대뇌에서 가장 높았으며, 회장에 대해서는 중등도였고, 심실에서 가장 낮았다. 이로 보아 oxomemazine은 M_1 receptor에 선택성이 있는 것으로 추정된다.