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Aceclidine and pilocarpine interact differently with muscarinic receptor in isolated rabbit iris muscle

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Abstract

The relationship between muscarinic receptor affinity states and the contractile response to the muscarinic agonists carbachol, aceclidine, and pilocarpine, has been examined in the isolated rabbit iris muscle. Contraction of the iris muscle by carbachol and aceclidine was more potent and/or more efficacious than the response to pilocarpine. Analysis of [^{3}H]-Quinuclidinyl benzilate (QNB) binding showed that while both carbachol and aceclidine bound to high- and low-affinity forms of the muscarinic receptor, pilocarpine bound to one affinity state. The efficacy of carbachol and aceclidine to stimulate contraction of the iris muscle was consistent with receptor occupancy theory only when considering the low-affinity state of the muscarinic receptor, and activation of the low-affinity rather than high-affinity binding state of the receptor is likely to mediate the contraction of iris muscle. Therefore, the typical anti-glaucoma muscarinic agonists aceclidine and pilocarpine may interact differently with their target receptors in isolated rabbit iris muscle.

Keywords: Aceclidine; Pilocarpine; Muscarinic receptor; Two-site binding; Iris

Introduction

Aceclidine and pilocarpine are the typical cholinomimetics used to treat glaucoma, the progressive degenerative neuropathy of the optic nerve that can lead to blindness (Khaw et al., 2004). Although systemic side effects of glaucoma alleviating cholinomimetics are rare, ocular side effects associated with concomitant contraction of the ciliary and iris muscles limit their usefulness in many patients. Dissociation of the effects of outflow facility increase with accommodation and miosis of muscarinic agonists may lead to the development of greatly improved antiglaucoma agents. Aceclidine is effective at lowering intraocular pressure, but produces less accommodation than other cholinomimetics, in part fulfilling separation of the responses (Erickson-Lamy and Schroeder, 1990; Gabelt and Kaufman, 1994; Poyer et al., 1994). Since much attention has been put on the difference of accommodation between aceclidine and pilocarpine, further investigations are needed on the characteristics of iris contraction associated with miosis. It is generally accepted that the main effects on iris contractility are mediated by muscarinic stimulation, and the M_3 receptor subtype appears to be the most abundantly expressed muscarinic receptor in the iris of humans and other mammals (Woldemussie et al., 1993; Gil et al., 1997, 2001; Ishizaka et al., 1998; Collison et al., 2000; Barilan et al., 2003).

Recently, data have emerged to suggest that carbachol and acetylcholine interact with both a high- and low-affinity state of the muscarinic receptor in various tissues or cells such as heart, cerebral cortex, hippocampus and cloned human muscarinic receptors stably expressed in Chinese hamster ovary (CHO) cells (Hou et al., 1998; Daeffler et al., 1999; Ladner and Lee, 1999; Christopoulos and Wilson, 2001; Shiozaki and Iseki, 2004; Rossi et al., 2005). Although the iris cholinergic neuromuscular system can be regarded as a classical example of a muscarinic apparatus (Ishizaka et al., 1998), there has been little investigation on the dual affinity state of the muscarinic receptors in this system. There has recently been renewed interest in the application of muscarinic-based therapies in the treatment of glaucoma since it now appears that acetylcholine influences both the production and drainage of aqueous

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humour and so there is the possibility of dual control from one drug (Duncan and Collison, 2003).

In the present report, we investigated the binding characteristics and the contractile responses of isolated rabbit iris muscle to carbachol, aceclidine, and pilocarpine and have revealed that carbachol and aceclidine stimulated the contraction of iris muscle with greater potency and/or efficacy than that of pilocarpine. In binding analysis, pilocarpine displayed one-site affinity, whereas carbachol and aceclidine interacted the muscarinic receptor in both high- and low-affinity forms. Further analysis indicated that the contractile effect of carbachol and aceclidine on rabbit iris muscle might be through activating low-affinity muscarinic receptors.

Materials and methods

Drugs

Aceclidine was purchased from Toronto Research Chemicals, North York, Canada; carbachol, pilocarpine, atropine and tris-[hydroxymethyl] amino methane (Tris) from Sigma, St. Louis, MO, USA; [^{3}H]-Quinuclidinyl benzilate (QNB) (spec. act. 43 Ci/mM) from Amersham, Buckinghamshire, England.

Animal and tissue preparation

New-Zealand rabbits weighing 2.0 to 3.0 kg (Certificate no. 02-23-4) were obtained from the Animal Center of Shanghai Second Medical University and were treated in accordance with the University Guide for the Care and Use of Laboratory Animals. Animals were sacrificed by injecting atmosphere air into the marginal ear vein. The eyes were immediately enucleated and the intact iris smooth muscle was excised.

Isolated iris contraction assay

The freshly prepared iris muscle was mounted in 10 ml organ chambers containing modified Krebs–Henseleit solution containing (in mM): NaCl, 118.0; KCl, 4.7; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; $MgSO_4$, 1.2; KH_2PO_4, 1.2; glucose, 11.0, EDTA·Na_2 0.5. The bath was continuously aerated with O_2:CO_2 mixture (95%:5%) and kept at a constant temperature of 37 °C. The preparation was connected vertically to a force-displacement transducer under a resting tension of 500 mg. Preparations were allowed to equilibrate for at least 60 min before drug addition, during which the buffer solution was refreshed every 15 min. Isometric contractions were recorded using a PowerLab 8sp life analysis system (AdInstruments Co.). In order to confirm the viability of the tissue, preparation was exposed to a high-potassium concentration (KCl 60 mM) following the stabilization period. After washout replacement with normal medium and return to the original baseline, cumulative concentration–response curves were obtained for carbachol, aceclidine, and pilocarpine, respectively. The contractile responses of iris muscle to each dose of the muscarinic agonists are expressed as percentages of that elicited to 10^{-4} M carbachol. In our pilot study, carbachol at this concentration could induce the maximum contraction. No significant desensitization was observed for at least two consecutive concentration–response curves for the muscarinic agonists; accordingly, no more than two complete curves were recorded for each tissue. In another set of experiments, after concentration–response curves for the agonists were obtained, preparations were incubated with a selective muscarinic receptor antagonist atropine for 20 min before challenge with the agonist.

Radioligand-receptor binding assay

The iris muscle was minced with scissors in ice-cold 50 mM Tris buffer (pH 7.4). The tissue was then homogenized in 1 g:20 ml (w:v) volume ice-cold 0.32 M sucrose in Tris buffer using a Waring blender and further disrupted with an Ultraturrax Tissuemizer. The crude homogenate was centrifuged for 10 min at 1000 $\times g$ and the resulting supernatant was centrifuged for 30 min at 20,000 $\times g$ to yield a membrane pellet. The pellet was resuspended in Tris buffer as a crude membrane fraction. All the procedures were performed at 4 °C. In the saturation binding assay, membranes (0.1 mg protein) were incubated vibrantly at 32 °C for 30 min with 0.05–1.1 nM [^{3}H]QNB with or without 10 μM atropine sulfate in a total volume of 0.4 ml. The reaction was terminated by rapid filtration through glass fiber filters, washed three times with ice-cold Tris buffer. Protein concentration was determined with the micro BCA kit (Pierce, Rockford, IL), using bovine serum albumin as the standard. For competition binding assays, iris muscle membranes (0.1 mg protein) were incubated with 0.4 nM [^{3}H]-QNB at 32 °C for 60 min with increasing concentrations of the agonists carbachol, aceclidine, or pilocarpine in total volume of 0.4 ml. All the dilutions for the agonists were made in Tris buffer. Assays were performed in duplicate.

Statistics and data analysis

For the iris contraction assay, EC_{50} values (concentration of agonists causing a half-maximal response) and the slopes of the log concentration–response curves were calculated by means of nonlinear curve fitting of sigmoidal dose-response (variable slope) logistic transformation using program GraphPad PRISM 4.0 (San Diego, CA, USA). pA_2 values for atropine were determined according to (Arunlakshana and Schild, 1959). In saturation binding tests, nonlinear curve fitting was used to generate affinity (K_D) and capacity (Bmax) values for [^{3}H]-QNB. The competition curves were also analyzed according to both one- and two-site mass action binding models, and the better model was determined by an extra-sum-of-squares test using PRISM. When a one-site model provided a better description of the data, the apparent dissociation constants (Ki) were calculated from IC_{50} values according to (Cheng and Prusoff, 1973). When a multiple affinity-site model provided a better description of the data, then the fraction of high-affinity states (%RH) as well as the apparent dissociation constants for the high- and low-affinity agonist binding sites, expressed as K_H and K_L, respectively, were calculated. The values were

expressed as pKi (−log Ki), pK_H (−log K_H) and pK_L (−log K_L). The only variables constrained in the analysis were those that were experimentally determined, namely, the dissociation constant for [^{3}H]-QNB and the nonspecific binding of [^{3}H]-QNB. Data were expressed as "mean±SEM" of three independent experiments unless otherwise stated.

The statistically significant differences were determined by Student's *t*-test or by analysis of variance (ANOVA) as appropriate. Unless otherwise stated, a probability (p) value of 0.05 was taken to indicate statistical significance.

Receptor occupancy values for carbachol, aceclidine, and pilocarpine were determined according to (Stengel and Cohen, 2001). Log concentration–response curves for carbachol, aceclidine, and pilocarpine-stimulated contraction of iris muscle were obtained as indicated above. For carbachol we assumed a maximal response of 100% stimulation on contraction of iris muscle. Fractional receptor occupancy for each contraction response was calculated from the following equation:

$$\frac{[RA]}{[R_T]} = \frac{[A]}{K_A + [A]}$$

where $[A]$ is the agonist concentration, K_A is the apparent agonist dissociation constant, [RA] is the concentration of receptor agonist complex, and $[R_T]$ is the total receptor concentration. The contraction of iris muscle as a percentage of the carbachol-induced maximum changes produced by each concentration of agonist was then plotted as a function of the percentage of receptors occupied ($[RA]/[R_T] \times 100$).

Results

Effect of muscarinic agonists on the contraction of isolated rabbit iris muscle

Cumulative addition of muscarinic agonists carbachol, aceclidine and pilocarpine to isolated iris muscle produced a log concentration-dependent contractile response (Fig. 1). The pEC_{50} values for carbachol, aceclidine and pilocarpine are shown in Table 1. Among the agonists tested, carbachol was the most potent, approximately 7- and 8-fold more potent than pilocarpine and aceclidine, while aceclidine and pilocarpine showed very similar potency. The efficacy of the three muscarinic agonists varied considerably. Carbachol stimulated the contraction of iris muscle with a maximum response of 0.44±0.13 g, and was the most efficacious of the three agonists. Pilocarpine was the least efficacious, producing maximum contraction less than 25% comparing to that of carbachol ($p<0.01$). The maximum response to aceclidine was 85.9% of that of carbachol ($p<0.01$) but greater than that of pilocarpine ($p<0.01$).

Table 1
Pharmacological activity of muscarinic agonists to contract the isolated rabbit iris muscle ($n=5–8$)

Agonist	pEC_{50}	Emax %	Hill coefficient
Carbachol	6.09±0.03	100	1.43±0.13
Aceclidine	5.19±0.04[a]	85.90±2.63[a]	1.44±0.14
Pilocarpine	5.21±0.16[a]	22.40±2.90[a,b]	1.24±0.44

pEC_{50}: negative logarithm of the concentration of agonists causing a half-maximal response.
Emax: maximal response, expressed relative to that of carbachol.
[a] $p<0.01$ vs. group of carbachol.
[b] $p<0.01$ vs. group of aceclidine.

Atropine shifted the agonists-induced response curves to the right, generating pA_2 values of 9.01±0.18, 9.23±0.13, and 8.97±0.11 against carbachol (Fig. 2), aceclidine and pilocarpine (Figures not shown), respectively.

[^{3}H]-QNB binding to iris muscle membranes

[^{3}H]-QNB binding studies were performed with a crude membrane fraction prepared from the rabbit iris muscle. The binding of [^{3}H]-QNB was saturable (Fig. 3), and best fit the one-site binding model indicating that [^{3}H]-QNB bound to a single population. The dissociation equilibrium constant (K_D) and receptor density (Bmax) values were 0.25±0.06 nM and 1.40±0.12 pmol/mg protein ($n=3$), respectively. As shown in Fig. 4, [^{3}H]-QNB binding to muscarinic receptors was completely inhibited by the muscarinic agonists in a log

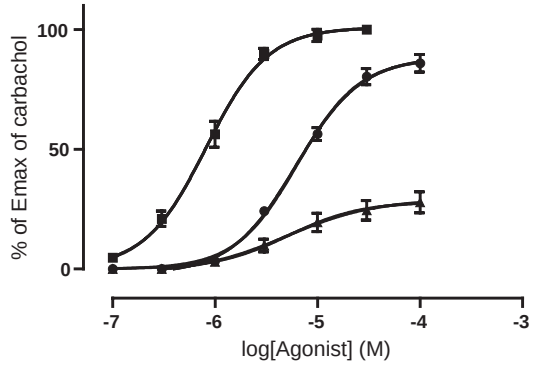

Fig. 1. Effects of muscarinic agonists carbachol (■), aceclidine (●), and pilocarpine (▲) on the contractile response of the isolated rabbit iris muscle. The contractile responses of iris muscle to each dose of the agonists are expressed as percentages of that induced by 10^{-4} M carbachol. The data points represent the mean±S.E.M. ($n=5–8$).

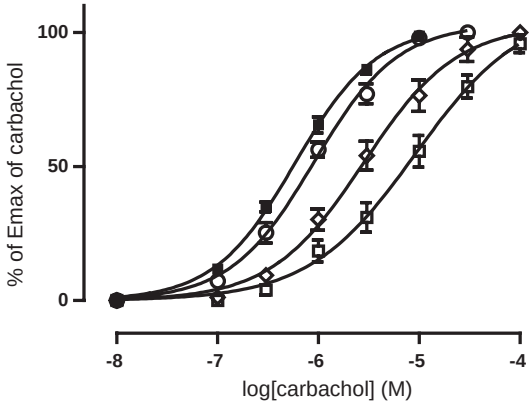

Fig. 2. Log concentration–response curves for carbachol in the isolated rabbit iris muscle in the absence (■) and presence of atropine, 1×10^{-9} M (○), 1×10^{-9} M (◇) and 1×10^{-8} M (□). The data points represent the mean±S.E.M. ($n=7–9$).

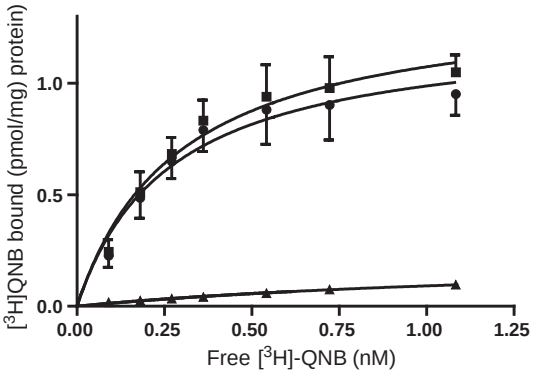

Fig. 3. Saturation isotherms of [^{3}H]-QNB binding to membrane fraction prepared from the rabbit iris muscle. Increasing concentrations of [^{3}H]-QNB were incubated with iris homogenates and specific binding (●) was defined as the difference between total binding (■) and nonspecific binding (▲) observed in the presence of 10 μM atropine. Points represent the mean ± S.E.M. of three experiments each performed in duplicate. In some points, the error deviation is hidden inside the symbol.

concentration-dependent manner. The competition curve of pilocarpine was best fitted to a one-site binding model (Fig. 4C). In contrast, aceclidine and carbachol competition curves could more readily be fitted to a two-site model in which the muscarinic receptor was proposed to be recognized by the agonists in both high and low affinity forms (Fig. 4A,B). The pK_i values for pilocarpine and pK_H, pK_L as well as the percentage of high affinity sites of carbachol and aceclidine are summarized in Table 2.

Receptor occupancy versus response characteristics of carbachol, aceclidine and pilocarpine

Given the pA_2 values determined with atropine, it is reasonable to assume that the contractions of iris muscle produced by carbachol, aceclidine, and pilocarpine were mediated by activation of muscarinic receptors; therefore the fractional muscarinic receptor occupancy was calculated for each agonist concentration (Fig. 5). This analysis indicated that carbachol was a full agonist requiring less than 10% of the receptors to be occupied for greater than 50% response only when carbachol was considered to interact with the low-affinity state of the muscarinic receptor in iris muscle. When the affinity of carbachol at the high-affinity state of the muscarinic receptor was used, the receptor occupancy calculation was not consistent with classical receptor theory regarding receptor occupancy for a full agonist (Fig. 4A). For example, if carbachol were interacting with the high-affinity state of the muscarinic receptor, then over 50% of the receptors must be occupied for a 20% response, an unlikely situation for a full agonist. Thus, carbachol-induced contraction must be associated with activation of the low-but not high-affinity state of the iris muscarinic receptor.

Aceclidine behaved like a full agonist or high efficacious partial agonist in contracting the rabbit iris muscle (see Table 1). If interacting with only the low-affinity state of the muscarinic receptor, then 20% of the receptors would need to be occupied by aceclidine to produce 60% of its own maximal

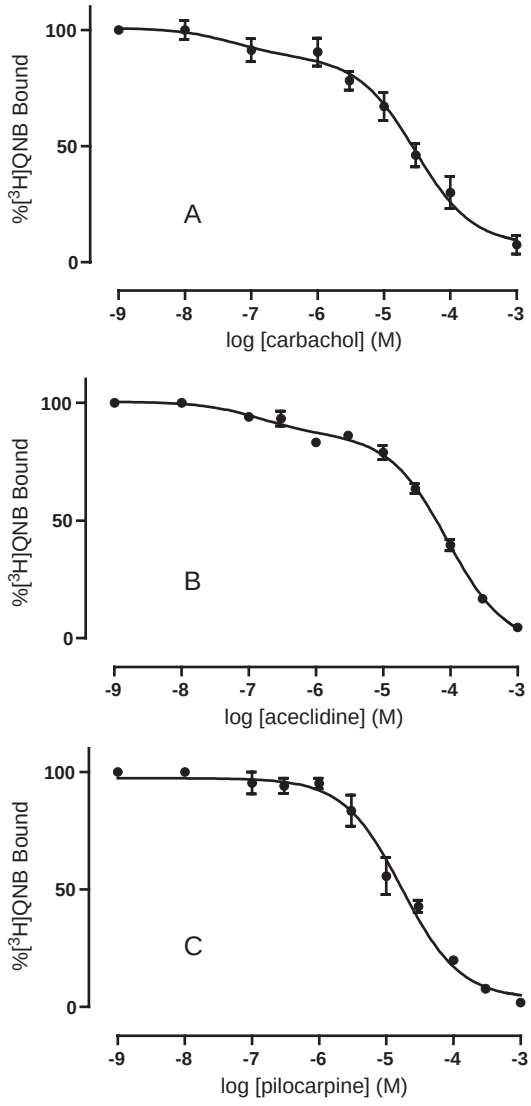

Fig. 4. Competition curves for carbachol (A), aceclidine (B) and pilocarpine (C). Rabbit iris muscle membranes were incubated with 0.4 nM [^{3}H]-QNB and increasing concentrations of agonists. Results are expressed as percentage of control maximal specific binding in the absence of agonists. Points represent the mean ± S.E.M. of three experiments each performed in duplicate.

contraction and to produce 50% of the maximal contraction to carbachol. Similarly, aceclidine would need to occupy 50% of receptors for 90% of its own maximal contraction and for 80%

Table 2
Binding parameters for muscarinic agonist-induced displacement of [^{3}H]-QNB in rabbit iris muscle membranes

	Best fit	pKi[a]	pK_H[b]	pK_L[c]	K_L/K_H	% RH[d]
Carbachol	2-site	–	7.73 ± 0.19	4.94 ± 0.02	506.9	13 ± 3
Aceclidine	2-site	–	7.17 ± 0.11	4.47 ± 0.02	614.3	13 ± 2
Pilocarpine	1-site	5.18 ± 0.03	–	–	–	–

Values are the mean ± S.E.M. of three experiments conducted in duplicate.
[a] Negative logarithm of the dissociation constant for the single binding site.
[b] Negative logarithm of the dissociation constant for the high-affinity agonist binding site.
[c] Negative logarithm of the dissociation constant for the low-affinity agonist binding site.
[d] Percentage of high affinity binding sites.

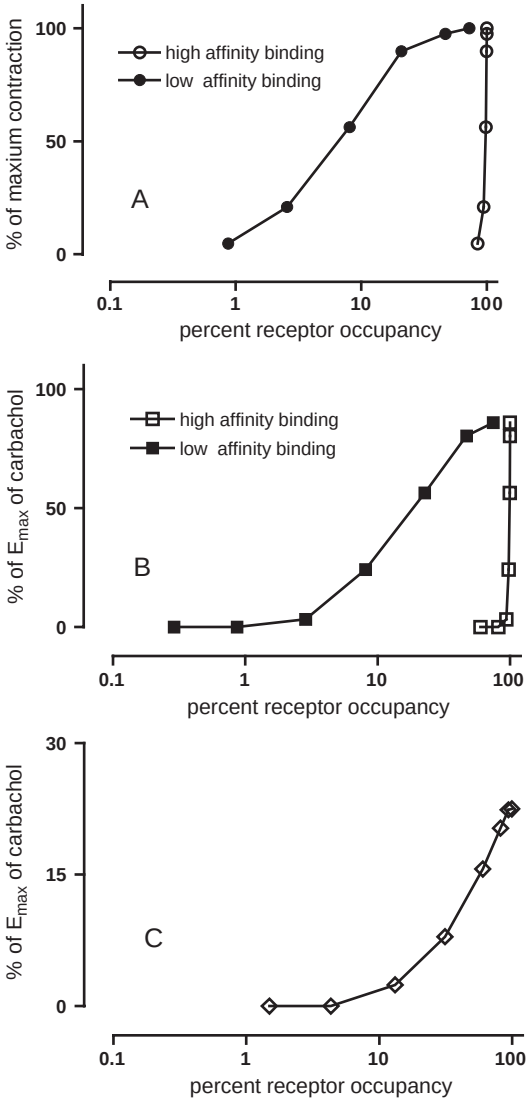

Fig. 5. Isolated rabbit iris muscle contraction in response to carbachol (A), aceclidine (B) and pilocarpine (C) as a function of the percentage of receptors occupied ($[RA]/[R_T] \times 100$). Curves for carbachol and aceclidine were generated using the apparent dissociation constants determined for interaction with the high- and low-affinity states (K_H and K_L) of the muscarinic receptors in the rabbit iris muscle. The curve for pilocarpine was generated using the apparent dissociation constant for interaction with the single state (K_i) of the muscarinic receptors. The negative logarithm of the dissociation constants is shown in Table 2.

of the maximal contraction seen with carbachol. In contrast, assuming an interaction of aceclidine with the high-affinity state of the muscarinic receptors, then it requires 94% of the receptors to be occupied for 4% of its own maximal contraction and 3% of the maximal contraction to carbachol; this is an unlikely situation for a full or high efficacious partial agonist (Fig. 4B). Thus, aceclidine-induced contraction may also be associated with activation of the low–but not high–affinity state of the iris muscarinic receptor.

Pilocarpine displayed the classical properties of a partial agonist, with 50% receptor occupancy producing less than 50% of its own maximal contraction and just 14% that seen with carbachol. Therefore, even when the occupancy reached 100%, the maximal contraction produced by pilocarpine was less than a quarter that of carbachol (Fig. 4C).

Discussion

Both aceclidine and pilocarpine stimulate iris contraction and subsequent miosis at similar or lower doses than those needed to stimulate outflow facility increase or accommodation (Gabelt and Kaufman, 1994; Kiland et al., 2000). We have shown here that cumulative addition of muscarinic agonists to isolated rabbit iris muscle produced a log concentration-dependent contractile response. Of the three muscarinic agonists analyzed, carbachol was the most potent and efficacious, and pilocarpine showed the lowest efficacy. Aceclidine moderately stimulated the contraction of iris muscle, with the maximum contraction less than that of carbachol and greater than that of pilocarpine. Aceclidine could be considered as a full agonist or high efficacious partial agonist in stimulating muscarinic receptor-dependent contraction of rabbit iris muscle.

Molecular cloning studies have revealed five distinct muscarinic acetylcholine receptors referred to as M1–M5. The odd-numbered muscarinic subtypes (M1, M3, and M5) are selectively linked to Gq/11 proteins, while the even-numbered subtypes (M2 and M4) are preferentially coupled to the pertussis toxin (PTX)-sensitive Gi/o family (Caulfield and Birdsall, 1998). Various techniques indicate that it is the M_3 subtype that is the most abundant muscarinic receptor expressed in the iris of humans and other mammals (Honkanen et al., 1990; Woldemussie et al., 1993; Gil et al., 1997; Ishizaka et al., 1998; Collison et al., 2000). For example, immunoprecipitation of muscarinic receptors in human iris revealed that 60% to 75% of all subtypes present were of the M_3 variety (Gil et al., 1997), and Quantitative Reverse Transcription PCR determinations support the finding that 84% of all subtypes present in iris were of the M_3 variety (Collison et al., 2000). Furthermore, functional analysis showed the contraction of iris by muscarinic agonists and subsequent miosis is also primarily mediated by M_3 receptors (Koss and Wally, 1995; Choppin et al., 1998). Stimulation of M_3 receptors induces inositol 1,4,5-trisphosphate (IP_3) production in iris muscle cells which increases intracellular calcium ($[Ca^{2+}]_i$) transient mobilization, recruits more $[Ca^{2+}]_i$ by accelerating Ca^{2+} influx, and thus triggers the contraction of iris muscle and consequently produces miosis (Ding et al., 1997; Ishizaka et al., 1998).

In competition binding assays with [^{3}H]-QNB, we found that carbachol and aceclidine also showed some differences compared with pilocarpine. All three agonists completely displaced [^{3}H]-QNB binding in a log concentration-dependent manner. However, while the competition curves of pilocarpine could readily be fitted to a one-site binding status, those for carbachol and aceclidine showed a systematic deviation from the curve predicted for the interaction of an agonist with a single affinity site, and the displacement data could be more readily fitted to a two-site model. To our knowledge, this is the first report that carbachol and aceclidine interact with both high- and low-affinity states of the muscarinic receptor in iris

muscle. One possible explanation is that carbachol and aceclidine interact with the different muscarinic receptor subtypes with different affinity, since although the M_3 subtype is likely to be the most abundant, the other four muscarinic receptor subtypes may also be present. However, this explanation is unlikely because: (1) carbachol and aceclidine are typically non-selective for subtypes of muscarinic receptor (Bymaster et al., 1998; Lind et al., 1998; Oberhauser et al., 2001), (2) the difference in binding affinity for low- and high-affinity states was over 100-fold, and such a large difference is unlikely to represent nonselective binding to different receptor subtypes, and (3) even in CHO cells expressing individual muscarinic receptor subtypes, the muscarinic agonists still recognize both high- and low-affinity states (Hou et al., 1998; Christopoulos and Wilson, 2001) with a large difference in affinity. Competition binding experiments assessing the affinity of muscarinic M_2 receptors (in pig atrial sarcolemma) coupled to G proteins (R^*), of combinations of coupled and uncoupled receptors (R^*+R), and of uncoupled receptors (R), revealed that the ranking of Ki values for the agonist carbachol was $R^* \ll R^*+R \ll R$ (0.95, 124 and 1017 nM) (Daeffler et al., 1999). In contrast, the antagonists atropine and AF-DX 116 showed similar affinities for all three binding conditions (0.34, 0.42, 0.41 and 19, 22, 32 nM, respectively), and the affinity of the inverse agonist pirenzepine was 174, 155, and 115 nM (i.e., $R^* > R^*+R > R$). Therefore, full muscarinic receptor agonists can display a variety of affinities depending on the state of the receptor-G protein complex. When interacting with agonist, muscarinic receptors change their conformation and couple to G proteins. The receptors coupled to G proteins (R^*) show a higher affinity for agonists, which can be thousands of times greater than their affinity as uncoupled receptors (R) (Breivogel and Childers, 2000; Shiozaki and Iseki, 2004). The large difference between the high- and low affinity states in our binding assay with carbachol and aceclidine is therefore likely to be due to the recognition by the two agonists of the different receptor states coupled to G proteins. The binding data for the weak partial muscarinic agonist pilocarpine was readily fitted to a one-site affinity model, suggesting that it might show such low intrinsic ability that the number of receptors induced to couple to G proteins by pilocarpine was low. Alternatively, the competition binding assay may not be able to differentiate minor change in affinity produced by partial agonists. Other studies also have suggested that multiple binding sites are associated with full agonists rather than partial agonists (Sharif et al., 1995; Ladner and Lee, 1999).

In general, it is the high-affinity muscarinic receptor site that is most often measured and considered to be physiologically relevant (Rossi et al., 2005). However, the present study shows that activation of the low-affinity rather than high-affinity binding state of the muscarinic receptor is likely to mediate the contraction of iris muscle induced by carbachol and aceclidine. Calculation of receptor occupancy for carbachol and aceclidine revealed that the efficacy of these agonists was consistent with receptor occupancy theory only when efficacy was calculated using affinity constants for the low-affinity rather than the high-affinity state of the muscarinic receptor. Our finding that the carbachol and aceclidine-stimulated contraction of rabbit iris muscle may be through low affinity muscarinic receptors is consistent with the report that the low-affinity state of the M_2 receptor is responsible for atrial bradycardia (Stengel and Cohen, 2001). Moreover, studies on cannabinoid agonists showed that high-affinity receptor binding did not appear to produce any stimulation of [^{35}S]GTPγS binding, whereas intermediate-and low-affinity receptor-binding sites appeared to correspond to the high-and low-affinity [^{35}S]GTPγS-stimulating sites (Breivogel and Childers, 2000). The full significance of activation of the low-affinity state of the muscarinic receptor mediating physiological responses to muscarinic agonists needs to be further investigated. In the meantime, it is also necessary to be cautious when evaluating the miosis effect of glaucoma-alleviating muscarinic agonists simply by assessing binding data.

In summary, our pharmacological studies have revealed that carbachol and aceclidine showed some differences compared with pilocarpine in their binding characteristic on the muscarinic receptors and in their ability to stimulate the contraction of isolated rabbit iris muscle. Although both carbachol and aceclidine could interact with both high- and low-affinity [^{3}H]-QNB binding sites, the efficacy of carbachol and aceclidine to stimulate contraction of the iris muscle was consistent with receptor occupancy theory only when considering the low-affinity state of the muscarinic receptor and activation of the low-affinity rather than high-affinity binding state of the receptor is likely to mediate the contraction of iris muscle.

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