Characterization of Subtype of Propylbenzilylcholine Mustard (PrBCM)-Sensitive and -Resistant Muscarinic Cholinceptors in Guinea Pig Ileal Muscle

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ABSTRACT—The subtype of propylbenzilylcholine mustard (PrBCM)-sensitive and -resistant muscarinic cholinceptors in guinea pig ileal muscle was examined using four selective muscarinic antagonists, pirenzepine, AF-DX 116, himbacine and 4-DAMP. The pA2 values of the four antagonists against pilocarpine were not different from their values against carbachol after the treatment with PrBCM and was identified with the values for the m3 subtype. These results suggest that the subtype of PrBCM-sensitive and -resistant muscarinic cholinceptors in guinea pig ileal muscle is the m3 subtype only and not other subtypes.

Keywords: Propylbenzilylcholine mustard (PrBCM) receptor, Muscarinic m3 subtype

Muscarinic cholinceptors have been pharmacologically divided into three major subtypes, M1, M2 and M3 subtypes, by differences in affinities for the selective competitive antagonists (1–4). However, differing from these subclassifications based on findings obtained from functional studies using selective competitive antagonists, we have proposed that in the intestinal smooth muscles, there are two types of cholinceptor mechanisms, one being sensitive to and the other being resistant to propylbenzilylcholine mustard (PrBCM), which is known to be an irreversible specific muscarinic cholinergic antagonist. Cholinergic partial agonists (e.g., pilocarpine) produce contractions of the intestinal smooth muscle through activation of PrBCM-sensitive receptors, while full agonists (e.g., carbachol), which contract it through an interaction with both types, are more effective at PrBCM-resistant receptors than at PrBCM-sensitive receptors. Pilocarpine, a partial agonist, behaves as a competitive cholinergic antagonist on PrBCM-resistant receptors in the intestinal smooth muscles treated with PrBCM (5–7). Furthermore, PrBCM-sensitive cholinceptors were found to play a physiological role (6). However, these two types may also provide clues for finding unknown subtypes.

Recently, molecular cloning of muscarinic receptors has demonstrated the presence of five related but distinct gene products (m1, m2, m3, m4 and m5) (8, 9). Pharmacological studies can differentiate muscarinic receptors into three types (M1, M2, M3), and this pharmacological classification most likely corresponds to the m1, m2 and m3 receptors, respectively. More recently, Dörje et al. (10) have determined the affinity profiles of several of the selective muscarinic antagonists at five cloned human muscarinic receptors (m1–m5) stably expressed in Chinese hamster ovary cells (CHO-K1).

In this paper, we tried to characterize PrBCM-sensitive and -resistant muscarinic cholinceptor subtypes in guinea pig ileal muscle using four selective muscarinic antagonists: pirenzepine (m1[M1]-selective); AF-DX 116 (11-((2-(diethylamino)methyl-1-piperidinyl)acetyl)-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepine-6-one) (m2[M2]-selective); himbacine (m2[M2], m4-selective); and 4-DAMP (4-diphenylacetoxy-N-methylpiperidine methiodide) (m1[M1], m3[M3]-selective).

Male guinea pigs, weighing 250 to 350 g, were killed by a blow on the head. A longitudinal muscle strip was isolated by carefully slipping an ileal segment over a tapered glass rod. A piece (about 3 cm) of the strip was suspended in a 20-m1 organ bath filled with a physiological solution of the following composition: 154 mM NaCl, 5.6 mM KCl, 2.1 mM CaCl2, 0.8 mM MgCl2, 6.0 mM NaHCO3 and 2.8 mM glucose, kept at 37°C and
gassed with a mixture of 95% O₂ and 5% CO₂. Responses to agonists were isotonically recorded under a tension of 0.7 g. Concentration-response curves of agonists were obtained cumulatively.

To exclude the effect of muscarinic cholinceptors, after the control concentration-response curve for carbachol were determined, the preparation was treated with PrBCM (3 × 10⁻⁶ M) for 50 min according to the method of Hisayama et al. (11). When PrBCM was applied for 50 min, it was renewed every 10 min. The preparation was then allowed to re-equilibrate for 30 min with washing every 10 min. The curve for carbachol was again determined after thorough washing every 10 min.

To estimate the dissociation constants of four competitive antagonists, the pA₂ value (a negative logarithm of the dissociation constant) can be calculated by the method of Arunlakshana and Schild (12) as modified by Tallarida et al. (13). After determining the control concentration-response curves for carbachol after the treatment with PrBCM (response mediated through PrBCM-resistant muscarinic cholinceptors) and pilocarpine (response mediated through PrBCM-sensitive ones), the preparation was equilibrated with a competitive antagonist for 30 min. A concentration-response curve for agonist was then obtained in the presence of the antagonist, and the procedure repeated with a high (either 3-fold or 10-fold) concentration in the same preparation. The pA₂ value was calculated from parallel shifts of the curve for the agonist.

The data are expressed as means with S.E., and Duncan's new multiple range test was used to calculate statistical significance where appropriate. A P value less than 0.05 was considered as a significant difference. The following drugs were used: Carbachol chloride and pilocarpine hydrochloride (Sigma, St. Louis, MO); PrBCM (New England Nuclear, Boston, MA); AF-DX 116 and himbacine (Dr. Karl Thomae GmbH, Biberach, Germany); pirenzepine and 4-DAMP (Research Biochemicals, Inc., Natick, MA).

Table 1. The pA₂ values and slopes for four muscarinic antagonists

| Antagonists      | pA₂       | slope    | pA₂       | slope    |
|------------------|-----------|----------|-----------|----------|
| PrBCM-sensitive receptors (against pilocarpine) | PrBCM-resistant receptors (against carbachol after treatment with PrBCM) |
| Pirenzepine      | 6.56 ± 0.04 | 1.00 ± 0.12 | 6.49 ± 0.02 | 0.94 ± 0.01 |
| AF-DX 116        | 6.45 ± 0.04 | 1.03 ± 0.10 | 6.30 ± 0.06 | 0.90 ± 0.10 |
| Himbacine        | 7.02 ± 0.03 | 0.97 ± 0.01 | 7.00 ± 0.02 | 0.96 ± 0.01 |
| 4-DAMP           | 9.05 ± 0.03 | 0.96 ± 0.01 | 9.00 ± 0.04 | 0.95 ± 0.02 |

Mean ± S.E. of 6 experiments.
differentiated the two mechanisms, PrBCM-sensitive and -resistant ones, in the presence of guanine nucleotide (GTP), but in its absence, PrBCM did not discern the two. In other words, one mechanism is PrBCM-sensitive, irrespective of the presence or absence of GTP, and the other is PrBCM-resistant only when GTP is present (15). G-Protein-linked receptors and G-protein are considered to be present separately, and the receptors are activated by their binding with an agonist; the receptors and G-protein will associate to elicit a final response. However, it is now not known how GTP regulates PrBCM-sensitive and -resistant cholinoreceptors. Since the subtype of muscarinic cholinoreceptors in guinea pig ileal muscle is only the m3 subtype, PrBCM may distinguish between two states of m3 with and without GTP, although further studies are needed to clarify this point.

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