Anticholinergic Action of Disopyramide in Intestinal Smooth Muscle of the Guinea Pig: Inhibition of Muscarinic Receptors (M₁ and M₂)

Yukisato ISHIDA, Masami MIZUKAMI, Takashi TANIGUCHI, Nobuhiro SATAKE, Motohatsu FUJIWARA and Shoji SHIBATA

Department of Pharmacology, School of Medicine, University of Hawaii, Honolulu, Hawaii 96822, U.S.A.
1Department of Pharmacology, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan

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Abstract—Antimuscarinic actions of disopyramide were investigated by measuring the contractile responses of intestinal smooth muscles and ligand binding in cardiac and intestinal membrane preparations. Disopyramide caused a parallel shift of the dose-response curves for acetylcholine, McN-A-343, and carbachol to the right in the guinea pig taenia caeci; pA₂ values were 5.4 for acetylcholine, 5.5 for McN-A-343 and 5.9 for carbachol. In the guinea pig ileum, disopyramide competitively antagonized acetylcholine in the contractile responses, having the pA₂ value of 6.1. In microsomal fractions of the guinea pig taenia caecum and heart, disopyramide was capable of replacing 3H-QNB; Kᵢ values were 7X10⁻⁶ M for the taenia and 2X10⁻⁶ M for the heart. These results suggest that disopyramide exerts antimuscarinic action through M₁ and M₂ receptors with a potency approximately 3 times greater for M₂ than M₁.

Disopyramide, (4-diisopropylamino-2-phenyl)-2(2-pyridyl)butylamide, has been used as an antiarrhythmic drug for atrial and ventricular arrhythmias (1). Presumably, the anticholinergic action of this drug is related to its antiarrhythmic activity (2).

In the isolated smooth muscle preparation disopyramide has been reported to inhibit contractile responses to cholinergic agonists but not those to other agonists such as histamine, bradykinin, etc., indicating the selective antimuscarinic action of the drug (3). It was also reported that the drug in high concentrations, more than 10⁻⁵ M, has an antinicotinic action in skeletal muscle and ganglionic preparations (4). Recently, for acetylcholine receptors, five distinct receptor proteins have been recognized in animal and human tissues (5-7). On the other hand, pharmacologically, at least three subtypes of the receptors have been proposed: M₁, M₂ and M₃ (8-10). Furthermore, both McN-A-343 and carbachol, having higher affinity for M₁ and M₂ subtypes, respectively, are active in producing contractile responses in the guinea pig taenia caeci (11). However, no precise studies on the effect of disopyramide on the muscarinic receptors (M₁ and M₂) have been attempted. Therefore, in the present experiments, the relaxing effect of disopyramide on carbachol- and McN-A-343-induced contractile responses of the taenia was tested. Also, effects of the drug on 3H-quinuclidinyl benzilate binding in the taenia as well as guinea pig heart were investigated.

Materials and Methods

Mechanical response: Male guinea pigs (Hartley, 300–500 g) were stunned and bled. Taenia caeci were isolated. In part of the experiments, the ileum was isolated and a longitudinal ileal preparation (approximately 2–3 cm long) was made. The smooth muscle preparations were suspended in a physio-
logical salt solution of the following composition: 136.8 mM NaCl, 5.4 mM KCl, 2.5 mM CaCl$_2$, 1.0 mM MgCl$_2$, 11.9 mM NaHCO$_3$ and 5.6 mM glucose. The solution was kept at 37°C and bubbled with 95% O$_2$, 5% CO$_2$ (pH 7.1–7.2). The mechanical activity was recorded isotonically via a transducer (IT-203, Riken Kaihatsu, Tokyo, Japan) with a load of 0.5 g.

**Preparation of membranes:** Membranes from the longitudinal ileal preparation or heart were prepared as follows: the ileal preparation or heart was minced with scissors and then homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) with a glass homogenizer. The homogenates were filtrated through two layers of gauze and then homogenized at setting 10 on a Polytron (Brinkmann Instruments) with 20 second bursts. The homogenate was centrifuged at 1,000 x g for 10 min, and the supernatant was carefully removed and then centrifuged at 100,000 x g for 60 min. The resulting pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.4). Protein concentrations were determined by the method of Lowry et al. (12).

**Binding assay:** The $^3$H-QNB binding assay was performed by incubating aliquots of the homogenates at 37°C for 60 min in 250 µl of the 50 mM Tris-HCl buffer, containing $^3$H-QNB in the absence or presence of a high concentration of atropine (10 µM). The assay was terminated by the addition of 3 ml of ice-cold buffer and rapid filtration through Whatmann GF/B glass fiber filters under suction. After washing twice with 3 ml of the buffer, the filters were dried in an oven and transferred to counting vials; then 8 ml of scintillation fluid was added. Radioactivity was counted in a Packard Tri-Carb scintillation spectrometer (Model 3295). The $^3$H-QNB bound in the presence of 10 µM atropine was termed nonspecific binding and was subtracted from that obtained in the absence of 10 µM atropine (total binding) to obtain the specific binding.

**Materials:** The following drugs were used: carbachol (Sigma, St. Louis, MO, U.S.A.), histamine dihydrochloride (Woko-Junyaku, Tokyo, Japan) and atropine sulphate (Tokyo Kasei, Tokyo, Japan). McN-A-343, (4-hydroxy-2-butynyl)-trimethylammonium chloride, was purchased from Research Biochemicals, Inc., Natik, MA, U.S.A. Disopyramide phosphate was a gift from Roussel Medical Japan, Tokyo, Japan.

**Data analysis:** The data were presented as the mean±S.E. The values of pA$_2$ were calculated according to Arunlakshana and Schild (13). Data were analyzed by Student’s t-test, analysis of variance, Dunnett’s test and regression analysis.

**Results**

Application of 10$^{-6}$ M acetylcholine or 3×10$^{-6}$ M histamine produced nearly maximum contraction of the longitudinal smooth muscles of the guinea pig ileum. In the presence of acetylcholine (10$^{-6}$ M), addition of disopyramide (10$^{-7}$ to 10$^{-5}$ M) elicited a dose-dependent inhibition of the muscle (Fig. 1). Its ID$_{50}$ value was 1.04±0.18×10$^{-6}$ M (n=3). Disopyramide at 10$^{-5}$ M nearly abolished the acetylcholine-induced contraction. On the other hand, the contractile response to histamine was apparently not affected by disopyramide at less than 10$^{-6}$ M (Fig. 1). In the presence of disopyramide at 10$^{-5}$ M, the histamine-induced contraction...
was slightly attenuated to 84.5±3.6% (n=3) of the control. Sixty minutes after wash out of the drug, application of acetylcholine produced the same degree of contraction as that before treatment with disopyramide, indicating that the inhibitory effect of disopyramide is readily removed by rinsing the muscle with normal solution. These results suggest that disopyramide selectively interferes with the acetylcholine action at the muscarinic receptor of the ileum.

Figure 2 shows the inhibitory effect of disopyramide on the dose-response curve for acetylcholine in the ileal longitudinal smooth muscle. Acetylcholine dose-dependently produced a contraction with the magnitude of half the maximum response at 1.34±0.17×10⁻⁷ M and the maximum at 3×10⁻⁶ M. Increasing concentrations of disopyramide, 3×10⁻⁷, 10⁻⁶ to 3×10⁻⁵ M, gradually shifted the response curves to high concentrations of acetylcholine in a parallel manner, indicating that disopyramide competitively antagonizes the action of acetylcholine. From the Arunlakshana-Schild plot of the data (Fig. 2 inset), the value of the negative logarithm of the apparent affinity for competitive inhibition (pA₂) was estimated to be 6.09 with a slope of 1.37 for disopyramide (Table 1). The pA₂ value was not significantly different (P<0.05) from the ID₅₀ value for disopyramide in the presence of 10⁻⁶ M acetylcholine, described above.

At 3×10⁻⁶ M disopyramide, the maximum response to acetylcholine was also attenuated by 22.1±5.3% (n=4) (Fig. 2). The value of the negative logarithm of the affinity for noncompetitive inhibition (pD'₂) was 5.09±0.05 (n=4). The pD'₂ value was 10 times greater than the pA₂ value for disopyramide.

In the guinea pig taenia caeci, cumulative application of acetylcholine, McN-A-343 and carbachol elicited dose-dependent contractions (Figs. 3 and 4). Values for 50% of Fig. 2. Inhibitory effect of disopyramide on the dose-response curve for acetylcholine in the guinea pig longitudinal ileum. Disopyramide, 3×10⁻⁷ M (●), 10⁻⁶ M (□) or 3×10⁻⁵ M (■), was applied 15 min before the cumulative application of acetylcholine. ○ represents the control response to acetylcholine. 100% of contraction stands for the control response to 3×10⁻¹¹ M acetylcholine. Vertical bar on the symbol indicates the S.E.M. (n=4). Inset: Schild plot for the inhibitory effect of disopyramide from the dose-response experiments. Ordinate: logarithm of (acetylcholine dose ratio (X) in the presence and absence of each concentration of disopyramide -1). Abscissa: logarithm of the disopyramide concentration. The slope of the regression line was 1.37±0.24 (n=4).

Table 1. Arunlakshana-Schild analysis of the effects of disopyramide in the ileum and taenia caecum of the guinea pig

| Disopyramide  | Agonist      | pA₂       | slope       | n  |
|---------------|--------------|-----------|-------------|----|
| ileum         | acetylcholine| 6.09±0.11 | 1.37±0.24   | 4  |
| taenia        | acetylcholine| 5.43±0.04 | 0.96±0.08   | 4  |
|               | McN-A-343    | 5.60±0.06 | 1.21±0.06   | 4  |
|               | carbachol    | 5.91±0.08 | 1.10±0.08   | 8  |
| Atropine      |              |           |             |    |
| taenia        | carbachol    | 8.80±0.31 | 1.21±0.14   | 4  |

Values are expressed by the mean±S.E.M.
the effective concentration (ED50) were $1.43 \pm 0.10 \times 10^{-7}$ M ($n=4$) for acetylcholine, $2.50 \pm 0.46 \times 10^{-6}$ M ($n=4$) for McN-A-343 and $7.65 \pm 0.63 \times 10^{-8}$ M ($n=12$) for carbachol. Maximum contractile response to $3 \times 10^{-5}$ M McN-A-343 was $72.9 \pm 5.37\%$ ($n=4$) of that to $3 \times 10^{-6}$ M carbachol. These values are comparable with those reported by Gardner et al. (11). Pretreatment with disopyramide ($10^{-6}$, $3 \times 10^{-6}$, $10^{-6}$, $10^{-5}$ and $3 \times 10^{-5}$ M) gradually shifted the response curves for acetylcholine, McN-A-343 and
carbachol to the right in a parallel manner (Figs. 3 and 4), apparently indicating the competitive antagonism of the drug. The Arunlakshana-Schild plot of the data gave a straight line with a slope of near unity (Table 1). The pA2 value for disopyramide was significantly (P<0.05) smaller in the responses to acetylcholine and McN-A-343 (approximately 5.5) than that in the responses to carbachol (6.91) in the taenia.

Atropine also elicited the parallel shift of the dose-response curve for carbachol in the taenia (Fig. 4). The slope of the Arunlakshana-Schild plot was not significantly different from unity, and the pA2 value was 8.80 (Table 1). These values are consistent with those reported by Takayanagi et al. (14).

Effects of disopyramide as well as other cholinergic agents on the QNB-binding to

Table 2. Properties for 3H-QNB binding to microsomal fractions of the guinea pig taenia caecum and heart

|                | $K_d$ (pM) | $B_{max}$ (fmol/mg protein) | n |
|----------------|------------|----------------------------|---|
| Taenia caecum  | 489±89     | 558±36                     | 3 |
| Heart          | 783±121    | 232±19                     | 3 |

Values are expressed by the mean±S.E.M. $K_d$: dissociation constant for QNB. $B_{max}$: amount of maximum binding of QNB.

Fig. 5. Displacement of 3H-QNB binding by various drugs in the membrane preparation of the guinea pig taenia caeci.
Fig. 6. Displacement of $^3$H-QNB binding by various drugs in the membrane preparations of the guinea pig heart.

Table 3. Displacement of $^3$H-QNB binding by various drugs in the membrane preparations of guinea pig, taenia caecum and heart

|             | IC50        | Hill        | $K_i$        |
|-------------|-------------|-------------|--------------|
| Taenia caecum|             |             |              |
| Atropine    | 4.0±0.6×10^{-9} M | 1.02±0.03 | 1.4±0.2×10^{-9} M |
| Acetylcholine| 4.8±0.5×10^{-9} M | 0.36±0.07 | —             |
| Carbachol   | 1.0±0.1×10^{-9} M | 0.42±0.02 | —             |
| Disopyramide| 1.9±0.1×10^{-9} M | 0.94±0.04 | 7.0±0.4×10^{-6} M |
| Heart       |             |             |              |
| Atropine    | 4.9±0.2×10^{-9} M | 1.06±0.03 | 2.3±0.1×10^{-9} M |
| Acetylcholine| 2.0±0.2×10^{-9} M | 0.47±0.03 | —             |
| Carbachol   | 2.3±0.4×10^{-9} M | 0.37±0.02 | —             |
| Disopyramide| 4.9±0.6×10^{-9} M | 0.97±0.04 | 2.3±0.1×10^{-6} M |

Values are expressed by the mean±S.E.M. of 5 experiments. $K_i$ is calculated from the equation: $K_i=IC50/(1+L/K_d)$. L, concentration of QNB, 910 pM; $K_d$, dissociation constant for QNB, 489 pM for the taenia and 783 pM for the heart. IC50 is the concentration of drugs that reduces the specific $^3$H-QNB binding by 50%.

The inhibitory effect of acetylcholine. Using obtained IC50 values for each agent, the rank order of inhibitory potencies in competing against $^3$H-QNB binding was atropine ≫ acetylcholine ≥ carbachol ≥ disopyramide in both membrane preparations of the taenia and heart (Table 3). $K_i$ values for atropine in Table 3 (taenia caecum, 1.4 nM; heart, 2.3 nM) in both preparations are similar to those reported (ileal longitudinal muscle, 1.9 nM; heart, 2.8 nM) by Choo and Mitchelson (19). $K_i$ values for disopyramide is three times smaller in the heart than in the taenia ($P<0.001$).

**Discussion**

In the longitudinal muscle of the guinea pig ileum, the apparent affinity of disopyramide estimated in the present experiments was 8×10^{-7} M, which is 7 times smaller than that reported by Bains et al. (20). Later, using the longitudinal ileum, optical isomers
of R(+) - and S(+) -disopyramide was reported to competitively antagonize the acetylcholine-induced contraction with pA₂ values of 6.25-5.74 (21), being similar to the present value. The difference may be due to the different preparations employed, ileal longitudinal muscle in the present experiments and whole ileum in the previous study.

A high concentration (10⁻⁵ M) of disopyramide slightly attenuated the histamine-induced contraction of the longitudinal ileum. Disopyramide at 10⁻⁵ M also inhibited the dose-response curve for acetylcholine insurmountably. Therefore, it is suggested that disopyramide in low concentrations, less than 10⁻⁶ M, elicits more selective antimuscarinic action.

In the guinea pig taenia caeci, the pA₂ value of disopyramide was greater against carbachol than against acetylcholine and McN-A-343; i.e., the affinity of the drug to receptor was estimated to be approximately three times greater when carbachol was employed as an agonist. Furthermore, disopyramide was capable of replacing ³H-QNB from the receptor approximately three times more potently in the heart than the taenia. McN-A-343 and carbachol are known to more preferentially interact with M₁ and M₂ receptors, respectively (11). It has been proposed that M₂ receptors are more predominant in the cardiac muscle (22, 23). Therefore, it is suggested that disopyramide exerts its antimuscarinic action through M₁ and M₂ receptors, presumably with a greater potency against M₂ than against M₁ subtypes in the guinea pig taenia caeci.

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