**Anticholinergic Effects of Disopyramide and Quinidine on Guinea Pig Myocardium**

**Mediation by Direct Muscarinic Receptor Blockade**

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**SUMMARY** We studied the interaction of disopyramide, quinidine, and procainamide with cardiac muscarinic receptors. In electrophysiological experiments, the effects of disopyramide, quinidine, procainamide, and atropine were determined on spontaneously depolarizing guinea pig right atria (GPRA) both in the presence and absence of pharmacologically induced (physostigmine) cholinergic stimulation. All four agents demonstrated a concentration-dependent antagonism of the negative chronotropic effects of physostigmine. The order of anticholinergic potency was atropine >> disopyramide > quinidine >> procainamide. The ability of disopyramide to antagonize the physostigmine-induced slowing was stereoselective, (+)-disopyramide > (-)-disopyramide. In contrast, the ability of quinidine to antagonize the negative chronotropic effects of physostigmine was non-stereoselective, quinidine = quinine. In parallel experiments, we studied the ability of disopyramide, quinidine, procainamide, and atropine to compete with the radiolabeled muscarinic receptor antagonist [3H]quinuclidinyl benzilate ([3H]QNB) for binding to muscarinic receptors in crude homogenates of GPRA and membrane vesicles from canine ventricular myocardium. All four agents inhibited [3H]QNB binding to muscarinic receptors. The order of anticholinergic potency determined by the receptor binding studies was identical to that determined by the physiological studies. The interaction of disopyramide with muscarinic receptors was stereoselective, (+)-disopyramide > (-)-disopyramide. Quinidine was only slightly more potent than quinine in inhibiting [3H]QNB binding to muscarinic receptors. Interaction of antiarrhythmic drugs with muscarinic receptors satisfied criteria for a competitive interaction. The data from this study localize the anticholinergic effects of disopyramide and quinidine to the muscarinic receptor. *Circ Res* 47: 855-865, 1980

THE antiarrhythmic agents disopyramide, quinidine, and procainamide produce similar direct electrophysiological effects in cardiac tissues. Classically, in vitro analysis of the effects of these agents has been conducted in isolated preparations devoid of autonomic influence. Based on these electrophysiological studies, disopyramide, quinidine, and procainamide all produce depressant effects on isolated atria, Purkinje fibers, and ventricular muscle strips (Weidmann, 1955; Hoffman, 1958; Vaughan Williams, 1958; West and Amory, 1960; Sekiya and Vaughan Williams, 1963; Nye and Roberts, 1966; Rosen et al., 1973; Kus and Sasyshin, 1975; Danilo et al., 1977; Rosen and Hoffman, 1973). Although these three compounds produce similar electrophysiological effects on isolated tissue preparations, their actions in intact animals and humans appear more complex (Mokler and Van Arman, 1962; James and Nadeau, 1964; Danilo and Rosen, 1977; Josephson et al., 1974a; Corr et al., 1978; Wallace et al., 1974; Josephson et al., 1973; Birkhead and Vaughan Williams, 1977). Presumably, this is because disopyramide and quinidine possess prominent anticholinergic properties in addition to their myocardial depressant action. The effects of disopyramide and quinidine observed clinically may be the result of both "excitatory" (anticholinergic) and depressant properties. Depending on the underlying cholinergic tone, the excitatory effects may predominate over the depressant effects. Thus, in spite of the direct depressant action of these drugs on sino-atrial pacemaker cells, disopyramide and quinidine actually may accelerate heart rate in intact animals and humans (Wallace et al., 1974; Josephson et al., 1973; Birkhead and Vaughan Williams, 1977).
Electrophysiological Experiments

Results of these studies suggest that the cardiac muscarinic receptors were examined directly using receptor binding assays. The electrophysiological effects of these agents were assessed in an isolated guinea pig right atrial preparation which could be modified pharmacologically to simulate the presence of cholinergic tone. The results of these studies suggest that the cardiac anticholinergic effects of disopyramide and quinidine are due to direct interaction of these compounds with muscarinic cholinergic receptors.

Methods

Electrophysiological Experiments

Guinea pigs of either sex, weighing 400-600 g, were injected intraperitoneally with heparin sulfate (500 U) 30 minutes prior to use. Each guinea pig was stunned with a blow to the head, after which the heart was removed rapidly and placed in cool oxygenated Tyrode’s solution. Unless stated otherwise, the right atrium, including the area of the sinus node, was excised and pinned to the floor of a wax-bottomed lucite muscle chamber (7-ml volume) which was superfused constantly with Tyrode’s solution that was gassed with 95% O2-5% CO2 and maintained at a temperature of 37 ± 0.5°C. The composition of the Tyrode’s solution was (mM): Na+, 138; K+, 4.0; Cl-, 128; Ca2+, 1.25; HCO3-, 20.0; H2PO4-, 0.9; Mg2+, 0.5; and glucose, 5.5. The osmolarity of this solution was 285 mOsm/l and the pH was 7.40 ± 0.05.

Conventional microelectrode techniques were used (Draper and Weidmann, 1951). Data were displayed on a Tektronix 5100 series oscilloscope, photographed with a Tektronix C-59 Polaroid oscilloscope camera and stored on an eight-channel Honeywell 7600 tape recorder. An action potential-triggered tachometer (MECA CLC-1) was used to record the rate of spontaneous electrical discharge of the right atria. The tachometer produced a linear ramp that was triggered by the upstream of each successive action potential. The tachometer was calibrated by stimulating the preparation at known constant basic cycle lengths and measuring the height of the ramp. Tissues were superfused in the muscle chamber for at least 15 minutes (while recording control spontaneous cycle length) prior to any experimental intervention. Two types of studies of automaticity in guinea pig right atria were performed. First, the direct depressant actions of disopyramide, quinidine, and procainamide were studied. For each agent, concentration-related slowing of atrial rate was quantified after 5 minutes of drug superfusion. In a second series of experiments, the effects of these agents on spontaneous atrial rate were analyzed in the presence of cholinergic stimulation. In these experiments, the cholinesterase inhibitor physostigmine was used to block degradation of endogenously released acetylcholine. Five minutes of superfusion with 10^-6 M physostigmine was chosen to simulate the presence of cholinergic tone. This method of activating muscarinic receptors elicited the most reproducible slowing of spontaneous atrial rate. This physostigmine-induced rate slowing was blocked by atropine (see Results, Fig. 3). Physostigmine (10^-6 M) was administered simultaneously with varying concentrations of atropine or antiarrhythmic drug and the slowing of atrial rate was assessed after 5 minutes of exposure to drug. A single right atrium was used for each experiment and 5 to 8 experiments were performed at each atropine and antiarrhythmic drug concentration. Experiments were also performed with (+)disopyramide, (-)disopyramide, and quinidine (1 optical isomer of quinidine) to determine the stereospecificity of the anticholinergic effects of these compounds. Experiments also were conducted with each optical isomer in the absence of physostigmine to analyze the direct negative chronotropic effects of these agents.

Membrane Preparations

Guinea pig right atria were superfused with Tyrode’s solution using techniques identical to those employed in the electrophysiological studies. Following 20 minutes of superfusion in Tyrode’s solution, each atrium was frozen rapidly in liquid nitrogen and subsequently pulverized with a pre-cooled mortar and pestle. A crude homogenate of guinea pig right atrium was prepared by homogenizing a suspension of 15 pulverized right atria 3 times for 15 seconds in medium of 0.25 M sucrose, 30 mM histidine using a Polytron PT-10 (Brinkman Instruments) at a setting of half maximal speed. Aliquots of this homogenate were stored frozen at −20°C until used.

To support the analysis of drug interactions with muscarinic receptors in guinea pig atria, a cardiac membrane vesicle fraction also was isolated from homogenates of canine ventricular myocardium as described previously (Jones et al., 1979). This preparation, which has been characterized extensively, is known to contain significant quantities of membrane vesicles derived from sarcolemma. Results of all binding studies performed in guinea pig right atrial homogenates were confirmed in this canine membrane vesicle preparation. These supplemental studies were undertaken to establish that the interaction of antiarrhythmic agents with muscarinic receptors was not species specific, and occurred in ventricular as well as atrial tissue. In addition, the specific nature of the interaction between antiarrhythmic agents and [3H]QNB at the level of the muscarinic receptor was examined in this membrane vesicle fraction. Protein determinations were performed by the method of Lowry (1951).

Receptor Binding Assays

The assay procedure for studying muscarinic cholinergic receptors was a modification of the
method originally described by Yamamura and Snyder (1974). Unless otherwise noted, homogenate (100–200 μg protein) was incubated in 1–5 ml of medium containing 0.05 M sodium/potassium phosphate buffer (pH 7.4), 80 μM [3H]quinuclidinyl benzilate ([3H]QNB), and relevant drugs. Incubations were for 60 minutes at 37°C, which allowed complete equilibrium of binding. Specific binding was measured as that radioactivity displaceable by 1 μM atropine. Bound [3H]QNB was separated from free [3H]QNB by filtration through Whatman GF/C filters.

The [3H]QNB binding assay was used to examine directly the interaction of antiarrhythmic agents with muscarinic receptors. A series of [3H]QNB competition curves were generated with antiarrhythmic drugs as well as conventional muscarinic antagonists. Binding studies were performed in homogenates of guinea pig right atria, allowing direct comparison of binding data with results of electrophysiological studies. To strengthen the analysis of drug-receptor interaction, all results obtained for crude homogenates were confirmed with a well-characterized sarcolemmal-enriched membrane vesicle fraction isolated from canine ventricle.

Beta-adrenergic receptors were assessed directly with [3H]dihydroalprenolol ([3H]DHA) as previously reported (Jones et al., 1979; Watanabe et al., 1978). Briefly, cardiac membrane vesicles (60–100 μg protein) were incubated for 10 minutes at 37°C in 200 μl of medium containing Tris (50 mM), MgCl₂ (9 mM), [3H]DHA (14 nM), and relevant drugs. Specific binding was measured as that radioactivity displaceable by 2 × 10⁻⁵ M dl-propranolol. Bound [3H]DHA was separated from free [3H]DHA by filtration through Whatman GF/C filters. All assays for both receptors were performed in triplicate. Radioactivity was counted on a liquid scintillation counter (Beckman Instruments) at a counting efficiency of 34%.

**Drugs**

Atropine sulfate, physostigmine sulfate, quinidine sulfate, quinine sulfate, and (−)scopolamine sulfate were purchased from Sigma Chemical Company. Procainamide HCl was purchased from E.R. Squibb and Sons, Inc. Racemic disopyramide, (+)-disopyramide, and (−)-disopyramide were gifts from G.D. Searle Company and Roussel Laboratories. Unlabeled and tritiated (±)quinuclidinyl benzilate (QNB) were obtained from New England Nuclear.

**Analysis of Data**

Analysis of electrophysiological data was designed to allow quantitative correlation with results of direct receptor binding studies. In the electrophysiological model, concentration-dependent drug effects were analyzed as follows: The mean slowing of spontaneous rate observed with 10⁻⁴ M physostigmine was arbitrarily designated as 100% cholinergic response. Attenuation of this cholinergic response was observed in the presence of antiarrhythmic agents, as well as muscarinic antagonists. Fractional physostigmine-induced slowing of rate seen in the presence of these agents was expressed as a percent of the cholinergic response observed with physostigmine alone. The concentration of antiarrhythmic drug or muscarinic antagonist that effected a 50% inhibition of the cholinergic response was defined as the EC₅₀ for each drug. The analysis of binding data was performed by the method described by Cheng and Prusoff (1974).

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K_i = (IC_{50A})/(1 + ([Q]/K_{app}))
\]

where \( K_i \) is the inhibition constant for drug A, IC₅₀, the concentration of drug A that inhibits specific [3H]QNB binding by 50%, [Q] the concentration of [3H]QNB, and Kₐₚp, the apparent dissociation constant for [3H]QNB defined by Scatchard analysis (30 pm).

**Results**

**Effects of Disopyramide, Quinidine, and Procainamide on Spontaneous Rate of Guinea Pig Atria**

Disopyramide, quinidine, and procainamide produced similar direct membrane effects. All three antiarrhythmic agents depressed atrial automaticity. Spontaneous atrial rate was reduced after 5 minutes of superfusion with 7 × 10⁻⁶ M, 1 × 10⁻⁵ M, and 1 × 10⁻⁴ M (±)disopyramide (Fig. 1, panel A). The direct atrial rate slowing effects of disopyramide appeared to be stereospecific. The (+)-stereoisomer appeared to produce greater slowing of atrial rate than the (−)-stereoisomer (Fig. 1, panel A). Quinidine also reduced spontaneous atrial rate when included in superfusate for 5 minutes at concentrations of 10⁻⁵ and 10⁻⁴ M (Fig. 1, panel B). However, this effect on atrial automaticity did not appear to be stereospecific: quinine appeared to produce similar rate slowing effects, as did quinidine (Fig. 1, panel B). Procainamide administered alone to isolated atria at concentrations of 10⁻⁵ M and 10⁻⁴ M also slowed atrial rate (Fig. 1, panel C). Thus, all these antiarrhythmic drugs depressed spontaneous automaticity of guinea pig right atria, this effect becoming apparent with concentrations in the range of 10⁻⁶ to 10⁻⁵ M.

**Effect of Physostigmine on Spontaneous Rate of Guinea Pig Right Atria**

Physostigmine was used in this study to produce cholinergic stimulation. During cholinesterase inhibition, muscarinic receptors in guinea pig atria were activated by endogenously released acetylcholine. In the presence of this cholinesterase inhibitor, a concentration-dependent reduction in spontaneous right atrial rate was observed (Fig. 2). Removal of the sinus node region did not alter the negative chronotropic effects of physostigmine. Be-
ORUG CONCENTRATION (M)

FIGURE 1 Effect of disopyramide, quinidine, and procainamide on spontaneous rate of guinea pig right atria. Values are means ± SE for 5-10 hearts after 5 minutes of drug superfusion. Panel A: (+)disopyramide (△), (±)disopyramide (●), (−)disopyramide (○). Panel B: quinidine (●), quinine (○). Panel C: procainamide (□). For each curve, control atrial rate (beats/min, expressed as a mean ± SE for the number of atria shown in parentheses) was: (+)disopyramide 232 ± 9.6 (n = 24); (±)disopyramide 233 ± 7.3 (n = 35); (−)disopyramide 204 ± 6.4 (n = 28); quinidine 207 ± 6.2 (n = 40); quinine 244 ± 12.8 (n = 23); procainamide 221 ± 7.1 (n = 38).

cause physostigmine (10⁻⁶ M) produced consistent and reproducible reductions in spontaneous atrial rate in this preparation, superfusion with this concentration of physostigmine was used as the method of simulating cholinergic stimulation in vitro. As shown in Figure 2, 10⁻⁶ M physostigmine superfused alone for 5 minutes slowed spontaneous atrial rate by 28% (sinus node intact). For the purpose of subsequent data analysis, this rate slowing effect of

10⁻⁶ M physostigmine was designated as the 100% cholinergic effect induced by physostigmine. All subsequent experiments with muscarinic antagonists were performed on atria (sinus node intact) in the presence of 10⁻⁶ M physostigmine.

Effects of Atropine, Disopyramide, Quinidine, and Procainamide on the Spontaneous Rate of Guinea Pig Right Atria in the Presence of Cholinergic Stimulation

Atropine, the prototypic muscarinic antagonist, produced a concentration-dependent antagonism of the negative chronotropic effects of physostigmine (Fig. 3). The EC₅₀ for this effect was 3 × 10⁻⁹ M. At concentrations of 10⁻⁶ and 10⁻⁵ M, atropine completely abolished the negative chronotropic effect of physostigmine. These results verified that the negative chronotropic effects of this cholinesterase inhibitor were mediated by endogenously released acetylcholine which stimulated atrial muscarinic receptors to slow spontaneous rate.

The muscarinic receptor blocking effects of disopyramide, quinidine, and procainamide were examined using the same experimental protocol outlined for atropine. Disopyramide induced a concentration-dependent antagonism of the maximal negative chronotropic effects of physostigmine (Fig. 4, panel A). The threshold for the anticholinergic effects of this compound appeared to be between 10⁻⁹ and 10⁻⁸ M, about 1000 times less than the minimum concentration required to slow directly spontaneous rate. Further, this effect appeared to be stereospecific. The concentration at which both (±) and
Figure 3  Antagonism of the maximal negative chronotropic effects of physostigmine by atropine. Values are means of 6–8 hearts expressed as a percent of the maximal rate slowing response observed with physostigmine (10⁻⁶ M) alone. SE < 4% at all points. Control atrial rate was 204 ± 6.7 beats/min (expressed as mean ± SE for 60 atria).

(−)disopyramide antagonized by 50% the maximal effect of phystostigmine was 3 × 10⁻⁷ M, whereas 3 × 10⁻⁸ M (+)disopyramide produced a similar degree of anticholinergic effect. Quinidine also produced a concentration-dependent antagonism of the maximal negative chronotropic effects of phystostigmine (Fig. 4, panel B). This effect of quinidine was non-stereospecific. Identical concentrations of quinine exerted the same anticholinergic effects. In contrast to the results observed with disopyramide and quinidine, procainamide did not significantly antagonize the maximal negative chronotropic effects induced by phystostigmine. As shown in Figure 4, panel C, even 10⁻⁴ M and 10⁻³ M procainamide administered for 5 minutes produced only a 20% antagonism of the maximal effect of phystostigmine.

In these electrophysiological experiments, disopyramide and quinidine produced significant muscarinic receptor blockade at concentrations which are thought to occur in the clinical setting (Niarchos, 1976; Sokolow and Edgar, 1950; Winkle et al., 1975). However, procainamide did not exert anticholinergic effects except at concentrations that would be considered to be toxic in patients (Winkle et al., 1975; Koch-Weser, 1977).

Effects of Atropine, Disopyramide, Quinidine, and Procainamide on [³H]Quinuclidinyl Benzilate Binding to Guinea Pig Right Atria and Canine Ventricular Myocardium

The [³H]QNB assay of muscarinic cholinergic receptors has been characterized in the guinea pig atrial homogenates and the canine ventricular myocardial preparation (Fig. 5). Radioligand affinity, as well as inhibition constants for a series of muscarinic agents, were virtually identical in the two preparations. All of the accepted criteria for establishing true ligand-receptor interaction were met,
and the data obtained corresponded with those reported by others (Yamamura and Snyder, 1974; Fields et al., 1978). The binding sites were saturable, and Scatchard analysis confirmed high affinity binding of \([^3H]QNB\) to a homogeneous population of receptors. Binding was displaceable by known muscarinic agonists and antagonists in accordance with their known pharmacological potencies. Stereospecificity of interaction of receptors with the stereoisomers of the potent muscarinic antagonist benzetimide also was confirmed.

Disopyramide and quinidine also inhibited \([^3H]QNB\) binding to atrial muscarinic receptors. In contrast, procainamide did not inhibit \([^3H]QNB\) binding to atrial muscarinic receptors until concentrations of \(10^{-4}\) M and \(10^{-3}\) M (Fig. 6). For purposes of comparison, atropine inhibition of \([^3H]QNB\) binding is also shown in Figure 6. As expected for pure antagonists, Hill coefficients calculated from \([^3H]QNB\) competition curves were approximately 1.0 for all agents studied.

The stereospecificity of the binding of disopyramide to cardiac muscarinic receptors is shown in Figure 7. In Panel A, (+)disopyramide was approximately three times as potent as (−)disopyramide in competing with \([^3H]QNB\) for binding to atrial muscarinic receptors in a crude homogenate of guinea pig right atria. Results were virtually identical in a membrane vesicle preparation obtained from canine ventricular myocardium (panel B).

The stereospecificity of muscarinic receptor interaction also was examined for quinidine and quinine, optical isomers of a cinchona alkaloid. Quinidine (Kᵢ 2.8 ± 0.2 μM) was only slightly more potent than quinine (Kᵢ 4.2 ± 1.1 μM) in competing with \([^3H]QNB\) for binding to muscarinic receptors, both in guinea pig atria (Fig. 7C) and canine membrane vesicles (Fig. 7D).

Further analysis of the nature of the interaction between antiarrhythmic agents and muscarinic receptors was pursued in the canine membrane vesicle fraction, a receptor-enriched preparation less contaminated by potentially interfering non-receptor material. Scatchard analysis of \([^3H]QNB\) saturation curves, generated in the absence and presence of disopyramide (Fig. 8A), quinidine (Fig. 8B), and procainamide (Fig. 8C), demonstrated identical total receptor number. Thus, \([^3H]QNB\) binding in the presence of these agents could not be accounted for by reduction in total receptor number. For all three
antiarrhythmic agents, inhibition constants ($K_I$) derived from Scatchard plots (Fig. 8) were in close agreement with those calculated from $[^3]H$QNB competition curves (Fig. 6). Inhibition constants ($K_I$) calculated from Figure 8 were (±)disopyramide $7.0 \times 10^{-7}$ M, quinidine $2.7 \times 10^{-6}$ M, and procainamide $5.2 \times 10^{-5}$ M. Thus, the results of this binding analysis are consistent with simple competition between antiarrhythmic agents and $[^3]H$QNB for binding to muscarinic receptors.


To evaluate further possible nonspecific effects of the antiarrhythmic agents on $[^3]H$QNB binding which might have fortuitously simulated a competitive drug-receptor interaction, binding to another membrane-bound receptor was investigated. $[^3]H$ DHA binding to $\beta$-adrenergic receptors was unaltered in the presence of antiarrhythmic drugs at concentrations ($10^{-4}$ M) which significantly inhibited $[^3]H$QNB binding to muscarinic receptors.

Correlation of Anticholinergic Potencies Determined by Electrophysiological Studies and Receptor Binding Studies

Because the concentration of acetylcholine at atrial muscarinic receptor sites could not be quan-
Figure 8 Scatchard analysis of \(^{3}H\)QNB saturation curves performed in the presence and absence of antiarrhythmic drug in membrane vesicles from canine ventricle. Panel A: \(^{3}H\)QNB alone (■), \(^{3}H\)QNB binding in the presence of (±)disopyramide \((1 \times 10^{-6} \text{ M})\) (○). Panel B: \(^{3}H\)QNB alone (■), \(^{3}H\)QNB binding in the presence of quinidine \((1 \times 10^{-5} \text{ M})\) (○). Panel C: \(^{3}H\)QNB alone (■), \(^{3}H\)QNB binding in the presence of procainamide \((2 \times 10^{-4} \text{ M})\) (□).

tified, calculation of a \(K_i\) from electrophysiological data could not be performed. Therefore, the EC\(_{50}\) was taken as an approximation of the anticholinergic potency determined physiologically. Correlation of electrophysiological EC\(_{50}\) with \(K_i\) derived from receptor binding studies is presented in Figure 9. Excellent correlation between the anticholinergic potency determined electrophysiologically and by receptor binding was observed for the antiarrhythmic agents as well as classic muscarinic antagonists.

**Discussion**

Disopyramide and quinidine possess important anticholinergic properties that contribute to their cardiovascular effects as well as to their effects on other organ systems. Mokler and Van Arman (1974) showed that disopyramide abolished the effect of vagal stimulation to slow atrial rate in anesthetized dogs. These investigators also observed that disopyramide blocked the negative chronotropic effects of acetylcholine in an isolated perfused rabbit heart. The vagolytic effects of disopyramide on sinoatrial (SA) nodal automaticity and atrioventricular (AV) nodal refractoriness have been documented in humans (Josephson, 1973; Birkhead and Vaughan Williams, 1977). In addition to these important cardiac effects, disopyramide causes significant side effects due to anticholinergic actions on organs other than the heart. These actions are the cause of the majority of side effects observed with disopyramide and frequently necessitate discontinuation of therapy with this drug (Danilo and Rosen, 1977; Niarchos, 1976; Peterson, 1955). Quinidine also has long been recognized to produce anticholinergic effects on cardiac tissues. James and Nadeau (1964) demonstrated that perfusion of canine sinoatrial node with quinidine prevented the sinus rate slowing effects of acetylcholine. Recently, clinical and experimental electrophysiological studies have documented important anticholinergic effects of quinidine on AV nodal refractoriness (Josephson et al., 1974a; Wallace et al., 1974). In humans, these anticholinergic effects are responsible for the enhancement of AV nodal conduction, which presents a problem in the management of supraventricular tachyarrhythmias (Josephson et al., 1974a). Thus, the anticholinergic properties of disopyramide and quinidine are manifested as significant cardiac and extracardiac side effects, thereby emphasizing the clinical importance of these anticholinergic properties. In intact animals, procainamide in high doses also has been shown to produce vagolytic effects (Corr et al., 1978; Peterson, 1955). However, electrophysiological studies have demonstrated variable evidence of these parasympatholytic effects on AV nodal refractoriness in man (Josephson et al., 1974b).

Results of the present investigation suggest that the anticholinergic properties of disopyramide and quinidine can be accounted for by a direct interaction of these agents with muscarinic receptors. That is, disopyramide and quinidine, although less potent, appear to possess muscarinic receptor-blocking properties similar to those of atropine. Both disopyramide and quinidine produced concentration dependent inhibition of \(^{3}H\)QNB binding to muscarinic receptors in guinea pig right atria. Procainamide was much less effective in inhibiting \(^{3}H\)QNB binding. To support these findings, experiments also were conducted in a more purified receptor-containing preparation isolated from canine ventricular myocardium. In this ventricular preparation, each antiarrhythmic agent inhibited \(^{3}H\)QNB binding with a potency identical to that seen in the atrial preparation.
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Several lines of evidence suggest that the inhibition of \[^3H\]QNB binding by the antiarrhythmic agents resulted from competition between the radiolabeled ligand and the added drug for binding to muscarinic receptors. Results of the receptor binding studies were consistent with a simple model of competitive drug-receptor interaction. Neither receptor number nor receptor affinity for \[^3H\]QNB was altered by the antiarrhythmic agents. Second, the lack of significant inhibition of \[^3H\]DHA binding to \(\beta\)-adrenergic receptors excluded a generalized derangement of membrane-localized receptors to account for the apparent muscarinic antagonism by these drugs. Finally, disopyramide and quinidine inhibited \[^3H\]QNB binding to muscarinic receptors at concentrations two to three orders of magnitude less than that required to decrease spontaneous automaticity, an action of these drugs presumably due to their direct membrane effects. Despite these lines of evidence suggesting simple competition, it is not possible to exclude unequivocally an allosteric interaction which quantitatively satisfies criteria for a competitive interaction. Inhibition of \[^3H\]QNB binding by antiarrhythmic agents has been noted previously. In homogenates of rabbit heart, Fields et al., (1978) identified concentration-related inhibition of \[^3H\]QNB binding by quinidine and procainamide. However, effects of disopyramide on \[^3H\]QNB binding have not been reported previously.

The present results suggest that the phenomena observed in the binding experiments were relevant to the results of the electrophysiological studies, and may indeed explain at the subcellular level the mechanism for the anticholinergic effects of disopyramide and quinidine. The (+)isomer of disopyramide was the most potent in blocking the rate slowing effect of physostigmine. Similarly, the (+)isomer was more potent than the (-)isomer in competing with \[^3H\]QNB for binding to the receptor. By contrast, the optical isomers quinidine and quinine were equipotent in the electrophysiological studies. Similarly, binding studies demonstrated only a slight difference in potency of quinidine and quinine in inhibiting \[^3H\]QNB binding in both the atrial and ventricular preparations. Finally, the anticholinergic potency of the antiarrhythmic agents (as assessed by \(EC_{50}\) estimates) correlated very well with their ability to inhibit \[^3H\]QNB binding as expressed by the \(K_i\). The established anticholinergic agents atropine, scopolamine, and QNB fit very well on the line describing the relationship between the electrophysiological and binding parameters for the antiarrhythmic agents. The excellent correlation between results of physiological and direct binding studies suggests that the anticholinergic properties of disopyramide and quinidine can be accounted for by direct interaction of these antiarrhythmic agents with muscarinic cholinergic receptors.

The actions of antiarrhythmic agents to produce alterations in spontaneous atrial rate are complex.
and multiple. Even in the isolated preparation used, two distinct effects were observed to alter spontaneous atrial rate. At concentrations of $10^{-8}$ M or greater, disopyramide and quinidine exerted effects that attenuated cholinergic-induced atrial rate slowing. At concentrations of $10^{-3}$ M or greater, the previously reported direct negative chronotropic effects of disopyramide, quinidine, and procainamide were observed (West and Amory, 1960; Sekiya and Vaughan Williams, 1963; James and Nadeau, 1964). These direct negative chronotropic effects were apparent both in the presence and absence of cholinergic stimulation. It should be noted that disopyramide and quinidine were much more potent as anticholinergic agents (these effects occurred over a concentration of $10^{-8}$ to $10^{-6}$ M) than as direct membrane active agents. In fact, the concentrations required to produce anticholinergic effects were well within the range thought to be achievable in plasma of patients treated with these drugs (Niarchos, 1976; Sokolow and Edgar, 1950; Winkle et al., 1975). However, procainamide did not exert significant anticholinergic effects except at concentrations which would be considered toxic in humans (Winkle et al., 1975; Koch-Weser, 1977).

A discussion of the limitations of the electrophysiological model used in this study is in order. Pilot studies demonstrated that the efficacy of exogenous acetylcholine could be augmented substantially by the simultaneous inhibition of cholinesterase. Further, it was observed that physostigmine alone produced pharmacological effects typical of muscarinic receptor stimulation. These two types of experiments demonstrated that significant quantities of endogenous acetylcholine were released and that this released acetylcholine activated muscarinic receptors and elicited physiological responses. In addition, it was observed that the tissue response was more consistent and reproducible when cholinesterase was inhibited than when exogenous acetylcholine alone was added. Therefore, the administration of physostigmine was adopted as the method for activating muscarinic receptors. A problem with this preparation is that the concentration of acetylcholine at the receptor level is unknown. Thus, a $K_i$ for inhibition of a cholinergic response could not be determined, and the anticholinergic efficacy of the antiarrhythmic drugs could be expressed only in terms of $EC_{50}$. Nevertheless, this estimate of drug receptor interaction correlated very well with the $K_i$ as determined by binding studies.

Another limitation with the physiological preparation stems from the fact that the antiarrhythmic agents exerted two distinct effects to produce alterations in spontaneous atrial rate. The presence of anticholinergic and direct atrial rate slowing effects may explain partially the contour of the concentration-response curves that suggested a complex drug receptor interaction. Hill coefficients derived from $[^3H]QNB$ competition curves for the antiarrhythmic agents were approximately 1.0, consistent with expectations for simple antagonist interaction with receptors. However, the Hill coefficients estimated from electrophysiological experiments for each antiarrhythmic drug were 0.5 or less, suggesting a complex interaction of these drugs with the isolated atrial tissues studied. Notably, the concentration-response curve obtained with atropine demonstrated similar shallow contour, corroborating the complex interaction of muscarinic antagonists in this preparation. Despite these limitations in the physiological preparations, there was a high degree of correlation between the anticholinergic potency of the antiarrhythmic drugs (as assessed by the $EC_{50}$) and the ability of these compounds to compete with $[^3H]QNB$ for binding to muscarinic receptors.

Clinical studies have documented previously the important cardiac and extracardiac anticholinergic effects of disopyramide and quinidine (Josephson et al., 1973, 1974a; Birkhead and Vaughan Williams, 1977; Niarchos, 1976). The present in vitro study establishes the direct interaction of disopyramide and quinidine with cardiac muscarinic receptors. The interaction of disopyramide and quinidine with muscarinic receptors occurs at concentrations achievable in humans. Although the effects of pharmacologic cholinergic stimulation in vitro should not be equated directly with effects of vagal stimulation in vivo, the results of the present investigation suggest a plausible subcellular mechanism by which these two antiarrhythmic drugs produce clinically important anticholinergic effects.

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