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Antimuscarinic action of quinidine on the heart? A study in myocardial preparations from cat hearts

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1 Quinidine exerts anticholinergic effects which have been ascribed to atropine-like properties of the drug. We have examined the effects of acetylcholine on the force of contraction in isolated heart muscle preparations from cats and compared the inhibitory effects of atropine with those of quinidine.

2 The effects of acetylcholine were antagonized competitively in the presence of atropine. The Schild-plot yielded a straight line; the slope was not significantly different from unity.

3 In the presence of quinidine, the concentration-response curve of acetylcholine was shifted to the right as with atropine, however, the Schild-plot yielded a regression line which was not linear; the slope was statistically different from unity.

4 The negative inotropic response to acetylcholine in cat ventricular heart muscle (revealed in the presence of the phosphodiesterase inhibitor, papaverine) was antagonized by atropine but not influenced by quinidine.

5 We conclude that the inhibitory action of quinidine on the effects of acetylcholine in atrial heart muscle is not merely antimuscarinic. The antagonistic effects of acetylcholine and quinidine on atrial heart muscle may also be due to the opposite effects of the drugs on potassium conductance of the myocardial cell membrane.

Introduction

Quinidine has long been recognized to antagonize the effect of vagus stimulation on conduction at the AV node (Lewis, Drury, Iliescu & Wedd, 1921). In his note on the reversal of vagal action by quinidine, as seen in the heart of the cat, Dale (1921) emphasized that the partial or complete paralysis of vagal action is not due to an atropine-like action but rather to an effect of the drug on the vagus path, as also seen during electrical stimulation of the nerve (Dale, Laidlaw & Symons, 1910).

Since then, numerous reports have appeared which are in agreement with the original observations that quinidine has anticholinergic properties (Nathanson, 1934; Hiatt, Brown, Quinn & MacDuffie, 1945; James & Nadeau, 1964; Wallace, Cline, Sealy, Young & Troyer, 1966; Josephson, Seides, Battsford, Weisfogel, Akhtar, Caracta, Lau & Damato, 1974; Mason, Winkle, Rider, Stinson & Harrison, 1977; Pérez, Ledea, Hermandez & Garcia-Barreto, 1977; Benfey, Yong, Belleau & Melchiorre, 1979). However, the mechanism by which the paralytic effect is produced by quinidine remained unclear. Fields, Roeske, Morkin & Yamamura (1978) and Mirro, Manalan, Bailey & Watanabe (1980) reported that quinidine inhibited [^{3}H]-quinuclidinyl benzilate (QNB) binding in heart muscle homogenates of various species. Since there was a significant correlation between the anticholinergic potencies determined by receptor binding studies and electrophysiological studies, the latter authors localized the anticholinergic effects of quinidine to postsynaptic muscarinic receptors. The purpose of the present study was to evaluate the effects of quinidine on the physiological response of the heart mediated by muscarinic receptors. The action of quinidine was assessed by evaluation of the effects of the drug on the force of contraction in atrial and ventricular heart muscle preparations from cats. The decrease in atrial tension by cholinergic stimulation has been described as the most sensitive and consistent parameter of postsynaptic muscarinic receptor activation in the rabbit heart (Fozard & Muscholl, 1972).

The classical evaluation for competitive antagonism according to Schild (1949) and Arunlakshana & Schild (1959) revealed significant differences be-

tween the action of atropine and quinidine. The results of the present study suggest that the cardiac anticholinergic effects of quinidine are complex and that they may be partially ascribed to a direct interaction of acetylcholine and quinidine at the myocardial cell membrane.

Methods

Hearts of young cats (either sex; body weight 800–1500 g) were rapidly excised under ether anaesthesia and immediately rinsed in warm oxygenated Tyrode solution. Both atria were dissected from the heart and cut in half to produce four preparations. The region of the sinus node was cut off and no spontaneous contractions were observed. In some experiments, right ventricular trabeculae or papillary muscles (diameter 0.4–0.9 mm, length 3.5–8.5 mm) were isolated by ligating both ends with a fine silk suture and dissecting them from the heart. The preparations were attached to the hook of a muscle holder and positioned next to two platinum electrodes built in the muscle holder. The preparations were then placed in organ baths containing 5 ml Tyrode solution and connected via steel wires to an inductive force displacement transducer which was fed by a carrier frequency of 5 kHz. The transducer output was fed to a Hellige frequency preamplifier, converted to d.c. signals and recorded on a Hellige pen recorder. The preparations were driven electrically by square wave pulses (Grass stimulator S4; 1 ms duration; 10–20% above threshold) at 1 Hz. Twitch responses were recorded under isometric conditions at the apex of the preload active tension curve.

The Tyrode solution was prepared with distilled deionized water and had the following composition (mmol l^{-1}): NaCl 136.9, KCl 5.4, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, $CaCl_2$ 1.8 and glucose 5.6. The Tyrode solution was continuously gassed with 95% O_2 and 5% CO_2; the temperature was kept at 37 ± 0.2°C. The experimental set up permitted a rapid exchange (1–2 s) of one solution for another.

Experimental protocol and evaluation of results

In most experiments, the preparations were treated as follows: (1) cumulative addition of acetylcholine (10^{-12}–10^{-3} mol l^{-1}); (2) washout in drug-free Tyrode solution for 60 min; (3) exposure for 30 min to atropine or quinidine; (4) cumulative addition of acetylcholine (10^{-12}–10^{-3} mol l^{-1}) in the presence of atropine or quinidine. The effects of acetylcholine on the force of contraction (F_c) were evaluated after steady state values had been reached (10–15 min) and expressed as a percentage of the values before the addition of acetylcholine. Results are expressed as means ± s.e. EC_{50} values were determined by regression analysis taking into account two points on the steep portion of each individual concentration-response curve. Confidence limits were calculated according to Documenta Geigy (1968). In control experiments, the responses to acetylcholine were repeatedly tested in the absence of any blocking agents. The resulting cumulative concentration-response curves were almost super-imposable: EC_{50} ratios obtained from EC_{50} values during second exposure over EC_{50} values during first exposure amounted to 0.91 ± 0.16 (means ± s.e.; $n = 12$). The exposure of the preparations to different concentrations of quinidine resulted in different changes of F_c. The responses to acetylcholine in the presence of quinidine were evaluated as % of the steady state values which had been established in the presence of quinidine alone. Concentration-ratios were obtained by dividing the EC_{50} values obtained in the presence of the blocking agents by the EC_{50} values obtained in the presence of acetylcholine alone. To test for competitive antagonism, plots of log (concentration ratio − 1) against log concentration of the blocking agents were constructed according to Arunlakshana & Schild (1959) using the method of least squares. The regression lines were tested for linearity and the slopes of regression lines were tested for difference from unity according to Documenta Geigy (1968).

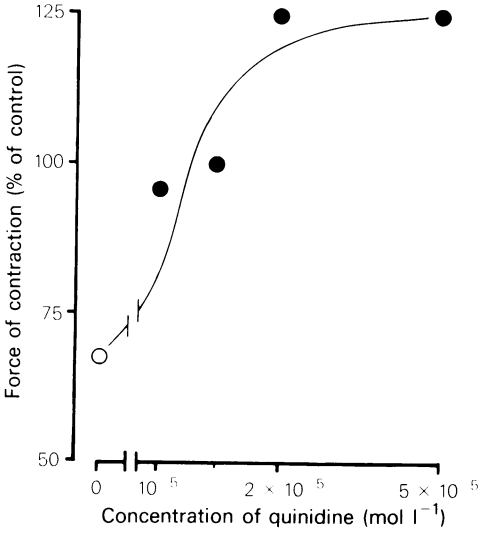

Figure 1 Concentration-response relationships of quinidine in cat atrial preparations in the presence of acetylcholine 10^{-6} mol l^{-1} as a percentage of control values (●). Each concentration of quinidine was tested in a different preparation ($n = 1$). Acetylcholine alone (○) depressed F_c to 68 ± 1.8% of control values (mean ± s.e.; $n = 4$). The effects of acetylcholine and quinidine were evaluated 15 min and 30 min after the addition of the drugs, respectively.

Where relevant, statistical comparisons were made, using Student's *t* test for paired data. A *P* value of less than 0.05 was considered significant.

Electrophysiological experiments

In some experiments, electrical and mechanical activity was recorded from atrial trabeculae which were mounted horizontally in a 2 ml tissue bath. The tissue bath was built into a perspex block that also contained a main reservoir of 40 ml Tyrode solution. Communication between these compartments was provided by connecting pores through which the fluids were driven by gas (95% O_2 and 5% CO_2). The transmembrane potential was detected intracellularly by the use of 10–20 MOhm glass microelectrodes filled with KCl 3 mol l^{-1}. The signals were amplified by means of a voltage follower with input capacity neutralization (WPI micro-probe system Model M 701). Action potential and F_c were recorded on FM tape and, for evaluation, played back to a digitizing oscilloscope (Nicolet Explorer I) the analog output of which was fed to an XY-recorder. The duration of the action potential (APD) was evaluated in ms both at 20% and 90% repolarization.

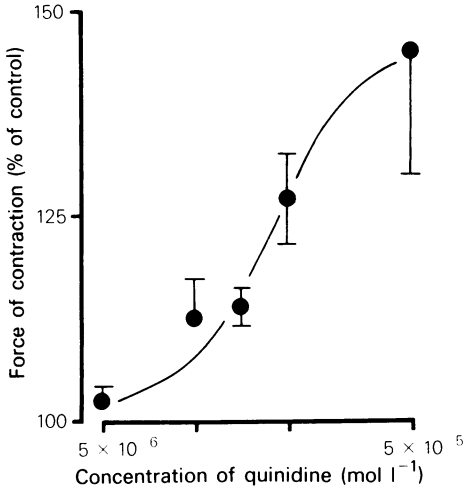

Figure 2 Concentration-response relationships of quinidine in cat atrial preparations. Means of 24 preparations as a % of control values; vertical lines are s.e. Each concentration of quinidine was tested in different preparations ($n = 4-6$). The effects of quinidine were evaluated 30 min after the addition of the drug.

Drugs

The following were used (sources in parentheses): acetylcholine chloride (Merck/Darmstadt); atropine sulphate (Merck/Darmstadt); papaverine hydrochloride (Serva/Heidelberg); quinidine sulphate (Merck/Darmstadt). The pH of the test solutions was 7.3–7.4 and did not change in the presence of the drugs.

Results

Quinidine antagonized the negative inotropic response to acetylcholine in cat atrial muscle. Figure 1 shows the results from an experiment where four

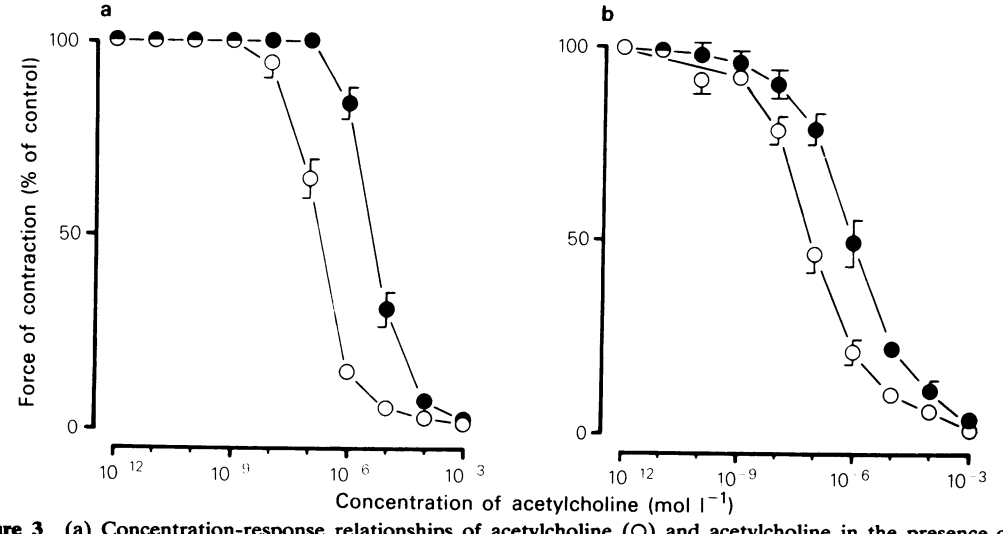

Figure 3 (a) Concentration-response relationships of acetylcholine (○) and acetylcholine in the presence of atropine 10^{-8} mol l^{-1} (●) in cat atrial preparations. Cumulative addition of acetylcholine. Means of 4 preparations; vertical lines are s.e. (b) Concentration-response relationships of acetylcholine (○) and acetylcholine in the presence of quinidine 5×10^{-5} mol l^{-1} (●) in cat atrial preparations. Cumulative addition of acetylcholine. Means of 4 preparations; vertical lines are s.e.

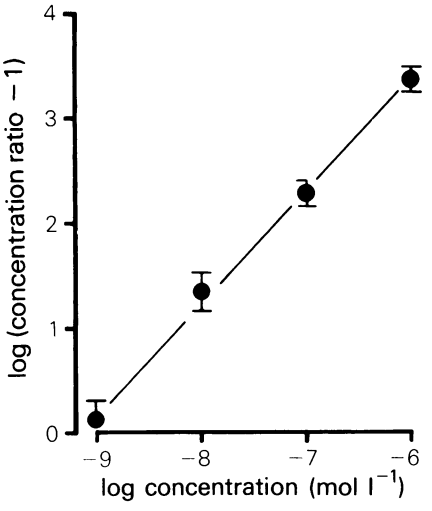

Figure 4 Schild plot with acetylcholine as agonist and atropine as antagonist on F_c in cat atrial preparations. Means of 16 preparations; vertical lines are s.e. Each concentration of atropine was tested in a different preparation ($n=4$).

preparations were first treated with acetylcholine 10^{-6} mol l^{-1}. F_c was depressed to 67.6 ± 1.8% of control values. Each preparation was then exposed to a different concentration of quinidine (acetylcholine still present). Upon the addition of quinidine, F_c recovered to values of about or above control. In the absence of acetylcholine, quinidine also exerted a concentration-dependent positive inotropic effect (Figure 2). The increase by quinidine of F_c may be related to the fact that endogenously stored acetylcholine can be released during electrical stimulation (Trautwein, Whalen & Grosse-Schulte, 1960). However, atropine in concentrations between 10^{-9} and 10^{-6} mol l^{-1} did not significantly alter F_c. The mean values ± s.e. of all preparations treated with atropine ($n=16$) were 98.5 ± 4.1% of control, 30 min after the addition of the drug.

The exposure of atrial preparations to acetylcholine resulted in a concentration-dependent negative inotropic effect: the EC_{50} was 5.2×10^{-8} mol l^{-1} ($n=110$). Concentration-response relationships obtained from repeated administration of acetylcholine were almost superimposable (see methods). In the presence of atropine, the concentration-response curves of acetylcholine were shifted to the right (Figure 3a), as was also seen in the presence of quinidine (Figure 3b).

The effects of increasing concentrations of atropine on the responses to acetylcholine are shown as Schild-plot in Figure 4. The data were best fitted by the following regression line: $y = 9.80 + 1.07x$ ($r=0.999$; 4 experiments for each concentration of atropine). The pA_2 value for atropine was 9.14 (confidence limits: 8.98–9.44). The slope of 1.07 was not different from unity ($P > 0.05$). Quite different results were obtained with quinidine. Although quinidine shifted the acetylcholine concentration-response curve to the right as did atropine, comparison of the Schild-plots revealed marked differences

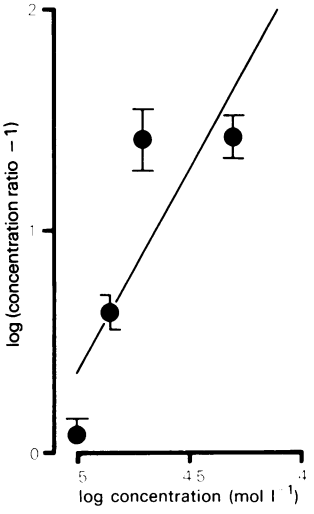

Figure 5 Schild plot with acetylcholine as agonist and quinidine as antagonist on F_c in cat atrial preparations. Means of 20 preparations; vertical lines are s.e. Each concentration of quinidine was tested in a different preparation ($n=4-6$).

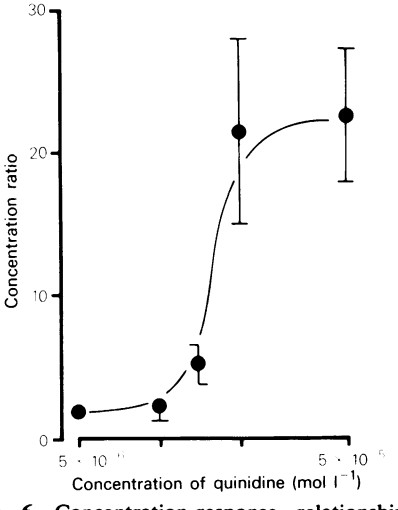

Figure 6 Concentration-response relationships of quinidine in cat atrial preparations. Means of 24 preparations: vertical lines are s.e. Each concentration of quinidine was tested in a different preparation ($n=4-6$). Values are concentration ratios, i.e.

$$\frac{EC_{50} \text{ in the presence of quinidine + acetylcholine}}{EC_{50} \text{ in the presence of acetylcholine}}$$

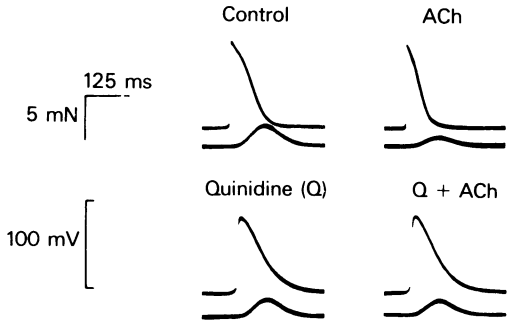

Figure 7 Influence of acetylcholine 10^{-6} mol l^{-1} (ACh), quinidine 10^{-5} mol l^{-1} (Q) and a combination of both substances on the action potential and F_c in a cat atrial trabecula. Original records. The preparation was first exposed to ACh for 5 min and, after washout, to Q for 15 min. The preparation was then again exposed to ACh for 5 min.

Figure 5 shows the Schild-plot with quinidine as inhibitor of acetylcholine responses. The regression line obtained with the method of least squares had a slope of 1.82 ($r=0.840$; 4 experiments for each concentration of quinidine, 8 experiments at 2×10^{-5} mol l^{-1} quinidine). However, the regression line was not linear ($P<0.05$) and the slope of 1.82 was different from unity ($P<0.05$); a pA_2 value was therefore not calculated.

It seems obvious from the statistical analysis that the data with atropine were best fitted in the Schild-plot (Figure 4), whereas the data with quinidine were better described as concentration-response relationships in a semi-log system (Figure 6). Figure 6 demonstrates flat concentration-response relationships at low and at high concentrations of quinidine with a steep portion between 10^{-5} and 2×10^{-5} mol l^{-1}. The relatively narrow concentration range of quinidine and the failure to fit adequately the data in a Schild-plot suggest that quinidine may exert its anticholinergic properties in a different or more complex way than atropine. If atropine and quinidine simply act as competitive antagonists of acetylcholine responses, the amount of inhibition should be simply additive in the presence of both atropine and quinidine. However, Table 1 shows that the combined administration of atropine and quinidine produces a much

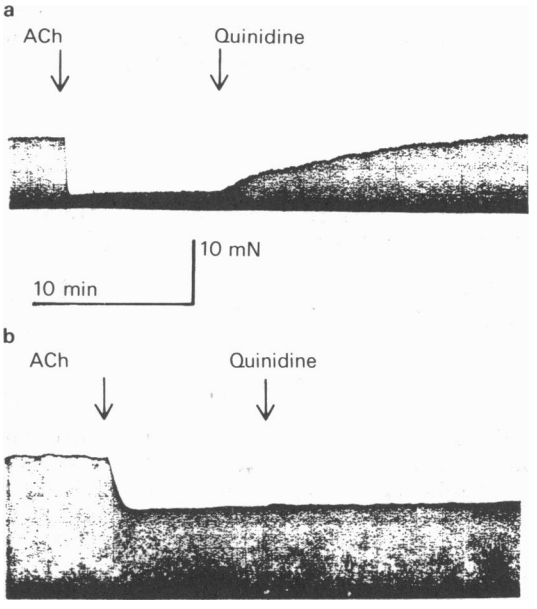

Figure 8 (a) Influence of acetylcholine 3×10^{-7} mol l^{-1} (ACh) and quinidine 2×10^{-5} mol l^{-1} on F_c in a cat atrial preparation. Original record. (b) Influence of acetylcholine 3×10^{-5} mol l^{-1} (ACh; in the presence of papaverine 2×10^{-5} mol l^{-1}) and quinidine 2×10^{-5} mol l^{-1} on F_c in a cat ventricular preparation. Original record.

greater inhibition of acetylcholine responses than expected from the addition of the inhibitory effects of each substance alone.

The effects of acetylcholine of F_c were accompanied by a decrease in APD. Quinidine exerted the opposite effect on the repolarization phase and, in the presence of acetylcholine + quinidine, both APD and F_c remained virtually unchanged (Figure 7). Table 2 summarizes the results of three experiments where the effects of acetylcholine, quinidine and a combination of both substances on APD were evaluated. The opposite effects of acetylcholine and quinidine on the repolarization phase of the action potential offer an alternative explanation for the antagonistic effects of both drugs on F_c (see discussion).

Table 1 Influence of atropine, quinidine and a combination of both substances on the mechanical response to acetylcholine in cat atrial preparations

	Concentration ratio	n
Atropine 3×10^{-9} mol l^{-1} (A)	6±3	8
Quinidine 2×10^{-5} mol l^{-1} (Q)	22±5	8
A+Q	231±49**	8

Values are means±s.e. Statistical significance was determined by Student's t test: **$P<0.01$ (231±49 versus 28±4)

Table 2 Influence of acetylcholine (10^{-6} mol l^{-1}), quinidine (10^{-5} mol l^{-1}) and a combination of both substances on the duration of the action potential in cat atrial preparations

	Control	*Acetylcholine*	*Quinidine*	*Quinidine + acetylcholine*
Action potential duration at 20% repolarization (ms)	41 ± 5	20 ± 2**	56 ± 6*	39 ± 2
Action potential duration at 90% repolarization (ms)	124 ± 12	94 ± 11*	177 ± 13**	161 ± 12**

Values are means ± s.e. ($n = 6$). Statistical significance was determined by Student's t test: *$P < 0.05$; **$P < 0.01$.

Finally, the interaction of acetylcholine and quinidine was also investigated in the ventricular myocardium. The negative inotropic response to acetylcholine in the ventricular myocardium was obtained in the presence of the phosphodiesterase inhibitor, papaverine (Nawrath, Gruschwitz & Zong, 1981) and easily reversed upon the addition of atropine 10^{-6} mol l^{-1}. In contrast to the reversal by quinidine of the action of acetylcholine in atrial muscle, no antagonizing effect of the drug against the response to acetylcholine was found in ventricular heart muscle. In atrial muscle, F_c was depressed to 53.5 ± 18.0% of control in response to acetylcholine 3×10^{-7} mol l^{-1}; upon the addition of quinidine 2×10^{-5} mol l^{-1}, F_c recovered to 118 ± 2.3% of control values (means ± s.e.; $n = 4$). In ventricular muscle, F_c was depressed to 62.4 ± 1.9% of control in response to acetylcholine 3×10^{-5} mol l^{-1} (in the presence of papaverine 2×10^{-5} mol l^{-1}); upon the addition of quinidine 2×10^{-5} mol l^{-1}, F_c amounted to 61.4 ± 6.3% of control values (means ± s.e.; $n = 4$). The time course of these drug effects in atrial and in ventricular heart muscle can be seen from the original records depicted in Figure 8.

Discussion

Quinidine exerts anticholinergic effects which may contribute to its cardiovascular as well as to its effects on other organ systems. The present study was undertaken to characterize, at the cellular level, the antagonizing effects of quinidine on cholinergic stimulation in the heart.

The evaluation of drug antagonism according to Arunlakshana & Schild (1959) was used in the present study as a test for competitive interaction at the receptor level. Schild-plots with acetylcholine as agonist and quinidine as antagonist were constructed earlier by Pérez *et al.* (1977) and Benfey *et al.* (1979). In both studies, competitive antagonism between acetylcholine and quinidine was suggested since the slopes of the regression lines in the plot of log quinidine concentration against log (concentration ratio − 1) of acetylcholine were not different from unity. However, the concentration range for which the Schild-plot was constructed was less than one decade and no statistical analysis of the data was presented.

Our results suggest that the anticholinergic effects of atropine are compatible with the concept of competitive inhibition, whereas the effects of quinidine cannot be solely explained by an interaction at the receptor level. The Schild-plot with quinidine did not result in a linear regression line; similar results were obtained by Fuder, Meiser, Wormstall & Muscholl (1981) who also found a complex relationship between the concentration of quinidine and its antimuscarinic activity on pre- and postsynaptic receptors in rabbit isolated heart. Furthermore, the presence of both atropine and quinidine resulted in a much greater than additive inhibition of the effects of acetylcholine. Paton & Rang (1965) emphasized that such an interaction is not compatible with the concept of competitive antagonism.

Part of the difficulty in assigning the mode of action of quinidine lies in the controversy on how the effects of acetylcholine on the heart are mediated. The studies of Harris & Hutter (1956) and Hutter & Trautwein (1956) indicated that the effects of acetylcholine receptor activation are mediated by an increase in the potassium conductance of the myocardial cell membrane. Voltage clamp studies in atrial heart muscle preparations from frogs (Garnier, Nargeot, Ojeda & Rougier, 1978) and warm-blooded animals (TenEick, Nawrath, MacDonald & Trautwein, 1976) confirmed and extended the earlier findings. Since the studies of Prokopczuk, Lewartowski & Czarnecka (1973) and Ikemoto & Goto (1975), it has been thought that the action of acetylcholine on potassium conductance is not sufficient to account for all of the inhibitory effects on the heart. Most authors think that the shortening of the action potential duration is also due to a change in calcium

conductance. Giles & Noble (1976) emphasized that the influence of acetylcholine on the calcium-dependent slow inward current is of major importance. TenEick *et al.* (1976) concluded that the enhancement of the potassium conductance is of major importance since the calcium-dependent slow inward current was only inhibited at very high concentrations of acetylcholine. Recently, Ochi & Hino (1978) and Hino & Ochi (1980) described the effects of acetylcholine in guinea-pig ventricular myocardium. These authors found that acetylcholine decreased the calcium-dependent slow inward current without any effect on the potassium conductance. In conclusion, all voltage clamp studies with acetylcholine revealed a decrease in the calcium-dependent slow inward current; the relative importance of this effect is still a matter of debate.

The actions of quinidine on the heart are commonly understood (1) in terms of membrane effects which may determine the antiarrhythmic potency and (2) as an interaction at muscarinic receptors which may determine the adverse effects of the drug on atrioventricular conduction. Numerous reports have been published concerning the actions of quinidine on the heart and the presumed underlying mechanism. There are two voltage clamp studies, one in frog atrial muscle (Ducouret, 1976) and the other in cat ventricular trabeculae (Nawrath, 1981) both showing that the action of quinidine on the heart is to reduce the ion conductances for sodium, calcium and potassium. A concomitant reduction of the calcium-dependent slow inward current and the outward potassium currents can produce oppositional changes of the repolarization phase, and different changes of the action potential configuration in response to quinidine have been described (Johnson, 1956; Hoffman, 1958; Vaughan Williams, 1958; Matsumura & Takaori, 1959; West & Amory, 1960; Vaughan Williams & Szekeres, 1961; Nawrath & Eckel, 1979; Nawrath, 1981).

The fact that the blocking effect of quinidine on acetylcholine responses was different from that of atropine suggests that additional or different mechanisms may be responsible for the anticholinergic effect of quinidine. The above described membrane effects of quinidine may not only determine the antiarrhythmic potency but also the anticholinergic properties. A possible membrane effect which may account for the inhibitory effects of quinidine is the depression of the potassium conductance. This assumption is supported by the finding that the effect of acetylcholine on F_c was blocked by quinidine in atrial but not in ventricular heart muscle. If the effects of acetylcholine in atrial heart muscle are mainly determined by an increase in the potassium conductance as suggested by TenEick *et al.* (1976), the inhibition by drugs of the potassium conductance will antagonize the effects of acetylcholine. The reversal by promazine of acetylcholine-induced changes in rat atrial action potentials (Landmark, Haffner & Lindberg, 1973) and the antagonistic effects of 4-aminopyridine (Freeman, 1979) were interpreted in the same way. The potassium system in ventricular heart muscle is obviously not affected by acetylcholine (Ochi & Hino, 1976; Hino & Ochi, 1980). This may explain why the effects of acetylcholine are inhibited by quinidine in atrial but not in ventricular heart muscle.

Mirro *et al.* (1980) localized the anticholinergic effects of quinidine to the muscarinic receptor on the basis of binding studies and their correlation to the change in physiological parameters. However, in a more recent study, Chassaing, Duchêne-Marullaz & Paire (1982) suggested that the action of quinidine on the heart rate in dogs is not due to a direct vagolytic action but rather to a mechanism involving β-adrenoceptors.

In summary, it is not excluded that part of the effects of quinidine may be explained by an interaction of the drug with muscarinic receptors. However, the findings of the present study suggest, that additional mechanisms, possibly direct membrane effects, may counteract the effects of acetylcholine.

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