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SHORT COMMUNICATIONS

Actions of some antiarrhythmic agents on heart sarcolemma*

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Quinidine, a well-known antiarrhythmic agent, is considered to depress myocardial contractility in high doses by decreasing calcium-uptake activities of sarcoplasmic reticulum [1–4] and mitochondria [3–5]. However, it is now becoming evident that sarcolemma also plays an important role in regulating myocardial contractility [6–8], and it is possible that the cardiodepressant action of quinidine may also be due to its effect on the heart sarcolemma. This view is supported by the findings of Silva-Graca and Van Zwieten [9] that this drug decreased the rate of transmembrane Ca^{2+} exchange during excitation. Therefore, it was the purpose of this study to investigate the actions of quinidine on calcium binding and Ca^{2+} ATPase, Mg^{2+} ATPase, Na^+–K^+ ATPase and adenylate cyclase activities of the heart sarcolemmal fraction. The functional effects of quinidine were also tested by employing isolated, perfused heart preparations. Other antiarrhythmic agents such as procaine amide and lidocaine, which depress myocardial contractility [3], were used for comparison. It should be mentioned that sarcolemmal Ca^{2+} ATPase, Mg^{2+} ATPase, Na^+–K^+ ATPase and adenylate cyclase are believed to be involved in the regulation of cation movements across heart sarcolemma [8, 10–12], and that cardiodepressant agents, such as propranolol, have been reported to decrease the activities of these membrane-bound enzymes [13].

Male New Zealand white rabbits (2–3 kg) were used in this study. The animals were sacrificed and the hearts quickly removed and placed in ice-cold Tris–HCl buffer, pH 7.4. The sarcolemmal fraction was isolated by the hypotonic shock-LiBr method described earlier [10] except that all solutions contained 1 mM dithiothreitol (Cleland's reagent) and the fraction was extracted with 0.6 M KCl for 15 min. The pellet was washed and suspended in 1 mM Tris–HCl, pH 7.0, and used within 1 hr. Marker enzyme activities and electron microscopic examination [10] revealed that this membrane fraction was essentially devoid of cytoplasmic contaminants.

The sarcolemmal fraction (0.2 mg protein/ml) was suspended in a medium containing 100 mM Tris–HCl, pH 7.4, and incubated for 3 min at 37° in the absence or presence of drugs before the addition of 0.1 mM $^{45}CaCl_2$. The reaction was terminated 5 min later by the Millipore filtration technique [12, 14]. The radioactivity in the protein-free filtrate, as well as in the filter, was analyzed and the amount of calcium bound to the membrane was calculated. The sarcolemmal Ca^{2+} ATPase (4 mM $CaCl_2$), Mg^{2+} ATPase (4 mM $MgCl_2$), and Na^+–K^+ stimulated ATPase (100 mM NaCl and 10 mM KCl) activities were determined according to procedures reported earlier [10, 11]. The sarcolemmal fraction (0.05 mg protein/ml) was preincubated at 37° in the presence or absence of drugs for 3 min followed by the addition of 4 mM Tris–ATP. After 10 min of further incubation, the reaction was terminated by adding 1 ml of 12% trichloroacetic acid containing 50 mg/ml of activated charcoal, and the P_i in the protein-free supernatant was determined by the method of Taussky and Shorr [15]. The treatment with activated charcoal was necessary to eliminate drug interference during color development when determining P_i [1]. The protein content was estimated according to the method of Lowry *et al.* [16]. The adenylate cyclase activity was assayed by the method of Drummond and Duncan [17]. The sarcolemmal fraction (50 μg protein/0.15 ml) was preincubated in a medium containing 50 mM Tris–HCl, pH 8.5, 8 mM caffeine, 5 mM KCl, 20 mM phosphoenol pyruvate, 15 mM $MgCl_2$, 130 μg/ml of pyruvate kinase, 2 mM cyclic AMP and 2 mM NaF with or without drugs for 3 min at 37° followed by the addition of 0.4 mM [^{14}C]ATP. It should be mentioned that the conditions for the adenylate cyclase and ATPase assays were for the optimal enzyme activities [10, 11].

The methods for perfusing hearts by the Langendorff technique and recording the contractile force were the same as described previously [3]. The coronary flow was maintained at 20 ml/min and the hearts were allowed to beat spontaneously for 20 min at 37° before perfusion with the medium containing the drug. The contractile force (developed tension) and the rate of change of the force development (dF/dt) were monitored on a Grass polygraph recorder with a force displacement transducer (FT 03). In some experiments, electrically paced heart preparations were also used; hearts were stimulated at 280 beats/min with a square-wave pulse just above the threshold.

As reported earlier [12], the heart sarcolemmal fraction used in this study bound a considerable amount of calcium

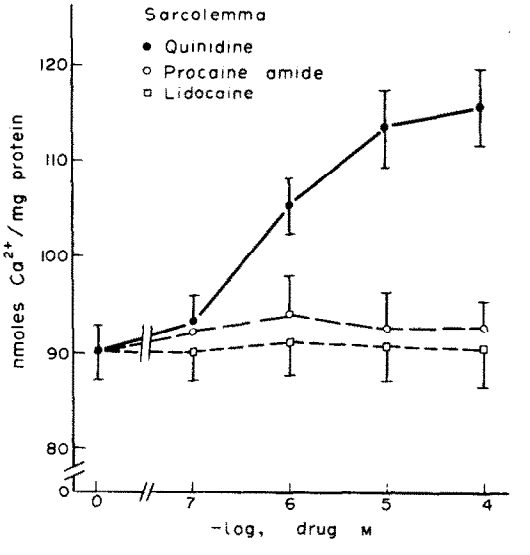

Fig. 1. Effects of various concentrations of quinidine, procaine amide and lidocaine on calcium binding by rabbit heart sarcolemmal fraction. Each value is the mean ± S.E.M. of six experiments.

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Table 1. Effects of various concentrations of quinidine, procaine amide and lidocaine on rabbit heart sarcolemmal adenylate cyclase and Na^+–K^+ ATPase activities

Concn. of drug (M)	Adenylate cyclase activity* (pmoles cyclic AMP mg^{-1} min^{-1})			Na^+–K^+ ATPase activity* (μmoles P_i Mg^{-1} hr^{-1})		
	Quinidine	Procaine amide	Lidocaine	Quinidine	Procaine amide	Lidocaine
Control	849 ± 56	876 ± 60	869 ± 54	10.8 ± 0.91	10.2 ± 1.1	11.0 ± 1.2
10^{-5}	773 ± 66	883 ± 69	857 ± 51	10.9 ± 1.9	12.4 ± 1.7	11.7 ± 1.0
10^{-4}	668 ± 42†	857 ± 48	869 ± 60	11.7 ± 2.0	11.2 ± 1.2	13.0 ± 1.7
10^{-3}	582 ± 52†	842 ± 53	852 ± 47	11.1 ± 1.6	11.1 ± 1.6	12.4 ± 1.3

* Each value is the mean ± S. E. M. of four to five experiments.
† Significantly different from the control value ($P < 0.05$).

in the absence of Mg ATP; the sarcolemma bound about 90 nmoles Ca^{2+}/mg of protein under the experimental conditions employed here. The effects of different concentrations of various antiarrhythmic agents on calcium binding by sarcolemma were studied and the data are shown in Fig. 1. Unlike procaine amide or lidocaine, quinidine in 10^{-6} to 10^{-4} M concentrations increased sarcolemmal calcium binding significantly ($P < 0.05$). In this regard, it should be pointed out that quinidine did not increase the nonspecific calcium binding by the rabbit microsomal or mitochondrial fractions [5].

The effects of different concentrations of quinidine, procaine amide and lidocaine were also studied on the adenylate cyclase, Na^+–K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase activities of the rabbit heart sarcolemmal fraction. From Table 1 it can be seen that the adenylate cyclase activity decreased ($P < 0.05$) with 10^{-4}–10^{-3} M quinidine only, whereas the Na^+–K^+ ATPase activity was unaffected ($P > 0.05$) by quinidine, procaine amide and lidocaine. It should be noted here that Lowry *et al.* [18] have shown an inhibitory effect of quinidine on the bovine heart Na^+–K^+ ATPase activity. This discrepancy in results may be due to differences in the methods employed for isolation or in the sources of preparations. The results given in Table 2 show that both Mg^{2+} ATPase and Ca^{2+} ATPase activities of the rabbit heart sarcolemma were significantly decreased ($P < 0.05$) by 10^{-4}–10^{-3} M quinidine, procaine amide or lidocaine.

In order to determine the possible significance of the observed membrane changes with antiarrhythmic agents, the effects of these drugs on myocardial function were studied by employing spontaneously beating rabbit hearts. It can be seen from Fig. 2 that 10^{-5} and 10^{-4} M quinidine decreased the contractile force and dF/dt of the rabbit heart. Quinidine, at 10^{-3} M, was found to inhibit the contractile force development completely. Likewise, 10^{-4} M and 10^{-3} M procaine amide and lidocaine decreased myocardial contractility by 40–100 per cent in rabbit heart, whereas these agents at 10^{-5} M had no effect. Similar results with all the three drugs were obtained in electrically paced preparations.

In this study, we have demonstrated that quinidine, procaine amide and lidocaine decreased rabbit heart sarcolemmal Ca^{2+} ATPase and Mg^{2+} ATPase activities. The sarcolemmal site of action of these antiarrhythmic agents is also apparent from the observation that quinidine and lidocaine depressed calcium influx in guinea pig atria [9]. Furthermore, different anesthetic agents, including lidocaine, have been shown to produce a blockade of the slow calcium channels in cardiac muscle [19]. At any rate, the actions of the antiarrhythmic agents on sarcolemmal Ca^{2+} ATPase and Mg^{2+} ATPase activities may be of a specific nature, since 10^{-4} M quinidine, procaine amide or lidocaine was found to have no effect on the mitochondrial or microsomal ATPase activities [4]. In addition, heart sarcolemmal Na^+–K^+ ATPase activity was not altered by these agents.

Quinidine has been observed to depress adenylate cyclase activity in rabbit heart sarcolemma. This can be interpreted to mean that less cyclic AMP would be formed after exposing the heart to quinidine. Since cyclic AMP has been implicated in increasing calcium influx into the myocardium [20], it is likely that quinidine may be partly affecting the myocardium through this mechanism. In this regard, quinidine seems to affect sarcolemmal sites which are different from those for lidocaine and procaine amide because these agents did not alter the sarcolemmal adenylate cyclase activity. The difference between the sites of action of these antiarrhythmic agents is also apparent from the experiments in which quinidine was observed to increase calcium binding by heart sarcolemma whereas procaine amide and lidocaine had no effect. It should be pointed out that high concentrations of propranolol, which depress myocardial contractility, were found to decrease calcium binding by heart sarcolemma [13]. Thus, the significance of changes in calcium binding in terms of the

Table 2. Effects of various concentrations of quinidine, procaine amide and lidocaine on rabbit heart sarcolemmal Mg^{2+} ATPase and Ca^{2+} ATPase activities

Concn of drug (M)	Mg^{2+} ATPase activity* (μmoles P_i mg^{-1} hr^{-1})			Ca^{2+} ATPase activity* (μmoles P_i mg^{-1} hr^{-1})		
	Quinidine	Procaine amide	Lidocaine	Quinidine	Procaine amide	Lidocaine
Control	23.4 ± 1.6	23.8 ± 1.2	23.5 ± 1.8	28.1 ± 1.2	28.4 ± 1.3	28.5 ± 1.6
10^{-5}	21.8 ± 1.6	24.9 ± 1.3	22.1 ± 0.9	25.6 ± 1.5	27.7 ± 1.3	25.9 ± 1.3
10^{-4}	15.8 ± 1.4†	19.2 ± 1.0†	15.7 ± 0.6†	24.1 ± 1.4†	23.7 ± 1.5†	22.2 ± 2.0†
10^{-3}	13.7 ± 0.9†	19.0 ± 0.8†	15.6 ± 0.8†	21.8 ± 1.2†	22.4 ± 1.7†	20.3 ± 1.5†

* Each value is the mean ± S. E. M. of four to five experiments.
† Significantly different from the control ($P < 0.05$).

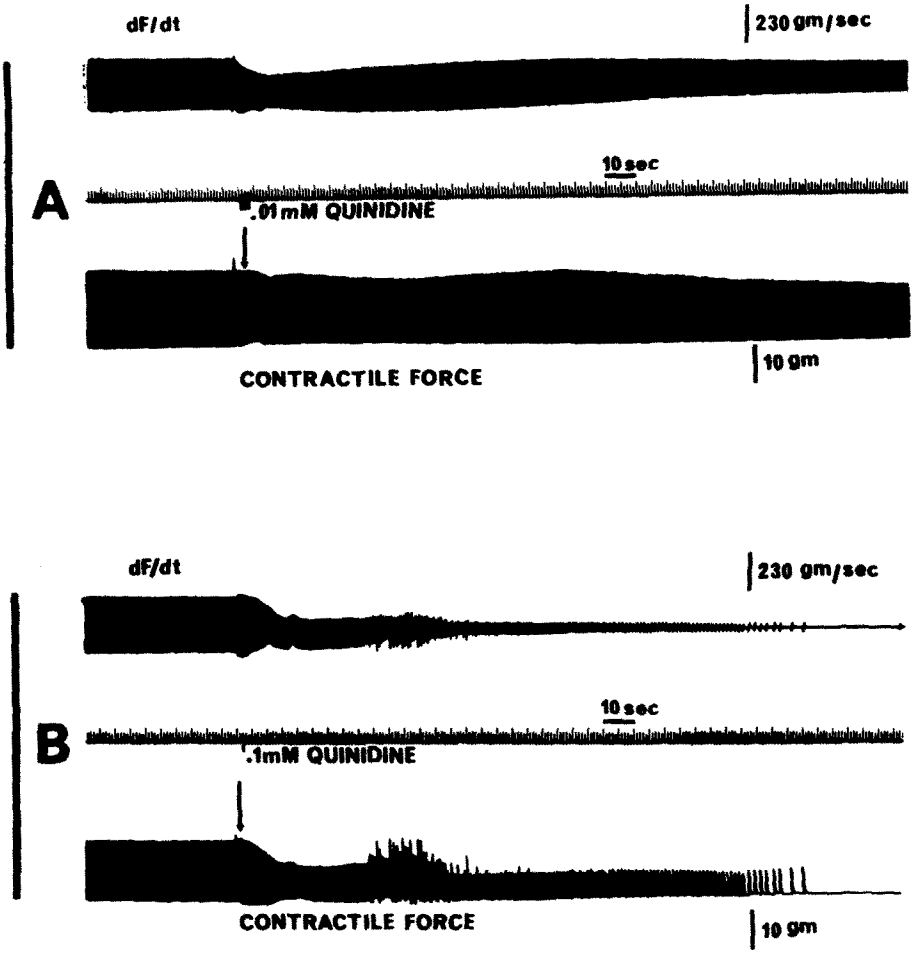

Fig. 2. Effects of quinidine on contractile force and rate of change of contractile force development (dF/dt) of the isolated perfused rabbit heart. Panels A and B show the effects of 0.01 and 0.1 mM quinidine respectively. The upper portion of each panel shows dF/dt, whereas the lower portion shows contractile force. These figures are typical of three experiments carried out for each concentration.

cardiodepressant actions of these agents is not clear at present. It is, however, possible that quinidine exerts its stabilizing action by increasing sarcolemmal calcium in a manner similar to external calcium [21].

From the data reported here it appears that the inhibitory actions of quinidine on sarcolemmal Ca^{2+} ATPase, Mg^{2+} ATPase and adenylate cyclase activities may contribute to decreasing myocardial contractility. It should also be noted that 10^{-4} to 10^{-3} M quinidine has been reported to decrease calcium uptake by fragments of sarcoplasmic reticulum and mitochondria from rabbit heart [3, 4]. Thus, the negative inotropic effect of quinidine in rabbit heart may be due to complex mechanisms involving different membrane systems such as those of mitochondria, sarcoplasmic reticulum and sarcolemma. On the other hand, both procaine amide and lidocaine do not affect calcium transport by mitochondria or sarcoplasmic reticulum [3, 4] but have been shown to depress sarcolemmal Ca^{2+} ATPase and Mg^{2+} ATPase activities. These results suggest some differences in the modes of cardiodepressant action by quinidine and procaine amide or lidocaine.

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