The Effect of Quinidine on Calcium Accumulation by Isolated Sarcoplasmic Reticulum of Skeletal and Cardiac Muscle

FRANKLIN FUCHS, EDWARD W. GERTZ, and F. NORMAN BRIGGS

From the Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

ABSTRACT Quinidine potentiates twitch tension and (at higher concentrations) causes contracture of skeletal muscle whereas the same drug reduces tension development of cardiac muscle. To gain insight into the possible differences in the excitation-contraction coupling mechanism of the two types of muscle the effect of quinidine on calcium accumulation by isolated sarcoplasmic reticulum from skeletal and cardiac muscle was investigated. In a medium containing ATP, Mg++, oxalate, and 45Ca, pharmacologically active concentrations of the drug inhibited calcium accumulation by both skeletal and cardiac sarcoplasmic reticulum. The inhibition of the rates of calcium uptake by the skeletal muscle preparation ranged from 11% with 10^-4 M quinidine to 90% with 10^-6 M quinidine. With the cardiac muscle preparation the inhibition ranged from 16% with 3 X 10^-5 M quinidine to 100% with 10^-4 M quinidine. With both preparations the inhibition of calcium transport was accompanied by an inhibition of the Ca++-activated ATPase activity of the sarcoplasmic reticulum. The effect of quinidine on the skeletal sarcoplasmic reticulum supports the hypothesis that this compound produces twitch potentiation and contracture by interfering with intracellular calcium sequestration. Its effect on cardiac sarcoplasmic reticulum has been interpreted in terms of the hypothesis that cardiac contractility is a function of the amount of calcium released from the sarcoplasmic reticulum which is in turn dependent upon the absolute calcium content of the reticulum. Hence, following inhibition of calcium transport there would be less calcium available for coupling.

INTRODUCTION

The alkaloid quinidine (and its optical isomer quinine) has opposite effects on skeletal and cardiac muscle. Low concentrations of quinidine (10^-5 to 10^-4 M) cause a potentiation of twitch tension of skeletal muscle, whereas higher concentrations (≈ 10^-3 M) cause contracture (1-4). In contrast, the depressant...
effect of this drug on cardiac contractility has been well-established (5–8). On the basis of studies with isolated rabbit atria (5) and cat papillary muscle (7) it would appear that quinidine concentrations of \( \sim 10^{-6} \) M or greater cause a significant reduction in myocardial tension development. Thus in its pharmacological effects quinidine bears at least a superficial resemblance to NO\(_3^–\), Zn\(^{++}\), and UO\(_2^{++}\), all of which have been reported to enhance skeletal muscle tension (9) but depress cardiac contractility (10–12).

It has been established that in both skeletal and cardiac muscle contraction is coupled to excitation through the release of calcium from the sarcoplasmic reticulum (13–16). There is a growing body of evidence suggesting that drugs which alter the contractile states of both types of muscle do so through their ability to act on the intracellular calcium stores of the muscle, in particular those of the sarcoplasmic reticulum (16).

In the case of skeletal muscle it has been suggested that drugs which cause twitch potentiation and contracture (e.g. caffeine, quinidine) act directly on the sarcoplasmic reticulum to cause a release of calcium or inhibition of the calcium transport system, thereby raising the free myoplasmic calcium concentration (13). This hypothesis is supported by tracer studies carried out with living frog muscles which show that these agents do indeed cause a release of intracellular calcium (4, 17). There are also reports indicating that these drugs can release a fraction of the calcium bound by isolated sarcoplasmic reticulum (4, 18, 19).

Much less information is available on the regulation of intracellular calcium movement in cardiac muscle. According to the hypothesis of Gertz et al. (20) cardiac contractility is directly related to the absolute calcium content of the sarcoplasmic reticulum. In terms of this hypothesis any agent which inhibits sarcotubular calcium transport will reduce the amount of calcium available for coupling and thus cause depression of tension development.

As suggested earlier by Sandow (9), an agent such as quinidine, which has well-defined but opposite effects on skeletal and cardiac muscle, might be a useful tool for delineating the differences in the coupling mechanisms in the two types of muscle. With this end in mind we have investigated the effects of quinidine on the ATP-dependent calcium accumulation by sarcotubular vesicles isolated from rabbit skeletal muscle and canine myocardium.

METHODS

Preparation of Sarcotubular Fraction

Skeletal muscle sarcotubular vesicles were prepared from minced back and leg muscles of the rabbit. After being chilled on ice, the muscle tissue was homogenized for 1 min in a Waring Blendor in 4 volumes of 0.3 M sucrose, 10 mM imidazole, pH 7.0. Following centrifugation to remove myofibrils (1500 g \( \times \) 20 min) and mitochondria (10,000 g \( \times \) 20 min), the sarcotubular fraction was collected by centrifugation at 30,000 g for
Effect of Quinidine on Sarcoplasmic Reticulum

1 hr. The vesicles were washed once with sucrose solution and finally suspended, with the aid of a glass Teflon homogenizer, in a small volume of sucrose solution to a final protein concentration of 4–6 mg per ml. The entire procedure was carried out in a cold room at 4°C. The protein concentration of the suspension was determined according to Lowry et al. (21), using bovine serum albumin as a standard.

For the preparation of cardiac sarcotubular vesicles, an approximately 20 kg dog was anesthetized with sodium pentobarbital (25 mg/kg) and maintained on 100% oxygen by endotracheal intubation. The heart was removed as quickly as possible, rinsed with ice-cold saline, cut into small pieces, and cooled in crushed ice. After removing fat and connective tissue the ventricular muscle was homogenized in a Servall Omnimixer for 40 sec with 4 volumes of 0.3 M sucrose, 10 mM imidazole, pH 7.0. Following centrifugation for removal of myofibrils (1000 g × 15 min) and mitochondria (12,000 g × 25 min), the sarcotubular vesicles were collected by centrifugation at 65,000 g for 70 min. The vesicles were washed by suspending them in fresh sucrose solution and recentrifuging them at 150,000 g for 30 min. The vesicles were finally suspended in a small volume of sucrose solution to a protein concentration of ~20 mg per ml. Some of the preparations were suspended in 45% sucrose to stabilize them (22). High concentrations of sucrose have been found to inhibit the destruction of the vesicles by lysosomal enzymes (23).

Measurement of Calcium Accumulation

The rate of calcium uptake by sarcotubular vesicles was measured by the Millipore filtration technique (24) as described in the paper of Gertz et al. (20). Filters of 0.45 μm pore diameter were used throughout. The incubation medium for the skeletal muscle preparation contained 100 mM KCl, 20 mM imidazole, pH 7.0, 4 mM MgCl₂, 4 mM K oxalate, 0.1 mM CaCl₂, 0.1 μc per ml ⁴⁶Ca, 4–5 mM ATP, and 0.02 mg per ml protein. Incubations were carried out at room temperature (20–22°C). The incubation medium for the cardiac muscle preparation contained 104 mM KCl, 19 mM imidazole, pH 7.0, 5 mM MgCl₂, 1.8 mM K oxalate, 0.09 or 0.18 mM CaCl₂, 0.05 μc per ml ⁴⁶Ca, 5 mM ATP, and 0.15 mg per ml protein. The incubation temperature was 37°C.

Measurement of Adenosinetriphosphatase Activity

ATPase activity was determined from the rate of liberation of inorganic phosphate. Following the addition of ATP, 2 ml aliquots of the incubation mixture were removed at various times and pipetted into test tubes containing 2 ml of ice-cold 10% trichloroacetic acid and 200 mg of acid-washed Norit A. The charcoal was added to adsorb the quinidine (25), since the latter compound interferes with the phosphate determination. The charcoal and denatured protein were removed by filtration and the phosphate concentration of the filtrate was determined according to the method of King (26). The skeletal muscle preparations were incubated at 25°C in the same medium used for the measurement of calcium uptake. The cardiac muscle preparations were incubated at 37°C in either the same medium used for the measurement of calcium uptake by the cardiac vesicles or in the skeletal muscle medium. The results were the same in either case. The ATPase activity was assayed by measuring the rate of phosphate liberation before and after the addition of 0.1 mM CaCl₂ (27).
activated ATPase associated with calcium transport (27) was calculated from the difference in slopes of the curves before and just after the calcium addition.

**Solutions and Reagents**

All solutions were prepared with distilled, deionized water. The ATP was a product of P-L Biochemicals, Milwaukee, Wis., and quinidine sulfate was obtained from Sigma Chemical Co., St. Louis, Mo.

![Figure 1](image)

**RESULTS**

**Skeletal Muscle Sarcoplasmic Reticulum**

The effect of quinidine on the kinetics of calcium transport by isolated sarcoplasmic reticulum of skeletal muscle is illustrated in Fig. 1. The initial rates of calcium uptake of the controls were in the range of 1–2 μmoles Ca++ per mg per min, in good agreement with values recorded by others (14) for oxalate-dependent calcium transport. As seen from the data in Fig. 2 quinidine exerted no discernible effect on calcium uptake at concentrations less than 10^{-4} M. With higher concentrations there was an inhibition of calcium uptake which was a linear function of the logarithm of the quinidine concentration. An average inhibition of 90% was obtained with 10^{-3} M quinidine.

The effect of quinidine on the sarcotubular ATPase activity is shown in Figs. 3 and 4. As first observed by Hasselbach and Makinose (27), the addition of calcium (0.1 mM) to a suspension of sarcotubular vesicles in the presence

---

1 Abbreviation used: ATP, adenosine triphosphate.
of ATP, Mg++, and oxalate caused an immediate burst of ATP hydrolysis associated with the transport of calcium into the interior of the vesicles. On the average there was an approximately 10-fold increase in the rate of ATP hydrolysis following the addition of calcium. In the presence of quinidine the degree of calcium activation was markedly reduced (Fig. 3). In the range of quinidine concentrations which produced inhibition of calcium transport (10^{-4}-10^{-3}) there was an increasing inhibition of Ca++-activated ATPase activity as the quinidine concentration was increased (Fig. 4). Inasmuch as the free calcium concentrations were not controlled with a calcium buffer system and calcium uptake and ATPase measurements were made on different samples of vesicles, these data can only be considered to be semiquantitative. Further studies are needed to determine the precise relationship between ATPase inhibition and inhibition of calcium transport.

**Cardiac Muscle Sarcoplasmic Reticulum**

The effect of varying concentrations of quinidine on the calcium uptake of isolated cardiac sarcoplasmic reticulum is illustrated in Fig. 5. The initial rates of calcium accumulation by the controls were in the range of 0.2–0.4 μmole
Ca++ per mg per min, in good agreement with previous values obtained in this laboratory (20). Inhibition ranged from an average of 16% with $3 \times 10^{-6}$ M quinidine to 100% with $10^{-8}$ M quinidine. Thus the effect of quinidine was qualitatively similar to that seen with the skeletal muscle preparation. The main difference was in the greater sensitivity of the cardiac preparation to lower concentrations ($10^{-6}$-$10^{-4}$ M) of quinidine.

Also shown in Fig. 5 is the effect of quinidine on the isometric tension development of cat papillary muscle, taken from the data of Parmley and Braunwald (7). The curve relating tension to quinidine concentration follows the same general shape as the curve relating calcium uptake to quinidine concentration but is shifted somewhat to the right. Considering the species difference and the inevitable difficulty in comparing experiments made with intact
The effect of quinidine on the Ca\(^{++}\)-activated ATPase of the cardiac sarcotubular vesicles was essentially the same as that observed with the skeletal muscle preparations (Figs. 6 and 7).

![Graph showing relative rates of calcium uptake by cardiac sarcoplasmic reticulum as a function of quinidine concentration.](image)

**DISCUSSION**

The data presented in this report clearly show that quinidine strongly inhibits the transport of calcium into the sarcoplasmic reticulum of both skeletal and cardiac muscle. Quinidine readily passes through cell membranes, including those of muscle (28). The concentrations of quinidine which inhibited the sarcotubular calcium pump in this study closely approximated the pharmacologically active concentrations. It seems safe to conclude that in the intact cell the calcium pump is inhibited under conditions in which the drug alters the contractility of the cell. Since the intracellular free calcium concentration is the critical determinant of contractile force development in both types of muscle, the question which must be raised is how inhibition of the sarcotubular calcium pump can be associated with increased force development in skeletal muscle and decreased force development in cardiac muscle. There would appear to be a fundamental difference between the two types of muscle as...
regards the regulation of intracellular calcium movements. A similar conclusion was reached by Nayler (12) on the basis of her studies of the effects of certain inorganic ions (NO₃⁻, Zn²⁺, UO₂²⁺) on cardiac contractility.

According to the hypothesis of Isaacson and Sandow (4) alkaloids such as

![Graph](image)

**Figure 6.** The effect of 10⁻⁸ M quinidine on the Ca²⁺-activated ATPase of cardiac sarcoplasmic reticulum. See Methods section for experimental conditions. At time indicated by arrow CaCl₂ was added to concentration of 0.1 mM. Protein concentration, 0.1 mg per ml.

![Graph](image)

**Figure 7.** The effect of variation in quinidine concentration on the Ca²⁺-activated ATPase activity of cardiac sarcoplasmic reticulum. See Methods section for experimental conditions. Protein concentration, 0.1 mg per ml.

quinine (or quinidine) and caffeine cause twitch potentiation and contracture of skeletal muscle by virtue of their ability to cause an increased calcium flux from sarcoplasmic reticulum to myoplasm. They suppose that low concentrations of quinidine (10⁻⁴ M or less), acting directly on the sarcoplasmic reticulum, cause the release of an amount of calcium which by itself is subthreshold for the activation of contraction but which, when added to the calcium normally released by the action potential, causes a prolongation of the active
state and, hence, an increase in twitch tension. With higher concentrations (> $5 \times 10^{-4} \text{M}$) the amount of calcium released by the drug would be sufficient to activate the contractile mechanism and thus produce contracture. The data presented here are compatible with this hypothesis. Thus in the concentration range associated with twitch potentiation ($10^{-5}$-$10^{-4} \text{M}$) quinidine had little or no effect on calcium accumulation. This would not rule out small in situ effects since in a study of the type reported here an average inhibition of, say 5%, could easily have escaped detection. All other factors remaining equal, the same degree of in situ inhibition would ultimately lead to an elevation of the free intracellular calcium concentration. With $10^{-4} \text{M}$ quinidine we observed an average inhibition of 11% whereas at $5 \times 10^{-4} \text{M}$ there was an average inhibition of 64%. In comparison, Isaacson and Sandow (4) found that $10^{-4} \text{M}$ quinine, which under their conditions only produced twitch potentiation, caused a 20% increase in the rate coefficient for $^{46}\text{Ca}$ efflux from frog sartorius muscle whereas $5 \times 10^{-4} \text{M}$ quinine, which was roughly the threshold concentration for contracture, caused a 320% increase in the rate coefficient. Considering the differences in experimental materials and conditions the two sets of data correspond reasonably well and lend support to the hypothesis that the twitch potentiation and contracture of skeletal muscle induced by quinidine (or quinine) are causally related to the same mechanism, namely inhibition of sarcotubular calcium accumulation. To what extent the rise in myoplasmic calcium concentration produced by low concentrations of quinidine would in fact account for twitch potentiation is not clear in the absence of quantitative information on the intracellular calcium concentrations and fluxes. Even if no additional calcium were released by the drug, inhibition of the calcium pump would prolong the active state by virtue of the longer period of time after the action potential in which the myoplasmic calcium concentration remains elevated above the threshold level. In other words, the important factor in prolongation of the active state is not the presence of a supernormal free calcium concentration but the delay in the reaccumulation of calcium following the action potential.

In considering the effects of quinidine on the heart we encounter several new problems. We have seen that the effect of the drug on isolated cardiac sarcoplasmic reticulum was essentially the same as that observed with skeletal muscle preparations. Yet, contrary to its effect on intact skeletal muscle, quinidine causes a reduction in myocardial tension development. Since the activity of the contractile protein complex of cardiac muscle, like that of skeletal muscle, is a function of the free intracellular calcium concentration (16), we might assume that any agent which inhibited the sarcotubular calcium pump would raise the free calcium concentration and thus cause an increase in tension development. This assumption is implicit in Lee's (29) attempt to explain the positive inotropic effect of cardiac glycosides in terms
of an inhibition of sarcotubular calcium transport. The validity of this assumption is open to question. As pointed out by Gertz et al. (20), the extent to which an increased calcium flux from sarcoplasmic reticulum to myoplasm leads to an increased myoplasmic calcium concentration will depend upon how well other potential calcium transport systems in the cell (e.g. mitochondria, sarcolemma) compete with the myofibrils for the available free calcium. Thus different responses of skeletal and cardiac muscle to the same agent may reflect differences in calcium compartmentalization within the cell. The mitochondrial content of skeletal muscle, especially the amphibian muscle used in most experiments, is considerably less than that of mammalian heart muscle (30). In view of the fact that the mitochondria can accumulate large quantities of calcium (31), this difference may have important implications for the regulation of intracellular calcium movement. The role of the sarcolemma in calcium transport is not clear. The recent radioautographic experiments of Winegrad (32) suggest that in skeletal muscle the main site of calcium exchange between intracellular and extracellular space is not the sarcolemma but the junction between the terminal cisternae of the sarcoplasmic reticulum and the transverse tubules. There also seems to be some functional specialization of different parts of the sarcoplasmic reticulum of skeletal muscle (32). At present even less is known about intracellular calcium compartments in cardiac muscle. It is quite conceivable that in skeletal muscle changes in myoplasmic calcium concentration are an accurate reflection of calcium fluxes out of the sarcoplasmic reticulum whereas in cardiac muscle the cooperative action of several different compartments must be considered. Since the identification of these compartments and their kinetic parameters are unknown further speculation on this point is probably not warranted.

Recent studies of cardiac muscle mechanics provide another line of evidence that the excitation-contraction coupling mechanism in this tissue differs in some fundamental respects from that of skeletal muscle. In the electrically stimulated skeletal muscle the intensity of the active state is essentially invariant (33). Alterations in muscle tension, whether in response to repetitive stimulation or drugs, are mediated through changes in the duration of the active state. In contrast, the studies of Sonnenblick (34) and Edman et al. (35) have shown that alterations of cardiac contractility such as those produced by changes in frequency of stimulation, catecholamines, and cardiac glycosides are mediated through changes in intensity of the active state. We assume that active state intensity in both skeletal and cardiac muscle is a function of the myoplasmic calcium concentration (13, 35). This in turn depends on the amount of calcium released from the sarcoplasmic reticulum. It follows that in skeletal muscle a single action potential releases a "saturation-
ting” amount of calcium. If the rate at which this calcium is restored to the sarcoplasmic reticulum is decreased the active state will be prolonged and the twitch tension increased. The active state intensity of cardiac muscle can be considerably less than maximum depending on the inotropic state of the heart (34, 35). On the basis of the above assumption the amount of calcium released from the sarcoplasmic reticulum during excitation is a variable which reflects the various physiological and pharmacological parameters known to alter cardiac contractility. The basic problem in the regulation of cardiac contractility is to define the factors which determine the amount of calcium released from the sarcoplasmic reticulum during excitation.

The hypothesis currently under consideration in this laboratory is that the amount of calcium released during excitation-contraction coupling is a function of the calcium content of the sarcoplasmic reticulum and that changes in the inotropic state of the heart are mediated through changes in the calcium content of the reticulum. This hypothesis is based on experiments of Briggs et al. (36) and Gertz et al. (20) demonstrating a depression of sarcotubular calcium transport associated with both barbiturate-induced heart failure and spontaneous failure (heart-lung preparation). This depression of calcium transport, like the contractile failure, was reversible with ouabain. Gertz et al. (20) proposed that if the calcium pump is inhibited calcium would flow out of the reticulum and possibly be shunted to compartments which are not responsive to electrical stimulation. Thus less calcium would be available for coupling.

The fact that another negative inotropic substance in addition to the barbiturates, namely quinidine, inhibits calcium transport, provides support for the above hypothesis. Recently it has been shown that the β-blocking drugs, propranolol and pronethalol, both of which also depress cardiac contractility (7, 37), cause a marked inhibition of sarcotubular calcium accumulation (38, 39). Further systematic studies with a variety of positive and negative inotropic drugs would clearly be of interest in evaluating the validity of this hypothesis.

In a recent paper Scales and McIntosh (40) noted that $2 \times 10^{-4}$ M quinidine caused a small but significant increase in the total ATP-dependent calcium binding by skeletal muscle sarcoplasmic reticulum (in the absence of oxalate). However, Isaacson and Sandow (4) cite several brief observations in the literature suggesting that quinidine and quinine can displace bound calcium from the sarcoplasmic reticulum. It is difficult to relate these observations to our own since the experimental conditions and the quantities being measured were quite different. Because oxalate was present in all our experiments the data obtained were the unidirectional fluxes across the sarcotubular membrane (14). There is reason to believe that part of the ATP-dependent
calcium uptake in the absence of oxalate may represent binding to the membrane rather than transport across the membrane (19, 41). The relationship between this binding and transport remains to be elucidated.

As to the mechanism of action of quinidine, little can be said beyond the fact that the inhibition of transport is accompanied by an inhibition of the Ca$^{++}$-activated ATPase of the sarcotubular membrane. Ells and Faulkner (25) have similarly observed a quinidine inhibition of skeletal muscle microsomal ATPase in the presence of low concentrations of calcium. Nayler (42, 43) has shown that epinephrine and norepinephrine potentiated the binding of calcium to sarcotubular phospholipids. Thus alterations in calcium-phospholipid affinities may be of importance in the molecular mechanism of action of cardioactive drugs. Inhibition of the Ca$^{++}$-activated ATPase activity is probably related to the finding that phospholipids are essential for sarcotubular ATPase activity (44).

**Note Added in Proof** After this paper was submitted for publication a study by Carvalho (J. Gen. Physiol. 1968. 52:622) appeared describing the effects of various drugs on the binding of calcium to sarcotubular membranes. He found that quinine and quinidine (1–5 mm) displaced both the passively bound calcium and the calcium bound in the presence of ATP.

Dr. Fuchs is the recipient of a Lederle Medical Faculty Award and Dr. Briggs is the recipient of a Research Career Award (1 K6-HE-5290) from the National Institutes of Health. Dr. Gertz was supported by training grant GM-01404 from the National Institutes of Health.

This work was supported by research grants AM-10551 and HE-06782 from the National Institutes of Health.

**REFERENCES**


Med. 122:373.
38. Scales, B., and D. A. D. McIntosh. 1968. The effects of propranolol and its optical iso-
mers on the radiocalcium uptake and adenosinetriphosphatases of skeletal and cardiac
blocking agents on the calcium pump of the cardiac sarcoplasmic reticum. Nature.
220:79.
40. Scales, B., and D. A. D. McIntosh. 1968. Studies on the radiocalcium uptake and the
adenosinetriphosphatases of skeletal and cardiac sarcoplasmic reticulum fractions (SRF).
42. Nayler, W. G. 1966. An effect of quinidine sulfate on the lipid-facilitated transport of
43. Nayler, W. G. 1966. The effect of pronethalol and propranolol on lipid-facilitated trans-
44. Martonosi, A., J. Donley, and R. A. Halpin. 1968. Sarcoplasmic reticulum. III. The
role of phospholipids in the adenosine triphosphatase activity and Ca++ transport.