Gentamicin Blockade of Slow Ca^{++} Channels in Atrial Myocardium of Guinea Pigs

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ABSTRACT Cardiac dysfunction is occasionally detected in patients undergoing treatment with aminoglycoside antibiotics; however, the mechanism responsible for the negative inotropic effect of these agents has not been identified. In the present investigation, electrically driven left atria of guinea pigs were used to study the effects of gentamicin on calcium ion (Ca^{++})-dependent contractile events in heart muscle isolated from in vivo influences. When atria were first inactivated by excess potassium ion (K^+; 22 mM) and contractions were then restored by isoproterenol (an experimental model that accentuates the contractile dependence of myocardial fibers on influx of Ca^{++} through specific "slow channels" of the sarcolemma), the cardiac depressant activity of gentamicin (0.1 mM) was profoundly augmented. Conversely, the negative inotropic effect of tetrodotoxin (23.5 μM) was abolished by the same experimental conditions. Also, gentamicin (1 mM) and La^{+++} (0.5 mM) markedly decreased the positive inotropic response to increased frequency of stimulation; whereas, D_{600} (1.05 μM) converted the positive frequency-force relationship to a negative relationship. Present data indicate a direct cardiac depressant action of gentamicin, and suggest that this antibiotic adversely affects either the transport system responsible for Ca^{++} movement through slow channels of the sarcolemma, the availability of Ca^{++} for translocation to these sites, or both.

INTRODUCTION

Among the clinically useful antibiotic drugs, the aminoglycosides (i.e., neomycin-streptomycin group) are quite unique. These agents, in addition to their useful antibacterial activity, also (*a*) inhibit synaptic transmission at various neuroeffector junctions (1, 2), (*b*) depress cerebral neuron responses to catecholamines (3), (*c*) depress cardiovascular function (4–8), and (*d*) relax different types of smooth muscles (5, 9). Although the precise mechanisms responsible for these varied events have not been completely resolved, a Ca^{++} antagonistic property of the aminoglycosides has been proposed for many years (1). Indeed, inhibitory effects of these antibiotics on Ca^{++} metabolism have now been directly or indirectly demonstrated in a variety of tissues (1–3, 5, 6, 9); however, little is known concerning the membrane sites susceptible to aminoglycoside action. In view of the essential role of Ca^{++} in the linkage of cell membrane excitation to subsequent mechanical (e.g., smooth muscle, myocardium) or secretory (e.g., neurons, endocrine glands) functions (10, 11), the interaction between aminoglycosides and Ca^{++} represents an exceptionally important aspect of the pharmacology-toxicology of these antibiotics. We have attempted to identify membrane loci of aminoglycoside action by using a heart muscle model that depends upon a transsarcolemmal influx of Ca^{++} through activated "slow Ca^{++} channels", cell membrane passageways quite distinct from the tetrodotoxin-sensitive "fast Na^+ channels" (12–14). Present findings indicate that the cardiac depressant effects of gentamicin, a representative aminoglycoside antibiotic, are profoundly augmented in myocardium contracting under "slow Ca^{++}-activated" conditions.

METHODS

Left atria of male albino guinea pigs were prepared for monitoring isometric contractile tension and its first derivative (dT/dt) according to a method previously reported in detail (15). Electrical stimulation of the atria with single square wave impulses was accomplished with an AEL stimulator (American Electronic Laboratories, Colmar, Pa.) and a miniature bipolar electrode. Voltage was greater-than-threshold, pulse duration was 2 ms, and stimulus frequency was one pulse per second (1 Hz), unless otherwise designated. The physiological saline solution (pH 7.4) contained in millimolar: NaCl, 154; KCl, 5.4; $CaCl_2$, 2.5; $MgSO_4$, 0.012; glucose, 11; and Tris, 6 (7). A "high K^+ solution" was similarly prepared, except additional KCl was added to yield a final concentration of 22 mM K^+ (NaCl was decreased to maintain isotonicity). During exposure of atria to the high K^+ medium,

Received for publication 11 November 1977 and in revised form 2 February 1978.

stimulation frequency was decreased to 0.2 Hz, voltage intensity was increased severalfold, and l-isoproterenol hydrochloride (Sigma Chemical Co., St. Louis, Mo.) was added to the solution to yield a final concentration (supramaximal) of 50 nM (12–14). Solutions were aerated with 100% oxygen and maintained at 27±0.5°C.

Inotropic effects of gentamicin sulfate (Schering Corp., Kenilworth, N. J.) were studied in heart muscle maintained in the control solution (5.4 mM K^+) and compared with effects observed in muscle beating under the "slow-Ca^{++} activated" (i.e., 22 mM K^+-isoproterenol) conditions. Tests were also made with tetrodotoxin (TTX; Calbiochem, San Diego, Calif.),[1] an agent that selectively blocks the fast Na^+ membrane channels (without depolarization) (14), and with the well-characterized Ca^{++} antagonists D_{600} hydrochloride (A. G. Knoll Co., W. Germany) and lanthanum chloride (La^{+++}) (16, 17). To ensure that release of endogenous norepinephrine was not a modifying influence on tension responses to inotropic interventions, studies were also made with *dl*-propranolol hydrochloride (Sigma Chemical Co.). All concentrations refer to the base.

Contractile tension and *dT/dt* were measured and expressed as grams of developed tension (peak systolic tension minus resting tension) and grams per second, respectively, or values obtained in the presence of a drug were expressed as a percentage of predrug (control) values obtained in that muscle. Measurements are expressed as the mean±1 SEM and the difference between two means was evaluated statistically by Student's *t* test.

RESULTS

Contractile responses to cumulative increases in the concentration of gentamicin (0.05–1.5 mM) were first measured in atria maintained in the normal solution (i.e., 5.4 mM K^+, no isoproterenol) to quantitate the inotropic effects of the antibiotic under standard conditions. As summarized in Fig. 1, gentamicin produced a concentration-dependent decrease in contractile tension and *dT/dt*. The maximal depressant effect of each concentration was obtained within 15 min after its addition to the bathing medium and was reversed within 20 min after placing the tissue in gentamicin-free medium, in agreement with previous temporal studies in rat atria (7).

After verification of the negative inotropic effect of gentamicin, a concentration that produced a 20–30% decrease in tension was selected for comparing the activity of the antibiotic in atria contracting under normal conditions (i.e., 5.4 mM K^+) with its activity in atria contracting under slow channel activation conditions (i.e., 22 mM K^+-isoproterenol). Effects of 0.1 mM gentamicin are shown as representative tracings in Fig. 2, and summarized as mean values in Fig. 3. Upon exposure of beating heart muscle to the high K^+ solution, contractions ceased; however, contractile activity was restored to control strength by isoproterenol (Fig. 2*A*). When added before isoproterenol, 0.1 mM gentamicin prevented restoration of contractions by

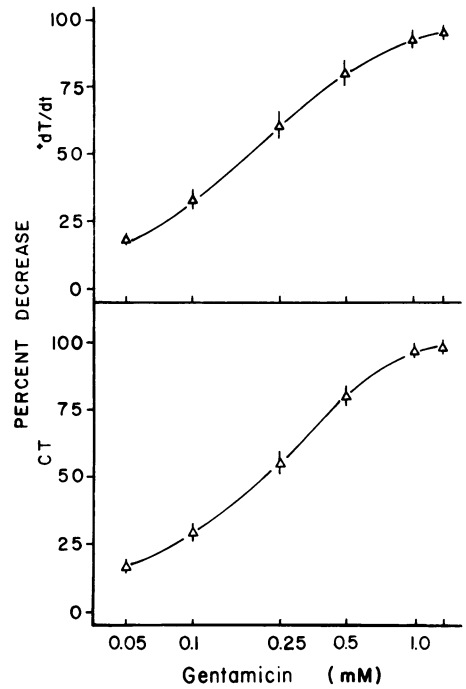

FIGURE 1 Effects of gentamicin on isometric contractile tension (CT) and its first derivative (*dT/dt*) in electrically driven left atria of guinea pigs. Each value is the mean±SE of data from five to seven atria maintained in normal K^+ (5.4 mM) medium and stimulated at 1 Hz.

isoproterenol (four tissues) (Fig. 2*A*). Similarly, when gentamicin was added after contractile responses had been restored by isoproterenol, contractile amplitude was markedly depressed (Fig. 2*B*). Two basic patterns of the depressant effects of 0.1 mM gentamicin were observed: either a progressive depression of contractile strength that reached a maximum effect (>95% decrease in developed tension and *dT/dt*) in about 10 min, or a sudden cessation of contractions within a few minutes that was often preceded by several arrhythmic beats. However, the same concentration of the antibiotic had no inotropic effect in isoproterenol-treated atria contracting in the control (5.4 mM K^+) solution (Fig. 2*B*).

The pronounced depressant effect of gentamicin in high K^+-isoproterenol-treated atria is contrasted in Fig. 3 with the lack of inotropic effect of the antibiotic in control atria (5.4 mM K^+) exposed to isoproterenol. If gentamicin was not added to the bathing media, contractile responses to isoproterenol were well maintained in both control and high K^+-treated atria, as also summarized in Fig. 3. Also sulfate ion (1 mM Na_2SO_4) had no discernible effect on contractile tension in either control or high K^+-isoproterenol-treated tissues.

Unlike gentamicin, TTX did not decrease contrac-

[1] *Abbreviation used in this paper*: TTX, tetrodotoxin.

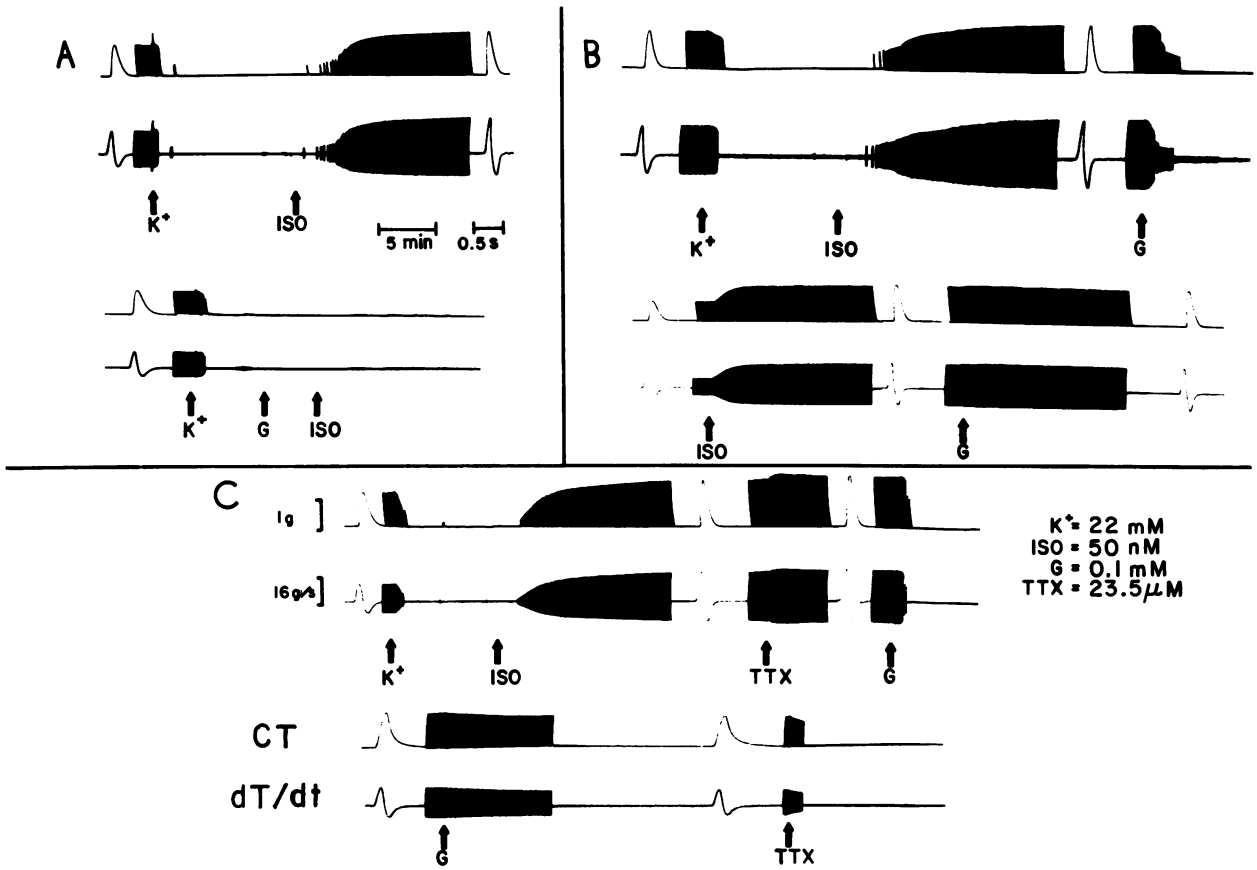

FIGURE 2 Effects of gentamicin (G) in guinea pig left atria contracting under "normal" (5.4 mM K^+) or "slow channel activated" (22 mM K^+-isoproterenol) conditions. (*A*) Contractions (CT) ceased after 22 mM K^+, but were restored by isoproterenol (ISO) (upper tracings). Pretreatment with G (0.1 mM) prevented restoration of contractions by ISO (lower tracings). (*B*) G abolished contractions of muscle beating under high K^+-ISO conditions (upper tracings); however, G had no effect in the ISO-treated atria beating under normal K^+ (5.4 mM) conditions (lower tracings). (*C*) TTX did not stop contractions of atria beating under high K^+-ISO conditions; whereas G blocked contractions (upper tracing). Conversely, under control conditions (5.4 mM K^+; no ISO), G had little effect whereas TTX stopped contractions (lower tracings). The time markers in *A* and the calibration markers and drug concentrations in *C* apply to all tracings. Stimulation frequency is 0.2 Hz.

tions of atria contracting under the high K^+-isoproterenol conditions (Fig. 2*C*). Conversely, TTX completely stopped contractions of heart muscle maintained in the control medium; whereas, 0.1 mM gentamicin had minimal negative inotropic effects (about 20% reduction of tension, see Fig. 4) under the same conditions (Fig. 2*C*).

To determine if the effects of gentamicin were related to a β-adrenoceptor blocking action, comparisons were made between the antibiotic and propranolol in atria bathed in the normal (5.4 mM K^+) medium. These atria were stimulated at 0.2 Hz to correspond with the stimulation frequency of preparations studied under the high K^+ conditions. Effects of 0.1 mM gentamicin and 1 μM propranolol were measured (*a*) after the positive inotropic response of atria to 50 nM isoproterenol reached steady state (15 min after addition of isoproterenol), or (*b*) in atria not treated with isoproterenol. As shown in Fig. 4, gentamicin decreased tension and *dT/dt* by 23 and 22%, respectively, in atria not treated with isoproterenol, but had no measurable inotropic effect during isoproterenol treatment. Conversely, propranolol had a pronounced depressant effect in heart muscle exposed to isoproterenol (decrease in tension and *dT/dt* by 65 and 75%, respectively), but had little effect in atria not treated with isoproterenol (Fig. 4). These experiments differentiate the activities of gentamicin and propranolol, and also confirm (*a*) the β-blocking activity of 1 μM propranolol in these tissues, and (*b*) that release of endogenous

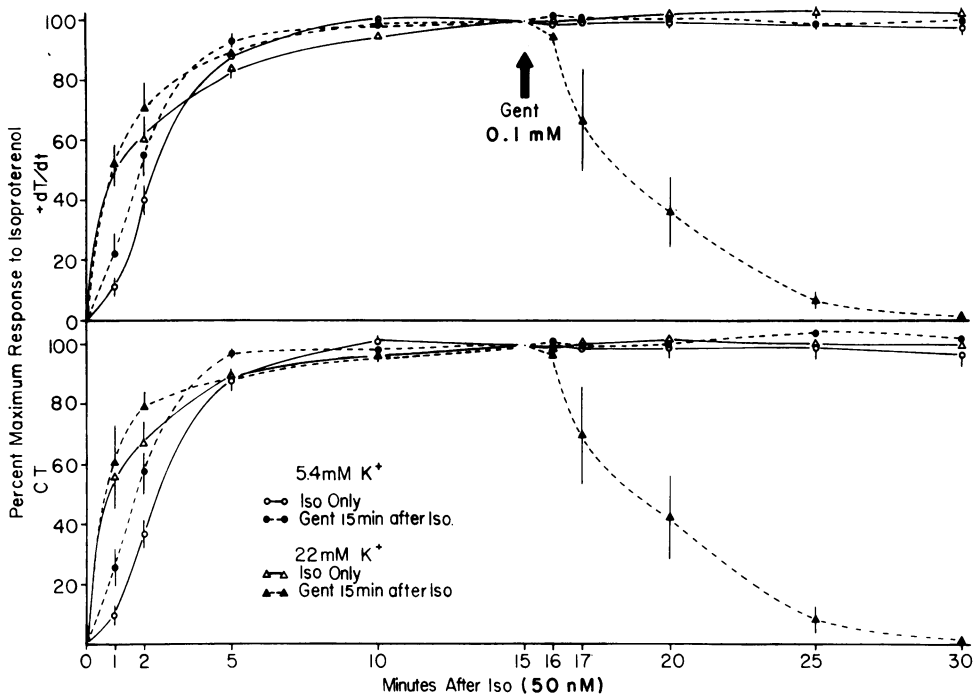

FIGURE 3 Inotropic effects of gentamicin (Gent) in guinea pig left atria exposed to isoproterenol (ISO) in either a normal K^+ (5.4 mM) or high K^+ (22 mM) medium. Each value is the mean±SE of data from five different muscles. Atria in either the 5.4 mM K^+ (●---●) or 22 mM K^+ (▲---▲) solution were exposed to 0.1 mM Gent 15 min after ISO. Paired control atria in the 5.4-mM K^+ (○---○) and 22-mM K^+ solutions (△---△) were not treated with Gent. Gent values in the high K^+ medium (▲---▲) were significantly different than corresponding values of the other three groups at all time intervals after 16 min ($P < 0.001$). Stimulation frequency is 0.2 Hz.

catecholamines is not a major characteristic of these preparations (15).

The inotropic response to increased rate of stimulation (i.e., treppe, Bowditch effect) was used to compare the negative inotropic activity of gentamicin with the activities of D_{600} and La^{+++} in atria bathed in the normal medium. Frequency-tension relationships (0.1–2.2 Hz) were determined under control conditions (no drugs) and again (after rinsings, restabilization at 1 Hz) after the negative inotropic effects of equipotent concentrations of La^{+++} (0.5 mM), D_{600} (1.05 μM), or gentamicin (1 mM; a 10-fold greater concentration than that used in the high K^+-isoproterenol-treated atria) had reached a steady state. Representative myograms of the changes in contractile tension are shown in Fig. 5; mean values for tension and *d*T/*dt* are summarized in Fig. 6. As stimulation frequency was increased, a rate-dependent increase in contractility occurred in each control atrium that reached a maximal effect at about 2.0 Hz. The magnitude of the frequency-force curves was markedly depressed by both gentamicin and La^{+++}; however, contractile tension characteristically increased, even in the presence of pronounced inotropic depression induced by either of these drugs (Figs. 5 and 6). Conversely, in atria depressed by D_{600}, contractile strength actually decreased as frequency was increased (Figs. 5 and 6).

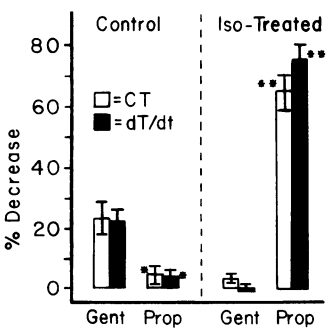

FIGURE 4 Effects of gentamicin (Gent) and propranolol (Prop) on contractile tension (CT) and *d*T/*dt* of guinea pig left atria before or during isoproterenol (ISO) treatment. Each value is the mean±SE of data from four or five different preparations. Effects of Prop (1 μM) or Gent (0.1 mM) were determined in tissues not exposed to ISO (control) or in tissues exposed for 15 min to 50 nM ISO (ISO-treated). Normal K^+ (5.4 mM) medium; stimulation frequency is 0.2 Hz. □ = CT; ■ = *d*T/*dt*. Mean value of Prop group is significantly different than corresponding value of Gent group, $^*P < 0.05$; $^{**}P < 0.001$.

Propranolol (1 μM) did not affect frequency-force relationships (Fig. 6), indicating that release of endog-

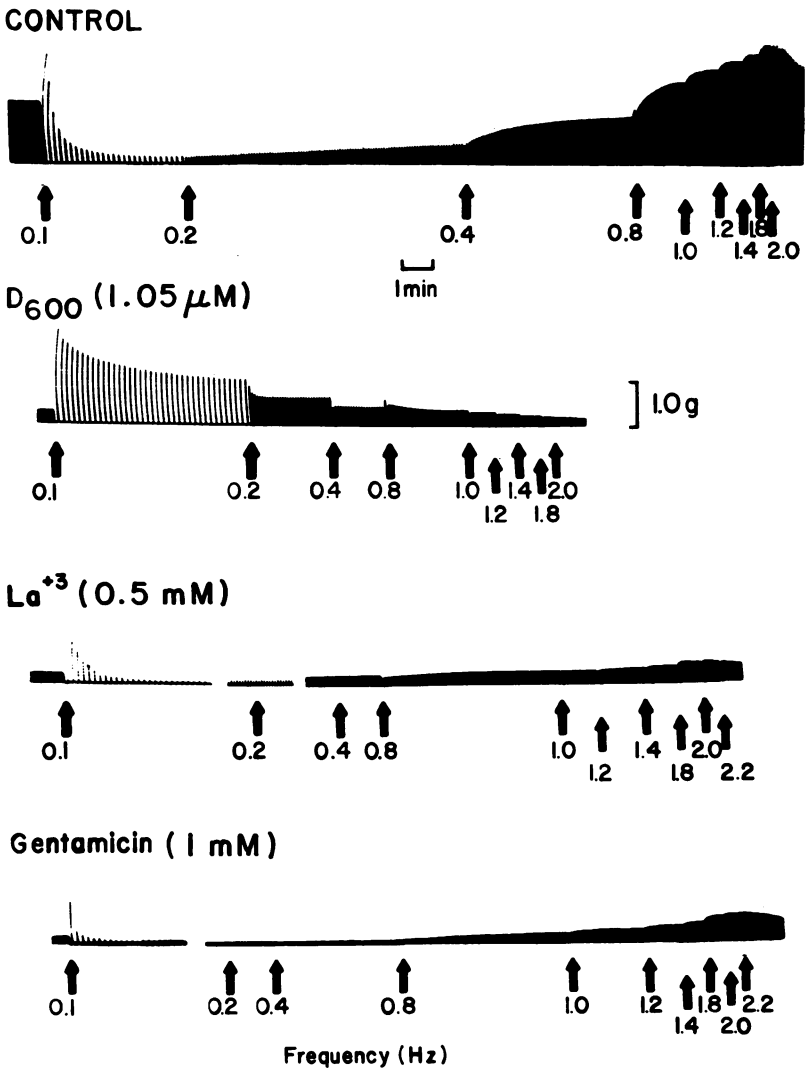

FIGURE 5 Effects of D_{600}, La^{+++}, and gentamicin on frequency-force responses in guinea pig left atria. Typical myograms of contractile responses to increased stimulation frequency (0.1–2.2 Hz) before (control) or after the negative inotropic effects of D_{600}, La^{+++}, or gentamicin reached a steady state at the control frequency of 1 Hz.

enous norepinephrine is not a complicating factor in inotropic responses of these preparations to changes in stimulus frequency.

DISCUSSION

Present findings provide a previously unrecognized and rather surprising pharmacodynamic aspect of an aminoglycoside antibiotic. Evidence was obtained indicating that gentamicin inhibits the influx of Ca^{++} across the sarcolemma of contracting myocardial cells, thereby decreasing the amount of Ca^{++} available for the interior of the myofiber.

The dependence of heart muscle contraction on an increase in the intracellular concentration of free Ca^{++} is unequivocal, and an inward movement of this cation through specific slow channels of the sarcolemma directly activates the contractile proteins and(or) triggers a regenerative release of additional Ca^{++} from cellular storage sites (10, 17). Activation of Ca^{++} movement through the slow channels is dependent, under normal conditions, upon a preceding rapid influx of Na^{+} through fast channels of the sarcolemma. The rapid Na^{+} influx, reflected as the upstroke phase (rapid depolarization) of the cardiac action potential, lowers membrane potential to the threshold level for slow channel activation. However, independence from the fast Na^{+} channels can be gained by selectively inactivating these fast channels (e.g., with TTX or partial depolarization by high K^{+}) and activating the slow channels (e.g.,

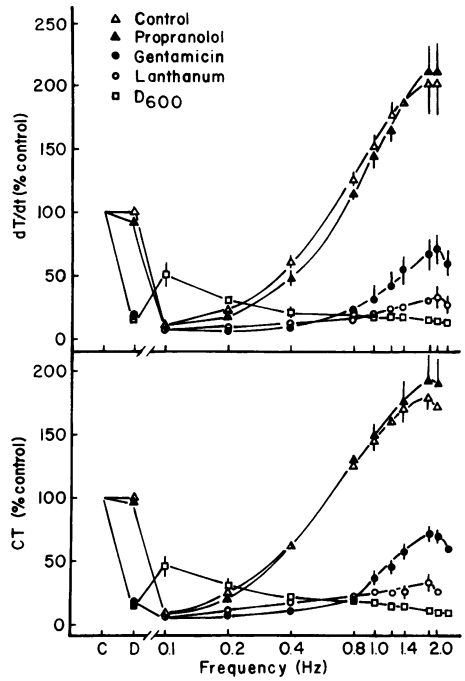

FIGURE 6 Effects of propranolol, D_{600}, La^{+++}, and gentamicin on frequency-force responses in guinea pig left atria. Each value of contractile tension (CT) and *dT/dt* is the mean±SE of data from three to six preparations. Contractile responses to increased stimulation frequency (0.1–2.2 Hz) were determined before (control, △) or during exposure to a drug. C = value at control (1 Hz) predrug level; D = value after effects of propranolol (1 μM; ▲), gentamicin (1 mM; ●), La^{+++} (0.5 mM; ○), or D_{600} (1.05 μM; □) reached steady state at the control frequency of 1 Hz.

with isoproterenol) (12–14). The resulting action potential resembles the plateau portion of the normal cardiac action potential and is due to an inward "slow Ca^{++} current". The slow channels are insensitive to TTX but can be inhibited by D_{600}, Mn^{++}, La^{+++}, Co^{++}, Ni^{++}, and Ca^{++}-free solution (12–14, 16, 17); present data indicate that gentamicin should be recognized as a putative member of this list.

In the present study, a concentration of gentamicin that had minimal inotropic effect on control heart muscle preparations markedly depressed or even arrested contractions of the high K^+-isoproterenol-treated atria. Thus, the negative inotropic activity of gentamicin was profoundly augmented when heart muscle contractions were characterized by accentuated dependence upon inward movement of Ca^{++} through slow channels of the sarcolemma. This suggests that the antibiotic in some way affects either the transport system(s) responsible for Ca^{++} influx through the slow channels, the availability of Ca^{++} for these channels, or both. Since TTX selectively blocks the fast Na^+ channels, this toxin has been used to test for the dependence of myocardial contractions on slow Ca^{++} current in K^+-depolarized preparations (12, 14). We, too, found that although TTX abruptly stopped mechanical activity in tissues bathed in control medium, it had no negative inotropic effect in the high K^+-isoproterenol-treated preparation. This result provides additional evidence that the mechanical activity blocked by gentamicin was selectively dependent upon slow channel activation, and also differentiates the action of gentamicin from that of TTX.

A β-adrenoceptor blocking action seems untenable as an explanation for the depressant effect of gentamicin in isoproterenol-activated myocardium since comparative studies with the antibiotic and propranolol indicated dissimilar effects of these two drugs on inotropic responses to isoproterenol in the normal bathing medium. Previous work by others has shown that β-receptor agonist-antagonist interrelationships are not changed during exposure of cardiac preparations to high K^+ media (14).

Based on frequency-force relationships as a test inotropic intervention, a basic dissimilarity between the effects of D_{600} and gentamicin was identified. Whereas gentamicin and La^{+++} only decreased the magnitude of the frequency-force responses, D_{600} converted the positive relationship to a negative relationship. Inversion of the frequency-force relationship by D_{600} has previously been explained by a rate-dependent inhibitory effect on Ca^{++} movement through the slow channels, thereby preventing refilling of other Ca^{++} binding sites (17, 18). On the other hand, La^{+++} displaces and replaces Ca^{++} at various superficial membrane sites, thereby reducing net availability of Ca^{++} for translocation (17, 19). We have previously reported that aminoglycosides decrease the uptake and increase the efflux of ^{45}Ca in vascular smooth muscle by affecting superficial membrane sites and, through this Ca^{++}-dependent action, inhibit contractile responses of arteries to a variety of vasoconstrictor agents (5, 6, 20). Based on present mechanical findings, it seems that gentamicin also disrupts the excitation-contraction coupling process in heart muscle, perhaps by inhibiting the binding of Ca^{++} at superficial membrane sites responsible for availability of Ca^{++} for transsarcolemmal systolic influx.

If present findings with an isolated heart muscle model relate to other excitable tissues, then an interference by aminoglycoside antibiotics with Ca^{++} influx across cell membranes may well explain the depressant properties of these drugs previously observed in a variety of functionally related and unrelated tissues in both man and lower animals (1–9). Importantly, present observations also provide a mechanistic explanation for cardiac irregularities occasionally detected in patients treated with aminoglycoside antibiotics (4, 21–23).

ACKNOWLEDGMENTS

The authors wish to thank Doctors Janet L. Parker and James Willerson for suggestions and manuscript critique. The D_{600} was a generous gift from A. G. Knoll Co., W. Germany.

This work was supported by the U. S. Public Health Service, the Food and Drug Administration, Department of Health, Education, and Welfare grant 223-76-7312, and by the National Institutes of Health, grant 07062 from the National Institute of General Medical Sciences.

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