Effect of Changes in Cycle Length on Diastolic Depolarization Produced by Ouabain in Canine Purkinje Fibers

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ABSTRACT

Isolated canine papillary muscle–false tendon tissue preparations stimulated at a cycle length of 630 msec were treated with ouabain (2.1 X 10⁻⁸ M) until an increase in the slope of diastolic depolarization of Purkinje fibers was produced. Then the effects of changes in cycle length on the slope were tested. Sustained shortening of the cycle length increased the slope, and sustained lengthening had the opposite effect. An abrupt decrease in cycle length caused by a stimulus during phase 3 of a driven beat induced an increase in the slope of diastolic depolarization for one or more subsequent cycles. This procedure occasionally led to the spontaneous generation of an action potential which propagated into surrounding tissue. Suspension of stimulation often was followed by spontaneous beats. Shortening of the cycle length decreased the time of onset and increased the number and frequency of spontaneous beats. These effects were correlated with increases in the slope of diastolic depolarization. The diastolic depolarization of Purkinje fibers in false tendons was increased earlier and to a greater degree by ouabain than was the depolarization of Purkinje fibers on the muscle surface. These findings suggest that enhancement of diastolic depolarization of Purkinje fibers to the point of spontaneous discharge of action potentials may be a common means by which digitalis produces a variety of ventricular arrhythmias observed in intact animals.

KEY WORDS pacemaker potential arrhythmias cardiac glycosides transmembrane potentials phase-4 depolarization microelectrodes ventricular muscle fibers spontaneous action potentials

Ventricular tachyarrhythmias are a common sign of digitalis toxicity, and many studies have been done both with intact hearts and with single cardiac fibers to define the mechanisms responsible for their occurrence. One possible mechanism for the onset of such arrhythmias involves the enhanced automaticity of ventricular pacemakers: digitalis compounds do increase the slope of phase-4 depolarization of Purkinje fibers (1–6). However, not all types of digitalis-induced arrhythmias observed in intact animals are clearly the result of spontaneous generation of action potentials. Vassalle et al. (7) conducted a detailed analysis of arrhythmias produced by ouabain infusion in dogs. They divided the observed disorders in rhythm into two groups. The first group was characterized by activity of ventricular foci which functioned independently of the sinus node. The cellular basis for this type of arrhythmia could reasonably be a sufficient enhancement of phase-4 depolarization to the point of spontaneous discharge of action potentials. The second group was composed of arrhythmias which arose only in response to an initiating beat which usually was of sinus origin. These arrhythmias often were single extrasystoles coupled to a normal sinus beat and were termed reentry arrhythmias. However, the coupling intervals were variable rather than constant, and occasionally runs of extrasystoles were produced by a single initiating beat. Also, the frequency of occurrence of such arrhythmias and the number of beats composing them were greater when the basic sinus rate was fast or above normal. The cellular basis for these arrhythmias was not apparent.

More recently Lown and co-workers (8–10) studied the effects of single premature excitations on the cardiac rhythm of dogs before and during digitalization. While digitalis was being given, a single stimulus applied to the ventricle elicited one or more spontaneous beats. This phenomenon was termed a repetitive ventricular response, and was
enhanced by increases in heart rate (11). The mechanisms responsible for repetitive ventricular response have not been elucidated. The purpose of the present experiments was to study the cellular mechanisms by which the above types of arrhythmias might be produced.

Methods

Papillary muscle—false tendon tissue preparations were isolated from the hearts of dogs anesthetized with sodium pentobarbital (30 mg/kg, iv). A preparation was mounted in a tissue chamber perfused with oxygenated Tyrode's solution (12) at 37.5–38°C. Contraction at 93/min were produced by electrical stimuli. Glass microelectrodes were used to record the transmembrane potentials of Purkinje fibers. Occasionally two microelectrodes were used to record from different fibers simultaneously. Details of the perfusion and recording techniques have been described previously (12).

In all experiments photographic records were obtained during a control perfusion; then ouabain (2.1 x 10^{-7}M) was administered to produce an increase in the slope of phase-4 depolarization. In most experiments, treatment with ouabain was stopped and control perfusion was reinitiated after a moderate increase in magnitude of diastolic depolarization (10–20 mv) had occurred. Ouabain-induced diastolic depolarization persisted for 30 or more minutes after removal of ouabain from the perfusate (6). This procedure yielded a period of relatively steady-state conditions in which to test the effects of various changes in cycle length. In other experiments, ouabain was administered continuously, and the fiber was depressed to a greater degree as evidenced by decreases in maximum diastolic potential, amplitude, and duration of the action potential. Tests of the effects of alterations in cycle length were made during the progress of the ouabain treatments. A detailed description of the effects of ouabain on Purkinje fibers as determined in this laboratory has been given previously (6).

Sustained increases or decreases in the rate of response were produced by changing the frequency of the drive stimuli. These rates can be indicated either by stating the number of contractions per minute or by giving the cycle length in milliseconds. Thus, the control cycle length was 630 msec (approximately 93/min). An abrupt shortening in the cycle length which persisted for a single beat was produced by applying a suprathreshold stimulus during phase 3 of a driven response. The use of these stimulating techniques in specific experiments will be described in Results.

Results

Initial experiments present the effects of steady rates of stimulation on the slope of ouabain-induced diastolic depolarization. In eight experiments, a steady-state increase in the rate and the magnitude of diastolic depolarization was produced with ouabain, and then the effects of rate changes were determined on the same fiber within a short period. Figure 1A–C shows the results from a fiber made to beat steadily for a minute or longer at each of three cycle lengths. Dashed lines have been drawn tangent to the slope of diastolic depolarization on each record, and it is apparent that shortening of the cycle length caused an increase in the slope of diastolic depolarization and that lengthening had the opposite effect. The superimposed records of Figure 1D are from a different preparation and illustrate the same effect. Also, there was a small decrease in the maximal diastolic potential at the higher rates of stimulation. In addition, the rate of diastolic depolarization sometimes was not steady throughout the diastolic interval but tended to decline during the latter portion (Fig. 1A). Thus the level of membrane potential at the time of excitation was determined by the interaction of several factors.

In similar experiments, it was not possible to detect changes in diastolic depolarization at cycle lengths of less than about 400 msec, since the diastolic intervals at these faster rates were too short to allow a depolarization phase. However, the tendency of the fiber to develop slow depolarization at fast rates could be detected from experiments in which rapid stimulation was administered steadily for about a minute and then abruptly terminated. The ability of the fiber to undergo diastolic depolarization was evident in the period immediately following cessation of the driven responses.
Effects of cycle length on the slope and the magnitude of depolarization after the cessation of driven responses recorded by moving the film past a vertically deflecting oscilloscope beam. A-D are from the same fiber responding at the indicated cycle lengths (msec). Termination of driven responses is evident. The slope and the magnitude of the depolarization phase at this time should be noted. E and F are from a different fiber in the same preparation. A single premature excitation marked by the black bar was given in F immediately before termination of driven responses, but in E premature excitation was not produced. Time and voltage calibrations are 100 msec and 100 mv for all records.

Figure 2 shows the results from such an experiment. Figure 2A–D are from the same fiber and show the effects of the termination of the drive stimuli after different cycle lengths had been applied for 1 minute. Both the rate and the magnitude of the diastolic depolarization phase increased with shortening of the cycle length. Figure 2E and F are from a different fiber in this preparation stimulated at the control frequency. In Figure 2F, a single premature excitation was produced immediately before cessation of stimulation. The rate and the magnitude of the depolarization phase were greater than those observed when a premature excitation was not produced, as in Figure 2E.

It was unnecessary to discontinue drive stimulation to observe this latter type of response. Figure 3 illustrates results obtained from four fibers from different hearts. In all experiments, drive stimuli were administered at the control cycle length, and a
single premature excitation was produced without alteration in the driving frequency. This procedure resulted in an increase in the slope of diastolic depolarization during the first normal-length diastolic interval following the test stimulus. Lines were drawn tangent to the diastolic phase on a cycle before the test stimulation and on the first normal-length diastolic interval after the test stimulation to aid visualization of the changes in the slope. These experiments demonstrated that an abrupt decrease in cycle length which persisted for only a short time could cause an increase in the rate of diastolic depolarization. In some of these experiments the maximal effect on diastolic depolarization was not produced until the second diastolic interval following the premature excitation. Also, the effects of the excitation sometimes persisted for two or more cycles following the test stimulation. Figure 4 illustrates these observations. In Figure 4A, the cell developed the characteristics of a true pacemaker fiber: a smooth progressive transition from phase-4 depolarization to phase 0 occurred on the second response following the test stimulation. This effect will be illustrated in greater detail (see Fig. 8).

In the experiment depicted in Figure 2, the fiber did not discharge spontaneously in the period of suspended drive stimulation, but in other experiments of this series spontaneous beats were observed during this time. Figure 5 shows such responses in two experiments. Two microelectrodes were used to record from Purkinje fibers simultaneously. In Figure 5A, B, and D, spontaneous excitations occurred on cessation of the drive stimuli. Shortening of the cycle length resulted in an earlier onset of the excitations and increased their number and frequency. Also, the slope of diastolic depolarization declined as the spontaneous rate decreased.

In several preparations, some cells appeared to generate action potentials by the normal pacemaker mechanism. Figure 6 illustrates results from four such cells. In A, when the drive stimulus was turned off, the cell underwent rapid depolarization but failed to discharge an action potential. However, if a single premature excitation preceded termination of the drive stimulation (Fig. 6B) the fiber discharged spontaneously with the characteristics of a true pacemaker cell. Figure 6C and D illustrate a similar experiment with a different preparation. Figure 6D and F show the action potentials of a Purkinje fiber in the top trace and those of a ventricular muscle fiber in the bottom trace. Cessation of the drive stimuli resulted in a

![Figure 4](image-url)

Effects of a single premature excitation on the slope of ouabain-enhanced diastolic depolarization. The procedure was the same as in Figure 3. The single test excitation has been marked by a vertical bar; its effects persisted for more than a single cycle. In A, the cell developed the characteristics of a true pacemaker for a single beat. Time and voltage calibrations are 100 msec and 100 mv.
Effects of cycle length on the diastolic depolarization and the occurrence of spontaneous beats following cessation of stimulation. A and B are from the same cells in one preparation. C and D are from a different heart. In each experiment two microelectrodes were used to record from different Purkinje fibers. In A and B the top action potentials are from a cell in the false tendon and the bottom action potentials from a cell on the surface of the muscle. In C and D this positioning is reversed. The fibers were stimulated at the cycle lengths indicated for 1 minute or more and then drive stimulation was terminated. See text for discussion. Time and voltage calibrations are as in Figure 4.

depolarization of relatively large magnitude in the Purkinje fiber (Fig. 6E), but there was no propagation to the ventricular recording site since this fiber did not respond. However, when a single premature excitation was produced before stopping the drive stimuli (Fig. 6F), an action potential was generated in the Purkinje cell which propagated to the ventricular muscle fiber. Very similar results are shown in Figure 6G and H, for which both microelectrodes were located in Purkinje fibers. The cell whose action potentials are shown in the bottom tracing discharged spontaneously, and propagation to the other recording site occurred only when a premature excitation preceded termination of the drive stimuli.

During the course of these experiments, the preparations responded regularly to the drive stimuli until the trials of test stimulation were initiated; at this time arrhythmias sometimes occurred which persisted for variable times. Although the precise mechanisms responsible for these arrhythmias are not known with certainty, it seems possible that spontaneous generation of action potentials might be involved. In many experiments, the fiber under study was not behaving as a pacemaker; in fact, its rate of diastolic depolarization was relatively slow, and the contour of its action potential at these times often was unaltered to any marked degree. Still the above hypothesis would be reasonable if other cells in the preparation were altered more. To study this possibility, two microelectrodes were used to record simultaneously from different fibers. One electrode was located in a fiber of the false tendon, and the second electrode was inserted into a Purkinje fiber on the surface of the muscle. Care was taken to impale a cell on the immediate surface at both sites. In control solution the characteristics and the magnitude of the membrane potential of both cells were normal for Purkinje fibers. Treatment with ouabain usually (8 of 11 experiments) produced changes earlier in the fiber of the false tendon than in the fiber on the muscle surface. In 1 experiment, this effect was reversed and, in 2 experiments, there was no significant difference between cells. Figure 7 illustrates a typical experiment, and similar effects can be observed in Figures 5 and 6G–H.

The final observations describe the characteristics of certain spontaneous arrhythmias. Numerous dysrhythmias are shown in Figure 8. The actual mechanisms responsible for their occurrence are not known, and they admittedly cannot be produced at
Ability of a single premature excitation to evoke pacemaker discharge following the cessation of the drive stimuli. A and B are from a Purkinje fiber in one preparation and C and D are from a fiber in another preparation. Premature excitation before cessation of drive stimulation (B and D) caused generation of an action potential by the true pacemaker mechanism. E and F show the action potentials of a Purkinje fiber on the top trace and those of a ventricular muscle fiber on the bottom trace. The experiment was as described for A-D. The spontaneous action potential generated in F was propagated to the ventricular recording site. G and H illustrate an essentially similar experiment. Here the top trace is from a false tendon fiber and the bottom trace is from a Purkinje fiber on the muscle surface. The voltage calibration equals 100 mv, and the time calibration equals 500 msec for A-D and 100 msec for E-H.

Differential response of two Purkinje fibers during exposure to ouabain. The same fibers were recorded from throughout the experiment. The top action potentials are from a fiber in the false tendon and the bottom action potentials are from a fiber on the surface of the muscle. A is from the control solution. B, C, D, and E were recorded at 30, 33, 38, and 45 minutes respectively, during the treatment with ouabain. It is apparent that the cell in the false tendon responded earlier and to a greater degree to ouabain than did the cell on the muscle surface. Time and voltage calibrations are 100 msec and 100 mv.

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Examples of various spontaneous dysrhythmias observed during treatment with ouabain. Only D and E are from the same preparation. The drive stimuli were given steadily during some of these records and have been marked by black dots. Discussion of specific records is in the text. The black bars in D, E, G, and H mark the application of a single test stimulus.

Discussion

The results presented in this paper on the effects of relatively long-duration changes in the rate of stimulation on the slope of ouabain-induced diastolic depolarization confirm those of Hogan et al. (13) and Gandel et al. (14) reported in preliminary communications. In an earlier study, a dependency of the slope of diastolic depolarization on heart rate was described in experiments in which increases...
in slope were produced by the elevation of calcium concentration (12). In the latter study, spontaneous beats did not occur on cessation of drive stimulation; but in the present experiments with ouabain, such beats frequently were observed during this time. Furthermore, the onset was earlier, the rate faster, and the number greater when the rate of response was increased. These effects were correlated with increases in the slope of diastolic depolarization. The slope always was greatest immediately following the last driven response, and it sometimes led to excitation. When two or more spontaneous beats occurred after the cessation of stimulation (Fig. 5A, B, and D), the slope of depolarization in each diastolic interval declined progressively and was accompanied by a slowing of the rate. Thus it is evident that in the presence of ouabain fast rates of stimulation enhance the tendency of a Purkinje fiber to undergo diastolic depolarization and may lead to spontaneous discharge.

This finding may illustrate the cellular mechanism for certain observations made in intact dogs injected with ouabain (7, 15, 16). In these studies, the transient acceleration of ventricular rate produced by electronic pacing resulted in enhanced automaticity of the ventricles as evidenced by a shortening in the time for onset of spontaneous ventricular beats when the origination of or the conduction of atrial impulses into the ventricles was prevented. In addition, the number of spontaneous beats and their frequency were increased with an increase in the pacing rate. Therefore, the responses in intact dogs probably arise from spontaneous generation of action potentials by Purkinje fibers with enhanced diastolic depolarization.

The design of certain experiments done in this paper allows discussion of the possible mechanisms involved in repetitive ventricular response. This phenomenon has been investigated extensively by Lown and co-workers (8-11) and consists of one or more ventricular beats elicited by a single premature ventricular excitation in the digitalized dog. The onset of repetitive ventricular response and the number of spontaneous beats composing it are dependent on the degree of digitalization and the heart rate. In the present study, a single premature excitation of a Purkinje fiber with ouabain-enhanced diastolic depolarization resulted in an increase in the slope of diastolic depolarization for one or more subsequent cycles. On rare occasions, this procedure caused an apparently true pacemaker discharge by the fiber when the drive stimuli continued without pause (Figs. 4A and 8H). However, spontaneous generation of action potentials was observed more frequently when the drive stimulus was discontinued. This procedure more closely simulates the condition in the intact dog caused by premature ventricular excitation which results in a compensatory pause. It was shown that the spontaneously generated action potentials were propagated into surrounding Purkinje and ventricular muscle tissues. Thus the repetitive ventricular response might be the result of enhanced diastolic depolarization of Purkinje fibers to the point of spontaneous discharge. In addition, a similar mechanism might be operative in the onset of extrasystoles appearing as the result of an initiating beat (7). At least it is not necessary to postulate a disturbance of conduction as the causative agent, although this possibility cannot be ruled out for reasons noted below.

Rapid rates of discharge that increased the slope of diastolic depolarization were especially evident in experiments in which spontaneous dysrhythmias developed (Fig. 8). Often a period of rapid erratic discharge resulted in enhanced diastolic depolarization which progressed smoothly into an action potential. This phenomenon is the basic mechanism by which known cardiac pacemaker fibers function, and thus it provides additional support for the theory that ectopic focal discharge is the mechanism for digitalis-induced ventricular arrhythmias (5, 6). However, a reduction in the rising velocity of phase 0 and in the amplitude of the action potential coincides with the development of diastolic depolarization (17). These changes slow the conduction velocity along the fiber. Slowing of the conduction velocity favors the onset and the maintenance of circuitous movement and the reentry of excitation (18, 19). Therefore, it is impossible to accept exclusively either of the two classical theories accounting for the tachyarrhythmias.

Not all fibers in any given preparation responded to ouabain at the same time or to the same degree. Usually fibers in a false tendon were more sensitive than those on the muscle surface. The reasons for this difference are not known, but two of the possibilities can be ruled out. First, only cells on the surface of the preparation were impaled; therefore, presumably each cell was exposed equally to the ouabain. Second, cells near the cut edges of the preparation which might have been damaged were avoided. Also, damage is not a likely factor since in
the control solution the magnitude of the maximum diastolic potentials and the amplitude and configuration of the action potentials were normal. Whatever the reasons for the differential response the fact that it occurs has some practical implications for relating isolated tissue data to phenomena observed in intact dogs. Some of the responses to changes in cycle length were not observed in all cells or in all preparations. This finding was especially true for the spontaneous generation of action potentials by the fiber under study. However, isolated tissue preparations contain only a fraction of the total Purkinje tissue of the intact ventricles and only one or two fibers are recorded from during an experiment. Therefore, the lack of uniform response does not weigh heavily against the proposal that spontaneous discharge is a common means for onset of a variety of ventricular arrhythmias in the intact dog receiving digitalis.

References
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