Triggered Activity in Cardiac Muscle Fibers of the Simian Mitral Valve

ANDREW L. WIT, PH.D., AND PAUL F. CRANEFIELD, M.D., PH.D.

SUMMARY The action potential of cardiac fibers in the anterior mitral-valve leaflet of the monkey heart is followed by an after-hyperpolarization. The addition of catecholamines causes a delayed after-depolarization to follow the after-hyperpolarization. The amplitude of the after-depolarization increases as the stimulus cycle length is decreased, or after premature stimulation, and as a result can reach threshold to yield nondriven, sustained rhythmic activity which we term triggered activity. This sustained rhythmic activity can be terminated by a single, appropriately timed, premature stimulus. The amplitude of the action potentials of mitral valve fibers is increased by catecholamines; the amplitude and rate of depolarization are depressed by verapamil. The amplitude of the action potentials is little affected by tetrodotoxin (TTX) but the maximum rate of depolarization is reduced by TTX. The delayed after-depolarization induced by catecholamines is abolished by verapamil, as is triggered activity. These observations suggest that mitral valve fibers generate slow response action potentials, that triggerable sustained rhythmic activity may be a property of the slow response and that such activity may cause the types of cardiac arrhythmias that usually are attributed to reentry.

WE HAVE described, in Purkinje fibers exposed to sodium-free solutions, a form of sustained rhythmic activity that can be initiated and sometimes terminated by a single stimulus and that is probably not reentrant.1-4 The driven action

Supported by U.S. Public Health Service Grants HL-12738-06 and HL-14899 from the National Heart and Lung Institute.

Dr. Wit was a Senior Investigator of the New York Heart Association during this study and is a Career Scientist of the Irma T. Hirschl Trust.

Received April 3, 1975; accepted for publication October 13, 1975.

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From the Department of Pharmacology, Columbia University College of Physicians and Surgeons, and The Rockefeller University, New York, New York.
The delayed after-depolarization reaches threshold and evokes the next action potential. Without an initial driven action potential a quiescent fiber remains quiescent. Triggered activity also has been described for sinoatrial nodal fibers exposed to low [Na⁺], embryonic fibers, and Purkinje fibers exposed to digitalis. Sustained rhythmic activity that depends on depolarizing afterpotentials is of obvious interest as a possible cause of arrhythmias. We now report that fibers of the mitral valve of the monkey heart show this behavior when exposed to catecholamines and a normal ionic environment. Sustained rhythmic activity can be triggered in such fibers by a gradual increase in drive rate, by a single premature stimulus interposed in a train of regular driven impulses, or by a single driven impulse evoked in a quiescent fiber; this triggered rhythmic activity can be arrested by a premature impulse. In addition, the upstrokes of the action potentials of fibers of the mitral valve of the monkey heart are less resistant to verapamil; they are more resistant to tetrodotoxin (TTX) than are the upstrokes of action potentials in ordinary atrial fibers; and they persist at high levels of [K⁺], when the resting membrane potential is markedly reduced; these action potentials thus depend on the slow inward current. These findings, together with previous reports on triggered activity, suggest that the phenomenon of triggered activity may be a property of the slow response.

Methods

Isolated anterior mitral valve leaflets or leaflets attached to the anteromedial atrial wall were obtained from the hearts of 30 pigtail monkeys. The monkeys (weighing 5–8 kg) were anesthetized with sodium pentobarbital (30 mg/kg, iv); the hearts were rapidly removed through a thoracotomy and dissected at room temperature in a modified Tyrode’s solution, as previously described for the canine heart. The preparation was pinned to the wax base of a 50-ml tissue superfusion chamber with the atrial surface of the mitral valve and endocardial surface of the atrial wall facing upward. The tissue was superfused at a rate of about 15 ml/min with the modified Tyrode’s solution that was saturated with a 95% O₂-5% CO₂ mixture. Temperature was maintained at 36 ± 0.5°C.

The preparation was stimulated through bipolar Teflon-coated silver electrodes on the atrial wall or on the valve leaflet by rectangular pulses 1.5x–2x threshold and 3 msec long. Premature impulses could be applied to the preparation at any given interval after the preceding basic drive stimulus; the basic drive following the premature stimulus could be omitted to determine whether nondriven action potentials occurred. When the effects of premature stimuli were studied the stimuli were induced after every 10th basic drive stimulus. Action potentials were recorded from atrial wall fibers and mitral valve fibers through intracellular electrodes filled with 3 M KCl. Atrial muscle fibers usually extend only 1-4 mm into the simian mitral valve near its attachment to the atrial wall and not into the midvalve leaflet or more distal regions as in the dog.

Epinephrine or norepinephrine was added either directly to the bath without stopping the inflow, to achieve an estimated transient concentration of 0.5–2.0 μg/ml, or to the reservoir of Tyrode’s solution to give the desired concentration. When catecholamines were added to the reservoir ethylenediaminetetraacetate (EDTA) was also added to give a concentration of 10⁻⁴ M. In three experiments norepinephrine was applied to the surface of the mitral valve through a micropipette with a tip diameter of about 30 μ and connected to a syringe system. The concentration of epinephrine or norepinephrine in the injection fluid was 2 μg/ml; the concentration that reached the tissue was probably much lower. When the effects of verapamil or TTX were studied, the drugs usually were added to the reservoir to give the desired concentration, but to attain a very high concentration of TTX it sometimes was added to the tissue bath with the flow stopped.

In three experiments the sequence of excitation was determined during sustained rhythmic activity; an extracellular electrogram recorded from the atrial wall served as a time reference. The time of activation of sites on the atrial leaflet and atria relative to the reference site was determined at a recording speed of 5–20 msec/cm. We determined the location of each site by using the stage movement on the micromanipulator and plotted the times of activation on a diagram of the preparation, as we have previously described.

TERMINOLOGY

In this report we have used several recently introduced terms, defined as follows:

After-hyperpolarization. An afterpotential that is continuous with phase 3 repolarization but that carries the membrane potential to a level more negative than that seen later in diastole.

Delayed After-depolarization. A depolarizing afterpotential that occurs after repolarization has brought membrane potential to the level characteristic of diastole. The delayed after-depolarizations described in the present report invariably followed an early after-hyperpolarization.

Spontaneous or Automatic Activity. The activity that arises in the absence of an external cause, i.e., activity that does not have to be triggered by a stimulated action potential. An example of such activity is that of the sinoatrial node. In fibers that remain quiescent until driven at least once, and thereafter initiate nondriven action potentials, the resultant activity does not arise spontaneously even though it persists once it is evoked. We call this persistent activity sustained rhythmic activity (see below).

Triggered Activity. Activity in which nondriven action potentials are initiated by one or more driven action potentials. The term triggered underlines the fact that a quiescent fiber will remain quiescent until driven either by a locally evoked action potential or by an action potential that propagates into the fiber from a distant site.

Sustained Rhythmic Activity. This is a purely descriptive term that embraces both triggered and truly spontaneous activity as well as repetitive activity arising from sustained circus movement of excitation. It implies sustained generation of action potentials in the absence of artificial stimulation.
Results
CHARACTERISTICS OF THE ACTION POTENTIAL OF FIBERS OF THE SIMIAN MITRAL VALVE

In atrial muscle fibers of the anterior atrial wall the maximum diastolic potential was $83 \pm 3$ mV, action potential amplitude was $98 \pm 5$ mV, overshoot was $15 \pm 2$ mV and $V_{max}$ (maximum value for the first time derivative of transmembrane voltage) of phase 0 was $125 \pm 15$ V/sec (mean $\pm$ SD, values from 50 cells). In contrast, the maximum diastolic potential of mitral valve fibers was $65 \pm 5$ mV, the amplitude of the action potential, measured from maximum diastolic potential to the peak of the overshoot, was $80 \pm 4$ mV, the overshoot was $15 \pm 2$ mV, and $V_{max}$ of phase 0 was $5 \pm 2$ V/sec (mean $\pm$ SD, values from 150 cells). The action potentials of mitral valve fibers of the simian heart thus resemble those of fibers of the canine mitral valve but differ markedly from those of atrial cells. As in the canine heart, the action potentials showed a marked early after-hyperpolarization after which the potential declined to a steady resting level (Fig. 1, top). Neither triggered nor automatic impulse initiation could be demonstrated in the mitral valve SF during superfusion with Tyrode's solution which did not contain a catecholamine.

TRIGGERED ACTIVITY

Delayed After-depolarization. In the presence of epinephrine or norepinephrine (0.5–2.0 $\mu$g/ml) the after-hyperpolarization of all fibers increased and a delayed after-depolarization appeared 600–800 msec after the upstroke of the action potential (Table 1, Fig. 1). The magnitude of the increase in the after-hyperpolarization was not related to the amplitude of the delayed after-depolarization. The amplitude of the delayed after-depolarization, defined as the difference between the afterpotential at its peak and the steady diastolic potential, varied greatly from fiber to fiber in the same valve and from valve to valve and ranged from 2 to 30 mV at a drive rate of 30/min (Table 1). The addition of catecholamine (0.5–2.0 $\mu$g/ml) to unstimulated, quiescent preparations never caused them to become spontaneously active. The addition of catecholamine in this concentration to fibers driven at rates of 30/min or less rarely caused them to show triggered or sustained rhythmic activity.

The delayed after-depolarization that occurred in the presence of catecholamine was not caused by electrotonic spread from a second impulse blocked in the vicinity of the recording electrode; detailed exploration of each of 10 preparations never revealed such a second action potential. In three of these experiments, a solution containing a catecholamine also was applied locally to mitral valve fibers through a micropipette; the after-depolarization invariably appeared only in fibers within a distance of 1 mm from the site of application of catecholamine (Table 1, Fig. 1). Fibers outside this zone did not show a delayed after-depolarization. In all of these experiments the amplitude of the after-depolarization was maximal immediately beneath the tip of the micropipette used to apply the catecholamine and gradually decreased with increasing distance from this site (Fig. 1).

Triggering by Increasing Stimulation Rate. In 18 experiments we superfused the isolated valve leaflet with epinephrine or norepinephrine (0.5 to 2.0 $\mu$g/ml) and determined the effects of the interval between stimuli (cycle length) on the delayed after-depolarization. The responses to epinephrine and norepinephrine were identical. An initial cycle length of greater than 3 seconds was decreased first in steps of 200 msec and later in steps of 50 msec. Fifteen impulses were initiated at each cycle length and then stimulation was interrupted. After the drive had been stopped, we measured the amplitude of the delayed after-depolarization that followed the last driven impulse. Nondriven activity sometimes supervened either during the test period or at its end (Fig. 2).

A low initial rate was chosen so that each driven action potential occurred long after completion of the preceding after-depolarization (Fig. 3). As the cycle length was reduced the delayed after-depolarization occurred at the same time relative to the preceding action potential, although there were slight changes from one cycle to the next, and its amplitude increased. In five preparations, reduction of the stimulus cycle length did not increase the amplitude of the after-depolarization unless each driven action potential occurred during the repolarization phase of the preceding.

FIGURE 1  Epinephrine-induced delayed after-depolarizations in mitral valve fibers. Panels IA and IB show recordings from the same fiber. Only the lower part of the action potential (more negative than $-35$ mV) is shown. In IA, in the absence of catecholamines, there is a prominent after-hyperpolarization but no after-depolarization. IB shows the after-depolarization (arrow) during superfusion with epinephrine (1.0 $\mu$g/ml). IIA shows the effect of local application of epinephrine on a mitral valve fiber. The epinephrine was ejected through a micropipette (see Methods) and the action potential in the bottom trace was recorded at the site of ejection. The action potential in the middle trace was recorded at a site 5 mm away. Note the development of the delayed after-depolarization (solid arrow) recorded from the fiber at the site of epinephrine ejection (no after-depolarization was present prior to epinephrine). No after-depolarization developed in the fiber remote from this site (open arrow). Calibrations for IIA: vertical = 50 mV, horizontal = 200 msec.
after-depolarization. This occurred at cycle lengths of 1500–750 msec (Figs. 2 and 3). Further reduction of the cycle length resulted in a further increase in the amplitude of the delayed after-depolarization both during stimulation and after the final driven action potential. In five other preparations a reduction in cycle length caused an immediate increase in amplitude of the after-depolarization, even when each driven action potential occurred after completion of the preceding after-depolarization (Fig. 2, Fig. 4-111). Five to 10 trials were performed in each study and the response always was reproducible.

In fibers showing either type of response, in 10 of the 18 experiments shortening the cycle length to a critical value caused single nondriven action potentials to occur either before the next stimulus or after stimulation stopped (Fig. 3). This critical cycle length varied widely among the different preparations (2,500–600 msec) (Fig. 2). The amplitude of the after-depolarization often increased gradually over the first 5–10 impulses during stimulation at this critical cycle length until, when the amplitude of the delayed after-depolarization reached a critical value (18–22 mV), the nondriven action potentials occurred (Fig. 4-1B). The nondriven action potential arose from the peak of the delayed after-depolarization: therefore the coupling interval between the nondriven action potential and the preceding driven action potential always was the same as the interval between the upstroke of the driven action potentials and the peak of their after-depolarizations. Further reduction in the cycle length beyond that necessary to elicit single nondriven action potentials triggered sustained rhythmic activity at a rate more rapid than the rate at which the fiber was being driven; this activity continued for several seconds to 15 minutes after stimulation was terminated (Fig. 4). The cycle length of sustained rhythmic activity ranged from 350 to 600 msec for the 10 preparations but was constant during repeated trial in each preparation. Although the first nondriven action potential occurred at a coupling interval corresponding to that of the after-depolarization, the rate of nondriven activity increased during the first few beats of sustained rhythmic activity. The same acceleration is seen after triggering by a premature stimulus (Fig. 4-III) but we do not know why it occurs.

Four valves in which triggered activity could be produced were cut into small pieces, some no larger than 2–4 mm². Stimulation induced triggered activity in 75–80% of the small pieces; the upstrokes of action potentials recorded simultaneously from different cells in a given fragment occurred virtually simultaneously; this suggests that reentry was not taking place (see Discussion).

In eight of the 18 preparations, nondriven action potentials could not be elicited in mitral valve fibers by rapid drive or premature stimulation. In three of these preparations muscle fibers did not extend into the mitral valve leaflet. In the remaining five preparations the amplitude of the after-depolarizations after catecholamine superfusion was only 2–8 mV, and in four of these the after-depolarizations did not increase in amplitude when cycle length was shortened, or did so only slightly, even though we used concentrations of catecholamine of up to 5.0 µg/ml (Fig. 5).

Triggering by a Premature Stimulus. In four experiments, when mitral valve fibers were driven at a fixed rate that did not lead to triggering the delayed after-depolarizations that followed premature impulses were larger than those following the driven action potentials and triggered nondriven activity. As the coupling interval between the basic and premature impulse was decreased, the after-depolarization of the premature impulse progressively increased in amplitude until triggering occurred (Fig. 2; Fig. 4-III). In two fibers sustained rhythmic activity was triggered by premature impulses that occurred after the peak of the preceding after-depolarization; in two others, the impulse that triggered rhythmic activity occurred before the expected peak of the preceding after-depolarization. In all cases the first triggered action potential arose from the peak of an after-depolarization (Fig. 4).

Initiation of Rhythmic Activity by a Single Applied Stimulus. Superfusion of quiescent mitral valve fibers with catecholamine (0.5–2.0 µg/ml) never caused spontaneous activity. In each of 10 separate trials on two preparations during such superfusion, a single electrical stimulus resulted in a single driven action potential that was followed by sustained rhythmic activity which consisted of 5–500 impulses (Fig. 4).

Spontaneous and Induced Termination of Sustained Rhythmic Activity. Once sustained rhythmic activity had been triggered, it either stopped spontaneously or could be
stopped by a single premature impulse. When sustained rhythmic activity stopped spontaneously, the last action potential was followed by one or more subthreshold depolarizations at intervals corresponding to that of the preceding rhythmic activity (Fig. 4). A premature impulse terminated sustained rhythmic activity if it was induced either very early in the cycle (two of five experiments) or very late in the cycle (five of five experiments). Early premature impulses that terminated sustained rhythmic activity occurred while the fibers were partially refractory and evoked only an abortive response, after which the after-depolarization did not reach threshold (Fig. 6A). The coupling intervals of these premature impulses ranged from 250 to 350 msec. Action potentials evoked slightly later showed a normal amplitude and did not terminate the sustained rhythmic activity, but often demonstrated an increased after-hyperpolarization of 2-5 mV. This increased the time needed for depolarization to reach threshold and initiate the next impulse. As a result the

![Diagram of Figure 2](http://circres.ahajournals.org/)

**Figure 2** Effects of stimulus cycle length on the amplitude of the delayed after-depolarization of mitral valve action potentials. All preparations are superfused with a catecholamine. In both panels each symbol designates a different preparation. Each point represents the mean ± SD for 5-7 cells in the preparation. The top panel shows the effects of decreasing the basic cycle length (abscissa) on after-depolarization amplitude (ordinate). Note that there are two general types of responses: in five preparations after-depolarization amplitude increased progressively as cycle length decreased and in five preparations cycle length was decreased to 750-1,750 msec before after-depolarization amplitude began to increase. The bottom panel shows the effects of premature stimuli induced at a basic stimulus cycle length of 2,800 msec: the coupling intervals of the premature impulses are on the abscissa and the after-depolarization amplitude of the premature impulses is on the ordinate. In both panels the shortest cycle length shown for each experiment is the cycle length which induced triggered impulses.
FIGURE 3 The effect of decreasing stimulus cycle length on the amplitude of the delayed after-depolarization. Recordings from the same fiber are shown in each panel. Only the lower part of the action potential is shown. The preparation was superfused with epinephrine (1.0 ng/ml). The last several driven potentials of a series of 15 are shown in each panel. Cycle length in A is 2,600 msec. In B (cycle length, 1,300 msec) the amplitude of the delayed after-depolarization is increased. In C (cycle length, 850 msec) each action potential is elicited prior to complete repolarization of the previous after-depolarization; the amplitude of the after-depolarization has increased further. In D (cycle length, 800 msec) a nondriven action potential occurs (arrow) after the stimulus has been turned off. The nondriven action potential is followed by a delayed after-depolarization.

The next upstroke was delayed (Fig. 6B). Premature impulses induced still later in the cycle were followed by a slightly greater after-hyperpolarization; the subsequent after-depolarization failed to give rise to an action potential, and quiescence ensued (Fig. 6C). Late premature impulses that terminated sustained rhythmic activity occurred at coupling intervals of 350–430 msec.

Absence of Triggered Activity in Atrial Wall Fibers. None of the phenomena described above could be produced in the isolated anteromedial atrial wall when it was not attached to the mitral valve leaflet. In five experiments unstimulated preparations of the atrial wall remained quiescent during superfusion with catecholamine, and stimulation did not produce either afterpotentials or sustained rhythmic activity. Spontaneous activity occurred in the atrial wall in five other preparations at cycle lengths of 800 ± 85 msec, i.e., at rates much slower than those seen during triggered activity of fibers in the mitral valve. We were unable to find cells showing phase 4 depolarization in the atrium during this spontaneous activity.

Activation of the Atrium from the Mitral Valve during Sustained Rhythmic Activity. We studied the sequence of activation in five preparations in which the atrial wall was stimulated and sustained rhythmic activity was induced in the mitral valve fibers by impulses that propagated from the atrium into the valve (Fig. 7). Once rhythmic activity had been triggered in the mitral valve leaflet, the spontaneously occurring action potentials propagated back to activate the atrium (Fig. 7) and the sequence of activation was identical to that described previously. That the impulse originated in the mitral valve was confirmed in three of the preparations when, during the course of sustained rhythmic activity, conduction block developed between the valve and atrium. At such times rhythmic activity occurred only in the valve leaflet and not in the atrium (Fig. 7).

IS THE ACTION POTENTIAL OF MITRAL VALVE FIBERS A SLOW RESPONSE?

Triggered activity previously has been demonstrated in fibers in which the action potential probably is a slow response, i.e., in fibers in which action potential upstroke depends on inward current flowing through the slow channel. The slow response can be characterized by the effects of catecholamines, verapamil, and TTX and by effects of resting potential on the upstroke of the action potential.
Effects of Catecholamines. In addition to enhancing the delayed after-depolarization, epinephrine or norepinephrine (0.5–2.0 ng/ml) increased the amplitude of the mitral valve action potential, often without affecting the maximum diastolic potential (Table 2). This effect was particularly pronounced when the action potential amplitude was low (<65 mV) and the overshoot absent even though resting potential was normal (~65 to ~70 mV). In such depressed fibers an increase in action potential amplitude after exposure to catecholamine resulted from an increase in overshoot. In fibers whose action potentials initially had a normal amplitude of 75–85 mV and overshoots of 10–15 mV, the addition of catecholamine increased the amplitude only slightly by increasing the overshoot (Table 2).

Effects of Verapamil. In four studies we were able to obtain action potential recordings from the same single fiber during a control period and during superfusion with verapamil (0.1–1.0 mg/liter). In the absence of catecholamine the upstroke of the action potential of the mitral valve was slowed by verapamil and the overshoot and amplitude were diminished. Maximum diastolic potential was not changed (Table 3, Fig. 8). Similar concentrations had no effect on the maximum diastolic potential, action potential upstroke velocity, or overshoot of fibers of the atrial wall, but they depressed the level at which the plateau occurred (results from three fibers in which impalement was maintained during control and drug superfusion) (Table 3). The depressant effects of verapamil on the upstroke of the mitral valve action potential could be partially reversed by the addition of norepinephrine or epinephrine (0.5–2.0 μg/ml) to the superfusate.

Verapamil depressed the delayed after-depolarization seen during catecholamine superfusion (five experiments) (Table 3, Fig. 9). During superfusion with catecholamine (0.5–2.0 μg/ml) verapamil (0.5 mg/liter) reduced the rate of depolarization and amplitude of the action potential less than in the absence of catecholamine but almost abolished the delayed after-depolarization (Table 3). Verapamil thereby abolished the ability of a decrease in cycle length or premature stimulation to trigger activity. This was true whether the stimulus was applied directly to the valve or to the atrium (Fig. 9). If the catecholamine concentration in the superfusate was increased after verapamil had suppressed the delayed after-depolarization, the amplitude of the after-depolarization increased and triggered activity once again could be induced.

![Figure 4](http://circres.ahajournals.org/)

**Figure 4** Triggering of sustained rhythmic activity. Only the lower part of the action potential is shown in each panel. Horizontal lines in I and II are at minus 30 mV, top trace in III is at −20 mV. Vertical calibration is 30 mV for I and II and 60 mV for III. Horizontal calibration = 1,400 msec is for I and II only. Time pips in top trace of III are at 100-msec intervals. Panels IA and B show triggered activity induced by decreasing the basic cycle length. In IA cycle length is 3,400 msec and a delayed after-depolarization is present. In IB cycle length is 1,750 msec and there is a progressive increase in the after-depolarization which follows each of the first four driven action potentials. The last driven action potential (arrow) is followed by a nondriven action potential and by sustained rhythmic activity at a rate greater than the driven rate. Panels IIA and B show triggering of sustained rhythmic activity in a mitral valve fiber by a single driven impulse in a preparation superfused with epinephrine (1.0 μg/ml). In the absence of electrical stimulation there are no spontaneous action potentials. In IIA, after a period of quiescence, a single stimulated action potential (arrow) is followed by an after-depolarization and is present. In IB, after a subsequent 2-minute period of quiescence another single action potential is stimulated (arrow) and is followed by sustained rhythmic activity which lasted for 5 minutes. Panels IIIA and B show triggered activity induced by premature stimulation in the presence of epinephrine (1.0 μg/ml). The basic cycle length is 2,800 msec. In IIIA, when the premature impulse (arrow) was induced on the repolarization phase of the previous after-depolarization, the amplitude of the after-depolarization of the premature impulse was increased. In IIIB the premature impulse (small arrow) was induced prior to the time at which the after-depolarization of the preceding basic impulse would have occurred, a nondriven action potential arises from the after-depolarization (large arrow), and nondriven activity is sustained. The bottom right panel shows this sustained rhythmic activity terminating in a subthreshold after-depolarization (arrow).
Figure 5 Effect of basic stimulus cycle length (abscissa) on the amplitude of the delayed after-depolarization (ordinate) of mitral valve action potentials in preparations in which triggered activity could not be induced. Each symbol designates a different preparation. Each point represents the means ± SD of 5-7 cells in the preparation. All preparations are superfused with a catecholamine.

Figure 6 Termination of sustained rhythmic activity by a premature stimulus. Each panel shows the last two nondriven action potentials which preceded the premature stimulus at the arrows. Recordings from the same fiber are shown in each panel. In panel A, the premature stimulus is induced prior to complete repolarization of the preceding action potential, and induces only an abortive response which is followed by a low amplitude oscillation and termination of the rhythmic activity. In B, a premature impulse induced later in the cycle is followed by a slight lengthening of the cycle, but sustained rhythmic activity continues. In C, a premature impulse occurring still later in the cycle is followed by an after-depolarization which fails to reach threshold, and sustained rhythmic activity is terminated. Time pips in top trace of each panel are at 100-msec intervals.

Effect of Tetrodotoxin (TTX). We studied the effect of TTX on mitral valve action potentials in five preparations. We were not able to record mitral valve action potentials from the same fiber both during the control period and after exposure to TTX; therefore recordings were obtained from five or six fibers consecutively at each period during the experiment. We were able to obtain recordings from three atrial fibers during control and drug superfusion. Mitral valve fibers were more resistant to the effects of TTX than were muscle fibers of the anterior atrial wall (Table 4). The steepness of the upstroke and the amplitude of the action potential of atrial muscle fibers were progressively depressed at concentrations of TTX greater than 2.0 mg/liter. At 10.0 mg/liter atrial fibers became inexcitable, although the level of resting membrane potential was unaltered (Table 4). The slope of the action potential upstroke of mitral valve fibers also was progressively depressed by TTX in concentrations greater than 2.0 mg/liter; this occurred both in the presence and absence of catecholamine (Table 4, Fig. 8). However, the amplitude of depolarization was not greatly affected by TTX, and action potentials with nearly normal amplitude but greatly reduced upstroke velocity could be elicited after exposure to TTX (5.0 mg/liter) and, in two experiments, after 10.0 mg/liter (Fig. 8).

The effect of TTX on atrial muscle fibers was not altered by the presence of catecholamine. However, TTX caused less decline in steepness and amplitude of mitral valve action potentials in the presence of either epinephrine or norepinephrine (Table 4). TTX (5.0 mg/liter) did not have any
Figure 7. Propagation of triggered activity from the anterior mitral valve leaflet to the adjacent atrial wall in the presence of epinephrine, 1.0 μg/ml. Top trace, bipolar atrial electrogram; bottom trace, action potential of mitral valve muscle fiber. Stimulus electrodes are on the atrial wall. At cycle lengths greater than 1,200 msec triggering did not occur (not shown). The first two action potentials shown in the top panel were initiated after the cycle length was reduced to 1,150 msec. Atrial activity precedes activation of the valve fiber, and an after-hyperpolarization and a delayed afterdepolarization follow the first driven action potential. The afterdepolarization following the second driven action potential progresses into the upstroke of a nondriven action potential which now precedes the atrial electrogram, suggesting that the impulse is now propagating in the opposite direction (from valve to atrium). Sustained rhythmic activity continues in the valve, and the atrium is activated after each valve action potential. After several minutes complete conduction block developed between the valve leaflet and the atrial wall, as shown in the bottom panel. Sustained rhythmic activity continues in the valve fiber but there is no evidence of electrical activity in the atrial electrogram.

Effect on the delayed after-depolarization that occurred in the presence of catecholamine; the effects of changes in rate and rhythm on the amplitude of this afterpotential were not altered, and sustained rhythmic activity could be triggered by stimulation patterns similar to those that induced it before exposure to TTX (Table 4). Also, the rate at which sustained rhythmic activity appeared was not affected.

Effects of Resting Potential on the Rate of Depolarization and Overshoot. The resting potential of atrial fibers in the atrial wall and mitral valve was reduced by increasing $[K^+]_o$ in the absence of catecholamine. In five experiments, increasing $[K^+]_o$ from 4 mM to 12, 20, 30, 50, and 80 mM resulted in a progressive decline in maximum diastolic potential of atrial wall fibers. The upstroke velocity, amplitude, and overshoot of the action potential fell as the fibers depolarized. When $[K^+]_o$ was 12 mM the resting potential fell to less than -70 mV and these cells became inexcitable (Fig. 10). Further elevation of $[K^+]_o$ to 20, 50, and 80 mM caused additional decreases in membrane potential and continued inexcitability.

In four experiments increasing $[K^+]_o$ above 4 mM also caused a loss of resting membrane potential of mitral valve fibers and a decrease in action potential upstroke velocity, but the overshoot did not decrease as much as for atrial wall fibers (Fig. 10). When $[K^+]_o$ was 12-15 mM, resting potential fell to about -55 mV and action potentials could not be evoked by extracellular stimulation. However, when $[K^+]_o$ was 20 mM and resting potential was about -50 mV, action potentials with a nearly normal overshoot of 15-15 mV once more could be elicited (Figs. 10 and 11). These action potentials were not followed by an after-hyperpolarization. Action potentials with overshoot still could be elicited when $[K^+]_o$ was 50 mM and resting potential -30 mV.

In mitral valve fibers elevation of $[K^+]_o$ to 30 mM in the absence of catecholamine resulted in the appearance of nondriven activity at a cycle length of 1,100 ± 75 msec (mean ± SD for the four preparations) (Fig. 11). This activity seemed to be spontaneous; that is, it appeared without being triggered by a driven or propagated impulse. It was not affected by cutting the valve into several small pieces, and therefore probably was not reentrant. As $[K^+]_o$

### Table 2: Effects of Catecholamines on Mitral Valve Fibers

<table>
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<th>Condition</th>
<th>$n$</th>
<th>MDP (mV)</th>
<th>APA (mV)</th>
<th>Overshoot (mV)</th>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td>68 ± 4</td>
<td>64 ± 3</td>
<td>-4 ± 1</td>
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<td>Catecholamines (0.5-2.0 μg/ml)</td>
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<td>85 ± 4</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>6</td>
<td>70 ± 4</td>
<td>81 ± 4</td>
<td>12 ± 2</td>
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<tr>
<td>Catecholamines (0.5-2.0 μg/ml)</td>
<td>6</td>
<td>72 ± 3</td>
<td>89 ± 5</td>
<td>17 ± 2 &lt;0.001</td>
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<tr>
<th>All values are mean ± SD.</th>
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</thead>
<tbody>
<tr>
<td><em>p</em> values are derived from comparison of electrophysiological values after drug perfusion with control values.</td>
</tr>
</tbody>
</table>

| Values under overshoot are the difference between the level of membrane potential attained at the peak of the action potential and zero transmembrane potential. Positive values therefore indicate a true overshoot or reversal and negative values indicate the degree to which the action potential fell short of zero. |
**TABLE 3** Effects of Verapamil on Mitral Valve and Atrial Action Potentials

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>MDP (mV)</th>
<th>APA (mV)</th>
<th>Overshoot* (mV)</th>
<th>V&lt;sub&gt;max &lt;/sub&gt; (V/sec)</th>
<th>ADA (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MV&lt;sub&gt;a&lt;/sub&gt;</td>
<td>AT</td>
<td>MV&lt;sub&gt;a&lt;/sub&gt;</td>
<td>AT</td>
<td>MV&lt;sub&gt;a&lt;/sub&gt;</td>
<td>AT</td>
</tr>
<tr>
<td>No catecholamine in superfusate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>65 ± 2</td>
<td>85 ± 3</td>
<td>70 ± 5</td>
<td>+ 5 ± 1</td>
<td>+18 ± 2</td>
</tr>
<tr>
<td>Verapamil 0.1 mg/liter</td>
<td>4</td>
<td>64 ± 2</td>
<td>85 ± 3</td>
<td>62 ± 3†</td>
<td>- 8 ± 2†</td>
<td>+18 ± 2</td>
</tr>
<tr>
<td>Verapamil 1.0 mg/liter</td>
<td>4</td>
<td>64 ± 2</td>
<td>86 ± 2</td>
<td>35 ± 2†</td>
<td>-10 ± 5†</td>
<td>+18 ± 2</td>
</tr>
<tr>
<td>Catecholamine (0.5-2.0 μg/ml) in superfusate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>32</td>
<td>69 ± 4</td>
<td>89 ± 5</td>
<td>19 ± 3</td>
<td>-</td>
<td>9 ± 2.0</td>
</tr>
<tr>
<td>Verapamil, 0.5 mg/liter</td>
<td>35</td>
<td>66 ± 5</td>
<td>70 ± 4†</td>
<td>3 ± 11</td>
<td>-</td>
<td>4 ± 1.0†</td>
</tr>
</tbody>
</table>

n = number of impalements; MV<sub>a</sub> = mitral valve fibers; AT = atrial fibers; MDP = maximum diastolic potential; APA = action potential amplitude; ADA = amplitude of after-depolarization; T = triggering.

In studies without catecholamine a single impalement was maintained during the control and after verapamil perfusion. In studies with catecholamine action potentials were recorded from several different cells within a small circumscribed area before and after verapamil perfusion and not from the same cells throughout the experiment.

*For explanation of overshoot values, see Table 2.
†P < 0.01.
‡P < 0.001.
§Cycle length = 1.575 ± 0.30 msec.

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**Discussion**

**MIGHT THE SUSTAINED RHYTHMIC ACTIVITY BE REENTRANT?**

The triggered activity described in this study does not appear to be caused by reentry. Triggered action potentials occurred in the mitral valve leaflet even when it was detached from the atrium, indicating that a reentrant pathway was not responsible. Furthermore, when the valve leaflet was divided into small pieces, many of the pieces exhibited triggered activity. It is possible that reentry involving only a few fibers occurred within each piece; however, the upstrokes of action potentials recorded from different fibers within each piece were virtually simultaneous and thus did not show the pattern of conduction expected from circus movement or reentry.

The occurrence of spontaneous action potentials in the mitral valve fibers is due to a combination of factors. These factors include the presence of catecholamines, the high potassium concentration in the superfusate, and the altered membrane properties resulting from verapamil perfusion. The combination of these factors leads to the development of triggered activity, which in turn results in the sustained rhythmic activity observed in the mitral valve leaflet.

**Figure 8**

The effects of verapamil and tetrodotoxin (TTX) on mitral valve fibers. The left panels show the control mitral valve action potential (top) and the action potential recorded from the same fiber after superfusion with verapamil (bottom). The right panels show the control mitral valve action potential (top) and the action potential recorded from the same fiber after superfusion with TTX (bottom).
mitral valve leaflet of canine hearts has previously been reported. The phenomenon of triggering was not noticed in this earlier study.

**THE ACTION POTENTIAL OF MITRAL VALVE FIBERS AS A SLOW RESPONSE**

The slow response is a propagated action potential the upstroke of which results from inward current through the slow channel. The slow response can occur in atrial, ventricular, or Purkinje fibers in which a low resting potential has inactivated the fast inward current and may be the normal response of the sinus and atrioventricular nodes. The upstroke of the slow response is insensitive to TTX but is depressed by verapamil, an agent that appears to block the slow channel. Catecholamines can increase the magnitude of the inward current in the slow channel and thereby increase the amplitude of the slow response without changing the resting potential. The action potential of mitral valve fibers appears at low resting potentials, is enhanced by catecholamines, and is depressed by verapamil; it therefore resembles the slow response. On the other hand, the upstroke is depressed by TTX and may arise at a membrane potential somewhat negative to the generally accepted threshold for the slow inward current. Nor is the response of mitral valve fibers to elevation of \([K^+]_o\) and loss of resting potential that expected of either the pure fast response or the pure slow response. As resting potentials between \(-60\) mV and \(-55\) mV the fibers cannot be excited by extracellular stimuli, but when resting potential falls to \(-50\) mV extracellular stimuli elicit action potentials with overshoots. It is possible therefore that the upstroke depends both upon the slow channel and upon a partially inactivated, TTX-sensitive fast channel. Depolarization to \(-55\) mV would wholly inactivate the fast channel. The fact that the fibers are more easily excited from a resting potential of \(-50\) mV merely indicates that a smaller stimulus-induced depolarization is adequate to bring such fibers to their threshold potential. It is known that slow response activity cannot be elicited in fibers exposed to Na-free solutions if the resting potential is too high, but can be elicited if resting potential is reduced. Mitral valve fibers also become spontaneously active if markedly depolarized by increased \([K^+]_o\). We know of no other cardiac fiber in which spontaneous activity is caused by elevation of \([K^+]_o\). Purkinje fibers that are quiescent when exposed to Na-free solutions show repetitive activity when depolarized by an applied current, as may frog atrial fibers and guinea pig ventricular fibers, but such fibers do not become spontaneously active when exposed to high \([K^+]_o\); elevation of \([K^+]_o\) causes normal Purkinje fibers to lose spontaneous activity even in the presence of catecholamines. It is conceivable that elevation of \([K^+]_o\) releases catecholamines from adrenergic nerves in the mitral valve and thereby enhances the slow inward current, although it is not clear why this would occur only at such high levels of \([K^+]_o\).

**THE MECHANISM OF TRIGGERED ACTIVITY**

Triggered activity differs from spontaneous activity in that triggered activity is dependent on the prior occurrence of an action potential. The delayed after-depolarization that follows a driven action potential may or may not reach the threshold potential. If it does reach threshold (because of a decrease in stimulus cycle length or a premature stimulus) a nondriven action potential occurs. If this nondriven action potential is followed by a delayed after-depolarization that reaches threshold, and if each subsequent action potential also is followed by a delayed after-depolarization that reaches threshold, sustained rhythmic activity occurs. Spontaneous activity does not occur after catecholamines are added to a nonstimulated, quiescent mitral valve. Truly automatic fibers presumably develop phase 4 depolarization and spontaneous activity without prior activation, although Vassalle and Carpentier found that electrical stimulation can induce automaticity in Purkinje fibers exposed to low concentrations of norepinephrine.

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**FIGURE 9** The effects of verapamil on the delayed after-depolarization of fibers of the isolated mitral valve superfused with epinephrine. 0.5 μg/ml. Reference 0 line is shown for first action potential in each panel. A, cycle length = 4,000 msec; delayed after-depolarizations follow each action potential but there is no triggered activity. B, cycle length = 1,500 msec; the third driven action potential is followed by a nondriven response (arrow) and by sustained rhythmic activity. C, a different fiber of the same mitral valve, superfused with verapamil (0.2 mg/liter) for 30 minutes, is driven at a cycle length of 1,500 msec. No delayed after-depolarization was evident in this fiber or in 15 other fibers from which recordings were obtained. Sustained rhythmic activity could not be triggered in the presence of verapamil, which also reduced the upstroke velocity and amplitude of the driven action potential.
The ionic mechanism for the delayed after-depolarization that causes triggered and sustained rhythmic activity is unknown. The effects of verapamil, TTX, and catecholamines on the delayed after-depolarization suggest that it may be caused, at least in part, by inward current in the slow channels. The delayed after-depolarization may result from a phasic increase in slow-channel inward current during diastole or it may arise because inactivation of that inward current flowing during the depolarization phases of the action potential lags behind complete repolarization. If the terminal phase of repolarization is caused by a transient increase in K conductance and the slow-channel inward current persists after K conductance has returned to its normal value (or possibly even below the value characteristic of diastole) an afterpotential should result. The absence of the after-depolarization in the presence of elevated [K+]o and catecholamines suggests that a low K conductance is important for its generation. The occurrence of the afterpotential at levels of membrane potential more negative than the generally accepted threshold for activation of slow inward current may be explained by this mechanism or alternatively by a catecholamine-induced shift of threshold to more negative values. In view of the proposed mechanism it is also possible that abolition of the after-depolarization by verapamil might be due to effects of this agent on plateau currents other than the slow-channel inward current.26

The cause of the increase in amplitude of the after-depolarization of mitral valve fibers in response to an increase in the frequency of activation is unknown.

TRIGGERED ACTIVITY AS A CAUSE OF CARDIAC ARRHYTHMIAS

Fibers that are anatomically similar to those described in this article exist in the human heart, and the occurrence of triggered activity in such fibers is at least possible.27 The addition of catecholamines in vitro may merely restore the fiber to the state in which it exists in situ; it is also possible that such activity occurs in situ only in the presence of increased levels of catecholamines or of increased activity of adrenergic nerves that richly innervate the valve leaflet.28 Scherf and Schott29 have long maintained that nondriven action potentials arising from after-depolarizations might cause extrasystoles or tachycardia. Fibers in the mitral valve that exhibited triggerable activity might account for several types of atrial arrhythmias. For example, paroxysmal atrial tachycardia is often initiated by a premature atrial impulse, can be terminated by a premature impulse, and is generally thought to result from circus movement of excitation in a pathway part of which passes through the atrioventricular node.30 However, some of these arrhythmias may be triggered, because triggered arrhythmias also can be evoked by a single critically timed premature impulse and interrupted in the same way. In fact, it is widely assumed that spontaneous or evoked premature impulses or rapid stimulation may initiate or terminate only reentrant rhythms. In view of our data and the data of others suggesting that sustained rhythmic activity which is not reentrant can be induced in much the same manner, perhaps this criterion should be reevaluated.
TRIGGERED MITRAL VALVE ACTIVITY/Wit and Cranefield

Figure 10. Relationship of overshoot and phase 0 $V_{\max}$ (ordinate) of atrial wall fibers (left panels) and mitral valve fibers (right panels) to membrane potential at which the upstroke is initiated (abscissa). Each symbol represents a different single fiber from a different preparation. Resting membrane potential was reduced by increasing $[K]$ in the superfusate. For the atrial wall fibers, overshoot and phase 0 $V_{\max}$ decreased in each experiment as membrane potential declined when $[K]$ was increased. For the mitral valve fibers, the overshoot was not affected when membrane potential decreased, although phase 0 $V_{\max}$ was reduced. The shaded column in the right panels denotes the membrane potentials at which most mitral valve fibers were transiently inexcitable.

Figure 11. The effects of increasing $[K^+]_o$ on mitral valve action potentials. At the top, action potentials were recorded from the same cell at $K = 4, 10,$ and $30$ mM concentrations, and from a different cell at $K = 50$ mM. As $[K^+]_o$ was increased, there was a decrease in resting membrane potential from 78 mV at $[K^+]_o = 4$ mM to 50 mV at $[K^+]_o = 30$ mM, but action potential overshoot remained unchanged. At a $[K^+]_o = 50$ mM resting membrane potential of a different cell was 36 mV and an action potential with a slight overshoot could still be elicited. The bottom three panels show the occurrence of nonstimulated action potentials after graded depolarization caused by increasing $[K^+]_o$. At $K = 10$ mM stimulated action potentials occurred but when $[K^+]_o$ was increased to 15 mM no action potentials could be elicited and there was no spontaneous activity (membrane potential is still declining and has not yet equilibrated in the trace which is shown). At $[K^+]_o = 30$ mM nondriven action potentials occur, and the spontaneous rate increases with further depolarization at $[K^+]_o = 50$ mM.

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Triggered activity in cardiac muscle fibers of the simian mitral valve.

A L Wit and P F Cranefield

doi: 10.1161/01.RES.38.2.85

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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