Interaction of Epinephrine and Ouabain on Automaticity of Canine Purkinje Fibers

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ABSTRACT
The interaction of ouabain and epinephrine on diastolic depolarization of canine Purkinje fibers was studied using conventional microelectrode techniques. When the Purkinje fibers were treated with ouabain alone, they developed slow diastolic depolarization accompanied by a proportionate decrease in the maximum diastolic potential. When these digitalized Purkinje fibers were further treated with epinephrine, the rate of these changes was greatly accelerated, resulting in rapid spontaneous activity in some Purkinje fibers. The rate of development of increased automaticity observed in the preparations treated with both ouabain and epinephrine was two to three times faster than that observed in the preparations exposed to ouabain alone. Epinephrine alone at the same concentration caused minimal changes in diastolic depolarization of these preparations. Propranolol in a beta-receptor blocking dose prevented the potentiating effect of epinephrine but did not interfere with the effect of ouabain on diastolic depolarization of Purkinje fibers. It is concluded that epinephrine potentiates the ouabain-induced increase in automaticity of Purkinje fibers by stimulating beta receptors.

KEY WORDS
adrenergic activity
diastolic depolarization
adrenergic blockade
digitalis
propranolol

It is well established that lethal doses of digitalis result in ventricular fibrillation in both experimental and clinical situations. Digitalis has been shown to increase idioventricular pacemaker activity in intact animals (1-3) and the slope of diastolic depolarization of Purkinje fibers in in vitro studies (4-6). Catecholamines, both norepinephrine and epinephrine, are noted for their ability to increase automaticity of Purkinje fibers. Seevers and Meek (7) have observed that ephedrine, a sympathomimetic amine, frequently results in ectopic ventricular activity when it is administered to dogs together with digitalis. Roberts et al. (8) have shown that reserpine, which depletes catecholamine stores in adrenergic nerve endings, reduces the capacity of cardiac glycosides to induce extrasystoles in dog hearts. Erlij and Mendez (9) have also found that cardiac arrest rather than ventricular fibrillation is the common terminal event in reserpinized dogs. Mendez et al. (10) and Erlij and Mendez (9) have shown that dogs subjected to cardiac denervation and adrenalectomy usually go into cardiac arrest rather than ventricular fibrillation after administration of a toxic dose of digitalis.

Since sympathetic activity is constantly present in individuals and varies in degree at different times, the question of whether catecholamines contribute to the development of digitalis-induced cardiac arrhythmias arises. In the present study, microelectrode techniques were used to study the effect of epinephrine on ouabain-induced changes in automaticity of canine Purkinje fibers.

Methods
Hearts were excised from dogs anesthetized with sodium pentobarbital (30 mg/kg, iv). Papillary muscles with attached false tendons were dissected from the right or the left ventricle and stored in oxygenated Tyrode's solution. The preparation was then pinned under slight tension to a paraffin block in a tissue bath. Modified Tyrode's solution equilibrated in a reservoir with 95% O₂-5% CO₂ was infused into the bath at 5 ml/min. Temperature in the bath was maintained between 37 and 38°C and remained constant during each experiment. The millimolar composition of the Tyrode's solution was NaCl 137, dextrose 5.5, KCl 2.7, CaCl₂ 2.7, MgCl₂ 0.5, NaHPO₄ 0.9, and NaHCO₃ 24.0. Transmembrane potentials of Purkinje fibers were registered with conventional microelectrode techniques. Glass microelectrodes were filled with 2.7M KCl, and the resistance of these electrodes ranged from 20 to 30 megohms. A silver-wire indifferent electrode was in contact with the Tyrode's solution in the bath. Action potentials were continuously monitored on an oscilloscope and photographed with a Grass camera. A series of Tektronix wave-form and pulse generators was used to deliver pacing stimuli to a pair of
bipolar electrodes attached to the preparation. The preparation was driven by applying suprathreshold rectangular pulses at 95/min.

Results obtained in our laboratory and also those obtained by Davis (6) have indicated that Purkinje fibers in false tendons are more sensitive to ouabain than those on the endocardial surface. In the present study, therefore, only the Purkinje fibers in false tendons were impaled with microelectrodes to obtain more uniform results. Ouabain octahydrate was dissolved in 0.9% saline to make a stock solution; the ouabain concentration of this solution was 1 mg/ml. The working solution had a ouabain concentration of 2 x 10^{-6}M and was prepared by diluting the stock solution with Tyrode's solution. Epinephrine was diluted with the saline to a concentration of 20 µg/ml, and 0.3 ml of this solution (or 6 µg of epinephrine) was injected rapidly into the inflow of the tissue bath. The volume of the tissue bath was 15 ml, and Tyrode's solution flowed through the bath at a rate of 5 ml/min. The preparations were exposed fairly abruptly and transiently to epinephrine at a concentration of about 0.4 µg/ml. When propranolol hydrochloride was used, it was diluted with Tyrode's solution to a concentration of 0.2 mg/liter.

Results

In the control experiments, ouabain alone consistently increased the slope of diastolic depolarization of Purkinje fibers in all studies. This change occurred gradually and was associated with a gradual decrease in the amplitude of the maximum diastolic potential. The experiment was usually terminated when the Purkinje action potential amplitude had been reduced to about 50 mv or when spontaneous activity had developed. The time required to reach one of these end points ranged from 40 to 70 minutes. In 6 of 18 studies, the increase in diastolic depolarization led to spontaneous activity. Activity was considered to be spontaneous when the transmembrane potential had developed the configuration of a pacemaker potential, which has a steep slope of diastolic depolarization and a smooth transition from phase 4 to phase 0, and overcome the selected driving rate.

In the experiments in which the interaction of epinephrine and ouabain was studied, the preparations were first superfused with ouabain; then 6 µg of epinephrine was injected into the inflow of the tissue bath. Epinephrine was applied either when diastolic depolarization had increased about 2-3 mv over the control or 25 minutes after the beginning of superfusion of ouabain if no obvious increase in diastolic depolarization was observed. The magnitude of diastolic depolarization was measured at the end of diastole while the preparation was driven at a constant rate of 95/min. In some preparations, epinephrine was applied as early as 15 minutes after the beginning of ouabain superfusion. At this level of digitalization, the configuration of transmembrane potentials of Purkinje fibers usually showed minimal changes compared with the control. Phase 2 repolarization had usually shortened slightly, but the amplitude of the action potential was not yet significantly decreased. Epinephrine applied to the preparation in this manner consistently increased diastolic depolarization; in most studies, the increase was from about 3 mv to approximately 15 mv within 3 minutes. When epinephrine was applied prior to the development of these selected conditions in six preparations, it resulted in only a 2-3-mv increase in diastolic depolarization.

Figure 1 illustrates the results of a typical experiment on the interaction of epinephrine and ouabain. Tracing A is a control recording of transmembrane potentials of a Purkinje fiber. Tracing B shows that no obvious change in the slope of diastolic depolarization was observed 25 minutes after the beginning of ouabain superfusion. At the arrow labeled E, epinephrine (6 µg) was injected into the inflow of the tissue bath. In tracing C, recorded 26 minutes after the beginning of ouabain superfusion or about 1 minute after the application of epinephrine, the slope of diastolic depolarization was increased and the maximum diastolic potential was decreased. In tracing D, recorded 1 minute later, the slope of diastolic depolarization was further increased and spontaneous activity had developed. As the experiment continued, further increases in diastolic depolarization or the rate of spontaneous activity usually progressed more slowly, possibly because of the continuous effects of ouabain. In 10 of 16 preparations studied in this...
manner, spontaneous activity developed. The patterns of increase in diastolic depolarization that occurred in preparations treated with ouabain alone and those treated with both ouabain and epinephrine were qualitatively similar; the primary difference between these two situations was in the time course. In the studies in which preparations were treated with both ouabain and epinephrine, the increase in diastolic depolarization and the development of spontaneous activity were greatly accelerated.

In four other experiments, the effect of epinephrine alone and the combined effect of ouabain and epinephrine on diastolic depolarization were studied on the same Purkinje fibers. Each preparation was first treated with 6 μg of epinephrine; it was allowed to rest for about 30 minutes and then it was treated with ouabain and epinephrine as described earlier. In these studies, epinephrine alone caused a 2–3-mv increase in diastolic depolarization, which returned to the control level in about 5 minutes. However, the combination of ouabain and epinephrine again greatly accelerated the rate of increase in diastolic depolarization from about 3 mv to 15 mv, and spontaneous activity developed in two of these preparations.

Some of the control studies also indicated that preparations from different hearts had different degrees of sensitivity to ouabain. The time taken by Purkinje fibers to increase their diastolic depolarization by about 2–3 mv after the beginning of superfusion of ouabain ranged from 15 to 30 minutes in various preparations. In six preparations in which spontaneous activity occurred, the time of onset of spontaneous activity after the beginning of ouabain superfusion ranged from 28 to 50 minutes. Preparations obtained from the same heart usually showed less variation in their sensitivity to ouabain. Therefore, in some experiments, two preparations from the same heart were simultaneously studied to demonstrate the accelerated rate of increased automaticity in the presence of epinephrine. In other words, both preparations were superfused with ouabain, but only one of them was also treated with epinephrine. Both preparations from the same heart were placed in a tissue bath, which was divided in half by a plastic septum, and independently superfused with ouabain. The addition of Evans blue dye to one side of the bath indicated that there was no obvious leak to the other side. Both preparations were paced simultaneously through two pairs of bipolar stimulating electrodes connected to the same pulse generator. Effects of both ouabain and epinephrine were studied on one preparation, and these effects were compared with the effects of ouabain alone on the other preparation at any phase of the experiment. Results of six experiments studied in this manner again showed that epinephrine consistently accelerated the rate of increase in the slope of diastolic depolarization of digitalized Purkinje fibers.

Figure 2 depicts the results of one of the experiments in which the effect of epinephrine was studied in the manner described in the preceding paragraph. To facilitate the description of these results, the preparation from which the top tracing of action potentials was recorded is referred to as the top preparation, and that from which the bottom tracing was recorded is the bottom preparation. Tracing A is a control recording of transmembrane potentials of a Purkinje fiber. At the arrow labeled O, ouabain was superfused onto both preparations independently. Tracing B shows that no obvious change in the slope of diastolic depolarization was observed in either preparation 22 minutes after the start of ouabain superfusion. Epinephrine was then injected into the bottom preparation at the arrow labeled E. In tracing C, recorded 1 minute after the application of epinephrine, the slope of diastolic depolarization of the Purkinje fiber of the bottom preparation had increased. In tracing D, recorded 3 minutes after epinephrine was infused or 25 minutes after the beginning of ouabain superfusion, spontaneous activity had developed in the bottom preparation. This change contrasts with the relatively small changes in the slope of diastolic depolarization of the Purkinje fiber of the top preparation. In tracing E, recorded 28 minutes after the beginning of ouabain superfusion, pacing stimuli to both
preparations were stopped. The arrow indicates the last stimulus artifact. The bottom preparation evinced sustained spontaneous activity, but the top preparation only developed one spontaneous action potential followed by a low-amplitude potential. The results of this experiment clearly demonstrate that epinephrine potentiates the ouabain-induced increase in automaticity of Purkinje fibers.

In some experiments, the onset of spontaneous activity was used as the end point of the experiment; therefore, the exact time course of its occurrence could be compared in two preparations from the same heart. Hence, only the studies in which spontaneous activity occurred in both preparations were considered. The results of one of these studies is illustrated in Figure 3. The time required for the preparation treated first with ouabain and then with epinephrine to develop spontaneous activity was 22 minutes (tracing A–C). The time required for the preparation treated with ouabain alone to develop spontaneous activity was doubled to 41 minutes (tracing D–G). The results of two other studies showed a similar pattern.

Propranolol was used to determine whether the potentiating effect of epinephrine on the rate of diastolic depolarization of digitalized Purkinje fibers could be prevented by a beta-receptor blocking agent. Propranolol at higher concentrations also reverses digitalis-induced electrophysiological changes in Purkinje tissue. Therefore, a concentration that would exert a beta-blocking effect but would not interfere with the effect of digitalis

![Figure 3](image)

Comparison of the time required for the development of spontaneous activity in two digitalized preparations from the same heart. One preparation was further treated with epinephrine. Abbreviations and calibrations are the same as in Figure 1. See text for details.

![Figure 4](image)

Beta-receptor blocking effect of propranolol. P = propranolol, E' = first application of epinephrine, and E'' = second application of epinephrine. Calibrations are the same as in Figure 1. See text for details.

needed; propranolol at a concentration of 0.2 mg/liter was found to meet these requirements. Its effectiveness as a beta-receptor blocking agent was tested by determining its ability to prevent the epinephrine-induced increase in the slope of diastolic depolarization of Purkinje fibers. The results of one of these studies are illustrated in Figure 4. Tracing A is a control recording. In tracing B, after 6 µg of epinephrine was applied to the preparation at E', the magnitude of diastolic depolarization was increased 2–3 mv. Propranolol (0.2 mg/liter) was then superfused onto the preparation, and tracing C shows the transmembrane potentials recorded 5 minutes after the beginning of the superfusion. Epinephrine was again injected onto the preparation at E'' Tracing D shows that the epinephrine-induced increase in the slope of diastolic depolarization was abolished during propranolol superfusion. Similar results were obtained in seven other experiments.

In five experiments, the efficacy of propranolol blockade of the potentiating effect of epinephrine on the rate of diastolic depolarization of digitalized Purkinje fibers was studied. The results of a typical experiment are depicted in Figure 5. Tracing A is a control recording, and tracing B shows the transmembrane potentials 20 minutes after the beginning of ouabain superfusion. The preparation was then superfused with a mixture of ouabain and propranolol for the rest of the experiment. Tracing C shows the transmembrane potentials 27 minutes after the beginning of ouabain superfusion or 7 minutes from the start of superfusion with the mixture of ouabain and propranolol. Epinephrine was then injected onto the preparation. Tracing D shows the transmembrane potentials recorded 3
minutes after the application of epinephrine. The potentiating effect of epinephrine on the rate of diastolic depolarization was prevented in the presence of propranolol. Tracing E shows that a slow but progressive increase in the slope of diastolic depolarization and a decrease in the amplitude of transmembrane potential were observed 16 minutes after the application of epinephrine. These changes were probably due to the direct effect of ouabain on Purkinje fibers. In four other studies in which the preparations were superfused with the mixture of ouabain and propranolol, the transmembrane potentials showed similar changes, and the time course of these changes was comparable with that observed in studies in which ouabain alone was used.

Discussion

In the present study, ouabain increased the slope of diastolic depolarization of Purkinje fibers. This change was usually slow and was associated with a proportionate decrease in the maximum diastolic potential. These findings are similar to those reported by others (4-6). When digitalized Purkinje fibers were exposed to epinephrine, the rate of these changes was greatly accelerated. The diastolic depolarization often increased from about 3 mv to approximately 15 mv, and in many instances spontaneous activity developed within 3 minutes after the application of epinephrine. The rate of change in diastolic depolarization under these conditions was too rapid to be attributed to the effect of ouabain or epinephrine alone. In the preparations treated with epinephrine alone, the increase in diastolic depolarization within the same length of time was much smaller, e.g., approximately 3 mv. These results indicate that ouabain and epinephrine are synergistic in increasing the slope of diastolic depolarization in Purkinje fibers. This view is further supported by the results of our experiments in which two preparations from the same heart were studied simultaneously. This experimental arrangement permitted the effects of the combination of ouabain and epinephrine on one preparation to be precisely compared with those of ouabain alone on the other preparation. In this experiment, the rate of increase in diastolic depolarization of digitalized Purkinje fibers was consistently accelerated by the exposure to epinephrine. The time course of onset of spontaneous activity observed under the influence of both ouabain and epinephrine was at least twice as fast as that observed with ouabain alone. These observations, therefore, indicate that epinephrine influences the effect of digitalis on automaticity of Purkinje fibers. This finding agrees with the observations in intact animals made by Roberts et al. (8), Erlij and Mendez (9), and Mendez et al. (10).

The results of the present study further suggest that epinephrine modifies the rate of electrophysiological changes in Purkinje fibers induced by ouabain but does not produce any new changes. This effect of epinephrine on digitalized Purkinje fibers is probably synergistic rather than additive. An increase in diastolic depolarization of only about 3 mv was observed when Purkinje fibers were treated with epinephrine alone. A comparable degree of change was also observed in the preparations treated with ouabain alone just prior to the application of epinephrine. The sum of these values or approximately 6 mv of diastolic depolarization would be expected if the effects of the two agents were additive. However, epinephrine applied to digitalized Purkinje fibers often induced an increase of about 15 mv in diastolic depolarization. Furthermore, this potentiating effect of epinephrine depended on the level of digitalization. When epinephrine was applied prior to the 3-mv increase in diastolic depolarization, it usually resulted in a small increase in diastolic depolarization (2-3 mv); its potentiating effect became more prominent as the level of digitalization was increased.

Propranolol, a beta-receptor blocking agent, was used to determine whether the potentiating effect of epinephrine could be prevented. This agent has been shown to exert some direct effects on transmembrane potentials of Purkinje fibers (11) and to be effective in reversing digitalis-induced electrophysiological changes in Purkinje fibers (5). However, the dose required to exert these effects is several times larger than that needed to achieve beta-receptor blocking action. Therefore, an appropriate dose would exert only effective beta-receptor blocking action. In the present study, propranolol at a concentration of 0.2 mg/liter was found to be adequate in preventing epinephrine
from increasing the slope of diastolic depolarization of Purkinje fibers. This dose of propranolol did not alter the effects of ouabain on transmembrane potentials of Purkinje fibers. It was further demonstrated that propranolol at this concentration prevented the epinephrine-induced increase in diastolic depolarization of digitalized Purkinje fibers. The observed effect of propranolol, therefore, suggests that the potentiating effect of epinephrine on digitalized Purkinje fibers is mediated by stimulation of beta receptors.

Our observation that epinephrine potentiates the effect of ouabain on diastolic depolarization and automaticity of Purkinje fibers suggests that catecholamines might contribute to the development of digitalis-induced arrhythmias in man. Sympathetic activity of various degrees is present constantly in individuals taking digitalis, and the intensity of this sympathetic activity at any moment probably has a great bearing on the effects of digitalis. A possible clinical implication of this view is that patients who receive an adequate dose of digitalis could suddenly develop cardiac arrhythmias when they are subjected to any form of stress or to an increase in sympathetic activity.

References
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