Effects of Acetylcholine on Automaticity and Conduction in the Proximal Portion of the His-Purkinje Specialized Conduction System of the Dog

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ABSTRACT
Conventional intracellular recordings from the bundle of His and right bundle branch of the canine heart demonstrated that the slope of diastolic depolarization is markedly depressed by superfusion with relatively small concentrations (4–8 μg/ml) of acetylcholine. As the cells become less automatic, take-off potential increases, rise time of phase 0 is reduced, action potential amplitude increases, and conduction proceeds more rapidly.

KEY WORDS
right bundle branch
determinants of conductivity
spontaneous phase 4 depolarization
transmembrane action potential
innervation of the heart

Early investigations indicated that cells of the specialized conduction system below the atrioventricular (AV) junction are unaffected by small concentrations of acetylcholine (1). Specifically, the slope of spontaneous phase 4 depolarization from cells of the bundle of His, bundle branches, and peripheral Purkinje fibers is reported to be essentially unchanged by the infusion of acetylcholine. A method of exposing the entire proximal portion of the specialized AV conduction system of the canine heart in vitro was devised by one of the authors (M.V.E.), and the regular observation of automaticity afforded the opportunity to reexamine certain aspects of this property.

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The purpose of this paper is to report our studies of the effects of small doses of acetylcholine on spontaneity and conduction within the proximal portion of the His-Purkinje conduction pathways.

Methods
Adult mongrel dogs of either sex weighing 15–20 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and intubated; respiration was controlled with a Harvard respirometer. The hearts were excised rapidly through a right thoracotomy, and the portion of the septum containing the AV junction, the bundle of His, and both bundle branches was removed, placed in a Lucite muscle chamber, and superfused with oxygenated Tyrode’s solution maintained at a temperature of 31 ± 0.2°C. Then the entire bundle of His was exposed from the nodal His (NH) region of the AV junction to the origins of both bundle branches. Portions of the proximal part of the specialized AV conduction system were isolated, when appropriate, by transection. The tissue was then allowed to stabilize for several hours until muscular contraction ceased and to minimize changes in diastolic depolarization as a function of time and independent of acetylcholine. All control recordings were obtained no sooner than 3 hours after dissection. Locations of stimulating and recording electrodes varied and are identified in the figure legends and text. When electrical stimulation was used, the...
frequency was slightly greater than the spontaneous frequency of discharge. A conventional microelectrode technique was employed (2, 3). Acetylcholine chloride was administered either by superfusion with a solution containing 4 or 8 μg/ml or by injection of a 250-μg bolus into the solution bathing the preparation (to yield a final bath concentration of 4 μg/ml). Rate of superfusion was 4 ml/min.

**Results**

Figure 1 is a representative recording of action potentials from the branching portion of the bundle of His. The control tracing shows relatively rapid spontaneous phase 4 depolarization. A 250-μg bolus of acetylcholine chloride injected directly into the bath caused an immediate pronounced reduction in the slope of diastolic depolarization and the rate of spontaneous discharge. Maximal effect was attained at 0.8 minutes; at 3.2 minutes there was partial recovery, and at 28 minutes recovery was complete.

Figure 2 illustrates the results of an experiment in which the proximal portion of the isolated right bundle branch was impaled with a microelectrode. During superfusion with acetylcholine, 4 μg/ml, in normal Tyrode’s solution, the slope of spontaneous phase 4 depolarization decreased, as did the rate of discharge. Concomitantly, take-off potential increased, the upstroke became more rapid, and total amplitude and overshoot increased. Following reinfusion of normal Tyrode’s solution, the slope of phase 4 depolarization and the spontaneous discharge rate increased to control values.

Because of the well-known relationship between automaticity and conduction depression (4), a series of ten experiments was performed to determine how the abolition of spontaneous diastolic depolarization with acetylcholine might affect conduction. Figure 3 is an example of one such study. After exposure of the proximal portion of the His-Purkinje system, stimulating electrodes were placed in the NH portion of the AV junction. The penetrating portion of the His bundle and the right bundle branch approximately 2 cm from its origin were then impaled with microelectrodes. The preparation was stimulated at a rate slightly greater than its spontaneous discharge rate so as not to affect the slope of diastolic depolarization. The control tracing was obtained 15 minutes after initiation of stimulation. During superfusion with acetylcholine, there was significant foreshortening of conduction times from the stimulating electrode to the proximal recording electrode and...
between the two recording electrodes. Furthermore, there was a gradual increase in take-off potential of the proximal cell, a more rapid upstroke, and a greater total amplitude. No such changes were recorded from the right bundle branch, indicating that this cell responded maximally to the stimulus, albeit after varying conduction times.

Figure 4 depicts total action potential and phase 4 configuration during control stimulation, acetylcholine superfusion, and at recovery. The frequency of stimulation was constant. Although relative activation times are not readily apparent from this figure, there are obvious changes in the configuration of the proximal action potential. Specifically, the slope of phase 4 depolarization decreased, and take-off potential, total amplitude, and overshoot increased during superfusion with acetylcholine.

Figure 5 was recorded during an experiment in which the right bundle branch was transected at its origin. Stimulating electrodes were placed just distal to the cut, and the bundle branch was impaled at the level of the conus papillary muscle and just proximal to the severed false tendon to the anterior papillary muscle. Simultaneous rise times and maximal rising velocities (dV/dt) were recorded. During control stimulation there was profound conduction depression between the stimulating and proximal recording electrodes, and the rising velocity of the proximal
response was too slow to measure at the gain that was used. However, during superfusion with acetylcholine, 8 \( \mu \)g/ml, there was an increase in the proximal take-off potential, maximal rising velocity, and total amplitude coincident with more rapid conduction between the stimulating and recording electrodes. These parameters returned to control values after normal Tyrode’s solution was rein infused. It is noteworthy that the distal cell demonstrated only a foreshortening of activation times, but the proximal cell showed variation in take-off potential and action potential configuration. This observation implies that an area of depressed conduction was impaled by the proximal microelectrode and hence that the cell from which the recording was made demonstrated electrophysiological alterations consistent with such depression of impulse propagation. On the other hand, the distal microelectrode may have impaled a cell in a relatively “healthy” area where the monophasic action potential appeared more normal and constant in configuration. An alternative explanation is that, because of longitudinal dissociation and block, the responses in the two microelectrodes were independent, suggesting functionally separate pathways. However, in either instance, acetylcholine enhanced conduction.

Figure 6 illustrates parallel enhancement of conduction between the stimulating and proximal recording electrodes and between the two recording electrodes while take-off potentials, rising velocities, and total amplitudes increase simultaneously. This experiment, again involving the isolated right bundle branch, was similar in design to the study depicted in Figure 5. The results suggest that conduction depression which results in graded responses distally may be alleviated by acetylcholine. Certainly, specific determinants of conductivity are enhanced by small doses of this drug.
FIGURE 5
Changes in conduction within right bundle branch during acetylcholine, 8 μg/ml, superfusion. Note gradual increase in dV/dt of proximal cell. See Figure 3 for explanation of sketch (bottom right). N.T. = normal Tyrode’s solution.

Discussion
Spontaneous diastolic depolarization has been demonstrated to be, under certain circumstances, a normal property of cells of the specialized conduction system from the NH region of the AV junction through the peripheral Purkinje network (1). The characteristic phase 4 decline of the transmembrane potential of these cardiac cells may be the consequence of decreased potassium conductance during electrical diastole (5-8). A slow influx of sodium concomitant with the reduced potassium conductance results in a net inward current of positively charged ions which gradually reduces the transmembrane potential to threshold. Acetylcholine, by increasing potassium conductance (9), permits a stable resting diastolic potential.

The actions of this drug on atrial and AV junctional cells are well documented and widely recognized (10). Considerable anatomical evidence exists for the presence of cholinergic receptors in the ventricles (11-17). Furthermore, acetylcholinesterase activity has been documented in the canine ventricles (16). The physiological evidence for direct vagal or acetylcholine-induced effects on the ventricles has been reported. Eliakim et al. (18) noted that intravenous injections of acetylcholine more profoundly depressed the idioventricular rate than did vagal stimulation in dogs with heart block induced by injection of formalin into the bundle of His. They surmised that most of the vagal fibers accompanying the main bundle were destroyed by the technique employed to produce heart block. Others have reported a significant reduction in ventricular rate as a result of vagal stimulation in adrenalectomized dogs after removal of the upper vertebral and stellate ganglia and section of the bundle of His (19). Fisch and co-workers (20) have presented electrocardiographic evidence that acetylcholine consistently alters ventricular repolarization in the intact dog. Greenspan et al. (21) have demonstrated similar alterations consequent to vagal stimulation. Others (22) have reported potent negative inotropic effects of vagal stimulation in canine hearts, although still others have demonstrated a direct positive inotropic effect of acetylcholine on ventricular

FIGURE 6
Another example of changing conduction within the right bundle branch consequent to acetylcholine, 4 μg/ml. Note that take-off potential, rising velocity of phase 0, and total amplitude increase in both cells. P = recording from proximal electrode; D = recording from distal electrode.

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myocardium, an apparent paradox which has led to the postulation of multiple cholinergic receptors in the heart (23). Changes in the configuration of ventricular action potentials have been reported as a result of large doses of acetylcholine (24), and a recent study describes the effects of acetylcholine on ionic currents in calf Purkinje fibers subjected to voltage clamping (25).

Latent pacemakers below the AV junction become the dominant automatic focus when relieved of suppression by higher, more rapidly discharging pacemakers (26-30). Anatomical isolation of each segment of the specialized AV conduction system removes the influence of more rapid pacemakers. Thus, recording of spontaneous activity within such a segment represents the inherent pacemaker potential of that segment. Our studies indicate that specialized conducting cells below the AV junction, when rendered automatic by anatomical isolation, are responsive to a significant degree to the negative chronotropic effects of acetylcholine.

Singer et al. (4) demonstrated that phase 4 depolarization in Purkinje fibers results in varying degrees of conduction depression. Thus, as impulses arrive at a cell progressively later during spontaneous phase 4 depolarization, take-off potential will be decreased, and hence total action potential amplitude, maximal rising velocity of phase 0, and conduction velocity will fall. Conversely, abolition of diastolic depolarization should result in improved conduction since impulses are arriving when the diastolic potential is greater because of slowed phase 4 depolarization or complete disappearance of pacemaker behavior. Our experiments indicate that acetylcholine, by depressing the slope of spontaneous phase 4 depolarization, permits conduction through the proximal portion of the His-Purkinje system to proceed more rapidly because of the progressive increase in transmembrane potential at the moment of excitation.

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