Effect of acetylcholine on automaticity of canine Purkinje fibers

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TSE, Warren W., Jaok Han, and Myung S. Yoon. Effect of acetylcholine on automaticity of canine Purkinje fibers. Am. J. Physiol. 230(1): 116-119. 1976. — The effect of acetylcholine on automaticity of Purkinje fibers was studied in isolated canine false tendon preparations with conventional microelectrode techniques. Of 15 preparations with the control spontaneous rate of 12–60 beats/min, acetylcholine in a concentration of 0.5 μg/ml decreased the spontaneous rate by 20–87% in 13 preparations. This decrease in automaticity was due to a decrease in the slope of phase 4 depolarization and an increase in the maximum diastolic potential. The inhibitory effect of acetylcholine could be reversed by atropine in a concentration of 3 μg/ml in six preparations and prevented by pretreatment with atropine in another six preparations. Atropine per se did not have any appreciable effect on automaticity of Purkinje fibers. The results indicate that acetylcholine significantly suppresses automaticity of canine Purkinje fibers through its muscarinic action.

Whether or not vagal stimulation of acetylcholine (ACh) has electrophysiologic effects on the mammalian ventricles has been controversial. Earlier studies failed to show any appreciable effect of vagal stimulation on the idioventricular pacemaker rate (7, 11). Hoffman and Cranefield (12) also reported that ACh in relatively large concentrations does not decrease the spontaneous discharge of Purkinje fibers. More recent studies, however, indicate that cholinergic stimulation can exert significant effects on idioventricular automaticity in mammalian hearts. For example, Benforado (2) demonstrated that ACh decreases the ventricular rate of isolated rabbit hearts, and Eliakim et al. (6) showed that ACh and vagal stimulation decrease the rate of idioventricular rhythm in dog hearts in which the atrioventricular (AV) transmission was interrupted. The present study was conducted to investigate the effect of ACh on spontaneously discharging Purkinje fibers with conventional microelectrode techniques.

Methods

Hearts were excised from dogs anesthetized with pentobarbital sodium (30 mg/kg iv). False tendons, each with a piece of ventricular muscle attached, were dissected from the right or the left ventricle and stored in oxygenated Tyrode solution. The preparation was then pinned to a paraffin block in a tissue bath. Modified Tyrode solution equilibrated in a reservoir with 95% O₂ and 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 solution was as follows: 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 recorded with conventional microelectrode techniques. Glass microelectrodes were filled with 2.7 M KCl and the resistance of these electrodes ranged from 20 to 30 MΩ. A silver-wire indifferent electrode was in contact with the Tyrode solution in the bath. Action potentials were displayed on an oscilloscope and photographed with a Grass camera.

The effects of ACh and atropine were studied in only those Purkinje preparations that exhibited spontaneous activity with the prominent phase 4 depolarization. Acetylcholine chloride was diluted with the Tyrode solution in a concentration of 0.5 μg/ml (2.8 x 10⁻⁹ M) and infused into the bath. Atropine was also diluted with the Tyrode solution and infused in a concentration of 3 μg/ml (4.4 x 10⁻⁹ M). In the present study, only those experiments in which a microelectrode remained in the same fiber throughout were analyzed.

Results

Effect of acetylcholine. The effect of ACh in a concentration of 0.5 μg/ml was studied in 15 preparations. Acetylcholine consistently reduced the spontaneous rate by decreasing the slope of phase 4 depolarization in 13 preparations. These changes began to occur slowly in about 1–3 min after the beginning of superfusion of ACh, and the maximum effect was seen in another 2–8 min. The duration of such inhibitory effect lasted for a considerable length of time. It took about 20–40 min for the spontaneous rate to return to the control level after the preparation was returned to the Tyrode solution. Table 1 summarizes the results of the 15 experiments. In 13 experiments, ACh decreased the spontaneous rate of Purkinje fibers by 20–87%. In two experiments, no appreciable change in the spontaneous rate was observed. Figure 1 depicts the results of a representative experiment. Record A shows the control spontaneous rate of about 43 beats/min with the maximum diastolic potential of −55 mV. The prominent phase 4 depolarization
TABLE 1. Effect of acetylcholine on spontaneous rate of Purkinje fibers

<table>
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<tr>
<th>Exp No</th>
<th>Control, beats/min</th>
<th>Lowest Spontaneous Rate during ACh, beats/min</th>
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<tr>
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</table>

Mean 31.3 17.5 -49

P < 0.02

and smooth transition from phases 4 to 0 suggest that the Purkinje fiber impaled by the microelectrode was the pacemaker of the preparation. At the arrow, ACh was superfused to the preparation. The spontaneous rate began to decrease about 1 min after the beginning of the superfusion in record B. The rate continued to decrease slowly to 20 beats/min and the maximum diastolic potential increased to -65 mV in record C, about 8 min after the onset of the superfusion. Record D, a superimposition of records A and C, shows that the maximum diastolic potential was increased and the slope of phase 4 depolarization was decreased in the presence of ACh. The arrows indicate the action potentials from record C. Record E shows that the spontaneous rate increased to about 51 beats/min (a value near control). The increase in rate was largely due to an increase in the slope of phase 4 depolarization.

In the second group of six experiments, the preparations were first treated with atropine and then with the mixture of atropine and ACh. The results indicated that atropine per se had no significant effect on the spontaneous activity of Purkinje fibers. The subsequent superfusion of ACh also failed to produce any apparent inhibition on the spontaneous activity. Figure 3 depicts the results of one of these experiments. Record A shows the control rate of 17 beats/min. At the solid arrow, atropine was superfused to the preparation. Record B, 10 min after the beginning of the superfusion, shows no significant change in the spontaneous rate (18 beats/min). At the open arrow, the mixture of ACh and atropine was superfused to the preparation. Record C shows that a slight increase in the rate (21 beats/min) was observed.
10 min after the beginning of this superfusion. The important observation is that the inhibitory effect of ACh was prevented by atropine.

DISCUSSION

In the present study ACh was shown to inhibit automaticity of Purkinje fibers, and this finding is in agreement with the results of Benforado (2) and Eliakim et al. (6) that ACh decreases the dioviodural rate of isolated rabbit hearts and dog hearts in situ. The present study showed that the inhibition of automaticity of Purkinje fibers by ACh was primarily due to a decrease in the slope of phase 4 depolarization. The observed increase in the maximum diastolic potential may have also contributed to a decrease in the spontaneous discharge. The mode of action of ACh in decreasing the spontaneous discharge of Purkinje fibers is similar to that of other automatic fibers in the higher pacemaker sites. Weet et al. (17) showed that ACh decreases the spontaneous rate of the sinoatrial node of rabbit hearts. Bailey et al. (1) showed that ACh decreases the spontaneous rate of the His bundle of dog hearts. Trautwein and Kassebaum (15) and Vassalle (16) suggested that the increase in phase 4 depolarization of Purkinje fibers is mediated by the same ionic mechanism that increases potassium conductance. The finding that ACh consistently increases the maximum diastolic potential probably reflects the increased potassium conductance and a shift of the membrane potential toward the potassium equilibrium potential.

The results of the present study also indicate that the action of ACh on Purkinje fibers is slow. The onset of the inhibitory effect on the spontaneous activity was about 1–3 min after the beginning of the superfusion and reached the maximum effect in another 2–8 min. A similar observation was described by Bailey et al. (1) on the proximal portion of the His-Purkinje system. They showed that ACh at 4 μg/ml progressively suppresses the rate of spontaneously beating His bundle fibers. Another characteristic action of ACh on Purkinje fibers is its prolonged effect. It required about 40 min for the preparation to return to a control rate after returning to the Ach-free Tyrode solution. This can probably be attributed to the lower concentration of enzyme cholinesterase in the ventricles. The distribution of the enzyme cholinesterase activity in the canine ventricles is said to be about one-third of that in the atria (4).

The present experiments indicate that atropine alone does not have any significant effect on the spontaneous rate of Purkinje fibers. However, in the preparation in which the spontaneous rate was first suppressed by ACh, atropine increases the spontaneous rate to a level near the control. These findings suggest that the effect of atropine is due to blocking action on the cholinergic receptors and not due to a possible direct effect. This view is further supported by the fact that ACh could not suppress the spontaneous rate of Purkinje fibers pre-treated with atropine. Benforado (2) and Eliakim et al. (6) showed a similar effect in isolated rabbit hearts and dog hearts in situ, respectively. Bucino et al. (3) postulated that two types of cholinceptive receptors are present in the ventricular tissues. The first type is muscarinic and responds to a small dose of ACh (0.3 μg/ml), resulting in mild negative inotropism. The second type is nonspecific and responds to a large dose of ACh (30 μg/ml), resulting in positive inotropism. The former effect is blocked and the later is not blocked by atropine.

Since the inhibitory effect of ACh on the spontaneous activity of Purkinje fibers was exerted by a small dose of ACh (0.5 μg/ml) and was reversed or prevented by atropine, it is logical to assume that the cholinceptive receptors that mediate this effect are muscarinic.

Evidence is also available to suggest that the vagus nerves influence idioventricular rhythm and other ventricular electrophysiologic properties. Eliakim et al. (6) demonstrated that vagal stimulation suppresses the idioventricular rate. Greenspan et al. (9) showed that vagal stimulation increases the amplitude of the T wave of normo- and hyperkalemic dog hearts. These findings suggest that vagal activity might have some physiological or clinical implication. First, vagal activity probably has some regulatory function on the idioventricular rhythm during complete AV block. Second, vagal stimulation was shown to delay or prevent the development of ventricular arrhythmias and to increase the ventricular fibrillation threshold (5, 13). The demonstrated effect of ACh on phase 4 depolarization of Purkinje fibers may be in part responsible for these antiarrhythmic effects. A decrease in phase 4 depolarization and an increase in the maximum diastolic potential are considered to improve impulse conduction and lead to electrical stability in the ventricle.

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REFERENCES

