Acetylcholine Reverses Effects of \( \beta \)-Agonists on Pacemaker Current in Canine Cardiac Purkinje Fibers but Has No Direct Action

A Difference Between Primary and Secondary Pacemakers

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We have investigated the actions of acetylcholine in the absence and presence of the \( \beta \)-agonist isoproterenol in cardiac Purkinje fibers. \( \beta \)-Agonists, like isoproterenol, increase the magnitude of the pacemaker current (I\( _p \)) in cardiac myocytes by shifting its activation voltage more positive on the voltage axis. We find that acetylcholine has no effect on I\( _p \) in the absence of isoproterenol. However, if I\( _p \) is first increased by \( \beta \)-agonist stimulation, acetylcholine can then return I\( _p \) to control levels. This effect on I\( _p \) is exerted through muscarinic receptors since atropine prevents this action of acetylcholine. Functionally, this action of acetylcholine can guarantee the maintenance of ventricular pacemakers when there is high parasympathetic tone but can also prevent extra ventricular beats when sympathetic and parasympathetic tone are both high. *(Circulation Research 1990;66:633–636)*

The pacemaker current (I\( _p \)) is present in the sinus node\(^1\) and in cardiac Purkinje fibers.\(^2\)

In both preparations it activates on hyperpolarization, is largely nonselective for Na\(^+\) over K\(^+\), is blocked by low concentrations of Cs\(^+\), and is shifted in the positive direction on the voltage axis by \( \beta \)-agonists.\(^3\)–\(^6\)

Recent work by DiFrancesco and Tromba\(^7\)–\(^9\) has investigated the actions of acetylcholine on myocytes from the rabbit sinoatrial node. In these studies, acetylcholine shifted the activation of I\( _p \) in the negative direction on the voltage axis. Similar studies investigating the actions of acetylcholine on I\( _p \) in Purkinje fibers have indicated that in this preparation I\( _p \) is essentially insensitive to acetylcholine or may shift the position of its activation curve in the positive direction by only a few millivolts on the voltage axis.\(^10\),\(^11\) These previous studies were performed in the absence of Ba\(^{2+}\), which blocks background K\(^+\) permeability and the acetylcholine-induced K\(^+\) current. Because I\( _p \) is highly K\(^+\) dependent,\(^3\) [K\(^+\)]\(^+\) fluctuations in narrow intercellular spaces caused by opening of K\(^+\) channels may interfere with the measurement of I\( _p \).\(^2\) If acetylcholine had no effect in Purkinje fibers, this might suggest the absence of muscarinic receptors coupled either directly or indirectly to I\( _p \). An alternative interpretation hypothesized by DiFrancesco and Tromba\(^8\) is based on the observation that in sinoatrial node myocytes the acetylcholine-induced I\( _p \) inhibition is due to a reduction of cyclic AMP levels. Since in Purkinje fibers the I\( _p \) activation threshold is more negative than in the sinoatrial node cell, DiFrancesco and Tromba\(^8\) suggested that in the Purkinje fibers basal adenyl cyclase activity and cyclic AMP levels are much lower than in the sinoatrial node. Thus, in Purkinje fibers cyclic AMP levels may be too low to be further reduced by acetylcholine under normal conditions. If this is correct, then application of acetylcholine after \( \beta \)-adrenergic stimulation (when cyclic AMP levels are elevated) should result in a reduction of I\( _p \) due to a negative shift on the voltage axis. The results presented below test this hypothesis.

**Materials and Methods**

The two-microelectrode voltage-clamp technique was applied to canine Purkinje fibers of narrow radius (<0.15 mm) and short length (<1.5 mm). The method for recovering the fibers and the electronic setup were as reported previously by Cohen and Mulrine.\(^13\) To reduce our use of animals, fibers were stored overnight, in some cases, in medium 199 (HEPES buffered to pH 7.2–7.4, Sigma Chemical, St.

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Supported by National Institutes of Health grants HL-20558, HL-28958, HL-36075, and HL-35064.

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Received June 21, 1989; accepted September 15, 1989.
Louis, Missouri) bubbled with 95% O₂-5% CO₂ with 100 units penicillin and 100 μg streptomycin/ml. Healthy fibers on the second day responded well to both isoproterenol and acetylcholine. The control Tyrode’s solution contained (mM) NaCl 140, KCl 8, CaCl₂ 2, MgCl₂ 1, BaCl₂ 4, MnCl₂ 2, dextrose 8, and HEPES 5 buffered to pH 7.4. BaCl₂ was added to eliminate background K⁺ permeability and the associated [K⁺] fluctuations. Acetylcholine chloride, isoproterenol HCl, and atropine sulfate (all from Sigma Chemical) were added to the control solution in the concentrations indicated. The data were collected at temperatures between 34° and 36° C and were constant to 0.5° C in any experiment. As in our previous studies on canine Purkinje fibers, activation curves were not constructed because of slow relaxations of the pacemaker current, which could take more than a minute to reach steady state. Instead, we measured current amplitude and obtained information on the voltage dependence of activation with a three-pulse protocol.₈,₉

Results

Figure 1 illustrates our basic findings. In response to a hyperpolarizing voltage step from −50 to −88 mV, acetylcholine (1 μM) has no effect on the Iₛ in the absence of β-adrenergic stimulation (see Figure 1A). β-Adrenergic stimulation (10 μM isoproterenol) increases the amplitude of Iₛ and decreases the activation time constant (Figure 1B). When acetylcholine is added in the presence of isoproterenol, there is a reversal of the changes in membrane current and the time course of Iₛ in the presence of acetylcholine, and isoproterenol superimposes on that in the control solution (Figure 1C). This action of acetylcholine is muscarinic since addition of atropine, which is a muscarinic receptor blocker, eliminates it (Figure 1D). Removal of the atropine again results in the ability of acetylcholine to reverse the effects of isoproterenol (Figure 1E). Similar results on the interaction between acetylcholine and isoproterenol were observed in seven additional experiments, and the block by atropine of the acetylcholine effects was observed in a total of four experiments. In Figure 1F, the amplitude of Iₛ, as modified by application of the various agents, is plotted against time. The ordinate is the amplitude of time-dependent current, Iₛ, and the abscissa is the time in minutes of the experimental protocol. The points labeled A through E correspond to the data tracings above. The
plot shows that 1 µM acetylcholine has no effect by itself while isoproterenol increases the amplitude of \( I_f \). This effect of isoproterenol can be reversed by acetylcholine, but only if atropine, a muscarinic blocker, is not present.

This action of acetylcholine to reverse the effects of isoproterenol might simply be a blockade of \( I_f \) that fortuitously returns \( I_f \) to roughly control levels. To investigate this alternative, we used a three-pulse protocol to examine the voltage dependence of activation of \( I_f \). The results of a sample experiment are shown in Figure 2. The protocol involves two hyperpolarizing pulses, one to the middle of the \( I_f \) activation curve (−82 mV) and one to the top of the activation curve (−102 mV), followed by a depolarizing pulse (to −20 mV) to rapidly deactivate \( I_f \). Isoproterenol shifts the activation curve for \( I_f \) to more positive potentials on the voltage axis and, thus, increases the amplitude of \( I_f \) in response to the first hyperpolarizing voltage step and reduces the amplitude of \( I_f \) in response to the second voltage step. If acetylcholine blocks \( I_f \), it should reduce the amplitude of \( I_f \) in response to both pulses as compared with their amplitude in isoproterenol. However, if acetylcholine is shifting the activation curve back to the control position, it should return the time-dependent current in response to both hyperpolarizing steps to control levels. The results clearly indicate that acetylcholine reverses the voltage shift in \( I_f \) activation induced by isoproterenol and does not block the \( I_f \) current. Similar complete reversals of the effects of isoproterenol by acetylcholine in the three-pulse protocol were observed in two experiments; partial reversals were observed in two additional experiments.

Discussion

Our results demonstrate that acetylcholine has no action on \( I_f \) in canine Purkinje fibers in the absence of \( \beta \)-adrenergic stimulation; however, when the preparation is stimulated by isoproterenol, acetylcholine can reverse the effects of the \( \beta \)-agonist on \( I_f \).

These results support the hypothesis that acetylcholine may not act on \( I_f \) in unstimulated Purkinje fibers because of low basal levels of cyclic AMP. If low levels of cyclic AMP are present in Purkinje fibers, it could explain why the voltage range of \( I_f \) activation is more negative in Purkinje fibers than in sinoatrial nodal preparations.1,3

Reversal of \( \beta \)-adrenergic activation by acetylcholine is not unique to \( I_f \), but is similar to that observed on the calcium current in frog ventricular myocytes.14 In this preparation, acetylcholine reduced calcium current only when it was first increased by \( \beta \)-adrenergic stimulation.

The absence of a strong action of acetylcholine to directly suppress \( I_f \) and to inhibit Purkinje fiber automaticity could be of some functional value. In the presence of high vagal tone, the sinus rate can drop dramatically. However, the absence of a marked reduction in Purkinje fiber automaticity guarantees a ventricular pacemaker that can sustain an adequate cardiac output. On the other hand, in the presence of both high sympathetic and parasympathetic tone, it is important not to generate a higher rate of firing in Purkinje fibers than in the sinus node (whose rate would then be more than sufficient to maintain cardiac output). It is worth pointing out that this action of acetylcholine on \( I_f \) is only one of several actions of acetylcholine in Purkinje fibers.10,11,15–17 To understand the sum total of the effects of this neurotransmitter on automaticity, it is necessary to fully consider all acetylcholine-mediated transport changes. Nevertheless, present results support a dynamic interaction between sympathetic and parasympathetic stimulation in canine Purkinje fibers.

Acknowledgments

We would like to thank Joan Zuckerman for excellent technical assistance. We would also like to thank Judy Samarel for manuscript preparation.

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KEY WORDS • acetylcholine • Purkinje fibers • pacemaker current • isoproterenol
Acetylcholine reverses effects of beta-agonists on pacemaker current in canine cardiac Purkinje fibers but has no direct action. A difference between primary and secondary pacemakers.
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doi: 10.1161/01.RES.66.3.633

_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

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circres.ahajournals.org/content/66/3/633

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