Acetylcholine Antagonism of the Electrophysiological Effects of Isoproterenol on Canine Cardiac Purkinje Fibers

JOHN C. BAILEY, AUGUST M. WATANABE, H. R. BESCH, JR., AND DAVID A. LATHROP

SUMMARY The purpose of these experiments was to determine whether or not acetylcholine modulated the electrophysiological effects of isoproterenol on canine cardiac Purkinje fibers. Conventional microelectrode techniques were used. Predictably, isoproterenol produced shortening of action potential duration; acetylcholine significantly blunted this effect of isoproterenol. Isoproterenol restored excitability to fibers exposed to 22 mM potassium solutions, and acetylcholine abolished this isoproterenol-restored excitability. Both of these antagonistic effects of acetylcholine were blocked by atropine. Acetylcholine alone did not affect action potential duration in polarized fibers or excitability in potassium-depolarized fibers. Furthermore, acetylcholine had no effect on the decrease in action potential duration induced by premature electrical stimulation or by acetylcholinesynthetase. These data demonstrate a direct cellular basis for cholinergic antagonism of the electrophysiological effects of β-adrenergic stimulation of canine cardiac specialized intraventricular conducting tissue. Circ Res 44: 378-383, 1979.

CONTRARY TO long-established physiological concepts, several lines of evidence during the past two decades indicate that cholinergic nerves innervate the ventricles of mammalian hearts (Higgins et al., 1973). The evidence includes histological demonstration of cholinergic nerve endings (Kent et al., 1974), histochemical localization of choline acetyltransferase [the enzyme that catalyzes the synthesis of acetylcholine (Jacobowitz et al., 1967; Roskoski et al., 1975)], and demonstration of the presence of acetylcholine in the ventricular myocardium (Brown, 1976). In addition to the evidence establishing the presence of cholinergic nerves in the ventricles, recent studies have shown that postsynaptic muscarinic cholinergic receptors exist on ventricular myocardial cells. By interacting with muscarinic cholinergic receptors, cholinergic agonists increase cardiac tissue cyclic guanosine 3':5' monophosphate concentrations (George et al., 1973; Watanabe and Besch, 1975a). Muscarinic cholinergic receptors on myocardial cells also have been directly demonstrated with ligand-binding assays using [3H]quinuclidinyl benzilate to label the receptors (Fields et al., 1978).

Studies on the regulation of ventricular function by the autonomic nervous system support the conclusion that cholinergic nerves innervate the ventricles and, in addition, suggest that this innervation is functionally significant (De Geest et al., 1965; Daggett et al., 1967; Randall et al., 1968; Wildenthal et al., 1969). Stimulation of vagal nerves produced small reductions in contractility of dog hearts. When this stimulation was applied during simultaneous adrenergic stimulation, the negative inotropic effects were magnified (Levy et al., 1966; Dempsey and Cooper, 1969; Levy and Zieske, 1969; Levy, 1971, 1977). It thus appears that the cholinergic effects on ventricular contractile function are di-
directly and indirectly mediated, the latter occurring by modulation of sympathetic effects on the ventricles (Levy, 1971, 1977).

Although it has been demonstrated that the cholinergic nerves that innervate the ventricles most richly supply the ventricular septum and the areas surrounding the specialized conducting tissues (Kent et al., 1974), relatively few studies (Higgins et al., 1973) have examined the cardiac electrophysiological effects of stimulation of the vagal nerves or administration of cholinergic agonists. No studies have been reported that assessed directly the interaction between sympathetic and cholinergic nerves in regulating the electrophysiological properties of the specialized His-Purkinje system. The purpose of the present study was to examine the modulation by acetylcholine of adrenergic effects on the electrophysiological properties of canine Purkinje fibers. The fibers were studied in vitro to allow assessment of direct interaction, at the tissue level, between sympathetic and cholinergic effects. Using microelectrode techniques, we studied the modulation by acetylcholine of two well-recognized effects of catecholamines on cardiac Purkinje fibers: (1) shortening of the action potential duration in electrically paced, normally polarized fibers, and (2) restoration of excitability in potassium (22 mM) depolarized fibers (Watanabe and Besch, 1974).

Methods

Adult mongrel dogs of either sex, weighing 10-15 kg, were anesthetized with secobarbital (30 mg/kg, iv). Their hearts were removed rapidly through a right thoracotomy and immersed in cool, oxygenated Tyrode's solution. Free-running Purkinje tendons were excised from either ventricle, affixed to the paraffin floor of a 10-ml Lucite muscle chamber, and superfused with Tyrode's solution maintained at 37°C and gassed with 95% O2-5% CO2.

The composition of the Tyrode's solution was (in mM): Na+, 150; K+, 4.0; Cl-, 147; Ca2+, 2.7; HCO3-, 120; PO43-, 0.9; Mg2+, 0.5; and glucose, 5.5.

In the experiments with normally polarized fibers, the tissues were electrically stimulated at a constant basic cycle length of 800 msec. Action potential duration was measured at 50% and 100% repolarization in untreated fibers and during the following interventions: administration of isoproterenol alone or combined with various concentrations of acetylcholine; administration of isoproterenol, acetylcholine, and atropine; administration of isoproterenol, acetylcholine, and physostigmine; administration of acetylcholine alone or atropine alone; administration of acetylstrophanthidin and acetylcholine; and administration of acetylcholine during premature electrical stimulation.

In experiments with depolarized fibers, the tissues were stimulated at a constant cycle length of 1000 msec. After the electrophysiological recordings were stable, the potassium concentration of the superfusant was increased to 22 mM. When the tissue became inexcitable, isoproterenol (10^-7 M) was added to the superfusant, and an extracellular stimulus 10 msec in duration and of sufficient intensity to produce a response to each stimulus was delivered to the tissue. Acetylcholine then was added to the superfusant while isoproterenol was continued, and the effects of the choline ester on excitability were noted. An action potential-triggered tachometer was used to record whether a delivered stimulus resulted in a transmembrane voltage change. The tachometer produced a linear ramp triggered by the upstroke of each successive action potential of at least 30 mV. The tachometer was calibrated by delivering stimuli at known constant cycle lengths while recording the heights of the ramps. Thus, when the tachometer output was displayed at slow sweep speeds on the oscilloscope, the ramps appeared as a continuous solid bar, the height of the bar being directly proportional to the frequency of depolarization of the preparation.

In other fibers, the effects of acetylcholine on action potentials generated in a Na+-free solution containing 128 mM tetraethylammonium and 5 mM Tris were studied (Cranefield et al., 1972). Conventional microelectrode techniques were used. Continuous impalement of a single cell was maintained throughout an experiment. A t-test for paired groups was used to analyze the statistical significance of changes in the parameters measured (Dixon and Massey, 1969).

Results

Polarized Cardiac Purkinje Fibers

Isoproterenol (10^-7 M) consistently produced shortening of action potential duration, as expected. Analog recordings of a typical experiment are

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** The effects of acetylcholine on the shortening of action potential duration. A: A control cardiac Purkinje fiber action potential. B: Action potential duration shortening produced by isoproterenol (10^-7 M). C: The addition of acetylcholine (10^-5 M) partially reversed the shortening of action potential duration elicited by isoproterenol. D: Atropine (10^-4 M) attenuated the effects of acetylcholine, resulting in an action potential duration resembling that produced by the superfusion of isoproterenol alone. Zero potential indicated in each panel. Calibrations: horizontal bar = 50 msec; vertical bar = 25 mV.
shown in Figure 1. Acetylcholine antagonized this shortening induced by isoproterenol (Fig. 1C), and the antagonism was attenuated by atropine (Fig. 1D), suggesting that the effects of the choline ester were specifically mediated via muscarinic cholinergic receptors. A summary of the data pertaining to the effects of isoproterenol on total action potential duration and its modification by various concentrations of acetylcholine is presented in Table 1. In every instance, isoproterenol (10^{-7} M) effected a significant (approximately 12-14%) shortening of action potential duration at both the 50% and 100% level of repolarization. Addition of acetylcholine (10^{-7} M or 3 \times 10^{-7} M) to the Tyrode’s solution containing isoproterenol tended to attenuate the action potential duration shortening effects of the catecholamine, but the decreases were not statistically significant. When the concentration of acetylcholine was increased to 10^{-6} or 10^{-5} M, the shortening effects of isoproterenol on both 50% and 100% action potential duration were significantly reduced. The addition of atropine (10^{-6} M) significantly attenuated the effects of the latter concentrations of acetylcholine.

Physostigmine (10^{-6} M) significantly potentiated the effects of acetylcholine. Thus, acetylcholine (10^{-7} M), when given with physostigmine, significantly antagonized the action potential shortening induced by isoproterenol (Table 2). Physostigmine alone had no effect on action potential duration. Acetylcholine alone (10 fibers) or atropine alone (10 fibers) had no effect on action potential duration (data not shown). The shortening of action potential duration of the premature action potential during premature electrical stimulation (10 fibers) was not modified by 10^{-3} M acetylcholine (data not shown). Likewise, the shortening of action potential duration induced by acetylstrophanthidin (10 fibers) was not affected by 10^{-3} M acetylcholine. Results of a typical experiment are shown in Figure 2.

K⁺-Depolarized Fibers

Exposure of 10 Purkinje fibers to Tyrode’s solution containing 22 mM K⁺ resulted in reduction of

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**Table 1** Effect of Acetylcholine on Action Potential Duration Shortening Produced by Isoproterenol

<table>
<thead>
<tr>
<th></th>
<th>10^{-7} M ACh</th>
<th>10^{-6} M ACh</th>
<th>10^{-5} M ACh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (NT)</td>
<td>364 ± 50</td>
<td>377 ± 33</td>
<td>358 ± 61</td>
</tr>
<tr>
<td>+ Iso 10^{-7} M</td>
<td>317 ± 45*</td>
<td>307 ± 44*</td>
<td>304 ± 66†</td>
</tr>
<tr>
<td>+ Iso 10^{-7} M and ACh</td>
<td>325 ± 43</td>
<td>332 ± 32†</td>
<td>334 ± 54‡</td>
</tr>
<tr>
<td>+ Iso 10^{-5} M, ACh and atropine 10^{-6} M</td>
<td>314 ± 26</td>
<td>314 ± 32§</td>
<td>301 ± 47§</td>
</tr>
</tbody>
</table>

Values are mean ± SD of total action potential duration, n = number of experiments, Iso = isoproterenol, ACh = acetylcholine, NT = drug-free Tyrode’s solution.

* P < 0.01 compared to control.
† P < 0.05 compared to control.
‡ P < 0.06 compared to Iso response.
§ P < 0.01 compared to Iso/ACh response.

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**Table 2** Potentiation of the Electrophysiological Effects of Acetylcholine by Physostigmine

<table>
<thead>
<tr>
<th></th>
<th>10^{-7} M ACh</th>
<th>10^{-6} M ACh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (NT)</td>
<td>371 ± 52</td>
<td>367 ± 25</td>
</tr>
<tr>
<td>Physyo</td>
<td>375 ± 47</td>
<td>364 ± 27</td>
</tr>
<tr>
<td>Physyo + Iso</td>
<td>327 ± 44*</td>
<td>317 ± 26*</td>
</tr>
<tr>
<td>Physyo + Iso + ACh</td>
<td>346 ± 43†</td>
<td>322 ± 22</td>
</tr>
</tbody>
</table>

Values are mean ± SD of total action potential duration, n = number of experiments. Physostigmine (Physyo) was given in a concentration of 10^{-7} M. The concentration of isoproterenol (Iso) was 10^{-7} M. ACh = acetylcholine. NT as in Table 1.

* P < 0.01 compared to Physyo.
† P < 0.05 compared to Physyo + Iso.

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**Figure 2** The effects of acetylcholine on action potential shortening effected by the superfusion of acetylstrophanthidin, 5 \times 10^{-7} M. A: Control Purkinje fiber action potential. B: Action potential duration shortened by superfusion of acetylstrophanthidin. C: Addition of acetylcholine (10^{-6} M) produced no effect on action potential duration shortening due to acetylstrophanthidin. Administration of acetylstrophanthidin was continued during superfusion of acetylcholine. Zero potential lines and calibrations as in Figure 1.
The effects of acetylcholine on an isoproterenol-dependent "slow response." A: Control Purkinje fiber action potential in 4 mM potassium. B: Generalized depolarization and loss of excitability produced by superfusion of Tyrode's solution containing 22 mM potassium. C: Restoration of excitability by the addition of 10^{-7} M isoproterenol. D: The addition of acetylcholine (10^{-5} M) abolishes the "slow response" generated by the addition of isoproterenol. Zero potential lines and calibrations as in Figure 1.

The transmembrane potential to approximately −45 to −50 mV and loss of excitability. Analog recordings from a typical experiment are shown in Figure 3. The addition of 10^{-7} M isoproterenol restored excitability to these fibers and produced low-amplitude, slowly rising responses to stimulation (Fig. 3C). Acetylcholine (10^{-5} M) abolished the restoration of excitability by isoproterenol (Fig. 3D). Removal of acetylcholine from the superfusant regularly restored excitability. Figure 4 illustrates the results of a typical experiment which was continuously monitored by the tachometer recording. Isoproterenol restored excitability to this K^+-depolarized fiber so that each delivered stimulus (cycle length of 1000 msec) resulted in a "slow response," indicated by the solid black bar. Addition of acetylcholine (10^{-7} M) resulted initially in blockade of every other stimulus, and then in 3:1, and finally 4:1 responses to the applied stimuli. Wenckebach-type periodicity also was noted. The concentration of acetylcholine was then increased to 10^{-6} M, and after approximately 1 minute the preparation became inexcitable. When acetylcholine superfusion was terminated, excitability returned toward control values. In five other experiments of this type, atropine (10^{-6} M) prevented the acetylcholine-induced blockade of excitability of these K^+-depolarized, isoproterenol-treated fibers. Low-amplitude responses generated in Na^-free, Ca^{2+}-rich media (to which isoproterenol was not added) were not abolished by the addition of acetylcholine (10^{-5} M) (Fig. 5).

Discussion

The results of the present study demonstrate that acetylcholine can antagonize the electrophysiological effects of isoproterenol on canine cardiac Purkinje fibers. Acetylcholine antagonized both the effects of isoproterenol on action potential duration and the catecholamine-induced restoration of excitability to potassium-depolarized fibers. Inui and Imamura (1977) also have reported recently that acetylcholine suppresses electrical and mechanical activity in guinea pig papillary muscle partially depolarized by elevated extracellular potassium and then treated with isoproterenol. Several lines of evidence generated in the present study suggest that this action of acetylcholine was specific antagonism of isoproterenol-induced effects, and not simply a result of nonspecific membrane alterations induced by the choline ester. First, acetylcholine...
alone had no effect on action potential duration of paced Purkinje fibers. Second, shortening of action potential duration induced by premature electrical stimulation or by administration of acetylcholine, two treatments that shorten action potential duration by mechanisms that are fundamentally different from catecholamine administration, was unaffected by acetylcholine. Finally, the electrophysiological response induced by superfusing fibers with zero sodium, high calcium solution was not affected by acetylcholine. Thus, it would appear that the present results demonstrate another manifestation of adrenergic-cholinergic interaction in regulating the physiological state of the ventricles. Like what has been observed for ventricular contractility (Levy and Zieske, 1969; Levy, 1971, 1977), the antagonistic effect of acetylcholine was accentuated during simultaneous adrenergic stimulation, thus suggesting that the effect of acetylcholine occurred via a modulation of the action of isoproterenol.

The presence of tissue acetylcholinesterase probably reduced the effective concentration of acetylcholine at the cholinergic receptor, accounting for the relatively high concentrations of acetylcholine required to produce the antagonistic electrophysiological effects. The experiments with physostigmine support this conclusion. In the presence of this inhibitor of acetylcholinesterase, acetylcholine (10^-7 M) significantly attenuated the isoproterenol-induced shortening of action potential duration. Thus, the actual threshold concentration for the effects of acetylcholine may be substantially lower than that suggested by the experiments in which the neurotransmitter was given alone.

Several studies have shown that vagus nerve stimulation in dogs prevents the ischemic or normochemic heart against the development of ventricular fibrillation or repetitive ventricular extrasystoles (Kent et al., 1973; Kolman et al., 1975). In addition, it has been shown that the protective effect of vagal stimulation is more pronounced in the setting of high sympathetic activity, which would be expected to increase the propensity for developing ventricular arrhythmias (Kolman et al., 1975; Lown et al., 1977). These latter results suggest that the cholinergic effects on the electrophysiological properties of the in situ dog heart are at least partially mediated by modulation of the effects of the sympathetic nervous system. The results of the present study, which show cholinergic antagonism of sympathetic effects on isolated cardiac Purkinje fibers, provide a possible mechanism for the electrophysiological observation in dogs.

In addition to the cholinergic antagonism of adrenergic effects demonstrated in the present study, we have previously reported (Bailey et al., 1972) that acetylcholine exerts a direct negative chronotropic effect on automaticity of the proximal, partially depolarized His-Purkinje specialized conduct-}

ing system. Similarly, Tae et al. (1976) showed that acetylcholine caused a significant slowing of the rate of spontaneous discharge of more peripheral canine cardiac Purkinje fibers. Recently, Gadsby and colleagues (1978) have reported that acetylcholine increases resting potential, decreases action potential duration, and decreases spontaneous activity in canine cardiac Purkinje fibers. Thus vagal stimulation may antagonize, both directly and indirectly, potentially arrhythmogenic alterations of cardiac electrophysiological function.

Cholinergic antagonism of the inotropic effects of sympathetic stimulation in intact animals is well established (Levy, 1971, 1977). It has been shown that, among the possible mechanisms for this interaction, acetylcholine released from vagal nerve endings can interact with muscarinic cholinergic receptors on sympathetic nerve endings and thereby inhibit the release of norepinephrine from these nerve terminals (Löffelholz and Muscholl, 1969). We have shown previously that, in addition to such presynaptic interaction between vagal and sympathetic nerves, the cholinergic system can directly modulate adrenergic effects on the heart at the postsynaptic level (Watanabe and Besch, 1975a). Thus, acetylcholine markedly attenuated the positive inotropic effects of isoproterenol in isolated perfused guinea pig hearts (Watanabe and Besch, 1975a). We and others have also shown that muscarinic cholinergic agonists inhibited catecholamine-induced activation of glycogen phosphorylase in isolated hearts (Watanabe et al., 1978a; Gardner and Allen, 1977). Cholinergic agents thus can regulate the inotropic, metabolic, and electrophysiological effects of sympathetic stimulation of the heart directly at the tissue level, independently of presynaptic nerve terminals.

The subcellular mechanism by which acetylcholine antagonized the cardiac electrophysiological effects of β-adrenergic receptor stimulation remains to be established. However, based on results of previously published studies, several possibilities seem worthy of consideration. Evidence suggests that cyclic adenosine 3':5'-monophosphate (cyclic AMP) mediates the effects of catecholamines on the slow inward current (Watanabe and Besch, 1974, 1975b). Cyclic AMP may also mediate the isoproterenol-induced shortening of action potential duration, although this is less well established. It is likely that the cholinergic modulation of sympathetic effects involves cyclic AMP. It has been shown by several groups of investigators that acetylcholine can attenuate the amount of cyclic AMP generated in response to catecholamines in the heart (Gardner and Allen, 1977; Kuo et al., 1972; LaRaia and Sonnenblick, 1971). In addition, we have recently found that cholinergic agonists regulate β-adrenergic receptor affinity for catecholamines and inhibit catecholamine-induced stimulation of myocardial adenylate cyclase activity (Watanabe et
al., 1978b). Thus, one possible explanation for the observed electrophysiological interaction is that, in the presence of acetylcholine, less cyclic AMP was generated in the myocardial cells in response to β-adrenergic receptor stimulation.

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