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Muscarinic Activation Causes Biphasic Inotropic Response and Decreases Cellular Na^+ Activity in Canine Cardiac Purkinje Fibers

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Key Words
Purkinje fiber · Muscarinic receptor · Na^+, cellular

Abstract
In this study, the effects of carbachol (CCh) on twitch tension, intracellular Na^+ activity (a^i_{Na}), and action potential were simultaneously measured in canine cardiac Purkinje fibers in order to examine the regulation of inotropy through muscarinic receptors and its relation to a^i_{Na}. In fibers driven at 1 Hz, CCh (10 μM) initially and transiently decreased and then increased the twitch tension by 36 ± 8%. The action potential showed a significant elevation of the plateau and a significant shortening of the duration at 90% repolarization (APD_{90}), from 403 ± 7 to 389 ± 7 ms. The a^i_{Na} decreased from 7.4 ± 0.4 to 6.7 ± 0.3 mM ($n = 23$, $p < 0.05$). Atropine (1 μM) decreased the twitch tension by 21 ± 6% ($n = 7$, $p < 0.05$) without significant effects on the action potential and a^i_{Na}, and inhibited the effects of CCh. Cs^+ (20 mM) increased the plateau height and APD_{90}, enhanced the twitch tension by 66 ± 24%, but decreased a^i_{Na} from 7.3 ± 0.3 to 6.3 ± 0.4 mM ($n = 6$, $p < 0.05$). In the presence of 20 mM Cs^+, some fibers generated slow responses. The addition of 10 μM CCh further increased the twitch tension and APD_{90}, and decreased a^i_{Na} from 6.3 ± 0.4 to 5.3 ± 0.3 mM. Ouabain (0.3 μM) increased the twitch tension and a^i_{Na}, and inhibited the CCh-induced decrease of a^i_{Na}. In the presence of ouabain, 20 mM Cs^+ depolarized the fiber and generated slow responses with a decreased a^i_{Na}. The addition of 10 μM CCh enhanced the slow action potential, and increased a^i_{Na} although there was a transient decrease during early exposure. These results suggest that activation of muscarinic receptors in canine Purkinje fibers results in an enhancement of the Na^+-K^+ pump activity and a biphasic inotropic response, probably via different receptor subtypes. The inhibitory effect, most likely through M_2 receptors, is associated with the activation of K^+ channels. The stimulatory effect, on the other hand, is probably due to the action on the M_1 receptors, resulting in increases in Ca^{2+} currents.

Introduction

Activation of muscarinic receptors is known to decrease cAMP-dependent cellular response and to enhance the turnover of membrane phosphoinositide, a precursor to a certain cellular signal that produces two intermediates, diacylglycerol and inositol trisphosphate, to regulate cellular function [1, 6, 9, 22]. In cardiac tissues, cholinergic agonists can produce various effects through the action on muscarinic receptors. Reported results of mus-

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carinic receptor activation on inotropy and cardiac action potential have been diverse, depending on species and tissues studied. In pharmacological and molecular cloning studies, muscarinic receptors comprise a family of at least five distinct subtypes (M_1–M_5) [7, 24, 25]. Only two of these subtypes, M_1 and M_2, have been identified in cardiac muscles of adult rats and guinea pigs. Immunofluorescent detection of muscarinic receptor subtypes has demonstrated that M_2 receptors are the predominant type [9, 23]. The main signal transduction pathway used by M_2 muscarinic receptors involves the activation of K^+ channels and the inhibition of adenylyl cyclase through a pertussis-toxin-sensitive G protein, resulting in negative inotropy and chronotropy [7, 14]. Stimulation of M_1 muscarinic receptors, requiring high concentrations of agonist, can elicit positive inotropic and chronotropic effects most likely through a mechanism involved in a greater turnover rate of membrane phosphoinositide [9, 21].

The positive inotropic effect has been hypothesized to occur through contributions of increased cellular Na^+ concentrations (a^i_{Na}), Ca^{2+} currents and/or intracellular Ca^{2+} transients, as well as heightened calcium sensitivity of myofilaments [9, 19, 20, 27]. In sheep cardiac Purkinje fibers and guinea pig ventricular papillary muscles, activation of the muscarinic receptors can increase intracellular Na^+ activity (a^i_{Na}) that is assumed to be responsible for increases in contractile force [11, 13]. In the Purkinje fibers of young dogs, stimulation of the muscarinic receptors increased automaticity, which was antagonized by pirenzepine, an M_1 antagonist, suggesting the involvement of M_1 muscarinic receptors [21]. As for the muscarinic action on a^i_{Na}, carbachol (CCh) activated Na^+ currents in guinea pig ventricular myocytes, presumably through M_2 muscarinic receptors in a study with selective antagonists [18]. However, high concentrations of CCh were required to produce this effect.

The present work was undertaken to investigate the muscarinic receptor-mediated inotropic effect and its relation to a^i_{Na} in canine cardiac Purkinje fibers. High concentrations of CCh were used to examine the contribution of a^i_{Na} to the inotropic changes, and regulation of the membrane currents and Na^+-K^+ pump activity. Ouabain was used to block the Na^+-K^+ pump activity, and cesium was used to block the potassium and funny currents and to reactivate the ATP-dependent Na^+-K^+ pump during the presence of ouabain [4, 12]. Our results showed that the muscarinic receptor-mediated positive inotropic effect was not associated with an increase of a^i_{Na} in canine cardiac Purkinje fibers. An alternative role of changes of a^i_{Na} in the regulation of the contractile force is discussed.

Materials and Methods

Mongrel dogs of either sex (10–15 kg) were sacrificed after being anesthetized with sodium pentobarbital (40 mg/kg, i.p.). A strand of fine Purkinje fiber from the ventricle of the heart was carefully dissected and was fixed in a perfusing chamber, superfused with oxygenated Tyrode solution, and maintained at 37 °C. The composition of the solution was (in m*M*) NaCl 135, KCl 5.4, $CaCl_2$ 1.8, $NaHCO_3$ 12, $MgCl_2$ 1.1, NaH_2PO_4 0.5, glucose 5.0. The perfusate was equilibrated with a mixture of gas (97% O_2 and 3% CO_2) to give a pH of 7.4. One end of the fiber was fixed and driven with a Grass stimulator (model S44) at a rate of 60 beats/min. The other end was tied to a force displacement transducer (Cambridge, 403A) to measure twitch tension. The measurements of a^i_{Na} and action potential were made using methods similar to those described previously [26, 27]. Conventional microelectrodes were made from borosilicate micropipettes that had a tip resistance in the range of 10–40 MΩ when backfilled with 3 *M* KCl solution. The Na^+-selective microelectrode was made from thick-wall pipettes (1.8 mm outside diameter, 1.1 mm inside diameter), and was beveled and backfilled with 100 m*M* NaCl after silanization with a tiny amount of n-tributylchlorosilane [17]. A 100- to 300-μm column of Na^+-selective liquid sensor (Fluka) was drawn into the tip of the microelectrode. The sodium electrode was calibrated with standard solutions containing NaCl (100, 10 and 1 m*M*), KCl (100 m*M*), and $CaCl_2$ (1 m*M*), separately, before and after each experiment as previously described [16]. The potential response of Na^+ electrodes was about 60 mV per 10-fold change of sodium activity at 37 °C. The selectivity coefficients for K^+ (k_{NaK}) and Ca^{2+} (k_{NaCa}) were less than 0.02 and 2, respectively. The signals, from the Na^+-selective and conventional microelectrodes, passed through two identical low-pass filters (A.P. Circuit). The filtered potentials (E^i_{Na} and $\overline{V}m$) and their difference (a^i_{Na}) were recorded on a chart recorder. The action potential signal and the twitch tension were displayed and recorded using a digital oscilloscope (Gould 1604). The intracellular Na^+ activity of muscle fibers was calculated using the following modified Nicolsky equation [16]:

$$E^i_{Na} - \overline{V}m = E_o + S \log[a^i_{Na} + k_{NaK}\, a^i_K + k_{NaCa}\, (a^i_{Ca})^{1/2}]$$

where E^i_{Na} and $\overline{V}m$ are the respective filtered potentials of the sodium electrode and conventional electrode in cells; E_o is the constant potential of the electrometric system; S is the slope of potential response in calibration solutions for each electrode; a^i_K (120 m*M*) and a^i_{Ca} (110 n*M*) are the intracellular activities of K^+ and Ca^{2+}, respectively, as previously reported for canine cardiac Purkinje fibers [15]. The possibility of small differences in a^i_K and a^i_{Ca} values was neglected in this work [2, 15].

CCh, atropine sulfate, and ouabain (Sigma) were dissolved in distilled water as a stock solution (0.1 *M*). The cesium-containing solution was prepared by adding a desired amount of cesium chloride. In beating fibers, a long exposure to ouabain easily elicited ectopic rhythms, making it very difficult to obtain a stable recording. Also, electrodes were often dislodged before a^i_{Na} and twitch tension reached a steady state. Therefore, we chose arbitrarily to measure the effects of CCh on the rate of the increases of twitch tension and a^i_{Na} in the 10 min after the administration of ouabain. Student-Newman-Keuls or Student's t test was employed as appropriate in the analysis of the experimental data. A difference was considered statistically significant if the p value was less than 0.05.

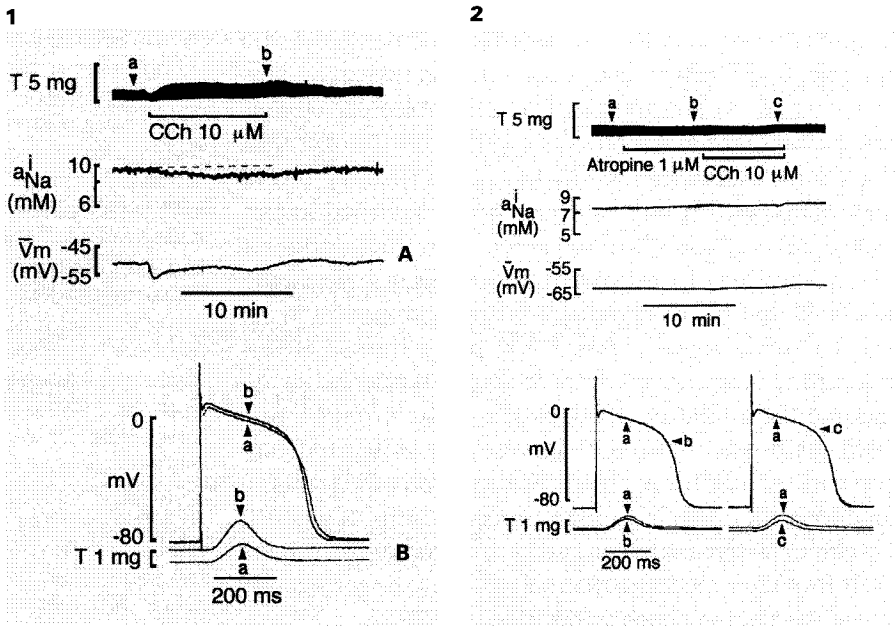

Fig. 1. Effects of CCh on a canine cardiac Purkinje fibers. **A** Slow recordings of twitch tension (T), intracellular Na^+ activity (a^i_{Na}) and filtered membrane potential ($\bar{V}m$). **B** Fast recordings of the superimposed action potential and the twitch tension were taken at points a and b as indicated in **A**. Note that CCh produced a biphasic response in the twitch tension. The filtered membrane potential was initially hyperpolarized by CCh.

Fig. 2. Effects of CCh in the presence of atropine on a canine cardiac Purkinje fiber. Abbreviations and recordings are the same as in figure 1. Atropine caused a decrease in twitch tension and completely abolished the electrical effect of CCh.

Results

Effects of CCh and Atropine

The effects of CCh and atropine on the canine cardiac Purkinje fiber are shown in figures 1 and 2. As shown in figure 1, CCh at a concentration of 10 μ*M* initially decreased and then increased the twitch tension. The initial decrease was transient and not always observed. During the decrease of the twitch tension, there was no change in a^i_{Na} while an increase in the filtered membrane potential was observed with nearly 5 mV of hyperpolarization, and with marked decrease in the duration of the action potential at 50 and 90% repolarization (APD_{50} and APD_{90}). Subsequently, the twitch tension and the plateau height showed a definitive and sustained increase with a decreased a^i_{Na}. Figure 2 shows that pretreatment with atropine antagonized the effects of CCh. Atropine (1 μ*M*) decreased the twitch tension without changing the action potential or a^i_{Na}. The effects of 10 μ*M* CCh were completely inhibited by atropine. Changes of the parameters in twenty-three fibers tested with CCh and seven fibers tested with atropine and CCh are summarized in table 1. On average, 10 μ*M* CCh significantly increased the twitch tension by nearly 36% of control, and decreased a^i_{Na} by nearly 9.3% and the APD_{90} by 14 ms of control. Atropine significantly reduced the twitch tension by nearly 21% and completely abolished the electrical and mechanical effects of CCh.

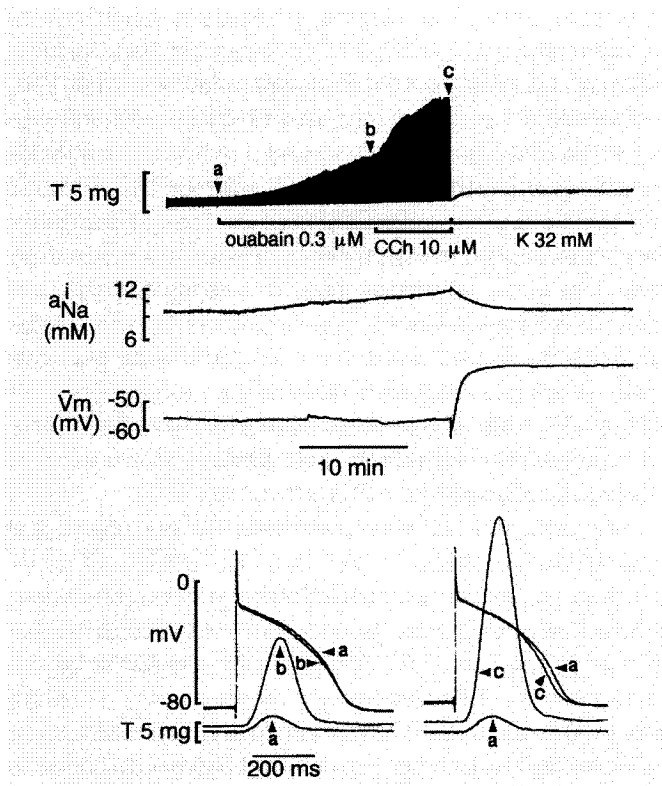

Fig. 3. Effects of CCh in the presence of ouabain in a canine cardiac Purkinje fiber. Abbreviations and recordings are the same as in figure 1. Note that CCh did not change the rate of the increase of a^i_{Na} induced by ouabain, but significantly increased the twitch tension and the rate of repolarization of the action potential.

Table 1. Effects of CCh on canine cardiac Purkinje fibers in the presence and absence of atropine

	APA, mV	APD_{50}, ms	APD_{90}, ms	a^i_{Na}, m*M*	TT, %
Control (n = 23)	115±1	301±10	403±7	7.4±0.3	100
CCh 10 μ*M*	117±1*	297±8	389±7*	6.7±0.3*	136±8*
Control (n = 7)	104±3	358±29	436±31	6.9±0.4	100
Atropine 1 μ*M*	105±3	352±28	431±33	7.0±0.5	79±6*
Atropine 1 μ*M* + CCh 10 μ*M*	105±3	342±26	421±31	7.0±0.5	73±7*

Values are mean ± SEM. * $p < 0.05$ vs. control.

APA = Amplitude of action potential; APD_{50} and APD_{90} = respective durations at 50 and 90% repolarization; a^i_{Na} = intracellular Na^+ activity; TT = twitch tension.

Table 2. Effects of CCh on canine cardiac Purkinje fibers in the presence of ouabain or Cs^+

	APA, mV	APD_{50}, ms	APD_{90}, ms	a^i_{Na}, m*M*	TT, %
Control (n = 6)	115±5	296±25	411±18	9.8±0.7	100
Ouabain 0.3 μ*M*	114±2	291±32	417±22	11.8±0.8*	272±52*
Ouabain 0.3 μ*M* + CCh 10 μ*M*	107±3*	284±34	397±37	12.8±0.8*	375±111
Control (n = 6)	108±4	287±26	390±16	7.3±0.3	100
Cs^+ 20 m*M*	106±4	344±24*	480±12	6.3±0.4*	166±24*
Cs^+ 20 μ*M* + CCh 10 μ*M*	107±3	348±22*	487±12*,+	5.3±0.3*,+	349±98*,+
Control (n = 6)	107±1	263±26	409±24	7.6±0.4	100
Ouabain 0.3 μ*M*	104±2*	258±23	405±34	9.4±0.4*	490±144*
Ouabain 0.3 μ*M* + Cs^+ 20 m*M*				7.8±0.2	
Ouabain 0.3 μ*M* + Cs^+ 20 m*M* + CCh 10 μ*M*				8.5±0.6*,+	

Values are mean ± SEM. * $p < 0.05$ vs. control; + $p < 0.05$ vs. Cs^+- and/or ouabain-pretreated groups.

APA = Amplitude of action potential; APD_{50} and APD_{90} = respective durations at 50 and 90% repolarization; a^i_{Na} = intracellular Na^+ activity; TT = twitch tension. In the group treated with Cs^+ and CCh, except for a^i_{Na}, the values in action potential and twich tension were calculated in four fibers because two fibers became slow action potential. In the group treated with ouabain and Cs^+, fibers depolarized to regular or irregular slow responses after 20 m*M* Cs^+. Only the value of a^i_{Na} was calculated.

Effects of Ouabain

The effects of ouabain and CCh on canine Purkinje fibers are shown in figure 3. Ouabain (0.3 μ*M*) increased the twitch tension as well as a^i_{Na}. The action potential showed a slight decrease of the plateau and APD_{90}. Addition of CCh (10 μ*M*) in the presence of ouabain enhanced the rate of increase of the twitch tension, but did not change the rate of increase of a^i_{Na} induced by ouabain. The action potential showed a slight elevation of the plateau and a shortening of the APD_{90}. Similar results were found in another five fibers. The average changes of these variables are summarized in table 2.

Effects of Cs^+

The effects of Cs^+ and CCh on the twitch tension, a^i_{Na} and action potential are shown in figure 4. As shown, 20 m*M* Cs^+ increased the twitch tension and decreased a^i_{Na}. The plateau height and APD_{90} were increased. Subsequent addition of 10 μ*M* CCh further increased the twitch tension, plateau height and APD_{90}. Six fibers were tested. Two of these generated slow responses after the addition of Cs^+. On average, the twitch tension increased by 66% and a^i_{Na} decreased by 1.0 m*M* after exposure to 20 m*M* Cs^+. Addition of CCh further significantly decreased a^i_{Na} by 1.0 m*M* and increased twitch tension to 348% of control. The action potential in four fibers

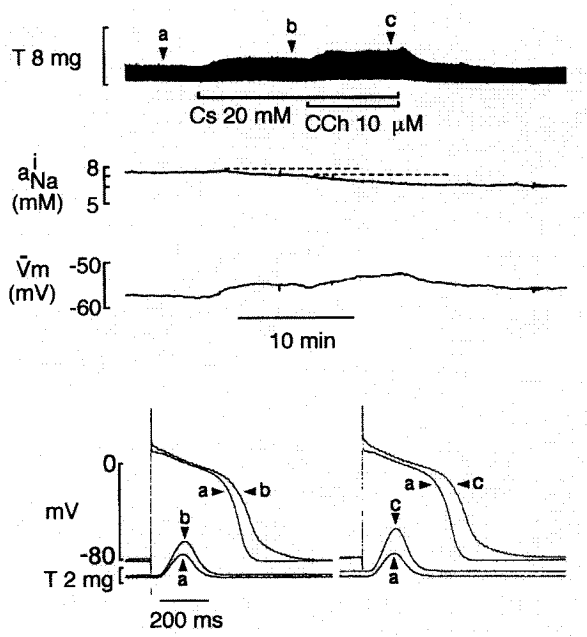

Fig. 4. Effects of CCh in the presence of Cs^+ in a canine cardiac Purkinje fiber. Abbreviations and recordings are the same as in figure 1. Note that Cs^+ decreased a^i_{Na} and increased the twitch tension and the action potential duration. These results were enhanced by the subsequent addition of CCh.

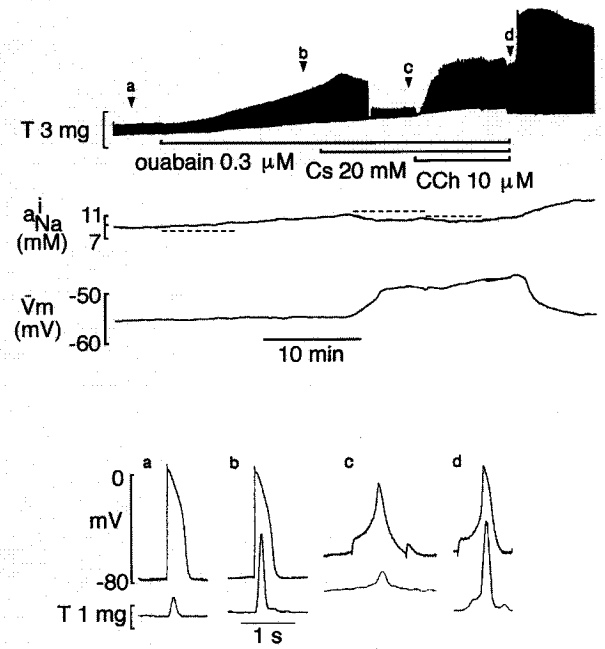

Fig. 5. Effects of CCh in the presence of ouabain and Cs^+ in a canine cardiac Purkinje fiber. Abbreviations and recordings are the same as in figure 1. Note that ouabain increased the twitch tension and increased a^i_{Na}. Cs^+ depolarized the ouabain-treated fiber, and induced an intermittent slow action potential in response to electrical stimulation. CCh increased the excitability and the contractile force, and transiently decreased the a^i_{Na}. However, the a^i_{Na} eventually gradually increased. A rebound increase in the twitch tension and a^i_{Na} was noted after washing out of the drugs.

showed that APD_{90} increased from 390 ± 16 to 480 ± 12 ms after 20 m*M* Cs^+ and CCh further increased APD_{90} to 486.7 ± 12 ms.

Figure 5 shows the effects of CCh in the presence of both Cs^+ and ouabain. As shown, the twitch tension as well as a^i_{Na} increased in the presence of ouabain. Cs^+ (20 m*M*) depolarized the fiber to induce an intermittent slow response with decreased a^i_{Na} after a transient increase in the twitch tension. In the presence of both ouabain and Cs^+, the addition of 10 μ*M* CCh increased a^i_{Na} in the 10 min after a transient decrease. The twitch tension and slow action potential, however, were enhanced. Similar results were observed in another five fibers. The changes in a^i_{Na} are summarized in table 2.

Discussion

In the present study, CCh at rather high concentrations produced biphasic inotropic effects and decreased the action potential duration and a^i_{Na}. The biphasic inotropic responses consisted of a transient negative inotropy followed by a sustained positive inotropy. This response is thought to be mediated through muscarinic receptors since all the effects were completely abolished by atropine. In some fibers, the initial transient decrease was obscure, with only an increase in tension observed. The biphasic nature of the response suggests that activation of muscarinic receptors elicits different signal transduction pathways most likely through different subtypes of muscarinic receptors [5]. The involvement of the M_1 or M_2 subtypes of muscarinic receptors in the positive inotropic effect remains unclear in the mammalian heart [9, 18, 19, 23]. In the present study, the initial decrease in the contractile force probably can be explained by an activation

of K^+ channels, and a reduction of the synthesis and action of basal cAMP through M_2 muscarinic receptors [1, 14]. The increased twitch tension most likely occurs through stimulation of the M_1 muscarinic receptor subtype, which involves the activation of calcium currents and the stimulation of phosphoinositide breakdown [1, 5, 9, 21]. The maximal rate of rise of the action potential upstroke has been found to be unchanged in response to muscarinic stimulation, suggesting that the decreased a^i_{Na} in this study was not due to inhibition of fast Na^+ channels [10]. In this study, after inhibition of the Na^+-K^+ pump with ouabain, the decrease of a^i_{Na} by activation of the muscarinic receptor was abolished but the increased contractile force was still evident. Cs^+, which has been found to enhance the muscarinic response and the ATP-dependent Na^+-K^+ pump, may partially antagonize the inhibitory effect of ouabain [3, 8]. This may explain the finding that a^i_{Na} transiently decreased and later on increased after the addition of CCh in the presence of ouabain and high concentrations of Cs^+. These results suggest that the decreased a^i_{Na} during stimulation of the muscarinic receptors is associated with activation of the Na^+-K^+ pump, possibly through the stimulation of phosphoinositide metabolism. Because the addition of CCh decreases a^i_{Na} after blockade of K^+ channels by Cs^+, the increase of the Na^+-K^+ pump activity is unlikely due to the effect of accumulation-depletion of intercellular K^+.

The decreased a^i_{Na} in response to the addition of CCh in canine Purkinje fibers found in this study is contrary to observations in guinea pig papillary muscles and in sheep Purkinje fibers [11, 13]. In these cardiac tissues, CCh increases a^i_{Na} which thereby contributes to the increase of contractile force and intracellular calcium through Na^+-Ca^{2+} exchange. However, in our previous study CCh increased the contractile force and decreased a^i_{Na} after the muscle fiber was depolarized by Cs^+ in guinea pig ventricular papillary muscles [27]. Thus, changes of a^i_{Na} are not a prerequisite for the inotropic effect, and the present results suggest that, during muscarinic activation, a decrease of a^i_{Na} is not pertinent to the increase in contractile force. CCh causes increase in the inotropy of cardiac tissues of different species despite diverse changes in a^i_{Na} [11, 13, 27]. In this study, activation of muscarinic receptors in canine Purkinje fibers decreased a^i_{Na} resulting in an increase in the electrochemical gradient of Na^+ and helping Ca^{2+} extrusion by a mechanism of Na^+-Ca^{2+} exchange, avoiding Ca^{2+} overload and promoting muscle relaxation.

Changes in the configuration of the action potential depend on the intracellular and extracellular milieus and the activity of ionic channels. CCh has been shown to increase the rate of repolarization by the activation of K^+ channels, which would secondarily reduce Ca^{2+} inward currents and the twitch tension [14]. This effect can be inhibited by high concentrations of Cs^+, which can completely block K^+ channels [12]. In the present study, CCh increased the plateau height and decreased the APD_{90}. However, after blockade of K^+ channels by Cs^+, CCh increased both the plateau height and APD_{90}. Even in Cs^+-depolarized fibers, CCh enhanced the excitability and the amplitude of the slow action potential in parallel with the increase of twitch tension. These results strongly suggest that CCh can increase the slow Ca^{2+} inward current in canine Purkinje fibers. This increase in Ca^{2+} inward currents during activation of muscarinic receptors apparently contributes to the increased twitch tension in canine cardiac Purkinje fibers.

In conclusion, stimulation of the muscarinic receptors by CCh produces a biphasic inotropic response with a decrease of a^i_{Na} in canine cardiac Purkinje fibers. Two different subcellular signal transduction pathways, which appear to occur through two subtypes of muscarinic receptor, may account for this biphasic inotropic response. One of these signal transductions involves activation of K^+ channels through M_2 muscarinic receptors that increases the rate of repolarization of action potential to reduce Ca^{2+}-inward currents and the contractile force. Another pathway through M_1 muscarinic receptors involves activation of the Na^+-K^+ pump and Ca^{2+} channels resulting in a decreased a^i_{Na} and a positive inotropy, respectively. Diverse changes in a^i_{Na} are seen in different species, and appear to be independent of the changes in inotropy.

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