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Effect of α_2-adrenoceptor stimulation on isolated canine Purkinje fiber contraction

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Abstract

We have recently identified the presence of postjunctional α_2-adrenoceptors in canine Purkinje fibers. In this study, we examined the effects of α_2-adrenoceptor stimulation on the contraction strength of isolated Purkinje fibers. Exposure to the α_2-adrenoceptor specific agonist and antagonist, UK 14,304 (5-bromo-*N*-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine) and yohimbine (17-hydroxyyohimban-16-carboxylic acid methyl ester hydrochloride) alone at 0.1 μM respectively, did not produce any significant effect on Purkinje contraction strength. Purkinje contraction strength was augmented by isoproterenol (0.1 μM), forskolin (0.1 μM), or 8-bromo-adenosine cyclic 2′,3′-monophosphate (8-bromo-cAMP, 10 μM). UK 14,304 significantly reversed the effects of isoproterenol and forskolin but not those of 8-bromo-cAMP on Purkinje contraction strength. After incubation with pertussis toxin, the positive inotropic effect of forskolin on Purkinje contraction strength remained intact, but the forskolin effect could no longer be reversed by UK 14,304. These results suggest that the postjunctional α_2-adrenoceptors in canine Purkinje fibers are coupled to a pertussis toxin-sensitive G protein, probably G_i. Stimulation of the α_2-adrenoceptor antagonizes the effect of β-adrenoceptor stimulation on Purkinje contraction strength in an accentuated antagonism manner. © 1998 Elsevier Science B.V.

Keywords: α_2-Adrenoceptor; Purkinje fiber; UK 14,304; Contraction strength; Antagonism accentuated; Pertussis toxin

1. Introduction

α_2-Adrenoceptors are ubiquitous and are known to be present in the central nervous system as well as various peripheral and humoral tissues (Ruffolo, 1991). α_2-Adrenoceptor stimulation regulates a wide range of physiological phenomena including modulation of sympathetic outflow from the central nervous system and regulation of vascular tone and circulation (Nichols and Ruffolo, 1991). At the cellular level, stimulation of the α_2-adrenoceptor could lead to increase in transmembrane Ca^{2+} influx resulting in arterial and venous vasoconstriction (Medgett and Rajanayagam, 1984; Matthews et al., 1984). In addition, activation of the α_2-adrenoceptor has been shown to enhance phospholipase A_2 activity through stimulation of membrane-bound Na^+/H^+ exchange activity (Sweatt et al., 1985). More recently, α_2-adrenoceptors have been found to activate protein kinase C in human platelets (Nieuwland et al., 1994). However, the most widely established α_2-adrenoceptor-mediated signal transduction mechanism is the inhibition of adenylyl cyclase through coupling with an inhibitory GTP-binding protein, G_i (Nichols, 1991; Bylund, 1992). All α_2-adrenoceptor subtypes have been shown to attenuate adenosine cyclic 2′,3′-monophosphate (cAMP) production in intact cells (Blaxall et al., 1991; Bylund and Ray-Prenger, 1989; Murphy and Bylund, 1988) and may have profound effects on the regulation of tissue physiology (Nichols, 1991).

Studies examining the expression of α_2-adrenoceptors using radioligand binding (Hoffman and Lefkowitz, 1980) or molecular techniques (Eason and Liggett, 1993; Handy et al., 1993) have demonstrated that postjunctional α_2-adrenoceptors are widely distributed in human and other mammalian tissues. Conspicuously missing is knowledge regarding the role of postjunctional or extrajunctional α_2-

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adrenoceptors in the regulation of cardiac physiology. The apparent absence of α_2-adrenoceptor effects in heart has become clear by recent findings that postjunctional α_2-adrenoceptors are present in Purkinje fibers but absent in myocardium (Lee et al., 1996a). Autoradiographic examination showed the presence of both α_1- and α_2-adrenoceptor binding in isolated Purkinje fibers, but only α_1-adrenoceptor binding could be detected in ventricular myocardium. Radioligand binding examination showed the presence of high affinity α_2-adrenoceptor specific binding in Purkinje membranes but not in ventricular myocardium (Lee et al., 1996a). Stimulation of the α_2-adrenoceptors resulted in prolongation of the Purkinje action potential in isolated Purkinje fibers (Samson et al., 1995) as well as prolongation of the relative Purkinje refractory period in the intact dog heart (Cable et al., 1994). The current studies were conducted to examine the role of postjunctional α_2-adrenoceptors in the regulation of contraction strength of cardiac Purkinje tissue and to determine the functional interaction between β- and α_2-adrenoceptors.

2. Materials and methods

2.1. Animals

Male and female Mongrel dogs weighing 24 to 28 kg were sedated with ketamine (20 mg/kg, i.m.) and then anesthetized by intravenous administration of pentabarbitol (30 mg/kg i.v.). The dogs were intubated and stabilized on a ventilator. Anticoagulation with 10,000 units of Heparin was administered intravenously. The heart was excised through an intercostal incision and transferred immediately to ice-cold Tyrode's solution equilibrated with 95% O_2–5% CO_2. The Tyrode's solution contained (in mM): 125 NaCl, 24 $NaHCO_3$, 4 KCl, 2 $CaCl_2$, 0.5 $MgCl_2$, 0.25 NaH_2PO_4, and 5.5 glucose, pH 7.40. Free-running strands of Purkinje fibers were dissected from both ventricles as well as from the septum and placed in oxygenated Tyrode's solution at room temperature (21–23°C).

2.2. Contraction measurements

A Purkinje fiber was mounted in a bath chamber on the stage of a dissection microscope. The bath temperature was maintained at 37.0 ± 0.5°C by a water jacket with continuous circulating water from a heated bath. The bath chamber had a volume of 10 ml and was perfused with Tyrode's solution at a rate of 15 ml/min, allowing the bath contents to be exchanged 1.5 times per minute. One end of the Purkinje fiber was pinned to the Sylgard-coated (Dow Corning, Midland, MI) floor of the chamber and the other end was connected to an electronic microtransducer (Model BG-10, Kulite Semi-conductor Products, Leonia, NJ) using a 0.125-mm diameter silver wire.

The Purkinje fiber was stimulated at 2 Hz using a bipolar electrode with square wave pulses of 2 ms in duration at two times threshold (A300 pulse generator, WPI, New Haven, CT). The fiber was gradually stretched using a micromanipulator until maximum tension was obtained. Tension was then reduced to decrease the amplitude of contraction to 80% of that maximally achieved. The contraction signals were recorded on a strip-chart recorder (Model #30-V7606-00, Gould, Rolling Meadows, IL) and simultaneously acquired and stored on a 80386-based personal computer using pCLAMP 5.5 software (Axon Instruments, Foster City, CA). Data were sampled at 2 kHz and analyzed using CLAMPAN software. Ten consecutive contractions were digitally averaged. Contraction amplitudes were calibrated using standard weights and the system was sensitive to changes in contraction strengths of < 0.1 mg.

2.3. Protocols

Baseline contraction measurements were recorded following at least 30 min of equilibration. Experiments were started only after Purkinje contraction strength remained stable for at least 5–10 min. The following drug protocols were performed and only one protocol was performed on each Purkinje fiber.

2.3.1. (A)

Direct effects of 5-bromo-*N*-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine (UK 14,304), an α_2-adrenoceptor agonist, and 17-hydroxyyohimban-16-carboxylic acid methyl ester hydrochloride (yohimbine), an α_2-adrenoceptor antagonist, on Purkinje contraction strength were examined over a range of drug concentrations of 1 nM to 1 μM.

2.3.2. (B)

To assess the α_2-adrenoceptor effects on β-adrenoceptor stimulation of Purkinje contraction strength, activation of the β-adrenoceptor pathway was achieved by: (i) activation of the β-adrenoceptor with isoproterenol (0.1 μM); (ii) direct activation of adenylyl cyclase with forskolin (0.1 μM); and (iii) activation of adenosine cyclic 2′,3′-monophosphate (cAMP)-dependent protein kinase with the cell-permeant 8-bromo-adenosine cyclic 2′,3′-monophosphate (8-bromo-cAMP, 10 μM). Drug effects were allowed to reach steady-state before UK 14,304 (0.1 μM) was added. The ability of UK 14,304 to antagonize the effects of β-adrenoceptor stimulation at different levels of activation of the signalling pathway was determined.

2.3.3. (C)

To determine the effects of pertussis toxin on the α_2-adrenoceptor-mediated inhibition of Purkinje contraction strength, isolated strands of Purkinje fibers were incubated at 37°C in tissue culture medium (M-199) supple-

mented with 20% fetal bovine serum, 1% Penicillin/Streptomycin solution, and 1 μg/ml pertussis toxin for 6 h as previously described (Samson et al., 1995). The Purkinje fibers were then used for contraction studies. After 30 to 40 min of equilibration, the ability of forskolin (0.1 μM) to augment the Purkinje contraction strength and the ability of UK 14,304 (0.1 μM) to antagonize the forskolin effects were determined.

2.4. Data analysis

Contraction strengths of Purkinje fibers were measured in milligram and in each experiment were normalized to baseline values to adjust for the variability of diameter and length of the fibers examined. Data were expressed as the percentage of contraction strength compared to baseline and summarized as mean ± S.E.M. Data were analyzed using Repeated Measures Analysis of Variance and pairwise comparisons among groups were performed using Scheffe's *F*-test. Statistical significance was met at $P < 0.05$. The absolute magnitudes (in mg) and variabilities of Purkinje contraction strength were also included in the legend of each figure.

2.5. Pharmacological agents

Yohimbine, isoproterenol, forskolin, pertussis toxin and 8-bromo-cAMP were purchased from Sigma Chemical, St. Louis, MO. UK 14,304 was obtained from Research Biochemicals, Natick, MA. All salts and buffer materials were obtained from Sigma. Yohimbine, isoproterenol, and 8-bromo-cAMP were solubized in water. UK 14,304 and forskolin were solubized in ethanol with a final concentration of ethanol at 0.01%. Ethanol at this concentration did not have any significant effects on Purkinje contraction strength.

3. Results

UK 14,304 and yohimbine had no significant effects on Purkinje contraction strength over the range of drug concentrations studied (1 nM to 1 μM, Table 1). Based on the results of previous studies (Samson et al., 1995; Lee et al., 1996b), UK 14,304 and yohimbine at 0.1 μM were chosen for these experiments. At this concentration, UK 14,304 produced significant prolongation of the Purkinje action potential duration and inhibition of phase 1 magnitude that were blocked by equimolar concentration of yohimbine, and there were no direct UK 14,304 or yohimbine electrophysiological effects independent of the α_2-adrenoceptor. The effect of UK 14,304 at 0.1 μM on the Purkinje contraction strength is shown in Fig. 1.

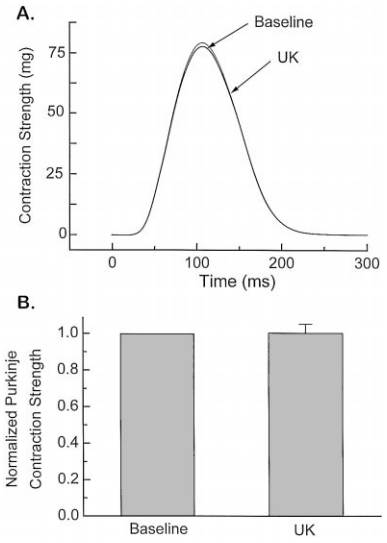

Fig. 1. Effect of UK 14,304 (UK, 0.1 μM) on Purkinje contraction strength. (A) Results from a representative experiment. (B) Summary of the results from five experiments. Results are expressed as Purkinje contraction strength normalized to baseline values (normalized mean ± SEM), (P = N.S. vs. baseline). The absolute magnitudes of the Purkinje contraction strength are 72.9 ± 10.5 mg at baseline, and 71.8 ± 7.8 mg with UK.

3.1. Effects of α_2-adrenoceptor stimulation on Purkinje contraction in the presence of isoproterenol

β-Adrenoceptor stimulation with isoproterenol (0.1 μM) resulted in a significant increase in Purkinje contraction strength. The mean Purkinje contraction strength increased to 117 ± 3% (mean ± S.E.M., $n = 6$, $P < 0.05$ vs. baseline) of baseline values. Addition of UK 14,304 (0.1 μM) in the presence of isoproterenol completely reversed the β-adrenoceptor-mediated augmentation of Purkinje con-

Table 1
Dose–response relationships of UK 14,304 and yohimbine on the contraction strength of isolated canine Purkinje fibers

	Baseline	0.001 μM	0.01 μM	0.1 μM	1 μM
UK 14,304 (n = 5)					
Normalized contraction strength	100%	101 ± 2.5%	102 ± 9.3%	101 ± 4.7%	100 ± 5.2%
Yohimbine (n = 5)					
Normalized contraction strength	100%	101 ± 2.0%	101 ± 2.0%	98 ± 2.3%	83 ± 8.0%

The values in the table represent contraction strength of isolated canine Purkinje fibers normalized to baseline values (100%). P = N.S. vs. baseline for UK 14,304 and yohimbine at the concentrations studied.

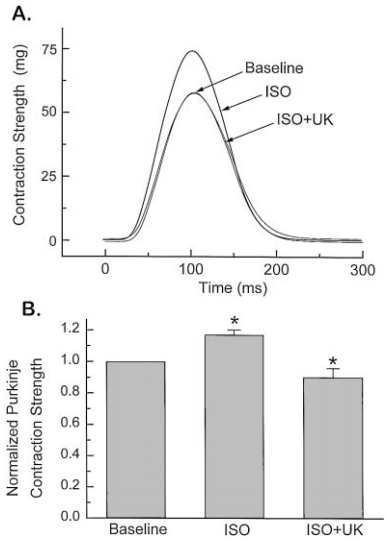

Fig. 2. Effect of UK 14,304 (UK, 0.1 μM) on Purkinje contraction strength in the presence of isoproterenol (ISO, 0.1 μM). (A) Results from a typical experiment in which ISO produces a significant increase in Purkinje contraction strength and the ISO effects are completely reversed by the addition of UK. (B) Summary of the results from six experiments. Results are expressed as Purkinje contraction strength normalized to baseline values (normalized mean $\pm$ SEM). $*$ $P < 0.05$ vs. preceding intervention. The absolute magnitudes of the Purkinje contraction strength are 48.6 ± 10.3 mg at baseline, 56.2 ± 11.4 mg with ISO, and 44.3 ± 10.2 mg with ISO + UK.

traction, decreasing the mean Purkinje contraction strength to $91 \pm 6\%$ of baseline ($P < 0.05$ vs. isoproterenol; $P =$ N.S. vs. baseline)(Fig. 2).

3.2. Effects of α_2-adrenoceptor stimulation on Purkinje contraction in the presence of forskolin

Forskolin, a diterpene extracted from the root of the Indian plant *Coleus forskohlii*, is known to directly stimulate the activity of adenylyl cyclase independent of the β-adrenoceptor and its associated G-protein, G_s (Zalups and Sheu, 1987; Metzger and Lindner, 1981; Seamon and Daly, 1981). Forskolin at 0.1 μM has been shown to significantly increase contraction strength in isolated sheep Purkinje fibers (Zalups and Sheu, 1987) and to increase adenylyl cyclase activities in rat heart tissue (Metzger and Lindner, 1981). In our experiments, exposure of dog Purkinje fibers to 0.1 μM forskolin increased contraction strength to $183 \pm 15\%$ ($n = 6$, $P < 0.05$ vs. baseline). Addition of 0.1 μM UK 14,304 reversed the effects of forskolin by almost 60%, decreasing Purkinje contraction strength to $135 \pm 10\%$ of baseline ($P < 0.05$ vs. forskolin alone). The effects of UK 14,304 was completely blocked by equimolar concentration of yohimbine, returning Purkinje contraction strength to $171 \pm 21\%$ ($P < 0.05$ vs. forskolin + UK 14,304, $P =$ N.S. vs. forskolin alone). These results are summarized in Fig. 3.

3.3. Effects of α_2-adrenoceptor stimulation on Purkinje contraction in the presence of 8-bromo-cAMP

To examine the ability of α_2-adrenoceptor stimulation in antagonizing the effects of cAMP on Purkinje contraction strength, isolated Purkinje fibers were exposed to the cell-permeant 8-bromo-cAMP (10 μM). Studies performed on rat cardiac myocytes showed that 8-bromo-cAMP at 10 μM produced significant augmentation in intracellular $[Ca^{2+}]_i$ and contraction (Yu et al., 1994). Exposure of isolated dog Purkinje fibers to 10 μM 8-bromo-cAMP increased contraction strength to $163 \pm 22\%$ of baseline ($n = 5$, $P < 0.05$ vs. baseline). Addition of 10 μM UK 14,304 did not produce any significant changes on the 8-bromo-cAMP-mediated effects with Purkinje contraction strength remained augmented at $157 \pm 26\%$ (Fig. 4). These results suggested that α_2-adrenoceptor stimulation antagonizes β-adrenoceptor stimulation at the receptor and adenylyl cyclase levels but does not alter cAMP-activated protein kinase effects.

3.4. Effect of α_2-adrenoceptor stimulation on Purkinje contraction after incubation with pertussis toxin

Pertussis toxin uncouples receptors from G-proteins and their associated signalling pathways in cultured cells and tissue preparations (Kaslow and Burns, 1992). After incubation of Purkinje fibers with pertussis toxin (1 μg/ml)

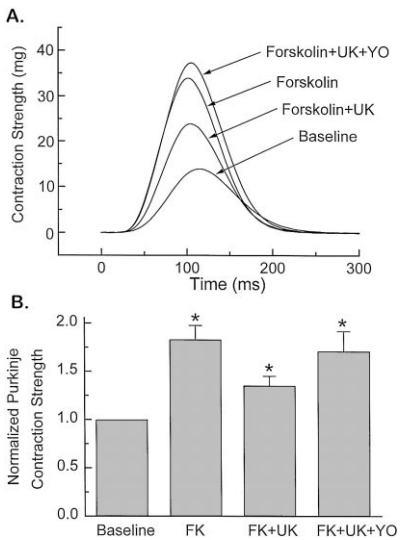

Fig. 3. Effect of UK 14,304 (UK, 0.1 μM) on Purkinje contraction strength in the presence of forskolin (FK, 0.1 μM). (A) Results of a typical experiment in which FK produces dramatic augmentation of Purkinje contraction strength but the FK effects are significantly reversed by the addition of UK. The UK effects are, in turn, completely blocked by equimolar concentrations of yohimbine (YO), rendering the FK effects uninhibited. (B) Summary of the results from six experiments. Results are expressed as Purkinje contraction strength normalized to baseline values (normalized mean $\pm$ SEM). $*$ $P < 0.05$ vs. preceding intervention. The absolute magnitudes of the Purkinje contraction strength are 21.6 ± 2.7 mg at baseline, 39.2 ± 5.1 mg with FK, 29.1 ± 3.6 mg with FK + UK, and 36.2 ± 5.2 mg with FK + UK + YO.

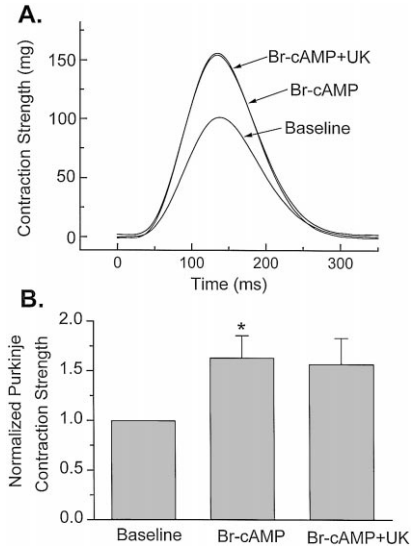

Fig. 4. Effect of UK 14,304 (UK, 0.1 μM) on Purkinje contraction strength in the presence of 8-bromo-cAMP (Br-cAMP, 10 μM). (A) Results from a typical experiment in which Br-cAMP produces a significant increase in Purkinje contraction strength. The addition of UK, however, fails to counteract the effects of Br-cAMP. (B) Summary of the results from five experiments. Results are expressed as Purkinje contraction strength normalized to baseline values (normalized mean $\pm$ SEM). $*$ $P < 0.05$ vs. preceding intervention. The absolute magnitudes of the Purkinje contraction strength are 95.1 ± 17.3 mg at baseline, 144.7 ± 18.0 mg with Br-cAMP, and 139.1 ± 20.2 mg with Br-cAMP + UK.

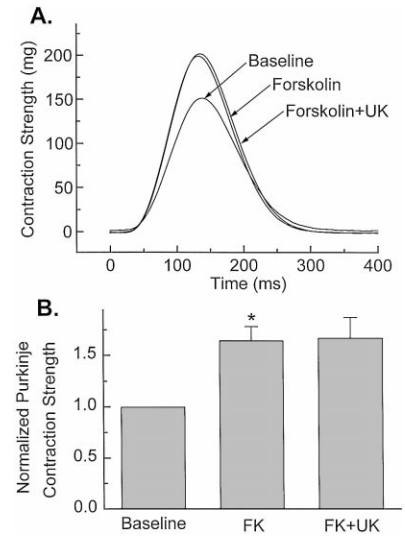

Fig. 5. Effect of UK 14,304 (UK, 0.1 μM) on Purkinje contraction strength in the presence of forskolin (FK, 0.1 μM) after incubation with pertussis toxin (1 μg/ml for 6 h). (A) Results from a typical experiment in which FK produces significant enhancement in Purkinje contraction strength. However, after treatment with pertussis toxin, UK can no longer reverse the effects of FK, suggesting that the α_2-adrenoceptors are uncoupled after incubation with pertussis toxin. (B) Summary of the results from three experiments. Results are expressed as Purkinje contraction normalized to baseline values (normalized mean $\pm$ SEM). $*$ $P < 0.05$ vs. preceding intervention. The absolute magnitudes of the Purkinje contraction strength are 108.0 ± 21.5 mg at baseline, 174.9 ± 27.6 mg with FK, and 178.4 ± 32.3 mg with FK + UK.

for 6 h, stimulation of adenylyl cyclase with 0.1 μM forskolin remained intact increasing Purkinje contraction strength to $165 \pm 14\%$ of baseline ($n = 3$, $P < 0.05$ vs. baseline). Addition of 0.1 μM UK 14,304 did not alter Purkinje contraction strength which remained augmented at $167 \pm 20\%$ (P = N.S. vs. forskolin alone, $P < 0.05$ vs. baseline). These results suggested that the α_2-adrenoceptors were uncoupled from its signal transducing G-protein, probably G_i, after incubation with pertussis toxin (Fig. 5). These results confirmed that the α_2-adrenoceptors present in cardiac Purkinje tissue mediate important physiological functions and serve to counteract the effects of β-adrenoceptor stimulation in an accentuated antagonism manner.

4. Discussion

In this study, we have demonstrated that activation of α_2-adrenoceptors alone has no direct effect on Purkinje contraction but inhibits the contraction strength of isolated Purkinje fibers that has been previously enhanced by β-adrenoceptor stimulation. The α_2-adrenoceptor-mediated antagonism can be demonstrated against stimulation of the β-adrenoceptor and adenylyl cyclase but fails to exert any modulation on cAMP-mediated effects. In addition, the α_2-adrenoceptor effects are dependent on a pertussis toxin-sensitive guanine nucleotide-binding protein. These results confirm that the post-junctional α_2-adrenoceptors play an important role in the regulation of cardiac Purkinje tissue physiology.

Post-junctional α_2-adrenoceptors were thought not to be present in mammalian hearts (Buxton and Brunton, 1986) until Purkinje fibers were examined separately. Rosen et al. (1984) reported that automaticity changes in dog Purkinje fibers by clonidine were not blocked by yohimbine and concluded that the clonidine effects were independent of α_2-adrenoceptors. On the other hand, Mugelli et al. (1986) reported the change in automaticity in sheep Purkinje fibers exposed to norepinephrine under hypoxic conditions were blocked by yohimbine but not by prazosin or practolol, suggesting the presence of α_2-adrenoceptor effects under those conditions. Cable et al. (1994) demonstrated in intact dog hearts in vivo, the Purkinje relative refractory period was prolonged by both α_1- and α_2-adrenoceptor stimulation. These results were confirmed by direct determination in isolated canine Purkinje fibers that α_2-adrenoceptor stimulation prolongs the Purkinje action potential (Samson et al., 1995) as well as by α_2-adrenoceptor binding studies (Lee et al., 1996a). In this study, we showed that the effects of α_2-adrenoceptor stimulation can be substantial, producing greater than 50% changes in Purkinje contraction strength that has been previously augmented by β-adrenoceptor stimulation.

The mechanism through which α_2-adrenoceptor stimulation modulates Purkinje contraction strength is consistent with an accentuated antagonism scheme, which was first observed in the sympathetic/parasympathetic interaction

on cardiac contraction (Levy, 1978). Levy observed that when the existing level of cardiac sympathetic nervous activity was low, the depressant effect of increased parasympathetic activity on the ventricular myocardium was relatively feeble; however, against a background of tonic sympathetic activity, the negative inotropic effect produced by vagal stimulation was considerably more prominent. Receptor-mediated inhibition of β-adrenoceptor effects in the heart, therefore, is not confined to α_2-adrenoceptors. We have previously reported that β-adrenoceptor stimulation of the cardiac voltage-sensitive Na^+ currents in isolated rabbit ventricular myocytes was inhibited by acetylcholine (Matsuda et al., 1993). Acetylcholine has no direct effects on the Na^+ currents but could significantly reverse the Na^+ current enhancement by isoproterenol or forskolin but not that by cAMP. Likewise, activation of the A_1-adenosine receptor has been shown to antagonize the afterdepolarizations and triggered activity induced by isoproterenol and forskolin, but not those induced by dibutyryl cAMP in guinea pig ventricular myocytes (Song et al., 1992). The physiological behaviour of the α_2-adrenoceptors in canine Purkinje fibers is evidently very similar to those demonstrated by the acetylcholine and A_1-adenosine receptors in cardiac myocytes, and all three types of receptors are known to be coupled to a pertussis toxin-sensitive inhibitory G-protein, G_i. A major function of the postjunctional α_2-adrenergic receptors, therefore, appears to counter-balance the effects of β-adrenoceptor stimulation by inhibition of cAMP production. The α_2-adrenoceptor signalling pathway, however, is different from its α_1-adrenoceptor counterpart, which can also counteract β-adrenoceptor effects in the heart, but the α_1 effects are mediated via activation of protein kinase C. α_1-Adrenoceptor activation is known to inhibit L-type Ca^{2+} currents stimulated by 8-bromo-cAMP (Chen et al., 1996). The postjunctional α_2-adrenoceptor signal transduction mechanism is also different from that of the prejunctional α_2-adrenoceptor which is known to be coupled to G-proteins that are not pertussis toxin-sensitive (Nichols et al., 1988).

Our finding that UK 14,304 does not counteract the augmentation of Purkinje contraction strength by 8-bromo-cAMP suggests that UK 14,304 acts at or proximal to adenylyl cyclase activation. There are several possible mechanisms through which α_2-adrenoceptor activation of G_i could lead to inhibition of adenylyl cyclase. First, $\beta\gamma$ subunits dissociated from G_i on receptor-mediated activation could bind activated $G_{s\alpha}$ rendering them unavailable for stimulation of adenylyl cyclase (Gilman, 1984). Second, activated $G_{i\alpha}$ could inhibit cAMP production by competing with $G_{s\alpha}$ at the catalytic binding site of adenylyl cyclase (Kataka et al., 1986). Third, activated $G_{i\alpha}$ can directly inhibit adenylyl cyclase activity and accumulation of cAMP (Katada et al., 1984; Wong et al., 1991). Fourth, $\beta\gamma$ subunits of G-proteins have been shown to directly modulate adenylyl cyclase activity (Tang and Gilman, 1991). The accentuated antagonism demonstrated by α_2-against β-adrenoceptor stimulation should be differentiated from the α_1-adrenoceptor supersensitivity sometimes seen in cardiac tissue after chronic exposure to heightened β-adrenoceptor stimulation (Butterfield and Chess-Williams, 1993). Chronic β-adrenoceptor stimulation is associated with down-regulation of both β- and α_1-adrenoceptors in the rat heart and yet the α_1-adrenoceptor responses are enhanced. The mechanism for such α_1 supersensitivity is not clear but could be due to signal transduction 'cross-talk' (Manolopoulos et al., 1991; Lee et al., 1994).

The cardiac Purkinje fiber is a contractile tissue containing myofilaments and is thought to have the same embryonic origin as cardiac muscle, derived from the same mesodermal precursors (Wenink, 1976). Free-running Purkinje fibers are easy to isolate and have been used for the examination of various cellular parameters in the regulation of cardiac contractility (Brill et al., 1987; Sonn and Lee, 1988; Sprung et al., 1994). The results of our study could not be directly extrapolated to cardiac muscle regulation because myocardial tissue does not contain α_2-adrenoceptors. The possibility of differential regulation between Purkinje and ventricular myocardium in response to adrenoceptor influence is intriguing. Since the major function of the His–Purkinje system is impulse conduction, we speculate that the antagonistic α_2-adrenoceptor effect on β-adrenoceptor stimulation serves to maintain electrophysiological homeostasis of the heart. We have demonstrated previously that β-adrenoceptor induced afterdepolarizations and triggered activity are suppressed by α_2-adrenoceptor stimulation (Samson et al., 1995). In addition, enhanced automaticity produced by β-adrenoceptor stimulation could be inhibited by α_2-adrenoceptor stimulation. It is possible that by antagonizing the increase in contraction strength by β-adrenoceptor stimulation, the Purkinje tissue could operate at a lower metabolic state and allow the tissue to remain functional by preserving high energy phosphates and turnover of metabolites. Also, α_2-adrenoceptor stimulation may render the Purkinje tissue less susceptible to the development of mechanically-induced depolarizations and arrhythmias. However, further insight into the function of this complex interaction of receptors and their signal transduction pathways will require delineation by future studies.

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