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The positive inotropic effect of α_{1A}-adrenoceptor stimulation is inhibited by 4-aminopyridine

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Abstract

This study was designed to determine if 4-aminopyridine, a reported inhibitor of the transient outward K^+ current (I_{to}), alters the inotropic actions elicited via stimulation of WB4101- or chloroethylclonidine-sensitive receptors in rat myocardium. WB4101 (*N*-[2-(2,6-dimethoxyphenoxy)ethyl]-2,3-dihydro-1,4-benzodioxin-2-methanamine) is a competitive antagonist that is selective for α_{1A}- and α_{1C}-adrenoceptors, while chloroethylclonidine is an irreversible blocker that is reported to antagonize α_{1B}-, α_{1C}-, and α_{1D}-adrenoceptor binding. Inotropic effects of the α_1-adrenoceptor agonist phenylephrine were examined in isolated left atrial and papillary muscle before and after addition of 4-aminopyridine, and before and after addition of 4-aminopyridine in preparations pretreated with chloroethylclonidine or WB4101. In addition, effects of phenylephrine were examined before and after treatment with staurosporine (an inhibitor of protein kinase C) in chloroethylclonidine-pretreated preparations. Phenylephrine (10 μM) elicited a sustained positive inotropic response in left atria and a triphasic inotropic action in papillary muscle (transient positive and negative inotropic components preceding a sustained positive inotropic response). 4-Aminopyridine (1.0, 1.7, 3.0 mM) reduced the sustained positive inotropic responses in the absence of antagonists and in chloroethylclonidine-pretreated preparations. However, in the presence of 10 nM WB4101, 4-aminopyridine had no effect on the remaining inotropic actions of phenylephrine. The sustained positive inotropic response to the α_1-agonist in chloroethylclonidine-pretreated preparations was not inhibited by 100 nM staurosporine. These data suggest that the sustained positive inotropic actions of α_{1A}-adrenoceptor stimulation in rat atrial and ventricular myocardium are mediated via non-protein kinase C-associated reductions in I_{to}.

Keywords: 4-Aminopyridine; α_1-Adrenoceptor subtype; Chloroethylclonidine; Cardiac muscle, isolated; Staurosporine; WB4101

1. Introduction

It has been shown that α_1-adrenoceptor stimulation increases the force of contraction in hearts of most mammalian species (Benfey, 1990; Scholz, 1980) including man (Bruckner et al., 1984; Schumann et al., 1978). This effect is fundamentally different from the inotropic response elicited by β-adrenoceptors in that it is not associated with an enhanced rate of relaxation (Amrani et al., 1989) or increases in cyclic AMP (Schumann et al., 1975). Evidence suggests that the positive inotropic response to α_1-adrenoceptor stimulation may result from increased myofibrillar Ca^{2+} sensitivity (Endoh and Blinks, 1988; Puceat et al., 1990) and/or alterations in intracellular Ca^{2+} homeostasis due to changes in transsarcolemmal Ca^{2+} fluxes (Endoh et al., 1991; Fedida et al., 1990). It has been proposed that voltage-dependent Ca^{2+} influx is enhanced by a prolongation of the action potential which results from inhibition of the transient outward K^+ current (I_{to}) (Fedida et al., 1993).

Research has demonstrated the existence of several subtypes of α_1-adrenoceptors (see Minneman, 1988) that are linked to different second messenger systems (Han et al., 1990). In fact, molecular cloning studies suggest that there are as many as three or four α_1-adrenoceptor subtypes (Lefkowitz and Caron, 1990; Perez et al., 1991), while radioligand binding (Morrow and Creese, 1986), functional (Endoh et al., 1992; Takanashi et al., 1991; Williamson et al., 1994b) and electrophysiological (Williamson et al., 1993) studies suggest that there are at least two pharmacologically distinct subtypes. In the past, these two subtypes were often classified as α_{1A} and α_{1B} based

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on their affinity for the competitive α_{1A}-adrenoceptor-selective antagonist *N*-[2-(2,6-dimethoxyphenoxy)ethyl]-2,3-dihydro-1,4-benzodioxin-2-methanamine (WB4101) and their sensitivity to the irreversible α_{1B}-adrenoceptor antagonist chloroethylclonidine, an alkylating analog of clonidine (Han et al., 1987; Minneman et al., 1988). However, more recent data suggest that this classification may require further evaluation as chloroethylclonidine has been reported to irreversibly bind to sites identified as α_{1B}, α_{1C} and α_{1D}, and WB4101 has been reported to have a relatively high affinity for both α_{1A} and α_{1C} subtypes (Goetz et al., 1993).

α_1-Adrenoceptor stimulation has been shown to produce a monophasic increase in contractile force in rat atrial muscle (Endoh et al., 1991; Ertl et al., 1991). In rat ventricular myocardium, a biphasic (Endoh et al., 1991; Ertl et al., 1991; Terzic et al., 1992) or triphasic (Osnes et al., 1978; Otani et al., 1990; Tohse et al., 1987) response is observed with a transient negative inotropic component preceding a sustained positive inotropic action. Previous studies in this laboratory have demonstrated that both WB4101- and chloroethylclonidine-sensitive adrenoceptors are involved in the sustained positive inotropic responses elicited by the α_1-adrenoceptor agonist phenylephrine in isolated rat left atrial and papillary muscle (Williamson et al., 1994a). The present study was designed to determine: (1) if 4-aminopyridine, a reported blocker of the K^+-component of I_{to}, affects the inotropic actions of α_1-adrenoceptor subtype stimulation in rat heart; and (2) if effects found to be sensitive to 4-aminopyridine are mediated by protein kinase C.

2. Materials and methods

2.1. Isolated cardiac preparations

All protocols in this study were approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences and were in accordance with the Guide for the Use of Laboratory Animals put forth by the U.S. Department of Health and Human Services.

Male, Sprague-Dawley rats (300–400 g) were anesthetized. After thoracotomy, hearts were removed and immediately perfused through the aorta with a Krebs-Henseleit solution of the following composition (in mM): 118.0 NaCl, 27.1 $NaHCO_3$, 3.7 KCl, 1.4 $CaCl_2$, 1.2 $MgCl_2$, 1.0 KH_2PO_4, and 11.1 glucose. This solution was buffered to pH 7.4 by saturation with 95% O_2-5% CO_2 gas and maintained at 30°C.

After the heart was free of residual blood, left atrial and papillary muscle were dissected and hung vertically in muscle baths containing the oxygenated solution described above. Nadolol (3 μM; a β-adrenoceptor antagonist) was included in the buffer to prevent potential effects of endogenous catecholamines and to insure that phenylephrine was not acting via β-adrenoceptor stimulation. Preparations were paced via platinum contact electrodes at a frequency of 0.5 Hz by 1.0 ms square wave pulses set at 150% threshold voltage. Force of resting tension and isometric contraction were monitored by force-displacement transducers and recorded continuously on a polygraph. A length-tension relationship was determined for each preparation, and resting tension was subsequently maintained at the level that elicited 90% of maximum observed contractile force (approximately 0.5 g for papillary muscle and 0.7 g for atrial muscle). Tissues were equilibrated for 60 min, during which time the bathing solution was changed every 15 min.

Preparations were used initially to examine the inotropic actions of 4-aminopyridine. Concentration-response curves for effects of 0.3–10 mM 4-aminopyridine were obtained for each preparation by cumulative addition. The pH of the bathing solution was monitored during addition of 4-aminopyridine.

Preparations were also used to examine the inotropic actions of a near maximally effective concentration of phenylephrine (10 μM) before and after treatment with 1, 1.7 or 3 mM 4-aminopyridine, and before and after treatment with 4-aminopyridine in preparations pretreated with chloroethylclonidine or WB4101. Additional experiments utilized chloroethylclonidine-pretreated preparations to examine the inotropic effects of 10 μM phenylephrine before and after treatment with staurosporine. 4-Aminopyridine (1.0, 1.7 or 3.0 mM) or staurosporine (100 nM) was added to the bathing solution 20 or 60 min, respectively, before the second exposure to phenylephrine and remained in the bathing solution for the duration of the experiment. When used, WB4101 (10 nM) was added to the bathing solution 20 min before the second exposure to phenylephrine and remained in the bathing solution for the duration of the experiment. Tissues pretreated with chloroethylclonidine (300 μM) were exposed for 30 min before unbound chloroethylclonidine was removed by 3 consecutive additions of drug-free bathing solution at 5 min intervals.

2.2. Statistical evaluation

Data were analyzed by analysis of variance (ANOVA) with Duncan's multiple range test or by Student's *t*-test, as appropriate. Criterion for significance was a P value less than 0.05. All data are presented as means ± S.E.

2.3. Materials

Chloroethylclonidine dihydrochloride and WB4101 were purchased from Research Biochemicals (Natick, MA, USA). Staurosporine, 4-aminopyridine, phenylephrine hydrochloride and nadolol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals were of reagent grade. Stock solutions of phenylephrine

and chloroethylclonidine were prepared daily in Milli-Q water. 4-Aminopyridine and WB4101 were prepared daily in 0.01 N HCl, and staurosporine was prepared daily in dimethylsulfoxide (DMSO). Preliminary experiments indicated that the amount of HCl or DMSO added to the bathing solution had no effect on resting or developed tension, or observed inotropic responses.

3. Results

Under the experimental conditions utilized in this study, 10 μM phenylephrine produces near maximum increases in contractile force in both atrial and papillary muscle (Williamson et al., 1994a). This concentration elicits a monophasic positive inotropic response in left atria which reaches maximum in 15–20 min and a triphasic action in papillary muscle consisting of transient positive (0–0.5 min) and then negative (0.5–2.0 min) inotropic components followed by a sustained positive inotropic action which reaches maximum after 15–20 min (Williamson et al., 1994b). Following removal of the α_1-adrenoceptor agonist by three consecutive additions of drug-free bathing solution at 5 min intervals, a second exposure to 10 μM phenylephrine elicits inotropic effects that are not different from the initial responses.

3.1. Effects of 4-aminopyridine on contractile force

Initial experiments examined the effects of 4-aminopyridine on contractile force in isolated cardiac preparations. Cumulative addition of 0.03–10 mM 4-aminopyridine elicited concentration-dependent increases in contractile force in both left atrial and papillary muscle (Fig. 1). However, the effects of 4-aminopyridine differed somewhat between tissues (Fig. 2A). In left atria, the positive inotropic effect reached maximum values in 1–2 min with only a slight decline in developed tension observed during the remaining 20 min exposure. In contrast, in papillary muscle 4-aminopyridine elicited a biphasic positive inotropic response which reached a transient maximum after approximately 1 min and then rapidly declined to a relatively stable plateau. 4-Aminopyridine also produced transient increases in buffer pH (Fig. 2B) with values returning to control levels in 2–4 min.

3.2. Antagonistic effects of 4-aminopyridine

To determine if responses to α_1-adrenoceptor stimulation were altered by inhibition of I_{to}, experiments examined the inotropic effects of 10 μM phenylephrine before and after exposure to 1.0, 1.7, and 3.0 mM 4-aminopyridine. As in naive tissue, 4-aminopyridine elicited positive inotropic responses in left atrial and papillary muscle when added after preparations recovered from an initial exposure to phenylephrine. Subsequent addition of 10 μM phenylephrine in the continued presence of 4-aminopyridine resulted in a maximum contractile force which was not significantly different than that elicited by phenylephrine alone. Accordingly, 4-aminopyridine acted in a concentration-dependent manner to reduce the α_1-adrenoceptor-mediated increase in contractile force in left atria (Fig. 3) and the sustained positive inotropic response in papillary muscle (Fig. 4). The transient positive and negative inotropic components of the response to phenylephrine in papillary muscle were not altered by 4-aminopyridine (Fig. 4).

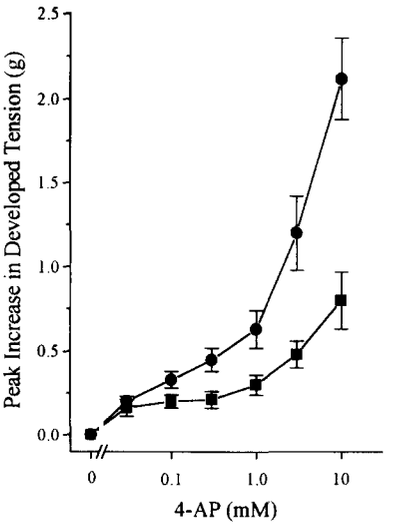

Fig. 1. Effects of 4-aminopyridine on developed tension in isolated rat left atria (circles) and papillary muscle (squares) ($n = 4$/group). Preparations were bathed in an oxygenated Krebs-Henseleit buffer (30°C) containing 3 μM nadolol and paced at 0.5 Hz. Actions of 4-aminopyridine were examined by cumulative addition; data represent the maximum increase in developed tension (observed after 30–90 sec) elicited by each concentration of 4-aminopyridine. Vertical bars represent S.E. Values for developed tension before addition of 4-aminopyridine were 0.95 ± 0.23 and 1.12 ± 0.18 g in atrial and papillary muscle, respectively.

In order to determine if this antagonistic effect of 4-aminopyridine was specific for α_1-adrenoceptor-mediated actions, experiments examined effects of 4-aminopyridine on the positive inotropic response to isoproterenol. The efficacy of this β-adrenoceptor agonist was not altered in either tissue. Maximum isoproterenol-induced increases in developed tension in left atrial muscle were 3.02 ± 0.22 and 2.83 ± 0.20 g in the absence and presence of 3.0 mM 4-aminopyridine, respectively; corresponding values in papillary muscle were 2.14 ± 0.22 and 2.05 ± 0.19 g ($n = 4$/tissue).

3.3. Antagonistic effects of 4-aminopyridine following chloroethylclonidine or WB4101 pretreatment

To determine which of the α_1-adrenoceptor subtypes is coupled to effects antagonized by 4-aminopyridine, the actions of 10 μM phenylephrine were compared before and after addition of 3.0 mM 4-aminopyridine in prepara-

tions pretreated with 300 μM chloroethylclonidine or 10 nM WB4101. These antagonists did not alter the positive inotropic actions elicited by 4-aminopyridine. However, as reported previously (Williamson et al., 1994b), both chloroethylclonidine and WB4101 reduced the magnitude of the sustained positive inotropic response to phenylephrine in both atrial and papillary muscle, and chloroethylclonidine antagonized the transient negative inotropic effect in papillary muscle. Following exposure to the irreversible antagonist chloroethylclonidine, the inotropic effect of phenylephrine in left atria and the sustained positive inotropic action in papillary muscle were further antagonized by 4-aminopyridine (Fig. 5). In contrast, in the presence of the competitive antagonist WB4101, the sustained positive inotropic actions of phenylephrine were not altered by 4-aminopyridine. In WB4101-treated atrial muscle, the phenylephrine-induced maximum increases in developed tension were 0.67 ± 0.08 and 0.72 ± 0.07 g in the presence and absence of 4-aminopyridine, respectively (n = 6/group). In WB4101-treated papillary muscle, the maximum sustained positive inotropic responses in the presence and absence of 4-aminopyridine were 0.19 ± 0.04 and 0.23 ± 0.10 g (n = 6/group).

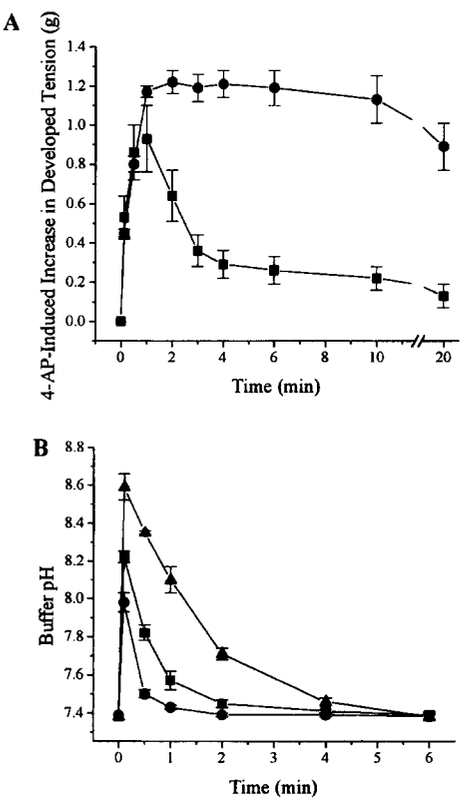

Fig. 2. (A) Time course for the inotropic effects of 3.0 mM 4-aminopyridine in isolated left atria (circles) and papillary muscle (squares) (n = 3–4/group). Preparations were bathed in an oxygenated Krebs-Henseleit buffer (30°C) containing 3 μM nadolol and paced at 0.5 Hz. Data represent the increase in developed tension elicited by 4-aminopyridine; vertical bars represent S.E. Values for developed tension before addition of 4-aminopyridine were 0.93±0.10 and 1.89±0.11 g for left atria and papillary muscle, respectively. (B) Time course for the effects of 1.0 (circles), 1.7 (squares) and 3.0 (triangles) mM 4-aminopyridine on buffer pH (n = 3/group). Vertical bars represent S.E.

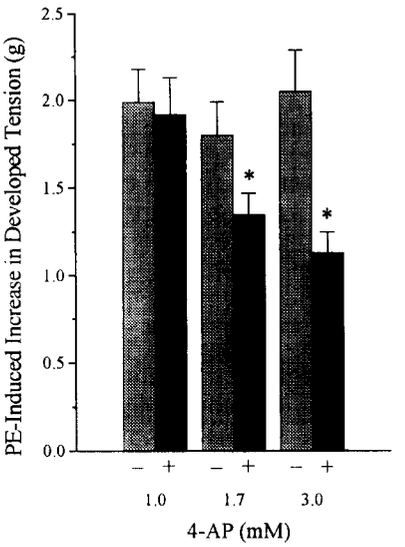

Fig. 3. Effects of 1.0, 1.7 and 3.0 mM 4-aminopyridine on the maximum increase in developed tension elicited by 10 μM phenylephrine (PE) in isolated left atrial muscle (n = 6–8/group). Preparations were bathed in an oxygenated Krebs-Henseleit buffer (30°C) containing 3 μM nadolol and paced at 0.5 Hz. Inotropic actions of phenylephrine were examined before (light bars) and 20 min after (dark bars) addition of 4-aminopyridine. Values for developed tension before phenylephrine exposure were 0.72±0.04 and 1.18±0.07 g before and after addition of 1.0 mM 4-aminopyridine; 0.62±0.09 and 1.24±0.13 g before and after addition of 1.7 mM 4-aminopyridine; and 0.71±0.10 and 1.54±0.16 g before and after addition of 3.0 mM 4-aminopyridine. Vertical bars represent S.E. * $P < 0.05$ vs. respective values observed before treatment with 4-aminopyridine.

3.4. Antagonistic effects of staurosporine following chloroethylclonidine pretreatment

Continued experiments were designed to determine if protein kinase C is involved in the inotropic actions elicited by WB4101-sensitive adrenoceptor stimulation; effects of 10 μM phenylephrine were compared before and after addition of 100 nM staurosporine in preparations pretreated with 300 μM chloroethylclonidine. As reported previously, staurosporine itself elicited a negative inotropic effect in both atrial and papillary muscle (Williamson et al., 1994b). Following treatment with chloroethylclonidine, staurosporine did not alter the sustained increases in contractility elicited by phenylephrine in either tissue. In atrial muscle, the phenylephrine-induced maximum increases in developed tension were 0.19 ± 0.05 and 0.21 ± 0.04 g in the presence and absence of staurosporine, respectively (n = 5–6/group). In papillary muscle, the maximum sustained positive inotropic responses in the presence and absence of staurosporine were 0.04 ± 0.05 and 0.01 ± 0.05 g (n = 5–6/group).

4. Discussion

As reported previously (Ertl et al., 1991; Williamson et al., 1994a), current results indicate that α_1-adrenoceptor stimulation elicits different inotropic responses in rat atrial and ventricular muscle. In atrial preparations, phenylephrine produced a monophasic positive inotropic effect, while the response in papillary muscle was triphasic. Previous studies in our laboratory have shown that the monophasic response in left atria and the sustained positive inotropic action in papillary muscle are antagonized by both chloroethylclonidine and WB4101. The transient negative inotropic component in papillary muscle is inhibited by chloroethylclonidine but not by WB4101 (Williamson et al., 1994b). According to studies suggesting that there are two functional α_1-adrenoceptor subtypes (Morrow and Creese, 1986; Endoh et al., 1992; Takanashi et al., 1991), these data suggest that the sustained positive in-

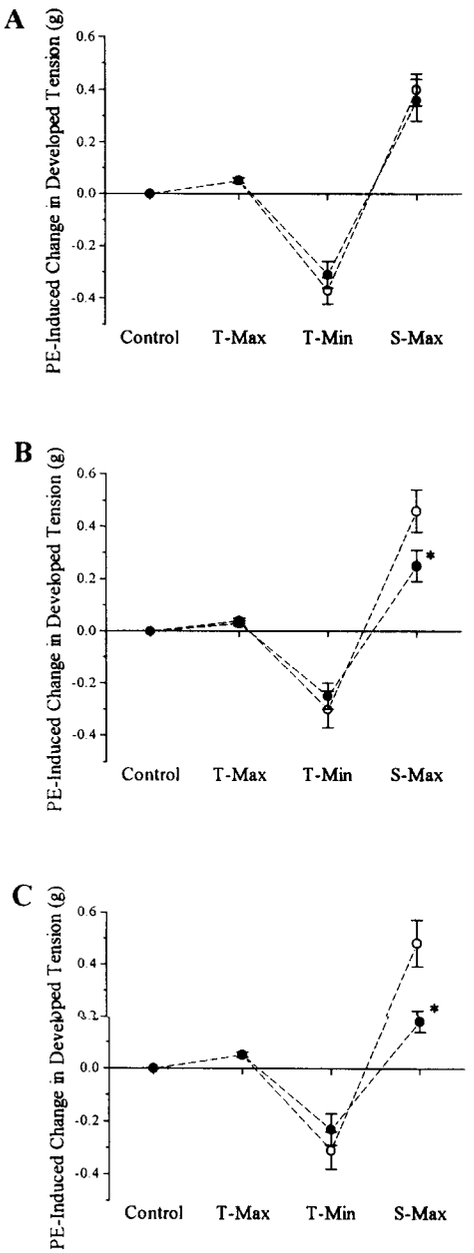

Fig. 4. Effects of 1.0 (A), 1.7 (B) and 3.0 (C) mM 4-aminopyridine on the maximum transient positive (T-Max), transient negative (T-Min) and sustained positive inotropic (S-Max) responses elicited by 10 μM phenylephrine (PE) in isolated papillary muscle ($n = 7$/group). Preparations were bathed in an oxygenated Krebs-Henseleit buffer (30°C) containing 3 μM nadolol and paced at 0.5 Hz. Inotropic actions of phenylephrine were examined before (open circles) and 20 min after (filled circles) addition of 4-aminopyridine. Values for developed tension before phenylephrine exposure were 1.34 ± 0.09 and 1.47 ± 0.12 g before and after addition of 1.0 mM 4-aminopyridine; 1.46 ± 0.21 and 1.58 ± 0.23 g before and after addition of 1.7 mM 4-aminopyridine; and 1.48 ± 0.18 and 1.52 ± 0.16 g before and after addition of 3.0 mM 4-aminopyridine. Vertical bars represent S.E. * $P < 0.05$ vs. respective values observed before treatment with 4-aminopyridine.

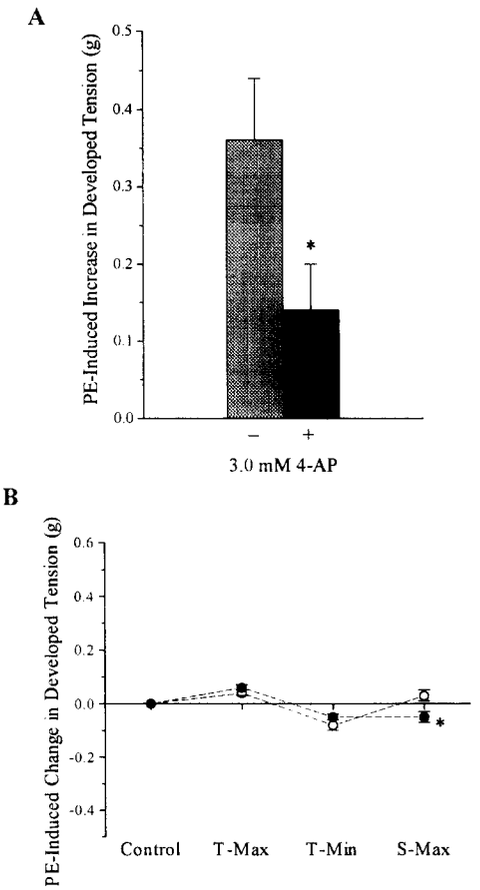

Fig. 5. Effects of 3.0 mM 4-aminopyridine on (A) the maximum increase in developed tension elicited by 10 μM phenylephrine (PE) in 300 μM chloroethylclonidine-pretreated left atria, and (B) the maximum transient positive (T-Max), transient negative (T-Min) and sustained positive inotropic (S-Max) responses elicited by 10 μM phenylephrine (PE) in 300 μM chloroethylclonidine-pretreated papillary muscle ($n = 6$–8/group). Preparations were bathed in an oxygenated Krebs-Henseleit buffer (30°C) containing 3 μM nadolol and paced at 0.5 Hz. Inotropic actions of phenylephrine were examined before (light bars, open circles) and 20 min after (dark bars, filled circles) addition of 4-aminopyridine. Values for developed tension before phenylephrine exposure were 0.85 ± 0.14 and 1.24 ± 0.13 g before and after addition of 4-aminopyridine in left atria, and 1.23 ± 0.17 and 1.33 ± 0.16 g before and after addition of 4-aminopyridine in papillary muscle. Vertical bars represent S.E. * $P < 0.05$ vs. respective values observed before treatment with 4-AP.

otropic responses in atrial and ventricular muscle are mediated via both α_{1A} and α_{1B} receptors, while the transient decline in developed tension observed in papillary muscle is the result of α_{1B} stimulation. However, recent data indicate that this classification may be oversimplified (Lefkowitz and Caron, 1990; Perez et al., 1991). Chloroethylclonidine has been reported to bind irreversibly to sites identified as α_{1B}, α_{1C} and α_{1D}, and WB4101 has been reported to have a relatively high affinity for both α_{1A} and α_{1C} subtypes (Goetz et al., 1993).

Multiple signal transduction pathways are involved in the inotropic effects produced by α_1-adrenoceptor stimulation (see Fedida et al., 1993). Evidence suggests that the positive inotropic response is mediated in part via increases in action potential duration that result from reductions in I_{to}, a transient outward current (Fedida and Bouchard, 1992). By prolonging the action potential, this reduction in I_{to} has been postulated to increase voltage-dependent Ca^{2+} influx. This hypothesis is supported by studies which show that 4-aminopyridine, a selective blocker of a K^+ component of I_{to} (Josephson et al., 1984; Thompson, 1977), attenuates the positive inotropic effect of α_1-adrenoceptor agonists in isolated rat ventricular myocytes (Tohse et al., 1990). In addition, this hypothesis is consistent with the observation that blockade of the slow inward current antagonizes the positive inotropic response but not the action potential prolongation produced by α_1-adrenoceptor stimulation (Handa et al., 1982).

4-Aminopyridine is known to produce a positive inotropic effect in isolated cardiac muscle (Frank et al., 1978; Shahid and Rodger, 1989; Yanagisawa and Taira, 1979). Current results indicate that the sustained positive inotropic action of 4-aminopyridine is more pronounced in atrial than in papillary muscle. This is consistent with data by Ertl et al. (1991) which show that the action potential prolongation elicited by α_1-adrenoceptor agonists is greater in atrial myocardium. 4-Aminopyridine is thought to selectively inhibit I_{to} by binding to the channel in its closed state (Castle and Slawsky, 1992), and this inhibition of I_{to} is postulated to be the mechanism underlying the increase in contractility. However, there is evidence to suggest that the inotropic response to 4-aminopyridine may be mediated by other mechanisms. For example, this compound has been shown to block other K^+ currents such as the delayed rectifier current in neurons (Pelhate and Pichon, 1974) and a $K_v1.2$ delayed rectifier current expressed in *Xenopus* oocytes (Russell et al., 1994), and it is possible that effects of 4-aminopyridine on contractile function were mediated via inhibition of K^+ currents other than I_{to}.

In addition, it is possible that some of the observed inotropic actions of 4-aminopyridine resulted from changes in pH. Shahid and Rodger (1989) observed a weak positive inotropic effect of 4-aminopyridine hydrochloride in isolated rabbit papillary muscle but a strong inotropic effect of the base form, thus suggesting that the increase in contractility was mediated primarily by an increase in extracellular pH. In current experiments, 4-aminopyridine produced significant increases in buffer pH which peaked in 10–30 s and then returned to control levels in 2–4 min. Since elevations in extracellular pH can increase intracellular pH and thus contractility in isolated cardiac muscle (Bountra and Vaughan-Jones, 1989), it is possible that the observed positive inotropic effect of 4-aminopyridine in papillary muscle was mediated in part by its effect on buffer pH. The decrease of the peak positive inotropic effect of 3.0 mM 4-aminopyridine to subsequent steady-state levels in papillary muscle followed closely the time course of observed changes in buffer pH. However, it is important to note: (1) that there was a sustained positive inotropic response to 4-aminopyridine in papillary muscle even after buffer pH returned to physiological levels; and (2) that atrial muscle showed a monophasic increase in contractility which reached relatively stable maximum levels in 1–2 min. This suggests that the sustained positive inotropic actions of 4-aminopyridine were not mediated by changes in pH. Furthermore, studies in our laboratory have shown that comparable pH changes in Krebs-Henseleit buffer elicited by NaOH show a similar time course of recovery with no sustained inotropic action in either atrial or papillary muscle (data not shown).

Current data indicate that the phenylephrine-induced sustained positive inotropic effects in both atrial and papillary muscle were antagonized in a concentration-dependent manner by 4-aminopyridine. This antagonistic action of 4-aminopyridine seems to be selective for α_1-adrenoceptor responsiveness since experiments demonstrated that 3.0 mM 4-aminopyridine does not alter the inotropic efficacy of isoproterenol. Current data also indicate that inhibitory effects of 4-aminopyridine were observed after pretreatment with chloroethylclonidine but were not detected in the presence of WB4101. This suggests that the α_{1A}-adrenoceptor subtype is coupled to reductions in I_{to} in rat heart.

The signal transduction mechanism involved in this reduction in I_{to} is unknown. Data from the present study indicate that staurosporine, an inhibitor of protein kinase C (Tamaoki et al., 1986), does not antagonize the sustained increases in contractility elicited by phenylephrine in atrial and papillary muscle pretreated with chloroethylclonidine. Since our previous studies have shown that staurosporine attenuates the positive inotropic action of phenylephrine in the absence of chloroethylclonidine (Williamson et al., 1994a), current results suggest that the sustained positive inotropic effects elicited by α_{1A}-adrenoceptor stimulation are mediated via non-protein kinase C-dependent reductions in I_{to}. This is consistent with observations of Tohse et al. (1990) who reported that 12-*O*-tetradecanoylphorbol-13-acetate, an activator of protein kinase C (Nishizuka, 1984), has no significant effect on I_{to} in rat ventricular cells. Similarly, Braun et al. (1990) showed methoxamine-induced reductions in I_{to} in rabbit atrial myocytes that were not altered by pre-treatment with staurosporine. How-

ever, present data are not in agreement with a report by Apkon and Nerbonne (1988) which demonstrated suppression of I_{to} in rat ventricular myocytes by phorbol 12-myristate 13-acetate and 1-oleoyl-2-acetylglycerol, two other agents known to activate protein kinase C (Kaibuchi et al., 1983; Leach et al., 1983). In addition, current results are not in complete agreement with a report by Wang et al. (1991) which suggested that both α_{1A}- and α_{1B}-adrenoceptor stimulation decreases I_{to}. This disparity among results is not understood but may reflect differences in preparations, α_1-adrenoceptor subtype selective antagonists or protein kinase C isoforms.

In summary, results of the current study suggest that α_{1A}-adrenoceptor stimulation elicits sustained positive inotropic responses in rat atrial and ventricular myocardium via non-protein kinase C-dependent reductions in I_{to}.

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