

Anisodamine inhibits cardiac contraction and intracellular Ca^{2+} transients in isolated adult rat ventricular myocytes

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Abstract

Increased cardiac workload often leads to serious complications during cardiac surgery such as pericardiopulmonary bypass. Various agents have been applied to lower peripheral resistance and cardiac workload, one of which, anisodamine, is widely used in Asia. However, the direct action of anisodamine on cardiac contractile property is essentially unknown. This study was designed to examine the influence of anisodamine on ventricular contractile function at the single cardiac myocyte level. Ventricular myocytes from adult rat hearts were stimulated to contract at 0.5 Hz, and mechanical and intracellular Ca^{2+} properties were evaluated using an IonOptix Myocam system. Contractile properties analyzed included peak shortening (PS), time-to-PS (TPS), time-to-90% relengthening (TR_{90}), maximal velocity of shortening/relengthening ($\pm \text{dL}/\text{dt}$), intracellular Ca^{2+} fluorescence intensity change (ΔFFI) and decay (τ). Anisodamine exhibited a concentration-dependent (10^{-12} – 10^{-6} M) inhibition in PS and ΔFFI , with maximal inhibitions of 44.7% and 47.2%, respectively. Anisodamine inhibited $\pm \text{dL}/\text{dt}$, lowered resting FFI but elicited no effect on TPS/ TR_{90} and τ . Pretreatment with the nitric oxide synthase (NOS) inhibitor *N*^ω-nitro-L-arginine methyl ester (L-NAME, 100 μM) abolished the inhibitory effect of anisodamine in cell shortening. In addition, anisodamine prevented cholinergic agonist carbachol-induced positive cardiac contractile response. This study demonstrated a direct cardiac depressive action of anisodamine at the myocyte level, which may be related to, at least in part, NO production and cholinergic antagonism. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Anisodamine; Myocyte shortening; Ca^{2+} intracellular; Nitric oxide (NO); Cholinergic

1. Introduction

Anisodamine (6-[*s*]hydroxyhyoscyamine), an alkaloid first extracted from the Chinese herb *Hyoscyamus niger* L. (Fig. 1), is very similar to atropine in structure and is considered a new cholinergic antagonist but with a weaker effect on the central nervous system (Department of Pharmacology, 1973). Anisodamine has been used as a vasoactive drug in China for decades to improve microcirculation, especially during septic shock (Xiu, 1980; Xiu et al., 1982; Su et al., 1983; Zhang et al., 1987). The microcirculatory effects of anisodamine have been demonstrated

to be associated with its ability to inhibit the aggregation of platelet and synthesis of thromboxane, malondialdehyde and 6-keto-prostaglandin $\text{F}_{1\alpha}$ (Xiu et al., 1982; Xu et al., 1989). In addition to its beneficial effects on microcirculation, anisodamine has also been used to protect against arrhythmias, myocardial ischemic reperfusion injury and cardiopulmonary bypass surgery-induced hypertension (Hu et al., 1986; Wang et al., 1988; Fu et al., 1993). To date, the mechanisms of action behind anisodamine-induced cardiovascular effects have been largely attributed to its antagonism against cholinergic and adrenoceptors (Verma and Yue, 1986). With the increased use of anisodamine in cardiovascular diseases (Wang et al., 1988; Fu et al., 1993; Yao et al., 1995), its direct impact on cardiac contractile function has not been reported. Therefore, the aim of the present study was to elucidate the effect of anisodamine on cardiac contractile function at the cellular level by evaluating myocyte shortening and intracellular Ca^{2+} properties in isolated ventricular myocytes.

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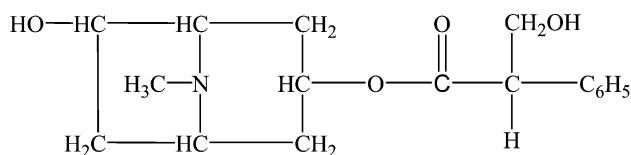
2. Materials and methods

2.1. Isolation of ventricular myocytes

The experimental procedures described in this study were approved by the animal investigation committee of the University of North Dakota (Grand Forks, ND, USA). Single ventricular myocytes were isolated from adult male Sprague–Dawley rats (200–225 g) as described previously (Ren, 2000). Briefly, hearts were rapidly removed and perfused (at 37 °C) with oxygenated (5% CO₂–95% O₂) Krebs–Henseleit bicarbonate (KHB) buffer (in mM: NaCl 118, KCl 4.7, CaCl₂ 1.25, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, *N*-[2-hydro-ethyl]-piperazine-*N'*-[2-ethanesulfonic acid] (HEPES) 10 and glucose 11.1, pH 7.4). Hearts were subsequently perfused with a nominally Ca²⁺-free KHB buffer for 2–3 min followed by a 20-min perfusion with Ca²⁺-free KHB containing 223 U/ml collagenase (Worthington Biochemical, Freehold, NJ, USA) and 0.1 mg/ml hyaluronidase (Sigma, St. Louis, MO, USA). After perfusion, the left ventricle was removed, minced and further digested with trypsin (Sigma) before being filtered through a nylon mesh (300 µm) and collected by centrifugation. Cells were initially washed with Ca²⁺-free KHB buffer to remove remnant enzyme, and extracellular Ca²⁺ was added incrementally back to 1.25 mM.

2.2. Myocyte shortening and relengthening

The mechanical properties of ventricular myocytes were assessed by an IonOptix Myocam detection system (IonOptix, Milton, MA, USA). Cells were placed in a chamber mounted on the stage of an inverted microscope and superfused (at 25 °C) with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 CaCl₂, 1 MgCl₂, 10 glucose and 10 HEPES at pH 7.4. The cells were field-stimulated at a frequency of 0.5 Hz. Cell shortening and relengthening were assessed using the following indices: peak shortening (PS), time-to-90% PS (TPS) and time-to-90% relengthening (TR₉₀), maximal velocities of shortening (+dL/dt) and relengthening (–dL/dt) (Ren, 2000). To test the effect of anisodamine on cardiac contraction, cell shortening was recorded before and 5 min after anisodamine administration. Anisodamine was purchased from Hangzhou Drug Inc., (Hangzhou, China). The chemical structure of anisodamine is shown in Fig. 1.



Anisodamine

Fig. 1. Chemical structure of anisodamine.

2.3. Intracellular Ca²⁺ fluorescence measurement

Myocytes were loaded with fura-2/AM (0.5 µM) for 10 min, and fluorescence measurements were recorded with a dual-excitation fluorescence photomultiplier system (Ionoptix) as described (Ren, 2000). Myocytes were plated on glass cover slips on an Olympus IX-70 inverted microscope and imaged through a Fluor 40 × oil objective. Cells were exposed to light emitted by a 75-W lamp and passed through either a 360- or a 380-nm filter (bandwidths were ± 15 nm), while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected between 480 and 520 nm after first illuminating the cells at 360 nm for 0.5 s, then at 380 nm for the duration of the recording protocol (333-Hz sampling rate). The 360-nm excitation scan was repeated at the end of the protocol. Qualitative changes in intracellular Ca²⁺ levels were inferred from the ratio of the fluorescence intensity at two wavelengths (360/380) and were presented as the fura-2 fluorescent intensity (FFI). Intracellular Ca²⁺ removal was evaluated as the rate of fluorescence decay (τ).

2.4. Data analysis

Data were presented as means ± S.E.M. The statistical significance ($p < 0.05$) for each variable was estimated by analysis of variance (ANOVA) or *t*-test where appropriate.

3. Results

3.1. Effect of anisodamine on myocyte shortening (PS)

The average cell length used in this study was 102 ± 2 µm ($n = 85$). Acute exposure of anisodamine did not affect the resting myocyte cell length over the range of concentrations tested. A representative trace depicting the effect of anisodamine (10^{-7} M) on myocyte shortening (PS) is shown in Fig. 2A. At the end of a 5-min exposure to this concentration of anisodamine, PS was decreased by 41.5%. Anisodamine exhibited little effect on the duration of shortening (TPS) and relengthening (TR₉₀). Anisodamine (10^{-12} – 10^{-6} M) elicited a concentration-dependent depression of PS, with a maximal inhibition of 44.7%. The threshold of inhibition was between 10^{-12} and 10^{-11} M (Fig. 2B). Anisodamine-induced inhibition on cell shortening was maximal within 4 min of exposure and was reversible upon washout (data not shown). The inhibitory effect of anisodamine was associated with depressed maximal velocities of shortening/relengthening (\pm dL/dt) with little response on TPS and TR₉₀ (Table 1).

3.2. Effect of anisodamine on intracellular Ca²⁺ transients

To determine whether the anisodamine-induced inhibition of PS was due to the reduced availability of intracellular

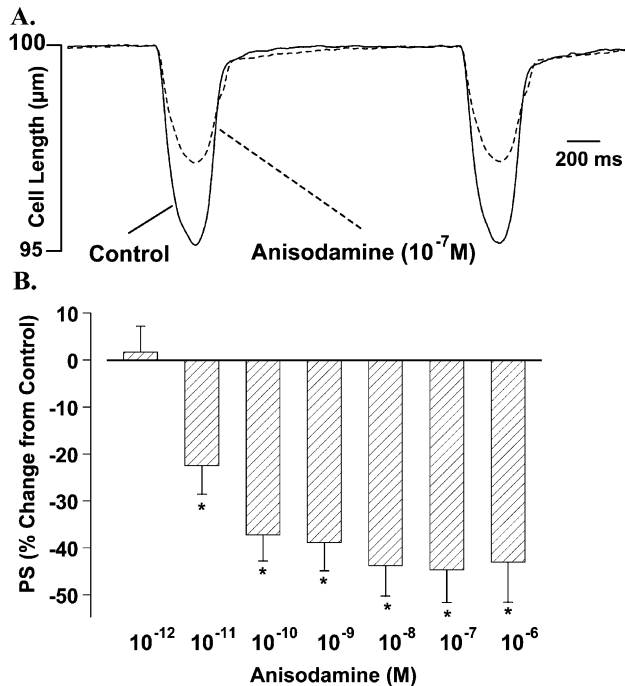


Fig. 2. (A) Representative traces depicting the effect of anisodamine (10^{-7} M) on cardiac contraction in ventricular myocytes. (B) Concentration-dependent response of anisodamine (10^{-12} – 10^{-6} M) on peak cell shortening. Data are presented as percent change from basal PS which was $4.2 \pm 0.6\%$. Means \pm S.E.M., $n=23$ /data group. * $p<0.05$ vs. baseline value.

Ca^{2+} , the influence of anisodamine on the changes of intracellular fluorescent intensity (ΔFFI) was examined. Representative traces of intracellular Ca^{2+} transients shown in Fig. 3A depict that 10^{-7} M anisodamine decreased ΔFFI

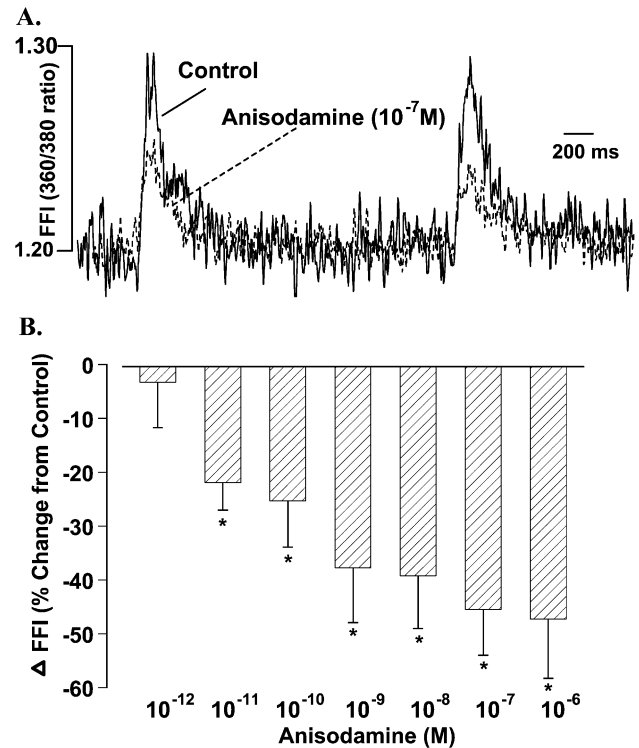


Fig. 3. (A) Representative traces depicting the effect of anisodamine (10^{-7} M) on intracellular Ca^{2+} transient changes (ΔFFI) in ventricular myocytes. (B) Concentration-dependent response of anisodamine (10^{-12} – 10^{-6} M) on ΔFFI . Data are presented as percent change from respective basal ΔFFI value. Means \pm S.E.M., $n=20$ /data group, * $p<0.05$ vs. baseline value.

Table 1
Effect of anisodamine on the duration of myocyte shortening and relengthening in cells from adult rat hearts

	TPS (ms)	TR ₉₀ (ms)	+dL/dt (μm/s)	−dL/dt (μm/s)
Control	151 ± 10	373 ± 42	69.2 ± 11.6	−57.6 ± 13.6
Anisodamine 10 ^{−12} M	166 ± 8	360 ± 43	65.3 ± 9.9	−52.9 ± 12.3
Anisodamine 10 ^{−11} M	155 ± 11	446 ± 48	39.5 ± 4.7 ^a	−23.4 ± 3.7 ^a
Anisodamine 10 ^{−10} M	148 ± 10	480 ± 48	31.7 ± 3.5 ^a	−17.6 ± 2.8 ^a
Anisodamine 10 ^{−9} M	150 ± 11	473 ± 47	30.2 ± 3.5 ^a	−17.0 ± 2.9 ^a
Anisodamine 10 ^{−8} M	148 ± 9	503 ± 47	29.4 ± 4.2 ^a	−15.5 ± 2.8 ^a
Anisodamine 10 ^{−7} M	148 ± 10	464 ± 37	31.2 ± 4.1 ^a	−17.2 ± 3.0 ^a
Anisodamine 10 ^{−6} M	140 ± 8	451 ± 44	35.6 ± 5.8 ^a	−19.5 ± 4.8 ^a

Time-to-peak shortening (TPS), time-to-90% relengthening (TR₉₀), maximal velocities of shortening and relengthening (\pm dL/dt). Data represent means \pm S.E.M., $n=20$ cells.

^a $p<0.05$ vs. baseline value.

by 44.8%. Anisodamine (10^{-12} – 10^{-6} M) elicited concentration-dependent inhibitions of ΔFFI , with a maximal inhibition of 47.2% (Fig. 3B). The threshold of inhibition was between 10^{-12} and 10^{-11} M (Fig. 3B), consistent with that of the cell shortening. The inhibitory response of ΔFFI suggests that a decrease in intracellular free Ca^{2+} is likely to be responsible for the anisodamine-induced depressive action on myocyte shortening. The resting intracellular Ca^{2+} level was also depressed by anisodamine although the fluorescence decay rate was not affected (Table 2).

3.3. Effect of anisodamine on myocyte shortening in the presence of nitric oxide synthase (NOS) inhibitor L-NAME

NO is an important regulator in cardiac contractile function. Constitutive NOS (cNOS) and inducible NOS (iNOS) are both present in cardiac myocytes (Kelly et al., 1996). To examine the potential mechanism of action for anisodamine, the effect of anisodamine on myocyte shortening was re-examined in the presence of the NOS inhibitor, *N*^ω-nitro-L-arginine methyl ester (L-NAME, 100 μM). L-NAME alone had no effect on cell shortening over 30 min (data not shown). As shown in Fig. 4, the anisodamine-induced decrease in PS was completely abolished by L-

Table 2

Effect of anisodamine on intracellular Ca^{2+} transient properties in cells from adult rat hearts

	Resting level (360/380 ratio)	ΔFFI (360/380 ratio)	Ca^{2+} decay rate (ms)
Control	1.20 ± 0.02	0.087 ± 0.010	410 ± 53
Anisodamine 10^{-12} M	1.17 ± 0.02	0.074 ± 0.013	463 ± 69
Anisodamine 10^{-11} M	1.14 ± 0.02^a	0.064 ± 0.006^a	470 ± 67
Anisodamine 10^{-10} M	1.12 ± 0.02^a	0.063 ± 0.007^a	458 ± 72
Anisodamine 10^{-9} M	1.11 ± 0.02^a	0.056 ± 0.008^a	498 ± 69
Anisodamine 10^{-8} M	1.10 ± 0.02^a	0.050 ± 0.008^a	536 ± 91
Anisodamine 10^{-7} M	1.10 ± 0.02^a	0.050 ± 0.008^a	536 ± 91
Anisodamine 10^{-6} M	1.11 ± 0.02^a	0.048 ± 0.007^a	593 ± 66

Data represent means \pm S.E.M., $n=20$ cells.

^a $p < 0.05$ vs. baseline value.

NAME, suggesting a role of NO in anisodamine-induced cardiac depressive response.

3.4. Effect of anisodamine on carbachol-induced myocyte shortening

Muscarinic receptor agonists at high concentrations produce stimulant effects within the heart (Colecraft et al., 1999), and anisodamine has been shown to be an antagonist for the muscarinic receptor (Verma and Yue, 1986). To examine the potential role of muscarinic receptor antagonism in anisodamine-induced cardiac response, the effect of the muscarinic receptor agonist carbachol (10^{-6} and 10^{-5} M) on cell shortening was evaluated in the presence or absence of anisodamine (10^{-7} M). Carbachol alone elicited an increase in PS. However, the carbachol-induced increase in PS was completely prevented by anisodamine, suggesting a role of

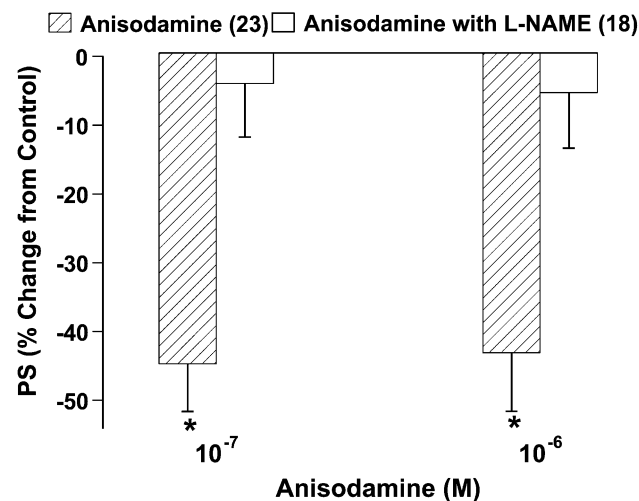


Fig. 4. Effect of the NOS inhibitor L-NAME on anisodamine-induced inhibition of myocyte shortening. L-NAME (100 μM) was applied prior to the addition of anisodamine (10^{-7} and 10^{-6} M). Means \pm S.E.M., * $p < 0.05$ vs. baseline value.

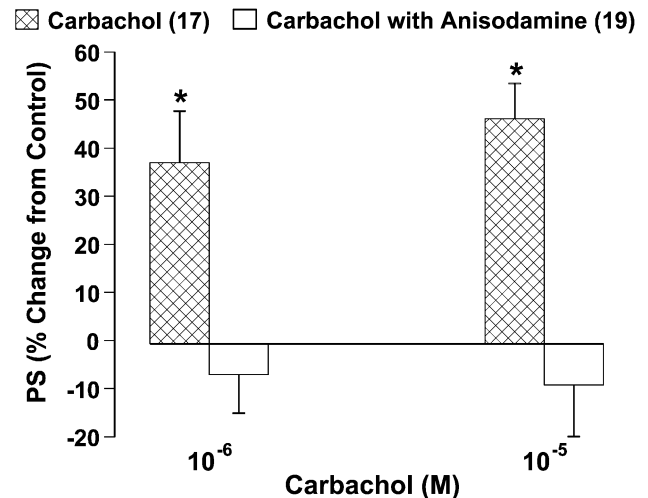


Fig. 5. Effect of the muscarinic receptor agonist carbachol (10^{-7} and 10^{-6} M) on myocyte shortening in the absence or presence of anisodamine (10^{-7} M). The myocytes were pre-treated with anisodamine prior to carbachol addition, and cell shortening taken in the presence of anisodamine (without carbachol) was used as control for the carbachol response). Means \pm S.E.M., * $p < 0.05$ vs. baseline value.

muscarinic receptor antagonism in anisodamine-induced cardiac depression (Fig. 5).

4. Discussion

Our study has provided evidence, for the first time, that the atropine-like microcirculatory vasodilator anisodamine directly inhibits ventricular contraction in isolated cardiac myocytes. The anisodamine-evoked cardiac depressant response was associated with reduced basal—as well as stimulated—intracellular Ca^{2+} levels. The NOS inhibitor L-NAME abolished anisodamine-induced cardiac depression, suggesting the involvement of NO in its cardiac action. In addition, the fact that anisodamine blunted carbachol-induced positive contractile response indicated a potential role of cholinergic antagonism in anisodamine-induced cardiac response.

Anisodamine is able to improve microcirculation in septic shock by eliciting vasodilation and reduce cardiac after-load by antagonizing the elevated blood pressure in certain surgeries such as cardiopulmonary bypass (Xiu, 1980; Xiu et al., 1982; Wang et al., 1988). In our current study, anisodamine directly depressed PS and $\pm \text{dL/dt}$ in a concentration-dependent manner, revealing its cardiac depressant property of anisodamine. This cardiac depression may allow anisodamine to work synergistically with its vasodilatory property in reducing the overall pre-load, after-load and energy expenditure in the heart. The reduced cardiac contractility and energy expenditure or metabolism may also be consistent with anisodamine-elicited protection against myocardial ischemic reperfusion injury and lipid peroxidation (Fu et al., 1993; Yao et al., 1995). However,

our result also provides information regarding the clinical application of anisodamine and that certain caution must be taken in patients with already compromised heart conditions such as congestive heart failure. It is worth mentioning that neither the duration of contraction (TPS) nor the duration of relaxation (TR₉₀) was affected by anisodamine administration, indicating the existence of potential “selectivity” of anisodamine on cardiac contractile proteins.

Our results indicate that anisodamine may depress cardiac contractile function through the inhibition of intracellular Ca²⁺ rise. Although the mechanism(s) of action underneath the reduced intracellular Ca²⁺ recruitment following anisodamine administration is/are not clear at this time, several speculations may be made. First, the observation that the NOS inhibitor L-NAME abolished anisodamine-induced cardiac depression suggests that anisodamine may elicit its depressant effect through the accumulation of NO in the hearts. Ambient NO levels are known to regulate cardiac contractile function (Kelly et al., 1996). NO has been reported to elicit a biphasic effect on cardiac contractile function with a potentiation at lower NO levels and an attenuation at higher NO levels (Mery et al., 1993). Our earlier study showed that the NO donor *S*-nitroso-*N*-penicillamine (SNAP, 100 μM) inhibited the myocyte contraction by ~ 40% (Nickola et al., 2000), similar to the degree of inhibition elicited by anisodamine. Secondly, the observation that anisodamine prevented carbachol-induced positive cardiac contractile response confirmed the cholinergic antagonistic property of anisodamine. However, since there is no acetylcholine in the *in vitro* cardiac myocytes preparation, the anisodamine-induced depressant response may be due to a possible constitutively stimulatory muscarinic receptor. The antagonism against intrinsic cholinergic activity may contribute to the cardiac depressant action of anisodamine, as the cholinergic activation has been shown to evoke an atropine-sensitive positive inotropic effect in ventricular tissue (Mubagwa et al., 1992). The anisodamine-elicited response on the NO system may be either down-stream or independent of its cholinergic antagonistic action although further study is warranted. Nevertheless, NO accumulation has been demonstrated to inhibit Ca²⁺ influx (reviewed by Kelly et al., 1996) and therefore, the rise of intracellular Ca²⁺, consistent with the finding from the present study where both resting and electrically stimulated increase in intracellular Ca²⁺ were observed.

In conclusion, our study demonstrates a direct cardiac depressive response of anisodamine at the ventricular myocyte level, possibly through the NO and/or cholinergic-mediated mechanism. The precise nature of cardiac contractile effects of anisodamine is still far from clear. Future studies should focus on its action on cardiac excitation–contraction coupling and membrane ion channels. These approaches will be essential to understand the cellular effects and pharmacological profiles of this herbal compound.

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