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Effects of amrinone on the transmembrane action potential of rabbit sinus node pacemaker cells

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1 Effects of amrinone on the membrane action potential and spontaneous activity were investigated in sinus node pacemaker cells of rabbit heart by use of microelectrode techniques.

2 Amrinone (1×10^{-4}M to 6×10^{-6}M) caused a shortening of cycle length of spontaneous firing (SPCL) accompanied by an increase in the maximum upstroke velocity at phase 0 ($\dot{V}_{max}$) and amplitude of action potential (AAP), while it did not affect the maximum diastolic potential (MDP).

3 All the effects of amrinone on sinus node pacemaker cells were markedly attenuated or abolished in a low calcium medium (Ca^+ 0.1 mM or 0.3 mM) or in the presence of the slow channel blocking agent, verapamil (5×10^{-7}M, 2×10^{-6}M).

4 The effects of amrinone were not antagonized by the β-adrenoceptor blocking agent, pindolol (2×10^{-7}M).

5 These results indicate that amrinone has an intrinsic effect on sinus node pacemaker cells, increasing their spontaneous firing activity.

6 It is also assumed that the effects of amrinone on sinus node cells are probably mediated by an augmentation of the slow calcium and/or sodium inward current through the cell membrane.

Introduction

Amrinone [5-amino-3, 4'-bipyridine-6-(1h)-one], a new cardiotonic agent, was reported to exert a marked positive inotropic action in a variety of *in vitro* and *in vivo* preparations (Farah & Alousi, 1978; Alousi 1982). The mechanisms of the positive inotropic action were shown to be quite different from cardiac glycosides and catecholamines (Honerjäger, Schafer-Korting & Reiter, 1981; Alousi, 1982; Adams, Rhody & Sutko, 1982). Nevertheless, the precise mode of action of amrinone has yet to be elucidated. The vasodilator action, lack of arrhythmogenic effect and oral availability of this drug (Alousi, 1982) suggest a high therapeutic potential in the treatment of heart failure. However, there is some discrepancy as to the effect of amrinone on heart rate among investigators. In either anaesthetized or unanaesthetized dogs, the intravenous administration of amrinone produced only a slight or negligible change in the heart rate (Farah & Alousi, 1978). In addition, no meaningful change in the heart rate was demonstrated in patients receiving reasonably high doses of amrinone (Le Jemtel, Keung, Sonnenblick, Ribner, Matsumoto, Davis, Schwartz, Alousi & Davolos, 1979; Benotti, Grossman, Braunwald & Carabello, 1980), yet all of these patients were also receiving cardiac glycosides. On the other hand, amrinone was shown to have a positive chronotropic effect on guinea-pig Langendorff perfused heart (Onuaguluchi & Tanz, 1981). Also, in a series of 20 normal male volunteers, amrinone increased the heart rate significantly (de Guzman, Munoz, Palmer, Davolos & Alousi, 1978). The present experiments were undertaken to ascertain whether or not amrinone has a positive chronotropic action by testing it on rabbit sinus node pacemaker cells.

Methods

Twenty five rabbits of either sex weighing 1.5 to 2.0 kg were killed by a blow on the head. The hearts were quickly excised and dissected in Krebs-Ringer solution. The ventricles and left atrium were removed and discarded. After exposing the endocardial surface of the right atrium by longitudinal dissection, a small specimen of tissue (2 × 2 mm) was iso-

lated from the central area of the sinus node. The preparation was mounted in a tissue bath of 0.5 ml volume and superfused with Krebs-Ringer solution at 30°C, gassed with 95% O_2 and 5% CO_2. The composition of the Krebs-Ringer solution was as follows (mM); NaCl 120.3, KCl 4.8, $CaCl_2$ 1.2, $MgSO_4 \cdot 7H_2O$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 24.2 and glucose, 5.5 (pH 7.4). In experiments with low calcium medium, the concentration of $CaCl_2$ was reduced to 0.3 or 0.1 mM. Transmembrane action potentials were recorded from a dominant pacemaker cell in the preparation through a glass microelectrode filled with 3M KCl. Action potentials and their first derivatives were displayed simultaneously on a storage oscilloscope (Tektronix 7163) as well as on a paper recorder (Nihon Kohden RJG-4004). The parameters measured were the maximum upstroke velocity at phase 0 ($\dot{V}_{max}$), the maximum diastolic potential (MDP), amplitude of action potential (AAP), and cycle length of spontaneous firing (SPCL). SPCL was determined by measuring the interval between two successive spikes of the first derivative of action potentials. The criteria used to identify the dominant pacemaker cell are smooth transition from diastolic depolarization (phase 4) to phase 0, and slow upstroke velocity ($\dot{V}_{max}$ was less than 10 V s^{-1}) under the control condition.

Drugs used were amrinone (Sterling Winthrop Research Institute, New York), verapamil (Knoll, Whippany, NJ), and pindolol (Sandoz, Switzerland). A stock solution of amrinone was prepared by dissolving 50 mg in 1.0 ml of 0.5 N lactic acid. After 1 h of equilibration, control measurements were performed, and then the preparations were superfused with Krebs-Ringer solution containing these drugs at various concentrations for 10 to 60 min.

Statistical analysis was performed using Student's t test, and significance was established at $P < 0.05$.

Results

Effects of amrinone on the transmembrane action potential

Effects of amrinone on the membrane action potentials were examined in five preparations. Amrinone at 1×10^{-4}M caused a shortening of SPCL (increase

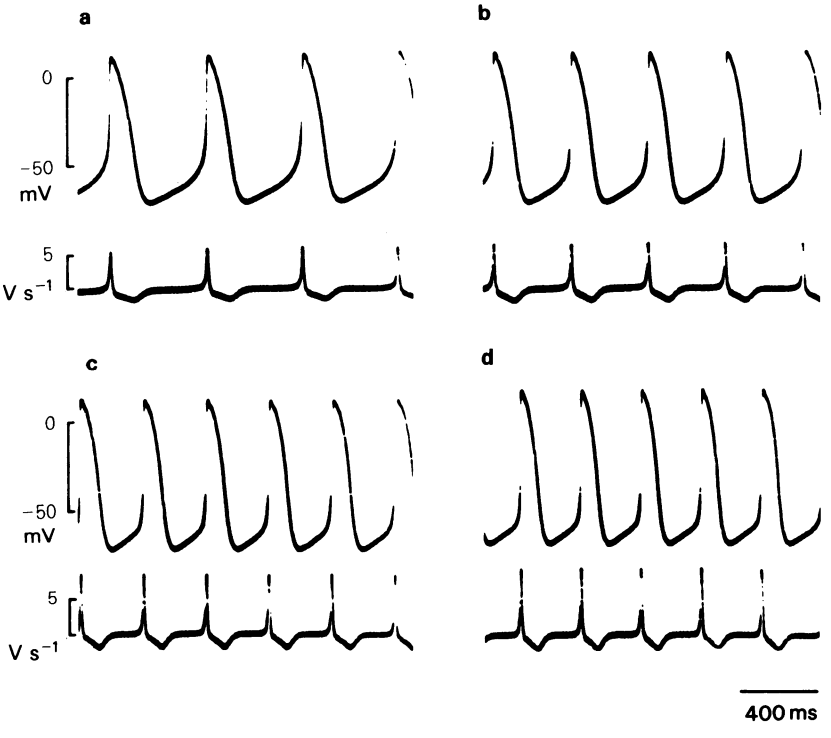

Figure 1 Effects of amrinone on the membrane action potential of sinus node pacemaker cells. (a) Control; (b) 1×10^{-4}M amrinone; (c) 3×10^{-4}M amrinone; (d) 6×10^{-4}M amrinone. panels (b) (c) and (d) were recorded 10 min after application of amrinone at each concentration. Upper trace is membrane action potential and lower trace shows the first derivative of action potential.

Table 1 Effects of amrinone on the membrane action potentials of sinus node pacemaker cells

	AAP (mV)	*MDP* (mV)	$\dot{V}_{max}$ (Vs^{-1})	*SPCL* (ms)
Control	79.8±1.4	-66.4±1.4	8.8±0.4	509±31
Amrinone (1×10^{-4} M)	81.7±1.0	67.1±0.3	9.6±0.6	392±30*
Amrinone (3×10^{-4} M)	82.4±1.5	66.1±1.1	10.7±0.6*	369±17*
Amrinone (6×10^{-4} M)	85.4±1.3	65.2±0.9	12.4±0.4*	368±21*

Values are means±s.e. ($n = 5$) before and 10 min after application of amrinone; AAP: action potential amplitude; MDP: maximum diastolic potential; $\dot{V}_{max}$: maximum upstroke velocity at phase 0; SPCL: cycle length of spontaneous firing. *Significantly different from the values of control at $P < 0.05$.

of firing rate) and a slight increase in $\dot{V}_{max}$ (Figure 1, Table 1), but did not change the other parameters (AAP and MDP). Amrinone at higher concentrations (3×10^{-4}M, 6×10^{-4}M) caused further shortening of SPCL and greater increase in $\dot{V}_{max}$ accompanied by a significant increase in AAP, but had no effect on the MDP.

The onset of action of amrinone was approximately 1 min after application of the drug and the maximum effect was observed within 10 min. After washing out the drug, all the parameters of membrane action potential tested returned to their control levels within 30 min.

Effects of the vehicle (lactic acid) were examined in three preparations. It caused no change in membrane action potential and in spontaneous activity even at the highest concentration, corresponding to 6×10^{-4}M amrinone.

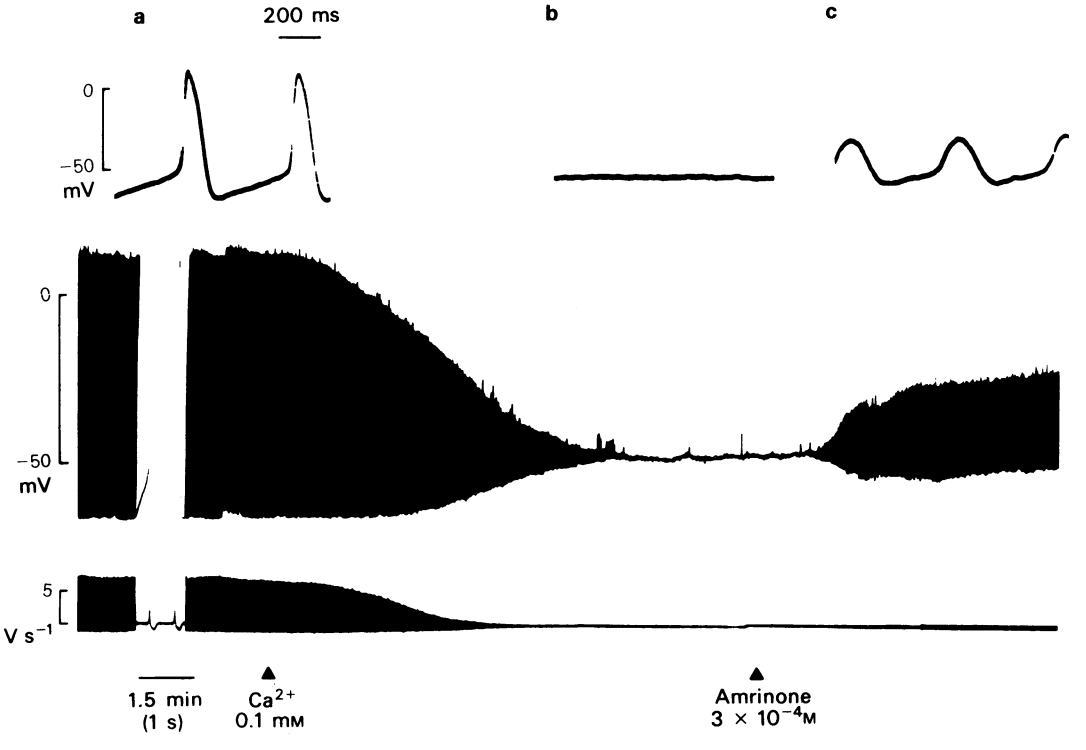

Figure 2 Effects of amrinone on the membrane action potential of sinus node pacemaker cells in a low calcium medium. (a) Control; (b) 15 min after exposure to a low calcium medium (Ca^{2+} 0.1 mM); (c) 10 min after application of amrinone (3×10^{-4}M) in a low calcium medium. Lower panel shows a continuous record of action potential (upper trace) and its first derivative (lower trace). The exposure to a low calcium medium (Ca^{2+} 0.1 mM) and the application of amrinone (3×10^{-4}M) are indicated by solid triangles below the record.

Table 2 Effects of amrinone (3×10^{-4} M) on the sinus node pacemaker cells in a medium containing various calcium concentrations.

$[Ca^{2+}]_o$(mM)	*AAP* (%)		$\dot{V}_{max}$ (%)		*SPCL* (%)	
	Before	*After*	*Before*	*After*	*Before*	*After*
1.2	100.0	104.0±1.6*	100.0	122.0±4.0*	100.0	74.0±6.9*
0.3	87.1±0.9	94.2±1.2*	69.2±5.6	88.3±5.1*	188±3.8	99.5±4.5*
0.1	0	35.1±3.4*	0	3.3±1.0*	———	101.0±6.0*

Values are mean±s.e. ($n=4$); percentage of control values (normal Ca^{2+} medium, 1.2 mM). Measurements were performed before and 10 min after application of amrinone (3×10^{-4} M). *Significantly different from the values before application of amrinone at $P < 0.05$. Abbreviations are the same as in Table 1.

Influence of low calcium medium and verapamil

In five preparations the effects of amrinone were examined in a low calcium medium. When the preparations were superfused with Krebs-Ringer solution containing 0.3 mM Ca^{2+} for 15 min, AAP and $\dot{V}_{max}$ were decreased and SPCL was prolonged significantly. In such preparations, amrinone (3×10^{-4} M) still caused a significant increase in AAP and $\dot{V}_{max}$ and a shortening of SPCL.

When the preparations were exposed to Krebs-Ringer solution containing 0.1 mM Ca^{2+}, AAP and $\dot{V}_{max}$ were progressively decreased resulting in a complete cessation of spontaneous activity at resting potential around −40 mV. In such preparations treatment with amrinone (3×10^{-4} M) restored spontaneous firing with much smaller AAP and $\dot{V}_{max}$ than those under the control condition (Figure 2, Table 2).

The influence of verapamil was examined in the other four preparations (Figure 3, Table 3). The pretreatment with verapamil at 5×10^{-7} M for 30 min caused a marked decrease in AAP and $\dot{V}_{max}$ accompanied by a prolongation of SPCL, but regular spontaneous firing was still preserved. Under such conditions, treatment with amrinone for 10 min caused a significant increase in AAP and $\dot{V}_{max}$, and a shortening of SPCL. Thus the inhibitory effects of verapamil at 5×10^{-7} M on the action potential and spontaneous activity of sinus node cells were partially overcome by amrinone at 3×10^{-4} M.

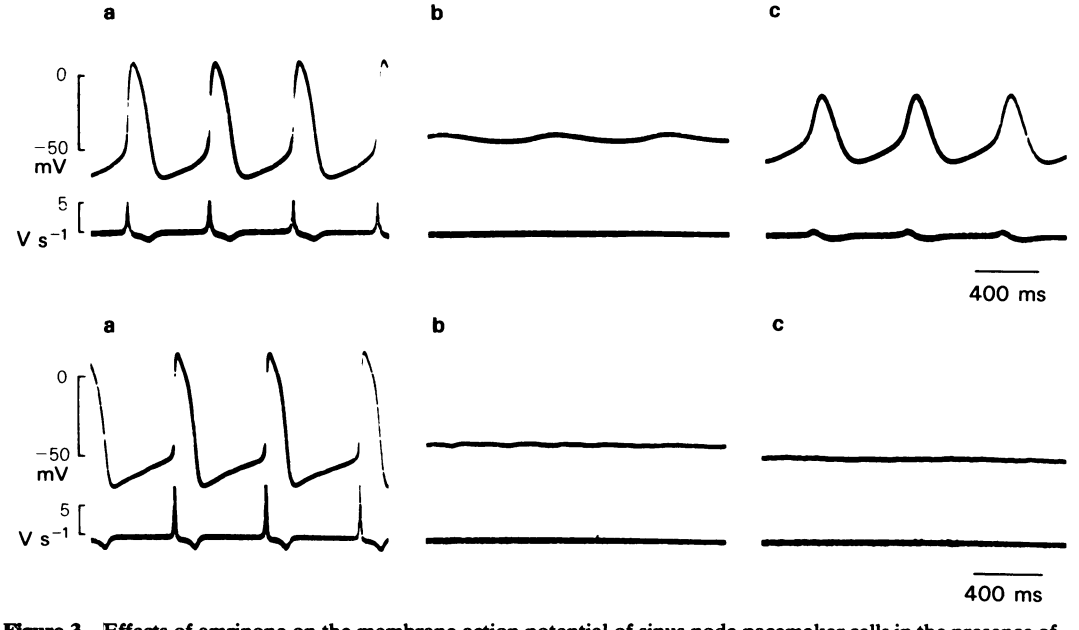

Figure 3 Effects of amrinone on the membrane action potential of sinus node pacemaker cells in the presence of verapamil. Upper and lower panels show experiments with verapamil at lower (5×10^{-7} M) and higher (2×10^{-6} M) concentrations respectively. (a) Control; (b) 30 min after treatment with verapamil; (c) 10 min after the application of amrinone (3×10^{-4} M) in the presence of verapamil.

Table 3 Effects of amrinone (3×10^{-4} M) on the sinus node pacemaker cells in the presence of verapamil

	AAP (%)		$\dot{V}_{max}$ (%)		*SPCL* (%)	
	Before	*After*	*Before*	*After*	*Before*	*After*
Untreated	100	105 ± 1.7*	100	120 ± 5.4*	100	73.2 ± 4.4*
Verapamil (5×10^{-7} M)	5.8 ± 4.5	44.6 ± 6.3*	0.7 ± 0.4	7.2 ± 2.1*	130 ± 2.5	107 ± 1.1*
Verapamil (2×10^{-6} M)	0	0	0	0		

Values are means ± s.e. ($n = 4$); percentage of control values in the absence of verapamil (Untreated). Measurements were performed before and 10 min after application of amrinone (3×10^{-4} M). *Significantly different from the value of untreated preparation at $P < 0.05$

Pretreatment with verapamil at the higher concentration (2×10^{-6} M) abolished the spontaneous firing. In such preparations, amrinone (3×10^{-4} M) failed to restore the spontaneous activity.

Influence of pindolol

Effects of amrinone were examined in three preparations pretreated with a β-adrenoceptor antagonist, pindolol. Representative experiments are shown in Figure 4. The pretreatment with pindolol at 2×10^{-7} M for 30 min did not cause any change in the configuration of action potential and spontaneous activity (Figure 4b). In the presence of pindolol, the effects of amrinone on $\dot{V}_{max}$, AAP and SPCL were not affected at all (Figure 4c). Similar results were observed in all three preparations.

On the other hand, the positive chronotropic effect of isoprenaline at 1×10^{-8} M on these preparations was completely blocked by pretreatment with pindolol at 2×10^{-7} M (not shown in the figures).

Discussion

Amrinone at concentrations above 1×10^{-4} M caused a significant shortening of cycle length of spontaneous firing (SPCL) and increase in $\dot{V}_{max}$ as well as in amplitude (AAP) of action potentials in sinus node pacemaker cells. The upstroke phase of sinus node cells is almost entirely dependent on the slow inward current through the cell membrane (Strauss, Prystowski & Scheiman, 1977; Irisawa, 1978). The slow inward current was also shown to play an important role in the development of diastolic depolarization especially at its later phase (Noma, Kotake & Irisawa, 1980). In the present experiments, the effects of amrinone were markedly attenuated or abolished in a low calcium medium or in the presence of the slow channel blocking agent, verapamil. These facts may suggest that the effects of amrinone on spontaneous activity and membrane action potential are most probably explained by an augmentation of the slow inward current.

The maximum diastolic potential (MDP) was not affected even by the highest concentration of amrinone. Therefore, a change in potassium outward current or in background currents, which would be reflected in the level of MDP (Carmeliet & Vereecke, 1979), seems unlikely as a possible mechanism for the drug action on sinus node cells.

Amrinone (3×10^{-4} M) restored spontaneous ac-

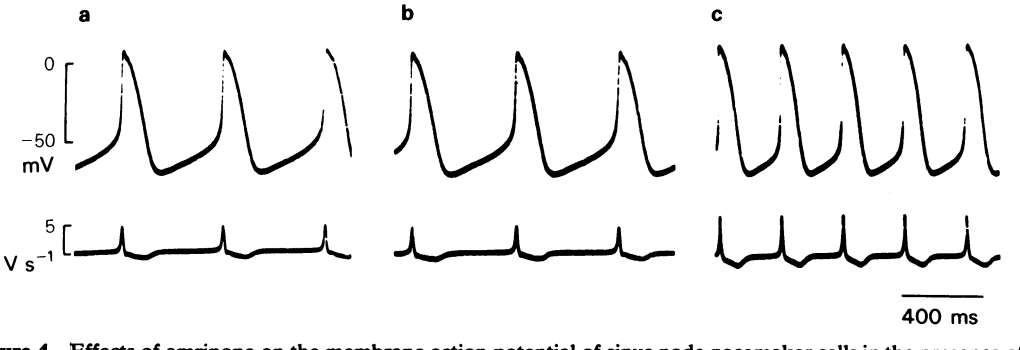

Figure 4 Effects of amrinone on the membrane action potential of sinus node pacemaker cells in the presence of pindolol. (a) Control; (b) 30 min after the treatment with pindolol ($2 \times ^{-7}$ M); (c) 10 min after application of amrinone (3×10^{-4} M) in the presence of pindolol. Upper trace is the membrane action potential and lower trace shows the first derivative of the action potential.

tivity in a reduced calcium medium (Ca^{2+} 0.1 mM) but failed to restore the activity in the presence of verapamil at 2×10^{-6} M. Thus the inhibitory action of a low calcium medium on the effects of amrinone was apparently less than that of verapamil. This could be explained by ion selectivity of the slow channel in sinus node cells.Noma & Irisawa (1976) and Noma, Yanagihara & Irisawa (1977) suggested that the slow inward current in sinus node cells is carried by Na^+ as well as by Ca^{2+}. If this is so, amrinone may have enhanced the slow inward current carried by Na^+ in the presence of a low extracellular calcium concentration. The interaction between verapamil and amrinone on the slow channels seems competitive since amrinone could not overcome the inhibitory effects of verapamil at the higher concentration (2×10^{-6} M).

The electrical effects of amrinone were not antagonized by pindolol, a β-adrenoceptor blocking agent (Singh & Vaughan Williams, 1971; Shimizu, Iwamura, Kodama, Toyama & Yamada, 1978) suggesting that the β-adrenoceptors do not play a role in the action of amrinone on sinus node cells.

On the basis of the present results, it is concluded that amrinone has an intrinsic effect on sinus node pacemaker cells, increasing their spontaneous firing activity. Also it is assumed that the effect of amrinone is probably mediated by an augmentation of the slow calcium and/or sodium inward current through the cell membrane. In our previous voltage clamp experiments in guinea-pig ventricular muscles (Kondo, Shibata, Kodama & Yamada 1983), amrinone at 6×10^{-4} M caused a marked increase in the peak value of the slow inward current, while it did not influence the net outward currents. The augmentation of the slow inward current in ventricular muscles by amrinone was also suggested by other investigators (Adams *et al.*, 1982). Honerjäger *et al.* (1981) demonstrated that amrinone can inhibit myocardial cyclic nucleotide phosphodiesterase and thereby increase cyclic adenosine monophosphate (cyclic AMP) concentration in the heart. Since the increase in intracellular concentration of cyclic AMP was shown to increase the slow inward current of cardiac cells (Mirro, Bailey & Watanabe, 1980; Li & Sperelakis, 1983), the present assumption would also be consistent with such a possible mechanism.

The concentrations of amrinone used in the present experiments were similar to the effective concentrations on the contractile force of cardiac muscles (Onuaguluchi & Tanz, 1981; Honerjäger *et al.*, 1981; Kondo *et al.*, 1983). Therefore, it is reasonable that the high doses of amrinone should have both a positive inotropic and positive chronotropic effect on the heart. It is presumed that the discrepancies in previous reports regarding the effects of amrinone on heart rate may be related to the autonomic reflex or to the enhanced parasympathetic tone caused by concomitantly administered cardiac glycosides (Hariman & Hoffman, 1982) to the patients.

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