Amrinone Activates K+-Depolarized Atrial and Ventricular Myocardium of Guinea Pigs

H. Richard Adams, Jeffery Rhody, and John L. Sutko
From the Departments of Pharmacology and Internal Medicine, Southwestern Medical School, The University of Texas Health Science Center at Dallas, Dallas, Texas

SUMMARY. Amrinone is a new synthetic drug that increases contractile strength of mammalian heart muscle; however, its mechanism of positive inotropic action has not been determined. We now report that amrinone (0.053-5.3 mM) consistently restores typical slow response electromechanical activity to K+-depolarized atrial and ventricular myocardial preparations from guinea pigs. This action was blocked in both tissues by D-600 (1 μM), but it was not significantly inhibited by either tetrodotoxin (23.5 μM), d,l-propranolol (1 μM), or phentolamine (10 μM). Cimetidine (3 μM) or metiamide (10 μM) slightly inhibited amrinone’s effect only in the ventricle, whereas pyrilamine (10 μM) slightly inhibited amrinone’s response only in the atrium. These data indicate that amrinone’s positive inotropic action may involve augmented Ca++ influx via the slow inward Ca++ current, and that although this action is independent of adrenoceptor mechanisms, it seems to include a small histaminergic component. (Circ Res 51: 662-665, 1982)

AMRINONE (5-amino-3,4′-bipyridine-6-(IH)-one) is a new nonglycoside and noncatecholamine drug that increases contractile strength of heart muscle without evoking cardiac dysrhythmias. This compound has already proven efficacious in preliminary clinical trials with patients experiencing heart failure refractory to standard cardiotonic therapies (Benotti et al. 1980). However, the mechanism of positive inotropic action of amrinone has yet to be elucidated. To determine whether enhanced Ca++ influx via the slow inward Ca++ current might play a role in the effects of this agent, we evaluated the ability of amrinone to restore electromechanical activity to myocardium depolarized by 22 mM extracellular K+. Such restoration is believed to be the result of a net increase in the slow inward Ca++ current (Pappano, 1970; Watanabe and Besch 1974).

Methods

Left atria and left ventricular papillary muscles of male guinea pigs were suspended under isometric conditions in glucose, 11 (pH = 7.4). The solution containing elevated K+ was aerated with 95% O2-5% CO2, and recorded photographically (Sutko et al., 1980). Temperature was maintained at 30 ± 0.5°C throughout all experiments.

The muscles were stimulated via punctate electrodes with single square-wave pulses of suprathreshold voltage, 0.2 Hz frequency, and 2-5 msec duration. After contractions were initiated, the muscles were allowed to stabilize for 45-60 minutes under the control K+ (5.9 mM) conditions. During exposure of the tissues to the 22 mM K+ medium, stimulus intensity was increased several-fold and maintained at this new setting throughout the remainder of the experiment.

Amrinone was dissolved in either 0.5 N lactic acid or 0.5 N HCl and added in small aliquots to the tissue bath to give final concentrations of 0.053-5.3 mM. Neither vehicle exerted positive inotropic action, but volumes of acid equivalent to that delivered with the larger concentrations of amrinone (eg., 1.6 and 5.3 mM) caused transient decreases in contractile function which correlated with transient declines in media pH (Alousi et al., 1979).

Results

Contractile responses to cumulative increases in the concentration of amrinone were first measured in six atrial muscles maintained in the normal K+ solution. In agreement with previous observations by others (Alousi et al., 1979), we found that amrinone consistently augmented contractile strength of isolated myocardium in a concentration-dependent manner; absolute increases in peak developed tension and +dT/dtmax observed after treatment with amrinone (0.053-5.3 mM) are summarized in Figure 1. The maximal response to amrinone occurred within 3-6 minutes, lasted for at least 30 minutes, and was reversed upon washing the tissues with drug free (normal) medium. Similar results were also obtained with ventricular muscle preparations (n=3).
Having confirmed the concentration-dependent positive inotropic activity of amrinone in normally polarized myocardium, i.e., under normal K⁺ (5.9 mM) conditions, we then tested the effects of this drug in heart muscle exposed to elevated extracellular K⁺ ([K⁺]o) concentration. Exposure to the 22 mM K⁺ medium produced the expected depolarization of the resting membrane potential (from $-81 \pm 3$ to $-42 \pm 2$ mV, $P < 0.05$; $n=6$ papillary muscles; cf. Fig. 2); this was accompanied by mechanical quiescence and lack of response to electrical stimulation, even after stimulation intensity was increased several-fold. Amrinone, however, consistently restored electromechanical activity to the K⁺-depolarized muscles. In atrial preparations inactivated by 22 mM K⁺ ($n=5-11$), developed force increased from zero to 247 ± 117, 791 ± 120, and 1,563 ± 206 mg after treatment with 0.53, 1.6, and 4.2 mM amrinone, respectively. The onset of action of amrinone in K⁺-depolarized tissues was usually within 2-5 minutes; maximal effects occurred in 15-30 minutes and persisted for at least a 60-minute observation period. Data in Figure 1 summarize the effects of amrinone on developed tension and $+dT/dt_{max}$ in atrial muscles maintained in either the normal or high K⁺ medium.

Figure 2 typifies the effects of amrinone in left ventricular papillary muscle, showing (Part A) normal action potential and muscle twitch, (Part B) depolarization and electromechanical quiescence after 22 mM K⁺, and (Part C) typical slow response action potential and accompanying twitch in K⁺-depolarized muscle after treatment with amrinone. Maximum rates of depolarization are not displayed, but they varied from 180-200 V/sec in normal K⁺- and from 10-20 V/sec in high K⁺-amrinone-treated tissues.

Having verified the capability of amrinone to restore slow response activity in K⁺-depolarized myocardium, we next examined the inotropic effects of selected pharmacologic compounds on this action. Since both histamine and catecholamines are present in the guinea pig heart, and since these agents can enhance the slow inward current (Watanabe and Besch, 1974; Shigenobu et al., 1979), it was important to determine whether amrinone's slow response activ-
ity depended upon catecholaminergic or histaminergic mechanisms.

However, neither the $\beta_1$-$\beta_2$ adrenoceptor antagonist propranolol (1 $\mu$m) nor the $\alpha_1$-$\alpha_2$ adrenoceptor antagonist phentolamine (10 $\mu$m) significantly affected the response to amrinone in K$^+$-depolarized muscle (Fig. 3). Similarly, the H1 histamine antagonist pyrilamine (10 $\mu$m) was without significant effect in papillary muscles treated with amrinone, and had only a slight depressant effect in atrial muscle; whereas, in contrast, the H2 histamine antagonists, metiamide (10 $\mu$m) or cimetidine (3 $\mu$m), slightly reduced amrinone’s effect in papillary muscle, but not in atrial preparations (Fig. 3). An increase in the cimetidine concentration to 15 $\mu$m did not further reduce amrinone’s slow response action (data not shown). Also, the response of K$^+$-depolarized heart muscle to amrinone was not inhibited by tetrodotoxin (TTX; 23.5 $\mu$m), the fast Na$^+$ channel-blocking agent (Fig. 3). On the other hand, the slow channel-blocking agent D600 (1 $\mu$m) virtually abolished the positive inotropic response to amrinone in K$^+$-depolarized muscle (Fig. 3).

Test experiments were done to ensure that concentrations of antagonists that we studied were appropriate for selective inhibition of corresponding agonistic activity at the intended receptor. Propranolol (1 $\mu$m), for example, reduced developed force from 1,470 ± 20 to 1,100 ± 50 mg ($P < 0.001$) in five atrial muscles depolarized by 22 mM K$^+$ and then reactivated by isoproterenol (50 nM). In contrast, the same concentration of propranolol has little effect on basal contractility when the tissue is not exposed to $\beta_1$-$\beta_2$ antagonist propranolol (Adams and Durrett, 1978). Similar stimulatory-inhibitory relationships were observed with phenylephrine (3 $\mu$m)-phenolamine (10 $\mu$m), histamine (0.1 $\mu$m)-pyrilamine (10 $\mu$m) in left atrium, and histamine (0.1 $\mu$m)-cimetidine (3 $\mu$m) or metiamide (10 $\mu$m) in papillary muscle.

Discussion

Amrinone consistently restored excitability and contractile function to K$^+$-depolarized atrial and ventricular myocardium of the guinea pig. Electromechanical changes elicited by amrinone under the high[K$^+$]o conditions were consistent with typical slow response activity resulting from activation of the slow inward Ca$^{2+}$ current (Pappano et al., 1974). Furthermore, amrinone’s effects were completely suppressed by the slow channel-blocking agent D600 (Fig. 3). Thus, amrinone’s mechanism of positive inotropic action may be explained, at least in part, by an augmentation of the slow inward current carried predominantly by Ca$^{2+}$.

Amrinone’s effect in K$^+$-depolarized cardiac muscle cannot be explained by a reactivation of the fast Na$^+$ current. Indeed, the lack of inhibitory action of TTX under these conditions further supports our conclusion that amrinone-restored functions of K$^+$-depolarized heart muscle are dependent upon slow channel activation. Previous study with similar preparations showed that the concentration of TTX used in the present experiments was sufficient to completely block contractile activity of heart muscle beating under control [K$^+$]o conditions, i.e., in the presence of operating fast Na$^+$ channels (Adams and Durrett, 1978).

Present experiments also demonstrated that neither the $\beta_1$-$\beta_2$ antagonist propranolol nor the $\alpha_1$-$\alpha_2$ antagonist phentolamine antagonized amrinone’s effects in K$^+$-depolarized heart muscle. These findings are important because they indicate that amrinone’s restoration of slow response activity does not occur directly through a release of endogenous catecholamine stores, and that it also does not depend upon direct activation of cardiac $\beta$- or $\alpha$-adrenergic receptors.

In the guinea pig heart, slow channel activation by histamine is subserved predominantly by H1-type histamine receptors in the left atrium and by H2-type histamine receptors in the left ventricle (Shigenobu et al., 1979). In the present study, an H3-type antihistamine (pyrilamine) selectively reduced the magnitude of amrinone-restored contractions only in the atrium, whereas an H2-type antihistamine (cimetidine or metiamide) selectively reduced amrinone’s effects only in the ventricle. Larger concentrations of pyrilamine were not studied because of the nonspecific depressant activity associated with the local anesthetic properties of this and other H1 blockers. The H2 blockers are devoid of significant local anesthetic activity (Shigenobu et al., 1979), and the inability of larger concentrations of cimetidine to depress amrinone’s slow channel action further indicates that only a portion of the latter may be mediated indirectly through histamine receptors. Nevertheless, the selectivity of inhib-

**Figure 3.** Effects of antagonists on amrinone-restored contractions of K$^+$-depolarized heart muscles. Values are expressed as the percent change (mean ± se) in developed tension (CT) after exposure of high K$^+$-amrinone-treated tissues to tetrodotoxin (TTX), D-600, dl-propranolol (prop), phenolamine (phen), metiamide (Met), pyrilamine (pyr), or cimetidine (Cim). Effects of D600, prop, phen, and TTX were similar in atrial muscle (AM) and papillary muscle (PM); the number of muscles are enclosed in parentheses within the appropriate bars.
itory effects of H₁ and H₂ blockers on amrinone’s action in atrial and ventricular muscle, respectively, opens the question of whether or not amrinone may release histamine under certain conditions, e.g., in the presence of high [K⁺]₀ concentrations. Previous studies demonstrated little, if any, effect of antihistamines on the positive inotropic response to amrinone when tested in myocardium exposed to normal K⁺ conditions (Alousi et al., 1979; Millard et al., 1980).

In conclusion, present data indicate that at least a portion of amrinone’s positive inotropic action may be explained by augmented entry of Ca²⁺ into the myocardial cell via the slow inward current. Although this action was found to be independent of adrenergic mechanisms, it seems to include a small histaminergic component.

Our findings are in complete agreement with those of Honerjager et al. (1981) which appeared while the present report was in preparation. These workers did not examine atrial tissue, slow channel blockade, or H₁-antihistamines but, identical to our findings, they observed that slow response activity by amrinone was only partially inhibited by cimetidine in guinea pig ventricle. Moreover, their demonstration that amrinone can inhibit myocardial cyclic nucleotide phosphodiesterase and thereby increase cyclic adenosine monophosphate (cAMP) concentrations in the heart would be consistent with the ability of amrinone to augment the slow inward Ca²⁺ current. Finally, a recent abstract of voltage clamp data from ferret papillary muscles (Arlock and Katzung, 1982) similarly supports the contention that amrinone’s positive inotropic action involves an increase in the slow inward current.

We thank Dr. A. A. Alousi for the generous supply of amrinone. A preliminary account of our data was published in abstract form (Adams et al., 1981) and presented in part at the American Heart Association Meetings, 1981, Dallas, Texas. Supported by the Texas Affiliate, American Heart Association (HRA), NIH Grant HL-26810 (JLS), and the Burroughs Wellcome Fund. J. Rhody was the 1980 recipient of the Burroughs Wellcome Summer Fellowship in Veterinary Pharmacology.

Address for reprints: Dr. H. Richard Adams, Department of Pharmacology, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235.

Received April 21, 1982; accepted for publication August 5, 1982.

References

Shigenobu K, Tatsuno H, Matsuki N, Oshima T, Kasuma Y (1979) Electrophysiological and mechanical studies on the cardiac effects of a histamine H₂ receptor antagonist, cimetidine, in the isolated guinea pig myocardium. J Pharm Dyn 2: 141–150

INDEX TERMS: Amrinone • Inotropic actions • Myocardium
Amrinone activates K+-depolarized atrial and ventricular myocardium of guinea pigs.
H R Adams, J Rhody and J L Sutko

Circ Res. 1982;51:662-665
doi: 10.1161/01.RES.51.5.662

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/51/5/662