Cardiotonic Activity of Amrinone—Win 40680 [5-Amino-3,4'-bipyridin-6(1H)-one]

ADAWIA A. ALOUSI, ALFRED E. FARAH, GEORGE Y. LESHER, AND CHESTER J. OPALKA, JR.

SUMMARY  The cardiotonic activity of a new, noncatechol, nonglycoside agent, amrinone, was investigated in vitro and in anesthetized and unanesthetized dogs. Amrinone (3-1000 μg/ml) caused a dose-dependent increase in papillary muscle developed tension and df/dt without significant changes in duration of the contractile cycle or time-to-peak tension. Amrinone induced slight increases in right atrial rate with no changes in electrophysiological properties of the cat papillary muscle or dog Purkinje fibers. In anesthetized dogs, intravenous bolus injections of amrinone at doses ranging from 1 to 10 mg/kg caused increases in cardiac contractile force and left ventricular dp/dt max with relatively small changes in heart rate and blood pressure. No significant changes in lead II ECG were observed. In unanesthetized dogs, intravenous infusion of amrinone (10-100 μg/kg per min) caused increases in left ventricular dp/dt max and only small changes in heart rate and blood pressure. Amrinone, tested orally in this model at doses of 2-10 mg/kg, produced a positive inotropic effect with a rapid onset and long duration of action. The inotropic response to amrinone was not blocked by propranolol, dibenzylne, chlorisondamine, atropine, metiamide, or reserpine. Amrinone's inotropic response was not associated with significant alterations in cardiac norepinephrine, phosphodiesterase, cyclic AMP, or Na⁺,K⁺-activated ATPase.

A LARGE number of agents have positive inotropic effects on the heart. However, of these, only two classes of compounds have been found to be useful in the treatment of heart failure in man. The cardiac glycosides have enjoyed a valued position for nearly 200 years in spite of the fact that they have a narrow therapeutic range, and digitalis intoxication is one of the most common drug-induced adverse reactions in this country (Mason et al., 1971; Beller et al., 1971; Doherty and Kane, 1975). The catecholamines have limitations in that they are arrhythmogenic, are chronotropic, have a short duration of action, and cannot be given orally (Goldberg, 1968). Dopamine (Goldberg, 1974; Beregovich et al., 1974) and the synthetic catecholamine, amine dobutamine (Tuttle and Mills, 1975; Goldberg et al., 1977), both of which have been introduced recently for clinical use, suffer from most of the same drawbacks as the older catecholamines. Other inotropic agents, such as glucagon (Farah and Tuttle, 1969; Glick et al., 1968), anthopleurin-A (Shibata et al., 1978), theophylline (Rall and West, 1963; Blinks et al., 1972), ionophores (Schwartz et al., 1974), imidazole (Knope et al., 1973), and others, have been studied but have not found general clinical application for the treatment of heart failure. More recently, the combination of afterload reduction with a vasodilator and a positive inotropic agent has proved of value in the management of congestive heart failure (Cohn and Franciosa, 1977; Mehta, 1977). The development of an orally active non-catechol and non-glycoside cardiotonic agent has been the goal of this laboratory for several years (Alousi and Farah, 1975; Farah et al., 1978; Farah and Alousi, 1978). Amrinone (Win 40680), a 5-amino-3,4'-bipyridine-6(1H)-one, is a novel chemical entity with rather unique cardiac inotropic properties (Alousi et al., 1978; Farah and Alousi, 1978). In this report, the action of amrinone on the heart is studied in several models, and in light of these results, the possible therapeutic implications of its use are discussed.

Methods

Isolated Cat Atria and Papillary Muscle

Cats of either sex weighing 1.4 ± 0.05 kg were anesthetized with sodium pentobarbital, 30 mg/kg, iv, and exsanguinated. The chest was opened rapidly, the heart excised, rinsed with Tyrode's solution, and the right atrium (weight = 3.0 ± 0.1 g) and one or more small thin papillary muscles from the right ventricle (weight = 7.0 ± 0.6 mg; length = 5.1 ± 0.2 mm; mean cross-sectional area = 1.3 ± 0.1 mm²) were excised. The tissues then were transferred to a Petri dish filled with cold modified Tyrode's solution oxygenated with 95% O₂, 5% CO₂. A silver wire was attached to each of two opposite ends of the papillary muscle or the atrial preparation. The wire from the nontendonous end of the papillary muscle was hooked to a glass-covered silver wire electrode connected to the cathode end of a Grass model SD5 stimulator, and one pole of
the atrium was hooked to a curved end of a glass rod. Both preparations were mounted in a 40-ml organ bath filled with modified Tyrode’s solution at 37°C and oxygenated with 95% O₂, 5% CO₂ mixture. The wire on the tendinous end of the papillary muscle was looped and tied to a force-displacement transducer and then connected to the anodal terminal of the stimulator via an alligator clamp. The second wire on the atrium was tied to a force-displacement transducer and allowed to beat spontaneously. The transducers were connected to a Grass model 7 polygraph, and the resting tension on the muscles was adjusted to produce a maximum contractile force. Resting tension applied to a papillary muscle was 1.5 ± 0.1 g and to the right atrium was 2.2 ± 0.1 g. The rate of tension development (df/dt) in papillary muscle was derived from the developed tension signal, using a Grass model 7P20 differentiator. Control values for papillary muscle-developed tension were 0.47 ± 0.05 g/mm² and for df/dt max were 7.5 ± 0.6 g/sec. The spontaneous atrial rate was 133 ± 3 beats/min with a developed tension of 1.93 ± 0.1 g. The papillary muscle was stimulated electrically at a rate of 120/min by a suprathreshold (1.5 X threshold) rectangular pulse, 0.5 msec in duration. The papillary muscles demonstrated a bell-shaped force-frequency curve (1-200 beats/min).

The modified Tyrode’s solution bathing the preparation had the following composition (in mM): NaCl, 136.9; KCl, 5.4; NaH₂PO₄, 0.4; CaCl₂, 1.8; MgCl₂, 6H₂O, 1.0; NaCHO₃, 11.9; glucose, 5.5; and EDTA, 0.04. The solution was equilibrated with a gas mixture consisting of 95% O₂ and 5% CO₂, and the pH was 7.3-7.4.

The preparation was left to equilibrate for 1 hour before any drug was added, and the bathing fluid was changed three or four times during the equilibration time. The drug, dissolved in vehicle (100 mg of amrinone in 2 ml of 0.5 n lactic acid, pH 1.8) or the vehicle alone (2.4 μl to 0.8 ml of 0.5 n lactic acid, pH 1.8), was added to the tissue bath, and the response was recorded. The addition of vehicle control in a volume of 2.4-80 μl of 0.5 n lactic acid caused no significant changes in pH of the bath or in muscle resting or developed tension. The addition of a larger volume of vehicle (0.24-0.8 ml of 0.5 n lactic acid) caused immediate depression in muscle-developed tension, which was of short duration (about 1 minute), and developed tension recovered to control levels within 2-3 minutes. The addition of high doses (0.2-0.8 ml) of 0.5 n lactic acid caused a lowering in bath pH to about 7.0. The tissues were washed between doses until predrug control values of rate and force of contraction were obtained. Unless otherwise stated, three to four doses were given to the same preparation over a period of 4-6 hours. Muscle developed tension declined gradually (32% ± 2%) during 6-7 hours of the experiment. Viability of the tissue was determined by its response to a standard dose of dopamine (30 μg/ml) before amrinone administration and at the end of the experiment. No significant change in inotropic response to dopamine was observed at the end of the experiment, i.e., the initial response to 30 μg/ml of dopamine was reproduced after three to four doses of amrinone.

**Electrophysiologist Studies**

The effects of amrinone were studied in dog Purkinje fibers and cat papillary muscle. These were removed rapidly from the anesthetized animals (pentobarbital, 30-35 mg/kg), washed in cold oxygenated Kreb’s solution, mounted in a 10-ml trough-like bath, and superfused (7 ml/min) with warm Kreb’s solution. Bath temperature was kept constant at 36°C and constantly monitored by means of a thermistor probe. The Kreb’s solution had the following composition (in mM): NaCl, 137; KCl, 5; NaH₂PO₄, 0.4; CaCl₂, 1.8; MgCl₂, 1.0; NaHCO₃, 11.9; glucose, 5.6. The gas phase was 95% O₂ and 5% CO₂.

Microelectrodes were machine pulled from clean 2-mm borosilicate glass tubing and filled with 3 m KCl. The microelectrode was mounted on a chlorided silver wire in a manner similar to that described by Woodbury and Brady (1956) and Hoffman et al. (1957) for floating electrodes. A small drop of mineral oil at the open end of the microelectrode prevented drying and permitted individual electrodes to provide stable readings for several hours. Satisfactory electrodes had an impedance of 6-18 MΩ to a 1-kHz signal. The microelectrode was connected to a high impedance amplifier with variable input capacity neutralization (W-P Instruments) mounted to the micro drive. The indifferent electrode was either a chlorided silver wire or a commercial Ag-AgCl-sintered electrode positioned downstream to the tissue and was connected to ground through a variable DC source to compensate for DC junction potential. A 100-mV, 1-kHz square wave and a variety of ramp voltages up to 1200 V/sec could be injected through the indifferent electrode for calibration. The signal was DC coupled to a 3A9 Tektronix amplifier and displayed by a Tektronix 565 oscilloscope; signals >30 kHz were attenuated. Differentiation of phase 0 was accomplished by using an "Analogue Devices" board and model 118A operational amplifiers. The differentiator circuit was linear between 100 and 1200 V/sec. Stimuli, provided by 160 series Tektronix waveform generators, were isolated from ground by Grass Instruments SIU5 R.F. isolation units.

The tissues were allowed to equilibrate for 30-60 minutes and were sampled at 10-minute intervals. Usually five cells were monitored during the control period to give numerical pretreatment values for each tissue. During drug exposure (10⁻⁴ and 10⁻³ M amrinone), similar readings were made, and composite values were compared to control values. Average and standard errors were calculated, and the Student’s t-test was employed to evaluate the sig-

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nificance of percent changes between test and control values.

Purkinje fibers either were stimulated (60/min; 2x threshold) through the bipolar silver wire electrodes or allowed to beat spontaneously. Effective refractory periods (ERP) were determined by measuring the shortest possible time interval between two propagated action potentials. The S2 stimulus was interposed at a desired interval between two driving stimuli (S1) at 4x threshold and a duration of 1 msec. At least 10 S2 beats were allowed between each S2 beat. An extracellular bipolar low impedance probe monitored the effects of S2 at the distal end of the fiber and was used to assess ERP and conduction time.

Intact Anesthetized Dog Preparation

Mongrel dogs of either sex, weighing 9-15 kg, were anesthetized with sodium pentobarbital, 30 mg/kg, iv. Supplemental doses of pentobarbital were administered whenever necessary. An intratracheal cannula was inserted, and ventilation was carried out by means of a Harvard constant-volume positive pressure pump, using room air. The right femoral vein was cannulated and used for intravenous administration of drug. The right femoral artery was cannulated, and the cannula was attached to a Statham P23A pressure transducer to measure arterial blood pressure. Pin electrodes were attached to the right forelimb, right hindlimb, and left hindlimb, and a lead II ECG was monitored.

For the direct measurement of myocardial contractile force, a ventrodorsal incision at the 4th intercostal space was made, the heart was exposed, and a Walton-Brodie strain gauge was sutured to the wall of the right ventricle (Walton et al., 1950). Diastolic tension was adjusted to obtain a maximum response under control conditions.

To measure left ventricular pressure (LVP) and its first derivative (dp/dt), the left carotid artery was isolated, and a Millar Mikro-Tip catheter was inserted. The catheter was advanced slowly into the left ventricle for the measurement of LVP and dp/dt, using a Grass 7P20 polygraph differentiator.

Cardiac output was determined by the dye dilution method. The left jugular vein was cannulated, and the cannula was advanced into the right ventricle for Cardio-Green injection (1.25 mg in 1 ml of solvent). The left carotid artery was cannulated, and the cannula was advanced into the ascending aorta just outside the left ventricle for blood sampling. Aortic blood samples, drawn at a rate of 38 ml/min by means of a Harvard pump, were passed through a cuvette transducer connected to a model DCR-702 Densitometer (Waters Instruments, Inc.). Blood samples were reinfused back into the dog after each measurement. Cardiac output was recorded graphically on a chart and displayed numerically on a digital computer board.

In all of the above experiments, the parameters measured were recorded continuously on a multichannel Grass polygraph. A small test dose of dopamine (20 μg/kg, iv) was given to test the cardiovascular responsiveness of the dog before the test drug was administered. After completion of the surgical procedures, the dogs were allowed to recover for 30 minutes before drug administration. Control cardiovascular parameters in 16 anesthetized (11.9 ± 0.7 kg) dogs were: systolic blood pressure = 147 ± 5.1 mm Hg, diastolic blood pressure = 104 ± 4.3 mm Hg, heart rate = 156 ± 6.2 beats/min, contractile force = 26.4 ± 1.1 g, left ventricle pressure = 135 ± 4.0/0, dp/dt max = 1468 ± 121 mm Hg/sec, and cardiac output = 2.0 ± 0.1/min. All drugs were prepared fresh, immediately before administration. Amrinone solution was prepared by dissolving 100 mg of drug in 2 ml of 0.5 N lactic acid and by diluting to the appropriate concentration with normal physiological saline. An equal volume of vehicle control (0.4-4.0 ml of 0.25 N lactic acid, pH 2.1) was administered before each dose of drug, and the response to amrinone was corrected accordingly.

Unanesthetized Instrumented Dog Preparation

Mongrel dogs of either sex with body weights ranging from 9.5 to 15.2 kg were anesthetized with sodium pentobarbital, 30 mg/kg, iv. An endotracheal tube was inserted and ventilation instituted with a Harvard constant-volume, positive pressure pump, using room air. All surgery was done aseptically.

In experiments in which direct measurement of myocardial contractile force was performed, the chest was opened through a ventrodorsal incision made in the 4th intercostal space. The heart was exposed and a Walton-Brodie strain gauge (Walton et al., 1950) was sutured to the right ventricle. The wires from the strain gauge were run subcutaneously and exteriorized through a stab wound below the midscapular area of the back. The wound was closed, and negative intrapleural pressure was reestablished. The left femoral artery and vein were cannulated with polyethylene tubes filled with heparinized saline, and the tubes were run subcutaneously to the same stab wound on the back and exteriorized.

To measure LVP and dp/dt, a Millar Mikro-Tip catheter was inserted into the left ventricle via the left carotid artery. The right femoral artery and left jugular vein were cannulated and used for the measurement of arterial blood pressure and for the administration of drugs, respectively. The proximal ends of the cannulas and that of the Millar Mikro-Tip catheter were passed under the skin and out through a stab wound in the back of the neck. An intramuscular injection of 250,000 U of penicillin G (Longicil Fortified) was administered and the dog returned to its cage.

The next day the conscious dog was placed in a sling, the strain gauge or Millar catheter wires were
The effects of norepinephrine (NE) and amrinone on cyclic AMP and GMP levels were determined in isolated cat left atria stimulated at 120 beats/min. At the appropriate time after drug addition, the atria were quick-frozen by means of a Wollenberger clamp, pulverized over dry ice, and stored at −70°C. Radioimmunoassay kits supplied by New England Nuclear were used for the nucleotide determinations as adapted from the procedure of Steiner et al. (1972). The atrial tissue was homogenized with 6% trichloroacetic acid (TCA), and the supernatant was extracted four times with diethyl ether to remove TCA. The supernatant was acidified to pH 7.4, and an aliquot was added to Hydromix scintillation fluid and counted in a Packard scintillation spectrometer by standard methods.

Biochemical Analysis

The effects of norepinephrine (NE) and amrinone on cyclic AMP and GMP levels were determined in isolated cat left atria stimulated at 120 beats/min. At the appropriate time after drug addition, the atria were quick-frozen by means of a Wollenberger clamp, pulverized over dry ice, and stored at −70°C. Radioimmunoassay kits supplied by New England Nuclear were used for the nucleotide determinations as adapted from the procedure of Steiner et al. (1972). The atrial tissue was homogenized with 6% trichloroacetic acid (TCA), and the supernatant was extracted four times with diethyl ether to remove TCA. The supernatant extract was dried under a stream of air, and the residue was dissolved in an acetate buffer for the assay of the nucleotides using as [32P]-labeled antigen which competed with an unlabeled antigen for antibody-binding sites. Because of the low concentrations of cyclic GMP, the nucleotides were acetylated to 2′-O-acetyl cyclic GMP (thus increasing the sensitivity 100-fold) (Steiner et al., 1972). All samples were assayed in quadruplicate and followed the procedure outlined in the New England radioimmunoassay kit.

The effect of amrinone on cardiac NE and phosphodiesterase (PDE) and on liver monoamine oxidase (MAO) was determined in six anesthetized dogs in which an amrinone solution was infused iv at a rate of 30 μg/kg per min. An equal number of control dogs received 0.01 N lactic acid, iv.

Ten minutes after peak contractility was observed due to the amrinone infusion, the apex of the heart was rapidly excised and rinsed in cold physiological saline. One part of the heart was frozen rapidly in a mixture of solid CO₂ and acetone, and the remaining portion was chilled on ice. Control dogs receiving lactate were treated in a similar manner at a time when maximal contractile effects due to amrinone would be expected. Approximately 1 g of liver tissue was excised rapidly from each dog, rinsed in saline, wrapped in foil, and frozen in solid CO₂. All frozen samples were stored at −70°C until assayed for NE by the method described by Crout (1961). PDE activity was determined by the method of Butcher and Sutherland (1962).

MAO activity was determined by a modified method of Wurtman and Axelrod (1963). The tissue was homogenized in cold isotonic potassium chloride, and a volume equivalent to 1 mg of liver tissue was added to a chilled pH 7.4 buffer. A final concentration of 19.4 μM 3H-tryptamine substrate was added, and the mixture was incubated at 37°C. The reaction was terminated by the addition of HCl, 5, 10, 15, and 20 minutes after the start of incubation. Zero-time values were obtained by reversing the order of substrate and acid addition. The deaminated radioactive products were extracted from the incubates with toluene, and an aliquot was added to Hydromix scintillation fluid and counted in a Packard scintillation spectrometer by standard methods.

Adenosine Triphosphatase

The in vitro effect of amrinone on cardiac sodium-potassium stimulated, magnesium-dependent, and ouabain-sensitive adenosine triphosphatase (Na⁺,K⁺-ATPase) was determined according to the method of Sulakhe et al. (1976). The enzyme was purified according to the Sulakhe et al. modification of the method of Matsui and Schwartz (1966).

Statistics

Student’s t-test for significance or analysis of variance was used throughout to compute statistical significance. All values are expressed as ± SEM.

Results

In Vitro Effects on Cardiac Contractility

The in vitro addition of amrinone (3–1000 μg/ml) to the isolated cat atria and papillary muscles caused dose-dependent increases in atrial and papillary muscle developed tension with relatively smaller changes in right atrial rate (Fig. 1A). The increase in papillary muscle-developed tension caused by all doses of amrinone was accompanied by a parallel increase in dP/dt (Fig. 1B). The inotropic response to amrinone was not associated with changes in total duration of isometric contraction or time-to-peak tension (Fig. 1C4D). Amrinone caused no significant changes in atrial or papillary muscle resting tension, and the addition of vehicle control up to a concentration of 100 μg/ml (2.4–80
In vitro effect of amrinone on isolated cat right atria and papillary muscle. A: Percent change from control in: right atrial rate $\circ$; right atrial developed tension $\Delta$; papillary muscle-developed tension (x). B: Absolute changes in papillary muscle-developed tension $\times$ and $\text{dp/dt}_{\text{max}}$ $\circ$. Each point and bar represents the mean $\pm$ SEM, $n = 8$-12. Isometric myogram for control (C) and drug-treated (D), fig ml of amrinone per ml.

$0.5$ N lactic acid) caused no significant changes in bath pH or in resting or developed tension of the tissues. The vehicle control in concentrations of 300 and 1000 $\mu$g/ml (0.2-0.8 ml of 0.5 N lactic acid) lowered the bath pH to 7.0 and caused a 30% depression in developed tension with recovery to control levels within 2-3 minutes.

The onset of the in vitro effect of amrinone was within 30-60 seconds after its addition to the incubation bath. The peak effect occurred after 2-3 minutes, and total duration was more than 1 hour. In four papillary muscle preparations, amrinone (30 $\mu$g/ml) was added to the bath, after a maximal positive inotropic effect had been attained, the drug was washed out, and 15-20 minutes later, the same dose of amrinone was added again. This was repeated seven times, and in every preparation the last addition of amrinone produced at least the same response as the first one. There was no statistically significant difference between the seven responses. Thus, amrinone does not produce tachyphylaxis under these experimental conditions.

Effects on Electrophysiology of the Heart

In driven canine Purkinje tissue, amrinone in concentrations of $10^{-4}$ to $10^{-3}$ M did not produce significant changes in resting membrane potential, action potential amplitude, effective refractory period, maximum upstroke velocity, or conduction velocity. A slight but statistically not significant 3% decrease in phase 2 amplitude (plateau) and slowing of the rate of phase 3 repolarization occurred when $10^{-3}$ M amrinone was superfused.

In spontaneously beating Purkinje fibers, an occasional fiber would show an increase in the rate of phase 4 depolarization (pacemaker), especially when the rate was less than 20 beats/min. However, no statistical significance could be discerned when comparing the experimental with the vehicle-superfused fibers.

In cat papillary muscle, amrinone $10^{-4}$ to $10^{-3}$ M increased contractile force significantly but did not cause an increase in resting membrane potential, action potential amplitude, phase 2 amplitude, or phase 0 maximum upstroke velocity. Small decreases in action potential duration (3-4%) occurred at both amrinone concentrations but did not achieve statistical significance from pretreatment or values obtained in vehicle-superfused preparations.

Intravenous Activity in Anesthetized Dogs

The cardiovascular effects of a single bolus injection of amrinone, 1 mg/kg, iv, in the anesthetized dog are shown in Figure 2. Amrinone caused an increase in cardiac contractile force and left ventricular dp/dt max with relatively small changes in maximum left ventricular systolic pressure, systolic and diastolic systemic pressure and in heart rate. The lead II ECG remained unchanged from control after the administration of this dose of amrinone. The onset of the inotropic effect of amrinone was within 1 minute of its administration, and the peak effect was reached after 2 minutes. The duration of

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action of this dose of amrinone was 30 minutes. Administration of vehicle control (0.4 ml of 0.25 N lactic acid, pH 2.1) caused no significant changes in cardiovascular parameters of the anesthetized dog.

Figure 3A is a dose-response curve of the inotropic effect of amrinone in anesthetized dogs. The intravenous administration of amrinone in doses of 0.3-10 mg/kg caused a dose-related increase in cardiac contractile force, a small increase in heart rate, and a lowering in the diastolic blood pressure. No cardiac arrhythmias were observed with the large doses of amrinone, as shown in Figure 3B; however, concomitant with the increase in heart rate, there was a shortening of the P-R interval. Vehicle control for the 10-mg/kg dose (4.2 ± 0.27 ml of 0.25 N lactic acid, pH 2.1) caused a 9 ± 3% reduction in cardiac contractile force and less than ±5% change in blood pressure and heart rate. Vehicles corresponding to lower doses of amrinone caused less than ±5% change in any of the cardiovascular parameters measured.

The effect of a bolus injection of amrinone (1 or 3 mg/kg, iv) or intravenous infusions (30 or 100 μg/kg per min for 60 minutes) on cardiac output of the anesthetized dog was studied in three separate experiments. Since these hearts were not in failure, no significant increase in cardiac output was observed, although there were significant changes in cardiac contractile force.

Intravenous Activity in the Unanesthetized Dog

The intravenous infusion of amrinone at a rate of 80 μg/kg per min caused a gradual increase in left ventricular dp/dt max that reached a maximum of 62% above control level within 2 hours and was maintained for the rest of the infusion time. The duration of the inotropic response to amrinone after termination of infusion was more than 2 hours. Amrinone caused relatively small changes in blood pressure and heart rate. The infusion of vehicle (1 ml/min of 0.016 N lactic acid) caused no significant changes in cardiovascular parameters of the unanesthetized dog.

Figure 4 is a dose-response curve of the cardiovascular effects of intravenously infused amrinone in the unanesthetized dogs. The dose-dependent increases in left ventricular dp/dt max caused by amrinone were accompanied by small but not significant changes in heart rate at rates of amrinone infusion below 100 μg/kg per min. The increase in heart rate observed with a dose of 100 μg/kg per min was significantly higher than control and was accompanied by a 16–18% reduction in systolic and diastolic pressure. No cardiac arrhythmias were observed with any of the doses of amrinone tested. The infusion of vehicle (1 ml/min of 0.02 N lactic acid) caused a 20% increase in diastolic blood pressure, and a 15% increase in systolic blood pressure and heart rate with no effect on dp/dt max.
Oral Activity in the Unanesthetized Dog

The oral activity of amrinone was tested in unanesthetized dogs chronically implanted with either a Miller Mikro-Tip catheter in the left ventricle or a Walton-Brodie strain gauge on the right ventricle. Figure 5 illustrates the effect of oral medication with amrinone on left ventricular dp/dt max, left ventricular maximum systolic pressure, and heart rate of the unanesthetized dog. The administration of amrinone (10 mg/kg) resulted in a 57% increase in dp/dt max within 45 minutes. The onset of action of amrinone was within 15 minutes of its administration, and the total duration of inotropic effect was more than 5 hours. At the time of peak inotropic response, left ventricular systolic pressure was increased by 16%, and heart rate was increased by 18% (14 beats/min).

Amrinone caused a dose-dependent increase in cardiac contractile force when given orally in single doses of 2-10 mg/kg. No appreciable changes in blood pressure and heart rate were observed up to a dose of 8 mg/kg. However, a dose of 10 mg/kg resulted in a 35% increase in heart rate and a 20% lowering in blood pressure. No cardiac arrhythmias were observed after oral medication with amrinone. The administration of no. 000 gelatin capsule as a placebo control resulted in no significant changes in cardiac parameters measured.

Effect of Reserpine Pretreatment

Pretreatment of the cat with reserpine (0.25 mg/kg, im, 48 and 24 hours prior to experiment) resulted in reduction in heart NE from a control level of 1.4 ± 0.06 to 0.01 ± 0.00 μg/g wet weight. In the dog, similar treatment with reserpine reduced heart NE from a control level of 0.97 ± 0.02 to 0.01 and 0.00 μg/g wet weight. No significant difference in the in vitro inotropic or chronotropic response to amrinone was observed between the normal and NE-depleted preparation. In the anesthetized dog, NE depletion caused no significant changes in the inotropic or chronotropic response to amrinone, but a statistically significant potentiation of the lowering in blood pressure was observed with amrinone, 3.0 mg/kg, iv (Table 1).

Effect of β-Adrenergic Blocking Agents

The effect of dl-propranolol on the positive inotropic response to amrinone was studied in the isolated preparations and in the whole animal. Table 2 shows the effect of 1 x 10^{-6} M dl-propranolol on the inotropic and chronotropic response of the isolated cat atria and papillary muscle to amrinone, 30 μg/ml. No significant reduction in atrial or papillary muscle response to amrinone was observed after a 30-minute incubation in propranolol. In this study, the response of the tissues to a test dose of isoproterenol (0.001 μg/ml) was completely blocked after the incubation in 1 x 10^{-6} M dl-propranolol.

<table>
<thead>
<tr>
<th>Amrinone (mg/kg, iv)</th>
<th>Control</th>
<th>Treated</th>
<th>Control</th>
<th>Treated</th>
<th>Control</th>
<th>Treated</th>
<th>Control</th>
<th>Treated</th>
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<td>0.3</td>
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<td>3.8±0.59</td>
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<td>7.3±0.95</td>
<td>5.4±0.01</td>
<td>5.5±0.01</td>
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<tr>
<td></td>
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<td>(6)</td>
<td>(13)</td>
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<td>14.5±0.94</td>
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<td>103.2±6.75</td>
<td>12.4±6.75</td>
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* Mean ± SEM.
† Numbers in parentheses = number of dogs.
‡ P < 0.05.
§ P < 0.001.
**Table 2** The In Vitro Effect of $1 \times 10^{-6}$ M Propranolol on the Cardiotonic Activity of Amrinone in Isolated Cat Right Atria and Papillary Muscle

<table>
<thead>
<tr>
<th>Amrinone concentration (µg/ml)</th>
<th>Right atrial rate (change in beats/min)</th>
<th>Right atrial force (change in g)</th>
<th>Papillary muscle force (change in g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Propranolol $P$</td>
<td>Control Propranolol $P$</td>
<td>Control Propranolol $P$</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.175±0.041</td>
<td>0.250±0.022</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>0.450±0.046</td>
<td>0.558±0.058</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>0.650±0.089</td>
<td>0.782±0.079</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>1.00±0.149</td>
<td>1.07±0.133</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>300</td>
<td></td>
<td>1.34±0.213</td>
<td>1.42±0.087</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
</tr>
</tbody>
</table>

* Mean ± SE.
† Numbers in parentheses = number of preparations.

In the anesthetized dog, pretreatment with dl-propranolol, 2.5 mg/kg, iv, 30 minutes prior to amrinone administration caused no significant changes in the inotropic response to amrinone, 3.0 mg/kg, iv [percent increases in cardiac contractile force before and after propranolol administration were 110.2 ± 18.6 and 88.8 ± 14.2 (mean ± SEM), respectively ($n = 6, P < 0.3$)]. The dose of propranolol used in this experiment caused complete block of an equipotent dose of isoproterenol.

**Effect of Histamine Receptor Blockade**

The effects of the H2 blocker, metiamide, on the cardiac effects of amrinone were determined on isolated cat atria and papillary muscles. The results obtained with histamine and amrinone are given in Table 3. Whereas metiamide partially blocked the effects of histamine on the heart muscle, the effects of amrinone were not significantly changed. Qualitatively, similar results were obtained in intact anesthetized dogs. Effects of histamine (10 µg/kg) on the heart were reduced markedly by metiamide, 5 mg/kg (over 90%), whereas the positive inotropic and chronotropic effects of amrinone (1 mg/kg) were not significantly changed by this dose of metiamide.

**Effects of Other Blockers**

Atropine (2 mg/kg), dibenzyline (2 mg/kg), or chlorisondamine Cl (a ganglionic blocking agent) (1 mg/kg) in intact dogs did not block the cardiac effects of amrinone although these doses did block the blood pressure fall due to iv acetyl choline (10 µg/kg), the blood pressure rise due to NE (0.4 µg/kg), and the response to 1,1-dimethyl-r-phenylpiparazinum (0.01 µg/kg), respectively.

**The Role of Cardiac Cyclic AMP, PDE, and Liver MAO**

NE increased both cyclic AMP and contractility 20 seconds after addition of the drug to isolated cat atria. Amrinone, in a dose causing an equivalent increase in contractility, did not significantly change cyclic AMP. Cyclic GMP was not changed by either NE or amrinone (Table 4). In Table 5 are given data describing the effects of amrinone on the NE content and PDE activity of the intact dog heart and MAO activity of the liver. Amrinone, in amounts which produced a significant increase in the force of the cardiac contractions, did not change the activity of cardiac cyclic AMP, PDE, or liver MAO.

**Effect on Na⁺,K⁺-ATPase**

The incubation of pig heart purified sarcolemmal Na⁺,K⁺-ATPase with an inotropic concentration dose of amrinone (2.7 × 10⁻⁵ to 2.7 × 10⁻³ µM) caused no significant inhibition of the Na⁺,K⁺-stimulated ATPase of heart muscle. In this study, the addition of ouabain in a concentration of 2 × 10⁻⁵ to 2 ×...
10\(^{-3}\) M caused significant inhibition in Na\(^+\),K\(^+\)-ATPase activity. The data are summarized in Table 6.

### Effects of Amrinone on the Failing Heart

Heart failure was induced in dogs anesthetized with pentobarbital. The vena cava and aorta were connected by means of cannulas, thus increasing the venous return to the heart. This was followed by the bolus injection (24-40 mg/kg) and a constant infusion of pentoarbital at 0.25 mg/kg per min. This procedure produced a relatively stable decrease in cardiac output, an increase in central venous pressure, and a decrease in cardiac contractile force. In control experiments, dogs receiving only the infusion of pentobarbital showed further deterioration of cardiac function with time. An amrinone bolus of 1 mg/kg followed by a constant infusion (100 /µg/kg per min) increased cardiac output, contractile force, and blood pressure and reduced central venous pressure. Cardiac rate was increased moderately. Table 7 presents results of a characteristic experiment (of a total of six experiments). Results with different types of heart failure will be reported in greater detail at a later date. Thus, contrary to its effects on the normal heart, amrinone increased the cardiac output of the failing heart.

### Toxicology of Amrinone

Some toxicological evaluation of amrinone has been completed, and further studies are in progress.

The intravenous LD\(_{50}\) of amrinone in albino mice was 150 mg/kg (confidence limits of 111-178 mg/kg) and for albino rats, 102 mg/kg (confidence limits 90.8-118 mg/kg).

The oral LD\(_{50}\) was 288 mg/kg (confidence limits 239-346) for albino mice and 363 mg/kg (confidence limits 244-1800 mg/kg) for albino rats.

In beagle dogs, amrinone was given on 12 consecutive days by intravenous infusion, each infusion period lasting 3 hours. The doses used were 12.5, 50, and 200 /µg/kg per day (four dogs per dose). With the highest dose, some of the dogs on various occasions vomited, and heart rate was increased (36-69%), P-R interval was decreased, and the mucous membrane of the eye became congested. No gross or microscopic changes in the heart, liver, kidney, and skeletal muscle attributable to amrinone could be observed. All usual hematological parameters (hematocrit, differential count, serum proteins, SGOT, SGPT, serum alkaline phosphatase, creatinine, and phosphokinase) were within normal limits and not significantly different from controls.

A 3-month oral toxicity study was conducted in beagle dogs. The doses of amrinone used were 5, 25, and 50 mg/kg body weight given daily (six dogs per dose). Only with the highest dose was there an occasional postmedication emesis and an increase in heart rate (up to 86%). Body weight gain and hematological and plasma ion values were within the normal range. Both gross and microscopic ex-
AMRINONE—CARDIOTONIC ACTIVITY/Alouisi et al. 675

**Table 6**  
In Vitro Effect of Amrinone and Ouabain on Na⁺,K⁺-stimulated ATPase

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (mol)</th>
<th>Na⁺,K⁺-stimulated ATPase specific activity (μmol Pi/hr per mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>5.88</td>
</tr>
<tr>
<td>Ouabain</td>
<td>2 x 10⁻⁵</td>
<td>0.51</td>
</tr>
<tr>
<td>Ouabain</td>
<td>2 x 10⁻⁴</td>
<td>0.51</td>
</tr>
<tr>
<td>Ouabain</td>
<td>2 x 10⁻⁵</td>
<td>0.87</td>
</tr>
<tr>
<td>Amrinone</td>
<td>2.7 x 10⁻⁵</td>
<td>5.71</td>
</tr>
<tr>
<td>Amrinone</td>
<td>2.7 x 10⁻⁴</td>
<td>5.80</td>
</tr>
<tr>
<td>Amrinone</td>
<td>2.7 x 10⁻⁵</td>
<td>5.83</td>
</tr>
</tbody>
</table>

amination of the heart, liver, kidneys, and skeletal muscle did not show any significant changes from the control group of dogs.

**Discussion**

The results of this study indicate that amrinone, a non-catechol and non-glycoside drug, is a potent, long-acting inotropic agent when given orally or intravenously to either anesthetized or unanesthetized dogs. The positive inotropic response to amrinone was demonstrated in isolated heart tissues and in the whole dog. The studies on the isolated cat atria and papillary muscles and intact dogs showed a wide separation between the positive inotropic and chronotropic response. The inotropic response to amrinone was characterized by an increase in total developed tension and its rate of development, without changes in total duration of the contractile cycle or the time-to-peak tension. No significant changes in the intracellular action potential or excitability in isolated cardiac tissue could be detected when amrinone was given in relatively high concentrations.

In either the anesthetized or unanesthetized dog, the intravenous bolus injection of amrinone caused dose-dependent increases in cardiac contractile force and left ventricular dp/dt max. The onset of action of the inotropic response to amrinone was within 1 minute of its administration, and the peak effect was reached within 2–3 minutes. The total duration of action of intravenously administered amrinone was 30–120 minutes, depending on the dose. Noticeable increases in cardiac contractile force were seen with doses of amrinone as small as 0.1 mg/kg, iv, and the first manifestation of side effects, namely, significant lowering in blood pressure and increase in heart rate, was observed at a dose of 3 mg/kg, iv. In the unanesthetized dog, orally administered amrinone was nontoxic up to a dose of 10 mg/kg. The toxicological studies in rodents and dogs also attest to the relatively low toxicity of amrinone. The wide therapeutic index of amrinone is in contrast with that of the cardiac glycosides, where the most commonly used glycosides, digoxin and digitoxin, have a therapeutic index of about 2–3, with life-threatening arrhythmias as a manifestation of toxicity (Mason et al., 1971; Beller et al., 1971; Doherty and Kane, 1975). The lowering in blood pressure, especially diastolic blood pressure, seen with the larger doses of amrinone might prove to be of value in patients with congestive heart failure, since this would reduce afterload and improve the competency of the heart.

The use of a vasodilator for afterload reduction in the treatment of pump failure has been a common practice in recent years (Cohn and Franciosa, 1977; Mehta, 1977). Therefore, the combined inotropic-vasodilator properties of amrinone might be of clinical values.

The nature of the vasodilatory response to amrinone is under investigation. Amrinone may act directly on smooth muscle in resistance vessels rather than on vascular adrenergic neurons, since

**Table 7**  
The Effect of Amrinone on a Pentobarbital-Induced Heart Failure in the Dog

<table>
<thead>
<tr>
<th></th>
<th>Cardiac output (liters/min)</th>
<th>Contractile force (g)</th>
<th>Central venous pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
<th>Blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.07</td>
<td>27</td>
<td>0.7</td>
<td>168</td>
<td>180</td>
</tr>
<tr>
<td>Pentobarbital failure induced</td>
<td>2.54</td>
<td>15</td>
<td>3.8</td>
<td>126</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>After infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 min</td>
<td>3.78</td>
<td>20</td>
<td>1.3</td>
<td>132</td>
<td>130</td>
</tr>
<tr>
<td>10 min</td>
<td>3.46</td>
<td>25</td>
<td>0.7</td>
<td>132</td>
<td>125</td>
</tr>
<tr>
<td>15 min</td>
<td>4.01</td>
<td>33</td>
<td>0.4</td>
<td>150</td>
<td>130</td>
</tr>
<tr>
<td>60 min</td>
<td>3.69</td>
<td>36</td>
<td>0.2</td>
<td>138</td>
<td>125</td>
</tr>
<tr>
<td>After 90 min</td>
<td>3.28</td>
<td>22</td>
<td>1.5</td>
<td>120</td>
<td>110</td>
</tr>
</tbody>
</table>

Anesthesia: 15.2 kg pentobarbital (30 mg/kg, iv); heart failure induced by bolus injections of 45 mg/kg, followed by constant infusion of pentobarbital (0.25 mg/kg per min).
pretreatment of the animal with adrenergic β-receptor blocking agents, such as propranolol, did not reduce the vasodilation caused by amrinone. Also, amrinone (1 and 3 mg/kg or 30 and 100 μg/kg per min) did not increase cardiac output of the normal, but increased that of the failing, heart. Therefore, it is unlikely that the vasodilation was due to reflex inhibition of a sympathetic vasoconstriction. The lack of significant effects by amrinone on cardiac output of the normal (nonfailing) heart was similar to that of the cardiac glycosides (Cotton and Stopp, 1958; Cotton and Moran, 1961). It is well established that increased inotropy alone would not lead necessarily to increased cardiac output, since other factors such as preload, afterload, and heart rate are important contributors to changes in this parameter (Fawaz, 1963). Amrinone produced a typical positive inotropic effect in pentobarbital-induced heart failure in dogs. The inotropic activity of amrinone also was demonstrated in the unanesthetized dog. In this preparation, the intravenous administration of amrinone resulted in pharmaco-logical responses qualitatively and quantitatively similar to those obtained in the anesthetized dog. Unmasking of side effects of amrinone, such as tachycardia, orthostatic hypotension, or behavioral changes, were not observed in the conscious dog. The inotropic response to intravenously administered amrinone could be sustained for several hours and at any level of improved cardiac performance by infusing the drug at appropriate rates. Increases in cardiac contractile force and left ventricular dp/dt max, ranging from 10 to 80% above control, were obtained by changing the rate of amrinone infusion from 10 to 100 μg/kg per min for 3 hours.

Amrinone was orally active with a rapid onset of action and duration of more than 5 hours. The ratio of intravenous to oral dose of amrinone was close to 1:2, indicating good absorption of the drug from the gastrointestinal tract. At this time, the mechanism of action of amrinone can be described only by the following exclusion criteria. Amrinone did not seem to act via a catecholamine mechanism, since its inotropic effect was not blocked by the β-receptor blocking agent, propranolol, or by depletion of cardiac NE by reserpine. Our data on the possible role of cyclic AMP (Sutherland et al., 1965) in the inotropic response to amrinone indicated that there are no significant changes in the level of cyclic AMP or PDE that could be considered responsible for the inotropic response to amrinone. Effects of amrinone on the heart were not blocked by metiamide, an agent which blocks the positive inotropic effect of histamine, nor did amrinone inhibit Na+,K+-stimulated ATPase. Thus, amrinone might represent a new class of inotropic agents, and the unravelling of its mechanism of action will have to be the subject of further studies.

The positive inotropic effect, the relatively wide margin of safety, the vasodilatory properties, and the oral activity have made amrinone a prime can-didate for clinical testing in patients with congestive heart failure. Preliminary results in patients (Bennotti et al., 1978; Le Jemtel et al., 1979) have confirmed our findings in the dog.

Acknowledgments

Grateful acknowledgment is made for the technical assistance of D. J. Fort and L. Fullem. Thanks are given to Drs. P. Hernandez, R. Perrari, R. W. Piontke, and H. P. Drobek for the biochemical, electrophysiological, and toxicological data presented in this study.

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Dynamic Changes in the Canine Mitral Regurgitant Orifice Area during Ventricular Ejection

Edward L. Yellin, Chaim Yoran, Edmund H. Sonnenblick, Shlomo Gabbay, and Robert W.M. Frater

SUMMARY We designed this study to test the hypothesis that in acute mitral regurgitation the mitral regurgitant area (MRA) is a dynamic quantity which varies with the time variation of ventricular volume. Mitral insufficiency was created in five open-chest dogs in which a portion of the anterior leaflet was excised. Phasic aortic and mitral flows were measured electromagnetically, along with left atrial and ventricular pressures. Filling, regurgitant, and stroke volumes, and systolic pressure gradient to give the temporal mean and the instantaneous value at three instants of time and at the time of peak flow (when inertia is negligible). Mean regurgitant fraction was 42 ± 12% with no indication of left ventricular failure due to volume overload. MRA decreased monotonically with time to 59% of its initial value and closely paralleled the decrease in ventricular volume during systole. In a control study using a tilting-disc prosthesis with a hole 5 mm in diameter in the occluder, the calculated MRA was time invariant and equal to the measured area for regurgitation. We conclude that in acute mitral regurgitation the MRA is a function of ventricular volume. Circ Res 45: 677-683, 1979

During mitral regurgitation, the distribution of flow between the forward and regurgitant paths is a dynamic process which depends on the driving pressure differences and the relative impedance of each path. Since energy losses across an incompetent valve vary with the square of the flow, the impedance of the regurgitant path is dominated by the effective A* of the mitral valve. It is therefore of major interest to examine the possibility that the regurgitant A* is not fixed. This study was designed to test the hypothesis that in acute mitral insufficiency the mitral regurgitant A* decreases during ventricular ejection.

Previous studies from this laboratory (Yoran et al, 1979a; 1979b) and elsewhere (Borgenhagen et al, 1977) have demonstrated that the mean MRA during ventricular systole varies directly with the size and shape of the left ventricle. We have shown...
Cardiotonic activity of amrinone--Win 40680 [5-amino-3',4'-bipyridine-6(1H)-one].
A A Alousi, A E Farah, G Y Lesher and C J Opalka, Jr

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