Negative Inotropic Effects of Amrinone in the Neonatal Piglet Heart

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Cardiac mechanical function and coronary flow (CF) were measured in isovolumically beating hearts from neonatal piglets 0.5 to 12 days of age. The hearts were perfused retrogradely at 70 cm H2O with a recirculating modified Krebs-Henseleit solution containing washed adult pig red cells (hematocrit, 25%). They were electrically paced (180 beats/min), and left ventricular developed pressure (∆P), maximum rate of rise of pressure (dP/dt), CF, and myocardial oxygen consumption were measured. These parameters were found to remain stable for at least 60 minutes. In one group, a single dose of amrinone was added to the perfusate to yield a concentration of 50 µg/ml. Within 5 minutes ∆P decreased from 104.0 ± 7.1 to 65.3 ± 8.6 mm Hg (p<0.001), and dP/dt fell from 1,160 ± 96 to 658 ± 78 mm Hg/sec (p<0.001). In a second group, successive doses of amrinone were added to yield concentrations ranging from 5 to 50 µg/ml. There was a progressive decrease in ∆P and dP/dt to 66.5 ± 4.2% and 57.6 ± 4.8% of initial values, respectively. CF increased progressively from 3.2 ± 0.2 to 6.8 ± 0.5 ml/min/g. In 3 experiments, amrinone was washed out after achieving the maximum concentration. Depressed mechanical function reversed and ∆P and dP/dt returned to control values in each heart. Additionally, CF decreased from 7.6 ± 0.3 to 5.0 ± 0.2 ml/min/g. It is concluded that amrinone has concentration-dependent negative inotropic actions in the neonatal piglet heart. Hence, the drug may not be useful in treating heart failure in the human neonate. (Circulation Research 1987;61:847-852)

Amrinone is a bipyridine derivative with positive inotropic and vasodilator actions and has been shown to substantially improve cardiac function in adults with congestive heart failure. Clinical trials for efficacy and toxicity have been performed only in adults, and the drug has not yet been approved for use in children. The majority of laboratory investigations have involved preparations using mature animals. However, recent studies with isolated canine muscle strips suggest that there are age-dependent differences in the magnitude of positive inotropic responses to amrinone. Strips from animals younger than 4 to 5 days actually showed a fall in developed tension when the drug was added to the muscle bath. This suggests that amrinone may have negative inotropic properties in the early newborn period.

The objective of the present study was to explore the actions of amrinone on the neonatal heart using a recently developed isovolumically beating piglet heart preparation. In this system, washed erythrocytes are added to the perfusate to provide adequate O2 delivery. Both myocardial and coronary vascular responses can be determined simultaneously. These hearts exhibited sustained metabolic and mechanical stability and levels of performance and O2 usage comparable to those found in the neonate in vivo. Moreover, coronary flow is within the expected physiologic range of 2–3 ml/min/g (wet weight). In contrast, coronary flow in isolated hearts perfused with crystalloid solutions characteristically averages 11–12 ml/min/g. Data from the adult isolated guinea pig heart preparation have demonstrated that amrinone has positive inotropic properties, which is consistent with findings from isolated cardiac muscle strips from other species. Comparison of changes in coronary vascular resistance and mechanical function with those found in the piglet indicates similarities in vascular smooth muscle responses in both age groups. However, inotropic effects contrast sharply with those described in the adult. The findings support the view that amrinone may not be a useful therapeutic agent in the neonate.

Materials and Methods

Isolated Perfused Hearts

Eleven piglets ranging from 0.5 to 12 days of age were obtained from a commercial breeder, and general anesthesia was induced by injection of sodium pentobarbital (25 mg/kg i.p.). The internal jugular vein was cannulated, and sodium heparin (1,000 U/kg i.v.) was given. A tracheostomy was performed, and mechanical ventilation was begun. The hearts were excised rapidly by transection of the pulmonary hila and great vessels and immediately placed in ice-cold saline. The aorta was cannulated and retrograde perfusion was initiated at 70 cm H2O pressure from a Langendorff column. A recirculating modified Krebs-Henseleit (KH) solution containing adult pig erythrocytes was used. The pulmonary artery was cannulated, and the pulmonary veins and venae cavae were ligated. The time elapsed...
between excision of the hearts and commencement of retrograde perfusion was less than 10 minutes. The perfusate temperature was maintained at 37°C by a heat exchanger, and arterial oxygenation was accomplished by bubbling a mixture of 95% O₂-5% CO₂ through the perfusate in the Langendorff column (Figure 1).

Isovolumically beating hearts were then prepared by passing a fluid-filled latex balloon into the cavity of the left ventricle via the left atrium and mitral valve. The distal tip of the balloon was brought through a stab wound in the apex of the heart and secured to a pledget. The proximal end of the balloon was connected to a Sanborn-267 pressure transducer (Waltham, Mass.) with a short length of double lumen polyethylene tubing. The balloon was maintained in the cavity of the left ventricle by placing a tie around the left atrium. This maneuver also prevented potential right-to-left shunting of coronary venous perfusate across a patent foramen ovale. Thus, venous return from the heart egressed via the pulmonary artery. Drainage through the Thebesian circulation was vented through the apical stab wound.

Left ventricular end-diastolic pressure was adjusted by changing the volume of the balloon and was maintained in most experiments at ~10 cm H₂O. No systematic volume adjustments were required to retain a constant diastolic pressure. Left ventricular pressure and the first derivative of left ventricular pressure (dP/dt) were recorded continuously. The isolated heart was enclosed in a water jacket to maintain myocardial temperature at 37°C. Heart rate was maintained at 180 beats/min with the use of a pacing electrode secured to the right atrium. An in-line Swank transfusion filter (Model IL-200, Extracorporeal Medical Specialties, Beaverton, Ore.; providing 13-μm exclusion) was incorporated into the system to filter microaggregates. Figure 1 depicts the overall configuration of the perfusion system.

The time from initiation of retrograde perfusion to the first measurements averaged 20 minutes. This period allowed for washout of cellular elements from the coronary vessels and recovery from ischemia associated with isolation of the heart.

**Preparation of Adult Pig Erythrocytes and Composition of Perfusate**

Fresh pig whole blood was collected in polyethylene bottles containing sufficient heparin for anticoagulation and refrigerated shortly after collection. Immediately prior to perfusion of the hearts, the whole blood was centrifuged in 500-ml polyethylene bottles at 2,500 rpm for 5 minutes. The plasma anduffy coats were separated from the erythrocytes. The erythrocytes were then washed 3 times with 0.9% NaCl containing 0.5% bovine serum albumin (BSA) (Cohn Fraction V, Sigma Chemical Co., St. Louis, Mo.) and finally with a 4% BSA-KH solution.

The perfusate consisted of a modified KH solution containing 4% BSA, 5.5 mM glucose, 1.5 mM lactate, 200 μU/ml insulin, and the following ions (in mM): NaCl 118, KCl 4.7, MgSO₄ 2.4, KH₂PO₄ 1.2, NaHCO₃ 25, and CaCl₂ 2.5. The washed erythrocytes were suspended in this solution to provide a hematocrit of 25% (approximately the hematocrit of neonatal piglets). The pH of the final solution was adjusted to 7.40 by titration with 0.1 N NaOH.

**Coronary Flow and Myocardial Oxygen Consumption**

Coronary flow was measured by timed collections of the pulmonary arterial (coronary venous) effluent. Samples were obtained anaerobically in oiled syringes simultaneously from the pulmonary artery and the aortic perfusion line. Myocardial oxygen consumption was calculated from the product of the measured arteriovenous oxygen content difference and CF. Oxygen content was measured directly with a Lex-O₂-CO₂ analyzer (Lexington Instruments Corp., Lexington, Mass.).

**Statistical Analysis**

All data were analyzed with the aid of a Digital Rainbow 100 computer and statistical program by Northwest Analytical, Inc. (Portland, Ore.). The data are presented as mean ± SEM. The Student’s paired or unpaired t test was employed to assess differences between two groups. One-way analysis of variance followed by the Newman-Keul test was used for multiple comparisons. Differences were considered significant when the p value was less than 5%.
**Results**

**Stability of the Preparation**

The body weight (BW) of the animals increased from 0.7 kg at 0.5 days to 4.09 kg at 12 days of age ($r=0.893$). Total heart weight (HW) ranged from 6.1 g to 27.6 g. The HW-to-BW ratio ranged between 10.1 and 4.4 and correlated inversely with increasing age ($r=-0.860$, $p<0.001$).

Physiologic characteristics of 5 hearts perfused for 60 minutes following the initial recovery period (~20 minutes) were determined to assess stability. The hearts were well-oxygenated throughout each experiment. This is judged by mean values for the arterial perfusate, which included a hematocrit of 26.0±3.2%, pH of 7.39±0.04, $P_{O_2}$ of 432±45 mm Hg, and oxygen content of 12.2±1.7 ml/dl. Values of $\Delta P$ and $(+\Delta P/\Delta t)_{max}$ were determined over the 60-minute period. Initially, developed pressure averaged 110.4±8.9 mm Hg, and $(+\Delta P/\Delta t)_{max}$ averaged 1,347±113 mm Hg/sec. After 1 hour, the respective values were 103±7 mm Hg and 1,270±103 mm Hg/sec. Average $\Delta P$ decreased 6.7%, $(+\Delta P/\Delta t)_{max}$ decreased 5.7%, and CF increased 26% from initial values. Thus, despite variations in absolute values at the onset of the perfusion period, left ventricular systolic function of each heart remained quite stable. Similarly, $M_{Vo2}$ showed no significant change and averaged 66.5±0.9 /ml/min/g during the 1-hour study period.

In 5 experiments, left ventricular systolic function was studied over a range of end diastolic pressure. The latter was adjusted by varying the volume of the fluid-filled balloon. Results from two such experiments are shown in Figure 2. A function curve was obtained initially and repeated 60 minutes after completing the first curve; total time required to make these measurements spanned about 90 minutes. As expected, peak systolic pressure in the 1-day-old heart was substantially less than that in the 11-day-old heart. However, in each case curves obtained after 1 hour were virtually identical with those obtained initially. Thus, there was no significant deterioration in left ventricular performance. Figure 2 also demonstrates a typical positive inotropic response following infusion of norepinephrine (NE, 1 /Ag/min) in the older heart. Peak systolic pressure generated at a given end diastolic pressure was about 20 mm Hg higher with NE administration.

**Effects of Amrinone**

Following 60 minutes of stable left ventricular function, amrinone was added to the perfusate reservoir to yield a concentration of 50 /mu/ml. As soon as the drug reached the hearts, left ventricular performance began to deteriorate, and concomitantly, CF increased. Figure 3 shows the results for these experiments. Left ventricular peak systolic pressure decreased from 110.5±7.0 to 74.8±8.7 (SEM) mm Hg (32.3%), $\Delta P$ decreased from 104.0±7.1 to 65.3±8.6 mm Hg (37.2%), and $(+\Delta P/\Delta t)_{max}$ decreased from 1,159.7±95.6 to 657.5±77.9 mm Hg/sec (43.3%). All changes were statistically significant ($p<0.001$). Coronary flow increased from 3.6±0.3 to 5.5±0.5 ml/min/g ($p<0.01$).

In 2 experiments in this group, a 2-day-old and a 10-day-old heart, left ventricular function curves were run immediately before and following the administration of amrinone. As shown in Figure 4, in both instances amrinone significantly depressed left ventricular performance, indicated by a sharp downward displacement of the curve. Infusion of NE (0.5 /Ag/min)
into the aortic perfusion line completely reversed the negative inotropic effect of amrinone in the younger heart (Figure 4, left panel).

Concentration-Response Relations

Successive doses of amrinone were added to the perfusate in 6 experiments to yield concentrations ranging from 5 to 50 μg/ml. The corresponding dose-response curves are shown in Figure 5. There was a decrease in ΔP and (+)dP/dt_max with each successive increase in amrinone concentration. The ΔP decreased from an initial value of 97.3 ± 7.2 to 64.5 ± 7.5 mm Hg at the maximum concentration of 50 μg/ml; (+)dP/dt_max decreased from 1,035.5 ± 94.3 to 602.3 ± 87.3 mm Hg/sec. Statistically significant negative changes in both ΔP (p < 0.05) and (+)dP/dt_max (p < 0.01) appeared at an amrinone concentration of 10 μg/ml. Positive inotropic responses were not identified at any of the concentrations studied.

Recovery of Left Ventricular Function Following Removal of Amrinone

In 3 of the dose-response experiments described above, recovery of left ventricular function was assessed following washout of amrinone. After achieving the maximum concentration of 50 μg/ml, which required a period of approximately 60 minutes, the amrinone was washed out of the preparation with fresh perfusate of identical composition. This was accomplished by not recirculating the new perfusate until the entire apparatus was estimated to be free of the drug. After approximately 20 minutes, ΔP, (+)dP/dt_max, and CF were again measured. Thereafter, amrinone was added to the new perfusate to obtain a concentration of 50 μg/ml and the measurements were repeated.

Figure 6 summarizes the results of these experiments. ΔP is expressed as percent of baseline and shown in the left panel. With successive increases in amrinone concentration, there was a linear decline in ΔP to 65.2 ± 6.1 of initial value at the maximum concentration. After amrinone was washed out of the preparation, ΔP returned to 103.3 ± 3.9% of control. Although not shown in the figure, (+)dP/dt_max decreased to 55.6 ± 9.9%. On removal of the drug, this measure returned to 107.3 ± 5.4% of initial values. Thus, after approximately 90 minutes of perfusion, including time for washout, there was complete recovery of left ventricular function. When amrinone was added to the new perfusate at a concentration of 50 μg/ml, ΔP and (+)dP/dt_max once again fell to 65.5 ± 0.9% and 58.8 ± 3.9% of baseline values, respectively.

Concomitant changes in CF are shown in the right panel of Figure 6. CF rose with successive increases in amrinone concentration from initial values of 2.0 ± 0.2 to 7.6 ± 0.3 ml/min/g. Following washout, CF diminished to 5.0 ± 0.2 ml/min/g. With reintroduction of the drug, flow (measured in 2 hearts) again increased.

The possibility was considered that changing to a fresh perfusate might itself have exerted some undefined positive inotropic action and, thus, influenced left ventricular recovery. This hypothesis was tested in a control experiment without amrinone by changing to a fresh perfusate of identical composition after about 60 minutes of recirculating perfusion. Thirty minutes thereafter, ΔP and (+)dP/dt_max were found to have increased by no more than 6% from values just prior to introducing the fresh perfusate. Amrinone was then introduced to yield a concentration of 50 μg/ml; ΔP and (+)dP/dt_max decreased by 28% and 43%, respectively.

Figure 5. Dose-response curves from 6 hearts showing developed pressure (upper panel) and (+)dP/dt_max (lower panel) in relation to amrinone concentration in the perfusate. Filling pressure and heart rate were held constant. *p<0.05, **p<0.01, ***p<0.001. Vertical brackets, mean ± SEM.
Discussion

The Preparation

Isovolumically beating heart preparations have been useful for studying mechanical function and metabolism in the adult heart. Our findings indicate that this model is also well suited for investigating neonatal cardiac function. Since there can be no mixing of coronary arterial and venous perfusates, coronary flow can be measured directly and $\text{MVO}_2$ calculated from the measured arteriovenous content differences. The model is especially useful for pharmacologic studies since there can be precise control of perfusate constituents. An added advantage is that drugs not tightly bound can subsequently be removed from the system with return to baseline values. This provides improved confidence that observed changes, as shown in Figure 6, for example, are independent of other unrecognized factors.

Recently, Paradise et al. have shown that isovolumically beating adult rabbit hearts, when perfused with conventional salt solution, are hypoxic and hypodynamic at physiologic workloads and temperatures. When the perfusate was enriched with sheep erythrocytes, these hearts exhibited improved mechanical function and metabolic stability. Adult rabbit hearts typically weigh less than 10 g; with one exception, our piglet hearts weighed more than 15 g. Therefore, it is important to maintain a well-oxygenated preparation to ensure optimal performance and stability. We employed a perfusate with added adult pig erythrocytes to yield a hematocrit of 25%. This approximates in vivo hematocrit values in neonatal piglets. The hearts developed left ventricular peak systolic pressures comparable to the aortic systolic pressures measured in vivo by Werner et al. Moreover, measurements of $\text{MVO}_2$ yielded values similar to those obtained by Bergman et al. for the adult rabbit heart and to those for the newborn lamb reported from our laboratory.

Effects of Amrinone

These experiments demonstrate that amrinone uniformly exerts a significant negative inotropic effect on neonatal piglet hearts. Left ventricular function, as represented by peak systolic pressure, $\Delta P$, and $(+\text{dP/dt})_{\max}$, was consistently depressed in a concentration-dependent fashion. In the younger animals (~3 days of age), both $\Delta P$ and $(+\text{dP/dt})_{\max}$ tended to be lower than in the older animals. However, as illustrated in Figure 4, the relative extent to which left ventricular function was impaired after exposure to amrinone was similar regardless of age.

The most convincing evidence for a specific negative inotropic action by amrinone was seen with the washout experiments in piglets 0.5, 4, and 12 days old. In each heart, there was rapid improvement in left ventricular function as amrinone was removed from the preparation and $\Delta P$ and $(+\text{dP/dt})_{\max}$ returned to preamrinone control values. Moreover, recovery of left ventricular function occurred after a minimum of 90 minutes of perfusion. This provides further evidence supporting the stability of the preparation.

Amrinone consistently increased CF in a concentration-dependent manner. This is in agreement with its known vasodilator effects. After washout with drug-free perfusate, CF decreased but not entirely to control values. There are likely several reasons why complete reversal did not take place. These could include a nonspecific loss of coronary tone with time, hence a shifting baseline. Tighter binding of the drug by vascular smooth muscle or differences in $\text{Ca}^{2+}$ movements in vascular muscle from those in cardiac muscle might be considered. However, the present experiments were not designed to explore these possibilities in detail.

Developmental Considerations

These and earlier studies indicate that the action of amrinone differs in immature and mature myocardium. Although the specific mechanisms of action remain to be elucidated, amrinone is believed to enhance contractility by inhibiting phosphodiesterase fraction III, thus increasing intracellular cyclic AMP and promoting calcium entry into myocytes. There is also evidence that influx through the slow inward calcium

![Figure 6. Effects of incremental increases of amrinone concentration and removal on developed pressure (left panel) and coronary flow (right panel).](http://circres.ahajournals.org/_download.png)
channels is increased directly. Recently, attention has focused on the role of the sarcoplasmic reticulum. Amrinone has been found to be less active in cardiac tissues in which the T tubular system is underdeveloped. Examples include newborn and adult animals of particular species and atrial tissue and Purkinje fibers. Binah et al, for example, have reported that the positive inotropic effect of amrinone is age-dependent in isolated canine heart muscle. These investigators found that the drug has negative inotropic properties until 3 days of age. Thereafter, the responses become positive and increase through the ensuing 3 months of development. However, these workers concluded that the effects were unrelated to developmental changes in phosphodiesterase activity.

In our study, a pronounced negative inotropic action was exhibited in all hearts, and these ranged to 12 days of age. It is possible that development of the T tubular system in the piglet is delayed with respect to other species or that phosphodiesterase inhibition is more age-dependent. Inotropic properties of amrinone have not been studied in older pig hearts. NE elicited pronounced increases in contractile function in our preparation regardless of age and was able to reverse the negative inotropic effect of amrinone. This supports findings of other investigators that this agent acts through a different mechanism than do the catecholamines. While these findings imply that amrinone may not be useful in treating the human neonate, species differences must be considered. It should also be recognized that the experimental hearts were not in failure prior to drug administration. The inotropic action in such circumstances might differ.

Acknowledgments

The authors are grateful for the expert technical assistance provided by William Stronk and Veronika Walton.

References


Key Words • amrinone • norepinephrine • LV contractility • newborn heart • isolated heart • coronary flow • coronary resistance • myocardial O2 consumption • isovolumic heart perfusion technique
Negative inotropic effects of amrinone in the neonatal piglet heart.
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Circ Res. 1987;61:847-852
doi: 10.1161/01.RES.61.6.847

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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/61/6/847

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