BRIEF COMMUNICATIONS

Developmental Changes in the Cardiac Effects of Amrinone in the Dog

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SUMMARY. Amrinone, a bipyridine compound, has been shown to exert a positive inotropic effect on the heart, without producing cardiac arrhythmias. Because of preliminary observations suggesting that the actions of amrinone might change significantly with growth and development, we studied its effects on the contractility and electrophysiology of isolated cardiac muscle of 0- to 96-day-old beagles and Purkinje fibers of 5-year-old beagles. Amrinone’s effects on ventricular muscle contractility were age-related. A significant decrease in contractility of right ventricular trabeculae and papillary muscles (not associated with changes in action potential characteristics) was observed in the 0- to 3-day newborn, whereas, by day 4-10, amrinone increased contractility. The magnitude of the increase became greater through day 96 of life. The negative inotropic effect of amrinone is unassociated with changes in the action potential plateau, suggesting that the slow inward current is not involved in this mechanism. (Circ Res 52: 747-752, 1983)

STUDIES of patients with congestive heart failure undergoing digitalis therapy have shown that amrinone, a bipyridine compound, improves cardiac performance (Benotti et al., 1978; Lejemtel et al., 1979). In studies of the mechanisms responsible for amrinone’s action, Alousi et al. (1979) demonstrated it increases the contractility of isolated feline atrial and papillary muscle in a dose-dependent fashion. Subsequently, it was shown that amrinone increased the contractility of guinea pig and ferret papillary muscle (Honerjager et al., 1981; Arlock and Katzung, 1982) and cardiac contractile force and left ventricular dP/dt of anesthetized dogs (Alousi et al., 1979).

Preliminary studies of experimental animals have suggested that amrinone might not be positively inotropic in the fetus and newborn (Katz et al., 1980; Alousi and Farah, 1980). We therefore performed a pilot study in which we determined the effects of amrinone on arterial pressure and dP/dt in newborn, young, and adult dogs (Binah et al., 1982). These preliminary results suggested that the effects of amrinone on cardiac contractility change with growth and development, and led us to study its actions on the contractility of ventricular papillary and trabecular muscles from dogs aged 0-96 days. As shall be demonstrated, the actions of amrinone on contractility change with growth and development from negatively to positively inotropic. Moreover, because it has been suggested that the t-tubule might be a site of action of amrinone (Binah and Rosen, 1981; Rosenthal and Ferrer, 1982), we performed experiments on isolated adult Purkinje fiber bundles to test this possibility.

Methods

We studied male and female beagles, 0 to 96 days old, as well as adult beagles and mongrels. The dogs were anesthetized with pentobarbital sodium, 30 mg/kg, iv, or ip. The hearts were rapidly removed, and Purkinje fiber bundles and papillary or trabecular muscles were excised and placed in iced Tyrode’s solution gassed with 95% O₂-5% CO₂.

In both the electrophysiological and the mechanical experiments, the preparations were superfused at a rate of 11 ml/min with Tyrode’s solution warmed to 36–37°C. The Tyrode’s solution contained (mM): NaCl, 131; NaHCO₃, 18; NaH₂PO₄, 1.8; MgCl₂, 0.5; CaCl₂, 2.7; dextrose, 5.5; and KCl, 4. The preparations were stimulated using standard techniques (Rosen et al., 1973) to deliver rectangular pulses through bipolar silver electrodes insulated to their tips with Teflon. The tissue bath was connected to ground using a salt bridge and silver-silver chloride junction.

For measurements of Purkinje fiber and ventricular muscle contractility, Purkinje fiber bundles and thin (<1 mm in diameter) right ventricular papillary and trabecular muscles were dissected from the heart, and both ends of each preparation were tied by silk thread to a fine gold chain. Muscle preparations were obtained only from dogs ≤96 days of age. The preparations were mounted horizontally in a Lucite tissue bath having a volume of 6 ml. One end of the preparation was attached to a Statham UC-2 force transducer and the other end to a stainless steel post affixed to a moveable mount. This mount, controlled by a micro-manipulator, enabled us to change the length of the preparations. In these studies, the muscle preparations were stimulated at a rate of 0.3 Hz, and the Purkinje fiber preparations at a rate of 0.6-1 Hz. All preparations were studied at the peak of their length tension curve. The output of the force transducer was carried through a Gould trans-
ducer coupler to a Gould model 220 recorder. The diameters of the preparations were measured using a graticule placed in the dissecting microscope. We used the measurements of diameter to express muscle and Purkinje fiber contraction as g/mm².

Transmembrane action potentials were recorded from muscles and from Purkinje fibers, using 3 KCl-filled microelectrodes and standard microelectrode techniques (Rosen et al., 1973). The transmembrane potentials were recorded on a Tektronix oscilloscope, and we measured the amplitude of phase 0 and phase 2, maximum diastolic potential, duration at 50% repolarization (APD₅₀), and maximum upstroke velocity of phase 0 (Vₘₐₓ) using previously described methods (Rosen et al., 1973).

After we had placed the preparations in the tissue bath, they were permitted to equilibrate for 60 minutes in Tyrode’s solution. Lactic acid (2 ml/liter of a 0.5 N solution) then was added to the superfusate. This induced no change in pH. In the studies of contractility, once resting and active tension stabilized in lactic acid for 10-20 minutes (until attainment of a steady state effect), amrinone (kindly supplied by Drs. A. Alousi and A. Farah) 1, 10, and 100 mg/liter, was added. Amrinone superfusion then was followed by a washout in Tyrode’s containing lactic acid, alone, until a steady state was attained (10-15 minutes).

In the electrophysiological experiments, after a 60-minute equilibration period in Tyrode’s solution, the preparations were superfused with Tyrode’s containing lactic acid, then with amrinone, 100 mg/liter, and again with Tyrode’s containing lactic acid for 20 minutes.

In preliminary experiments, we determined the contractile properties of ventricular trabeculae and papillary muscles to ascertain whether significant deterioration occurred during the time intervals required by the amrinone protocol. Such deterioration did not occur. Moreover, the trabecular and papillary muscles used in the actual studies of amrinone were evaluated ultrastructurally by means of previously described techniques (Rosen et al., 1981). No differences were seen in preparations that were studied immediately on excision from the heart and those studied at the end of the drug protocol.

Data Analysis
For all experiments, statistical analysis was done using the raw data. Depending on the design of the experiments, either a paired t-test or ANOVA was used. Where the latter showed a significant F value, Scheffe’s test was used to compare individual data points (Snedecor and Cochran, 1980).

Results
Age-Related Changes in Twitch Variables
We first studied changes in twitch variables as a function of age in 22 papillary and 17 trabecular muscles. There were no age-related changes from days 0–96 in twitch tension (papillary muscle, 0.90 ± 0.10; trabecular muscle, 0.97 ± 0.12 g/mm²), the rate of tension development (papillary muscle, 7.2 ± 1.3; trabecular muscle, 7.4 ± 0.6 g/mm² per sec), the rate of tension relaxation (papillary muscle, 7.4 ± 0.6 g/mm² per sec), and twitch duration (papillary muscle, 331 ± 25; trabecular muscle, 306 ± 26 msec). Moreover, for none of the twitch variables was there a significant difference between papillary and trabecular muscles. This permitted us to use either papillary muscle or trabecular muscle in our subsequent studies of amrinone.

Effects of Amrinone on Cardiac Muscle Contractility
We used papillary and trabecular muscles from dogs aged 0–96 days to study the effects of amrinone on contractility. We found that, in ventricular muscle from newborn dogs, amrinone induced a marked decrease in active tension.

Figure 1. Panel A: representative experiment showing the effects of amrinone on the active tension in a 1-day-old muscle. Record A (unfilled circles); tension is changed minimally during 90 minutes of superfusion with Tyrode’s solution containing lactic acid (LA). Record B (filled circles); superfusion of another day 1 muscle with 1, 10, and 100 mg/liter amrinone induces a concentration-dependent decrease in tension. The decrease is only partially reversed by 20 minutes of superfusion with Tyrode’s-containing lactic acid. Panel B: experimental record showing the effects of amrinone on active tension of a 1-day muscle. Amrinone induces a concentration-dependent decrease in active tension.

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TABLE 1

<table>
<thead>
<tr>
<th>Amrinone</th>
<th>Control</th>
<th>Lactic acid</th>
<th>1 mg/liter</th>
<th>10 mg/liter</th>
<th>100 mg/liter</th>
</tr>
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<tbody>
<tr>
<td>A: Days 0-2 (n = 5)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Active tension (g/mm²)</td>
<td>0.51 ± 0.13</td>
<td>0.48 ± 0.13</td>
<td>0.40 ± 0.11</td>
<td>0.37 ± 0.11</td>
<td>0.27 ± 0.10*</td>
</tr>
<tr>
<td>dT/dt (g/mm² per sec)</td>
<td>3.76 ± 1.21</td>
<td>3.42 ± 1.09</td>
<td>3.09 ± 1.10</td>
<td>2.74 ± 0.96</td>
<td>2.17 ± 0.72*</td>
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<tr>
<td>Duration (msec)</td>
<td>400 ± 35</td>
<td>400 ± 24</td>
<td>393 ± 15</td>
<td>388 ± 20</td>
<td>348 ± 13</td>
</tr>
<tr>
<td>B: Days 27-41 (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active tension (g/mm²)</td>
<td>0.91 ± 0.22</td>
<td>0.69 ± 0.18</td>
<td>0.72 ± 0.18</td>
<td>0.91 ± 0.24*</td>
<td>1.31 ± 0.36*</td>
</tr>
<tr>
<td>dT/dt (g/mm² per sec)</td>
<td>6.85 ± 0.68</td>
<td>5.09 ± 0.51</td>
<td>5.65 ± 0.67</td>
<td>7.14 ± 0.90*</td>
<td>9.39 ± 1.2*</td>
</tr>
<tr>
<td>Duration (msec)</td>
<td>395 ± 30</td>
<td>385 ± 32</td>
<td>389 ± 32</td>
<td>378 ± 35</td>
<td>370 ± 34</td>
</tr>
<tr>
<td>C: Days 71-96 (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active tension (g/mm²)</td>
<td>0.85 ± 0.19</td>
<td>0.63 ± 0.19</td>
<td>0.74 ± 0.20</td>
<td>1.04 ± 0.28*</td>
<td>1.42 ± 0.40*</td>
</tr>
<tr>
<td>dT/dt (g/mm² per sec)</td>
<td>6.36 ± 1.36</td>
<td>5.08 ± 1.08</td>
<td>4.35 ± 1.25</td>
<td>7.00 ± 1.45*</td>
<td>9.25 ± 2.19*</td>
</tr>
<tr>
<td>Duration (msec)</td>
<td>404 ± 31</td>
<td>404 ± 30</td>
<td>406 ± 30</td>
<td>393 ± 31</td>
<td>386 ± 30</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se.
* P < 0.05 of lactic acid.

concentration-related decrease in active tension (Fig. 1). This was associated with decreases in the rate of tension activation, the rate of tension relaxation, and twitch duration (Table 1). The concentration-dependent decrease in contractility induced by amrinone was reversed partially by washout for 20 minutes (Fig. 1).

In contrast to the negative inotropic effects that were induced by amrinone in the newborn, the drug was positively inotropic after 5 days of age. A representative experiment on a muscle from a 50-day-old dog is shown in Figure 2, and data from animals >27 days of age are summarized in Table 1. Amrinone now increased tension in a concentration-dependent fashion, an effect that reached a steady state within 10 minutes and could be washed out entirely in 15 minutes. Paralleling the increased force of contraction were increases in the rate of tension activation and relaxation, while twitch duration was unchanged (Table 1). It is important to note that none of the positive or negative inotropic effects of amrinone referred to in Figures 1 and 2 and in Table 1 was accompanied by changes in resting tension.

Whereas Table 1 demonstrates changes that occurred in active tension, dT/dt, and twitch duration at three periods during the first 3 months of life, Figure 3 summarizes the effects of amrinone on active tension in all experiments performed on animals between 0 and 96 days of age. Note that the negative inotropic effects of amrinone, 10 and 100 mg/liter, at days 0–4 was replaced by a positive inotropic effect by day 5. The magnitude of the positive inotropic effect increased with further changes in age. At amrinone, 10 mg/liter, the tension decreased by 22 ± 3% (mean ± se) on days 0–2. By days 80–91 of age, this same concentration increased tension by 53 ± 19%. With amrinone, 100 mg/liter, tension on days 0–2 decreased by 41 ± 7%, the increase on days 80–91 was 148 ± 23%. All these changes were significant (P < 0.05).

In summary, the inotropic effects of amrinone were both age- and concentration-related. A significant amrinone-induced decrease in contractility was seen at ages 0–4 days, whereas a positive inotropic effect commenced between days 5 and 10, and continued to increase through the 96th day of life. These results indicate that the positive inotropic response to amrinone increases with growth and development.

In prior studies of the mechanism of action of amrinone, Alousi et al. (1979) tested the possibility that a change in Ca++ current mediates the positive inotropic effects of amrinone in adult tissues, by studying amrinone's action on the transmembrane action potential. They found no effect of amrinone on the action potential, including the plateau, and concluded that amrinone was not acting by increasing transmembrane Ca++ current. In the present study, we considered that the negative inotropic effect of amrinone in the newborn might be induced by de-
expression of transmembrane Ca$^{++}$ current. For this reason, we studied its effects on neonatal Purkinje fiber action potentials. As shown in Table 2, amrinone had no effect on the transmembrane potential, including plateau height and duration. Hence, it appears unlikely that amrinone is acting to depress the slow inward current.

We also considered the possibility that, as suggested previously (Binah and Rosen, 1981; Rosenthal and Ferrier, 1982), the t-tubule, which is present in adult but not neonatal tissues (Legato, 1979), might be a site of action of amrinone. If this is the case, then amrinone should not have an effect on adult tissues that have no t-tubule. For this reason, we studied the effects of amrinone on the contractility of adult Purkinje fibers, in which t-tubules are not present (Johnson and Sommer, 1967).

An example of the effects of amrinone, 1, 10, and 100 mg/liter, on Purkinje fiber contractility is presented in Figure 4. As shown in panel A, amrinone induced a concentration-dependent decrease in tension and, in panel B, a decrease in the rate of tension development and tension relaxation. Twitch duration was unchanged (inset). Comparable effects were seen in seven adult Purkinje fiber bundles: For the seven, control active tension was 0.63 ± 0.15 g/mm². This decreased to 0.38 ± 0.09 g/mm² at amrinone, 100 mg/liter (P < 0.005). Hence, the effects of amrinone on contractility of adult Purkinje fiber bundles were not comparable to its actions on adult ventricle, but, rather (with the exception of its action in twitch duration), were comparable to its actions on newborn myocardium.

Discussion

Our studies of twitch tension, the rate of tension development, the rate of tension relaxation, and twitch duration from 0-96 days of age suggest that these variables are not age-dependent within this age range. In no way does this suggest, however, that age dependence would not be seen if a greater range of ages were studied. This result is superficially at variance with reports of age related changes in the mechanical properties of isolated cardiac preparations. Friedman (1972) studied moderator bands from fetal and adult sheep hearts and concluded that "at all muscle lengths along the length tension curve, there is a significant reduction in the active tension generated by fetal muscle when compared to the adult." These findings are not necessarily contradictory to ours, as Friedman used a different species (the sheep) and compared age groups (fetal and adult) that are clearly very different from the ages of 0-96 days that we studied in the dog. A second study (Davies et al., 1975) showed that, at Lmax, the adult feline papillary muscle develops more active tension than the neonatal

| TABLE 2 |
| Effects of Amrinone on Newborn Papillary Muscle Action Potentials |

<table>
<thead>
<tr>
<th></th>
<th>Action Potential (mV)</th>
<th>MDP (mV)</th>
<th>APD&lt;sub&gt;50&lt;/sub&gt; (ms)</th>
<th>Plateau amplitude (mV)</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (V/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrode's</td>
<td>94 ± 1</td>
<td>-66 ± 1</td>
<td>160 ± 6</td>
<td>27 ± 1</td>
<td>131 ± 21</td>
</tr>
<tr>
<td>Lactic</td>
<td>92 ± 1</td>
<td>-65 ± 1</td>
<td>150 ± 6</td>
<td>26 ± 1</td>
<td>135 ± 13</td>
</tr>
<tr>
<td>Amrinone, 100 mg/liter</td>
<td>92 ± 1</td>
<td>-66 ± 1</td>
<td>147 ± 3</td>
<td>26 ± 2</td>
<td>150 ± 32</td>
</tr>
</tbody>
</table>

AP = action potential; MDP = maximum diastolic potential; APD<sub>50</sub> = AP duration to 50% repolarization; V<sub>max</sub> = maximum rate of phase 0 depolarization.

Results are expressed as mean ± se.
Developmental Changes in the Effects of Amrinone

Figure 4. Representative experiment showing the effects of amrinone on contractility of a Purkinje fiber bundle. Panel A: active tension; after stabilization in lactic acid (LA), amrinone (1, 10, and 100 mg/liter) was superfused for 10–20 minutes at each concentration. Lactic acid then was superfused and, within 25 minutes, the decrease in tension was only partially reversed. Panel B: the effects of amrinone on the rate of tension activation (filled circles), the rate of tension relaxation (unfilled circles), and twitch duration (inset).

(<24 hours) muscle, whereas the 16- to 18-day-old cat occupies an intermediate position between the other two groups. The differences between our findings and those reported by Davies et al. might be explained by species variability. That species variability does, in fact, occur developmentally in feline and canine preparations was shown by Urthaler et al. (1978).

Considering the actions of amrinone, prior studies have shown that amrinone markedly increases the contractility of adult feline atrium and papillary muscle (Alousi et al., 1979), adult guinea pig papillary muscle (Honrjager et al., 1981), and adult canine papillary muscle and ventricular trabeculae (Rosenthal and Ferrier, 1982). However, as shown in the present study, the inotropic effect of amrinone depends on the age of the animal, as well.

It is also evident that amrinone induces both negative and positive inotropic effects on cardiac contractility, the transition between the two occurring over a 48-hour period. We considered several possible mechanisms for these effects: first, that the negative inotropic effect is mediated through depression of the slow inward current. However, the lack of an effect of amrinone on repolarization (Table 2) suggests that it does not depress the slow inward current. Second, we considered that the change from a negative to positive inotropic effect might be related to development of the t-tubular system. However, we could not relate the positive inotropic effect of amrinone to the development of the t-tubular system. In considering this possibility, it was of interest that an adult tissue that has no t-system (the Purkinje fiber) retains the same negative inotropic response to amrinone seen in the neonate, as was shown by us, and by Rosenthal and Ferrier (1982). Also of interest is the fact that adult tissues which have sparse t-systems—such as atrial muscle—show a far weaker positive inotropic response to amrinone than does ventricular muscle (Alousi et al., 1979). However, the fact that, in the dog ventricle, t-tubules are not developed at 2 months of age (Legato, 1979), and that, nevertheless, the positive inotropic response to amrinone commences at 4–5 days of age, makes the t-tubular site of action unlikely. One might speculate that, with development, the myocardial, but not the Purkinje, cell synthesizes a receptor for amrinone, and/or there may be maturation of a pathway that permits its effect to become positively inotropic. However, there is no direct evidence for this at present.

That there is a developmental change in the inotropic response of muscles to amrinone is, in itself, not surprising. Certainly, other non-catecholamine inotropic agents have an age-related component in their effects. For example, digitalis has a greater positive inotropic effect on adult tissues than it does in the neonate (Boerth, 1975). Such developmental changes in digitalis effect have been attributed in part to developmental changes in Na,K-ATPase (Inturrisi and Papaconstantinou, 1974). Glucagon, which exerts its positive inotropic action via a non-catecholamine-induced cAMP synthesis also shows an increase in its inotropic effect with age (Wildenthal et al., 1973). This action may be analogous to that of amrinone, in that recent studies have suggested the positive inotropic response to amrinone results from an increase in intracellular cAMP levels secondary to inhibition of phosphodiesterase (Honrjager et al., 1981). One might therefore consider whether developmental changes in phosphodiesterase activity might be the basis for developmental changes in the actions of amrinone. That there are developmental changes in the cyclic nucleotide system is already known; both basal adenylate cyclase activity, and the adenylate...
cyclase response to NaF stimulation increase with age, from the newborn to the 4-week-old dog (Vulliemoz et al., 1977).

Finally, our studies of developmental changes in the action of amrinone have important clinical implications. Whether the negative inotropic effect occurs in the human is uncertain. Consistent with the possibility that amrinone is species specific, it may be that its negative inotropy is limited to the dog. However, the possibility of a negative inotropic effect in the newborn should be weighed carefully at such time when the drug is to be considered for administration to very young children.

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