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# Effect of Milrinone on Myocardial Mechanical Function and Cyclic AMP Content in the Fetal Rabbit

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**ABSTRACT. The effect of milrinone on mechanical function was studied in the isolated arterially perfused heart of the fetal (28th day of gestation) and newborn rabbits. The inotropic effect of milrinone in the fetus was significantly less than in the newborn. After milrinone infusion, myocardial cyclic AMP levels increased significantly in the two age groups and the fetal values before and after milrinone infusion were not significantly different from the newborn values. In our previous study the inotropic effects of dibutyryl cyclic AMP and high extracellular calcium in the fetus were significantly less than in the newborn. These data suggest that the diminished inotropic effect of milrinone in the fetus may be due, at least in part, to the decreased inotropism of cyclic AMP and calcium. (*Pediatr Res* 22: 282–285, 1987)**

**Abbreviations**

**IBMX, isobutylmethylxanthin**
**DT, developed tension**
**RT, resting tension**
**+dT/dt max, maximum rate of tension development**
**½RT, half-time to relaxation**

Milrinone is a new analogue of amrinone and its inotropic effect has been evaluated mainly in the adult mammalian myocardium (1, 2). Although the precise mechanisms of the inotropic effect remain unclear, amrinone and milrinone may inhibit phosphodiesterase activity and increase myocardial cyclic AMP content (1–5). This may increase cytosolic calcium concentrations and cause a positive inotropic effect (2). Developmental changes in the effect of amrinone and milrinone have not been studied extensively. Binah *et al.* (6) showed that amrinone had a negative, rather than positive, inotropic effect in the newborn dog heart. Recently, a preliminary report of Binah *et al.* (7) showed that milrinone induced a minimal but significant positive inotropic effect in the newborn dog. The relationship between the inotropism of these drugs and cyclic AMP metabolism in the fetal and newborn myocardium remains unclear. Therefore, this study was designed to investigate the effect of milrinone on 1) mechanical function and 2) myocardial cyclic AMP content in the isolated fetal and newborn heart of the rabbit.

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## METHODS

The experiments utilized the fetus at the 28th day of gestation (term 31 days) and 3- to 5-day-old newborn New Zealand White rabbits. After the doe was killed by a sharp blow to the head the fetuses were delivered by cesarian section and used within 1 min after delivery. The fetal and newborn rabbits were killed by a sharp blow to the head. The heart was then excised from the chest cavity and used for mechanical function study.

*Perfusion solution.* The control Krebs-Henseleit solution contained in mM: NaCl, 118; KCl, 6; $CaCl_2$, 1.5; glucose, 6; $MgCl_2$, 1; $NaHCO_3$, 24; $NaH_2PO_3$, 0.436. KCl concentration was relatively high because at 6 mM KCl arrhythmia was observed less frequently than at 4 mM. Changing $K^+$ concentrations from 4 to 6 mM did not alter mechanical function. The control solution was equilibrated with 95% $O_2$ and 5% $CO_2$ yielding a final pH of 7.35–7.42. Milrinone (a gift from Sterling-Winthrop) was freshly prepared by dissolving the powder in 0.5 N lactic acid and an aliquot was added to the perfusate to achieve final concentrations of $10^{-5}$ to 5 $10^{-4}$ M. An aliquot of 0.5 N lactic acid was also added to the control perfusate to achieve the same concentrations as the perfusate containing milrinone. IBMX (Nakarai) was suspended in the perfusate to achieve final concentrations of $10^{-5}$ to $10^{-4}$ M.

*Experimental preparation.* Experiments were performed in the isolated, arterially perfused ventricular preparation as described previously (8–13). The aorta was canulated with PE-50 polyethylene canula and then perfused with oxygenated perfusate at a constant perfusion rate of 2.5 ml/g tissue per min using a Harvard pump. The base of the right and left ventricle was fixed between two Harmon forceps and the apex was attached to the Statham (UC 3) force transducer using a silk suture. The muscle was stimulated at 90 bpm, and its temperature was maintained at 37 ± 0.5° C. In some experiments, the muscle was stimulated at 40 bpm and its temperature was maintained at 27° C. The following parameters of mechanical function were monitored continuously: DT, RT, +dT/dt max, and ½RT.

In the present study a whole heart was suspended at three points and DT represents one of three force vectors. Because the muscle preparation was not cylindrical, parameters of mechanical function were normalized for the wet weight rather than for the cross-sectional area. Although the papillary muscle preparation may be more desirable for a mechanical function study, the whole heart preparation was used in the present study to perform mechanical and biochemical studies in the same tissue. It must be noted, however, that although the heart weights changed with growth, the geometry of the preparation remained similar. It must be also mentioned that since the heart was perfused retrograde via the aorta, this preparation is different from the working heart model described by Neely *et al.* (20).

*Experimental protocol.* Initially the muscles were perfused with a control solution containing 1.5 mM calcium for 60 min to

allow for stabilization of the mechanical function. During the initial 40 min of each experiment, the length of the muscle preparation was adjusted so that the tension was equal to 90% of the maximal tension. After this initial period, both resting tension and the length-tension relationship remained unchanged under control conditions. The following studies were then performed.

*Effect of Milrinone.* After stabilization of mechanical function, the heart was perfused with solutions containing $1 \times 10^{-5}$, $5 \times 10^{-5}$, and $1 \times 10^{-4}$ and $5 \times 10^{-4}$ M milrinone ($n = 7$ in each age group). The duration of perfusion was 20 min at each milrinone concentration. Mechanical function reached a new steady state within 10 min after switching to a new milrinone concentration, and all measurements were made at 10 min.

In additional experiments, hearts were perfused with solutions containing lactic acid of the same concentrations as the perfusate containing milrinone. The duration of perfusion was similar to that described above.

In another series of experiments, the heart was initially perfused with a solution containing $5 \times 10^{-4}$ M milrinone for 10 min. After a new steady state was obtained, the heart was perfused with a solution containing both milrinone ($5 \times 10^{-4}$ M) and 15 mM calcium for 10 min and whether in the heart perfused with solution containing milrinone contractile force can be increased further by an additional inotropic agent was studied.

*Effect of IBMX.* IBMX is a potent phosphodiesterase inhibitor (2, 3). The effect of IBMX was studied in a similar fashion described above ($n = 6$ in each age group).

Parameters describing mechanical function were expressed as a percentage of control values and a g/g tissue wet weight.

*Myocardial cyclic AMP content.* After stabilization of mechanical function, hearts were perfused with solutions containing $5 \times 10^{-4}$ M milrinone or IBMX for 10 min and then frozen with metal clamps in liquid nitrogen. Control hearts were perfused with solutions which did not contain milrinone or IBMX and frozen in liquid nitrogen. Muscles were then homogenized in ten volumes of 6% trichloroacetic acid using a ground-glass homogenizer and centrifuged at 0° C. The supernatant was neutralized by adding $CaCO_3$ (14) and centrifuged again. Cyclic AMP concentration in the neutralized supernatant was measured by radioimmunoassay methods (15) using cAMP [$^{125}$I] RIA Kit (New England Nuclear, Boston, MA).

*Statistical analysis.* Results were expressed as mean ± SE. Statistical significance of the difference between group means was determined using the Student's $t$ test (16). Percent changes were compared using nonparametric methods (Wilcoxon's rank sum test) (17). Statistical significance of response to milrinone and IBMX was analyzed using a paired $t$ test and repeated measurements of analysis of variance. The probability was considered to be significant if the $p < 0.05$.

## RESULTS

Mechanical function data under control conditions in the fetus were not significantly different from those in the newborn (Table 1). Although DT was slightly lower than the previous data (8), we thought that the present preparation was acceptable because myocardial high energy phosphate concentrations in muscles which showed mechanical function similar to the present data were not depressed (12). Furthermore, in a preliminary experiment the inotropic effect of high $[Ca]_o$ and isoproterenol using the present preparation were identical to the previously reported data (8, 12, 13).

Table 1. *Baseline data of mechanical function**

| Age | $n$ | RT (g/g tissue) | DT (g/g tissue) | +dT/dt max (g/s/g tissue) | ½RT (ms) |
|---|---|---|---|---|---|
| Fetus (28 days) | 11 | 3.5 ± 0.2 | 6.6 ± 0.4 | 31.8 ± 1.5 | 136 ± 6 |
| Newborn | 14 | 3.0 ± 0.2 | 7.0 ± 0.2 | 34.8 ± 0.7 | 133 ± 10 |

* Values are means ± SE. The data were obtained at 37° C. There was no significant difference between the 28-day fetus and newborn.

*Effect of milrinone.* Lactic acid used in the present study ($10^{-4}$ to $5 \times 10^{-3}$ M) had no significant effects on mechanical function. Typical data of the experiments using milrinone are shown in Figure 1. Milrinone infusion caused significant increases in DT in the newborn, but not in the fetus. Significant difference in DT between the newborn and fetus were observed at concentrations of $10^{-4}$ M and $5 \times 10^{-4}$ M (Fig. 2). After milrinone infusion +dT/dt(max) increased significantly both in the newborn and fetus but the increase in the fetus was minimal (Fig. 3). At $5 \times 10^{-4}$ M milrinone, +dT/dt(max) increased to 191 ± 10% of the

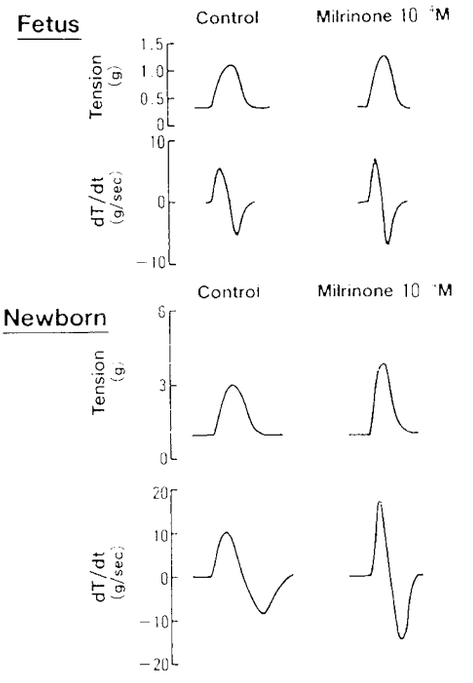


Fig. 1. Typical experiments that show the effect of milrinone on DT and dT/dt. The experiments were performed at 37° C. The inotropic effect of milrinone in the fetus (28th day of gestation) was less than in the newborn.

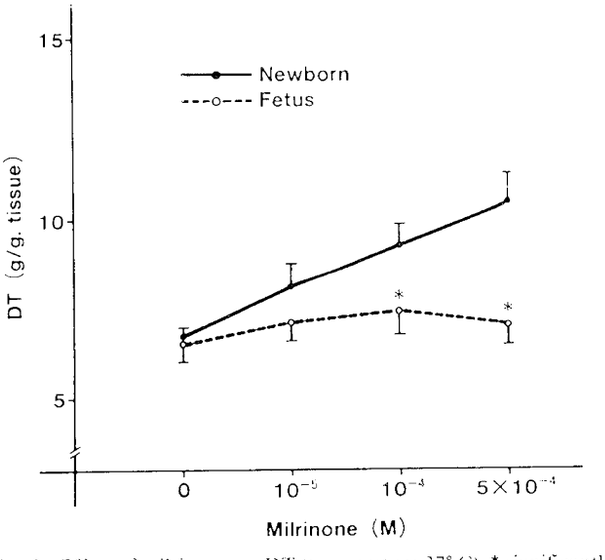


Fig. 2. Effect of milrinone on DT (temperature 37° C). * significantly ($p < 0.05$) different from the value in the newborn.

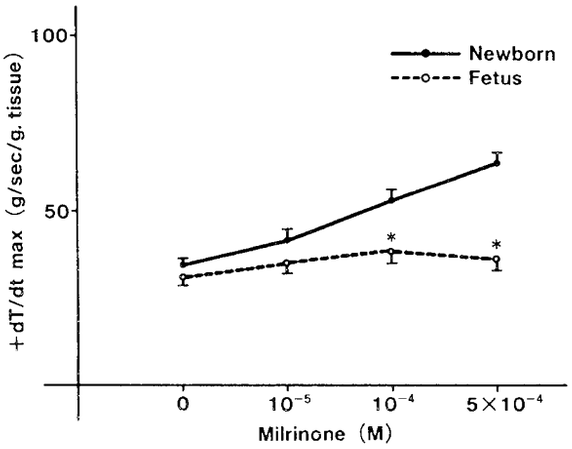


Fig. 3. Effect of milrinone on +dT/dt(max) (temperature 37° C). * significantly different from the newborn value.

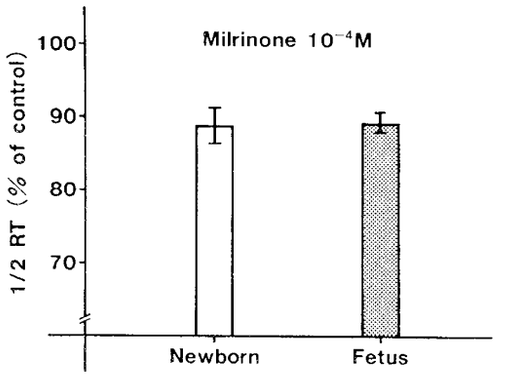


Fig. 4. Effect of milrinone on ½RT (temperature 37° C). Shortening of ½RT in the fetus was not significantly different from the newborn value.

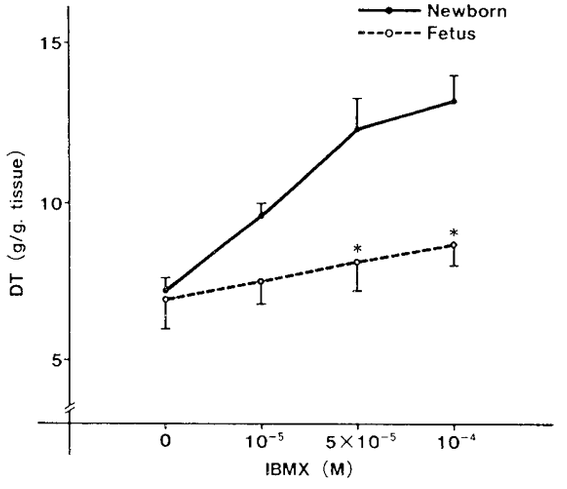


Fig. 5. Effect of IBMX on DT (temperature 37° C). * significantly different from the newborn value.

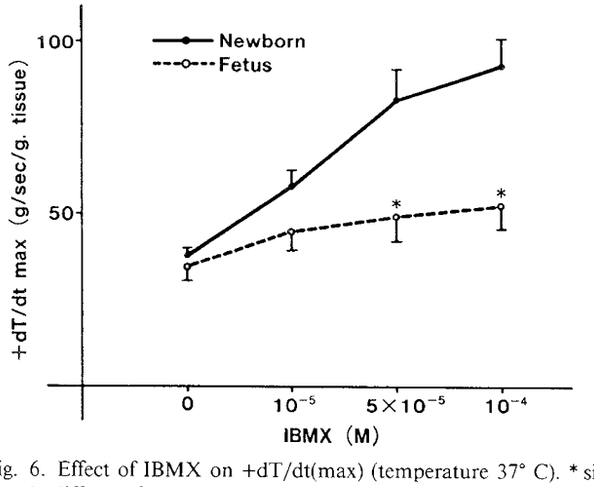


Fig. 6. Effect of IBMX on +dT/dt(max) (temperature 37° C). * significantly different from the newborn value.

control in the newborn and only to 126 ± 6% of the control in the fetus (significant difference between the newborn and fetus: $p < 0.001$). The inotropic effect of milrinone in the muscle maintained at 27° C was not significantly different from that at 37° C (Figs. 2 and 7). Milrinone ($10^{-5}$ M) decreased ½RT similarly in the newborn and fetus (Fig. 4).

Since the inotropic effect of milrinone was minimal in the fetus, the inotropic effect of milrinone and high $[Ca]_o$ was studied to examine whether other inotropic agent can increase +dT/dt(max) further in the fetus. High $[Ca]_o$ (15 mM) caused additional inotropic effect in the heart perfused with milrinone in the fetus (+dT/dt max = 185 ± 37% of control, $n = 4$) and newborn (265 ± 25%, $n = 4$).

*Effect of IBMX.* Whether the age-related difference in the inotropic effect of milrinone is observed in the other phosphodiesterase inhibitors was studied. After IBMX infusion, significant increases in DT and +dT/dt(max) were observed in the two age groups but the effect in the fetus was significantly less than in the newborn (Figs. 5 and 6).

*Myocardial cyclic AMP content.* The control value of myocardial cyclic AMP content was similar in the two age groups. Milrinone and IBMX infusion increased tissue cyclic AMP levels significantly, and the value after milrinone or IBMX infusion in the fetus was similar to the value in the newborn (Table 2).

## DISCUSSION

This study demonstrated that in the isolated heart preparation the increase of DT and +dT/dt(max) during milrinone infusion in the fetus was less than in the newborn. This finding is in agreement with the data of Binah *et al.* (7) who showed that the inotropic effect of milrinone increased with age in the dog heart.

The present study also showed that the effect of milrinone on myocardial cyclic AMP levels was similar in the fetus and newborn. Therefore, it is unlikely that the decreased inotropic effect of milrinone in the fetus is explained by the response of myocardial cyclic AMP levels. Cyclic AMP, by activating protein kinase, enhances calcium release from the sarcoplasmic reticulum and causes an increase in intracellular calcium and a positive inotropic effect. Cyclic AMP also accelerates the relaxation process by stimulating calcium uptake by the sarcoplasmic reticulum. Different inotropic effects of milrinone in the newborn and fetus may be due to the age-related differences in 1) the process from cyclic AMP to sarcoplasmic reticulum and/or 2) the inotropic effect of calcium per se.

In the present study, enhancement of the relaxation process by milrinone, expressed as a shortening of ½RT, was similar in the newborn and fetus. This suggests that the effect of milrinone on the sarcoplasmic reticulum is similar in the two age groups. One may argue that ½RT may not be related to the function of Ca sequestration by the sarcoplasmic reticulum but may be related to the function of the elastic elements of the preparation. However, when the heart was perfused with a solution containing ryanodine, an inhibitor of Ca uptake by the sarcoplasmic reticulum, ½RT prolonged significantly (data not shown). This suggests that ½RT is indeed related to the function of the sarcoplasmic reticulum.

Table 2. *Effect of milrinone and IBMX on tissue cyclic AMP contents**

| Age | Control | After IBMX (pmol/mg tissue wet wt) | After milrinone |
|---|---|---|---|
| Fetus (28-day) | 0.92 ± 0.23 (7) | 2.61 ± 0.43 (7) | 1.95 ± 0.28 (6) |
| Newborn | 0.83 ± 0.16 (6) | 2.55 ± 0.48 (6) | 2.00 ± 0.33 (9) |

* Values are means ± SE. Numbers in parentheses are numbers of measurements. Values after drug infusion were significantly ($p < 0.05$) greater than control in all age groups. There was no significant difference between the fetal and newborn values.

In the present study, the maximal inotropic effect of both milrinone and IBMX in the fetus was less than in the newborn. This suggests that the inotropic effect of phosphodiesterase inhibitors in general is diminished in the fetal myocardium. We have previously studied the inotropic effect of calcium (15 mM), isoproterenol ($10^{-5}$ M), and dibutyryl cyclic AMP ($10^{-4}$ M) under the identical experimental conditions used in the present study (8, 13). The inotropic effect of dibutyryl cyclic AMP, which passes the cell membrane and activates protein kinase directly (17), was less in the fetal heart than in the newborn (Fig. 7). This supports the hypothesis that the diminished response to milrinone in the fetus resulted from the difference in the process after the cyclic AMP increase.

Both milrinone and IBMX increase the amount of calcium reaching the myofilament and cause the inotropic effect. In our previous study, the inotropic effect of high $[Ca]_o$ in the fetus was diminished (Fig. 7). Therefore, it is likely that the decreased inotropic effect of milrinone in the fetus may be due, at least in part, to the age-related difference in the effect of calcium.

The precise mechanisms of the diminished inotropism of calcium in the fetus remained unclear but we postulated previously that premature development of the Ca-sequestering system *i.e.* sarcoplasmic reticulum (19), T tubular system, and mitochondria in the fetus resulted in the relatively high intracellular Ca concentration under control conditions (8). The higher cytosolic calcium in the fetus may enhance the contractility under control conditions but may prevent a further increase in contractile function at high $[Ca]_o$ (8, 18).

One may argue that if milrinone enhance contractile force to the maximal level that the muscle can generate and if the amount of contractile protein in the fetus is less than in the newborn, the inotropic effect of milrinone might be diminished in the fetus. Indeed, Nakanishi and Jarmakani (8) showed that the amount of myofibrils in the fetus was less than in the newborn and this may explain the lower DT (absolute value) in the fetus observed after administration of milrinone and other inotropic agents. In the present study, however, not only the absolute value of DT but also the relative change of DT after administration of inotropic agents in the fetus was less than in the newborn. We have shown previously that the sensitivity of myofibrillar ATPase to calcium does not change with development, suggesting that $[Ca]_i$-tension relationship is similar in the newborn and fetus (18). Furthermore, in the heart perfused with milrinone, DT increased further after high calcium infusion. This suggests that milrinone infusion did not increase the contractile force to the maximal level that the muscle can generate. These data suggest that the diminished inotropic effect (relative change of DT) of milrinone and other inotropic agents in the fetus (Fig. 7) cannot be explained by the amount of contractile protein.

In conclusion, the present data indicate that in the isolated heart preparation the inotropic effect of milrinone in the fetus is less than that in the newborn. The diminished inotropic effect of milrinone in the fetus is not due to the decreased response of myocardial cyclic AMP levels but may be due, at least in part, to the decreased inotropism of cyclic AMP and calcium.

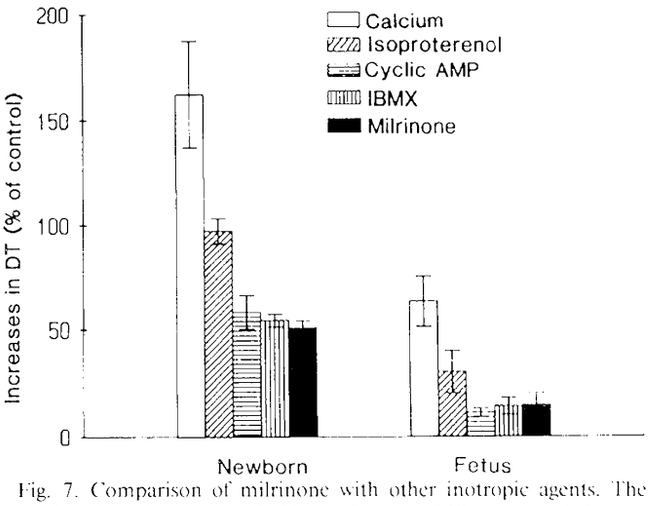


Fig. 7. Comparison of milrinone with other inotropic agents. The data of calcium, isoproterenol, dibutyryl cyclic AMP are from Reference 14. All experiments were performed at 27° C and the muscle was stimulated at 40 bpm. The effect of all inotropic agents studied in the fetus were approximately 30% of the newborn values.

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