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Mechanical Function and Metabolism of Working Rat Hearts Perfused with the Negative Chronotropic Agent Mixidine Fumarate (40816)

MICHAEL J. ROVETTO

Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Mixidine fumarate (2-[3,4-dimethoxyphenethyl)imino]-1-methylpyrrolidine fumarate, McNeil No. 1589-46) has been shown to act as a myocardial antiacceleratory agent in intact dogs (1). It antagonized increases in heart rate in response to aminophylline, glucagon, isoproterenol, atropine, poldine, treadmill exercise, and sympathetic nerve stimulation. Because mixidine did not alter the inotropic effect of isoproterenol, it appeared to be a specific chronotropic agent. It was thought to do this by acting directly on the sinoatrial node and by blocking myocardial sympathetic innervation.

Because of its therapeutic potential for use in myocardial ischemia and as an antianginal agent, the present studies were undertaken to determine its effect on heart rate, myocardial metabolism, and contractile force in isolated perfused working rat hearts. Mixidine slowed heart rate in a dose-dependent manner but was without effect on mechanical function and substrate utilization in electrically paced rat hearts.

Materials and methods. Hearts were removed from 225 to 275 g male Sprague–Dawley rats, the aortas cannulated and perfused at 55 mm Hg pressure. The perfusate employed was Krebs–Henseleit bicarbonate buffer containing 11 mM glucose equilibrated with 95% O_2 and CO_2 at 37°C. After 10 min of perfusion at this pressure, the pulmonary veins were cannulated for subsequent perfusion as working hearts (2, 3) and the pulmonary arteries were cannulated for collection of coronary venous outflow. The hearts were then perfused an additional 10 min as working hearts with Krebs–Henseleit buffer for studies of heart rate, or with Krebs–Henseleit buffer containing 3% bovine serum albumin and 0.4 or 1.0 mM palmitate for studies of mechanical function and metabolism. During these perfusion periods, left atrial pressure was 10 cm H_2O.

For studies of the effect of mixidine on heart rate, the perfusate was changed after an additional 10 min of perfusion to one containing a given concentration of mixidine. The heart rates were recorded 10 and 20 min after initiation of perfusion with mixidine. There were no differences in the heart rates at the two times.

For studies of ventricular mechanical function and metabolism, ventricular function curves were generated by perfusing at left atrial pressures of 5, 10, and 15 cm H_2O. These hearts were paced at 300 beats per minute. Aortic pressures and cardiac output were measured after 3 min at each filling pressure. This duration was found previously to be sufficient to obtain new steady state values (4). Coronary effluent was also collected at each pressure for determinations of oxygen tension, $^{14}CO_2$ and ^3H_2O content as discussed below. Following cardiac function measurements, the perfusate was switched to one containing 10^{-4} *M* mixidine. After 10 min, function curves were again generated. In some experiments, hearts were perfused only with mixidine to determine the influence of perfusion time on the results. The data from such hearts were identical to those obtained from hearts which were first perfused with control perfusate followed by perfusion with mixidine containing buffer.

Ventricular power was calculated according to the method of Bishop *et al.* (5), in g · cm per second per gram dry tissue by the formula: power = [aortic output (ml/sec) × (mean arterial pressure - left atrial pressure (cm H_2O)] ÷ gram dry tissue per heart.

Metabolism of glucose was determined by using uniformly labeled [^{14}C]glucose to assess oxidation and [2-^{3}H]glucose to esti-

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mate glycolysis. About 2×10^4 cpm [^{14}C]-glucose (60% counting efficiency) and 2×10^5 cpm [^{3}H]glucose (24% counting efficiency) were added per milliliter perfusate. The ^{3}H detection window of a Beckman LS230 liquid scintillation spectrometer was adjusted so that less than 7% of the counts due to ^{14}C were detectable. Two-milliliter samples of perfusate were collected in glass syringes from the pulmonary artery cannula and acidified to release CO_2. The CO_2 was trapped in Oxyflour (New England Nuclear, Boston, Mass.) soaked filter paper suspended in reaction flasks. The 0.5-ml samples of the collected coronary effluent were placed on small Dowex 1 borate form, anion exchange resin to separate unmetabolized glucose from ^3H_2O produced in glycolysis (6, 7). The columns were washed with three 0.5-ml vol of water to remove ^3H_2O, and the effluents were used for determination of radioactivity.

Results. Figure 1 shows the heart rate dependence on mixidine concentration. In Panel A the data are expressed in absolute rates, and in Panel B as a percentage of the initial heart rate during perfusion with control buffer. At 10^{-3} *M* mixidine, although the hearts maintained a steady rhythm, they could not be electrically stimulated to beat at a rate of 300 per minute. With 5×10^{-3} *M* mixidine the hearts were arrested and would not beat even with electrical pacing. At concentrations of mixidine less the 10^{-3} *M* the hearts could be electrically stimulated to beat at 300 beats per minute. The bradycardia produced by mixidine was maximal after 10 min exposure to mixidine. No further slowing was observed at 10^{-5} and 10^{-4} *M* when perfusion with mixidine was continued for 90 min.

Figure 2 shows the persistent bradycardia produced by mixidine after switching the perfusate to mixidine-free buffer. Even with continued washing with mixidine-free buffer the rates approached the initial rates only after 90 min perfusion.

Figure 3 is a representative aortic pressure tracing from a heart before and after treatment with 10^{-3} *M* mixidine. The rates for this heart were 220 and 128 beats per minute before and after exposure to mixidine, respectively. Although mixidine produced no visually detectable change in the rate of pressure development its effect on heart rate was reflected in mechanical function in unpaced hearts by decreased power development. The dose–response curve for ventricular power was nearly identical in shape to the dose–response curve for heart rate shown in Fig. 2B. At 10^{-4} *M* and 10^{-3} *M* mixidine the mean ventricular power was 59 and 41%, respectively, of the power developed in the absence of mixidine.

In contrast, when the hearts were perfused with 10^{-4} *M* mixidine and electrically paced at 300 beats per minute there was no difference in developed power between these and control hearts (Fig. 4).

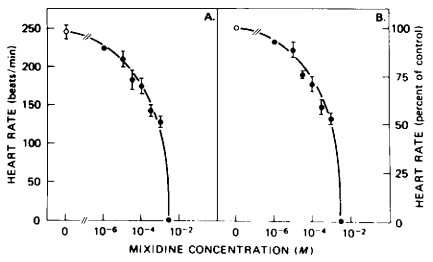

FIG. 1. The effect of mixidine on heart rate. Open circles represent the mean control heart rate and the solid circles represent the mean rates in the presence of mixidine for 8 to 12 hearts. The mean of 10^{-6} *M* mixidine is for two hearts.

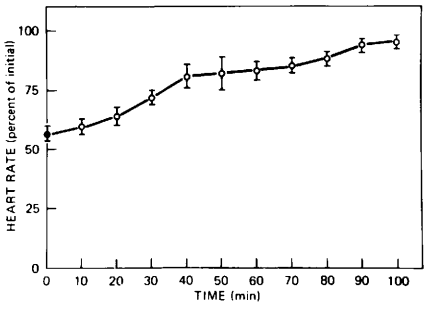

FIG. 2. Restoration of heart rate following exposure to mixidine. Following 15 min exposure of four hearts to 10^{-4} *M* mixidine the perfusate was switched to one without mixidine and heart rate was measured over the next 100 min. The coronary effluent was not recirculated.

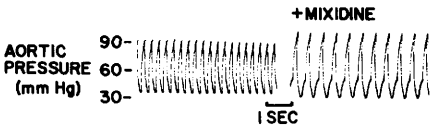

FIG. 3. Aortic pressure tracing before and after mixidine. The tracing on the right was obtained in the presence of 10^{-3} *M* mixidine.

Oxygen consumption for these hearts, expressed as a ratio to ventricular power is shown in Fig. 5. Although oxygen consumption increased with increased ventricular power, at any given level of power in both groups of hearts, mixidine did not alter the relationship between oxygen consumption and ventricular power. Furthermore, oxygen consumption was not different from that of control hearts at either concentration of palmitate. Glucose oxidation and glycolytic rates for these hearts are presented in Fig. 6. In Panel A are shown the data for hearts perfused with buffer containing 0.4 m*M* palmitate and in Panel B are the data for hearts perfused with 1 m*M* palmitate. As expected, increasing the fatty acid concentration from 0.4 to 1 m*M* depressed glucose utilization in control hearts. About 40% of the glucose utilized was oxidized in the presence of 0.4 m*M* palmitate at 10 and 15 cm water left atrial pressure. While more glucose was used per unit ventricular power at 5 cm than at 10 and 15 cm H_2O left atrial pressures, the same amount was oxidized at all three filling pressures. In the presence of 1 m*M* palmitate, the elevated ratio of glycolysis to ventricular power at 5 cm H_2O left atrial pressure was not observed. Only about 10%

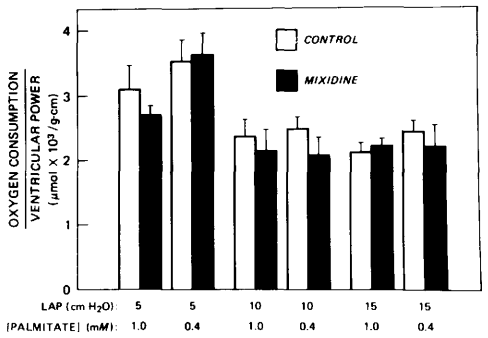

FIG. 5. The effect of mixidine on myocardial oxygen consumption. Oxygen consumption was measured as described in methods and expressed in micromoles per minute per gram dry tissue. This value was divided by ventricular power expressed in g · cm per minute per gram dry tissue. The bars represent the means for 12 hearts perfused with buffer containing 1.0 m*M* palmitate and 8 hearts perfused with buffer containing 0.4 m*M* palmitate. The mixidine concentration in both buffers was 10^{-4} *M* and the hearts were electrically paced at 300 beats per minute.

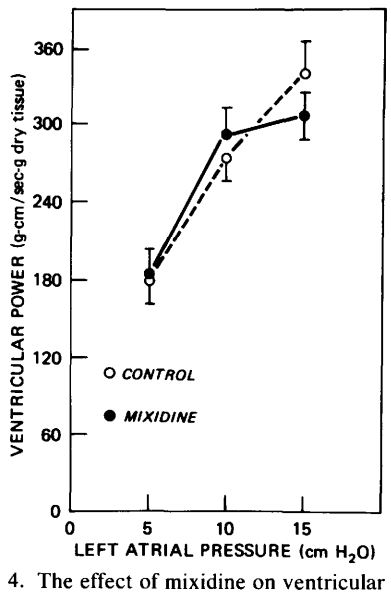

FIG. 4. The effect of mixidine on ventricular power in electrically paced hearts. The symbols represent the mean ± SEM for 12 hearts perfused with buffer containing 11 m*M* glucose, 10^{-4} *M* mixidine and 1 m*M* palmitate bound to 3% bovine serum albumin. The hearts were electrically paced at 300 beats per minute.

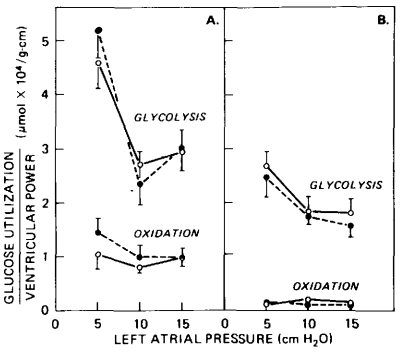

FIG. 6. The effects of mixidine on glycolysis and glucose oxidation. The data are from the same hearts as in Fig. 5. Panel A contains data from hearts perfused with buffer containing 0.4 m*M* palmitate and Panel B from hearts perfused with 1.0 m*M* palmitate.

of the glucose metabolized, however, was oxidized with 1 m*M* palmitate in the perfusate. From these data it can be calculated that glucose oxidation accounted for about 23 and 5% of the oxygen consumed with buffer containing 0.4 and 1.0 m*M* palmitate, respectively. The remainder of the oxygen was utilized for fatty acid metabolism. The addition of 10^{-4} *M* mixidine did not change the relationships between substrate utilization and ventricular power observed with control perfusates containing either 0.4 or 1.0 m*M* palmitate. Thus, mixidine did not alter the pattern of substrate utilization by the heart.

Discussion. The long-lasting selective reduction in heart rate produced by mixidine would appear to be desirable in a therapeutic agent for ischemic heart disease. Myocardial ischemia results when oxygen demand exceeds the supply. Adjustments in either supply or demand can restore this critical balance. The demand can be reduced by decreasing contractile force, rate, wall tension, or contraction velocity or by increasing carbohydrate metabolism or the efficiency of contraction. A change in any one of these factors which contribute to myocardial oxygen consumption would help rebalance the supply, demand equation.

Mixidine was an effective negative chronotropic agent at concentrations between 10^{-5} and 10^{-3} *M*. At the higher concentration, electrical pacing at higher rates was unsuccessful but hearts could be paced at 300 beats per minute at 10^{-4} *M*. As the mixidine concentration was increased between 10^{-5} and 10^{-3} *M* the slowing of the heart rate was accompanied by decreased ventricular power. The largest contribution to the decreased power was reduced cardiac output since developed pressure remained relatively constant. The reduction in heart rate and power also reduced coronary flow (data not shown) as would be expected by reduced oxygen demand. When the hearts were electrically paced, however, mechanical function and coronary flow were not affected by mixidine. Thus, mixidine primarily affected heart rate and did not directly depress myocardial force development.

Studies of substrate utilization failed to reveal any changes in substrate preference by the heart. Myocardial oxygen consumption, glycolysis and glucose oxidation were the same in control and mixidine treated hearts. Glucose utilization in the presence of fatty acids accounted for only a small percentage of total substrate utilization as expected (8) with the remainder of the oxygen consumption used for fatty acid metabolism. As the perfusate fatty acid concentration was increased from 0.4 to 1.0 m*M* the depression in glycolysis and the percentage of utilized glucose oxidized was the same regardless of whether or not mixidine was present. Furthermore, mixidine did not affect substrate utilization at any of the three myocardial work loads employed. Thus, mixidine did not have any direct actions on the enzymatic regulatory mechanisms in the glucose and fatty acid metabolic pathways. Had mixidine stimulated glucose utilization or inhibited fatty acid utilization this would have been an added benefit for reducing oxygen consumption above that due to slowing of heart rate. The efficiency of ATP production from oxidation of glucose is greater than that from fatty acid (8). Although mixidine did not alter myocardial metabolism, it had no apparent deleterious effects on the heart and may be useful in the experimental setting to control rate without directly depressing the heart and beneficial in reducing angina through its negative chronotropic action.

Summary. The experimental agent, mixidine fumarate, was studied in isolated perfused working rat hearts for effects on mechanical function and energy production. Mixidine had a potent negative chronotropic effect in this preparation. This in turn leads to decreased ventricular power development. The depression in mechanical function, however, was absent in mixidine treated hearts electrically paced at rates comparable to those of control hearts. Furthermore, mixidine produced no detectable changes in the myocardial energy pro-

ducing pathways. From these studies it appears that mixidine is a specific chronotropic agent.

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