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RAPID COMMUNICATIONS

MILRINONE PRODUCES A POSITIVE INOTROPY BUT IS NOT INHIBITORY TO GASTRIC ION TRANSPORT

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Abstract. The cardiac drugs ouabain and milrinone are positive inotropic agents. Since ouabain has inhibitory effects on ion transport in the gastrointestinal tract associated with vasoconstriction and hypoxia, milrinone needed to be tested also. This report indicates that therapeutic levels of milrinone on either side of the isolated stomach wall of the guinea pig has no significant effects on active chloride ion transport or the electrical parameters of the tissue. To verify that milrinone was active in the heart at these same levels, contractility of the isolated heart of the guinea pig was measured. Milrinone significantly increased ventricular pressure and pressure development. Thus milrinone may be expected to exert its inotropic stimulation of the heart during heart failure without compromising gastrointestinal functions.

Milrinone is a new cardiac bipyridine drug that may replace the older cardiac glycosides in the treatment of heart failure (1). The mechanism of its inotropic action in the heart is not fully understood, but milrinone has been reported to increase calcium availability in cardiac muscle cells (2). A similar mode of action in vascular smooth muscle cells of the intestine, however, could lead to vasoconstriction and hypoxia. These effects were reported to occur with the cardiac glycoside ouabain, although ouabain has a different inotropic mechanism of action, the inhibition of membrane Na^++K^+-ATPase. The pronounced vasoconstriction and hypoxia produced by ouabain in the mesenteric circulation (3) has been associated with an often fatal condition of nonocclusive mesenteric ischemia (4). Associated with the vasoconstriction and hypoxia in the gut produced by ouabain is a profound inhibition of active transport of ions, especially of chloride, in the isolated gastric mucosa (5).

This study was designed to test whether milrinone might have an effect similar to that of ouabain in the digestive tract at concentrations which are effective in the heart.

Materials and Methods

Hartley guinea pigs of either sex and weighing 280-330 g were fasted overnight and sacrificed by decapitation. The heart and attached thymus were removed first followed by the stomach.

Isolated heart: Hearts were isolated and perfused as previously described (6, 7). Varying concentrations of milrinone (0.5-64μg/ml) were infused into the hearts via the perfusing cannula with a sage syringe pump. Changes in heart rate, right ventricular pressure and rate of pressure change (dP/dt) were recorded on a Brush 2400 S physiological recorder.

Isolated stomach wall: The excised stomach was cut open along the lesser curvature and emptied of its contents with a brief water wash.

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After transfer to Ringer solution, the stomach wall was divided into bilateral halves and mounted in flux chambers for determination of electrical potential difference (PD), short-circuit current (Isc) and resistance (R) on a voltage-clamp system as described previously (8). For inclusion in the analyses, the stomach wall needed to attain a minimum PD of -7 mV, mucosal side negative, in the control period. The unidirectional and net fluxes of chloride (J^{Cl^-}) were traced by addition of 0.125 μCi of ^{36}Cl (ICN) to mucosal or serosal side of the paired stomach walls in the flux chambers. Chamber solution was 10 ml of Ringer solution buffered with 10 mM TES (Sigma), gassed with 100% O_2 and thermoregulated at 37°C.

Milrinone at a concentration 100 x the final therapeutic level (1) was added in a 100 μl aliquot and mixed by gas bubbling. Removal of the drug was accomplished by draining the half chamber, refilling with Ringer solution, draining and refilling again. Ouabain (Sigma) was added to the serosal compartment in a similar way.

Drugs: Milrinone (Win 47203, Sterling-Winthrop) (1,6-dihydro-2-methyl-6-oxo[3,4' bipyridine]-5-carbonitrile) was dissolved in 1 N HCl and diluted in Ringer solution for administration to the heart and stomach wall from the mucosal side. For serosal addition, the diluted milrinone was completely neutralized with 1 N NaOH. Milrinone solutions were made up fresh each day within 4 hr of use. Ouabain was also dissolved daily in warm Ringer solution.

Statistical significance of the treatments was determined by ANOVA at the 5% level and the Student range test applied post hoc. All results were expressed as $\bar{x} \pm$ SEM for n=6 or 7 animals.

Results

Milrinone had no significant effect on the active transport of chloride across the isolated stomach wall of the guinea pig (Fig. 1). The net flux of chloride under the condition of zero electrochemical potential difference, 4.7 ± 1.4 μEq/cm²h, was unaffected by sequential exposure to 1.5 x 10^{-5}M serosal or to 2.8 x 10^{-4}M mucosal milrinone. Exposure to 2.5 x 10^{-6}M serosal ouabain reduced the net chloride flux by 56% from 4.1 ± 1.5 μEq/cm²h at 90 min to 1.8 ± 1.0 μEq/cm²h at 120 min. Similarly, these exposures to milrinone had no significant effects on the unidirectional fluxes of chloride from serosa to mucosa, 15.6 ± 1.5 μEq/cm²h, or from mucosa to serosa, 10.9 ± 0.9 μEq/cm²h. Serosal ouabain reduced the unidirectional flux from serosa to mucosa to 11.9 ± 1.4 μEq/cm²h without affecting the opposite unidirectional flux.

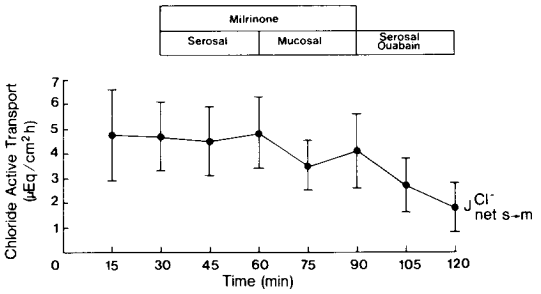
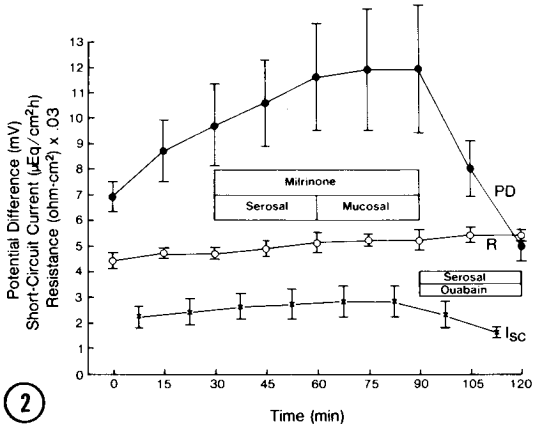


Figure 1. Active chloride ion transport (net flux from serosa to mucosa) across isolated, short-circuited guinea pig stomach wall in response to 1.5 x 10^{-5}M serosal milrinone, 2.8 x 10^{-4}M mucosal milrinone and 2.5 x 10^{-6}M serosal ouabain; n = 7.

The effects of the milrinone and ouabain treatments on the electrical parameters of the stomach wall are shown in Fig. 2. Neither serosal nor mucosal exposure to milrinone had any significant effects on the PD, Isc or R of the guinea pig stomach wall. Serosal exposure to ouabain reduced the PD by 58% from -11.9 ± 2.5 mV, mucosal side negative, at 90 min to -5.0 ± 0.6 mV at 120 min. Ouabain similarly reduced the Isc by 43% from 2.8 ± 0.5 to 1.6 ± 0.2 μEq/cm²h. The R at 90 min equal to 174 ± 12 ohm-cm² was unchanged by ouabain treatment.

In isolated hearts the mean resting right ventricular pressure was 33 ± 1 mmHg, the mean resting dP/dt was 895 ± 50 mm/sec and the mean resting heart rate was 206 ± 13 beats/min. Milrinone produced a concentration-related increase in



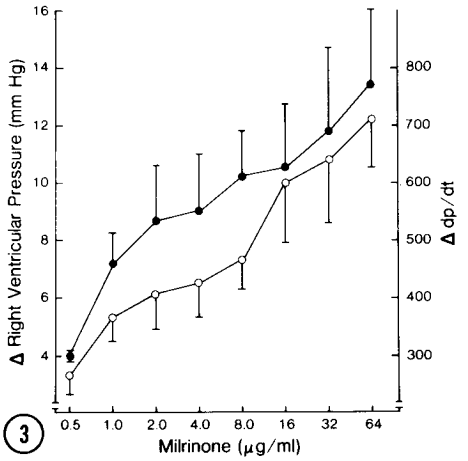


Figure 2. Responses in electrical potential difference (PD), short-circuit current (Isc) and resistance (R) in same isolated guinea pig stomach walls and at same concentrations of milrinone and ouabain used in Figure 1.

Figure 3. Change in right ventricular pressure (solid circles) and pressure development dP/dt (open circles) in isolated guinea pig hearts in response to milrinone; n = 5.

right ventricular pressure (12-51%) and dP/dt (30-117%) (Fig. 3). The increase in heart rate with milrinone was modest (maximum 22% at 32 μg/ml).

In one of five guinea pig hearts an apparent block of A-V conduction occurred at a milrinone concentration of 64 μg/ml (Fig. 4).

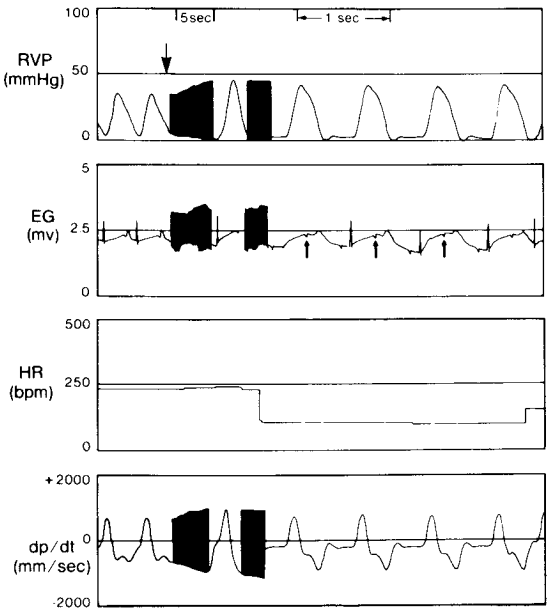


Figure 4. Atrio-ventricular (A-V) conduction block observed in one heart with the highest concentration of milrinone, 64μg/ml. Arrows at top indicates onset of milrinone infusion. Arrows below indicate P waves without ensuing QRS complexes.

Discussion

The results show that milrinone stimulates contractility of the heart while leaving gastric mucosal transport intact. Milrinone applied to either side of gastric mucosal tissue from the guinea pig had no significant effect on active chloride transport. Although the response of the less sensitive rat gastric mucosa was not reported here, the effect of milrinone treatment was also not significant in this species (unpublished observations). Ouabain, in contrast, has been found to inhibit acid secretion in the guinea pig gastric mucosa (9) and to inhibit active transport of hydrogen and chloride in the bullfrog (10) and rat gastric mucosa (8). Associated with inhibition of active sodium and chloride transport in dog gastric mucosa (5) is a condition of vasoconstriction and hypoxia in the gastrointestinal vasculature (3). The latter condition may lead to an often fatal, nonocclusive mesenteric vascular disease (4). Thus it is important to establish that milrinone, an inotropic agent like ouabain used to treat cardiac failure, does not inhibit gastric ion transport at therapeutic concentrations.

The levels of milrinone used were based on estimates of the concentrations achieved in the plasma and gastric juice of congestive heart patients treated with milrinone (1). After a short-term intravenous treatment with 25 μg/kg milrinone, these subjects received oral milrinone at 29 mg/day for 11 months. Assuming a plasma volume of 4% body weight and a gastric daily volume of 2.5 l yields the serosal and mucosal concentrations of milrinone, respectively, used on the stomach wall of the guinea pig. The concentration of ouabain used on the stomach wall was also very close to that known to achieve therapeutic positive inotropism in the heart (11).

Alousi et al (2) have previously reported the effects of the bipyridine compound milrinone on papillary muscle preparations and isolated left atria from several mammalian species. We felt it important to investigate the actions of milrinone on isolated hearts in order to determine its effects on the whole intact organ.

Milrinone produced a positive inotropic response in isolated guinea pig hearts with a modest chronotropic effect. The isolated rat heart was relatively insensitive to milrinone; there were no significant changes in right ventricular pressure, dP/dt or heart rate (unpublished observations). These effects are similar to those reported by Alousi et al (2), including the differing sensitivities between guinea pigs and rats. An action that was not reported in previous studies was an effect on A-V conduction observed in one guinea pig heart at the highest concentration of milrinone (64μg/ml). The significance of this single observation remains to be determined.

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