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Effects of intrarenal and intravenous infusion of the phosphodiesterase III inhibitor milrinone on renin secretion

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ABSTRACT

We have reported that administration of the phosphodiesterase III inhibitor milrinone increases renin secretion in conscious rabbits. The aim of the present study was to determine if the increase in renin secretion results from a direct renal action of milrinone, or from an indirect extrarenal effect of the drug. This was accomplished by comparing the effects of intrarenal and intravenous infusion of graded doses of milrinone on plasma renin activity in unilaterally nephrectomized conscious rabbits. Milrinone was infused into the renal artery in doses of 0.01, 0.1 and 1.0 μg/kg/min, and intravenously in the same rabbits in doses of 0.01, 0.1, 1.0 and 10 μg/kg/min. Each dose was infused for 15 min. No intrarenal dose of milrinone altered plasma renin activity or arterial pressure, although at the highest dose, there was a small increase in heart rate. Intravenous infusion of milrinone at 1.0 μg/kg/min increased plasma renin activity to 176 ± 55% of the control value ($P<0.05$). Heart rate increased but arterial pressure did not change. Intravenous infusion of milrinone at 10 μg/kg/min increased plasma renin activity to 386 ± 193% of control in association with a decrease in arterial pressure and an increase in heart rate. These results confirm that milrinone increases renin secretion, and indicate that the stimulation is due to an extrarenal effect of the drug.

INTRODUCTION

It is now generally accepted that cyclic AMP plays a major role in the control of renin secretion. Many investigators have reported that beta-adrenoceptor stimulation, which activates adenylate cyclase and increases cyclic AMP formation, stimulates renin secretion in vivo and in vitro (1-3). Renin secretion may also be increased by theophylline and other methylxanthines which inhibit the hydrolysis of cyclic AMP by phosphodiesterase (2-4). However, interpretation of this latter finding is complicated by the fact that methylxanthines exert other actions that are unrelated to cyclic AMP hydrolysis. For example, these drugs are known to block adenosine receptors, and adenosine has been implicated in the regulation of renin secretion (5).

It is now recognized that phosphodiesterase is not a single enzyme, but a group of at least five families of isozymes (PDE I-V) which differ in their substrate specificity and regulation (6,7). Specific inhibitors of the different isozymes are now available (6,7), but little information is available concerning their effects on renin secretion. We recently reported that milrinone, an inhibitor of phosphodiesterase III, increases resting renin secretion and enhances the renin secretory response to beta-adrenoceptor stimulation in conscious rabbits (8). However, in those experiments, milrinone was administered systemically and it was not clear if it increased renin secretion by an action in the kidneys, or by an extrarenal effect. This is an important distinction because beta-adrenoceptor stimulation can increase renin secretion by extrarenal as well as intrarenal actions (9,10).

The aim of the present investigation was to localize the site of action of milrinone on renin secretion. This was accomplished by comparing the effects of intrarenal and intravenous infusion of graded doses of the drug on renin secretion in conscious rabbits.

METHODS

Surgical Procedures

Surgery for unilateral nephrectomy and placement of vascular catheters was performed under aseptic conditions during anesthesia with intramuscular ketamine (Parke-Davis, Morris Plains, NJ; 35-50 mg/kg) and xylazine (Lloyd Laboratories, Shenandoah, Iowa; 5-10 mg/kg). After surgery, the rabbits were treated with intravenous ampicillin (Sigma Chemical Co., St. Louis, MO; 10 mg/day) for at least three days.

Unilateral Nephrectomy

The right kidney was removed using a retroperitoneal approach by way of a flank incision. The rabbits were allowed approximately three weeks to recover from this procedure before surgery for catheter placement.

Vascular Catheters

The left renal and adrenal arteries were exposed by way of a flank incision. A catheter consisting of PE10 tubing was inserted into the left adrenal artery and advanced into the left renal artery. Another catheter consisting of 10 cm of medical grade Silastic (Dow-Corning Corp., Midland, MI) connected to PE60 tubing was inserted into a femoral artery and advanced into the aorta to a point distal to the kidneys. Two Tygon catheters were inserted into a jugular vein and positioned near the heart. All catheters were led subcutaneously to a point between the scapulae where they emerged through a small skin incision and were protected in a pocket of a nylon mesh jacket. The rabbits were allowed to recover for at least three days following this procedure, during which time they were brought to the laboratory and accustomed to the experimental environment. The catheters were flushed with sterile heparinized isotonic saline (1000 U/ml) at least every other day. Using this preparation, it was possible to compare the effects of intrarenal and intravenous infusion of milrinone in the same animals. Moreover, because the infused kidney was the only kidney present, systemic arterial plasma renin activity could be used as an index of the rate of renin secretion by that kidney.

Experimental Procedures

On the day of an experiment, rabbits were brought to the laboratory and placed in a partly covered cage. Arterial blood pressure and heart rate were continuously monitored using a pressure transducer (Cobe Laboratories, Inc., Lakewood, CO) and a custom-built cardiovascular analyzer, and recorded on a Grass polygraph. Blood samples for analysis (volume = 1.2 ml) were collected from the femoral arterial catheter and replaced with an equal volume of sterile isotonic NaCl. Experiments were begun when blood pressure and heart rate had remained stable at their basal values for at least 15 min.

Effects of Intrarenal and Intravenous Milrinone

Blood pressure and heart rate were recorded during a 15 min control period at the end of which an arterial blood sample was collected. The phosphodiesterase III inhibitor milrinone (Primacor, Sanofi Winthrop Pharmaceuticals, New York, NY) was then infused into the left renal artery in a dose of 0.01 μg/kg/min for 15 min. The dose was then increased to 0.1 μg/kg/min, and after another 15 min, to 1.0 μg/kg/min. Blood samples were collected at the end of each 15-min infusion period. After a 30-min recovery period, the same procedure was repeated except that milrinone was infused intravenously instead of into the renal artery. Because each kidney normally receives approximately 10% of the cardiac output, the concentration of milrinone in the renal circulation during intravenous infusion would only be 10% of that during intrarenal infusion. Therefore, the effect of a higher intravenous dose of milrinone, 10 μg/kg/min, was also tested.

Plasma Renin Activity

Plasma renin activity was measured using a radioimmunoassay for angiotensin I, and expressed as nanograms angiotensin I generated per ml plasma during a two-hour incubation at 37° C and pH 6.5 (ng/ml/2h) (11).

Statistical Analysis

Results are expressed as the mean ± SE. Data were analyzed using analysis of variance for repeated measures (ANOVA) (12). When significant changes were detected by ANOVA, Dunnett's test (12) was used to make comparisons with the pre-infusion control value. Changes were considered to be statistically significant when $P < 0.05$.

RESULTS

The effects of all intrarenal and intravenous doses of milrinone on blood pressure, heart rate and plasma renin activity were tested in each of three

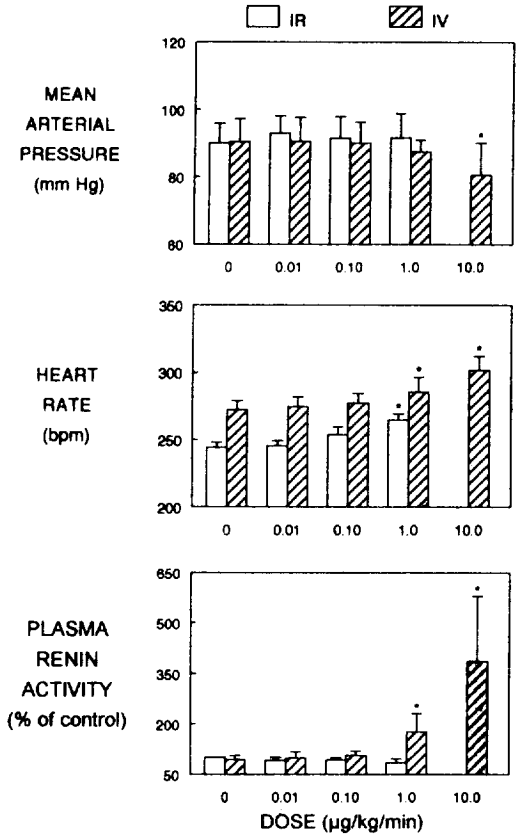

FIGURE 1.

Effects of intrarenal and intravenous infusion of milrinone on mean arterial pressure, heart rate and plasma renin activity. Plasma renin activity is expressed as a percentage of the initial control value (16.5 ± 8.4 ng/ml/2h). Results are expressed as the mean and standard error of observations made in three conscious rabbits.
* P<0.05 compared to the corresponding control value.

rabbits. The data are summarized in Figure 1. Note that plasma renin activity is expressed as a percentage of the initial control value which averaged 16.5 ± 8.4 ng/ml/2h. Mean arterial pressure and plasma renin activity did not change significantly during intrarenal infusion of any dose of milrinone, but there was a small increase in heart rate from 244 ± 4 to 265 ± 5 beats/min during infusion of the highest dose (P<0.05). Intravenous infusion of the lowest two doses of milrinone did not change any of the measured variables. Infusion at 1.0 µg/kg/min did not significantly change mean arterial pressure, but increased heart rate from 273 ± 7 to 286 ± 11 beats/min (P<0.05) and plasma renin activity to 176 ± 55% of control (P<0.05). Infusion at 10 µg/kg/min decreased mean arterial pressure from 90 ± 7 to 81 ± 10 mm Hg (P<0.05), increased heart rate from 273 ± 7 to 302 ± 11 beats/min (P<0.05), and increased plasma renin activity to 386 ± 193% of control (P<0.05).

DISCUSSION

Cyclic AMP is known to play an important role in the control of renin secretion. According to current concepts, renin secretion increases when cyclic AMP formation in the juxtaglomerular cells is increased by stimulating adenylate cyclase, or when cyclic AMP hydrolysis is decreased by inhibiting phosphodiesterase (1-3).

Phosphodiesterase is not a single enzyme, but a group of families of isozymes which differ in their substrate specificity and regulation by cyclic nucleotides and other factors (6,7). However, little information is available concerning the role of these enzymes in the control of renin secretion. The effects of some phosphodiesterase inhibitors on renin secretion have been investigated in patients with heart failure, but variable results have been obtained (13-16).

Recently we observed that administration of the phosphodiesterase III inhibitor milrinone stimulates renin secretion in conscious rabbits (8). However, because the milrinone was infused intravenously, it was not clear if it increased renin secretion directly by an action in the kidney, or indirectly by an extrarenal effect of the drug. The aim of the present investigation was to localize the site of action of milrinone on renin secretion by comparing the effects of intrarenal and intravenous infusion of graded doses of milrinone. The experiments were performed in conscious rabbits to avoid the confounding effect of anesthesia on renin secretion (2), and the effects of intrarenal and intravenous milrinone were studied under identical conditions in the same rabbits.

Intravenous infusion of the highest two doses of milrinone, 1.0 and 10 µg/kg/min, increased plasma

renin activity to 176 and 386% of control respectively. On the other hand, intrarenal infusion of milrinone at 0.1 and 1.0 μg/kg/min did not change plasma renin activity. Since each kidney normally receives approximately 10% of the cardiac output, the intrarenal infusion of milrinone at 0.1 and 1.0 μg/kg/min would have produced approximately the same increases in renal arterial milrinone concentration as intravenous infusion at 1.0 and 10 μg/kg/min respectively. These results therefore indicate that milrinone increases renin secretion by an extrarenal effect, rather than by a direct action on the kidney.

The mechanism by which intravenous administration of milrinone increases renin secretion remains to be determined. The highest intravenous dose of milrinone decreased arterial pressure and this may have contributed to the increase in renin secretion. However, other factors are apparently involved since in a previous study in a larger group of rabbits we observed that the same dose of milrinone increased plasma renin activity without decreasing blood pressure (8). Moreover, in the present study, intravenous infusion of milrinone at 1.0 μg/kg/min also increased plasma renin activity without decreasing blood pressure.

We (9) and others (10) have previously described an extrarenal effect of beta-adrenoceptor stimulation on renin secretion. Although the mechanisms and pathways involved have not been fully elucidated, it is likely that this extrarenal effect of beta-adrenoceptor stimulation, like the intrarenal action, is mediated by an increase in cyclic AMP concentration. Milrinone could mimic this effect by suppressing the hydrolysis of cyclic AMP at the extrarenal site.

Finally it is worth pointing out that phosphodiesterase III isozymes are inhibitable by cyclic GMP (6,7). This is of potential importance since guanylate cyclase is a major target for nitric oxide (17,18), which has recently been implicated in the control of renin secretion. For example, several investigators have reported that drugs which increase or decrease nitric oxide levels cause marked alterations in renin seretion (19-22). It is possible that these nitric oxide-induced changes in renin secretion are due, at least in part, to alterations in cyclic AMP metabolism.

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