Inhibition of Phosphodiesterase III with Milrinone Increases Renin Secretion in Human Subjects

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ABSTRACT
One of the major signaling molecules involved in the regulation of renin secretion is cyclic AMP (cAMP). The concentration of cAMP in cells is determined both by the rate of cAMP hydrolysis by several families of phosphodiesterases, especially the phosphodiesterase III family, but little is known about the roles of these enzymes in the control of renin secretion, particularly in humans. The aim of the present study was to investigate the effect of the phosphodiesterase III inhibitor milrinone on renin secretion in human subjects. Milrinone was infused i.v. in eight healthy normotensive subjects in a dose of 100 μg/kg. Immediately after the infusion, there was a transient increase in systolic pressure from 107 ± 5 to 116 ± 5 mm Hg (p < .01), but no significant change in diastolic or mean arterial pressure. Heart rate increased from 67 ± 2 to 86 ± 4 beats/min (p < .01) and remained elevated. Plasma renin activity increased in all subjects, the mean value increasing from 3.0 ± 0.5 to 6.0 ± 1.1 ng/ml/h at 15 min (p < .01). These results demonstrate that milrinone increases renin secretion in human subjects, thus providing evidence that phosphodiesterase III family participates in the control of renin secretion in humans. The increase in renin secretion does not appear to be mediated by major mechanisms that control renin secretion, and likely results from an increase in cAMP concentration in the juxtaglomerular cells.

It is now generally accepted that one of the major signaling molecules involved in the regulation of renin secretion is cAMP (cAMP; Reid et al., 1978; Keeton and Campbell, 1980; Hackenthal et al., 1990). The concentration of cAMP in cells is determined both by the rate of cAMP generation by adenylyl cyclase and cAMP hydrolysis by phosphodiesterases (PDEs). Adenylyl cyclase activity in the renal juxtaglomerular cells is stimulated by a variety of neurotransmitters and hormones. Of particular significance in this regard are the catecholamines epinephrine and norepinephrine, which stimulate β1-adrenergic receptors presumed to be located on the juxtaglomerular cells, thus increasing adenylyl cyclase activity and cAMP formation in these cells (Reid et al., 1978; Keeton and Campbell, 1980; Hackenthal et al., 1990; DiBona and Kopp, 1997). cAMP is, in turn, metabolized by a large group of enzymes, the PDEs. These enzymes have been grouped into families based on their biochemical characteristics including their substrate specificity (cAMP versus cyclic GMP), mode of regulation (e.g., calcium, cyclic GMP), kinetic properties, and response to selective inhibitors (Beavo and Reifsnyder, 1990; Conti et al., 1995; Manganiello et al., 1995; Sheth and Colman 1995). Seven families named PDE I-VII have been identified.

Little information is available concerning the role of the different PDEs in the regulation of renin secretion by the juxtaglomerular cells. PDE inhibitors such as theophylline, isobutylmethylxanthine, and papaverine increase renin secretion (Reid et al., 1972; Keeton and Campbell, 1980; Hackenthal et al., 1990), but these drugs are nonspecific in that they inhibit all PDE families and have additional actions unrelated to PDE inhibition (Keeton and Campbell, 1980; Beavo and Reifsnyder, 1990). However, specific inhibitors of most PDE inhibitors are now available and are useful for investigating the physiological functions of these enzymes (Pang, 1988; Wetzel and Hauel, 1988; Beavo and Reifsnyder, 1990; Palacios et al., 1995). Recent studies in this and other laboratories have shown that selective inhibitors of several PDE families, in particular PDE III and IV, increase renin secretion in conscious rabbits (Chiu et al., 1996; Chiu and Reid, 1996) and perfused rat kidneys (Kurtz et al., 1998).

Even less is known about the roles of the different PDEs in the control of renin secretion in humans. There have been reports that PDE III inhibitors such as milrinone alter plasma renin levels in patients with heart failure but the results are conflicting, plasma renin activity having been reported to increase (Packer et al., 1984; Smyth et al., 1986; Uretsky et al., 1986a,b), decrease (Jafri et al., 1990; Mager et al., 1991) or remain unchanged (Cody et al., 1986; Murali et al., 1987; Roth et al., 1987; Rauch et al., 1991).

The aim of the present study was to investigate the effect

ABBREVIATIONS: cAMP, cyclic AMP; PDE, phosphodiesterase.
of the PDE III inhibitor milrinone on renin secretion in healthy human subjects. Milrinone is a bipyridine derivative that selectively inhibits PDE III, the predominant PDE family in myocardium and vascular smooth muscle (Alousi et al., 1983; Colucci et al., 1986; Harrison et al., 1986). Although other mechanisms of action may be elicited at high concentrations in vitro, it appears that the predominant mechanism action of milrinone is the inhibition of PDE, resulting in increased accumulation of cAMP.

Materials and Methods

 Subjects. The studies were performed in eight healthy normotensive subjects (four male and four female) aged 20 to 55 years at the Y. J. Chiu General Hospital, Kaohsiung, Taiwan. All subjects gave their fully informed consent for the procedures. The subjects ingested a low-sodium diet (600 mg/day) for 7 days before the study. Plasma renin activity was measured at the beginning and end of the 7-day period.

 Procedures. The studies were performed with subjects in the supine position. A catheter was placed in an antecubital vein for collection of blood samples for analysis (volume = 3 ml) and administration of the PDE inhibitor or its vehicle. Blood pressure was measured at 5-min intervals with a sphygmomanometer. Mean arterial pressure was calculated as diastolic pressure + 1/3 pulse pressure. Studies were commenced after the subjects had rested in the supine position for 30 to 60 min.

The study began with a 15-min control period during which blood pressure and heart rate were monitored. Blood samples were collected at the beginning and end of the control period. Immediately after the control period, the PDE III inhibitor milrinone (Primacor; Sanofi Winthrop Pharmaceuticals, New York) was injected. The inhibitor was diluted in sterile isotonic saline and infused i.v. in a dose of 100 µg/kg over a 3- to 5-min period. Blood pressure and heart rate were monitored during the 45-min postinjection period and additional blood samples were collected at 15, 30, and 45 min.

On a different day, the procedure was repeated but isotonic saline was injected instead of milrinone. The two studies were performed in random order on successive days. The subject remained on the low-sodium diet until the study was completed.

 Analytical Methods. Plasma renin activity was measured using a radioimmunoassay for angiotensin I, and expressed as nanograms angiotensin I generated per ml plasma per hour incubation at 37°C and pH 6.5 (ng/ml/h) (Menard and Catt, 1972).

Plasma sodium and potassium concentrations were measured using standard techniques

 Data Analysis. Results are expressed as the mean ± S.E.M. In general, data were analyzed using ANOVA for repeated measures (Glantz and Slinker, 1990). When significant changes were detected by ANOVA, the Dunnett test (Glantz and Slinker, 1990) was used to compare experimental values to the control value. Single comparisons within groups were made using the paired t test. Changes were considered to be statistically significant when $p < .05$.

Results

 Low-Sodium Diet. During the 7-day period in which the subjects ingested a low-sodium diet, plasma renin activity increased from 0.9 ± 0.2 to 3.3 ± 0.6 ng/ml/h ($p < .01$; Fig. 1).

Milrinone. The effects of milrinone on systolic and diastolic arterial pressure and heart rate are shown in Fig. 2. Immediately after infusion of milrinone, there was an increase in systolic pressure from 107 ± 5 to 116 ± 5 mm Hg ($p < .01$) followed by a decrease to values not significantly different from the preinfusion values. There were no signifi-
Fig. 3. Effect of milrinone on plasma renin activity. Milrinone or saline vehicle was infused over 3 to 5 min starting at 0 min. Values represent mean ± S.E.M. of observations made in eight subjects. *p < .05; **p < .01 compared with corresponding 0-min value. +p < .05 compared with corresponding saline value.

(p < .05) after infusion of the saline vehicle. At all time points, plasma renin activity was significantly higher after milrinone than after saline.

Milrinone caused no significant changes in plasma sodium (140 ± 1 to 139 ± 1 mEq/l) or potassium (3.4 ± 0.1 to 3.5 ± 0.1 mEq/l) concentrations.

Discussion

Administration of milrinone produced the expected cardiovascular changes. There was a prompt and marked tachycardia, which persisted for the duration of the experiment. There was a transient increase in systolic pressure but no change in diastolic pressure or mean arterial pressure. These responses presumably reflect the combined positive inotropic, chronotropic, and vasodilatory actions of milrinone to increase cardiac output and decrease systemic vascular resistance.

The major finding in the present study was that milrinone increased plasma renin activity in all subjects. Overall, plasma renin activity increased 2-fold. This finding provides evidence that PDE III participates in the control of renin secretion in humans, as it apparently does in experimental animals (see below). The stimulation of the renin-angiotensin system by milrinone may protect against postural hypotension (Smyth et al., 1986).

There have been several reports concerning the effects of PDE III on renin secretion in patients with congestive heart failure but the results are conflicting and difficult to reconcile. Some investigators have observed increases in plasma renin activity in heart failure patients during treatment with PDE III inhibitors. Packer et al. (1984) found that amrinone did not change plasma renin activity during the first 48 h of treatment but caused a significant increase during long-term therapy. However, the increase did not reverse after drug withdrawal and was probably the result of worsening of cardiac performance rather than an effect of amrinone on renin secretion. Uretsky et al. (1986a,b) reported that another PDE III inhibitor enoximone increased plasma renin activity. They suggested that the increase resulted directly from an increase in cAMP levels in the renin-secreting cells and/or the small decrease in renal perfusion pressure. Finally, Smyth et al. (1986) observed that treatment with milrinone for 4 weeks increased plasma renin activity.

In marked contrast, other investigators have observed that treatment with PDE inhibitors suppresses renin secretion. Jafri et al. (1990) observed that the PDE III inhibitor ICI 153,110 decreased plasma renin activity, the largest decrease occurring in patients with high baseline plasma renin activity. The decrease in plasma renin activity preceded changes in cardiac index and systemic vascular resistance and may have contributed to those hemodynamic responses. Mager et al. (1991) also observed decreases in plasma renin activity during 24-h infusions of milrinone.

Finally, there have been reports that plasma renin activity does not change significantly during treatment with several different PDE inhibitors. Rauch et al. (1991) reported a heterogeneous response of plasma renin activity to the PDE inhibitor BM14.478. Plasma renin activity increased markedly in two patients but did not change in most of the group. Negative results have also been obtained during acute or long-term treatment with milrinone (Cody et al., 1986), CI-930 (Murali et al., 1987), and amrinone (Roth et al., 1987).

It is difficult to reconcile these disparate findings concerning the effects of PDE inhibitors on renin secretion. The wide variability in the plasma renin activity response to the drugs presumably reflects a number of factors including the clinical state of the patients, the choice and dose of the inhibitor, and the duration of treatment. The present study was designed to avoid some of these confounding factors by investigating the effect of acute administration of milrinone in healthy human subjects.

The present finding that milrinone increases renin secretion in humans is consistent with data obtained in experimental animals. For example, we have shown that milrinone increases renin secretion in conscious rabbits and also potentiates the renin secretory response to β-adrenergic stimulation (Chiu and Reid 1996). Similarly, inhibition of PDE III by milrinone or trequinsin increases renin release in an isolated perfused rat kidney preparation (Kurtz et al., 1998). Results obtained in conscious rabbits and perfused rat kidneys also indicate that PDE III plays a central role in the alterations in renin secretion produced by nitric oxide and nitric oxide synthase inhibitors (Chiu and Reid, 1996; Kurtz et al., 1998).

On the other hand, administration of olprinone, another PDE III inhibitor, in conscious pigs with heart failure, failed to increase plasma renin activity (Adachi and Tanaka, 1997).

The mechanism by which milrinone increased renin secretion was not investigated in the present study. Because there was no significant change in mean arterial pressure, participation of the renal baroreceptor can probably be ruled out. Sodium excretion was not measured in the present study but it has been reported that milrinone does not alter sodium excretion, at least in heart failure patients (Cody et al., 1986). This would argue against a role for the macula densa in the renin response to milrinone. Finally, there is general agreement in the literature that milrinone does not increase plasma norepinephrine levels, suggesting that it does not increase sympathetic tone (Cody et al., 1986; Uretsky et al., 1986a; Murali et al., 1987; Rauch et al., 1991). Thus it is unlikely that the renin response to milrinone is due to an increase in renal sympathetic nerve activity. It therefore seems reasonable to propose that the stimulation of renin...
secretion by milrinone results from an increase in cAMP levels in the renin-secreting juxtaglomerular cells.

In conclusion, these results demonstrate that milrinone increases renin secretion in healthy human subjects, thus providing evidence that PDE III participates in the control of renin secretion in humans. The increase in renin secretion does not appear to be mediated by the renal baroreceptor, macula densa, or sympathetic nervous system mechanisms regulating renin secretion, and it seems reasonable to propose that the increase results from an increase in cAMP concentration in the juxtaglomerular cells.

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References


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