Effect of Sildenafil on Renin Secretion in Human Subjects

YEONG JEN CHIU* AND IAN A. REID[1]†

*Y.J. Chiu General Hospital, Kaohsiung, Taiwan; and †Department of Physiology, University of California, San Francisco, San Francisco, CA

Sildenafil is a potent and selective inhibitor of the cyclic GMP-specific phosphodiesterase (PDE5) that is very effective in the treatment of male impotence. It inhibits breakdown of cyclic guanosine monophosphate (cGMP) formed in penile smooth muscle cells in response to stimulation by nitric oxide resulting in muscle relaxation. PDE5 is widely distributed in the body, being present in the vasculature, platelets, and kidneys. In the kidney, PDE5 is involved in the regulation of sodium excretion and renin secretion. The aim of the present investigation was to investigate the effect of sildenafil, in doses used clinically, on renin secretion in human subjects. The studies were performed in two groups of healthy normotensive subjects: one in which sodium intake was unrestricted, and one in which sodium intake was restricted to 600 mg/day. Blood pressure and heart rate were monitored throughout the study, and blood samples for the measurement of plasma cGMP and cAMP concentrations and plasma renin activity (PRA) were collected. After control measurements, the subjects ingested a capsule containing sildenafil or placebo. Cardiovascular measurements and blood sampling continued for the next 120 min. Sildenafil had only minor cardiovascular effects. Diastolic pressure tended to be lower and heart rate was generally higher after sildenafil than after placebo, but the differences were small. Sildenafil caused a prompt and sustained increase in plasma cGMP concentration and a more gradual increase in plasma cAMP concentration. After the subjects received placebo, there was a progressive decrease in PRA during the 2-hr observation period, presumably reflecting the circadian rhythm in renin secretion. In contrast, PRA failed to decrease after the subjects received sildenafil, thus indicating that sildenafil exerts a stimulatory action on renin secretion. This action on renin secretion may help explain why sildenafil only has minor effect on blood pressure despite the widespread distribution of PDE5 in vascular tissues.

[Exp Biol Med Vol. 227(8):620–625, 2002]

Key words: Viagra; sildenafil; phosphodiesterase; PDE5 inhibitor; renin secretion; cGMP; cAMP; blood pressure; heart rate

Sildenafil is a potent and highly selective inhibitor of the cGMP-specific phosphodiesterase (PDE5) that has proven to be very effective in the treatment of male impotence (1–3). Sildenafil blocks the breakdown of cGMP formed in response to nitric oxide released from the nonadrenergic, noncholinergic parasympathetic innervation of the corposa cavernosa during sexual stimulation. Cyclic GMP in turn causes relaxation of penile smooth muscle resulting in erection (4–6).

Cyclic GMP is distributed widely in the body and participates in many physiological functions, including stimulation of smooth muscle relaxation, inhibition of platelet aggregation, and initiation of visual signal transduction. These actions can account for some of the side effects of sildenafil, including transient visual disturbances and a slight lowering of arterial blood pressure.

Cyclic GMP has also been implicated in the control of renin secretion by the kidneys. At the present time, the effects of cGMP on renin secretion are incompletely understood: both stimulatory and inhibitory effects have been reported (7–11). There is preliminary evidence that PDE5 inhibitors have a stimulatory effect on renin secretion. For example, dipyridamole increases renin secretion *in vivo* (12), and zaprinast potentiates the stimulation of renin secretion by nitroprusside and 8-bromo-cGMP *in vitro* (13).

Based on these observations, it might be anticipated that sildenafil would alter renin secretion. The aim of the present investigation was to examine the effect of sildenafil on renin secretion in healthy human subjects.

Methods

Subjects. The studies were performed in normotensive subjects aged 20 to 55 years at the Y.J. Chiu General Hospital (Kaohsiung, Taiwan). The subjects were in good health and were not taking any medication. All gave their fully informed consent for the procedures.

Procedures. Two studies were performed. In the first study (n = 10), the sodium intake of the subjects was unrestricted. In the second study (n = 10), sodium intake was restricted to 600 mg/day starting 7 days before the study began.

Each subject received sildenafil and placebo, given in random order on successive days. The subjects did not know

[1] To whom requests for reprints should be addressed at Department of Physiology, Box 0444, University of California, San Francisco, CA 94143-0444. E-mail: ianreid@itsa.ucsf.edu

Received March 26, 2002.
Accepted April 18, 2002.

1535-3702/02/2278-0620$15.00

which treatment they were receiving. The studies were performed between 0800 and 1200 hr and were arranged so that each subject received sildenafil or placebo at the same time on the 2 days.

Unrestricted Sodium Intake. The study was performed with subjects in the supine position. A catheter was placed in an anticubital vein for collection of blood samples for analysis (volume = 3 ml). Blood pressure was measured at frequent intervals with a sphygmomanometer. Studies were commenced after the subjects had rested in the supine position for 30–60 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 end of the control period. Immediately after the control period, the subjects swallowed a capsule containing 50 mg of sildenafil citrate (Viagra; Pfizer, New York, NY). Blood pressure and heart rate were monitored during the following 120-min period and additional blood samples were collected at 30, 60, and 120 min.

On a different day, the procedure just described was repeated in the same subjects, but the capsules contained dextrose instead of sildenafil.

Low Sodium Intake. For this study, the sodium intake of the subjects was restricted to 600 mg/day and the dose of sildenafil was increased to 100 mg. The subjects remained on the low-sodium diet until the study was completed. In all other respects, the protocol was identical to that described for unrestricted sodium intake.

Analytical Methods. Plasma renin activity (PRA) was measured using a radioimmunoassay for angiotensin I, and is expressed as nanograms of angiotensin I generated per milliliter of plasma per hour of incubation at 37°C and pH 6.5 (14). Under most circumstances, measurement of PRA provides a reliable index of changes in renin secretion. The major factor that influences PRA other than renin itself is renin substrate concentration, but changes in substrate concentration are slow to develop and are unlikely to be a factor in the present study.

The concentrations of cAMP and cGMP in plasma were measured by ELISA using kits purchased from Linco Research Inc. (St. Charles, MO). Plasma samples were precipitated with trichloroacetic acid, extracted with water-saturated ethyl ether, evaporated to dryness, and reconstituted in assay buffer. Standards and samples were acetylated to allow detection of the nucleotides in the picomolar range.

Plasma sodium and potassium concentrations were measured using standard techniques.

Data Analysis. Results are expressed as the mean ± SE. In general, data were analyzed using analysis of variance (ANOVA) for repeated measures (15). When significant changes were detected by ANOVA, the Dunnett's test (15) was used to compare experimental values with the control value. Single comparisons within groups were made using the paired t test. Changes were considered to be statistically significant when $P < 0.05$.

Results

Unrestricted Sodium Intake. Sildenafil had only minor effects on blood pressure and heart rate in subjects on unrestricted sodium intake (Fig. 1). There were no significant differences in systolic pressure after sildenafil or placebo. Diastolic pressure tended to be lower after sildenafil than after placebo, but the differences were small. Heart rate increased from 65 ± 2 bpm to 72 ± 2 bpm 105 min after sildenafil ($P < 0.05$), but did not change after placebo.

The effects of sildenafil and placebo on plasma renin activity are summarized in Figure 2. After placebo, plasma renin activity decreased from 1.6 ± 0.3 ng/ml/hr to 1.0 ± 0.2 ng/ml/hr at 120 min ($P < 0.05$). In contrast, plasma renin activity did not change after sildenafil (1.5 ± 0.3 ng/ml/hr to 1.5 ± 0.5 ng/ml/hr).

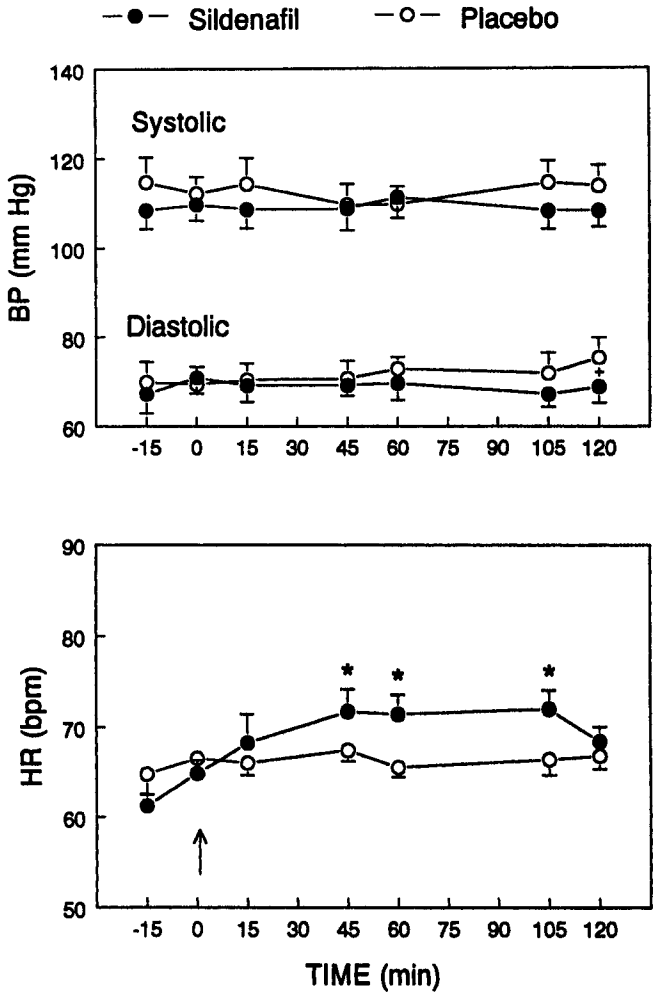

Figure 1. Effects of sildenafil and placebo on blood pressure and heart rate in subjects on unrestricted sodium intake. Sildenafil or placebo was administered at 0 min as indicated by the arrow. Values represent the mean ± SE of observations made in 10 subjects. $^{*}P < 0.05$ compared with the corresponding 0-min control value. $^{+}P < 0.05$ sildenafil compared with placebo.

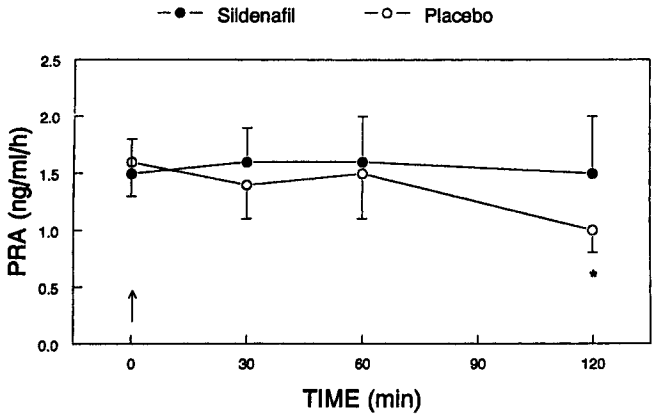

Figure 2. Effects of sildenafil and placebo on plasma renin activity in subjects on unrestricted sodium intake. Sildenafil or placebo was administered at 0 min as indicated by the arrow. *$P < 0.05$ compared with the corresponding 0-min control value.

There were no significant changes in plasma sodium or potassium concentrations after sildenafil or placebo (Table I).

Low Sodium Intake. In the subjects on a low-sodium diet, resting systolic pressure was lower than in the unrestricted sodium group (104 ± 3 mmHg vs 110 ± 4 mmHg, $P < 0.05$), but diastolic pressure was not significantly different (68 ± 4 mmHg vs 71 ± 4 mmHg; Figs. 1 and 3). Heart rate was higher in the low-sodium group (74 ± 3 bpm vs 65 ± 2 bpm, $P < 0.05$). Plasma renin activity was higher in the sodium-restricted group than in the unrestricted sodium group (3.8 ± 1.1 ng/ml/hr vs 1.5 ± 0.3 ng/ml/hr, $P < 0.05$; Figs. 2 and 4). Plasma sodium concentration was lower in the low sodium group, but plasma potassium concentration was not significantly different (Table I).

The effects of sildenafil on arterial pressure and heart rate in the low-sodium group are summarized in Figure 3. Systolic and diastolic pressures were not significantly different after sildenafil than after placebo. Heart rate tended to be higher after sildenafil than after placebo, but there was no significant difference.

The effects of sildenafil and placebo on plasma renin activity are summarized in Figure 4. As in the unrestricted sodium group, plasma renin activity decreased progressively after placebo (3.5 ± 1.4 ng/ml/hr to 1.9 ± 0.7 ng/ml/hr at 120 min, $P < 0.05$), but did not change significantly after sildenafil (3.8 ± 1.1 ng/ml/hr to 3.9 ± 1.4 ng/ml/hr).

The effects of sildenafil on plasma cGMP and cAMP concentrations are summarized in Figure 5. After sildenafil, plasma cGMP concentration increased from 8.4 ± 0.5 pmol/ml to 10.3 ± 0.8 pmol/ml at 60 min ($P < 0.05$) and to 10.6 ± 0.6 pmol/ml at 120 min ($P < 0.05$). There was a slower increase in plasma cAMP concentration from 3.8 ± 0.4 pmol/ml to 5.8 ± 1.3 pmol/ml at 120 min ($P < 0.05$). There were no significant changes in plasma cGMP or cAMP concentrations after placebo (Fig. 5).

There were no significant changes in plasma sodium or potassium concentrations after sildenafil or placebo (Table I).

Dicussion

Cardiovascular. In the present study, sildenafil had only minor effects on blood pressure and heart rate. Diastolic pressure was generally lower after sildenafil than after placebo, but the difference averaged only 2–5 mmHg and was generally not statistically significant. Sildenafil had even less effect on systolic pressure. These results are similar to those reported by others (16–18).

In the present study, sildenafil caused a small increase in heart rate in subjects on unrestricted sodium intake. Other investigators have reported that sildenafil has negligible effects on heart rate (16–18).

Cyclic Nucleotides. In the present study, sildenafil increased plasma cGMP concentration in subjects on restricted sodium intake. This agrees with the results of Jackson *et al.* (17) who observed increases in plasma cGMP concentration following intravenous or oral sildenafil in human subjects. Sildenafil also increases plasma cGMP concentration in awake lambs (19). The site of origin of this cGMP cannot be determined from these data. However, PDE5 is widely distributed in the body in the vasculature, platelets, kidneys, and other tissues (20–22) and is subject to inhibition by sildenafil. Thus, the increase in plasma cGMP could have resulted from inhibition of PDE5 in any or all of these tissues.

Sildenafil also increased plasma cAMP concentration. The increase was slower to develop than the increase in cGMP, reaching statistical significance only at 120 min. Because sildenafil is highly selective for PDE5 (2, 20, 22), it is unlikely that the increase in cAMP resulted from direct inhibition of other phosphodiesterases. One possibility that should be considered is that the increase in cGMP levels inhibited PDE3, the cGMP-inhibited phosphodiesterase, resulting in decreased breakdown of cAMP (23). PDE3 isoenzymes are widely distributed in the heart, vasculature, and kidneys (20–22).

Renin Secretion. In the present study, there were no significant changes in plasma renin activity after sildenafil in either the unrestricted or low-sodium groups. In contrast, there was a progressive decrease in plasma renin activity following placebo in both groups. This decrease presumably reflects the circadian rhythm in renin secretion, which is characterized by high plasma renin activity in the early morning, decreasing progressively to the late afternoon (24–26). The present study was performed during this time period. Because there was no reduction in plasma renin activity after sildenafil, these data indicate that sildenafil exerts a stimulatory effect on renin secretion.

PDE5 isoenzymes are present in the kidneys (27) and may participate in the regulation of sodium excretion under physiological conditions and in sodium retaining states in-

Table I. Effects of Sildenafil and Placebo on Plasma Sodium and Potassium Concentrations in Subjects on Unrestricted or Low Sodium Intake

Sodium intake	Concentration (m*M*)	Control	Placebo	Control	Sildenafil
Unrestricted	Na^+	141 ± 1	141 ± 1	143 ± 1	141 ± 1
	K^+	3.7 ± 0.1	3.8 ± 0.1	3.5 ± 0.1	3.7 ± 0.1
Low	Na^+	138 ± 1*	138 ± 1*	137 ± 1*	138 ± 1*
	K^+	3.5 ± 0.1	3.8 ± 0.1	3.5 ± 0.1	3.8 ± 0.1

Note. * $P < 0.01$ compared with unrestricted sodium intake.

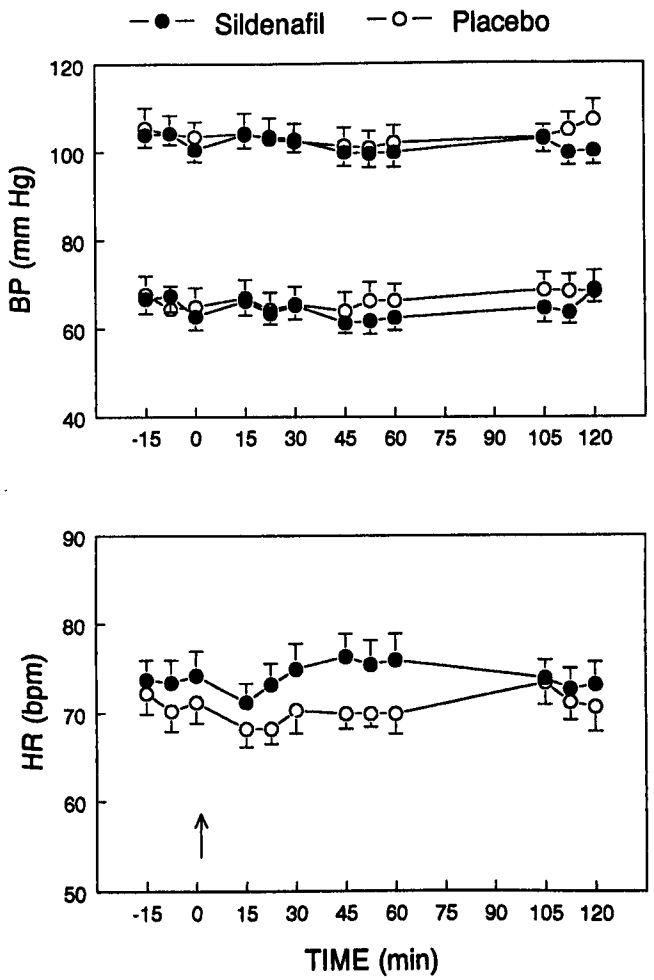

Figure 3. Effects of sildenafil and placebo on blood pressure and heart rate in subjects on restricted sodium intake. Sildenafil or placebo was administered at 0 min as indicated by the arrow.

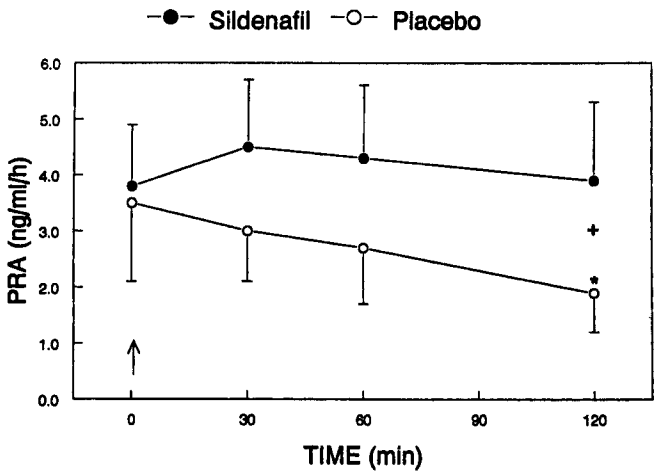

Figure 4. Effects of sildenafil and placebo on plasma renin activity in subjects on restricted sodium intake. Sildenafil or placebo was administered at 0 min as indicated by the arrow. *$P < 0.05$ compared with the corresponding 0-min control value. +$P < 0.05$ sildenafil compared with the corresponding placebo value.

cluding the nephrotic syndrome (21, 28). There is also evidence that PDE5 participates in the control of renin secretion. For example, we have observed that administration of the PDE5 inhibitor dipyridamole increases renin secretion in conscious rabbits (12). In addition, Noble *et al.* (13) have reported that the stimulation of renin secretion by nitroprusside and 8-bromo-cGMP in a dispersed rat kidney cortical cell preparation is potentiated by another PDE5 inhibitor, zaprinast. Thus, the present finding that sildenafil exerts a stimulatory effect on renin secretion is consistent with a role for PDE5 in the regulation of renin secretion in humans.

It seems reasonable to propose that the action of sildenafil to increase renin secretion is due to an increase in cGMP concentration. However, the role of cGMP in the control of renin secretion is somewhat enigmatic, evidence having been presented that cGMP stimulates, inhibits, or has no effect on renin secretion (7, 8, 10, 11). Furthermore, the mechanisms by which cGMP alters renin secretion are not known. Kurtz and Wagner (9) have proposed that cGMP exerts opposing actions on renin secretion: a stimulatory effect through inhibition of cAMP breakdown by PDE3, and an inhibitory effect via activation of cGMP-dependent protein kinase. The former action could account for the effect on renin secretion observed in the present study.

Recent studies by Phillips *et al.* (29) suggest another mechanism by which sildenafil could increase renin secretion. They reported that after a 100-mg dose of sildenafil in normal subjects, muscle sympathetic nerve activity increased by 141%. Sympathetic nerve activity during mental, physical, and cold stresses was also increased by sildenafil, as was plasma norepinephrine concentration. If sildenafil also increases renal sympathetic nerve activity, this could account for the stimulatory effect on renin secretion observed in the present study (30).

The significance of the action of sildenafil on renin secretion is not clear. However, it is interesting to speculate that the resultant increase in the activity of the renin-angiotensin system could serve to counteract or minimize the vasodilation resulting from increased cGMP (and

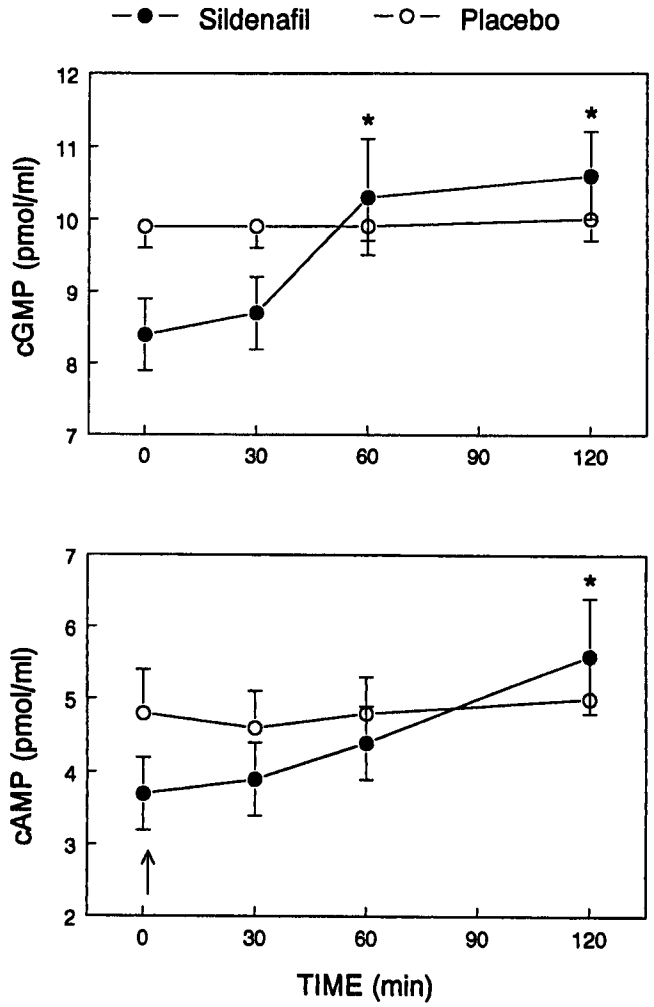

Figure 5. Effects of sildenafil and placebo on plasma cGMP and cAMP concentrations in subjects on restricted sodium intake. Sildenafil or placebo was administered at 0 min as indicated by the arrow. *$P < 0.05$ compared with the corresponding 0-min control value.

cAMP) concentration in the vasculature. This could partly account for the fact that sildenafil has such a minimal effect on blood pressure (16–18). In this regard, it would be of interest to determine if the mild hypotensive action of sildenafil would be amplified by inhibition of the renin-angiotensin system with angiotensin receptor antagonists or converting enzyme inhibitors.

In conclusion, the present results demonstrate that sildenafil exerts a stimulatory effect on renin secretion in humans. This action may help explain why sildenafil generally has only a small effect on arterial pressure.

The excellent technical assistance of Lance Chou and Dina San Juan is gratefully acknowledged.

1. Lue TF. A study of sildenafil (Viagra), a new oral agent for the treatment of male erectile dysfunction. J Urol **157**(Suppl):181, 1997.
2. Corbin JD, Francis SH. Cyclic GMP phosphodiesterase-5: target of sildenafil. J Biol Chem **274**:13729–13732, 1999.
3. Gibson A. Phosphodiesterase 5 inhibitors and nitrergic transmission: from zaprinast to sildenafil. Eur J Pharmacol **411**:1–10, 2001.
4. Ballard SA, Gingell CJ, Tang K, Turner LA, Price ME, Naylor AM. Effects of sildenafil on the relaxation of human corpus cavernosum tissue in vitro and on the activities of cyclic nucleotide phosphodiesterase isozymes. J Urol **159**:2164–2171, 1998.
5. Chuang AT, Strauss JD, Murphy RA, Steers WD. Sildenafil, a type-5 CGMP phosphodiesterase inhibitor, specifically amplifies endogenous cGMP-dependent relaxation in rabbit corpus cavernosum smooth muscle in vitro. J Urol **160**:257–261, 1998.
6. Moreland RB, Goldstein I, Traish A. Sildenafil, a novel inhibitor of phosphodiesterase type 5 in human corpus cavernosum smooth muscle cells. Life Sci **62**:PL309–PL318, 1998.
7. Hackenthal E, Paul M, Ganten D, Taugner R. Morphology, physiology, and molecular biology of renin secretion. Physiol Rev **70**: 1067–1116, 1990.
8. Kurtz A. Cellular control of renin secretion. Rev Physiol Biochem Pharmacol **113**:2–40, 1989.
9. Kurtz A, Wagner C. Cellular control of renin secretion. J Exp Biol **202**:219–225, 1998.
10. Peart WS, Quesada T, Tenyi I. The effects of cyclic adenosine 3′,5′-monophosphate and guanosine 3′,5′-monophosphate and theophylline on renin secretion in the isolated perfused kidney of the rat. Br J Pharmacol **54**:55–60, 1975.
11. Skott O, Jensen BL. Cellular and intrarenal control of renin secretion. Clin Sci **84**:1–10, 1993.
12. Coopersmith PF, Reid IA. Inhibition of phosphodiesterases IV and V increases renin secretion in conscious rabbits. FASEB J **10**:A395, 1996.
13. Noble AR, Abu-Kishk RA, D'Aloia M-AE, Williams BC, Lush DJ. Cyclic GMP-linked pathway for renin secretion. Kidney Int **46**: 1588–1590, 1994.
14. Menard J, Catt KJ. Measurement of renin activity, concentration and substrate in rat plasma by radioimmunoassay of angiotensin I. Endocrinology **90**:422–430, 1972.
15. Glantz SA, Slinker BK. Primer of Applied Regression and Analysis of Variance. New York: McGraw-Hill, 1990.
16. Cheitlin MD, Hutter AM, Brindis RG, Ganz P, Kaul S, Russell RO, Zusman RM, Forrester JS, Douglas PS, Faxon DP, Fisher JD, Gibbons RJ, Halperin JL, Hochman JS, Weintraub WS, Winters WL, Wolk MJ. Use of sildenafil (Viagra) in patients with cardiovascular disease. J Am Coll Cardiol **33**:273–282, 1999.
17. Jackson G, Benjamin N, Jackson N, Allen MJ. Effect of sildenafil citrate on human hemodynamics. Am J Cardiol **83**:13C–20C, 1999.
18. Kloner RA, Zusman RM. Cardiovascular effects of sildenafil citrate and recommendations for its use. Am J Cardiol **84**:11N–17N, 1999.
19. Weimann J, Ullrich R, Hromi J, Fujino Y, Clark MWH, Bloch KD, Zapol WM. Sildenafil is a pulmonary vasodilator in awake lambs with acute pulmonary hypertension. Anesthesiology **92**:1702–1712, 2000.
20. Conti M, Nemoz G, Sette C, Vicini E. Recent progress in understanding the hormonal regulation of phosphodiesterases. Endocrine Rev **16**:370–389, 1995.
21. Dousa TP. Cyclic-3′,5′-nucleotide phosphodiesterase isozymes in cell biology and pathophysiology of the kidney. Kidney Int **55**:29–62, 1999.
22. Wallis RM, Corbin JD, Francis SH, Ellis P. Tissue distribution of phosphodiesterase families and the effects of sildenafil on tissue cyclic nucleotides, platelet function, and the contractile responses of trabeculae carneae and aortic rings in vitro. Am J Cardiol **83**:3C–12C, 1999.
23. Reid IA. Role of phosphodiesterase isoenzymes in the control of renin secretion: effects of selective enzyme inhibitors. Curr Pharm Design **5**:725–735, 1999.
24. Gordon RD, Wolfe LK, Island DP, Liddle GW. A diurnal rhythm in plasma renin activity in man. J Clin Invest **45**:1587–1592, 1966.
25. Modlinger RS, Sharif-Zadeh K, Ertel NH, Gutkin M. The circadian rhythm of renin. J Clin Endocrinol Metab **43**:1276–1282, 1976.
26. Portaluppi F, Bagni B. degli Uberti E, Montanari L, Cavallini R,

Trasforini G, Margutti A, Ferlini M, Zanella M, Parti M. Circadian rhythms of atrial natriuretic peptide, renin, aldosterone, cortisol, blood pressure and heart rate in normal and hypertensive subjects. J Hypertension **8**:85–95, 1990.

27. Kotera J, Fujishige K, Omori K. Immunohistochemical localization of cGMP-binding cGMP- specific phosphodiesterase (PDE5) in rat tissues. J Histochem Cytochem **48**:685–693, 2000.
28. Sekizawa T, Shima Y, Yoshida K, Tanahashi M, Yoshida M, Suzuki-Kusaba M, Hisa H, Satoh S. Effects of zaprinast on renal nerve stimulation-induced anti-natriuresis in anaesthetized dogs. Clin Exp Pharmacol Physiol **25**:1008–1012, 1998.
29. Phillips BG, Kato M, Pesek CA, Winnicki M, Narkiewicz K, Davison D, Somers VK. Sympathetic activation by sildenafil. Circulation **102**:3068–3073, 2000.
30. Reid IA. Renin secretion: old concepts and new theories. In Husain A, Graham RM, Eds. Drugs, Enzymes and Receptors of the Renin-Angiotensin System: Celebrating a Century of Discovery. Amsterdam, The Netherlands: Harwood Academic Publishers, pp231–253, 2000.