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Evidence for β-Adrenergic Regulation of Renal and Extrarenal Plasma Prorenin and Renin in Dogs[1] (42329)

ESTHER A. WILCZYNSKI AND DANIEL H. OSMOND

Department of Physiology, Medical Sciences Building, Faculty of Medicine, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Abstract. Beta blockade with propranolol for 7 days in healthy normotensive dogs produced a sustained 20–25% drop in heart rate, but only a transient suppression of blood pressure. Plasma renin activity and prorenin were also suppressed transiently, suggesting that both are under β-receptor regulation. Bilateral nephrectomy (2NX) was followed by rapid clearance of renin from the circulation, at a rate that was minimally influenced by β blockade. In contrast, the plasma prorenin level rose markedly to a peak within an hour after surgery, leveled off during the next 24 hr, dropped almost toward the pre-2NX baseline by 48 hr, but proceeded to rise again between 48 and 120 hr. Propranolol administration before and during the 2NX period reduced the detectable prorenin, suggesting that its extrarenal source is under β-adrenergic regulation. The rapid increment of prorenin after 2NX suggests that extrarenal prorenin may have constituted part of the total plasma prorenin before 2NX, and/or had developed sufficiently quickly afterwards to replace and exceed the disappearing renal prorenin. Any fresh increment beyond 48 hr could presumably have been only extrarenal. These observations suggest the existence of a rich β-regulated extrarenal source of prorenin capable of rapidly supplying the plasma. However, no renin–angiotensin was apparently produced from this prorenin in the nephrectomized state, implying the lack of renal "convertase," without which the prorenin convertase mechanism as a whole was rendered ineffective. The source of the extrarenal prorenin and the identity of the renal convertase remain to be established.

The presence of circulating prorenin which can be activated to renin *in vitro* has been well documented for human plasma (1–3). More recently, relatively large quantities of activatable prorenin have also been reported in the plasmas of other species, such as dogs (4–6) and rats (6–9), suggesting that plasma prorenin is a more general occurrence, not one restricted only to humans. The questionable or small amounts of prorenin previously found in animal plasmas (10) seems attributable to the inadequacy of the methods employed. In essence, such animal plasmas do not cryoactivate as well as human plasmas and require higher concentrations of trypsin and modified incubation conditions (4–9, 11–15).

The physiology of plasma prorenin, especially its regulation, has not been well defined. β-adrenergic stimulation, induced by postural changes or isoprenaline administration, has shown that while active plasma renin levels are elevated in all cases, prorenin may increase slightly (16), decrease (17), or remain unchanged (18). A similar spread of apparently contradictory responses is seen when the β-blocker propranolol is administered. Whereas active renin dropped consistently, prorenin did not (19–21). Atlas *et al.* reported an increase in prorenin accompanying the decrease in active renin in 15 of 22 hypertensive patients on propranolol therapy (19). However, in anesthetized pigs, Bailie *et al.* demonstrated a decrease in both renin and prorenin during propranolol infusion (22).

Thus, a β-adrenergic component in the regulation of renal and/or extrarenal prorenin release is less well established than is the case for renal renin release (19, 21).

In regard to extrarenal prorenin, there have been several reports of activatable prorenin in the plasma of anephric humans (14, 23–27), dogs (28), and, more recently, rats (29, 30). Although active renin is generally undetectable in anephric humans (except perhaps when the method allows for some inadvertent activation of prorenin to occur during handling), prorenin remains at about 25–30% of the normal level (27). This suggests the existence of an

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extrarenal source of plasma prorenin from which active renin is poorly formed in the circulation when the kidneys are absent (27). Beyond this, little is known about extrarenal prorenin and its mechanism of endogenous activation in humans, or dogs. In rats, several organs and glands appear to have been excluded as production sites but the prime source of circulating prorenin was not pinpointed (29).

We have previously reported that extrarenal prorenin develops rapidly in dog plasma within a few hours of bilateral nephrectomy, reaching or exceeding prenephrectomy levels during the first 120 hr. Concurrently, active renin drops to very low levels within the first 48 hr, changing minimally thereafter, implying no formation of it from the abundant prorenin present (28).

The present study documents the effects of β-adrenergic blockade on plasma prorenin and renin in normal dogs before, and after, bilateral nephrectomy. The purpose was to assess, or re-assess, the role of the β-adrenergic system in renal and extrarenal prorenin/renin regulation.

Materials and Methods. Twelve healthy mongrel dogs (11 male, 1 female) with body weights ranging from 10 to 23 kg were maintained on a standard laboratory diet.

Propranolol (Inderal, Sp. 90559 L 6862-YD, donated by Ayerst Laboratories, St. Laurent, Quebec, Canada) was administered by mouth to 12 dogs, 25 mg/dog/day, in equally divided doses three times a day, at 8 AM, 4 PM, and 10 PM. In a crossover design, the same dogs served as their own controls (placebo), receiving the same volume of the vehicle (water) at the same stated hours. At least 7 days of recovery were allowed between the crossover from the control to the propranolol segments of the experiment.

Five dogs were bilaterally nephrectomized (2NX) after 7 days of placebo treatment, and 7 dogs after treatment with propranolol. These treatments were continued throughout the surgical and postnephrectomy periods. The bilateral nephrectomies (2NX) were performed under halothane anesthesia, both kidneys being removed through a single midline incision. Blood samples were collected at 1, 3, 6, 24, 48, 72, and 96 hr post-2NX in all dogs, and also at 120 hr in two dogs of each group. Pre-2NX samples were collected on Day 7 of the placebo/propranolol protocol from conscious dogs before surgery.

Venous blood samples were taken for prorenin and renin measurements between 2 and 3 PM on Days 1, 4, and 7 of each series. Blood pressure and heart rate determinations were made between 3 and 4 PM on Days 3 and 6.

The blood samples were collected by venipuncture from conscious, quiet dogs, into EDTA (ethylenediamine tetraacetate, ammonium form, 15% solution in water, 4.5 mg/ml blood). After centrifugation of the blood at 2000*g*, 4°C, for 20 min, the plasmas were separated and stored at −60°C.

Plasma renin activity (PRA) was determined by radioimmunoassay of angiotensin I using a kit (Dupont–New England Nuclear Medical Diagnostics Division, North Billerica, Mass.), as described previously (5, 14, 31). Briefly, plasma samples were incubated at 37°C, pH 6.0, for 15 and 30 min, and angiotensin I generation was expressed in nanograms (ng) per milliliter plasma per hour.

Prorenin was activated to renin by incubating with trypsin (3 mg trypsin/ml plasma, Sigma type III, 10,000 BAEE units/mg protein) for 10 min at 23°C (5), prior to PRA determination. Prorenin was calculated by subtracting the PRA of control unactivated plasma from the higher PRA obtained after activation.

For determination of blood pressure and heart rate, the dogs were previously trained and acclimatized in a quiet room so as to remain tranquil during the procedure. A photoelectric cell connected to a preamplifier–amplifier system (Sanborn 350-3200A) was tightly applied to a shaved portion of the animal's tail over the caudal artery. A blood pressure cuff (neonatal cuff, sized for circumference 3.5–7.0 cm, Critikon Inc., Tampa, Fla.) was placed proximal to the light source. Pressure was determined using a mercury manometer and blood flow was recorded on a 7700 series Sanborn chart recorder.

Statistical analysis was by two-way analysis of variance (ANOVA) available as a commercial computer program by Statistical Analysis System Institute Inc. (SAS, Cary, N.C.).

Results. *Intact dogs.* Propranolol at 25 mg/day produced a mean decrease of 20% in heart rate by Day 3, falling to 24% by Day 6, thereby

indicating the presence of significant β-blockade. Systolic blood pressure decreased slightly, but significantly, by the third day of propranolol treatment, returning to the normal control level by Day 6.

The effect of propranolol administration on PRA was variable; suppression was evident in 8 of 12 dogs on the first day, and in the remaining 4 by the fourth day. No blood samples were collected for PRA determinations on Days 2 and 3. The day on which such suppression of PRA was first apparent in each dog represented the "early" PRA, as shown in Fig. 1. By Day 7, PRA had returned to normal levels in all dogs ("late" PRA, Fig. 1). Thus, propranolol-induced suppression of renin release, as reflected in the plasma, was seen only in the early days of treatment of these normotensive dogs, with restoration to normal PRA by the seventh day.

A similar pattern was seen in the response of plasma prorenin, which fell in all the dogs by Day 4 of propranolol administration ($P < 0.05$), and returned to normal by Day 7 (Fig. 2). Thus, propranolol appeared to suppress circulating prorenin in parallel with its suppression of renin, suggesting that it, too, is under β-control.

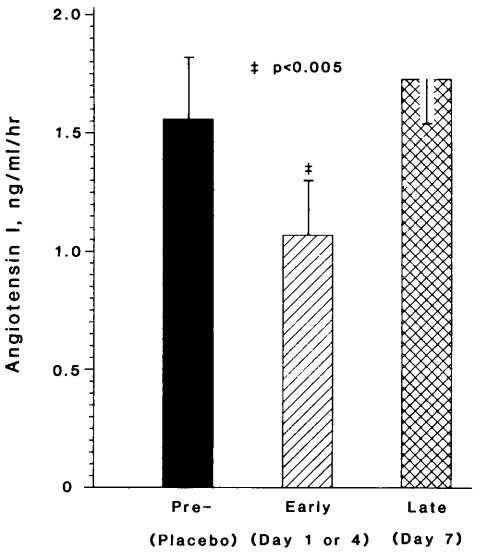

FIG. 1. Plasma renin activity in normotensive dogs, during either placebo or propranolol (25 mg/day) treatment. Average PRA (means ± SEM) in terms of angiotensin I generation, in untreated plasmas from 12 dogs. "Early" PRA represents minimum PRA values, obtained on either Day 1 or Day 4 of propranolol administration; "late" PRA represents values obtained on Day 7.

Nephrectomized dogs. Active renin (PRA) fell rapidly in both placebo-treated and propranolol-treated dogs, suggesting that β-blockade did not alter the rate of renin clearance from the circulation after 2NX (Fig. 3). The lowest PRA values were seen at 48 hr postnephrectomy. Between 48 and 120 hr, PRA tended to increase in the placebo, but not in the propranolol-treated group, causing a difference which appeared to reach its widest point at 72 and 120 hr postnephrectomy (Fig. 3).

Plasma prorenin increased markedly after nephrectomy in the placebo-treated dogs (Fig. 4). Here, the highest prorenin values were obtained at 1 hr post-2NX, with a return to pre-2NX levels by 48 hr, a pattern which substantially confirms our earlier observations (30). In the propranolol-treated group, however, this early "surge" in plasma prorenin activity was greatly attenuated, with prorenin remaining essentially unchanged throughout the 2NX period (Fig. 4). Between 48 and 120 hr, a slight additional "hump" of prorenin appeared in the placebo group, which must have come from an extrarenal source, and which further contributed to the maintenance of the prorenin level at, or above, the prenephrectomy baseline. This hump did not appear in the propranolol-treated dogs, suggesting that both the early and late release of extrarenal prorenin were considerably suppressed by β-blockade.

Discussion. The purpose of this study was to investigate β-receptor regulation of plasma prorenin, renal and extrarenal, in dogs. Very little information about such regulation in the dog is available because a satisfactory method for prorenin estimation in this species has only recently been established (4, 5). A higher concentration of trypsin is required for prorenin activation in dog plasma as compared with that in human plasma, and a higher prorenin estimate is obtained, namely a prorenin:renin ratio of 50:1, or greater, as compared with about 10:1 in humans (4, 5).

Propranolol, by mouth, to our conscious normotensive dogs produced an expected suppression of heart rate (approximately 20–25%) which was clearly evident within 3 days, and persevered into the sixth, and probably also the seventh day. Mean arterial blood

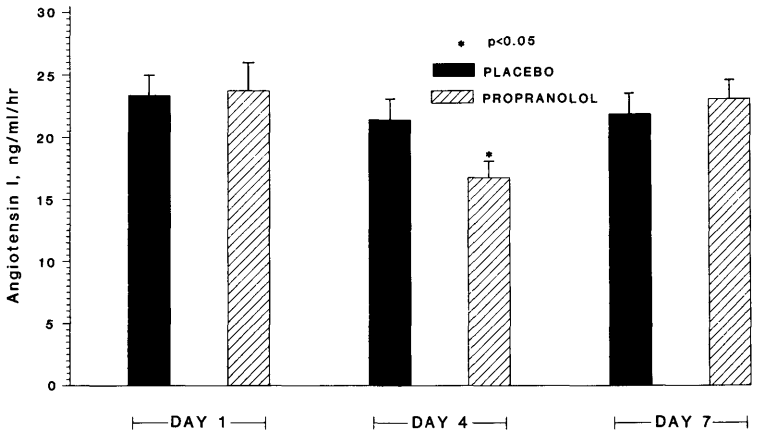

FIG. 2. Plasma prorenin in dogs during treatment with either placebo or propranolol. Prorenin was activated with trypsin, 3 mg/ml plasma, 10 min, 23°C, followed by PRA determination. Average values (±SEM) from 12 dogs.

pressure was only slightly lowered on Day 3. Although the drop was statistically significant, it did not last until Day 6. Plasma renin activity (PRA, active renin) also dropped early and then returned to normal (Fig. 1).

These results are in essential agreement with those of Nies *et al.* (32), using anesthetized dogs, in which propranolol induced no lasting drop in blood pressure in spite of the decrease in heart rate and stroke volume. Apparently, the effect of these cardiovascular changes was offset by a compensatory increase in total peripheral resistance—including renal vascular resistance—which would remove the homeostatic need for additional systemic renin, as reflected by an elevated PRA. Thus, PRA appears to normalize more or less in step with blood pressure in our experiment.

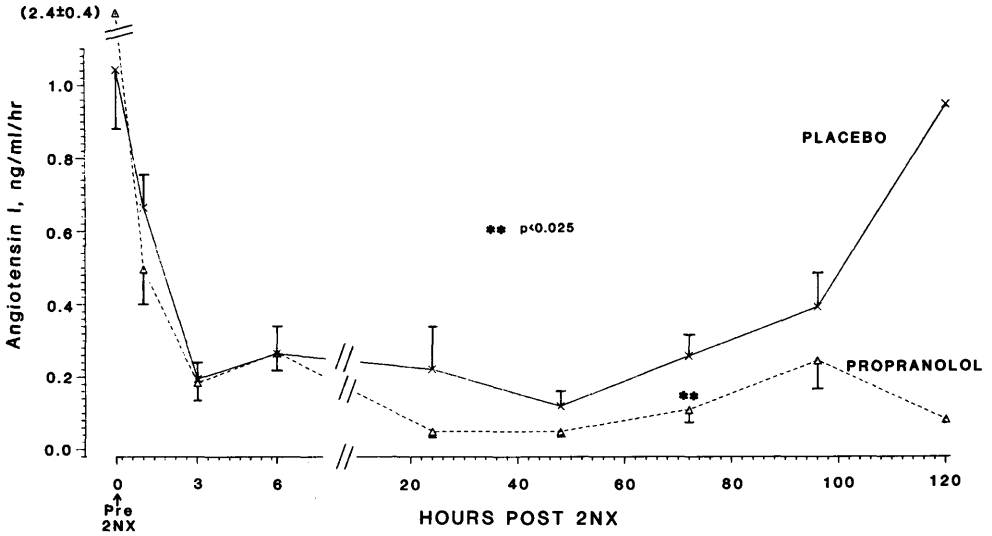

FIG. 3. Effect of binephrectomy (2NX) on plasma renin activity in normotensive dogs receiving placebo (n = 5) or propranolol (25 mg/day, n = 7). Pre-2NX values were obtained from Day 7 of prior placebo or propranolol treatment. Average PRA values (±SEM) are expressed in terms of angiotensin I generation.

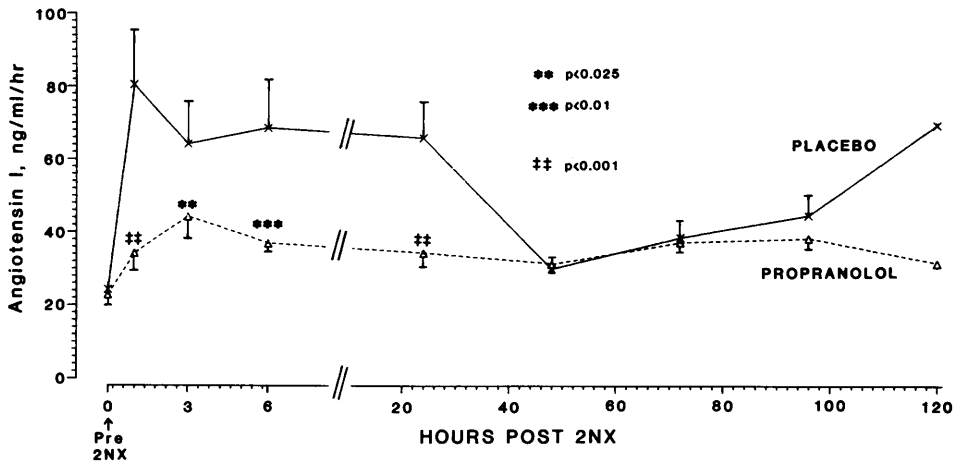

FIG. 4. Effect of binephrectomy on plasma prorenin (activated with trypsin, 3 mg/ml plasma, 10 min, 23°C) in normotensive dogs receiving placebo (n = 5) or propranolol (25 mg/day, n = 7). Average values (±SEM) are in terms of angiotensin I generation.

It is well known that β-blockade reduces renal renin release (33–36), but it is not as clear whether the same applies to prorenin (19–21). We observed that propranolol lowered plasma prorenin significantly in control (nephric) dogs within the first 4 days only, with a subsequent return to the normal baseline in parallel with renin (Fig. 2).

These data suggest a β-adrenergic-mediated control mechanism for renal prorenin release, analogous to that for renin, and are supported by similar observations in anesthetized pigs (22), while divergent findings are largely based on hypertensive human subjects, in whom the disease state may have modified the prorenin–renin response to propranolol (19–21).

The effect of bilateral nephrectomy is of special interest. We initially expected the disappearance of all prorenin from the circulation within a few hours of the operation. Instead, we observed a postoperative rise in plasma prorenin concentration, which we first thought might be the accumulated consequence of surgical stress experienced prior to actual removal of the kidneys (28, 37, 38). It now appears more likely that extrarenal prorenin either coexists with renal prorenin in the circulation before the kidneys are removed, and/or that its rate of release from an extrarenal source increases rapidly thereafter. Thus, whereas plasma renin falls to 25% of its original level within 3 hr of nephrectomy (Fig. 3, placebo group), prorenin rises significantly beyond the preoperative baseline at 1 hr after the nephrectomy and remains high during the subsequent 24 hr (Fig. 4, placebo group).

The interpretation of these data hinges partially on the relative clearance rates for renin and prorenin. The present renin clearance rate (Fig. 3) seems to agree with the 30 min half life reported previously in dogs (39), and the range 30–330 min reported in humans (40, 41). Renin activity drops more than 80% within 3 hr. The fourfold rise in prorenin within about 1 hr after 2NX (Fig. 4), and the fact that it was substantially maintained during the next 24 hr, suggests appearance of extrarenal prorenin rather than reduced clearance. Thus, the high plasma prorenin seems not to be merely a postsurgical stress phenomenon but rather a consequence of 2NX (43, 43). This argument is further strengthened by the second apparent rise in prorenin beyond the 48-hr mark (Fig. 4).

It is noteworthy that the production of renin after 2NX was minimal despite the availability of appreciable amounts of prorenin (renal and/or extrarenal). This lack of renin formation implies that plasma prorenin is minimally activated when the kidneys are absent, further confirming our previous observations regarding an important renal component necessary for systemic prorenin conversion (44).

Finally, the influence of propranolol on

renal and extrarenal prorenin release may be deduced from data in Fig. 4. First, the pre-nephrectomy prorenin level in the propranolol-treated group was lower than in the placebo group at 4 days (Fig. 2), but rose to normal by the seventh day. Second, the prorenin "surge" immediately after nephrectomy (whatever its source) was greatly attenuated. Third, propranolol blocked much of the expected progressive rise in prorenin between 48 and 120 hr after nephrectomy (compare placebo and propranolol groups, Fig. 4).

The effects of β-blockade with propranolol on renin are shown in Fig. 3. It did not influence the drop in renin activity significantly in the first 48 hr, but seemed to attenuate the unexplained rise that occurred in the later stages of 2NX, the significance of which is unclear. On the other hand, propranolol substantially prevented the rise in prorenin that took place after 2NX, but did not depress the level below the pre-2NX baseline (Fig. 4). This is either due to an inadequate dose of propranolol for complete blockade, or to a non-β-regulated component of prorenin release, which served to sustain the observed plasma level. Some support for our data derives from the observation that the β-agonist isoproterenol causes measurable plasma prorenin to rise in humans (45).

In conclusion, we have confirmed dynamic, physiologically responsive changes in plasma prorenin levels in the dog. Renal prorenin, like renin, appears to be substantially, but not entirely, under β-adrenergic regulation. An extrarenal supply of prorenin may coexist with the renal material before nephrectomy, and develop to an even greater extent after nephrectomy. This extrarenal prorenin is also β-regulated to a substantial degree, but it is not clear whether this applies also to the mechanism for its conversion to renin in the blood.

1. Overturf ML, Druilhet RE, Kirkendall WM. Renin: Multiple forms and prohormones. Life Sci **24**:1913–1924, 1979.
2. Leckie BJ. Inactive renin: An attempt at a perspective. Clin Sci **60**:119–130, 1981.
3. Inagami T, Murakami K. Prorenin. Biomed Res **1**: 456–475, 1980.
4. Wilczynski EA, Osmond DH. The dog as a model for the human "prorenin"/renin system. Physiologist **23**: 241, 1980.
5. Wilczynski EA, Osmond DH. Plasma prorenin in humans and dogs: Species differences and further evidence of a systemic activation cascade. Hypertension **5**:277–285, 1983.
6. Osmond DH, Wilczynski EA, Loh AY. Peculiarities of plasma "prorenin" measurements in man, dog, and rat, and their theoretical implications. Clin Exp Hypertens Part A **4**:2213–2230, 1982.
7. Barrett JD, Eggena P, Sambhi MP. Activation of rat plasma renin. Endocrinology **108**:778–785, 1981.
8. Barrett JD, Eggena P, Sambhi MP. Trypsin activable renin in rat plasma. In: Sambhi MP, ed. Heterogeneity of Renin and Renin-Substrate, New York, Elsevier–North Holland, p73–81, 1981.
9. Ioannou P, Loh AY, Osmond DH. Increased activation of rat plasma prorenin in a high renin state. IRCS Med Sci **11**:648–649, 1983.
10. Osmond DH, Cooper RM. Cryoactivation and tryptic activation of blood "prorenin" in normal man and animals. Canad J Physiol Pharmacol **56**:792–805, 1978.
11. Osmond DH, Ross LJ, Scaiff KD. Increased renin activity after cold storage of human plasma. Canad J Physiol Pharmacol **51**:705–708, 1973.
12. Gallagher JF, Laragh JH, Atlas SA, Sealey JE. Effect of trypsin or acid treatment on dog plasma renin activity measurements. Endocrinology **107**:147–154, 1980.
13. Cooper RM, Osmond DH, Scaiff KD, Ross LJ. Increase in angiotensin I production upon incubation of human plasma with trypsin. Fed Proc **33**:584, 1974.
14. Cooper RM, Murray GE, Osmond DH. Trypsin-induced activation of renin precursor in plasma of normal and anephric man. Circ Res **40**(Suppl I):171–179, 1977.
15. Osmond DH, Scaiff KD, Cooper RM, Ross LJ. Trypsin-induced increase in human plasma renin activity. Canad Fed Biol Soc **17**:24, 1974.
16. Rumpf KW, Schachterle B, Schmidt S, Necker K, Scheler F. Different responses of active and inactive plasma renin to various stimuli. Clin Sci Mol Med **55**:155s–157s, 1978.
17. Derkx FHM, van Gool JMG, Wenting GJ, Verhoeven RP, Man in 't Veld AJ, Schalekamp MADH. Inactive renin in human plasma. Lancet **2**:496–499, 1976.
18. Millar JA, Leckie BJ, Semple PF, Morton JJ, Sonkodi S, Robertson JIS. Active and inactive renin in human plasma: Renal arteriovenous differences and relationships with angiotensin and renin-substrate. Circ Res **43**(Suppl I):120–127, 1978.
19. Atlas SA, Sealey JE, Laragh JH, Moon C. Plasma renin and "prorenin" in essential hypertension during sodium depletion, beta-blockade, and reduced arterial pressure. Lancet **2**:785–789, 1977.
20. McKenzie JK, Reisin E. Acid-activated renin responses to hydrochlorthiazide, propranolol, and indomethacin. Clin Sci Mol Med **55**:151s–153s, 1978.
21. Birkenhager WH, De Leeuw PW, Falke HE, van Soest

GAW. Renin secretion by the human kidney. Clin Sci Mol Med **55**:147s–149s, 1978.
22. Bailie MD, Derkx FHM, Schalekamp MADH. Release of active and inactive renin by the porcine kidney. Circ Res **44**:32–37, 1979.
23. Sealey JE, Moon C, Laragh JH, Atlas SA. Plasma prorenin in normal, hypertensive, and anephric subjects and its effects on renin measurements. Circ Res **40**(Suppl I):41–45, 1977.
24. Sealey JE, White RP, Laragh JH, Rubin AL. Plasma prorenin and renin in anephric patients. Circ Res **41**(Suppl II):17–21, 1977.
25. Weinberger MH, Wade MB, Aoi W, Usa T, Dentino M, Luft F, Grim CE. An extrarenal source of "renin-like" activity in anephric man. Circ Res **40**(Suppl I): 1–4, 1977.
26. Purdon AD, Loh AY, Osmond DH. Activation of plasma prorenin in anephric men. Fed Proc **38**:1302, 1979.
27. Osmond DH, Loh AY. Effects of pregnancy, oral contraceptives, and binephrectomy, on human plasma "prorenin" and its activator(s). Clin Exp Hypertens Part A **4**:2385–2402, 1982.
28. Wilczynski EA, Osmond DH. Effects of binephrectomy on plasma prorenin-renin in the dog, and evidence of extra-renal prorenin. IRCS Med Sci **10**:756–757, 1982.
29. Doi Y, Franco-Saenz R, Mulrow PJ. Evidence for an extrarenal source of inactive renin in rats. Hypertension **6**:627–632, 1984.
30. Maddedu P, Glorioso N, Dessi-Fulgheri P, Oppes M, Tonolo G, Bandiera F, Rappeli A. Changes in circulating active and inactive renin after bilateral nephrectomy in the rat. IRCS Med Sci **12**:1143–1144, 1984.
31. Osmond DH, McFadzean PA, Scaiff KD. Renin activity by radioimmunoassay: The plasma incubation step. Clin Biochem **7**:52–63, 1974.
32. Nies AS, McNeil JS, Schrier RW. Mechanism of increased sodium reabsorption during propranolol administration. Circulation **44**:596–604, 1971.
33. Buhler FR, Laragh JH, Baer L, Vaughan ED, Brunner HR. Propranolol inhibition of renin secretion: A specific approach to diagnosis and treatment of renin-dependent hypertensive disease. N Engl J Med **287**: 1209–1214, 1972.
34. Michelakis AM, McAllister RG. The effect of chronic adrenergic receptor blockade on plasma renin activity in man. J Clin Endocrinol **34**:386–394, 1972.
35. Assaykeen TA, Clayton PL, Goldfein A, Ganong WF. Effect of alpha- and beta-adrenergic blocking agents on the renin response to hypoglycemia and epinephrine in dogs. Endocrinology **87**:1318–1322, 1970.
36. Yun JCH, Gill JR, Bartter FC, Kelly GD, Keiser HR. Effect of bradykinin on renal function in dogs treated with indomethacin or propranolol. Renal Physiol **5**: 31–43, 1982.
37. Wilczynski EA, Osmond DH. Effect of propranolol on plasma prorenin/renin in nephrectomized dogs. Fed Proc **42**:317, 1983.
38. Wilczynski EA, Osmond DH. Prorenin as part of the systemic renin-angiotensin system is responsive to β-blockade and nephrectomy. Clin Invest Med **6**(Suppl 1):40, 1983.
39. Staessen J, Lijnen P, Fagard R, Amery A. Active and inactive renin in dog plasma before and after bilateral nephrectomy. Experientia **37**:785–786, 1981.
40. Amery AKPC, Hannon RC. Plasma-renin after binephrectomy. Lancet **1**:679, 1969.
41. Derkx FHM, Wenting GJ, Man in 't Veld AJ, Verhoeven RP, Schalekamp MADH. Control of enzymatically inactive renin in man under various pathological conditions: Implications for the interpretation of renin measurements in peripheral and renal venous plasma. Clin Sci Mol Med **54**:529–538, 1978.
42. Wilczynski EA, Osmond DH. Evidence of a prorenin "convertase" which is stimulated by surgery and absent after bilateral nephrectomy in dogs. Canad J Physiol Pharmacol **63**:A31, 1985.
43. Wilczynski EA, Osmond DH. Extrarenal prorenin in plasma requires an activator of renal origin. Hypertension **7**:845, 1985.
44. Wilczynski EA, Osmond DH. Extrarenal prorenin in plasma requires an activator of renal origin. Hypertension **6** (Suppl II), June 1986.
45. Hsueh WA, Goldstone R, Carlson EJ, Horton R. Evidence that the β-adrenergic system and prostaglandins stimulate renin release through different mechanisms. J Clin Endocrinol Metab **61**:399–403, 1985.

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