Increase of Aldosterone Secretion in Adrenal Cortex Suspensions Derived from Pregnant Rats (44002)

MICHÈLE BROCHU,[1] JEAN-PIERRE GAUVIN, AND JEAN ST-LOUIS

Research Center, Sainte-Justine Hospital, and Department of Obstetrics-Gynecology, University of Montreal, Montreal, Quebec, Canada H3T 1C5

Abstract. **Plasma aldosterone levels increase markedly during pregnancy, but not in proportion to the rise in plasma renin activity (PRA). We have developed a reliable *in vitro* method to investigate aldosterone secretion during pregnancy. With this method, we have assessed the potency and effectiveness of ACTH and potassium to stimulate this secretion during pregnancy. Adrenal capsules from pregnant and nonpregnant rats were incubated in 1 ml of culture medium within wells of tissues culture plates. The cortex was transferred every 20 min to another well containing fresh medium with or without ACTH or potassium. Basal and stimulated aldosterone secretions were not significantly affected by time under our experimental conditions. The glands remained responsive to stimulants throughout the study period (360 min). Plasma aldosterone levels and PRA were increased during pregnancy. Basal aldosterone secretion in adrenal cortex suspensions from pregnant rats showed a 1.6-fold increment ($P < 0.001$) in comparison with nonpregnant controls. The dose-response curves of ACTH were not significantly different between pregnant and nonpregnant animals. However, sensitivity to potassium was significantly reduced during pregnancy, as demonstrated by an elevated ED_{50} (4.01 ± 0.08 vs 4.71 ± 0.07 m*M* for nonpregnant versus pregnant rats respectively, $P < 0.001$). These data indicate that adrenal cortex suspensions are a reliable and reproducible way to study aldosterone secretion during pregnancy. They reveal that, during pregnancy, sensitivity of potassium to stimulate aldosterone secretion is decreased while the response to ACTH is not affected.**

[P.S.E.B.M. 1996, Vol 212]

Pregnancy is associated with a substantial increase of circulating aldosterone and with changes in sodium and water homeostasis. Aldosterone is known to be one of the most potent steroids regulating electrolyte balance. Its secretion by the adrenal probably plays a role in volume homeostasis in normal pregnancy and in hypertensive states accompanying pregnancy. In women experiencing pregnancy-induced hypertension, however, aldosterone levels are reduced compared with those in the clinically normal pregnant women (1, 2). The increased activity of the renin-angiotensin system (RAS), in particular the increase in plasma renin activity (PRA), cannot alone explain the elevated aldosterone levels in the second and third trimesters of normal pregnancy (3–5). However, plasma aldosterone seems to be correlated with angiotensin II (Ang II) levels in preeclamptic pregnant women (6). The mechanism by which aldosterone secretion is regulated during normotensive pregnancy is still poorly documented.

In rodents, pregnancy is accompanied by decreased blood pressure (7) and increased plasma volume (8–10). In the rat, plasma aldosterone levels are augmented during pregnancy (11–13). Changes in PRA are, however, controversial, with some authors reporting increases (10, 13) when compared with nonpregnant values and others not (14, 15). In all instances, RAS activity did not match the rise in aldo-

[1] To whom requests for reprints should be addressed at Centre de recherche, Hôpital Ste-Justine, 3175 Côte Ste-Catherine, Montréal, Québec, Canada H3T 1C5.

Received July 20, 1994. [P.S.E.B.M. 1996, Vol 212]
Accepted January 18, 1996.

0037-9727/96/2122-0147$10.50/0

sterone levels. Aldosterone secretion by the adrenals is influenced by factors other than the RAS, such as ACTH, K^+, atrial natriuretic peptide (ANP), etc. Furthermore, these factors coupled with pregnancy may alter adrenal secretory activity.

The objective of the present report was to investigate the effect of gestation on aldosterone secretion stimulated by ACTH and potassium in the rat. To perform this study, we developed an *in vitro* method using rat adrenal capsule suspensions. This technique minimizes the time between sacrifice of the animal and the experimental procedure. Furthermore, under these conditions, adrenal capsular cells stay in their own tissue environment, making our protocols physiologically relevant.

Materials and Methods

Adrenal Capsule Suspensions. Female Sprague-Dawley rats (Charles River Canada, St-Constant, Quebec, Canada) weighing 225–250 g were mated with males. The day on which spermatozoa were found in morning vaginal smears was labeled Day 1 of pregnancy; the experiments were performed on Day 21–22 of gestation. Nonpregnant rats were randomly used during the estrous cycle to serve as controls. All animals were housed under controlled light and temperature. They were fed a normal synthetic diet containing 130 mEq/kg sodium and 190 mEq/kg potassium. Their adrenals were harvested immediately after decapitation. This study received approval from the local committee, which is accredited by the Canadian Council on Animal Care.

Animals were sacrificed by decapitation, and trunk blood was rapidly collected for plasma aldosterone and PRA measurements. Adrenal glands were isolated and freed of fat and adhering tissues. An incision was made in the adrenal capsule. This allowed the middle part of the gland to be removed by being pulled away, and the zona fasciculata/reticularis was then peeled away from the capsule. Adrenal capsules were weighed. Both capsules from each rat were equilibrated on a shaking plate, for 120 min in 5 ml of F12 medium (Gibco, Gaithersburg, MD) with 0.12% sodium bicarbonate, 0.2% BSA, and 1.25 mM Ca^{++}, pH 7.3, at 37°C in an atmosphere of 95% air and 5% CO_2. The F12 medium already contains 3.0 m*M* of potassium. The concentration of potassium expressed in this paper is the total concentration (i.e., including the 3.0 m*M* present in the medium). After equilibration, the different experimental protocols were followed in 12-well culture plates (Sarstedt, St-Laurent, Quebec, Canada). Every 20 min, the capsules were transferred to the next well, each containing 1 ml of the above medium with or without the stimulating agents (K^+ from Fisher (Montreal, Quebec, Canada), ACTH from Peninsula (Belmont, CA), or Ang II from Hukabel Scientific, St-Laurent, Quebec, Canada). The first two wells (time: 140 and 160 min) after the 120 min of equilibration were used to assure the stability of basal secretion before beginning the experimental protocols.

Aldosterone, Corticosterone, and PRA Measurements. Plasma aldosterone was measured by radioimmunoassay as described elsewhere (16). Plasma was extracted by the solid-phase procedure using C18 Sep-Pak cartridges (Millipore, Waters, Montreal, Quebec, Canada) before the assay. Media from adrenal cortex suspensions were measured directly. Plasma aldosterone concentration was expressed in pmol/ml while secretion in media was expressed in pmol/capsule/minute. Corticosterone secretion in adrenal cortex suspensions was measured directly with a radioimmunoassay kit (Immunocorp, Montreal, Quebec, Canada). PRA was determined indirectly by radioimmunoassay of angiotensin I generated during a 2-hr incubation period (17).

Analysis. Basal secretion with time was compared in the adrenals of pregnant and nonpregnant rats by two-way analysis of variance (ANOVA). Plasma aldosterone levels, PRA and weights of the capsules in pregnant and nonpregnant rats were compared by Student's *t* test. Dose-response curves were analyzed by weighted nonlinear least squares regression employing a four-parameter logistic equation. This method provides estimates of basal and maximal responses, the ED_{50} as well as the slope factor for each dose-response curve. ED_{50} values of the dose-response curves were compared by the partial *F* test (18).

The results are expressed as mean response and standard error of the mean (SEM) of at least three experiments (*n* given below).

Results

Basal Secretion. The weights of the adrenal cortex were not significantly different between pregnant and nonpregnant rats (11.3 ± 0.3 vs 11.4 ± 0.4 mg, respectively; $n = 36$ for each group). After equilibration, basal aldosterone secretion was measured for 180 min (time: 180 to 360 min). Figure 1 shows that basal aldosterone secretion from the adrenal capsules of pregnant and nonpregnant rats decreased with time. However, this diminution was not statistically significant by two-way ANOVA ($n = 11$ for each group) within each group. On the other hand, basal aldosterone secretion in the capsules of pregnant rats showed a 1.6-fold increase over the nonpregnant rats ($P < 0.001$) (Fig. 1). This difference in basal secretion persisted until 360 min after decapitation.

Stimulated Secretion. In early experiments, to assess the effect of potassium on aldosterone secretion, the adrenals were continuously stimulated for 200–360 min ($n = 3$) with 9 m*M* of potassium, a concentration that gave maximal response. The level of

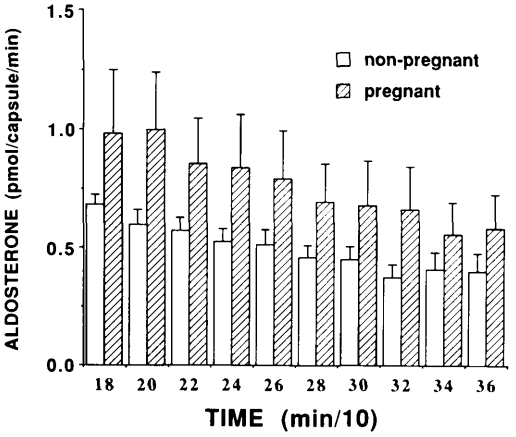

Figure 1. Basal aldosterone secretion with time in adrenal cortex suspensions derived from pregnant and nonpregnant rats. After equilibration, basal secretion was measured for 180 min in nonpregnant and pregnant rats (n = 11 for each group), whose adrenal glands were transferred to the next well every 20 min.

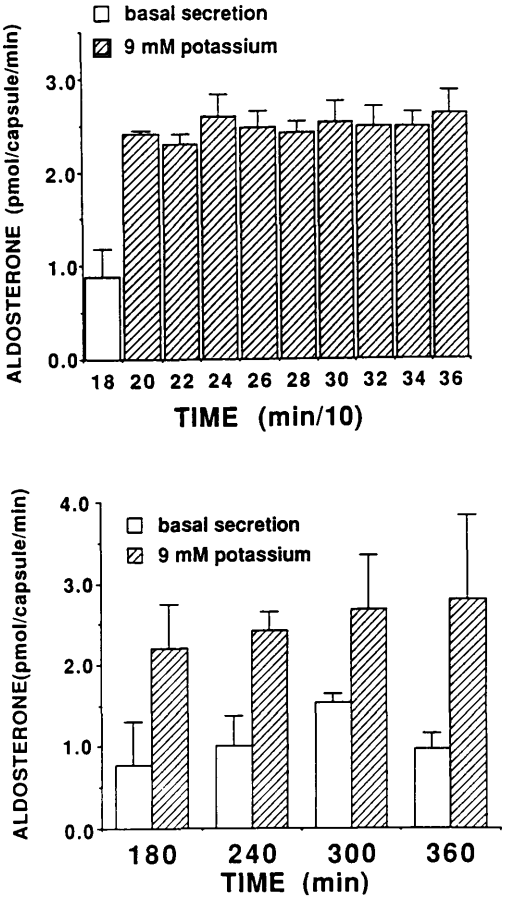

Figure 2. Aldosterone response to 9 mM of potassium in adrenal cortex suspensions derived from nonpregnant rats. (upper panel) Basal secretion was measured at 180 min. From 180 to 360 min, the capsules were continuously stimulated with 9 mM of potassium. (lower panel) Basal secretion (20 min) preceded each stimulation period (20 min) at 180, 240, 300, and 360 min. Between each stimulation, there was an unstimulated period of 40 min.

stimulation was maintained throughout the experiment in adrenal cortex suspensions derived from nonpregnant rats (Fig. 2, upper panel). In Figure 2 (lower panel), the glands were repeatedly stimulated at 180, 240, 300, and 360 min, (n = 3) with 9 mM of potassium, and separated by 40 min of incubation in control medium. Again, the aldosterone response to potassium remained unaltered for the entire experiment (180–360 min). Repeated stimulations with Ang II (1 μM of Ang II at 200 and 320 min; n = 4), an agent interacting with membrane receptors, also gives stable responses (Table I). Similar experiments performed with adrenal cortex suspensions derived from pregnant rats gave similar results. Stimulated secretion did not decline with time in these experiments. We also tested the secretory responsiveness of this capsule suspension with increasing concentration of Ang II. A dose-response curve can be obtained between 10^{-10} and 10^{-5} M of Ang II. The maximal secretion and the ED_{50} are not significantly different between the adrenal cortex suspensions derived from pregnant and nonpregnant rats (Table II).

Aldosterone Secretion during Pregnancy. Plasma aldosterone levels were increased during pregnancy from 1.53 ± 0.13 pmol/ml to 3.00 ± 0.32 pmol/ml (n = 24; $P < 0.001$). There was no correlation between plasma aldosterone or basal aldosterone secretion and the number of fetuses (data not shown). We also observed an increase in PRA from 1.01 ± 0.19 to 5.5 ± 1.5 pmol Ang I/ml/hr. However, no correlation was found between plasma aldosterone level and PRA. Corticosterone was measured in the medium suspensions to evaluate the presence of contamination by fasciculata cells. Since a corticosterone response to potassium was not detected, an increase of corticosterone cannot explain the increased aldosterone secretion (data not shown). To confirm that contamination

Table I. Aldosterone Secretion Stimulated by 1 μM of Angiotensin II (Ang II) at Two Different Times (200 and 320 min) in Adrenal Cortex Suspensions derived from Nonpregnant Rats

Time (min)	Basal secretion (pmol/capsule/min)	Stimulated secretion by 1 μM Ang II (pmol/capsule/min)
200	0.35 ± 0.08	1.71 ± 0.23
320	0.41 ± 0.06	1.37 ± 0.18

Note. Basal secretion (20 min) preceded each stimulation period. Between each stimulation, there was an unstimulated period of 100 min.

by zona fasciculata/reticularis was minimal, a Northern blot analysis for 11β hydroxylase was performed. This enzyme is absent in zona glomerulosa (19); if contamination were to occur, a substantial quantity of its mRNA would be found in zona glomerulosa (ZG), which was not the case here, as shown in Figure 3.

Concentration-response curves of ACTH and potassium were measured (time: 180 to 360 min). Pro-

Table II. Data of the Dose Response Curve of Angiotensin II (Ang II) Performed with Adrenal Cortex Suspensions Derived from Pregnant and Nonpregnant Rats (n = 3 for Each Group)

	Pregnant	Nonpregnant
Basal response (pmol of aldosterone/ capsule/min)	0.35 ± 0.05	0.31 ± 0.04
Maximal response (pmol of aldosterone/ capsule/min)	1.37 ± 0.47	1.59 ± 0.06
ED_{50} (nM of Ang II)	105 ± 62	55 ± 12

gressive increases in the concentration of these stimulating agents (3 to 9 mM of total potassium in the medium and 10^{-13}–10^{-8} M of ACTH) were tested. The dose-response curves of potassium (n = 11) are shown in Figure 4. The maximum response was not significantly altered by pregnancy, but the ED_{50} was statistically increased (from 4.01 ± 0.08 to 4.71 ± 0.07 mM K^+; $P < 0.001$), suggesting that sensitivity to potassium is diminished in this condition.

The dose-response curves of ACTH (n = 14) are shown in Figure 5. Aldosterone secretion was greater in pregnant rats, but this difference did not reach statistical significance. Furthermore, the ED_{50} also revealed no statistical difference (0.14 ± 0.08 vs 0.17 ± 0.13 nM ACTH for nonpregnant versus pregnant animals).

Discussion

It has been reported that plasma aldosterone levels decrease in the first 18 hr after parturition (15),

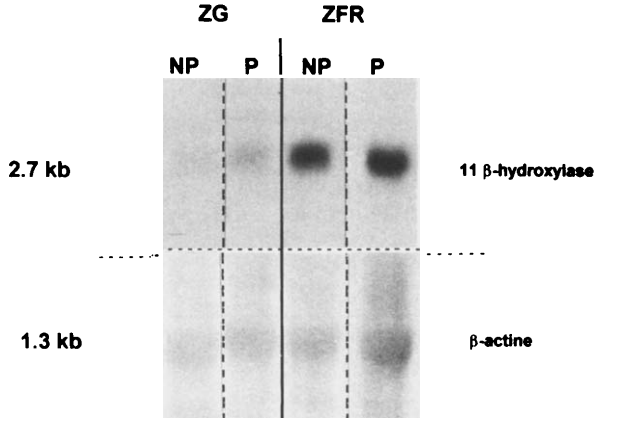

Figure 3. Northern blot analysis of total RNA from adrenal zona glomerulosa (ZG) and adrenal zona fasciculata-reticularis (ZFR) of nonpregnant (NP) and pregnant (P) rats. Total RNA (15 μg/ lane) were isolated by the guanidium isothiocyanate method and transferred to nylon membrane. Membranes were hybridized with (γ-^{32}P) d-adenosine triphosphate–labeled oligonucleotide probe specific to rat 11β-hydroxylase P-450. Length of mRNA is in kilobases (kb; right). Blots were also hybridized with a radiolabeled cDNA probe for β-actin to ensure that approximately equal amounts of RNA were contained in each sample.

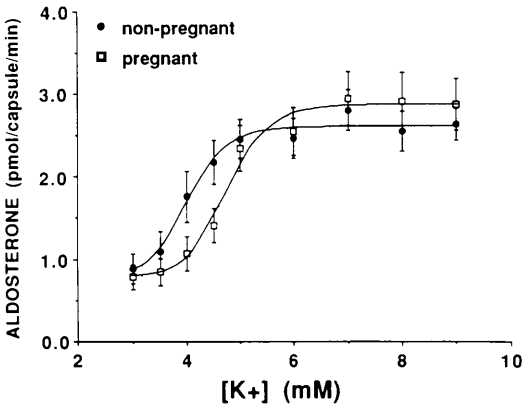

Figure 4. Dose-response curves of potassium in adrenal cortex suspensions derived from nonpregnant (n = 11) and pregnant (n = 11) rats.

suggesting that the use of cell cultures may not be reliable in the study of aldosterone secretion during pregnancy. The use of adrenal cell suspensions is also inappropriate, since low cell yields are obtained (data not shown). It has long been recognized that the amount of aldosterone secreted into the adrenal vein *in vivo* is considerably in excess of that measured *in vitro* in dispersed capsular cell preparations (20). Based on these considerations, the perfusion system was reported to mimic more closely the *in vivo* situation (21). We use a similar approach while suspending intact capsular preparations (which contain all zona glomerulosa and connective tissue) in tissue culture medium and changing the solution every 20 min (optimal time determined in preliminary experiments). We observed that the rate of aldosterone secretion was decreased after 50 min of incubation in the same well (data not shown).

It has already been reported that end product glucocorticoids manifest a direct suppressive action on their own secretion, suggesting the existence of a precise adjustment mechanism of steroidogenesis that operates in addition to the classical control exerted by the anterior pituitary (22). Such a mechanism could be

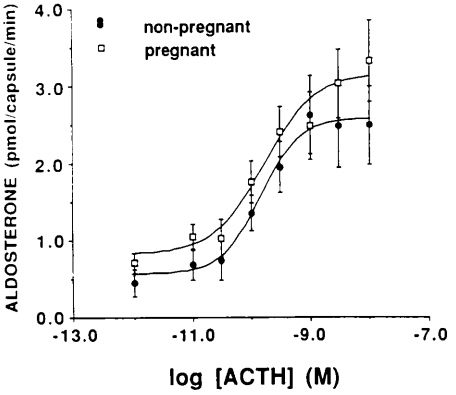

Figure 5. Dose-response curves of ACTH in adrenal cortex suspensions derived from nonpregnant (n = 14) and pregnant (n = 14) rats.

also present for aldosterone, although to our knowledge this has never been reported. By transferring the capsules to wells containing fresh medium every 20 min, this possible direct suppression should have been eliminated (see Fig. 1). Basal aldosterone output in our system was approximately 42 pmol/capsule/hr, which is comparable to the results obtained by other groups: 55 pmol/gland/hr in perfused adrenal gland (20) and 70 pmol/capsule/hr in perfused adrenal capsule (23). Results reported by Radke (24) differ, as she observed a basal aldosterone secretion of 4.8 pmol/capsule/hr in rats of 11–13 months. This difference could be due to the long period of preincubation that she used. In fact, she reported that basal aldosterone secretion stabilized after 8 hr of *in vitro* perifusion without showing the statistical analysis. In our experiments, the small decrease in basal aldosterone with time did not reach statistical significance. Maximal secretion in intact capsular preparations was about 150 pmol/capsule/hr, which is similar to the values obtained with perfused glands: 140 pmol/gland/hr (20). As seen in Figure 2 and Table I, maximal stimulation (by potassium or Ang II) occurred in the first 20-min period, in agreement with the data reported by others (20, 25). Sensitivity of the capsule suspension to ACTH (ED_{50}: 0.13 n*M*) corresponds to the range obtained in other studies in rat adrenal cortex (e.g., between 10^{-12} and 10^{-9} *M* ACTH using perifusion of whole capsules (26) and 50 p*M* (27), 0.31 n*M* (28), and 0.45 n*M* (29) in dispersed glomerulosa cells). The discrepancy with the results of one study (24), whose author reported much reduced sensitivity to ACTH, may be due to differences in strain of rat and in the longer preincubation period used. Although, the adrenal capsule suspensions were less responsive to physiological concentration of Ang II than can be expected, our results are comparable to those reported by Cunningham and Holzwarth (26), who used perifused cortex preparation. Others (24) have not found any response to Ang II by adrenal capsule perifusion, probably because of a long incubation time. As adrenal from rats contained high angiotensinase activity (30), it is possible that exogenous Ang II may be partly degraded in whole capsule preparations.

This method is easy to perform, inexpensive and reproducible. Our results clearly indicate that it is reliable method to study aldosterone secretion during pregnancy and for the elucidation of cellular mechanisms controlling steroidogenesis.

We have demonstrated that at the end of pregnancy in the rat, plasma aldosterone is increased by 2-fold, which is comparable to the 2.6-fold increment reported by Parent *et al.* (15) on the 21st day of gestation. Like other investigators (10, 13), we observed an elevation of PRA during gestation. We believe that the controversy surrounding PRA (14, 15) during gestation is due to the species of rat or sodium diet used, as already noted by Schneider and Mulrow (11). Since no correlation was found between plasma aldosterone and PRA, the RAS does not appear to play a major role in the aldosterone increase during pregnancy.

We have clearly shown that the weights of the capsules did not differ with pregnancy. Basal aldosterone secretion in adrenal cortex suspensions from pregnant rats was augmented by 1.6-fold, suggesting that elevated plasma aldosterone levels during pregnancy are partly the consequence of enhanced basal aldosterone synthesis by the adrenal. If so, the mechanism of this increased synthesis remains to be elucidated.

As mentioned earlier, potassium is a potent stimulus of aldosterone secretion. When circulating potassium concentration is increased from 3.9 to 4.5 m*M* by dietary potassium loading, a 2-fold increase in aldosterone secretion *in vivo* was observed (31). In intact capsules, we have observed (Fig. 4) that an elevation of potassium from 3.0 to 5.0 m*M* causes a 2.5-fold enhancement of aldosterone secretion. This aldosterone response to potassium is less sensitive during pregnancy, as indicated by the increased ED_{50} (Fig. 4). Schneider and Mulrow (11) found that plasma potassium concentration was significantly augmented in pregnant rats compared with nonpregnant controls (3.3 ± 0.1 vs 3.9 ± 0.2 m*M*, $P < 0.025$), while Churchill *et al.* (32) detected a small increase of plasma potassium (3.5 ± 0.1 vs 3.8 ± 0.2 m*M* in nonpregnant versus pregnant rats), which did not reach statistical significance. These elevations of plasma potassium cannot explain the enhanced basal secretion of aldosterone during pregnancy, since our data (Fig. 4) show that the threshold response to potassium was increased from 3.5 to 4.0 m*M* by pregnancy. The mechanism by which sensitivity of the aldosterone response to potassium is diminished during pregnancy remains to be elucidated.

In summary, adrenal capsule suspensions are a reliable method to study aldosterone secretion during pregnancy. A major finding of the present study is that basal aldosterone secretion by adrenal capsules increased during pregnancy while the response to ACTH was not affected. Another interesting point is the decreased sensitivity of the aldosterone response to a physiological range of potassium during pregnancy. The mechanism and the physiological relevance of this last observation are presently under study.

This research was supported by a grant from Fondation de l'hôpital Sainte-Justine. Michèle Brochu is a scholar of the Heart and Stroke Foundation of Canada. Jean St-Louis is a scholar of Fonds de la Recherche en Santé du Québec. The authors thank Mr. Serge Picard for his excellent technical assistance and Ms. Suzanne Beaudet for her secretarial work.

1. Stevens KJ, Paintin DB, Few JD. Aldosterone is secreted intermittently during pregnancy. Br J Obstet Gynaecol **96**:80–87, 1989.
2. Wacker J, E-Mistry N, Bauer H, Vecsei P, Stolz W, Bastert G. Mineralocorticoids and mineralocorticoid receptors in monocular leucocytes in patients with pregnancy-induced hypertension. J Clin Endocrinol Metab **74**:910–913, 1992.
3. Gallery EDM, Brown MA. Control of sodium excretion in human pregnancy. Am J Kidney Dis **9**:290–295, 1987.
4. McCance DR, McKnight JA, Traub AI, Sheridan B, Roberts G, Atkinson AB. Plasma atrial natriuretic factor levels during normal pregnancy and pregnancy complicated by diabetes mellitus and hypertension. J Hum Hypertens **4**:31–35, 1990.
5. Wu SJ, Tsai YL, Hsieh BS. Sequential changes in plasma renin activity and aldosterone level during pregnancy. J Formos Med Assoc **90**:932–935, 1991.
6. Hjertberg R, Belfrage P, Bremme K, Eneroth P. The renin-angiotensin-aldosterone system in pregnancy. Response to terbutaline provocation in preeclampsia. Clin Exp Hypertens **B10**:385–399, 1991.
7. Massicotte G, St-Louis J, Schiffrin EL. Blood pressure effects of angiotensin II and vasopressin in conscious pregnant normotensive and spontaneously hypertensive rats. Clin Exp Hypertens **B5**:135–138, 1986.
8. Barron WM. Volume homeostasis during pregnancy in the rat. Am J Kidney Dis **9**:296–302, 1987.
9. Baylis C. Renal hemodynamics and volume control during pregnancy in the rat. Semin Nephrol **4**:208–220, 1984.
10. Nadel AS, Ballermann BJ, Anderson S, Brenner BM. Interrelationship among atrial peptides, renin and blood volume in pregnant rats. Am J Physiol **254**:R793–R800, 1988.
11. Schneider G, Mulrow PJ. Regulation of aldosterone production during pregnancy in the rat. Endocrinology **92**:1208–1215, 1973.
12. Barron WM, Lindheimer MD. Renal sodium and water handling in pregnancy. Obstet Gynecol Annu **13**:35–69, 1984.
13. Garland HO, Atherton JC, Baylis C, Morgan MRA, Milne CM. Hormone profiles for progesterone, oestradiol, prolactin, plasma renin activity, aldosterone and corticosterone during pregnancy and pseudopregnancy in two strains of rat: correlation with renal studies. J Endocrinol **113**:435–444, 1987.
14. Conrad KP, Morganelli PM, Brinck-Johnsen T, Colpoys MC. The renin-angiotensin system during pregnancy in chronically instrumented, conscious rats. Am J Obstet Gynecol **161**:1065–1072, 1989.
15. Parent A, Schiffrin EL, St-Louis J. Receptors for Arg^8-vasopressin, angiotensin II, and atrial natriuretic peptide in the mesenteric vasculature of pregnant rats. Can J Physiol Pharmacol **69**:137–144, 1991.
16. Brochu M, Féthière J, Roy M, Ong H, De Léan A. Highly sensitive and rapid radioimmunoassay for aldosterone in plasma and cell culture medium. Clin Biochem **22**:289–292, 1989.
17. Gutkowska J, Boucher R, Genest J. Dosage radioimmunologique de l'activité rénine plasmatique. Union Med Can **106**:446–450, 1977.
18. De Léan A, Munson PJ, Rodbard D. Simultaneous analysis of families of sigmoidal curves: Application to bioassay, radioligand assay, and physiological dose-response curves. Am J Physiol **235**:E97–E102, 1978.
19. Ogishima T, Suzuki H, Hata JI, Mitani F, Ishimura Y. Zone-specific expression of aldosterone synthase cytochrome P-450 and cytochrome P-450 11β rat adrenal cortex: Histochemical basis for the functional zonation. Endocrinology **130**:2971–2977, 1992.
20. Hinson JP, Vinson GP, Whitehouse BJ, Price G. Control of zona glomerulosa function in the isolated perfused rat adrenal gland *in situ*. J Endocrinol **104**:387–395, 1985.
21. Oda H, Lotshaw DP, Franco-Saenz R, Mulrow PJ. Local generation of angiotensin II as a mechanism of aldosterone secretion in rat adrenal capsules. Proc Soc Exp Biol Med **196**:175–177, 1991.
22. Carsia RV, Malamed S. Acute self-suppression of corticosteroidogenesis in isolated adrenocortical cells. Endocrinology **105**:911–914, 1979.
23. Raven PW, McCredie E, McAuley M, Vinson GP. Origins of the differences in function of rat adrenal zona glomerulosa cells incubated as intact tissue and as collagenase prepared cell suspensions. Cell Biochem Funct **1**:17–24, 1983.
24. Radke KJ. Age-related impairment of K^+- and ACTH-stimulated aldosterone secretion by rat adrenal capsules. Am J Physiol **264**:E82–E89, 1993.
25. Vinson GP, Laird SM, Whitehouse BJ, Teja R, Hinson JP. The biosynthesis of aldosterone. J Steroid Biochem Mol Biol **39**:851–858, 1991.
26. Cunningham LA, Holzwarth MA. Vasoactive intestinal peptide stimulates adrenal aldosterone and corticosterone secretion. Endocrinology **122**:2090–2097, 1988.
27. Gallo-Payet N, Payet MD. Excitation-secretion coupling: involvement of potassium channels in ACTH-stimulated rat adrenocortical cells. J Endocrinol **120**:409–421, 1989.
28. Fakunding JL, Chow R, Catt KJ. The role of calcium in the stimulation of aldosterone production by adrenocorticotropin, angiotensin II, and potassium in isolated glomerulosa cells. Endocrinology **105**:327–333, 1979.
29. Fujita K, Aguilera G, Catt KJ. The role of cyclic AMP in aldosterone production by isolated zona glomerulosa cells. J Biol Chem **254**:8567–8574, 1979.
30. Bumpus FM, Smedy RR, Page IH, Khairallah PA. Distribution and metabolic fate of angiotensin II and various derivatives. Can Med Assoc J **90**:190–196, 1964.
31. Boyd J, Palmore WP, Mulrow PJ. Role of potassium in the control of aldosterone secretion in the rat. Endocrinology **88**:556–565, 1971.
32. Churchill S, Bengele HH, Melby JC, Alexander EA. Role of aldosterone in sodium retention of pregnancy in the rat. Am J Physiol **240**:R175–R181, 1981.