

Review

Roles of Bone Morphogenetic Protein-6 in Aldosterone Regulation by Adrenocortical Cells

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Aldosterone production occurs in the adrenal cortex, and is regulated primarily by angiotensin II (Ang II), potassium and adrenocorticotropin (ACTH). In the presence of the aldosterone stimulators, steroidogenesis is further governed by local autocrine and/or paracrine factors in the adrenal cortex. We reported the presence of functional bone morphogenetic protein (BMP) system in the adrenal cortex and also demonstrated that BMP-6 increases Ang II-induced aldosterone production, which could be involved in the "aldosterone breakthrough" phenomenon. Aldosterone breakthrough is the phenomenon by which circulating aldosterone concentrations increase above pre-treatment levels after long-term therapy with ACE inhibitors or Ang II type 1 receptor antagonists (ARB). This phenomenon may lead to important clinical consequences since increased aldosterone in a high-salt state facilitates cardiovascular and renal damage in hypertensive patients. We found that long-term ARB treatment reverses the reduction of aldosterone synthesis by adrenocortical cells, thereby causing "cellular aldosterone breakthrough". The availability of BMP-6 in the adrenal cortex may be at least partly involved in the occurrence of cellular escape from aldosterone suppression under chronic treatment with ARB.

Key words: adrenal cortex, angiotensin II, aldosterone, bone morphogenetic protein

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II (Ang II) type 1 receptor (AT1R) blockers (ARB) play major roles in the medication for hypertension. However, plasma aldosterone levels occasionally increase after an initial decline in some patients over the course of long-term therapy with ACE inhibitors and/or ARB. Aldosterone breakthrough is the phenomenon by which circulating aldosterone concentrations increase above pre-treat-

ment levels after long-term therapy with ACE inhibitors [1, 2] and/or ARB [3, 4]. Staessen *et al.* [1] first reported the aldosterone breakthrough phenomenon in 1981. As described in their report, the plasma concentration of aldosterone was followed in 7 hypertensive patients before and during long-term Ang II suppression with an orally active ACE inhibitor, captopril. The plasma concentration of aldosterone decreased initially from 74 to 21 pg/ml after 1 month of captopril administration. Thereafter the plasma concentration of aldosterone began to rise, reaching a level of 165 pg/ml after one year. During long-term captopril therapy the plasma renin activity remained elevated and the plasma Ang II concentration sup-

Received March 10, 2010; accepted April 12, 2010.

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pressed. A sizeable and lasting hypotensive effect was observed in all patients. Afterwards the aldosterone breakthrough phenomenon came to be widely known.

Based on a review of the literature [5], the incidence of the aldosterone breakthrough phenomenon ranges from 10% over 6 months to 53% over 1 year. This phenomenon could have important clinical consequences because increased aldosterone in a high-salt state may facilitate cardiovascular and renal damage in hypertensive patients [6, 7]. Two major studies, RALES (Randomized Aldactone Evaluation Study) [8] and EPHEsus (Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study) [9], indicated that aldosterone blockage prevents cardiovascular and renal damage. There is increasing evidence suggesting that aldosterone exerts physiologically and/or pathophysiologically critical effects on the cardiovascular system [10], kidney [11, 12], and central nervous system [13] in addition to its well-established role in regulating body electrolyte and water homeostasis.

Involvement of various *in vivo* factors such as adrenocorticotropin (ACTH), electrolytes, endothelins and Ang II type 2 receptor (AT2R) actions [14] have been proposed to explain the aldosterone breakthrough phenomenon; however, the detailed underlying mechanism remains unknown. We previously reported the presence of a functional bone morphogenetic protein (BMP) and activin system complete with ligands including BMP-6, activins, and their receptors in human adrenocortical H295R cells [15]. We also reported that BMP-6 enhances Ang II- but not potassium (K)-induced aldosterone production through activating the extracellular signal-regulated kinase (ERK) 1/2 pathway [16].

Furthermore, we investigated whether aldosterone breakthrough occurs *in vitro* using human adrenocortical cells [17]. Ang II stimulated aldosterone production in adrenocortical cells, which was dose-dependently blocked by the ARB candesartan. Interestingly, candesartan's effects in reducing Ang II-induced aldosterone synthesis and CYP11B2 expression were impaired in the course of candesartan treatment for 15 days. Levels of AT1R mRNA were not changed by chronic candesartan treatment, suggesting that down-regulation of AT1R was not critical in this phenomenon. We here discuss BMP-6 actions in aldosterone regulation, which is presumably involved in the aldos-

terone breakthrough occurring with long-term ARB treatment in human adrenocortical cells.

Biosynthesis of Aldosterone in the Adrenal Cortex

Aldosterone is synthesized from cholesterol in the zona glomerulosa of the adrenal cortex [18, 19] (Fig. 1). First, cholesterol is transferred into the mitochondria by the steroidogenic acute regulatory protein (StAR). StAR is present in all steroidogenic tissues and is considered to be the rate-limiting enzyme. Following translocation to the mitochondria, cholesterol is converted to aldosterone by a series of enzymatic reactions, many of which belong to the cytochrome P450 (CYP) superfamily. Cholesterol is converted to pregnenolone by the P450 side-chain cleavage enzyme (P450_{scc}), encoded by the CYP11A1 gene. Pregnenolone is released into the cytosol and is converted to progesterone by 3 β -hydroxysteroid dehydrogenase (3 β -HSD). There are two 3 β -HSD isoenzymes encoded by the HSD3B1 and HSD3B2 genes. Although HSD3B1 can carry out the same function as HSD3B2, these enzymes localize in different tissues including adrenal and gonadal tissues for type 2 and placental and nonsteroidogenic tissues for type 1 enzyme.

Progesterone is converted to 11 β -deoxycorticosterone (DOC) by 21-hydroxylase encoded by the CYP21A gene. P450 aldosterone synthase (P450_{aldo}) located in the mitochondria of the zona glomerulosa and encoded by the CYP11B2 gene, which converts DOC to corticosterone with 11 β -hydroxylation, corticosterone to 18-hydroxycorticosterone with 18-hydroxylation, and finally 18-hydroxycorticosterone to aldosterone with 18-oxidation. Recent studies have suggested the existence of extra-adrenal aldosterone production in the heart and blood vessels [20]. Nevertheless, there is still an argument against the pathophysiological significance of local aldosterone because of its very low production.

Regulation of Adrenocortical Aldosterone Production

Various factors including adrenaline, dopamine, vasoactive intestinal polypeptide, serotonin, ouabain, atrial natriuretic peptide, heparin and adrenomedullin

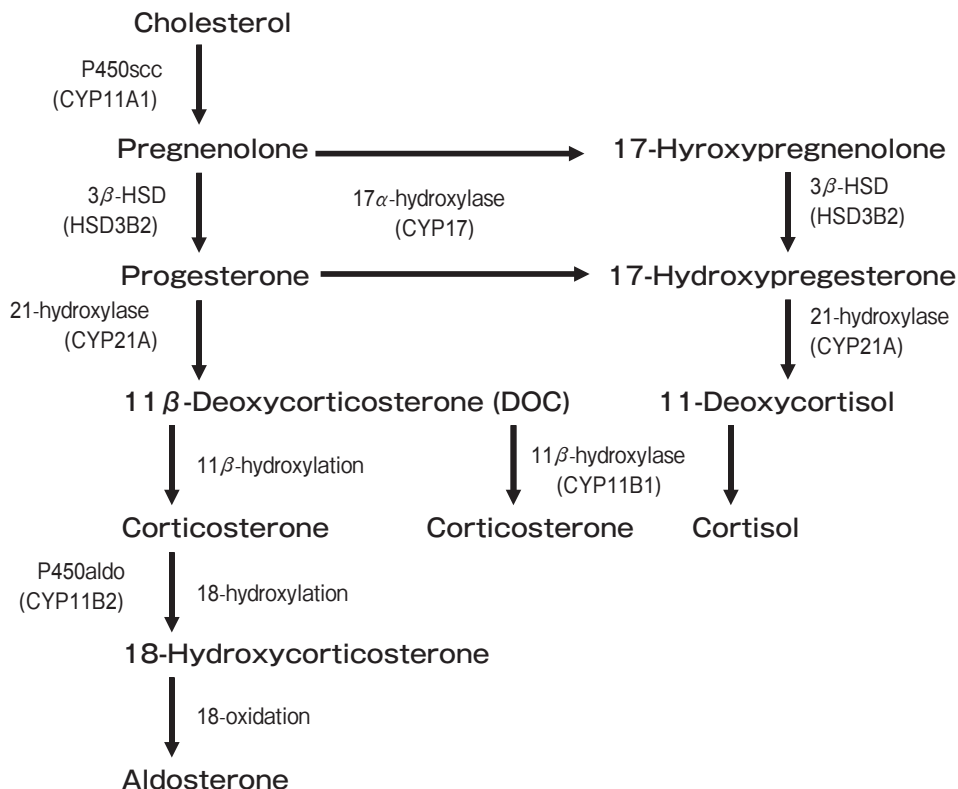


Fig. 1 Aldosterone biosynthesis in the adrenal cortex. Cholesterol is converted to pregnenolone by the P450 side-chain cleavage enzyme (P450scc), to progesterone by 3β -hydroxysteroid dehydrogenase (3β -HSD), and to 11β -deoxycorticosterone (DOC) by 21-hydroxylase. P450 aldosterone synthase (P450aldo), which is located in the zona glomerulosa, converts DOC to corticosterone, corticosterone to 18-hydroxycorticosterone, and finally 18-hydroxycorticosterone to aldosterone.

have been shown to be regulators of aldosterone production [21, 22]. However, production of aldosterone is regulated primarily by Ang II and K and, to a lesser degree, by ACTH.

Ang II action is transduced through the 1, 2-diacylglycerol (DAG)/protein kinase C (PKC) and the inositol 1, 4, 5-trisphosphate (IP3)/ Ca^{2+} signaling mechanism via AT1R [23]. IP3 increases intracellular Ca^{2+} influx, causing several Ca^{2+} /calmodulin-dependent protein kinases (CaM kinases) to phosphorylate and activate transcription factors such as activating transcription factor (ATF)-1, cAMP-response-element (CRE)-binding protein (CREB), and NURR-1/NGFIB. These bind CRE and other *cis*-acting elements such as Ad-5 and NBRE-1, which are unique to the 5'-untranslated region of the CYP11B2 gene. The NBRE-1 and Ad-5 *cis*-elements are specific to CYP11B2. Additionally, mitogen-activated protein kinase (MAPK) pathways have also been implicated in

Ang II-induced aldosterone production [24, 25].

Increased extracellular K causes cell membrane depolarization. K-induced depolarization activates voltage-dependent Ca^{2+} channels and increases Ca^{2+} influx. This activates calmodulin and CaM kinases, and finally stimulates CYP11B2 gene transcription [26]. ACTH acts primarily through cAMP/protein kinase A (PKA), but it also stimulates Ca^{2+} influx. In the early phase, ACTH stimulates aldosterone synthesis. In contrast, chronic exposure to ACTH suppresses aldosterone production. The mechanism of aldosterone reduction by chronic ACTH treatment is unclear but cAMP may downregulate the expression of Ang II receptors. Alternatively, ACTH may divert precursors from the mineralocorticoid to the glucocorticoid pathway [26].

BMP Actions in Regulating Aldosterone Production

Bone morphogenetic proteins (BMPs) were identified in 1965 by Urist as the active components in demineralized bone and bone extracts that are capable of inducing bone formation at ectopic sites [27]. Shortly after the cloning of the first BMPs, a number of new BMP family genes were identified using homology-based cDNA cloning. BMPs regulate cell growth, apoptosis, differentiation, and cell patterning and specification in numerous tissues [28]. Recent studies have shown that BMPs also exhibit multifunctional activities in many endocrine tissues [29] including ovary [30, 31], pituitary, hypothalamus [32], thyroid [33] and adrenal [15, 16] tissues.

BMP type I and type II receptors independently have certain affinity for the ligand (Fig. 2). BMP binds to the type II receptor, and then the phosphorylated type I receptors activate the downstream signaling molecules Smads. Among the receptor-regulated Smad antibodies (R-Smads), Smad1/5/8 is activated in response to BMPs, whereas Smad2/3 is activated in response to TGF- β and activin. They interact with a common-mediator Smad (Co-Smad: Smad4) to form a hetero-oligomeric complex with Smad1/5/8. The complex then translocates to the nucleus, where it

binds to target DNA and induces the transcription of specific genes. Inhibitory Smads (I-Smads: Smad6/7) compete with Smad4 and consequently regulate the transcription of its gene products.

We have recently reported the presence of a functional BMP and activin system complete with ligands including BMP-6 and activin $\beta A/\beta B$; receptors including activin receptor-like kinase (ALK)-2, -3, and -4, activin type II receptor (ActRII), and BMP type II receptor (BMPRII); and the binding protein follistatin in human adrenocortical H295R cells [15]. Activin and BMP-6 cause concentration-dependent increases in aldosterone production with increased expression of StAR, P450_{scc} and CYP11B2. BMP-6 enhances Ang II-induced but not ACTH-induced aldosterone production, whereas activin enhances ACTH-induced aldosterone production. Activin regulates aldosterone synthesis predominantly by modulating the ACTH-cAMP-PKA signaling pathway [15]. However, BMP-6 contributes to Ang II-induced aldosterone production by activating Smad1/5/8 after binding to ALK-2 and/or ALK-3 in combination with ActRII; this aldosterone production in turn sustains Ang II-induced extracellular signal-regulated kinase (ERK) activation [16].

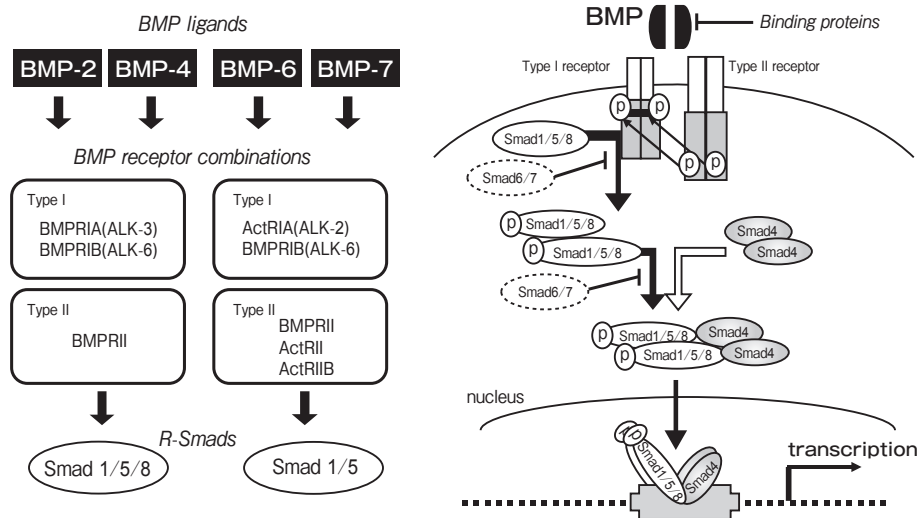


Fig. 2 BMP system and the signaling pathway. Each BMP ligand has preferential combinations of type I and type II BMP receptors. After ligand binding to the BMP receptors, phosphorylated type I receptors subsequently phosphorylate receptor-activated Smads (R-Smads), which associate with common-mediator Smad (Co-Smad) and enter the nucleus, where they regulate gene expression. Inhibitory Smads (I-Smads) prevent the association between R-Smads and Co-Smads.

Involvement of BMP-6 in Aldosterone Breakthrough Phenomenon

Ang II-induced aldosterone production in human adrenocortical cells is concentration-dependently blocked by ARB [17]. However, the ARB effects on reducing Ang II-induced aldosterone production and CYP11B2 expression are impaired in the course of ARB treatment for 15 days. We presumed that this phenomenon is a possible cellular aldosterone breakthrough (Fig. 3). Chronic ARB treatment has no effects on AT1R expression, suggesting that down-regulation of AT1R is not critical in this phenomenon. Ang II-induced ERK1/2 signaling is blocked by ARB treatment; however, the BMP-6 effects on Ang II-induced ERK1/2 become resistant to ARB in the chronic phase. BMP-6-induced Smad1/5/8 activation is in turn amplified in cells chronically treated with Ang II plus ARB compared with cells treated with Ang II alone. Cellular expression of BMP-6 and its receptor including ALK-2 and ActRII are decreased by chronic Ang II exposure but restored by co-treatment with ARB (Fig. 3). Collectively, changes in BMP-6

availability and response may be involved in the occurrence of cellular escape from aldosterone suppression under chronic treatment with ARB.

Conclusion

Various factors such as ACTH, electrolytes, endothelins and Ang II type 2 receptor actions have been proposed as the cause of aldosterone breakthrough. We here found that long-term ARB treatment reverses the reduction of aldosterone synthesis by adrenocortical cells, leading to “cellular aldosterone breakthrough”. Since *in vitro* breakthrough was attenuated by the neutralization of endogenous BMP-6 and ALK-2, BMP-6 availability in the adrenal cortex may be involved in the occurrence of cellular escape from aldosterone suppression under chronic treatment with ARB.

References

1. Staessen J, Lijnen P, Fagard R, Verschueren LJ and Amery A: Rise in plasma concentration of aldosterone during long-term angio-

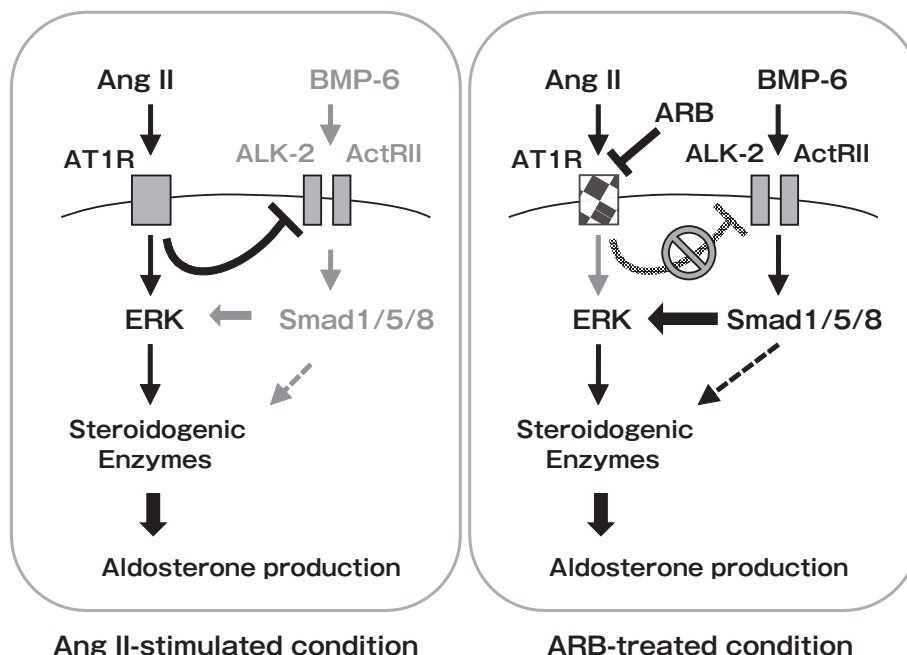


Fig. 3 The possible involvement of BMP-6 in the mechanism of cellular aldosterone breakthrough. BMP-6 stimulates Ang II-induced aldosterone production by sustaining Ang II-induced ERK1/2 phosphorylation. BMP-6, its receptors (ALK-2 and ActRII) and the following Smad1/5/8 activation are impaired by long-term Ang II exposure. However, in the presence of ARB, BMP-6/Smads signaling cannot be inhibited by Ang II, which may lead to the aldosterone escape phenomenon.

- tensin II suppression. *J Endocrinol* (1981) 91: 457–465.
2. Lijnen P, Staessen J, Fagard R and Amery A: Increase in plasma aldosterone during prolonged captopril treatment. *Am J Cardiol* (1982) 49: 1561–1563.
 3. Naruse M, Tanabe A, Sato A, Takagi S, Tsuchiya K, Imaki T and Takano K: Aldosterone breakthrough during angiotensin II receptor antagonist therapy in stroke-prone spontaneously hypertensive rats. *Hypertension* (2002) 40: 28–33.
 4. Horita Y, Taura K, Taguchi T, Furusu A and Kohno S: Aldosterone breakthrough during therapy with angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers in proteinuric patients with immunoglobulin A nephropathy. *Nephrology (Carlton)* (2006) 11: 462–466.
 5. Bomback AS and Klemmer PJ: The incidence and implications of aldosterone breakthrough. *Nat Clin Pract Nephrol* (2007) 3: 486–492.
 6. Sato A and Saruta T: Aldosterone escape during angiotensin-converting enzyme inhibitor therapy in essential hypertensive patients with left ventricular hypertrophy. *J Int Med Res* (2001) 29: 13–21.
 7. Sato A, Hayashi K, Naruse M and Saruta T: Effectiveness of aldosterone blockade in patients with diabetic nephropathy. *Hypertension* (2003) 41: 64–68.
 8. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J and Wittes J: The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med* (1999) 341: 709–717.
 9. Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J and Gatlin M: Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med* (2003) 348: 1309–1321.
 10. Rocha R and Stier CT Jr: Pathophysiological effects of aldosterone in cardiovascular tissues. *Trends Endocrinol Metab* (2001) 12: 308–314.
 11. Nishiyama A, Yao L, Nagai Y, Miyata K, Yoshizumi M, Kagami S, Kondo S, Kiyomoto H, Shokoji T, Kimura S, Kohno M and Abe Y: Possible contributions of reactive oxygen species and mitogen-activated protein kinase to renal injury in aldosterone/salt-induced hypertensive rats. *Hypertension* (2004) 43: 841–848.
 12. Otani H, Otsuka F, Inagaki K, Takeda M, Miyoshi T, Suzuki J, Mukai T, Ogura T and Makino H: Antagonistic effects of bone morphogenetic protein-4 and -7 on renal mesangial cell proliferation induced by aldosterone through MAPK activation. *Am J Physiol Renal Physiol* (2007) 292: F1513–1525.
 13. Gomez Sanchez EP: What is the role of the central nervous system in mineralocorticoid hypertension? *Am J Hypertens* (1991) 4: 374–381.
 14. Sato A and Saruta T: Aldosterone breakthrough during angiotensin-converting enzyme inhibitor therapy. *Am J Hypertens* (2003) 16: 781–788.
 15. Suzuki J, Otsuka F, Inagaki K, Takeda M, Ogura T and Makino H: Novel action of activin and bone morphogenetic protein in regulating aldosterone production by human adrenocortical cells. *Endocrinology* (2004) 145: 639–649.
 16. Inagaki K, Otsuka F, Suzuki J, Kano Y, Takeda M, Miyoshi T, Otani H, Mimura Y, Ogura T and Makino H: Involvement of bone morphogenetic protein-6 in differential regulation of aldosterone production by angiotensin II and potassium in human adrenocortical cells. *Endocrinology* (2006) 147: 2681–2689.
 17. Otani H, Otsuka F, Inagaki K, Suzuki J, Miyoshi T, Kano Y, Goto J, Ogura T and Makino H: Aldosterone breakthrough caused by chronic blockage of angiotensin II type 1 receptors in human adrenocortical cells: Possible involvement of bone morphogenetic protein-6 actions. *Endocrinology* (2008) 149: 2816–2825.
 18. Bassett MH, White PC and Rainey WE: The regulation of aldosterone synthase expression. *Mol Cell Endocrinol* (2004) 217: 67–74.
 19. Connell JM and Davies E: The new biology of aldosterone. *J Endocrinol* (2005) 186: 1–20.
 20. Ahmad N, Romero DG, Gomez-Sanchez EP and Gomez-Sanchez CE: Do human vascular endothelial cells produce aldosterone? *Endocrinology* (2004) 145: 3626–3629.
 21. Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA and Vinson GP: Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *Endocr Rev* (1998) 19: 101–143.
 22. Lumbers ER: Angiotensin and aldosterone. *Regul Pept* (1999) 80: 91–100.
 23. Kirschner LS: Signaling pathways in adrenocortical cancer. *Ann N Y Acad Sci* (2002) 968: 222–239.
 24. Chabre O, Cornillon F, Bottari SP, Chambaz EM and Vilgrain I: Hormonal regulation of mitogen-activated protein kinase activity in bovine adrenocortical cells: cross-talk between phosphoinositides, adenosine 3', 5'-monophosphate, and tyrosine kinase receptor pathways. *Endocrinology* (1995) 136: 956–964.
 25. Startchik I, Morabito D, Lang U and Rossier MF: Control of calcium homeostasis by angiotensin II in adrenal glomerulosa cells through activation of p38 MAPK. *J Biol Chem* (2002) 277: 24265–24273.
 26. Spat A and Hunyady L: Control of aldosterone secretion: a model for convergence in cellular signaling pathways. *Physiol Rev* (2004) 84: 489–539.
 27. Hogan BLM: Bone morphogenetic proteins: multifunctional regulators of vertebrate development. *Genes Dev* (1996) 10: 1580–1594.
 28. Kawabata M, Imamura T and Miyazono K: Signal transduction by bone morphogenetic proteins. *Cytokine Growth Factor Rev* (1998) 9: 49–61.
 29. Otsuka F: Multiple endocrine regulation by bone morphogenetic protein system. *Endocr J* (2010) 57: 3–14.
 30. Shimasaki S, Moore RK, Erickson GF and Otsuka F: The role of bone morphogenetic proteins in ovarian function. *Reprod Suppl* (2003) 61: 323–337.
 31. Shimasaki S, Moore RK, Otsuka F and Erickson GF: The bone morphogenetic protein system in mammalian reproduction. *Endocr Rev* (2004) 25: 72–101.
 32. Otani H, Otsuka F, Takeda M, Mukai T, Terasaka T, Miyoshi T, Inagaki K, Suzuki J, Ogura T, Lawson MA and Makino H: Regulation of GNRH production by estrogen and bone morphogenetic proteins in GT1-7 hypothalamic cells. *J Endocrinol* (2009) 203: 87–97.
 33. Suzuki J, Otsuka F, Takeda M, Inagaki K, Miyoshi T, Mimura Y, Ogura T, Doihara H and Makino H: Functional roles of the bone morphogenetic protein system in thyrotropin signaling in porcine thyroid cells. *Biochem Biophys Res Commun* (2005) 327: 1124–1130.