0031-6997/94/4604-417$03.00/0
PHARMACOLOGICAL REVIEWS

Vol. 46, No. 4
Printed in U.S.A.



# Role of Calcium and Other Mediators in Aldosterone Secretion from the Adrenal Glomerulosa Cells*

ARUNABHA GANGULY[1†] AND JOHN S. DAVIS[2]

*[1]Medical Service, James A. Haley Veterans Hospital and Department of Medicine, University of South Florida College of Medicine, Tampa, Florida, and [2]Department of Obstetrics and Gynecology and Internal Medicine, University of Kansas School of Medicine-Wichita and Veterans Affairs Medical Center, Wichita, Kansas*

I. Introduction and historical perspectives ..... 418
II. Aldosterone biosynthesis and physiological secretagogues ..... 418
A. Biosynthesis of aldosterone ..... 418
B. Aldosterone secretagogues and inhibitors ..... 418
III. Cellular signaling mechanisms for the secretagogues ..... 418
A. Adrenocorticotropin-induced aldosterone secretion ..... 419
1. Role of cyclic nucleotides ..... 419
2. Role of calcium ..... 420
3. Role of other possible mediators ..... 422
B. Angiotensin- and potassium-induced aldosterone secretion ..... 423
1. Role of calcium ..... 424
a. Extracellular calcium ..... 424
b. Calcium fluxes ..... 424
i. Effects of calcium antagonists or channel blockers ..... 424
ii. Effects of calcium ionophore and a calcium channel agonist ..... 425
iii. Estimation of calcium influx ..... 425
iv. Nature of calcium channels ..... 426
v. Calcium efflux ..... 427
c. Role of changes in cytosolic free calcium concentration ..... 427
d. Single cell calcium responses and calcium oscillations ..... 428
e. Intracellular calcium stores ..... 429
i. Calcium stores in endoplasmic reticulum and/or calciosome ..... 429
ii. Calcium stores in mitochondria ..... 430
iii. Calcium stores in multiple organelles ..... 430
f. Summary ..... 431
2. Role of calmodulin and calmodulin-dependent protein kinase ..... 431
3. Role of phosphoinositides and other mediators ..... 432
a. Phosphoinositides ..... 432
b. Other mediators ..... 434
4. Role of protein kinases ..... 435
C. Interactive effects of secretagogues and/or cellular mediators ..... 437
D. Inhibitors of aldosterone secretion ..... 438
1. Atrial natriuretic peptide ..... 438
2. Dopamine and somatostatin ..... 439
IV. Conclusions ..... 440
V. References ..... 440

* This work was supported by the Research Funds of the Veterans Administration and American Heart Association (Florida affiliate).
† Correspondence address Veterans Administration Hospital (111-M), 13000 Bruce B. Downs Boulevard, Tampa, FL 33612.

## I. Introduction and Historical Perspectives

The identification of aldosterone as a secretory product of the adrenal cortex was accomplished in the early 1950s (Simpson et al., 1952). The regulatory mechanisms involved in the secretion of aldosterone from the adrenal glomerulosa cells have since been investigated extensively. However, with the exception of one of the secretagogues of aldosterone, insights into the cellular signaling mechanisms involved in aldosterone secretion have come relatively recently. This aspect will be the subject of this review, particularly as it relates to the role of calcium in the adrenal cell signaling mechanism.

It has been recognized for quite some time that there is functional zonation of the adrenal cortex such that the secretory control of the inner zones (comprising fasciculata and reticularis cells) is dependent on the secretion of ACTH‡ from the anterior pituitary gland, whereas the outer zone (consisting of glomerulosa cells) is more or less independent of control from the pituitary gland (Long, 1975). Instead, renal renin secretion, leading to the formation of angiotensin II, is critical for the steroid secretion from the outer zone. Potassium ion also exercises significant influence on the steroid secretion from the outer zone, but not the inner zones, of the adrenal cortex.

Morphological features of the adrenal cortex also support the concept of dual dependence of the adrenal cortex for its control and trophic function. Thus, the secretion of glucocorticoid and sex hormones from the adrenal is primarily regulated by ACTH, and the secretion of mineralcorticoid hormone is controlled predominantly by the renin-angiotensin system. In recent years, it has been found that many tissues including the adrenal cortex possess all of the components of the renin-angiotensin system locally. The full significance of these local renin-angiotensin systems in extrarenal tissues remains unclear at present. After the discovery by Sutherland and Rall (1958) that cAMP was an intracellular mediator of hormones in cell function, it was observed that ACTH also increased cAMP in the adrenal cortex (Graham-Smith et al., 1967); thus, it became the first cellular mediator to be recognized in the adrenal cell.

## II. Aldosterone Biosynthesis and Physiological Secretagogues

### A. Biosynthesis of Aldosterone

Aldosterone is synthesized from cholesterol through a cascade of enzymatic reactions in the presence of mitochondrial and microsomal electron transfer systems consisting of NADPH-adrenodoxin reductase with or without adrenodoxin. The steroidogenic enzymes and the genes responsible for their expression and their control are now well-characterized in the adrenal glomerulosa cells (Waterman and Simpson, 1989; Lauber et al., 1990; Shibata et al., 1991). Two key steps in aldosterone biosynthesis are the conversion of cholesterol to pregnenolone and the conversion of deoxycorticosterone (previously thought to be corticosterone) to aldosterone (Kramer et al., 1980). Although the steps and the enzymes in the early pathway of aldosterone synthesis have been well-known for some time, new information relating to the late steps of aldosterone synthesis has led to a revision of the earlier concepts. From studies of the genes of the adrenocortical enzymes, it seems that there is a distinct enzyme with some homology in its nucleotide sequences to those of another adrenal steroidogenic enzyme, 11$\beta$-hydroxylase. This enzyme, now called aldosterone synthase, is believed to be responsible for the conversion of deoxycorticosterone to aldosterone (Curnow et al., 1991; Matsukawa et al., 1990).

### B. Aldosterone Secretagogues and Inhibitors

ACTH, angiotensin II, and potassium are the known physiological secretagogues of aldosterone, although there are other agents, such as serotonin and prostaglandins, that stimulate aldosterone secretion. Among these secretagogues, angiotensin II holds the dominant position in the regulation of aldosterone secretion. Sodium inhibits aldosterone secretion, both directly from the adrenal cells and indirectly by inhibiting peripherally the renin-angiotensin mechanism. The former effect of sodium is physiologically unimportant. A low-sodium diet enhances aldosterone secretion, and it seems to do so by increasing angiotensin II receptors (Lehoux et al., 1994) and perhaps by stimulating steroidogenic pathways as well. However, the latter could be mediated through angiotensin II receptors. Dopamine, somatostatin, and ANP also inhibit aldosterone secretion, but their physiological relevance remains unclear.

## III. Cellular Signaling Mechanisms for the Secretagogues

During recent years, our understanding of the mechanisms of cell signaling for endocrine and nonendocrine cells have been considerably broadened. The cellular membranes are no longer considered as inert structures but are considered dynamic constituents of cells actively participating in cellular function. Both physical and chemical changes occur in the cell membrane in response to cell stimulation. These changes involve (*a*) the membrane-anchored receptors; (*b*) activation and translocation of enzymes, lipids, and proteins (including receptors); (*c*) opening and closing of ion channels; and (*d*) alteration of membrane fluidity.

In most cells, the transduction of signals received by the cell surface receptors occurs through the plasma membrane, often by the mediation of specific G-proteins

‡ Abbreviations: ACTH, adrenocorticotropin; cAMP, cyclic adenosine 3′,5′-monophosphate; ATP, adenosine triphosphate; cGMP, cyclic guanosine monophosphate; $ED_{50}$, median effective dose; $InsP_3$, inositol trisphosphate; $InsP_4$, inositol tetrakisphosphate; TMB-8, 8-(N,N-diethylamino)-octyl-3,4,5-trimethoxy-benzoate; PtdIns, phosphatidylinositol; ANP, atrial natriuretic peptide; PLC, phospholipase C; $InsP_2$, inositol bisphosphate; InsP, inositol monophosphate.

(Casey and Gilman, 1988; Spiegel, 1987). These play major roles in coupling the receptor to signaling elements in the interior of the cell, thereby activating either ion channels or enzymes hydrolyzing nucleotide trisphosphates (ATP or GTP) or phospholipids. This then leads to sequential phosphorylation-dephosphorylation events (Krebs, 1985) involving various protein kinases and phosphatases, which complete the signaling process.

Excitable and some nonexcitable cells can also be activated by voltage changes through depolarization or hyperpolarization across the cell membrane, causing the opening or closing of ion channels. In addition to voltage-activation, the ion channels can be modulated by G-proteins or by second messengers generated in the cell, such as inositol phosphates, cAMP, cGMP, protein kinase, or even fatty acids. There are also cation-exchanging systems or pumps regulating the flux of various ions in the cells (Carafoli, 1987, 1991).

The earliest signaling mechanism identified in the adrenal cell was the adenylate cyclase-cAMP system, with its specific cAMP-dependent protein kinases (Sutherland and Rall, 1958). The activation of adenylate cyclase involves guanine nucleotide-binding proteins (Gilman, 1984).

Other intracellular signaling systems that generate second messengers and are implicated in the regulation of adrenal steroid secretion are as follows: guanylate cyclase-cGMP system, with its associated protein kinase (Waldman and Murad, 1987); PLC-induced cleavage of inositol phospholipids (Abdel-Latif, 1986; Berridge, 1984; Berridge and Irvine, 1984; Exton, 1985; Catt and Balla, 1989), generating inositol polyphosphates, particularly $InsP_3$ and $InsP_4$; 1,2-diacylglycerol with subsequent regulation of intracellular calcium and protein kinase C (Nishizuka et al., 1984; Nishizuka, 1986, 1988; Exton, 1985), respectively; phospholipase $A_2$-induced release of arachidonic acid and formation of eicosanoid metabolites (Bevan and Wood, 1987); phospholipase D-induced cleavage of phospholipids generating phosphatidic acid (Exton, 1990) and diacylglycerol with regulation of protein kinase C (Nishizuka et al., 1984); and calcium fluxes (Borle, 1981; Putney, 1986, 1990), with regulation of calcium calmodulin-dependent protein kinases (Kennedy and Greengard, 1981; Means, 1981).

### *A. Adrenocorticotropin-induced Aldosterone Secretion*

*1. Role of cyclic nucleotides.* That ACTH increases intracellular cAMP has been known for several decades. This increase of cAMP occurs concurrently with steroidogenesis, and in addition, exogenous cAMP and its analogues are able to stimulate steroidogenesis; thus, the role of cAMP as the second messenger of ACTH action in the adrenal fasciculata cells has been accepted. The importance of cAMP-dependent protein kinase in adrenal steroidogenesis has also been elegantly demonstrated in mutant cells lacking the kinase or its gene (Rae et al., 1979; Wong et al., 1986). There were, however, discrepancies noted between the effects produced by ACTH and by cAMP on steroidogenesis in some studies, prompting questions about the role of cAMP as the sole mediator of ACTH action. A lack of perceptible cAMP response in the adrenal fasciculata cells after low doses of ACTH (Beall and Sayers, 1972; Mackie et al., 1972; Moyle et al., 1973) or during the early phase of stimulation of the adrenal fasciculata cells by ACTH (Perchellet et al., 1978; Perchellet and Sharma, 1979), maximal stimulation of steroidogenesis without demonstrable increases of cAMP by some ACTH analogues (Finn et al., 1976; Seelig and Sayers, 1973), and steroidogenesis without a corresponding increase of protein kinase A activity (Richardson and Schulster, 1973) have raised the possibility of the participation of other mediators in the action of ACTH in the adrenal.

Some of the discrepancies observed between changes in steroidogenesis and changes in cAMP levels were, at least in part, attributable to the insensitivity of the available cAMP assays of the time such that small changes in cAMP concentration could not be appreciated. The evolution of sensitive competitive protein-binding assays and then radioimmunoassays later dispelled (although not completely), the earlier doubts about the role of cAMP. Even with such improvements in cAMP assays, it has been observed that maximal stimulation of steroidogenesis correlated with stimulation of cAMP-dependent kinase, but not with maximal cAMP generation or ACTH binding to the adrenal fasciculata cells. Small increases in cAMP could produce marked activation of protein kinase A (Saez et al., 1978), suggesting that very modest or unmeasurable changes in the mediators were adequate to produce the steroidogenesis in the adrenal fasciculata. These results have suggested that the binding of ACTH to a relatively small proportion of receptors could evoke maximal steroidogenesis, thereby implying that a greater proportion of ACTH receptors served as spare receptors. Such conclusions probably extend to the glomerulosa cells.

Rat glomerulosa cells might have more ACTH receptors than do the fasciculata cells of the same species (Gallo-Payet and Escher, 1985). Also, the number of ACTH receptors in rat glomerulosa cells have been reported to be greater than the corresponding numbers of angiotensin receptors (Gallo-Payet and Escher, 1985). Despite the difference in ACTH receptor numbers between the fasciculata and the glomerulosa cells in the rat adrenal, the threshold of steroid responses to ACTH seems to be similar in the two types of cells (Braley and Williams, 1977). There are almost certainly species differences in the numbers of ACTH receptors in adrenal glomerulosa cells. It is possible that a greater degree of binding of ACTH to the glomerulosa cells is required for steroid secretion from these cells than that from the fasciculata cells. Quantitatively, greater amounts of

phosphodiesterase, the enzyme responsible for the degradation of cAMP, seem to be present in the glomerulosa cells than in the fasciculata cells (Gallant et al., 1974; Koletsky et al., 1983). This observation could explain a requirement for greater numbers of ACTH receptors in the glomerulosa cells, because cAMP might be more rapidly degraded in the glomerulosa cells compared with the fasciculata cells.

Studies regarding cAMP generation in response to ACTH were extended to the glomerulosa cells. However, even in relatively recent studies of the aldosterone response to ACTH, it has been observed that the steroid response occurs usually at about a ten-fold lower concentration of ACTH than does the cAMP response. The $ED_{50}$ of the steroid response to ACTH is also less than that for cAMP. In rat glomerulosa cells (Fujita et al., 1979), the lowest concentration of ACTH able to stimulate aldosterone secretion was $10^{-11}$ M, whereas the peak steroid response was observed at $10^{-8}$ M of ACTH with an $ED_{50}$ of $4.5 \times 10^{-10}$ M. In contrast, significant changes in cAMP levels were generally not observed below ACTH concentrations of $10^{-10}$ M, and cAMP levels continued to increase at an ACTH concentration of $10^{-7}$ M. The $ED_{50}$ for the cAMP response was $6 \times 10^{-9}$ M ACTH. Likewise, in perifused calf adrenal glomerulosa cells (Kojima et al., 1985a), ACTH at a concentration of $10^{-11}$ M was able to stimulate a significant increase in aldosterone secretion with an $ED_{50}$ of $5 \times 10^{-10}$ M, i.e, higher than that seen in the rat cells. The cAMP response to ACTH in this study was again shifted to the right by an order of ten with an $ED_{50}$ of $5 \times 10^{-9}$ M, a response very similar to that in the rat cells. Compounds stimulating cAMP, such as choleragen and forskolin, also stimulated aldosterone secretion, and the increase of steroid production correlated with the cAMP production, although the magnitude of steroid production in comparison with that elicited by ACTH was decreased with each agent. Thus, there is little doubt that cAMP is the major mediator of ACTH-mediated aldosterone secretion.

Despite the strong evidence of cAMP as a mediator of ACTH action in the adrenal cell, some investigators have continued to either suspect this evidence or to present evidence for other candidates as possible (additional) mediator(s). A two-receptor model for an ACTH effect on the adrenal cells has also been proposed—one involving cAMP formation and the other not linked with cAMP (Bristow et al., 1980). Such two-receptor or transduction systems exist in other endocrine cell types (Davis et al., 1986; Lowik et al., 1985; Wakelam et al., 1986).

Cyclic GMP (cGMP) was implicated earlier as a possible mediator of ACTH-mediated steroidogenesis in the adrenal fasciculata cells (Harrington et al., 1978; Perchellet et al., 1978; Perchellet and Sharma, 1979; Rubin et al., 1977). It was reported that low concentrations of ACTH increased cGMP levels along with the increase in steroids, without an appreciable increase in cAMP levels (Harrington et al., 1978; Perchellet et al., 1978; Perchellet and Sharma, 1979). The increase in cGMP was shown to be transient (Perchellet and Sharma, 1979) and was not observed with high concentrations of ACTH. One group of investigators (Hayashi et al., 1979) demonstrated an increase in cGMP only extracellularly, but no increase in intracellular cGMP was evident. Activation of cGMP-dependent kinase by ACTH was also detected (Ahrens and Sharma, 1977). Additionally, cGMP was reported by several groups of investigators to stimulate steroidogenesis in vitro in adrenal segments or isolated cells (Kitabchi and Sharma, 1971; Mahaffee and Ney, 1970). This response has been observed both in the fasciculata and glomerulosa cells. However, the magnitude of stimulation of steroidogenesis by cGMP was substantially lower when compared with the steroid response to cAMP (Hayashi et al., 1979). Some studies even failed to show any steroidogenic effect of cGMP. In broken cells, ACTH did not seem to activate guanylate cyclase activity, either with soluble or particulate fractions (McMillan et al., 1971); this differed from the effects of ACTH on adenylate cyclase activity (Kelly and Koritz, 1971). In contrast, sodium nitroprusside increased guanylate cyclase activity markedly without stimulating steroidogenesis in the isolated adrenal cells (Laychock and Hardman, 1978).

It has been suggested that the transient nature of the increase of cGMP in the adrenal cells in response to ACTH might be related to an increase in cGMP-phosphodiesterase (Perchellet and Sharma, 1979). Because ACTH did not seem to activate guanylate cyclase per se in adrenal cells (McMillan et al., 1971), the mechanism of the increase of cGMP has also remained elusive. It might, however, be secondary to the increase of glucocorticoids in the adrenal cells as dexamethasone treatment increases cGMP in the adrenal of hypophysectomized rats (Guillemant and Guillemant, 1979). Taken together, these observations do not suggest that cGMP serves a major role in ACTH-evoked steroidogenesis.

*2. Role of calcium.* The presence of extracellular calcium is critically important for ACTH to elicit steroid secretion from the adrenal cells (Birmingham et al., 1953; Birmingham and Bartova, 1973; Farese, 1971; Haksar and Peron, 1972; Leier and Jungmann, 1973). This is not surprising, because calcium is important in many aspects of the signal transduction process. The requirement for optimal extracellular calcium concentrations seems to be more important at low concentrations of ACTH than at higher concentrations (Haksar and Peron, 1972). It has been demonstrated by binding studies with a labeled ACTH analogue that the duration of ACTH receptor occupancy is reduced at low extracellular calcium concentrations (Cheitlin et al., 1985). Thus, optimal concentrations of extracellular calcium increase receptor occupancy, enabling ACTH to produce sustained steroidogenesis.

Both in whole and in broken adrenal cells, calcium is required for the activation of adenylate cyclase (Podesta et al., 1980). However, calcium might have a biphasic effect on the enzyme itself such that both low and high calcium concentrations can inhibit adenylate cyclase activity, whereas intermediate concentrations of calcium stimulate the enzyme activity, at least in vitro. The stimulatory and inhibitory activity of calcium might be associated with its binding at different sites on the catalytic unit of the enzyme. In addition, calcium also seems to facilitate the association of ACTH receptors with G-proteins and the interaction of G-proteins with adenylate cyclase (Mahaffee and Ontjes, 1980). Calcium is also required for the activity of the steroidogenic enzymes, for protein synthesis, and for synthesis of RNA and DNA. The calcium requirement for the aldosterone-stimulating effect of ACTH and/or cAMP has been amply demonstrated by several investigators (Chiu and Freer, 1979; Fakunding et al., 1979; Kojima et al., 1985a; Muller, 1971; Saruta et al., 1972: Schiffrin et al., 1981; Schiebinger et al., 1985, 1986; Shima et al., 1979).

ACTH seems to produce an influx of calcium in the adrenal glomerulosa cells. Uptake of calcium in response to ACTH stimulation had been reported earlier in rat fasciculata cells (Leier and Jungmann, 1973; Yanagibashi, 1979). In bovine glomerulosa cells (Kojima et al., 1985c), ACTH increased calcium influx by 160% over baseline in one study, whereas in rat glomerulosa cells (Chartier and Schiffrin, 1987), a 135% increase in calcium influx induced by ACTH was observed. Alterations in extracellular potassium concentrations either abolished or enhanced such calcium flux (Kojima et al., 1985c). ACTH also induced a modest efflux of calcium from the glomerulosa cells, which was abolished in the presence of a calcium-free medium (Kojima et al., 1985c). Using an intracellular calcium indicator, Quin 2, no increases in the concentration of cytosolic free calcium could be demonstrated over a wide range of ACTH concentrations or cAMP concentrations in rat glomerulosa cells (Braley et al., 1986). However, when aequorin was used to measure levels of cytosolic free calcium (Kojima and Ogata, 1986), ACTH seemed to increase cytosolic free calcium signals in bovine glomerulosa cells in a dose-dependent manner. This effect was reduced by the removal of extracellular calcium and further decreased by the addition of EGTA, suggesting that calcium influx was the main determinant of the changes in the calcium signal.

In a study by Tremblay et al. (1991) that used Fura 2 in cultured rat or bovine glomerulosa cells, ACTH was reported to increase cytosolic free calcium concentration in single glomerulosa cells, although a relatively small proportion of cells (less than 30%) showed the response. In those cells that responded to ACTH, the cytosolic calcium concentration more than doubled. The pattern of increase of calcium in the adrenal cell was slow and gradual, unlike that induced by angiotensin II. BAY K8644, a calcium channel agonist, tended to potentiate the effect of ACTH. The ACTH-induced increase in cytosolic free calcium could be prevented by incubation of the cells in calcium-free medium, by calcium channel blockers, or by inhibition of cAMP-dependent kinase. Apparently, cAMP induced an increase of cytosolic calcium, which was similar to that produced by ACTH.

ACTH, at a concentration of $10^{-8}$ M, was observed to affect action potentials and calcium currents through both T and L channels in cultured rat and bovine adrenal glomerulosa cells (Durroux et al., 1991). T channels were initially inhibited, followed by some recovery. Significant increases in L channel currents were observed. These effects on calcium channels could be reproduced with a cAMP analogue. The activation of the calcium channels was postulated to be a consequence of potassium channel blockade, resulting in membrane depolarization and activation of voltage-related calcium channels. Calcium channels have also been identified in bovine adrenal fasciculata cells by an elegant technique involving reconstitution of lipid bilayer membrane in vitro (Coyne and Pinkey, 1991). From the magnitude of voltage changes, permeability to barium, and the effects of dihydropyridine compounds, these studies (Coyne and Pinkey, 1991) suggest that the calcium channels in bovine fasciculata cells are of the L type.

In an earlier report, ACTH-mediated aldosterone secretion in rat glomerulosa cells could not be inhibited by 10 $\mu$M nifedipine, a calcium channel inhibitor (Aguilera and Catt, 1986). Also a calcium channel agonist, BAY K8644 did not seem to potentiate the aldosterone-stimulatory effect of ACTH (Hausdorff and Catt, 1988). But, in perifused bovine glomerulosa cells, another calcium channel blocker (nitrendipine 10 $\mu$M) did inhibit aldosterone secretion to a significant degree, and the calcium ionophore, A23187 and calcium channel agonist, BAY K8644 potentiated the effect of forskolin on aldosterone secretion (Kojima et al., 1985a). Also, ACTH stimulation of "calcium-clamped" adrenal cells generated more cAMP when the intracellular calcium level was artificially increased than at a lower intracellular calcium concentration. Most of these observations support the contention that calcium plays a significant interactive role with cAMP in the steroidogenesis induced by ACTH in glomerulosa cells.

Calmodulin, a ubiquitous intracellular calcium-binding protein present in many cells (Cheung, 1980; Means and Dedman, 1980), has been detected in fetal adrenal cells as well as in adult adrenal fasiculata and glomerulosa cells. There is now good deal of evidence that calmodulin plays a significant role in adrenal steroidogenesis, and it is believed also to mediate adrenal steroidogenesis stimulated by ACTH (Carr et al., 1987; Carsia et al., 1982). This observation had been deduced from the inhibitory effects of various calmodulin inhibitors. Calmodulin

might influence the activity of adenylate cyclase (MacNeil et al., 1985) and has a role in the early pathway of steroidogenesis (Carr et al., 1987; Hall et al., 1981). Trifluoperazine, an agent often used to inhibit calmodulin, reduces steroid secretion from the fasciculata type of adrenal cells (Hall et al., 1981). Likewise, calmodulin inhibitors also inhibit ACTH-mediated aldosterone secretion (Balla et al., 1982; Wilson et al., 1984), although to a lesser extent than aldosterone secretion mediated by the other secretagogues, angiotensin and potassium. In mouse adrenal tumor cells, when calmodulin with or without calcium was introduced into the cells by means of liposomes, steroidogenesis was stimulated. Importantly, the stimulation of steroidogenesis was prevented by the presence of anticalmodulin antibodies or the calcium chelator, EGTA in the same liposomes (Hall et al., 1981). Introduction of calmodulin into the cells also stimulated transport of cholesterol to the mitochondria. Furthermore, calmodulin increased the activity of the cholesterol side chain cleavage enzyme in the mitochondria. These results further implicate calcium and calmodulin in the actions of ACTH.

*3. Role of other possible mediators.* Other mediators of ACTH action in the adrenal cells have been suggested. ACTH might produce arachidonic acid and eicosanoid metabolites, i.e., prostaglandins of the E and F series (Laychock and Rubin, 1975). Some investigators have reported the possible role of protein kinase C in ACTH-mediated steroid production (Farese et al., 1987; Vilgrain et al., 1984; Widemaier and Hall, 1985). In one report, ACTH, as well as cAMP, increased the protein kinase C activity in rat Y-1 adrenal cells as well as in rat fasciculata cells (Widemaier and Hall, 1985). The half-maximal concentration of ACTH for the increase of protein kinase C activity was less than that needed to increase steroidogenesis. Phorbol ester, an activator of protein kinase C, stimulated steroidogenesis. When the distribution of protein kinase C in these adrenal cells was examined, the major part of the increase in protein kinase C activity was found in the membranes, and no redistribution of the kinase activity could be detected. This lack of redistribution contrasts with the activation of protein kinase C in other tissues as well as that in rat adrenal fasciculata/reticularis cells in response to ACTH (Farese et al., 1987) or in bovine or rat adrenal glomerulosa cells in response to angiotensin II (Lang and Vallotton, 1987; Nakano et al., 1990) when the enzyme was shown to be redistributed from the cytosol to the membrane concomitantly with its activation.

Vilgrain et al. (1984) have reported that ACTH caused redistribution of protein kinase C from the membrane to the cytosol in the fasciculata cells. A similar redistribution of protein kinase C in response to ACTH was reported in cells from human aldosterone-producing tumors (Ishizuka et al., 1988). These varying observations by different investigators do not lead to clear-cut conclusions regarding a definitive role of protein kinase C in ACTH-mediated steroidogenesis in adrenal fasciculata cells. Interestingly, in cultured rat adrenal glomerulosa cells, phorbol ester caused mild inhibition of ACTH-induced aldosterone secretion, whereas in the protein kinase C-depleted cells as induced by chronic exposure to phorbol ester, ACTH-mediated aldosterone secretion was potentiated (Nakano et al., 1990). As judged by the effect of phorbol ester, protein kinase C activation might inhibit the stimulatory action of ACTH on the induction of some of the steroidogenic enzymes by ACTH (Bird et al., 1992; Ilvesmaki and Voutilainen, 1991; Nasseruddin and Hornsby, 1990).

The precise mechanism for the purported protein kinase C activation by ACTH is not wholly clear, but a role for ACTH-induced stimulation of synthesis of diacylglycerol de novo in the adrenal cells was suggested (Farese et al., 1987). Although ACTH generally does not cause breakdown of inositol phospholipids (Enyedi et al., 1985; Iida et al., 1986; Kojima et al., 1985a; Whitley et al., 1984), phosphoinositide hydrolysis has been reported to occur concomitantly with the increased steroidogenic response in some studies to only low concentrations of ACTH (Farese et al., 1986; Gallo-Payet and Payet, 1989) but not in other studies (Ganguly et al., 1989b) with either low or high concentrations of ACTH. In one study (Farese et al., 1987) in which phosphoinositide hydrolysis in the rat fasciculata/reticularis cells was reported, as induced by a low concentration of ACTH, an increase of cytosolic free calcium signal by Quin 2 was also observed.

Other propiomelanocortin-derived peptides (the peptides cleaved from the precursor peptide of ACTH) have not been shown to stimulate phosphoinositide metabolism during stimulation of aldosterone secretion either in rat (Ganguly et al., 1989b; Hyatt et al., 1986) or in bovine (Ganguly et al., 1989b) adrenal glomerulosa cells. Furthermore, ACTH could not be demonstrated to increase the content of phosphoinositides or $InsP_3$ in the adrenal glomerulosa cells (Underwood et al., 1988). Thus, it seems unlikely that the metabolites of phosphoinositides play a major role in ACTH-mediated steroidogenesis. This leaves the possible mechanism of activation of protein kinase C by ACTH an open question. Could the influx of calcium per se be responsible for the protein kinase C activation (Kobayashi et al., 1988) purported to occur in the adrenal cells in response to ACTH or could some other activator (other than diacylglycerol) of protein kinase C, such as arachidonic acid (McPhail et al., 1984), possibly be involved?

ACTH has long been thought to produce a labile protein in the adrenal cells during its steroidogenic effect. In this perspective, isolation of proteins by several groups of investigators (Krueger and Orme-Johnson, 1983; Pederson and Brownie, 1983; Yanagibashi et al., 1988) in ACTH-stimulated fasciculata cells are of interest and

such a protein is likely to be induced in the glomerulosa cells as well.

It has been proposed recently by two groups of investigators (Gallo-Payet and Payet, 1989; Milnar et al., 1993) that activation of potassium channels might lead to adrenal steroid secretion. The effect of ACTH on potassium flux was reported in bovine fasciculata cells earlier (Kenyon et al., 1985). On the basis of similarities between the effects of some potassium-channel blockers and ACTH on rat glomerulosa cells, inhibition of one type of potassium channels in steroid secretion by ACTH has been invoked (Gallo-Payet and Payet, 1989; Payet et al., 1987;). Calcium influx in the glomerulosa cells was also observed with both agents.

Another group of investigators (Milnar et al., 1993) reported the inhibition of a cholera toxin-sensitive potassium current by ACTH (as well as by angiotensin II) in bovine adrenal fasciculata cells and have suggested a possible role for such a mechanism involving potassium currents in both calcium entry into these cells and steroid secretion from such cells.

Thus, ACTH possibly exerts its steroidogenic effect on adrenal cells through several types of mediators (Kimura, 1986), but the relative importance or physiological relevance of the various mediators, other than cAMP and its protein kinases, remains to be fully defined.

### *B. Angiotensin- and Potassium-induced Aldosterone Secretion*

Angiotensin II is the principal regulator of aldosterone secretion, but the cellular mechanism by which it elicits the increase in aldosterone secretion has remained elusive until recently. It is now believed that alterations in intracellular calcium serve as the mediator of the steroidogenic action of angiotensin II. This subject will be the focus of the ensuing discussion.

Angiotensin II stimulates aldosterone secretion by activating specific well-characterized receptors (Aguilera et al., 1978; Glossman et al., 1974). It has become clear that there are subtypes of angiotensin II receptors (Chiu et al., 1989; Whitebread et al., 1989), one of which is probably involved in the stimulation of steroid secretion as shown in a hamster ovarian cell line (Ohnishi et al., 1992). Angiotensin II stimulates both early and late pathways of aldosterone synthesis (Kramer et al., 1980), the latter mediated by aldosterone synthase (Curnow et al., 1991; Matsukawa et al., 1990). The aldosterone synthase mRNA expression seems to be regulated by angiotenisin II and potassium ion (Lauber et al., 1990; Shibata et al., 1991). Angiotensin II, on the other hand, may inhibit 17$\alpha$-hydroxylase expression, perhaps through the mediation of protein kinase C (Bird et al., 1992; Nasseruddin and Hornsby, 1990).

Although some earlier studies (Albano et al., 1974; Petyreman et al., 1973) suggested that angiotensin might increase intracellular cAMP concentrations during the stimulation of aldosterone secretion, later studies did not support such a tenet. Angiotensin II could not be shown to activate adenylate cyclase in broken cell preparations (Woodcock and Johnston, 1984). These and other studies (Fujita et al., 1979; Ganguly et al., 1990; Marie and Jard, 1983; Woodcock and Johnston, 1984) quite clearly demonstrated that angiotensin II did not increase cAMP accumulation in adrenal glomerulosa cells. It has been demonstrated that angiotensin II actually inhibits adenylate cyclase through an inhibitory (pertussis-sensitive) G-protein (Hausdorff et al., 1987), and this inhibition of adenylate cyclase is independent of the stimulatory effect of angiotensin II on aldosterone secretion (Woodcock and McLeod, 1986).

As with angiotensin II, the signaling mechanism of potassium in aldosterone secretion was also enigmatic. Because potassium is principally an intracellular cation without cell surface receptors in the adrenal cells, it was felt that the intracellular localization of potassium in the glomerulosa cells was the sole mechanism responsible for the stimulation of aldosterone secretion (Baumber et al., 1971; Boyd et al., 1973; Braley and Williams, 1978). The glomerulosa cells also contained more $Na^+$-$K^+$-ATPase activity and had greater potassium content than did the fasciculata cells (Boyd et al., 1973). In one study (Mackie et al., 1977), the argument that increased intracellular potassium was responsible for steroidogenesis from the glomerulosa cell was disputed even though high potassium in the extracellular fluid increased the intracellular potassium content. In other studies (Boyd et al., 1973; Braley and Williams, 1978), it was suggested that the translocation of potassium in the glomerulosa cells required the participation of $Na^+$-$K^+$-ATPase and blockade of this enzyme resulted in a decrease of potassium content in such cells (Boyd et al., 1973) and reduced aldosterone secretion. Inhibitory effects of high concentrations of ouabain, a $Na^+$-$K^+$-ATPase inhibitor, on aldosterone secretion were observed in several studies (Boyd et al., 1973; Braley and Williams, 1978; Schiffrin et al., 1981), although the specificity of these effects remains unclear.

The role of potassium in aldosterone secretion has been clearly established. Potassium may serve several functions in influencing aldosterone secretion in addition to generating intracellular signals. The critical need for extracellular potassium in supporting aldosterone secretion has been demonstrated in vitro by altering media concentrations in incubations of acutely dispersed or primary cultures of adrenal glomerulosa cells (Fredlund et al., 1977; Hornsby and O'Hare, 1977). In the absence of adequate levels of potassium in the medium, glomerulosa cells seem to assume the characteristics of fasciculata cells (Hornsby and O'Hare, 1977). Extracellular potassium concentration also may (Douglas, 1980; Lehoux et al., 1994) or may not (Fredlund et al., 1977) influence angiotensin II receptor content/affinity of rat

glomerulosa cells. Potassium could be involved in the internalization of angiotensin II (probably associated with its receptors) in the adrenal glomerulosa cells (Hunyady et al., 1991), and it has been shown (Curnow et al., 1991; Lauber et al., 1990; Meuli and Muller, 1983) that potassium induces the expression of aldosterone synthase involved in the final step(s) of aldosterone biosynthesis. During the last few years, we have begun to understand the cellular basis of aldosterone secretion as mediated by angiotensin II and potassium and as discussed in the ensuing sections.

*1. Role of calcium.* It is now firmly believed that calcium acts as a major intracellular mediator of both angiotensin II- and potassium-stimulated aldosterone secretion. Intracellular calcium level is regulated by calcium entry, mobilization from and sequestration of calcium in organelles, and extrusion of calcium from the cell (Carafoli, 1987, 1991; Putney, 1986, 1990). Various aspects of the involvement of calcium and its interactions with other factors in aldosterone secretion became clearer through the years, and the evolution of the related concepts will be discussed in the ensuing sections in this historical perspective.

a. EXTRACELLULAR CALCIUM. Although the importance of calcium in adrenal steroid secretion had been appreciated as far back as 1953 (Birmingham et al., 1953), the role of extracellular calcium in aldosterone secretion from the adrenal glomerulosa cells only began to be delineated in the 1970s (Chiu and Freer, 1979; Fakunding et al., 1979; Muller, 1971; Saruta et al., 1972; Shima et al., 1978). The role of extracellular calcium in angiotensin II- and potassium-induced aldosterone secretion was suggested by several investigators in similar types of studies, and Fakunding and colleagues (1979) were the first to examine the question most exhaustively. They demonstrated that increasing extracellular calcium concentrations between 0 and 0.2 mM increased responsiveness of the rat glomerulosa cells to its secretagogues, including angiotensin II and potassium. Maximal increases in steroid production occurred when the extracellular calcium concentration was increased to 0.5 mM. Others (Chiu and Freer, 1979; Foster et al., 1981; Schiebinger et al., 1986; Schiffrin et al., 1981; Shima et al., 1978) have reached similar conclusions with regard to the threshold and maximal effective extracellular concentrations of calcium on steroidogenesis. When the aldosterone response to angiotensin II was examined (Fakunding et al., 1979) with varying concentrations of extracellular calcium, the latter was found not to shift the angiotensin II dose-response curve, but low concentrations of calcium did attenuate the maximal steroid response to both angiotensin II and potassium. This was observed with rat as well as dog glomerulosa cells. Fakunding et al. (1979) also demonstrated that in rat glomerulosa cells, varying extracellular calcium concentrations did not affect the binding of angiotensin II to its receptors.

b. CALCIUM FLUXES. The importance of extracellular calcium in aldosterone secretion suggested that calcium uptake by the adrenal cells could mediate the stimulatory effects of the aldosterone secretagogues, especially angiotensin II and potassium. Initially, the role of calcium entry into the adrenal cells was examined indirectly by using calcium antagonists or agents promoting calcium movement into the cells. Later, more specific calcium channel blockers were used, and finally, calcium fluxes and changes in cytosolic free calcium concentration were directly investigated.

**i. Effects of calcium antagonists or channel blockers.** If calcium influx is involved in angiotensin II-mediated aldosterone secretion from the glomerulosa cells, calcium antagonists should affect aldosterone secretion. With such a premise, the actions of a variety of calcium antagonists were studied.

Quite a few investigators (Balla et al., 1982; Fakunding and Catt, 1980; Foster et al., 1981; Schiebinger et al., 1986; Schiffrin et al., 1981; Shima et al., 1978;) examined the effects of calcium antagonists such as lanthanum, verapamil or methoxyverapamil (D-600) on basal as well as stimulated aldosterone secretion. Most investigators detected some decrease (from 20 to 50%) of basal aldosterone secretion with these agents. These agents also attenuated ACTH, angiotensin II, and potassium-induced aldosterone secretion (usually at high concentrations ranging from $10^{-5}$ to $10^{-3}$ M).

In the detailed studies in the laboratory of Catt (Fakunding and Catt, 1980), lanthanum produced a striking inhibition of basal and angiotensin II-induced aldosterone secretion from the rat glomerulosa cells. Aldosterone secretion was almost completely inhibited by 1 $\mu$M of lanthanum, and about the same relative degree of inhibition of aldosterone secretion occurred ($IC_{50}$ 0.5 to 1 $\mu$M) in the presence of a ten-fold range of angiotensin II concentrations, suggesting that lanthanum did not significantly shift the angiotensin II dose-response curve. Lanthanum produced a similar reduction in potassium-induced aldosterone secretion. Likewise, verapamil inhibited aldosterone secretion induced by angiotensin II and potassium with an $IC_{50}$ of 2 to 5 $\mu$M. In general, similar results were obtained by other investigators (Balla et al., 1982; Foster et al., 1981; Schiebinger et al., 1986; Schiffrin et al., 1981; Shima et al., 1978) using one or more of these compounds.

The clearest evidence for a role of calcium influx in aldosterone secretion has been obtained with the dihydropyridine compounds which are more specific calcium-channel blockers. These agents inhibited aldosterone secretion to a greater extent when stimulated by potassium than when stimulated by angiotensin II (Aguilera and Catt, 1986; Kojima et al., 1984b). This is consistent with the currently accepted mechanisms known to reg-

ulate the changes in the intracellular calcium concentration in glomerulosa cells—namely, mobilization of intracellular calcium—as well as influx of extracellular calcium associated with angiotensin II stimulation. Only calcium influx is associated with potassium stimulation. When intracellular calcium mobilization was blocked, angiotensin II-mediated aldosterone secretion was completely abolished with dihydropyridines (Kojima et al., 1984b).

Whether all of the dihihydropyridine compounds block the same types of calcium channels, and to the same degree, is far from clear as discussed later. Many investigators have used nifedipine, but some investigators have used nitrendipine. One group of investigators (Kojima et al., 1984b) have compared the effects of several dihydropyridine compounds—nitrendipine, nimodipine and nisoldipine. Seemingly, there was little difference between their effects. Both high- and low-affinity binding sites for labeled nitrendipine have been demonstrated in the rat adrenal glomerulosa cells; these binding sites can be considered the correlates of calcium channels (Aguilera and Catt, 1986), although it is not clear whether all types of calcium channels are recognized by such binding.

**ii. Effects of calcium ionophore and a calcium channel agonist.** Calcium ionophore, A23187, has been reported to increase aldosterone secretion (Foster et al., 1981; Fakunding and Catt, 1982; Kojima et al., 1983, 1984a). This finding has further supported the putative role of calcium in the adrenal cell signaling mechanism. The effect of the ionophore on aldosterone secretion, however, was modest and short-lived (Kojima et al., 1983, 1984a). The aldosterone-stimulating effect of the ionophore was shown to be dependent on extracellular cell calcium, and a reduction of extracellular calcium concentration to 0.4 mM shifted the A23187 dose-response curve to the right (Fakunding and Catt, 1982). The concentration of A23187 used in this report ranged from 0.1 to 4 $\mu$M with an $ED_{50}$ response of aldosterone response of 0.3 $\mu$M. The ionophore increased aldosterone secretion maximally at a concentration of 0.5 $\mu$M, with no additional increase occurring beyond this concentration. Kojima and colleagues (Kojima et al., 1983, 1984a) also observed a stimulatory effect of A23187 on perifused porcine and bovine glomerulosa cells at a concentration of 0.5 $\mu$M. However, higher concentrations of A23187 seem to inhibit angiotensin II- and potassium-stimulated aldosterone secretion, and this may be related to a number of potential confounding effects of this agent (Fakunding and Catt, 1982).

The dihydropyridine calcium channel agonist, BAY K8644, was also shown to stimulate aldosterone secretion in rat glomerulosa cells at a minimum and maximum concentration of 30 nM and 1 $\mu$M, respectively (Hausdorff et al., 1986). BAY K8644 did so, presumably by promoting calcium influx, because an actual increase in cytosolic calcium in response to BAY K8644 could not be demonstrated (Hausdorff and Catt, 1988). Unlike the calcium ionophore A23187, BAY K8644 potentiated the effects of angiotensin ii and potassium on aldosterone secretion. Paradoxically, the potentiating effect of BAY K8644 on potassium-induced aldosterone secretion was reduced at potassium concentrations exceeding 9 mM.

Finally, calcium itself was demonstrated to stimulate aldosterone secretion in permeabilized bovine glomerulosa cells at an $EC_{50}$ for calcium of 0.5 $\mu$M (Capponi et al., 1988). This effect of calcium was abolished by either W-7, a calmodulin inhibitor, or ruthenium red, an inhibitor of mitochondrial calcium uptake. These studies further support the involvement of calcium as an intracellular mediator of aldosterone secretion much in the same way as cAMP directly influences steroidogenesis. However, in contrast to cAMP, the magnitude of the calcium effect is quite modest. Whether persistent calcium influx or protein kinase C activation is the principal determinant of the greater and more sustained effect of calcium on aldosterone secretion is not totally clear.

**iii. Estimation of calcium influx.** Calcium influx into adrenal glomerulosa cells has been reported by several groups (Chartier and Schiffrin, 1987; Kojima et al., 1985a, 1985b; Takagi et al., 1988b), but it has been examined most extensively by Kojima and colleagues (1985a, 1985b). Most, but not all, observers have used a relatively simple technique (Mauger et al., 1984), using radiolabeled calcium, $^{45}Ca^{2+}$. By the use of such a technique, calcium influx was quantified in the bovine adrenal glomerulosa cells and was found to increase with time during the initial minute of stimulation of the cells with angiotensin II or potassium. The magnitude of calcium influx was greater with potassium than with angiotensin II (increases of 340 and 230%, respectively, compared with the unstimulated cell). Calcium influx in the glomerulosa cells was found to be sustained during extended periods of incubation of the adrenal cells with angiotensin II. Calcium influx in response to angiotensin II or potassium was dependent on the presence of optimal concentrations of extracellular calcium, and no stimulation of calcium influx could be demonstrated with either angiotensin II or potassium when the extracellular calcium concentration was decreased below 10 $\mu$M. The rate of calcium influx was found to be saturable or maximal at an extracellular calcium concentration of 1.25 mM. Predictably, the dihydropyridine calcium channel antagonist, nitrendipine, blocked both angiotensin II- and potassium-mediated calcium influx. The calcium channel agonist, BAY K8644, enhanced calcium influx additively with angiotensin II stimulation and potentiated potassium-mediated calcium influx.

In the above studies, aldosterone secretion was well correlated with calcium influx, and it was evident that, except for the initial increase of aldosterone secretion, angiotensin II-induced aldosterone secretion could not

be sustained without the calcium influx as judged by the effect of nitrendipine. The ability of angiotensin II to sustain aldosterone secretion was also dependent on the concentration of extracellular potassium, which would modify aldosterone secretion by reducing it or amplifying it several-fold, depending on the potassium concentration, ranging between 2 and 6 mM. This dependence of aldosterone secretion on extracellular potassium has been well-known (Fredlund et al., 1977; Hornsby and O'Hare, 1977). However, it is now clear that alteration of calcium influx is probably the main determinant of this effect of potassium, although other possible effects (Douglas, 1980; Hunyady et al., 1991) cannot be ruled out.

The need for a continual presence of extracellular calcium and calcium influx to maintain the stimulation of aldosterone secretion by angiotensin II and potassium has also been reported or alluded to by several investigators. In our own studies (Ganguly et al., 1992), we clearly demonstrated that the decrease of extracellular calcium concentration with EGTA anytime during stimulation of the adrenal cells markedly inhibited aldosterone secretion. The extent of inhibition was directly correlated with the extent of reduction in extracellular calcium concentration. Similarly, nitrendipine added to the medium at varying time points of incubation had a concentration-dependent and profound inhibitory effect on the aldosterone secretion evoked by both secretagogues, angiotensin II and potassium.

**iv. Nature of calcium channels.** Investigations of neurones initially have revealed several types of calcium channels of which L, T, and N channels have been best characterized (Nowycky et al., 1985; Miller, 1992; Spedding and Paoletti, 1992). Such classification may now be expanded to include other newly recognized channel(s) (Miller, 1992; Spedding and Paoletti, 1992). The structure of calcium channels has also been examined, and it seems to consist of several subunits (Miller, 1992). The calcium channels can be identified by their voltage-dependence, the size of conductance, duration of activity, the relative permeability of calcium and barium, and the effects of various channel blocking agents. The L channels are activated by large depolarizations, blocked by nifedifine and probably by nitrendipine as well, and are blocked by cadmium and $\alpha$-conotoxin among other agents. The T channels are activated by relatively small depolarization and are blocked selectively by nickel. The inactivation of T channels apparently might occur slowly and incompletely, permitting continued calcium influx. The N channels have characteristics in between those of T and L channels and are blocked by cadmium and gadolinium but not by nifedipine.

Earlier studies revealed that adrenal glomerulosa cells possess both L and T types of calcium channel characteristics (Matsunaga et al., 1987). High-affinity and low-affinity binding sites for labeled nitrendipine have been observed in the rat glomerulosa cells (Aguilera and Catt, 1986). Interestingly, the number of such binding sites correlated quite well with the number of angiotensin receptors. The binding of labeled nitrendipine could be inhibited completely by both unlabeled nitrendipine and nifedipine with similar potencies. As judged by the concentration of nifedipine required to inhibit aldosterone secretion, it seems that the action of nifedipine was mediated by the binding of nifedipine to the low-affinity, rather than the high-affinity, binding sites. It is conceivable that the low-affinity binding sites might represent T-type calcium channels.

Subsequent studies (Durroux et al., 1988) seem to suggest that all three types of calcium channels are present in the glomerulosa cells, although the existence of N channels in adrenal cells remains questionable. It is known that BAY K8644 promotes calcium influx through the opening of L channels but not of T or N channels. This was confirmed in rat glomerulosa cells by Durroux and colleagues (1988). Additionally, it was observed that BAY K8644, although facilitating calcium influx through L channels at lower concentrations, decreased calcium flux through the same channels at higher concentrations. Furthermore, BAY K8644 was shown to inhibit calcium influx through T channels at a concentration even lower than that needed to inhibit calcium flux through the L channels.

In bovine glomerulosa cells, other investigators have also identified both L and T types of calcium channels (Cohen et al., 1988). Examining the characteristics and voltage dependency of the calcium currents as well as the sensitivity to low levels of potassium, it was concluded (Cohen et al., 1988) that T-type channels, rather than L channels, were normally involved in aldosterone secretion induced by both potassium and angiotensin II. However, in a prior study (Matsunaga et al., 1987), potassium-induced aldosterone secretion was observed to be associated with activation of both T- and L-type channels in bovine glomerulosa cells. In contrast, only T-channel activation was observed in rat adrenal cells. Additionally, it has been noted (Spat et al., 1989) that potassium-induced aldosterone secretion could be inhibited by 1 $\mu$M nifedipine but not by nickel, and angiotensin II-mediated aldosterone secretion could not be inhibited by 1 $\mu$M nifedipine.

These findings pose more questions about the type of the calcium channels activated by the two secretagogues. Thus, there is still a good deal of ambiguity and confusion with regard to the nature of channels involved in calcium influx in adrenal glomerulosa cells. Some of the observations may be related to species differences in the regulation of calcium influx. The reported specificity of calcium channel blockers may also be questionable. It might be that the effects of low and high concentrations of potassium, and possibly angiotensin II, might be mediated by different types of calcium channels. This pos-

sibility is supported by the observation that potassium can clearly produce different levels of depolarization of adrenal glomerulosa cells.

**v. Calcium efflux.** Angiotensin II not only induces calcium influx, but it also causes calcium efflux from the glomerulosa cells. Calcium efflux from the glomerulosa cells in response to angiotensin II and/or potassium has been reported by several investigators (Elliott and Goodfriend, 1981; Foster et al., 1981; Kojima et al., 1984a,b; Mackie et al., 1978; Williams et al., 1981). The technique used to study calcium efflux generally uses cells preloaded with radiolabeled calcium as described by Borle (Borle, 1981) or a modification thereof. Calcium efflux is believed to be secondary to the increase in the intracellular calcium resulting from both mobilization of intracellular calcium as well as calcium influx.

When compared in a single study (Kojima et al., 1985f), angiotensin II elicited a 3.5-fold increase in calcium efflux, whereas potassium stimulated only a 1.5-fold increase. Calcium efflux preceded aldosterone secretion. In the absence of extracellular calcium or when strontium replaced calcium in the media and cells were also treated with dantrolene to inhibit mobilization of intracellular calcium, angiotensin II failed to increase either calcium efflux or aldosterone secretion.

Angiotensin II-mediated calcium efflux has been reported by other groups (Elliott and Goodfriend, 1981; Williams et al., 1981). In one such report (Elliott and Goodfriend, 1981), angiotensin II also inhibited calcium uptake but increased calcium efflux. As a result, the exchangeable calcium pool was decreased by 34%, and total cell calcium content was reduced in the bovine glomerulosa cells. A transient decrease in total calcium was also reported by Kojima and colleagues (Kojima et al., 1985a), and these investigators were also unable to demonstrate an increase of total cell calcium in response to angiotensin II but did observe an increase of total cell calcium after potassium stimulation. Calcium efflux is probably mediated by the actions of $Ca^{2+}$-ATPase and a $Na^{+}$-$Ca^{2+}$ exchanger. The activation of the latter by angiotensin II has been reported in adrenal glomerulosa cells (Hunyady et al., 1988; Van Der Bent et al., 1993).

**c. Role of changes in cytosolic free calcium concentration.** The capability of measuring intracellular free calcium concentration (Morgan and Morgan, 1982; Borle and Snowdowne, 1982; Tsien et al., 1982; Grynkiewicz et al., 1985) has added a new dimension to the investigations of calcium-related cell signaling mechanisms. Aequorin, a calcium-sensitive photoprotein, and several fluorescent dyes, such as Quin 2 and Fura 2, have been used extensively to monitor intracellular calcium.

Changes in cytosolic calcium concentration in the acutely dispersed adrenal glomerulosa cells were reported initially by two groups of investigators using the calcium chelating dye Quin 2 (Braley et al., 1984; Capponi et al., 1984). More extensive studies were reported later (Braley et al., 1986; Capponi et al., 1987). Resting calcium concentrations in the rat glomerulosa cells (Braley et al., 1986) ranged between 240 and 270 nM, whereas in bovine glomerulosa cells (Capponi et al., 1984), the resting mean cytosolic calcium concentration was 124 nM. In both rat (Braley et al., 1984, 1986) and bovine glomerulosa cells (Capponi et al., 1984, 1987), increasing the extracellular potassium concentration caused a progressive increase of cytosolic calcium concentration, with a plateau in the response being reached at a potassium concentration of 9 mM, even though membrane potential changes up to 28 mM potassiuim have been observed in the glomerulosa cells (Quinn et al., 1987).

In bovine glomerulosa cells (Capponi et al., 1984), the $ED_{50}$ of the cytosolic calcium response to potassium was 6.5 mM, and potassium-induced aldosterone secretion correlated remarkably well with the changes in cytosolic calcium concentration, with an $ED_{50}$ for aldosterone secretion of 6.8 mM. The actions of angiotensin II on the changes in intracellular calcium were also similar in the glomerulosa cells of the two species (Capponi et al., 1984; Braley et al., 1986). As little as $10^{-10}$ M angiotensin II increased intracellular calcium with maximal increases observed at $10^{-8}$ M angiotensin II. Saralasin, a partial angiotensin II antagonist, blocked angiotensin II-induced increase of cytosolic calcium at a ratio of saralasin to angiotensin II of 100:1 (Capponi et al., 1984).

The patterns of changes in cytosolic calcium were different in response to angiotensin II and potassium. Whereas the angiotensin II-induced increase in intracellular calcium was transient (with a return to baseline by 5 minutes), the potassium-provoked calcium response was sustained. Some investigators have referred to a "biphasic effect" of cytosolic calcium response to angiotensin II as an initial sharp, but transient, increase lasting for a few seconds followed by a lower, but sustained, elevation lasting over a longer period (up to 5 minutes or so). The initial phase presumably reflects intracellular calcium mobilization and the prolonged phase reflects calcium influx.

Effects of extracellular calcium, calcium channel blockers, and the calcium channel agonist BAY K8644 on cytosolic calcium were also investigated (Braley et al., 1986; Capponi et al., 1987). In both rat and bovine glomerulosa cells, removal or a reduction in extracellular calcium concentration was associated with a reduction in basal cytosolic calcium concentrations as well as reduction in the response to angiotensin II and potassium. The response to potassium was affected to a greater extent, especially by the calcium channel blocker, nifedipine, at a concentration of 1 μM. Restoring the extracellular calcium concentration to normal progressively increased the responsiveness of the adrenal glomerulosa cells. In the absence of extracellular calcium, angiotensin II produced a rapid transient peak (as occurred in the presence of extracellular calcium), but the calcium levels

were not sustained, and the signal dropped below the initial baseline (Capponi et al., 1987). Moreover, with time the glomerulosa cells became less responsive to angiotensin II, suggesting that depletion of intracellular calcium stores occurred when the cells were suspended in a calcium-free medium (Capponi et al., 1987).

The dihydropyridine calcium channel agonist, BAY K8644, by itself did not increase cytosolic calcium concentrations (Hausdorff et al., 1988), but it did potentiate the cytosolic calcium responses of potassium and angiotensin II in rat glomerulosa cells. In contrast, BAY K8644 had no effect on angiotesin II-induced changes in cytosolic calcium in the bovine glomerulosa cells. This has been interpreted to mean that in the bovine glomerulosa cells there are few or no L-type calcium channels. However, both calcium influx and potentiation of angiotensin II-mediated aldosterone secretion by BAY K8644 has been reported in bovine glomerulosa cells by another group (Kojima et al., 1985b).

Angiotensin II also increased cytosolic free calcium concentrations in cultured bovine glomerulosa cells loaded with Fura 2 with $10^{-13}$ M angiotensin II, consistently increasing cytosolic free calcium levels up to eightfold (Kramer, 1988a, 1988b). Thus, the cultured bovine glomerulosa cells seemed to be more responsive to low concentrations of angiotensin II than are acutely dispersed glomerulosa cells of both rat and bovine adrenals. The patterns of increase in cytosolic free calcium were different at low concentrations compared with high concentrations of angiotensin II stimulation. Aldosterone secretion correlated well with the initial phase of the increase of cytosolic calcium, but only at low concentrations of angiotensin II was the sustained phase of cytosolic calcium increase correlated with aldosterone secretion. Angiotensin II antagonists, $Sar^1Ile^8$ angiotensin and $Sar^1Ala^8$ angiotensin, inhibited the angiotensin II-mediated increase of cytosolic calcium at high concentrations but had different potencies in this regard.

In another study (Balla et al., 1990) with perifused rat glomerulosa cells, potassium produced concentration-dependent increases in the cytosolic calcium concentration as measured by Fura 2. Incremental effects of potassium on cytosolic calcium levels were observed up to a concentration of 18 mM, unlike concentrations seen in other studies. BAY K8644 potentiated the cytosolic calcium response of potassium concentrations up to 30 mM. In these studies, aldosterone secretion provoked by potassium peaked at 8.4 mM. The reason for the differences in the steroidogenic and calcium responses to potassium in this study, compared with other studies, are not clear but might be related to the fact that the former study was conducted in a dynamic perifusion system, whereas the other studies (Capponi et al., 1984; Braley et al., 1986) were performed in static incubations.

Angiotensin II similarly increased aequorin-related calcium signals in perifused bovine adrenal capsules (Appeldorf and Rasmussen, 1988; Appeldorf et al., 1988) as well as in acutely dispersed glomerulosa cells (Pratt et al., 1989). The effects of angiotensin II were inhibited by the angiotensin II antagonist, $Sar^1Ala^8$ angiotensin (Appeldorf and Rasmussen, 1988). Again, the initial increase of the aequorin signal in response to angiotensin II was unaffected by the presence or absence of extracellular calcium, but the subsequent sustained phase of the calcium signal was reduced by increasing extracellular calcium concentrations (Pratt et al., 1989). Nitrendipine caused the potassium-induced increase of cytosolic calcium to decline abruptly. Adequate concentrations of potassium in the media were required for an optimal cytosolic calcium signal in response to angiotensin II.

d. SINGLE CELL CALCIUM RESPONSES AND CALCIUM OSCILLATIONS. Changes in cytosolic calcium in response to angiotensin II and potassium have been examined in great detail by digital imaging of single rat glomerulosa cells loaded with Fura 2 (Connor et al., 1987). The responses in individual cells to potassium and angiotensin II were somewhat heterogenous, with regard to both intensity and spatial distribution of the changes of the signal within the cell. There were also differences in patterns of response observed with potassium and angiotensin II stimulation. Whereas the potassium-induced increase in cytosolic calcium concentration seemed to be generalized in the cell cytoplasm, angiotensin II-evoked changes were localized to certain areas of the cell.

Later, using microspectrofluorometry as well as digital imaging, changes in cytosolic free calcium concentrations were extensively characterized and quantitated in both single rat and bovine adrenal glomerulosa cells (Quinn et al., 1988a, 1988b; Johnson et al., 1989; Tremblay et al., 1991). Resulting studies provide interesting insights into cell calcium changes evoked by angiotensin II, potassium, as well as vasopressin. Again, elegant studies by Quinn from the laboratory of Williams (Quinn et al., 1988a, 1988b, 1991) revealed differences in the patterns of cytosolic calcium response in rat adrenal glomerulosa cells following the stimulation by angiotensin II or potassium. The effects of angiotensin II on the cytosolic calcium responses were rather complex. One major difference between the responses to angiotensin II and potassium was that angiotensin II (but not potassium) induced patterns of calcium oscillations in single cells. The frequency of the oscillations tended to occur more with the lower concentrations of angiotensin II than with the higher concentrations. Concentrations greater than 1 nM produced transient peaks followed by a plateau, whereas lower concentrations produced repeated oscillatory changes.

The responses to angiotensin II were also characterized by a concentration-related delay in the onset of the cytosolic calcium spike, with higher concentrations of angiotensin II causing a shorter delay. Peak levels of calcium were reached by about 15 seconds and showed

some concentration-dependence. Again, there was significant variability in the pattern of response between individual cells. The potassium-induced calcium response was quite prompt but lacked the oscillations seen with angiotensin II stimulation. Although paired experiments usually showed a greater magnitude of calcium response with the higher potassium concentrations, the changes in cytosolic calcium with varying concentrations of potassium could not be sharply differentiated. A calcium channel blocker, cadmium, or use of a calcium-free medium prevented the calcium response to potassium. Nifedipine also blocked the effect of potassium after a lag period.

Other investigators (Johnson et al., 1989; Tremblay et al., 1991) also have measured single bovine and/or rat adrenal glomerulosa cell responses to aldosterone secretagogues, using essentially similar techniques of microspectrofluorometry and digital imaging using Fura 2. Like Quinn, they have also shown different patterns of responses as well as varying responsiveness of individual cells to stimulation of bovine glomerulosa cells with angiotensin II and potassium. Oscillatory responses were seen only with angiotensin II but not with potassium. The effects of changes in extracellular calcium and use of calcium channel blockers also were in agreement with the previous studies.

Oscillatory patterns in cytosolic calcium responses to agonists have now been recognized in many different types of cells (Berridge, 1990; Fewtrell, 1993) including adrenal glomerulosa cells (Johnson et al., 1989; Quinn et al., 1988a; Tremblay et al., 1991), as alluded to earlier. These intriguing oscillations have been observed during cytosolic calcium measurements with aequorin, Fura 2, and microelectrodes. Various types of oscillations, namely sigmoid, transient, or asymmetric oscillations, have been observed. In the adrenal glomerulosa cells, such oscillatory behavior has been noted in response to angiotensin II stimulation but not with potassium stimulation.

Although the precise basis of such oscillations remains unclear, several possible mechanistic models have been proposed. Whereas in excitable cells oscillations can conceivably occur from fluctuating membrane potentials, this is not believed to account for the oscillations in nonexcitable cells, although some evidence suggests otherwise (Kukuljan et al., 1994). In such cells, the possible role of a receptor-operated or second-messenger-mediated mechanism, has been suggested. In this context, because of the purported effects of 1,4,5-$InsP_3$ (Harootunian et al., 1991), diacylglycerol, protein kinase C, and calcium itself (Finch et al., 1991) on calcium release, each might be assigned a putative role in different variations of the hypothetical models to explain the oscillatory behavior. Because each of the cellular mediators has been shown to produce oscillations in different experimental cell preparations, it will be difficult to determine their relative contribution to calcium oscillations in angiotensin II-stimulated cells. As potassium, unlike angiotensin II, did not produce the cytosolic oscillatory response in the adrenal glomerulosa cells, it can be inferred that potassium-mediated aldosterone secretion depends on amplitude-mediated changes in cell calcium concentrations, whereas angiotensin II-induced aldosterone secretion involves frequency-modulated cytosolic calcium changes.

e. INTRACELLULAR CALCIUM STORES. Intracellular calcium is located in various organelles, compartments or pools. Principal calcium storage sites include different parts of endoplasmic reticulum and mitochondria. A number of pharmacological agents have been used to probe the roles of various calcium stores as discussed in the ensuing sections.

**i. Calcium stores in endoplasmic reticulum and/or calciosome.** Dantrolene has been shown to inhibit angiotensin II-induced aldosterone secretion and/or increase of cytosolic calcium concentration (Braley et al., 1986; Kojima et al., 1984a, 1984b; Kramer, 1988b; Rossier et al., 1987), seemingly confirming the putative role of intracellular calcium mobilization from endoplasmic reticulum in adrenal glomerulosa cells. Surprisingly, dantrolene has also been reported to inhibit potassium-induced increase of cytosolic calcium concentration (Braley et al., 1986) and aldosterone secretion (Braley et al., 1986; Rossier et al., 1987), which is not accompanied by phosphoinositide hydrolysis and/or inositol polyphosphate formation (Ganguly et al., 1990; Hunyady et al., 1990; Kojima et al., 1985d). This suggests that intracellular calcium mobilization might also occur during potassium-evoked aldosterone secretion as reported for muscle cells (Meisheri et al., 1986). This type of mobilization of calcium could conceivably be triggered by calcium itself (known to potentiate $InsP_3$-induced calcium release (Finch et al., 1991; Iino and Endo, 1992) when calcium concentration increases during depolarization of the adrenal cell in response to potassium. It is of interest that dantrolene also inhibited the modest calcium ionophore-induced aldosterone secretion from the glomerulosa cells (Rossier et al., 1987). Dantrolene-mediated inhibition of the increase of cytosolic free calcium concentration in response to angiotensin II ($IC_{50}$ = 20 $\mu$M) occurred even in the absence of extracellular calcium (Kramer, 1988b), implying that dantrolene did not impede calcium influx. In one study (Rossier et al., 1987), dantrolene was unable to abolish 1,4,5-$InsP_3$-induced calcium release in bovine adrenal glomerulosa cells, even though it reduced angiotensin II-mediated aldosterone secretion markedly. This suggested that the aldosterone-inhibiting effect of dantrolene was not solely related to interference of calcium mobilization by 1,4,5-$InsP_3$.

Originally, the endoplasmic reticulum was thought to be the site of the stored calcium mobilized by 1,4,5-$InsP_3$. However, some evidence has been presented indicating

that the calcium-storing organelle sensitive to 1,4,5-$InsP_3$ is different from the endoplasmic reticulum and this putative calcium store has been called the calciosome, an organelle, situated close to the cell membrane (Rossier et al., 1989; Volpe et al., 1988). This conclusion was based on the findings that 1,4,5-$InsP_3$-binding sites were located on microsomes associated with the plasma membrane and that these binding sites in the microsomal fraction could be separated from the endoplasmic reticulum (Rossier et al., 1989, 1991). For example, when the microsomal components were fractionated on sucrose density gradients, the 1,4,5-$InsP_3$-binding sites or receptors could be separated from the endoplasmic reticulum. Also, various enzyme markers have been used to differentiate subcellular fractions containing endoplasmic reticulum from that containing calciosome. Furthermore, evidence obtained from immunocytochemical distribution of $Ca^{2+}$-ATPase, various calcium-binding proteins, calsequestrin, calsequestrin-like protein, and calreticulin, has been marshalled to support the possible existence of calciosomes as distinct organelles. Presently, the concept of the existence of calciosome as distinct from endoplasmic reticulum remains controversial at best (Koch, 1990; Krause et al., 1989; Meldolesi et al., 1990; Milani et al., 1990; Ross et al., 1989; Rossier et al., 1991 Villa et al., 1991). In various tissues, support or lack of it for this putative calcium-storing organelle has been presented. It is conceivable that calciosome could be part of the endoplasmic reticulum itself rather than a separate entity despite some of the evidence presented to the contrary.

Thapsigargin, a $Ca^{2+}$-ATPase inhibitor, increases basal aldosterone secretion and produces an additive or potentiating effect on angiotensin II and potassium-induced aldosterone secretion (Ely et al., 1991; Ganguly, 1994; Ganguly and Waldron, 1994; Hajnoczky et al., 1991). This aldosterone-stimulating effect seems to be related to an increase of cytosolic calcium concentration (Ely et al., 1991), resulting from the release of intracellular calcium from the endoplasmic reticulum (or calciosome) caused by $Ca^{2+}$-ATPase blockade (Thastrup et al., 1990) as well as calcium influx (Takemura et al., 1989). Thus the aldosterone-stimulating action of thapsigargin is consistent with the postulated role of calcium as a mediator of aldosterone secretion. The effect of thapsigargin on calcium influx also suggests an inherent cellular mechanism to stimulate calcium influx when certain intracellular calcium stores are depleted—the so-called capacitative entry mechanism (Putney, 1990).

**ii. Calcium stores in mitochondria.** Angiotensin II stimulation of glomerulosa cells not only releases calcium from a 1,4,5-$InsP_3$-sensitive calcium pool, it seems to decrease mitochondrial calcium as well (Kramer, 1990). Similar observations were reported by Balla et al. (1985), who also found a net decrease in total cell calcium following angiotensin II stimulation, an observation also made by other investigators (Elliott et al., 1985). How the mitochondrial pool is decreased by angiotensin II is not clear, but this pool presumably reflects a slowly exchangeable pool of calcium. Mitochondrial calcium pool is known to be unresponsive to stimulation by 1,4,5-$InsP_3$ (Rossier et al., 1987). Mitochondria may act as a sponge or a buffer, initially sequestering some of the cytosolic calcium increased by the agonists, but it then may help to restore slowly the cytosolic free calcium (in concert with the influx of calcium from the extracellular pool) to replace the calcium ejected from the cell by the efflux mechanism.

Some inhibitors of mitochondrial calcium uptake can modify the aldosterone response to angiotensin II and potassium. Ruthenium red, an inhibitor of mitochondrial calcium uptake, attenuates aldosterone secretion (Ganguly and Haxton, unpublished observations) probably caused by interruption of mitochondrial calcium flux (Rossier et al., 1987). Purported uncouplers of oxidative phosphorylation of the mitochondria (Balla et al., 1985; Kramer, 1990) have also been found to increase cytosolic calcium concentration (Kramer, 1991) or to mobilize calcium from adrenal glomerulosa cells (Balla et al., 1986). Calcium might serve as a modulator of steroidogenic enzymes located in the mitochondria (McCormack and Denton, 1986), and such a possibility can be extended to aldosterone synthesis in the glomerulosa cell, inasmuch as two of the key enzymes are mitochondria based.

**iii. Calcium stores in multiple organelles.** TMB-8 has been reported to inhibit adrenal steroid secretion including aldosterone (Braley et al., 1986; Lihrmann et al., 1987; Kramer, 1988b; Rossier et al., 1987), and it inhibited both the potassium-stimulated (Braley et al., 1986) and angiotensin II-stimulated (Braley et al., 1986; Kramer, 1988b) increase in cytosolic calcium concentration in rat and bovine glomerulosa cells in a dose-dependent ($IC_{50}$ = 50 $\mu$M) manner (Braley et al., 1986; Kramer, 1988b). Inasmuch as TMB-8-mediated inhibition of aldosterone secretion, as evoked by angiotensin II and potassium, is probably related to its effects on different cellular targets (Foster et al., 1990; Lihrmann et al., 1987), no firm conclusions about the basis of its effects on aldosterone secretion can be made.

After stimulation of the glomerulosa cells by angiotensin II, the angiotensin II-sensitive intracellular calcium stores are reduced, but the calcium stores are rapidly restored, as shown by an increase in total cell calcium, without any measurable change in the cytosolic free calcium concentration (Kojima et al., 1987). This replenishment of calcium stores required the presence of extracellular calcium and was not affected by a concentration of nitrendipine that blocked angiotensin II-induced (but not basal) calcium influx. Thus, the restoration of the 1,4,5-$InsP_3$-sensitive intracellular calcium pools most likely occurred as a result of calcium coming mainly from

an extracellular source. Immediately after restoration of the pool, there was some refractoriness of the pool to mobilization by angiotensin II, but with time, the pool regained responsiveness to the 1,4,5-$InsP_3$ stimulus. This suggests a temporary compartmentalization of the intracellular calcium stores during their replenishment. The initial unresponsiveness of calcium mobilization to 1,4,5-$InsP_3$ was probably not related to calcium sequestration in the mitochondrial pool (Rossier et al., 1987).

All of these studies underscore a complex interplay between extracellular calcium, intracellular cytosolic calcium and various calcium stores during and after stimulation of adrenal cells by the agonists.

f. SUMMARY. Both angiotensin II and potassium increase concentrations of cytosolic calcium concurrently or preceding the stimulation of aldosterone secretion. The evidence for a role of calcium as one of the critical cellular messengers for the two aldosterone secretagogues is quite persuasive inasmuch as calcium ionophore, a calcium channel agonist, and calcium itself are both capable of stimulating aldosterone secretion independently, and reduction of extracellular calcium or blockade of calcium influx is associated with marked inhibition of aldosterone secretion evoked by the two secretagogues. However, the precise mechanism by which calcium exerts this aldosterone-stimulating effect is not wholly clear, because there are potentially multiple targets of calcium action in adrenal glomerulosa cell leading to the steroidogenesis.

Despite the evidence that calcium is critically important for the steroidogenic response of both angiotensin II and potassium, there seem to be differences as regards the precise mode by which each secretagogue produces the qualitative and quantitative changes in the cytosolic calcium concentration. Potassium clearly causes depolarization of the glomerulosa cells (Quinn et al., 1987) preceding the calcium influx. Whether such a mechanism is also responsible for angiotensin II-mediated calcium influx is not entirely clear. Angiotensin II may also cause depolarization of adrenal cells (Natke and Kabela, 1979; Heschler et al., 1988). Changes in potassium fluxes observed during stimulation of glomerulosa cells by angiotensin II, which have been attributed to depolarization and hyperpolarization of such cells (Lobo and Marusic, 1986; Shepherd et al., 1991) provide additional evidence for angiotensin II-induced changes in membrane potential. On the other hand, it has often been suggested that angiotensin II might cause calcium influx by a receptor-activated mechanism. How does angiotensin II activate calcium channels through its receptors? Could it do so through a G-protein or other mediators such as 1,4,5-$InsP_3$, 1,3,4,5-$InsP_4$ or protein kinase C (Brown and Birnbaumer, 1990; Ferris and Snyder, 1992; Gomperts, 1983; Kaczmarek, 1987: Kuno and Gardner, 1987; Rosenthal et al., 1988)? It is of interest that one study showed that pertussis toxin does not inhibit angiotensin II-induced aldosterone secretion but blocks calcium influx (Kojima et al., 1986b). In Y-1 adrenal cells, pertussis toxin was also shown to inhibit angiotensin II-induced slowly inactivating calcium currents (Heschler et al., 1988), although the effect on aldosterone secretion was not mentioned; whereas in perifused bovine glomerulosa cells, pertussis toxin tended to attenuate angiotensin II-induced aldosterone secretion (Barrett and Isales, 1989). However, in other studies (Hausdorff et al., 1987; Woodcock and McLeod, 1986;), pertussis toxin treatment of rat adrenal glomerulosa cells did not affect angiotensin II-mediated aldosterone secretion. There are other as yet ill-defined mechanisms of calcium entry (Hoth and Penner, 1992; Luckhoff and Clapham, 1992) that could conceivably explain the basis of receptor-activated calcium influx. Such a mechanism might be triggered by depletion or decrease of intracellular calcium stores, as originally suggested by Putney (1990).

As dicussed earlier, calcium channel blockers, known to block voltage-dependent calcium channels, do inhibit aldosterone secretion induced by both angiotensin II and potassium. But there remain unanswered questions regarding differences of the effects of calcium channel blockers (especially nifedipine) on aldosterone secretion evoked by each secretagogue (Spat et al., 1989). However, in the presence of another dihydropyridine calcium channel blocker, nitrendipine, no differences in the sensitivity of aldosterone secretion induced by angiotensin II and potassium was observed by one group of investigators (Kojima et al., 1984b). Whether nitrendipine preferentially blocks T-type rather than L-type calcium channels in the concentrations used to inhibit aldosterone secretion is not clear. Finally, there also seems to be diversity within the same subtypes of calcium channels (Perez-Reyes et al., 1990). Could such a diversity perhaps explain the observed differences in the effects of some of the calcium channel blockers? Possibly, the current classification of the calcium channels is oversimplified. Alternatively, the purported specificity of the calcium channel blockers must be seriously questioned. Definitive answers to these issues are presently elusive.

*2. Role of calmodulin and calmodulin-dependent protein kinase.* Calmodulin-like activity has been detected in the glomerulosa cells (Koletsky et al., 1983). Interestingly, in the guinea pig adrenal cortex, significantly higher activity of calmodulin-dependent protein kinase was reported in the outer cortex compared with that in the inner cortex (Kubo and Strott, 1988). Also, substrate proteins for this protein kinase have been identified in the glomerulosa zone of the rat adrenal (Kigoshi et al., 1989).

A number of calmodulin inhibitors have been shown to inhibit aldosterone secretion induced by its secretagogues, especially angiotensin II and potassium. Trifluoperazine, pimozide, calmidazolium, W-5, and W-7, compounds which are known to inhibit calmodulin, also

inhibit aldosterone secretion (Balla et al., 1982; Wilson et al., 1984). We have demonstrated (Ganguly et al., 1990, 1992) that aldosterone secretion evoked by angiotensin II and potassium was extremely sensitive to inhibition by both W-7 and calmidazolium. Both compounds reduced aldosterone secretion to a similar degree. The effects of calmodulin inhibitors on aldosterone secretion might not necessarily be related to the specific inhibition of calmodulin. As with many pharmacological agents, calmodulin inhibitors are also not wholly specific with regard to their purported effects. However, because a variety of calmodulin antagonists have been demonstrated to inhibit aldosterone secretion, it is unlikely that their
effects, in major part, are nonspecific. Additionally, it has also been suggested that calcium and calmodulin influence enzymes in the steroidogenic pathway. Therefore, the evidence thus far seems to point to a major dependence of aldosterone secretion on calcium-calmodulin system.

*3. Role of phosphoinositides and other mediators.*

a. PHOSPHOINOSITIDES. About four decades ago, it was observed that cholinergic stimulation of pancreatic exocrine cells increased the incorporation of $^{32}P$ into PtdIns in such cells (Hokin and Hokin, 1953). Subsequently, a number of investigators observed a similar PtdIns turnover response in hormone- and neurotransmitter- sensitive tissues. Michell (1975) put forth a provocative hypothesis that cellular inositol phospholipid turnover was associated with a gating effect in the cell membrane, causing calcium influx. A large body of evidence has since linked inositol phospholipid metabolism with cell signaling in hormone and neurotransmitter action.

Phosphoinositides constitute a small portion of the cell membrane lipids (about 2 to 8%), and among the various phosphoinositides, PtdIns 4,5-bisphosphate and PtdIns 4-phosphate form a small minority. These are the principal phosphoinositides that are rapidly hydrolyzed during cellular stimulation. Research in this area led to a major discovery (Berridge, 1984; Berridge and Irvine, 1984) that two important metabolites of phosphoinositide hydrolysis—1,4,5-$InsP_3$ and 1,2-diacylglycerol—serve as intracellular second messengers. The metabolite, 1,4,5-$InsP_3$, was shown to mobilize intracellular calcium from nonmitochondrial stores and thereby measurably increase cytosolic calcium concentrations. About this time, a novel protein kinase had been discovered, and that was named protein kinase C (Nishizuka et al., 1984; Nishizuka, 1986, 1988). This serine/threonine protein kinase could be activated by phospholipids in the presence of calcium. Diacylglycerol was demonstrated to be an important cofactor, enabling the activation of this protein kinase at physiological calcium concentrations. The synthesis and metabolism of PtdIns (PtdIns, PtdIns4P, PtdIns4,5P2) as well as their hydrolytic products, inositol phosphates, have been extensively studied (Berridge, 1984; Berridge and Irvine, 1984; Majerus et al., 1986, 1988) and have proven to be quite complex.

It is generally recognized that the hormone-induced activation of PLC in the adrenal glomerulosa cells and hydrolysis of PtdIns polyphosphates (PtdIns4,5P2 and PtdIns4P) are independent of extracellular calcium or calcium influx and can take place at basal or resting levels of cytosolic free cell calcium. The enzyme is located both in the cytosol and in the plasma membrane of the cell. Subsequent to the initial hydrolysis of PtdIns4,5P2 and PtdIns4P by PLC at the basal calcium concentrations (about 0.1 $\mu$M), calcium-dependent breakdown of PtdIns may be catalyzed by PLC as a result of increased cytosolic calcium concentration (about 1 $\mu$M) caused by $InsP_3$-induced intracellular mobilization of calcium as well as calcium influx. Thus, the inositol phosphates, as well as diacylglycerol so formed during angiotensin II-induced activation of PLC, may have their origin from different phosphoinositides. It is not clear whether the cytosolic calcium concentration in the adrenal glomerulosa cell during angiotensin II stimulation can reach the level required to trigger the hydrolysis of PtdIns.

Several isoenzymes of PLC ($\beta$, $\gamma$, and $\delta$) have been identified (Rhee and Choi, 1992; Rhee et al., 1989), and they all hydrolyze the various phosphoinositides, although some of the phosphoinositides may be preferred over others (Rhee et al., 1989). PLC (especially PLC$\beta$) is probably activated by a GTP-binding protein (Baukal et al., 1988; Cockcroft, 1987; Litosch and Fain, 1986; Smrcka et al., 1991), which may be coupled to angiotensin receptors. The differential roles, localization, regulation, or unique characteristics, if any, of the various PLCs, in the regulation of phosphoinositide metabolism or cellular signaling in the adrenal, have not yet come to light.

In rat and bovine adrenal glomerulosa cells, angiotensin II has been clearly demonstrated (Baukal et al., 1988: Catt and Balla, 1989; Enyedi et al., 1985; Farese et al., 1984; Ganguly et al., 1986; Kojima et al., 1984a; Rossier et al., 1986, 1988; Woodcock et al., 1988), to cause the hydrolysis of phosphoinositides with the resultant production of the second messengers, inositol 1,4,5-$InsP_3$ and diacylglycerol. The calcium-mobilizing effects of potassium are not associated with increases in PLC activity, as judged by the lack of formation of inositol phosphates (Ganguly et al., 1990; Kojima et al., 1985d) or diacylglycerol (Hunyady et al., 1990). Although it is generally believed that angiotensin II-mediated hydrolysis of phosphoinositides by PLC is independent of calcium influx, this view is not shared by all investigators (Foster et al., 1990). After the initial hydrolysis of phosphoinositides, however, calcium influx seems to be a requisite for the continued action of PLC (Hunyady et al., 1990). Rapid hydrolysis of phosphoinositides in response to angiotensin II has been observed in bovine glomerulosa cells after radiolabeling of inositol phospho-

lipids with phosphorus, inositol, or arachidonic acid (Kojima et al., 1984a). Some differences were observed in the duration of the hydrolysis of the phosphoinositides or the restoration of their levels in cells labeled with the various labeled precursors, which might suggest compartmentalization of agonist-responsive phospholipid pools. The inositol polyphosphates formed in response to angiotensin II are degraded sequentially by a series of highly specific phosphatases (phosphomonoesterases) which cleave phosphate groups forming various $InsP_2$ and InsP in the process.

It is now clear (Balla et al., 1988; Rossier et al., 1986) that inositol 1,4,5-$InsP_3$ initially formed from PtdIns4,5P2, is rapidly converted to 1,3,4,5-$InsP_4$ and then dephosphorylated to inositol 1,3,4-$InsP_3$ (thought to be a relatively inert isomer of 1,4,5-$InsP_3$). The conversion of inositol 1,4,5-$InsP_3$ to 1,3,4,5-$InsP_4$ is mediated by the cytosolic 3-phosphokinase. The activity of this enzyme is dependent on calmodulin, and ATP and is stimulated by elevated levels of cytosolic free calcium (Rossier et al., 1986). The 1,4,5-$InsP_3$-kinase has a greater affinity for 1,4,5-$InsP_3$ than the 5-phosphatase has for 1,4,5-$InsP_3$. The latter enzyme degrades the $InsP_3$ to 1,4-$InsP_2$. Protein kinase C may regulate this phosphatase activity (Majerus et al., 1988). Thus, by mobilizing intracellular calcium, 1,4,5-$InsP_3$ favors its own metabolism to 1,3,4,5-$InsP_4$ rather than its degradation to 1,4-$InsP_2$. The higher affinity of 5-phosphatase for 1,3,4,5-$InsP_4$ than for 1,4,5-$InsP_3$ may also favor the metabolism of 1,4,5-$InsP_3$ to 1,3,4,5-$InsP_4$. As a result, several isoforms of $InsP_2$ and InsP are formed from the higher forms of the inositol polyphosphates. The various monophosphates are finally degraded by a nonspecific monophosphatase to free inositol. This enzyme is inhibited by lithium. Therefore, studies using lithium may amplify some of the inositol phosphate formation. The free inositol is reutilized for the synthesis of the various PtdIns. This synthesis whereby myo-inositol combines with cytidine diphosphodiacylglycerol is followed by incorporation of the phospholipid in the plasma membrane. There it is phosphorylated sequentially by kinases forming PtdIns4P and PtdIns4,5P2. A 3-PtdIns kinase has been identified in some cells (Stephens et al., 1991). Whether this enzyme is present in the adrenal glomerulosa cells and whether it is involved there in the synthesis of 3-phosphorylated PtdIns is unclear.

Among these inositol metabolites, the 1,4,5-$InsP_3$ has been clearly assigned a biological function in that it mobilizes intracellular calcium. The 1,3,4,5-$InsP_4$ may also serve a useful function in cell signaling (Irvine and Moor, 1986)—it may promote calcium influx or mobilize intracellular calcium (Ely et al., 1990) with or without the coparticipation of the 1,4,5-$InsP_3$. Two other tetrakis isomers of inositol phosphates, 1,3,4,6-$InsP_4$ and 3,4,5,6-$InsP_4$ have been identified in the adrenal glomerulosa cells (Balla et al., 1989b). Their functions, if any, are unknown presently. It is now known that higher forms of inositol phosphates ($InsP_5$ and $InsP_6$) may be formed in some tissues (including the adrenal) in response to agonist stimulation (Balla et al., 1989b; Heslop et al., 1985; Menniti et al., 1993). Their roles in cell signaling in general and adrenal in particular, are yet to be clearly defined.

It has been shown that in the cultured bovine glomerulosa cell, 1,4,5-$InsP_3$ in response to angiotensin II, is produced in a biphasic manner (Balla et al., 1989a). The secondary increase of 1,4,5-$InsP_3$ was accompanied by an increase of 1,3,4,5-$InsP_4$. When cell cytosolic calcium and 1,4,5-$InsP_3$ responses to varying doses of angiotensin II were compared, peak calcium and 1,4,5-$InsP_3$ responses correlated quite well, but the aldosterone response was more sensitive than calcium and 1,4,5-$InsP_3$ response, so that the dose-response relationship of aldosterone to angiotensin II was found to be on the left of the peak cytosolic calcium and 1,4,5-$InsP_3$ responses. This is similar to cAMP and aldosterone responses to ACTH and suggests the high degree of sensitivity of the steroid secretory response to very small changes in the intracellular mediators.

Receptors for the 1,4,5-$InsP_3$ have been found in the bovine adrenal microsomes (Guillemette et al., 1987). The binding of labeled 1,4,5-$InsP_3$ to the receptors is saturable, and is of high-affinity and specificity (Guillemette et al., 1987; Rossier et al., 1989; Ely et al., 1990). Initial studies suggested that the binding sites were in close approximation to the plasma membranes of the adrenal cells. Subsequent studies (Volpe et al., 1988), as discussed earlier, have pointed to a plasma membrane associated (nonendoplasmic reticulum) calcium store (named calciosome) as the site of 1,4,5-$InsP_3$-binding in the adrenal cortical cells (Rossier et al., 1989, 1991). The concept of the calciosome, however, remains controversial. The 1,4,5-$InsP_3$ receptors can be phosphorylated by any of several protein kinases: protein kinase A, protein kinase C, or calmodulin-dependent protein kinase (Ferris and Snyder, 1992), and this may be a necessary requisite for calcium mobilization by 1,4,5-$InsP_3$ from the stores. Increases in calcium may inhibit 1,4,5-$InsP_3$ binding to its receptors (Worley et al., 1987), and this may be an inherent mechanism to reduce or regulate additional calcium release. To add further complexity, subtypes of 1,4,5-$InsP_3$ receptors have been identified in some tissues (Nathanson et al., 1994), and such diversity of these receptors might involve differential mobilization of calcium from its stores. The 1,4,5-$InsP_3$ receptor protein may be similar to ryanodine receptor/$Ca^{2+}$ channel protein sarcoplasmic reticulum of skeletal muscle (McPherson and Campbell, 1993). Both are involved in mobilization of calcium from intracellular stores. Ryanodine receptors are present in muscle and some nonmuscle cells (but probably not in the adrenal glomerulosa cells).

Receptors for 1,3,4,5-$InsP_4$ have also been demon-

strated in the bovine adrenal cortical microsomes (Enyedi and Williams, 1988). These binding sites also seem to be quite specific and of high-affinity (mean $K_D$ about 4 nM) and are distinct from the binding sites of 1,4,5-$InsP_3$. The tetrakis isomer, 1,3,4,5-$InsP_4$, was able to bind to 1,4,5-$InsP_3$ receptors but at about 30 times lesser potency than 1,4,5-$InsP_3$. The tetrakisphosphate was also able to mobilize calcium from bovine adrenal microsomes even when the binding of 1,4,5-$InsP_3$ to its receptors and 1,4,5-$InsP_3$-induced calcium release were blocked by heparin (Ely et al., 1990). Thus, the calcium-mobilizing effect of 1,3,4,5-ImsP4 was mediated by receptors distinct from those of 1,4,5-$InsP_3$ receptors, and these findings further underscore the potential synergism between the two inositol polyphosphates in mobilizing intracellular calcium.

The formation of the other metabolite from phosphoinositide hydrolysis, diacylglycerol, in the glomerulosa cell in response to angiotensin II has been fairly well-characterized. A biphasic response to angiotensin stimulation was also seen, initially as a transient increase with a peak response of about 3.5-fold increase above the baseline at 20 seconds, followed by a sustained increase in diacylglycerol production (Hunyady et al., 1990; Bollag et al., 1991). The source of the diacylglycerol in the second phase may not be the same as that in the first phase (Underwood et al., 1993). This biphasic response is somewhat similar to the formation of 1,4,5-$InsP_3$, as described earlier. Diacylglycerol so formed from the phosphoinositides is further metabolized to monoacylglycerol, arachidonate, and glycerol by lipases, or to phosphatidic acid by diacylglycerol kinase. The latter is eventually utilized in the resynthesis of the phosphoinositides.

Whereas the effects of angiotensin II on phosphoinositides and calcium in the adrenal glomerulosa cells have been observed by a number of investigators (as discussed), curiously, angiotensin II provokes the same signaling mechanism in the adrenal fasciculata cells as well. But, there seem to be species differences with regard to the effect of angiotensin II on the fasciculata cells. Thus, angiotensin II seems to produce phosphoinositide hydrolysis concomitantly with cortisol secretion in bovine fasciculata cells (Bird et al., 1989) but not in the rat fasciculata cells. In the fasciculata/reticularis cells of the cow, but not in the rat, angiotensin II activates voltage-dependent calcium channels (Yanagibashi et al., 1990) and increases cytosolic calcium concentration (Walker et al., 1991). These findings are in agreement with the lack of binding sites for labeled nitrendipine as well as angiotensin receptors in rat fasciculata cells (Aguilera and Catt, 1986). Interestingly, in the ovine fasciculata cells, despite the presence of the same subtypes of angiotensin receptors as found in the bovine glomerulosa cells, angiotensin II fails to induce steroid secretion but provokes phosphoinositide hydrolysis, calcium influx, and efflux (Viard et al., 1990).

There are also differences with regard to the effect of potassium on the fasciculata and glomerulosa cells, and here too there may be species differences. In the rat fasciculata cells, increasing extracellular potassium concentrations produce the same magnitude of depolarization as in the glomerulosa cells, yet no calcium conductance or increase of steroid secretion by potassium is observed (Quinn et al., 1987). This may be explained by the absence of dihydropyridine calcium channels in the rat fasciculata cells (Aguilera and Catt, 1986). As a result, potassium fails to increase cytosolic free calcium concentration in such cells (Braley et al., 1986). In the rat fasciculata cells, calcium efflux is also not demonstrable in response to either angiotensin II (Williams et al., 1981) or potassium (Mackie et al., 1978). In mouse adrenal fasciculata cells, however, potassium has been reported to enhance corticosteroid production in response to ACTH (Robertson et al., 1984). In other studies (Yanagibashi, 1979), calcium uptake in rat fasciculata cells in response to ACTH has been reported, despite the reported lack of demonstrable effect of angiotensin II or potassium in such cells. The mechanism, however, remains obscure.

Thus, there seems to be differences in signaling mechanisms in adrenal fasciculata cells of different species in response to angiotensin II and potassium. The findings, as described, can explain the different steroidogenic responses of such cells to the two stimuli.

b. OTHER MEDIATORS. Other mediators of phospholipid metabolism have been proposed in aldosterone secretion. Diacylglycerol can potentially be derived from other sources such as phophatidylcholine (Exton, 1990) or by the action of phospholipase $A_2$ or phospholipase D. In vascular tissue, angiotensin II-induced diacylglycerol formation from phosphatidylcholine has been reported (Lassegue et al., 1991). Similarly, in adrenal cells, diacylglycerol may be derived from phosphatidylcholine (Bollag et al., 1991). Phospholipase $A_2$ may produce arachidonic acid, which, by itself or by its further metabolism might mediate aldosterone secretion. Evidence for that is the following. Inhibitors of phospholipase $A_2$ reduce aldosterone secretion, and phospholipase $A_2$ added to glomerulosa cells stimulate aldosterone secretion and efflux of radiolabeled calcium from these cells (Kojima et al., 1985e). The role of phospholipase $A_2$ activation during angiotensin II-mediated aldosterone secretion, however, remains somewhat uncertain. An earlier study (Enyedi et al., 1981) suggested that only high concentrations of arachidonic acid stimulated aldosterone secretion. If arachidonic acid is involved in aldosterone secretion, it might do so directly through stimulation of protein kinase C (McPhail et al., 1984) or indirectly through a metabolic product (Kojima et al., 1985e). Blockade of the lipoxygenase pathway by inhib-

itors of lipoxygenase (but not inhibitors of the cycloxygenase pathway) partially inhibited angiotensin II-mediated stimulation of aldosterone production (Kojima et al., 1985e).

Other studies have shown that angiotensin II (but not ACTH or potassium) increases a lipoxygenase-derived product, 12-hydroxyeicosatetranoic acid by activating the 12-lipoxygenase pathway (Nadler et al., 1987). This increase of 12-hydroxyeicosatetranoic acid might possibly occur through the involvement of protein kinase C (Shibata and Kojima, 1991). Angiotensin II has been reported to induce 12-lipoxygenase in the human adrenal glomerulosa cells (Gu et al., 1994). Both selective and nonselective inhibitors of 12-lipoxygenase pathway reduced aldosterone secretion and this could be restored by replacement or addition of the 12-lipoxygenase-derived product. Aldosterone secretion was not influenced by selective inhibitors of 5-lipoxygenase or 15-lipoxygenase pathways. Diacylglycerol seemed to be the source of the arachidonic acid that is metabolized to the eicasonoid metabolite, as judged by the effects of two different types of inhibitors of diacyglycerol metabolism (Natarajan et al., 1990). The role of cycloxygenase-derived metabolites in angiotensin II-induced aldosterone secretion remains controversial (Campbell et al., 1980; Enyedi et al., 1981).

Hydrolysis of phospholipids by phospholipase D results in the formation of phosphatidic acid and the release of phospholipid head groups (Exton, 1990). The resultant phosphatidic acid can be hydrolyzed to diacylglycerol. Angiotensin II has also been reported to activate phospholipase D (Bollag et al., 1990) and to activate formation of various diacylglycerols likely to be derived from both phosphoinositides and phosphatidylcholine, as judged by the sequential analysis of the composition of the diacylglycerols (Underwood et al., 1993). Angiotensin II increased phosphatidic acid along with diacylglycerol production, and, in the presence of ethanol, was associated with the formation of phosphatidylethanol from ethanol, a marker of phospholipse D activation. Addition of exogenous phospholipase D also resulted in an increase of diacylglycerol and aldosterone secretion from cultured bovine glomerulosa cells. The amount of diacylglycerol produced by the exogenous phospholipase D was about half of that produced in response to angiotensin II. The aldosterone-stimulating effect of phospholipase D was potentiated further by the presence of the calcium channel agonist, BAY K8644, which did not affect diacylglycerol (or inositol phosphate) formation. Thus, several mediators may be coparticipating or operating synergistically in cellular signaling of aldosterone secretion evoked by angiotensin II, although their relative importance requires more definitive studies.

Although the $Na^+$-$H^+$ antiporter system and $Ca^{2+}$-$H^+$ exchanger can be activated by angiotensin II, their precise roles in aldosterone secretion have not been adequately defined (Conlin et al., 1990; Hunyady et al., 1988). The activation of $Na^+$-$H^+$ exchanger by angiotensin II was thought to be mediated by protein kinase C (Conlin et al., 1991). Such an indirect action of protein kinase C could possibly explain the postulated role of protein kinase C in aldosterone secretion as discussed later. In a very recent study in which inhibitors of $Na^+$-$H^+$ and $Na^+$-$Ca^{2+}$ exchange mechanisms were examined further, it was concluded that the activation of these sodium exchangers did contribute to angiotensin II- and potassium-induced aldosterone secretion (Van Der Bent et al., 1993). Using a fluorescent probe for $Na^+$, these investigators demonstrated significant increase of cytosolic $Na^+$ (maximal increase five-fold) to increasing doses of angiotensin II. This increase could very well be mediated through one or both of the $Na^+$ exchanger system(s) thought to be stimulated by angiotensin II. Pharmacological blockade of each of the $Na^+$ exchanger mechanisms was associated with a decrease in angiotensin II-induced aldosterone secretion. Inasmuch as changes in cytosolic $Na^+$ concentration could not be measured in the presence of the pharmocolgical blockers of the $Na^+$ exchangers, the precise mechanism of action of these agents or the mode of increase of cytosolic $Na^+$ concentration remains elusive. A modest increase of cytosolic $Na^+$ level was also noted during potassium-mediated stimulation of aldosterone secretion in these studies.

*4. Role of protein kinases.* Phosphorylation-dephosphorylation cycles play critical roles in cellular signaling mechanisms (Cohen, 1982; Krebs, 1985). In this context protein kinases, phosphoproteins, and phosphatases are key elements. As discussed earlier, cAMP and its protein kinase(s) are the major intracellular mediators in ACTH-mediated aldosterone secretion (Garren et al., 1971; Gill, 1972). This aspect will not be discussed here any further. The role of other protein kinases (especially protein kinase C) associated with the newly identified mediators will be the subject of discussion in this section.

Nishizuka and his colleagues (Nishizuka et al., 1984) identified a new protein kinase that was named protein kinase C. This enzyme is distributed in many tissues and requires phospholipid and calcium for activation. This enzyme was first characterized on the basis of its cofactor requirements i.e., calcium, phospholipid and diacylglycerol. Diacylglycerol increases the affinity of the enzyme for the phospholipids at the usual physiological calcium concentrations, although an increment in cytosolic calcium concentration can produce a synergistic effect with diacylglycerol in activating the enzyme (Nishizuka et al., 1984).

Among the diacylglycerols, 1,2-diacylglycerols containing unsaturated fatty acids are believed to be capable of activating protein kinase C. The enzyme is generally present in inactive form in the cytosol and translocates to cellular membranes during cell stimulation. Protein kinase C can also be activated by unsaturated fatty acids such as arachidonic acid. Protein kinase C can also be

activated pharmacologically with the tumor-promoting phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (Blumberg et al., 1984), and with permeable diacylglycerol analogues. Phorbol esters apparently become intercalated in the cell membrane, whereby it becomes the receptor for the enzyme and its activator. These agents have been widely used in the investigations relating to protein kinase C in signaling as well as other biological processes.

It is now evident that there exists a family of protein kinase C isoforms (Coussens et al., 1986; Nishizuka, 1988), at least 12 in number, representing products of specific genes (Dekker and Parker, 1994). The protein kinase C isoforms show differential tissue distribution, substrate specificity and mode of activation. Thus, protein kinase C may conceivably subserve a variety of cellular functions and signaling mechanisms which remain to be clearly defined.

The metabolism phosphoinositides produces two chemically distinct classes of products: inositol phosphates and diacylglycerol. Whereas some of the inositol phosphates participate in cellular calcium changes, diacylglycerol causes physiological activation of protein kinase C, although it might also be involved in calcium influx (Kaczmarek, 1987; Shearman et al., 1989; Vivaudou et al., 1988). Recognition of the important role of diacylglycerol in the activation of protein kinase C prompted studies involving adrenal glomerulosa cell signaling in Rasmussen's laboratory (Kojima et al., 1983, 1984a), utilizing calcium ionophore A23187 and the protein kinase C agonists, a phorbol ester, and a diacylglycerol analogue. Having demonstrated the individual and concerted effects of these two types of agents in perifused porcine and bovine adrenal glomerulosa cells, these investigators deduced that in adrenal glomerulosa cells, the calcium-calmodulin system and protein kinase C might operate in concert or sequence to produce the initial and sustained stimulation of aldosterone secretion evoked by angiotensin II. This interesting hypothesis stimulated other investigators to examine the question further.

Angiotensin II has been reported (Lang and Vallotton, 1987) to cause a redistribution of protein kinase C from the cytosol to the plasma membrane (a concomitant of its activation) in bovine adrenal glomerulosa cells. In rat glomerulosa cells (Farago et al., 1988), angiotensin II failed to cause redistribution of protein kinase C under calcium-free conditions, but it was able to do so in the calcium-replete state. The significance of these findings is not clear. These investigators observed, however, that phorbol ester caused the expected redistribution of protein kinase C in the rat glomerulosa cells.

In another study (Nakano et al., 1990) using cultured rat glomerulosa cells, both angiotensin II and a phorbol ester acutely stimulated aldosterone secretion and caused a transient redistribution of protein kinase C from the cytosol to the cell membrane. However, when protein kinase C activity was down-regulated by extended exposure to a phorbol ester, angiotensin II-stimulated aldosterone secretion remained unimpaired, thus implying, as interpreted by Nakano et al. (1990), that protein kinase C activation was not an absolute requisite for cell signaling involved in angiotensin II-mediated aldosterone secretion. It has even been suggested on the basis of the effects of a phorbol ester and an inhibitor of protein kinase C that the stimulatory effects of angiotensin II and potassium on aldosterone secretion might involve inhibition of protein kinase C activity in the adrenal cells by these secretagogues (Hajnoczky et al., 1992).

These conflicting conclusions on protein kinase C activity and aldosterone secretion have to be considered in the perspective of various methods used to assess protein kinase C activity. In view of the heterogeneity of protein kinase C now known to exist and substrate specificity of the various isoforms of the enzyme, it is conceivable that substrates used or other variables in the assays could give different results, depending on the type(s) of protein kinase C activated during the experiments.

We have not been able to stimulate aldosterone secretion significantly with phorbol esters or diacylglycerol analogues in static incubations of acutely dispersed adrenal glomerulosa cells. In fact, somewhat higher concentrations of phorbol ester inhibited angiotensin II-mediated aldosterone secretion (Kojima et al., 1986a). Yet, inhibitors of protein kinase C do decrease aldosterone secretion in varying degrees (Ganguly et al., 1992a; Ganguly and Waldron, 1994). Whether such inhibition of aldosterone secretion is related definitively to protein kinase C inhibition is uncertain. Aldosterone secretion seems to be much more sensitive to the inhibition of calmodulin or manipulation of calcium than it is to the inhibition of protein kinase C (Ganguly et al., 1992; Ganguly and Waldron, 1994), although again the specificity of the various pharmacological inhibitors remains uncertain. If protein kinase C is indeed important for the maintenance of aldosterone secretion during angiotensin II stimulation, both the presence of extracellular calcium and continual calcium influx seem to be critical. In the absence of calcium influx, the sustained phases of the increase of cytosolic free calcium concentration and aldosterone secretion in response to angiotensin II decline to low levels, as shown by several investigators including ourselves (Braley et al., 1986; Capponi et al., 1987; Kramer, 1988b; Pratt et al., 1989; Ganguly et al., 1992).

If translocation of protein kinase C is a necessary concomitant of its activation, then potassium does not seem to activate protein kinase C in the bovine adrenal glomerulosa cells (Lang and Vallotton, 1987), nor does it increase diacylglycerol (Hunyady et al., 1990) or inositol phosphates (Ganguly et al., 1990; Kojima et al., 1985d). Its action on aldosterone secretion then must be me-

diated by calcium/calmodulin (Ganguly et al., 1990) and by activation of a specific protein kinase (Kennedy and Greengard, 1981), although a concomitant increase of cAMP in one study (Kojima et al., 1985a) and a reduction of pyridine nucleotides associated with potassium-induced aldosterone secretion in another study (Pralong et al., 1992) have been observed. Precise significance of each of those findings remains unclear.

Diacylglycerol could very well be involved in cellular signaling, i.e., through the activation of protein kinase C. But it is not easy to extrapolate the results of the effects of phorbol esters or diacylglycerol analogues to a physiological role for diacylglycerol. These protein kinase C agonists are metabolized slowly, unlike naturally produced diacylglycerol in the tissues, and therefore may not mimick the biological effect of diacylglycerol produced in the cell.

Prolonged stimulation by the synthetic protein kinase C agonists may produce confounding effects, such as depletion of specific protein kinase C isoforms or inhibition of phosphoinositide hydrolysis or calcium influx. These effects certainly would modify the signaling sequences and interactions between the mediators. But even with the natural events related to the hydrolysis of the phosphoinositides, the continual production of diacylglycerol, now known to proceed during angiotensin II-mediated cell stimulation (Hunyady et al., 1990; Bollag et al., 1991), may not be conducive to the purported sustained stimulation of protein kinase C, because the enzyme is expected to be down-regulated in a manner similar to that proposed during stimulation with a phorbol ester (Inagaki et al., 1986). Such desensitization can occur with diacylglycerol as well (Inagaki et al., 1986). Whether this effect does occur in vivo is by no means certain, because diacyglycerol is metabolized rapidly. However, it has been shown (Hunyady et al., 1990) that in adrenal glomerulosa cells stimulated by angiotensin II, formation of inositol phosphates and diacylglycerol are not affected initially by alteration of extracellular calcium concentration, but aldosterone secretion as well as inositol phosphate formation cannot be sustained if the extracellular calcium concentration is reduced. Aldosterone secretion declines despite the fact that two-thirds of the diacylglycerol production continues unabated under these conditions. This argues against an independent role of protein kinase C in the sustained phase of aldosterone secretion, as proposed by Kojima and colleagues (Kojima et al., 1983, 1984a). However, we do not know the composition of the diacylglycerol that is formed under low calcium conditions, presumably from sources other than phosphoinositide hydrolysis (Underwood et al., 1993). Whether diacylglycerol formed from phospholipids other than phosphoinositides such as phosphatidylcholine can also activate protein kinase C is uncertain (Leach et al., 1991). Depending on the nature of fatty acids, various diacylglycerols might have varying potencies in stimulating protein kinase C (Underwood et al., 1993). Thus, the attractive conceptual model of adrenal cell signaling proposed by Rasmussen's group (Kojima et al., 1983, 1984a) remains ambiguous at best. There is a strong possibility that protein kinase C might support other cellular functions such steroidogenic enzyme expression, cell growth and renewal. The inhibition or stmulation of steroid secretion could be secondarily related to those effects of protein kinase C.

Phosphorylation of proteins, which reflects the effects of activated protein kinases, has been reported (Barrett et al., 1986) in the bovine adrenal glomerulosa cells. Several different proteins were noted to be phosphorylated in response to stimulation of the cells by angiotensin. It was observed that several proteins were phosphorylated early (at 1 minute) and others were phosphorylated later (at 30 minutes). The concerted effects of phorbol ester and A23187 produced phosphorylation of the same proteins in a similar fashion as induced by angiotensin II. These findings were interpreted to mean that the early phosphorylation was probably mediated by a calcium-calmodulin-dependent protein kinase, and the proteins phosphorylated later were the products of the effects of protein kinase C. These observations seemingly support the original thesis of these investigators of a sequential participation of the calcium-calmodulin system and protein kinase C in angiotensin II-mediated steroidogenesis, but these observations provide only circumstantial evidence for the involvement of the two types of protein kinases during stimulation of aldosterone secretion. It is also not known whether those phosphorylated proteins, affected by the various agents, are actually involved in the glomerulosa cell steroidogenesis.

Recently, angiotensin II-induced activation of both tyrosine kinase and phosphatase has been reported in some cells (Huckle et al., 1992; Molloy et al., 1993; Duff et al., 1993). Serine-threonine related phosphtase(s) could also be involved in angiotensin II-mediated action (Ganguly et al., 1994). A myosin light chain kinase inhibitor has been reported to attenuate angiotensin II-mediated phosphoinositide hydrolysis in bovine adrenal glomerulosa cells (Nakanishi et al., 1994). The roles of all of these systems and their significance in the action of angiotensin II in the adrenal glomerulosa cells require further investigations.

### *C. Interactive Effects of Secretagogues and/or Cellular Mediators*

Limited studies have examined interactive effects of angiotensin II and potassium, of ACTH and potassium, or of ACTH and angiotensin II on the intracellular messengers or interactions between the messengers in the adrenal cells and steroid secretion. When two calcium-mobilizing secretagogues, angiotensin II and potassium, were used to stimulate rat adrenal glomerulosa cells, Balla et al. (1991) observed that angiotensin II, in

a dose-dependent manner, inhibited the increase of cytosolic free calcium as well as the initial phase of calcium influx induced by potassium without affecting aldosterone secretion. It is of interest that inhibition of calcium influx in bovine glomerulosa cells in response to angiotensin II has been reported previously (Elliott et al., 1985). In contrast, ACTH had no effect on the potassium-induced increment in cytosolic calcium concentration (Balla et al., 1991). The locus of the effect of angiotensin II was thought to be the voltage-dependent calcium channels but was mediated through protein kinase C.

Interactions between the second messengers formed by ACTH and angiotensin II administration have also been reported. Simultaneous stimulation of bovine adrenal fasciculata cells with angiotensin II and ACTH resulted in a greater cAMP response than that occuring with ACTH alone (Brami et al., 1987; Langlois et al., 1992). This effect of angiotensin II was transient and seemed to involve protein kinase C, inasmuch as phorbol ester and dioctanoylglycerol also produced the amplification of cAMP response. Forskolin and cholera toxin also stimulated more cAMP production when the adrenal cells were treated with phorbol ester. ACTH induced a greater cAMP response when pretreated with pertussis toxin (Brami et al., 1987). Thus, the amplifying effect of angiotensin II was thought to be mediated through the inhibition of $G_i$ protein.

On the other hand, angiotensin II-induced increases in inositol phosphate formation in the adrenal glomerulosa cells have been reported to be attenuated by concurrent stimulation with ACTH or with both ACTH and cAMP (Woodcock, 1989). However, Baukal et al. (1990) observed that in bovine adrenal glomerulosa cells, 8-bromo-cAMP had a stimulatory effect on the production of inositol 1,4,5-$InsP_3$ in response to angiotensin II; longer incubations with the cAMP analogue resulted in complex effects, including increased production of 1,3,4,5-tetrakisphosphates and 1,4,5,6-tetrakisphosphates as well as reduced metabolism of inositol polyphosphates by 5-phosphatase. In a different study (Yoshida et al., 1991), ACTH as well as an analogue of cAMP and forskolin, a stimulator of cAMP, were shown to reduce angiotensin II receptors as well as the angiotensin II-induced increase of cytosolic calcium and increased steroidogenesis in bovine glomerulosa cells. The effect of ACTH was attributed to cAMP generation, since as low doses of ACTH that did not increase cAMP also did not affect either angiotensin II receptors or angiotensin II-mediated aldosterone secretion. An earlier study (Andoka et al., 1984) had also suggested a decrease of angiotensin II receptors in adrenal cells in response to ACTH. Therefore, it seems that interactions among signaling systems occur, but the overall consequences of these interactions on steroidogenesis remain to be fully elucidated.

### *D. Inhibitors of Aldosterone Secretion*

*1. Atrial natriuretic peptide.* The effect of ANP on aldosterone secretion has been investigated extensively. ANP inhibits aldosterone secretion in vivo and in vitro. Such inhibition applies to aldosterone secretion evoked by all of the physiological secretagogues, indicating that ANP in all likelihood has a physiological role in aldosterone secretion. The mechanism by which ANP inhibits aldosterone secretion is far from clear, but many investigations have been carried out; these have been reviewed recently (Ganguly, 1992). The results point to a possible interference by ANP in the generation of appropriate cell signaling in the adrenal glomerulosa, although this is by no means certain at this time.

Binding of ANP to adrenal cells of various zones has also been demonstrated using autoradiography (Choi et al., 1986; Lynch et al., 1986). It is clear that glomerulosa cells have the highest density of the binding sites among the various adrenal cells. Specific receptors for ANP have been characterized in the adrenal. There are at least two types of high-affinity ANP receptors; they have apparent molecular weights of 135,00 and 62,000 Da (Takayanangi et al., 1987). The larger receptors are associated with guanylate cyclase activation (Takayanangi et al., 1987), whereas the smaller receptors might be linked with the adenylate cyclase (Anand-Srivastava et al., 1990). It appears that there might be a third type of ANP receptor characterized in other tissues, which has been referred to as a clearance receptor (Maack et al., 1985). ANP has been shown to increase cGMP in adrenal glomerulosa cells (Matsuoaka et al., 1985) and to activate guanylate cyclase in adrenocortical cell particulate fractions (Tremblay et al., 1986).

ANP has generally been shown to shift the dose-response curve of ACTH-mediated aldosterone secretion to the right (Ganguly, 1992), indicating that it alters the sensitivity of the glomerulosa cells to ACTH; this occurs without alterations in the binding of ACTH to its receptors. Dibutyrl cAMP-stimulated aldosterone secretion is also inhibited by ANP. ANP has been shown to inhibit adenylate cyclase in the adrenal and other tissues (Anand-Srivastava et al., 1984). This is probably mediated through the smaller of the ANP receptors in the adrenal cells (Anand-Srivastava et al., 1990) and presumably involves a pertussis-sensitive G-protein (Anand-Srivastava et al., 1987).

Both an increase of cytosolic calcium concentrations and activation of calcium channels have been reported in response to ACTH. Because calcium influx has been shown to be a concomitant of ACTH-induced aldosterone secretion, ANP could also affect this calcium-related signal (Chartier and Schiffrin, 1987).

Other mechanisms for ANP in the ACTH-induced aldosterone secretion are possible. These might include an increased degradation of cAMP resulting from a cGMP-dependent cAMP phosphodiesterase and relating

to an increase of cGMP levels in the adrenal glomerulosa cells in response to ANP (MacFarland et al., 1991). However, various cGMP analogues have failed to produce inhibition of aldosterone secretion in most studies (Ganguly, 1992), raising doubts about a role of cGMP in the aldosterone-inhibiting effect of ANP. If anything, cGMP tends to increase aldosterone secretion slightly. Other potential targets of ANP in the adrenal glomerulosa cells could be (*a*) the potassium channels, as reported regarding the anterior pituitary cells (Antoni and Dayanithi, 1990) and (*b*) protein kinase C. Both of these have been implicated in the modulation of ACTH-evoked aldosterone secretion.

ANP also produces an inhibitory effect on angiotensin II- and potassium-evoked aldosterone secretion (Ganguly, 1992). Again, a number of investigators have probed the possible mechanism of this ANP effect. As discussed earlier, angiotensin II is believed to increase cytosolic free calcium concentrations by a receptor-activated calcium influx and a 1,4,5-$InsP_3$-induced mobilization of intracellular calcium activation of calmodulin-dependent protein kinase and protein kinase C. The target of ANP could be any of these components of cellular signaling. ANP has been shown not to influence either phosphoinositide hydrolysis or initial cytosolic calcium transients in the adrenal glomerulosa cells (Appeldorf et al., 1988; Capponi et al., 1986; Ganguly, 1992).

In some studies (Chartier and Schiffrin, 1987) but not in others (Takagi et al., 1988), inhibition of either calcium influx or of modulation of a specific type of calcium channel by ANP (Barrett et al., 1991; McCarthy et al., 1990) associated with reduced aldosterone production has been reported. How ANP interrupts calcium influx or specific calcium channels, with the concomitant inhibition of aldosterone secretion, remains elusive. However, interference with a cellular calcium signal by ANP has also been reported for other tissues (Ganguly, 1992). The action of ANP may conceivably involve (*a*) cGMP or its kinase, as reported in vascular tissue, or involve (*b*) a G-protein. Because permeable cGMP analogues do not directly inhibit aldosterone secretion in response to angiotensin II, the role of cGMP remains doubtful. But, a cation channel could be a direct target of ANP through receptor-activated coupling through a G-protein. Whether ANP affects protein kinase C activity or calcium-calmodulin-dependent protein kinase activity has not to our knowledge been directly examined in the adrenal glomerulosa cells. However, phorbol esters or permeable diacylglycerol analogues have not been able to reverse the effect of ANP on aldosterone secretion (Ganguly et al., 1989a). In some studies, the effects of ANP on protein kinases have been investigated indirectly by examining the effects of ANP on the phosphorylation of proteins (Elliott and Goodfriend, 1986; Pandey et al., 1987; Takagi et al., 1988a; Ganguly et al., 1989a), but the results and the significance of these studies remain unclear.

Inasmuch as potassium-induced aldosterone secretion is associated with calcium efflux through a voltage-dependent calcium channel, the reported inhibitory effect of ANP on calcium influx in the glomerulosa cells could explain the ANP-mediated inhibition of potassium-evoked aldosterone secretion. Measurement of either cytosolic free calcium (Barrett et al., 1991) or calcium influx (Chartier and Schiffrin, 1987) in response to potassium in the presence of ANP seems to support such a tenet. However, the final curtain might not yet have come down on the modality by which ANP affects angiotensin II- and potassium-stimulated aldosterone secretion.

*2. Dopamine and somatostatin.* A dopaminergic mechanism of inhibition of aldosterone secretion has been proposed by Carey and colleagues (Carey et al., 1979; Ganguly, 1984). A dopamine antagonist, metoclopramide, stimulates aldosterone secretion, and this can be blocked by dopamine. Dopamine has also been reported to inhibit angiotensin II- and potassium-mediated aldosterone secretion from bovine glomerulosa cells (McKenna et al., 1979; Fitzpatrick and McKenna, 1989) in vitro in a concentration-dependent manner—but usually at a relatively high concentrations of dopamine. This seems to occur through a D-2 receptor (Missale et al., 1989), and glomerulosa cells have both D-1 an D-2 receptors (Dunn and Bosmann, 1981; Bevilacqua et al., 1982). The D-1 receptor seems to be linked with the adenylate cyclase system. The presence of two types of dopamine receptors may explain the variable direct effects of dopamine or related compounds on aldosterone secretion observed by different investigators. The mechanism by which dopamine inhibits aldosterone secretion induced by angiotensin II and potassium is not clear. In the pituitary, dopamine may or may not influence the turnover of PtdIns (Canonico et al., 1983, 1986). Dopamine is also reported to inhibit prolactin secretion by interfering with changes in intracellular calcium concentration, either through the inhibition of PLC-mediated hydrolysis of phosphoinositides (Malgaraoli et al., 1987) or calcium influx (Login et al., 1988), perhaps via a G-protein-mediated mechanism.

Dopamine has also been reported to inhibit both L and T calcium channels in the pituitary cell (Lledo et al., 1990). Similar mechanism(s) potentially can be operative in the adrenal glomerulosa cells. Indeed, in one study involving the frog interrenal gland, dopamine inhibited inositol phosphate formation and corticosteroid secretion through a pertussis-toxin sensitive G-protein (Morra et al., 1991). In a very recent study, dopamine has been reported to inhbit T calcium channels in rat adrenal glomerulosa cells and this inhibition seems to be mediated by the D-2 receptors (Ospenko et al., 1994). Dopamine has also been reported to activate potassium

channels in pituitary growth hormone-producing cells (Takano et al., 1994). This may also be of interest.

Somatostatin has also been inhibitory to aldosterone secretion evoked by its secretagogues (Aguilera et al., 1981). Somatostatin receptors have been identified in the adrenal cells using labeled somatostatin (Aguilera et al., 1982) or its analogue (Maurier and Reubi, 1986). Somatostatin immunoreactivity has been detected in the adrenal glomerulosa zone (Aguilera et al., 1981). Somatostatin might cause inhibition of aldosterone secretion through a $G_i$ protein inhibiting adenylate cyclase (Hausdorff et al., 1987) or through some other mechanisms, such as decreased calcium entry (Hsu et al., 1991), that could be mediated by a $G_i$ protein. In various tissues, somatostatin has been reported to inhibit cAMP formation (Litvin et al., 1986), increase of cytosolic calcium concentration (Hall et al., 1988), or calcium flux (Luini et al., 1986). It has also been demonstrated to inhibit voltage-dependent calcium currents (Nussinovitch, 1989). In the adrenal glomerulosa cells, the precise mechanism of action of somatostatin remains undefined but could very well involve one or more of the above mechanisms.

## IV. Conclusions

Our current understanding of the adrenal cellular mediators has been facilitated by crucial discoveries in cellular and molecular biology, by technical advances in the measurements of cytosolic calcium concentration using various probes, by digital imaging methods allowing direct visualization of such changes, by elegant patch-clamp techniques for studying electrophysiology of cells, and by the development of a variety of pharmacological and biochemical tools (as well as by creative methods to manipulate cells or introduce impermeable molecules inside the cells).

In this review, we have examined a large body of evidence relating to the cellular messengers involved in stimulus-secretion coupling leading to aldosterone secretion from the glomerulosa cells of the adrenal in response to its physiological secretagogues. The role of calcium and its interactions with other mediators were the major focus of this review. Whereas cAMP has been acknowledged to be the major second messenger for ACTH-induced steroidogenesis in the adrenal gland, cAMP was found not to serve a similar role in angiotensin II- or potassium-mediated aldosterone secretion. In contrast, calcium not only seems to serve as the chief mediator of angiotensin II- and potassium-induced aldosterone secretion, it also seems to participate as a comediator of ACTH-mediated aldosterone secretion. Like cAMP, which requires a class of protein kinases to fulfill its function as the cellular mediator of ACTH, calcium seems to exert its full effect in concert with specific protein kinases. Even though calcium is believed to act as the second messenger for both angiotensin II and potassium in provoking steroid secretion, there are significant mechanistic differences between the actions of the two secretagogues. Angiotensin II, unlike potassium, provokes hydrolysis of phosphoinositides, mobilizes intracellular calcium, and may utilize two different classes of protein kinases.

As part of the phosphorylation-dephosphorylation cycles in cell signaling, phosphatases must play an equally important role with the protein kinases to complete the sequence of events leading to the secretion of aldosterone from the glomerulosa cells. The sequence of events culminating in stimulus-secretion coupling in the glomerulosa cells might be more complex than originally thought; one must additionally consider the interplay of a variety of cation fluxes or exchange systems as well as shifts or movements of cations between subcellular organelles and the generation of additional mediators. The relative importance of each of the processes in the hierarchy of the various mechanisms remains to be defined. When the adrenal cells are stimulated by more than one secretagogue, there are complex interactions between the cellular mediators. The inhibitors of aldosterone secretion are likely to exert their effects by perturbing the normal cell signaling processes, but the precise mechanisms of their actions remain ill-defined.

## REFERENCES


ABDEL-LATIF, A. A.: Calcium-mobilizing receptors, polyphosphoinositides, and the generation of second messengers. Pharmacol. Rev. **38:** 227–272, 1986.

AGUILERA, G., AND CATT, K. J.: Participation of voltage-dependent calcium channels in the regulation of adrenal glomerulosa function by angiotensin II and potassium. Endocrinology **118:** 112–118, 1986.

AGUILERA, G., HAUGER, R. I., AND CATT, K. J.: Control of aldosterone secretion during sodium restriction: adrenal receptor regulation and increased adrenal sensitivity to angiotensin II. Proc. Natl. Acad. Sci. USA **78:** 975–979, 1978.

AGUILERA, G., HARWOOD, J. P., AND CATT, K. J.: Somatostatin modulates effects of angiotensin II in adrenal glomerulosa cells. Nature (Lond.) **292:** 262–263, 1981.

AGUILERA, G., PARKER, D. S., AND CATT, K. J.: Characterization of somatostatin receptors in the rat adrenal glomerulosa zone. Endocrinology **111:** 1376–1384, 1982.

AHRENS, H., AND SHARMA, R. L.: Guanosine 3′,5′-monophosphate-dependent protein kinase from bovine adrenal cortex: partial purification and characterization. J. Steroid Biochem. **11:** 1099–1105, 1977.

ALBANO, J. D. M., BROWN, B. L., EKINS, R. K., TAIT, S. A. S., AND TAIT, J. F.: The effects of potassium, 5-hydroxytryptamine, adrenocorticotropin and angiotensin II on the concentration of adenosine 3′,5′-cyclic monophosphate in suspensions of dispersed rat adrenal zona glomerulosa and zona fasciculata cells. Biochem. J. **142:** 391–400, 1974.

ANAND-SRIVASTAVA, M. B., FRANKS, D. J., CANTIN, M., AND GENEST, J.: Atrial natriuretic factor inhibits adenylate cyclase activity. Biochem. Biophys. Res. Commun. **121:** 855–862, 1984.

ANAND-SRIVASTAVA, M. B., SRIVASTAVA, A. K., AND CANTIN, M.: Pertussis toxin attenuates natriuretic factor-mediated inhibition of adenylate cyclase. J. Biol. Chem. **262:** 4931–4934, 1987.

ANAND-SRIVASTAVA, M. B., SAIRAM, M. R., AND CANTIN, M.: Ring-deleted analogs of atrial natriuretic factor inhibit adenylate cyclase/cAMP system. J. Biol. Chem. **265:** 8566–8572, 1990.

ANDOKA, G., CHAUVIN, M. A., MARIE, J., SAEZ, J. M., AND MORERA, A. M.: Adrenocorticotropin regulates angiotensin II receptors in bovine adrenal cells *in vitro*. Biochem. Biophys. Res. Commun. **121:** 441–447, 1984.

ANTONI, F. A., AND DAYANITHI, G.: Blocking of $K^+$ channels reverses the inhibitory action of atriopeptin on secretagogue-stimulated ACTH release by perifused isolated rat anterior pituitary cells. J. Endocrinol. **126:** 183–191, 1990.

APPELDORF, W. J., ISALES, C. M., AND BARETT, P. Q.: Atrial natriuretic peptide inhibits the stimulation of aldosterone secretion but not the transient increase in intracellular free calcium concentration induced by angiotensin II addition. Endocrinology **122:** 1460–1465, 1988.

APPELDORF, W. J., AND RASMUSSEN, H.: Simultaneous determination of intracellular free calcium and aldosterone production in bovine adrenal glomerulosa. Cell Calcium **9:** 71–80, 1988.

BALLA, T., BAUKAL, A. J., GUILLEMETTE, G., AND CATT, K. J.: Multiple pathways of inositol polyphosphate metabolism in angiotensin II stimulated adrenal glomerulosa cells. J. Biol. Chem. **263:** 4083–4091, 1988.

BALLA, T., HAUSDORFF, W. P., BAUKAL, A. J., AND CATT, K. J.: Inositol polyphosphates production and regulation of cytosolic calcium during the biphasic activation of adrenal glomerulosa cell by angiotensin II. Arch. Biochem. Biophys. **270:** 398–403, 1989a.

BALLA, T., HOLLO, Z., VARNAI, P., AND SPAT, A.: Angiotensin II inhibits $K^+$-induced $Ca^{2+}$-signal generation in rat adrenal glomerulosa cell. Biochem. J. **273:** 399–404, 1991.

BALLA, T., HUNYADY, L., BAUKAL, A. J., AND CATT, K. J.: Structures and metabolism of inositol tetrakisphosphates and inositol pentakisphosphate in bovine adrenal glomerulosa cells. J. Biol. Chem. **264:** 9386–9390, 1989b.

BALLA, T., HUNYADY, L., AND SPAT, A.: Possible role of calcium uptake and calmodulin in adrenal glomerulosa cells: effects of verapamil and trifluoperazine. Biochem. Pharmacol. **31:** 1267–1271, 1982.

BALLA, T., SZERENY, M., KANYAR, B., AND SPAT, A.: Angiotensin II and FCCP mobilizes calcium from different intracellular pools in adrenal glomerulosa cells: analysis of calcium fluxes. Cell Calcium **6:** 327–342, 1985.

BALLA, B., VERNAI, P., HOLLO, Z., AND SPAT, A.: Effects of high potassium concentration and dihydropyridine $Ca^{2+}$-channel agonists on cytoplasmic $Ca^{2+}$ and aldosterone production in rat adrenal glomerulosa cells. Endocrinology **127:** 815–822, 1990.

BARRETT, P. Q., AND ISALES, C. M.: The roles of cyclic nucleotides in atrial natriuretic peptide-mediated inhibition of aldosterone secretion. Endocrinology **122:** 799–806, 1989.

BARRETT, P. Q., ISALES, C. M., BOLLAG, W. B., AND MCCARTHY, R. T.: $Ca^{2+}$ channels and aldosterone secretion: modulation by $K^+$ and atrial natriuretic peptide. Am. J. Physiol. **261:** F706–F719, 1991.

BARRETT, P. Q., KOJIMA, I., KOJIMA, K., ZAWALICH, K., ISALES, C. M., AND RASMUSSEN, H.: Temporal patterns of protein phosphorylation after angiotensin II, A23187 and/or 12-*O*-tetradecanoylphorbol-13-acetate in adrenal glomerulosa cells. Biochem. J. **238:** 893–903, 1986.

BAUKAL, A. J., BALLA, T., HUNYADY, L., HAUSDORFF, W. P., GUILLEMETTE, G., AND CATT, K. J.: Angiotensin II and guanosine nucleotides stimulate formation of inositol 1,4,5-trisphosphate and its metabolites in permeabilized adrenal glomerulosa cells. J. Biol. Chem. **263:** 6087–6092, 1988.

BAUKAL, A. J., HUNYADY, L., BALLA, T., ELY, J. A., AND CATT, K. J.: Modulation of agonist-induced inositol phosphate metabolism by cyclic adenosine 3′,5′-monophosphate in adrenal glomerulosa cells. Mol. Cell. Endocrinol. **4:** 1712–1719, 1990.

BAUMBER, J. S., DAVIS, J. O., JOHNSON, J. A., AND WITTY, R. T.: Increased adrenocortical potassium ion association with increased biosynthesis of aldosterone. Am. J. Physiol. **220:** 1094–1099, 1971.

BEALL, R. J., AND SAYERS, G.: Isolated adrenal cells: steroidogenesis and cAMP accumulation in response to ACTH. Arch. Biochem. Biophys. **148:** 70–76, 1972.

BEVAN, S., AND WOOD, J. N.: Arachidonic-acid metabolites as second messengers. Nature (Lond.) **328:** 20, 1987.

BERRIDGE, M. J.: Inositol trisphosphate and diacylglycerol as second messengers. Biochem. J. **220:** 345–360, 1984.

BERRIDGE, M. J.: Calcium oscillations. J. Biol. Chem. **265:** 9583–9586, 1990.

BERRIDGE, M. J., AND IRVINE, R. F.: Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature (Lond.) **312:** 315–321, 1984.

BEVILACQUA, M., VAGO, T., SCARZA, D., AND NORBIATO, G.: Characterization of dopamine receptors by $^3$H-ADTN binding in calf adrenal zona glomerulosa. Biochem. Biophys. Res. Commun. **108:** 1661–1669, 1982.

BIRD, J. M., MEIKLE, I., WILLIAMS, B. C., AND WALKER, S. W.: Angiotensin II-stimulated cortisol secretion is mediated by a hormone-sensitive phospholipase C in bovine adrenal fasciculata/reticularis cells. Mol. Cell. Endocrinol. **64:** 45–53, 1989.

BIRD, I. M., MAGNESS, R. R., MASON, J. I., AND RAINEY, W. E.: Angiotensin-II acts via the type 1 receptor to inhibit 17$\alpha$-hydroxylase cytochrome P450 expression in ovine adrenocortical cells. Endocrinology **130:** 3113–3121, 1992.

BIRMINGHAM, M. K., AND BARTOVA, A.: Effects of calcium and theophylline on ACTH-and dibutyryl cyclic AMP-stimulated steroidogenesis and glycolysis by intact mouse adrenal glands in vitro. Endocrinology **92:** 743–749, 1973.

BIRMINGHAM, M. K., ELLIOTT, F. H., AND VALERO, P. H. L.: The need for the presence of calcium for the stimulation *in vitro* of rat adrenal glands by adrenocorticotropic hormone. Endocrinology **53:** 687–689, 1953.

BLUMBERG, P. M., JAKEN, S., KONIG, B., SHARKEY, N. A., LEACH, K. L., JENG, A. Y., AND YEH, E.: Mechanism of action of the phorbol ester tumor promoters: specific receptors for lipophilic liganda. Biochem. Pharmacol. **33:** 933–940, 1984.

BOLLAG, W. B., BARRETT, P. Q., ISALES, C. M., LISCOVITCH, M., AND RASMUSSEN, H.: A potential role for phospholipase-D in the angiotensin-II-induced stimulation of aldosterone secretion from bovine adrenal glomerulosa cells. Endocrinology **127:** 1436–1443, 1990.

BOLLAG, W. B., BARRETT, P. Q., ISALES, C. M., AND RASMUSSEN, H.: Angiotensin-II-induced changes in diacylglycerol levels and their potential role in modulating the steroidogenic response. Endocrinology **128:** 231–241, 1991.

BORLE, A. B.: Control, modulation, and regulation of cell calcium. Rev. Physiol. Biochem. Pharmacol. **90:** 13–153, 1981.

BORLE, A. B., AND SNOWDOWNE, K. W.: Measurement of intracellular free calcium in monkey kidney cells with aequorin. Science (Wash. DC) **217:** 252–254, 1982.

BOYD, J., MULROW, P. J., PALMORE, W. P., AND SILVA, P.: Importance of potassium in the regulation of aldosterone production. Circ. Res. **32**(Suppl. 1): I-39–45, 1973.

BRALEY, L., MENACHERY, A., BROWN, E., AND WILLIAMS, G.: The effects of extracellular $K^+$ and angiotensin II on cytosolic $Ca^{++}$ and steroidogenesis in adrenal glomerulosa cells. Biophys. Res. Commun. **123:** 810–815, 1984.

BRALEY, L. M., MENACHERY, A. I., BROWN, E. M., AND WILLIAMS, G. H.: Comparative effect of angiotensin II, potassium, adrenocorticotropin, and cyclic adenosine 3′,5′-monophosphate on cytosolic calcium in rat adrenal cells. Endocrinology **119:** 1010–1019, 1986.

BRALEY, L. M., AND WILLIAMS, G. H.: Rat adrenal cell sensitivity to angiotensin II, $\alpha$-$^{1-24}$ACTH, and potassium: a comparative study. Am. J. Physiol. **233:** E402–E406, 1977.

BRALEY, L. M., AND WILLIAMS, G. H.: The effect of ouabain on steroid production by rat adrenal cells stimulated by angiotensin II, $\alpha^{1-24}$ adrenocorticotropin, potassium. Endocrinology **103:** 1997–2005, 1978.

BRAMI, B., VILGRAIN, I., AND CHAMBAZ, E. M.: Sensitization of adrenocortical cell adenylate cyclase activity to ACTH by angiotensin II and activators of protein kinase C. Mol. Cell. Endocrinol. **50:** 131–137, 1987.

BRISTOW, A. F., GLEED, C., FAUCHERE, J.-L., SCHWYER, R., AND SCHULSTER, D.: Effect of ACTH (corticotropin) analogues on steroidogenesis and cyclic AMP in rat adrenocortical cells. Evidence for two different steroidogenically responsive receptors. Biochem. J. **186:** 599–603, 1980.

BROWN, A. M., AND BIRNBAUMER, L.: Ionic channels and their regulation by G protein subunits. Annu. Rev. Physiol. **52:** 197–213, 1990.

CAMPBELL, W. B., GOMEZ-SANCHEZ, C. E., AND ADAMS, B. V.: Role of prostaglandins in angiotensin-induced steroidogenesis. Hypertension **2:** 471–476, 1980.

CANONICO, P. L., JARVIS, W. D., JUDD, A. M., AND MACLEOD, R. M.: Dopamine does not attenuate phosphoinositide hydrolysis in rat anterior pituitary cells. J. Endocrinol. **110:** 389–393, 1986.

CANONICO, P. L., VALDENEGRO, C. A., AND MACLEOD, R. M.: The inhibition of phosphatidylinositol turnover: a possible post-receptor mechanism for the prolactin secretion-inhibiting effect of dopamine. Endocrinology **113:** 7–14, 1983.

CAPPONI, A. M., LEW, P. D., JORNOT, L., AND VALLOTTON, M. B.: Correlation between cytosolic free $Ca^{2+}$ and aldosterone production in bovine adrenal glomerulosa cells. J. Biol. Chem. **259:** 8863–8869, 1984.

CAPPONI, A. M., LEW, P. D., AND VALLOTTON, M. B.: Quantitative analysis of the cytosolic-free $Ca^{2+}$-dependency of aldosterone production in bovine glomerulosa cells. Biochem. J. **247:** 335–341, 1987.

CAPPONI, A. M., LEW, P. D., WUTHRICH, R., AND VALLOTTON, M. B.: Effects of atrial natriuretic peptide on the stimulation of angiotensin II of various target cells. J. Hypertens. **4**(Suppl. 2): 581–585, 1986.

CAPPONI, A. M., ROSSIER, M. F., DAVIES, E., AND VALLOTTON, M. B.: Calcium stimulates steroidogenesis in permeabilized bovine adrenal cortical cells. J. Biol. Chem. **263:** 16113–16117, 1988.

CARAFOLI, E.: Intracellular calcium homeostasis. Annu. Rev. Biochem. **56:** 395–433, 1987.

CARAFOLI, E.: Calcium pump of the plasma membrane. Physiol. Rev. **71:** 129–153, 1991.

CAREY, R. M., THORNER, M. O., AND ORTT, E. M.: Effects of metoclopramide and bromocriptine on the renin-angiotensin-aldosterone system in man. J. Clin. Invest. **63:** 727–735, 1979.

CARR, B. R., RAINEY, W. E., AND MASON, J. I.: The role of calmodulin antagonists on steroidogenesis by fetal zone cells of the human fetal adrenal gland. Endocrinology **120:** 995–999, 1987.

CARSIA, R. V., MOYLE, W. R., WOLFF, D. J., AND MALAMED, S.: Acute inhibition of corticosteroidogenesis by inhibitors of calmodulin action. Endocrinology **11:** 1456–1461, 1982.

CATT, K. J., AND BALLA, T.: Phosphoinositide metabolism and hormone action. Annu. Rev. Med. **40:** 487–509, 1989.

CASEY, P. J., AND GILMAN, A. G.: G protein involvement in receptor-effector coupling. J. Biol. Chem. **263:** 2577–2580, 1988.

CHARTIER, L., AND SCHIFFRIN, E.: Role of calcium in effects of atrial natriuretic peptide on aldosterone production in adrenal glomerulosa cells. Am. J. Physiol. **252:** E485–E491, 1987.

CHEITLIN, R., BUCKLEY, D. I., AND RAMACHANDRAN, J.: The role of extracellular calcium in corticotropin-stimulated steroidogenesis. J. Biol. Chem. **260:** 5323–5327, 1985.

CHEUNG, W. Y.: Calmodulin plays a pivotal role in cellular regulation. Science (Wash. DC) **207:** 19–27, 1980.

CHIU, A. T., AND FREER, R. J.: Angiotensin-induced steroidogenesis in rabbit adrenal: effects of pH and calcium. Mol. Cell. Endocrinol. **13:** 159–166, 1979.

CHIU, A. T., HERBLIN, W. F., MCCALL, D. E., ARDECKY, R. J., CARINI, D. J., DUNCIA, J. V., PEASE, L. J., WONG, P. C., WEXLER, R. R., JOHNSON, A. L., AND TIMMERANS, F. B. M. W. M.: Identification of angiotensin II receptor subtypes. Biochem. Biophys. Res. Commun. **165:** 196–203, 1989.

CHOI, S. Y., SAXTON, P. M., ALLEN, A. N., FIGDOR, AND MENDELSOHN, F. A. O.: *In vitro* autoradiographic localization of ANP receptors in rat kidney and adrenal gland. Am. J. Physiol. **250:** F753–F757, 1986.

COCKCROFT, S.: Polyphosphoinositide phosphodiesterase: regulation by a novel guanine nucleotide binding protein, Gp. Trends Biochem. Sci. **12:** 75–76, 1987.

COHEN, P.: The role of protein phosphorylation in neural and hormonal control of cellular activity. Nature (Lond.) **296:** 613–620, 1982.
COHEN, C. J., MCCARTHY, R. T., BARRETT, P. Q., AND RASMUSSEN, H.: Ca channels in adrenal glomerulosa cells: $K^+$ and angiotensin II increase T-type Ca channel currents. Proc. Natl. Acad. Sci. USA **85:** 2412–2416, 1988.
CONLIN, P. R., KIM, S. Y., WILLIAMS, G. H., AND CANESSA, M. L.: $Na^+$-$H^+$ exchanger kinetics in adrenal glomerulosa cells and its activation by angiotensin II. Endocrinology **127:** 236–244, 1990.
CONLIN, P. R., WILLIAMS, G. H., AND CANESSA, M. C.: Angiotensin II-induced activation of $Na^+$-$H^+$ exchange in adrenal glomerulosa cells is mediated by protein kinase C. Endocrinology **129:** 1861–1868, 1991.
CONNOR, J. A., CORNWALL, M. C., AND WILLIAMS, G. H.: Spatially resolved cytosolic calcium response to angiotensin II and potassium in rat glomerulosa cells by digital imaging techniques. J. Biol. Chem. **262:** 2919–2927, 1987.
COUSSENS, L., PARKER, P. J., RHEE, L., YANG-FENG, T. L., CHEN, E., WATERFIELD, M. D., FRANCKE, U., AND ULLRICH, A.: Multiple distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. Science (Wash. DC): **233:** 859–866, 1986.
COYNE, M. D., AND PINKEY, L.: Adrenocorticotropin activates barium-conducting channels from bovine adrenocortical zona fasciculata cells in lipid bilayers. Endocrinology **129:** 263–269, 1991.
CURNOW, K. M., TUSIE-LUNA, M.-T., PASCOE, R., GU, J.-L., NADLER, J. L., AND WHITE, P. C.: The product of the CYP11B2 gene is required for aldosterone biosynthesis in the human adrenal cortex. Mol. Endocrinol. **5:** 1513–1522, 1991.
DAVIS, J. S., WEAKLAND, L.-L., WEST, L. A., AND FARESE, R. V.: Luteinizing hormone stimulates the formation of inositol trisphosphate and cyclic AMP in rat granulosa cells. Biochem. J. **238:** 597–604, 1986.
DEKKER, L. V., AND PARKER, P. J.: Protein kinase C—a question of specificity. Trends Biochem. Sci. **19:** 73–77, 1994.
DOUGLAS, J. G.: Potassium ion as a regulator of adrenal angiotensin receptors. Am. J. Physiol. **239:** E317–E321, 1980.
DUFF, J. L., MARRERO, M. B., PAXTON, W. G., CHARLES, C. H., LAU, L. F., BERNSTEIN, K. E., AND BERK, B. C.: Angiotensin induces 3CH134, a protein-tyrosine phosphatase, in vascular smooth muscle cells. J. Biol. Chem. **268:** 26037–26040, 1993.
DUNN, M. G., AND BOSMANN, H. B.: Peripheral dopamine receptor identification: properties of a specific dopamine receptor in the rat adrenal zona glomerulosa. Biochem. Biophys. Res. Commun. **99:** 1081–1087, 1981.
DURROUX, T., GALLO-PAYET, N., AND PAYET, M. D.: Three components of the calcium currents in cultured glomerulosa cells from rat adrenal gland. J. Physiol. **404:** 713–729, 1988.
DURROUX, T., GALLO-PAYET, N., AND PAYET, M. D.: Effects of adrenocorticotropin on action potential and calcium currents in cultured rat and bovine glomerulosa cells. Endocrinology **129:** 2139–2147, 1991.
ELLIOTT, M. E., AND GOODFRIEND, T. J.: Angiotensin alters $Ca^{2+}$ fluxes in bovine adrenal glomerulosa cells. Proc. Natl. Acad. Sci. USA **78:** 3044–3048, 1981.
ELLIOTT, M. E., AND GOODFRIEND, T. J.: Atrial natriuretic peptide inhibits protein phosphorylation stimulated by angiotensin II in bovine adrenal glomerulosa cells. Biochem. Biophys. Res. Commun. **140:** 814–820, 1986.
ELLIOTT, M. B., SIEGEL, F. L., HADJOKAS, N. E., AND GOODFRIEND, T. L.: Angiotensin effects on calcium and steroidogenesis in adrenal glomerulosa cells. Endocrinology **116:** 1051–1059, 1985.
ELY, J. A., AMBROZ, C., BAUKAL, A. J., CHRISTENSEN, S. B., AND CATT, K. J.: Relationship between agonist-and thapsigargin-sensitive calcium pools in adrenal glomerulosa cells. Thapsigargin-induced Ca2+ mobilization and entry. J. Biol. Chem. **266:** 18635–18641, 1991.
ELY, J. A., HUNYADY, L., BAUKAL, A. J., AND CATT, K. J.: Inositol 1,3,4,5-tetrakisphosphate stimulates calcium release from bovine adrenal microsomes by a mechanism independent of the inositol 1,4,5-trisphosphate receptor. Biochem. J. **268:** 333–338, 1990.
ENYEDI, P., BUKI, B., MUCSI, I., AND SPAT, A.: Polyphosphoinositide metabolism in adrenal glomerulosa cells. Mol. Cell. Endocrinol. **41:** 105–112, 1985.
ENYEDI, P., SPAT, A., AND ANTONIONI, F. A.: Role of prostaglandins in the control of the function of adrenal glomerulosa cells. J. Endocrinol. **91:** 427–437, 1981.
ENYEDI, P., AND WILLIAMS, G. H.: Heterogenous inositol tetrakisphosphate binding sites in the adrenal cortex. J. Biol. Chem. **263:** 7940–7942, 1988.
EXTON, J. H.: Role of calcium and phosphoinositides in the action of certain hormones and neurotransmitters. J. Clin. Invest. **75:** 1753–1757, 1985.
EXTON, J. H.: Signaling through phosphatidylcholine breakdown. J. Biol. Chem. **267:** 12393–12396, 1990.
FAKUNDING, J. L., AND CATT, K. J.: Dependence of aldosterone stimulation in adrenal glomerulosa cells on calcium uptake: effects of lanthanum and verapamil. Endocrinology **107:** 1345–1353, 1980.
FAKUNDING, J. L., AND CATT, K. J.: Calcium-dependent regulation of aldosterone production in isolated adrenal glomerulosa cells: effects of the ionophore A-23187. Endocrinology **110:** 2006–2010, 1982.
FAKUNDING, J. L., CHOW, R., AND CATT, K. J.: 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.
FARAGO, A., SEPRODI, J., AND SPAT, A.: Subcellular distribution of protein kinase C in rat adrenal glomerulosa cells. Biochem. Biophys. Res. Commun. **156:** 628–633, 1988.
FARESE, R. V.: On the requirement for calcium during the steroidogenic effect of ACTH. Endocrinology **89:** 1057–1067, 1971.
FARESE, R. V., FANJUL, L. F., DE RUIZ GALLERTA, C. M., DAVIS, J. S., AND COOPER, D. R.: ACTH Iincreases diacylglycerol content and subcellular redistribution of protein kinase C in rat adrenal *in vivo*. Life Sci. **41:** 2631–2637, 1987.
FARESE, R. V., LARSON, R. E., DAVIS, J. S.: Rapid effects of angiotensin-II on phosphoinositide metabolism in the rat adrenal glomerulosa cells. Endocrinology **114:** 302–304, 1984.
FARESE, R. V., ROSIC, N., BABISCHKIN, J., FARESE, M. G., FOSTER, R., AND DAVIS, J. S.: Dual activation of the inositol-trisphosphate-calcium and cyclic nucleotide intracellular signaling systems by adrenocorticotropin in rat adrenal cells. Biochem. Biophys. Res. Commun. **135:** 742–748, 1986.
FERRIS, C. D., AND SNYDER, S. H.: Inositol 1,4,5-trisphosphate-activated calcium channels. Annu. Rev. Physiol. **54:** 469–488, 1992.
FEWTRELL, C.: $Ca^{2+}$ oscillations in non-excitable cells. Annu. Rev. Physiol. **55:** 427–454, 1993.
FINCH, E. A., TURNER, T. J., AND GOLDIN, S. M.: Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release. Science (Wash. DC) **252:** 443–446, 1991.
FINN, F. M., JOHNS, P. A., NISHI, N., AND HOFMANN, K.: Differential response to adrenocorticotropic hormone analogs of bovine adrenal membranes and cells. J. Biol. Chem. **251:** 3576–3585, 1976.
FITZPATRICK, S. C., AND MCKENNA, T. J.: Dopamine inhibition of potassium-stimulated aldosterone biosynthesis in bovine adrenal zona glomerulosa cells. J. Steroid Biochem. **32:** 715–724, 1989.
FOSTER, R. H., DAVIS, J. S., AND FARESE, R. V.: External calcium is required for activation of phospholipase C by angiotensin II in adrenal glomerulosa cells. Mol. Cell. Biochem. **95:** 157–166, 1990.
FOSTER, R., LOBO, M. V., RASMUSSEN, H., AND MARUSIC, E. T.: Calcium: its role in the mechanism of action of angiotensin II and potassium in aldosterone production. Endocrinology **109:** 2196–2201, 1981.
FREDLUND, P., SALTMAN, S., KONDO, T., DOUGLAS, J., AND CATT, K. J.: Aldosterone production by isolated glomerulosa cells: modulation of sensitivity to angiotensin II and ACTH by extracellular potassium concentration. Endocrinology **100:** 481–486, 1977.
FUJITA, K., AGUILERA, G., AND CATT, K. J.: The role of cyclic AMP in aldosterone production by isolated zona glomerulosa cells. J. Biol. Chem. **254:** 8567–8574, 1979.
GALLANT, S., KAUFMAN, P. C., AND BROWNIE, A. C.: Cyclic nucleotide phosphodiesterase activity in rat adrenal gland zones. Life Sci. **14:** 937–944, 1974.
GALLO-PAYET, N., AND ESCHER, E.: Adrenocorticotropin receptors in rat adrenal glomerulosa cells. Endocrinology **117:** 38–46, 1985.
GALLO-PAYET, N., AND PAYET, M. D.: Excitation-secretion coupling: involvement of potassium channels in ACTH-stimulated rat adrenocortical cells. J. Endocrinol. **120:** 409–421, 1989.
GANGULY, A.: Dopaminergic regulation of aldosterone secretion: how credible? Clin. Sci. (Lond.) **66:** 631–637, 1984.
GANGULY, A.: Atrial natriuretic peptide-induced inhibition of aldosterone secretion: a quest for mediator(s). Am. J. Physiol. **263:** E181–194, 1992.
GANGULY, A.: Thapsigargin increases as well as potentiates aldosterone secretion: effects dependent on calcium influx (abstract). Clin. Res. **42:** 114A, 1994.
GANGULY, A., CHIOU, S., AND DAVIS, J. S.: Intracellular mediators of potassium-induced aldosterone secretion. Life Sci. **46:** 173–180, 1990.
GANGULY, A., CHIOU, S., FINEBERG, N. S., AND DAVIS, J. S.: Greater importance of $Ca^{2+}$-calmodulin in maintenance of Ang II- and $K^+$-mediated aldosterone secretion: lesser role of protein kinase C. Biochem. Biophys. Res. Commun. **182:** 254–261, 1992.
GANGULY, A., CHIOU, S., WEST, L. A., AND DAVIS, J. S.: Aldosterone secretagogues and inositol trisphosphate as intracellular mediator. J Hypertens. **4**(Suppl.): S361–S363, 1986.
GANGULY, A., CHIOU, S., WEST, L. A., AND DAVIS, J. S.: Atrial natriuretic factor inhibits angiotensin-induced aldosterone secretion: not through cGMP or interference with phospholipase C. Biochem. Biophys. Res. Commun. **159:** 148–154, 1989a.
GANGULY, A., CHIOU, S., WEST, L. A., AND DAVIS, J. S.: Proopiomelanocortin-derived peptides, phosphoinositides, cAMP, and aldosterone secretion. J. Steroid Biochem. **33:** 1143–1148, 1989b.
GANGULY, A., LI, L., HAXTON, M.: Calmodulin-dependent protein kinase II and serine/threonine-specific phosphatase(s) are involved in angiotensin and potassium mediated aldosterone secretion (abstract). Program of the 76th Annual Meeting of the Endocrine Society, Anaheim, California, 1994. Abstract no. 994.
GANGULY, A., AND WALDRON, C.: Comparative effects of a highly specific protein kinase C inhibitor, calphostin C and calmodulin inhibitors on angiotensin-stimulated aldosterone secretion. J. Steroid Biochem. Mol. Biol. **50:** 253–260, 1994.
GARREN, L. D., GILL, G. N., MASUI, H., AND WALTON, G. M.: On the mechanism of action of ACTH. Recent Prog. Horm. Res. **27:** 433–474, 1971.
GILL, G. N.: Mechanism of ACTH action. Metabolism **21:** 571–588, 1972.
GILMAN, A. G.: Guaninine nucleotide binding regulatory proteins and dual control of adenylate cyclase. J. Clin. Invest. **73:** 1–4, 1984.
GLOSSMAN, H., BAUKAL, A., AND CATT, K. J.: Angiotensin II receptors in bovine

adrenal cortex. Modifications of angiotensin binding by guanyl nucleotides. J. Biol. Chem. 249: 664–666, 1974.
GOMPERTS, B. D.: Involvement of guanosine nucleotide-binding protein in the gating of $Ca^{++}$ by receptors. Nature (Lond.) 306: 64–66, 1983.
GRAHAM-SMITH, D. G., BUTCHER, R. W., NEY, R. L., AND SUTHERLAND, E. W.: Adenosine 3′,5′-monophosphate as the intracellular mediator of the action of adrenocorticotropic hormone on the adrenal cortex. J. Biol. Chem. 242: 5535–5541, 1967.
GRYNKIEWICZ, G., POENIE, M., AND TSIEN, R. Y.: A new generation of $Ca^{2+}$ indicators with greatly impaired fluorescence properties. J. Biol. Chem. 260: 3440–3450, 1985.
GU, J.-L., NATARAJAN, R., BEN-EZRA, J., VALENTE, G., SCOTT, S., YOSHIMOTO, T., YAMAMOTO, S., ROSSI, J. J., AND NADLER, J. L.: Evidence that a leukocyte type of 12-lipoxygenase is expressed and regulated by angiotensin II in human adrenal glomerulosa cells. Endocrinology 134: 70–77, 1994.
GUILLEMANT, J., AND GUILLEMANT, S.: Elevated cyclic GMP concentration in rat adrenal cortex after dexamethasone administration. Biochem Biophys. Res. Commun. 88: 163–169, 1979.
GUILLEMETTE, G., BALLA, T., BAUKAL, A. J., SPAT, A., AND CATT, K. J.: Intracellular receptors for inositol 1,4,5-trisphosphate in angiotensin II target tissues. J. Biol. Chem. 262: 1010–1015, 1987.
HAJNOCZKY, G., VARNAI, P., BULAY, L., FARAGO, A., AND SPAT, A.: The role of protein kinase C in control of aldosterone production by rat adrenal glomerulosa cells: activation of protein kinase C by stimulation with potassium. Endocrinology 130: 2230–2236, 1992.
HAJNOCZKY, G., VARNAI, P., HOLLO, Z., CHRISTENSEN, S. B., BALLA, T., ENYEDI, P., AND SPAT, A.: Thapsigargin-induced increase in cytoplasmic $Ca^{2+}$ concentration and aldosterone production in rat adrenal glomerulosa cells: interaction with potassium and angiotensin-II. Endocrinology 128: 2639–2644, 1991.
HAKSAR, A., AND PERON, F. G.: Comparison of the $Ca^{++}$ requirement for the steroidogenic effect of ACTH and dibutyryl cyclic AMP in rat adrenal cell suspensions. Biochem. Biophys. Res. Commun. 47: 445–450, 1972.
HALL, P. F., SAWA, S., AND THOMASSON, C. L.: A role of calmodulin in the regulation of steroidogenesis. J. Cell Biol. 90: 402–407, 1981.
HALL, R. W., THORNER, M. O., AND LEONG, D. A.: Intracellular calcium concentration and growth hormone secretion in individual somatotropes: effects of growth hormone-releasing factors and somatostatin. Endocrinology 122: 2927–2932, 1988.
HAROOTUNIAN, A. T., KAU, J. P. Y., PARANJAPE, S., AND TSIEN, P. Y.: Generation of calcium oscillations in fibroblasts by positive feedback between calcium and IP3. Science (Wash. DC) 251: 75–78, 1991.
HARRINGTON, C. A., FENIMORE, D. C., AND FARMER, R. W.: Regulation of adrenocortical steroidogenesis by cyclic 3′,5′-guanosonine monophosphate in isolated rat adrenal cells. Biochem. Biophys. Res. Commun. 85: 55–63, 1978.
HAUSDORFF, W. P., AGUILERA, G., AND CATT, K. J.: Selective enhancement of angiotensin II- and potassium-stimulated aldosterone secretion by calcium channel agonist BAY K8644. Endocrinology 118: 869–874, 1986.
HAUSDORFF, W. P., AND CATT, K. J.: Activation of dihydropyridine-sensitive calcium channels and biphasic cytosolic calcium response by angiotensin II in rat adrenal glomerulosa cells. Endocrinology 123: 2818–2826, 1988.
HAUSDORFF, W. P., SEKURA, R. D., AGUILERA, G., AND CATT, K. J.: Control of aldosterone production by angiotensin II is mediated by two guaninie nucleotide regulatory proteins. Endocrinology 120: 1668–1678, 1987.
HAYASHI, K., SALA, S., CATT, K., AND DUFAU, M. L.: Regulation of steroidogenesis by adrenocorticotropic hormone in isolated adrenal cells. The intermediate role of cyclic nucleotides. J. Biol. Chem. 254: 6678–6683, 1979.
HESCHLER, J., ROSENTHAL, W., HINSCH, K-D., WULFERN, M., TRAUTWEIN, W., AND SCHULTZ, G.: Angiotensin II-induced stimulation of voltage-dependent $Ca^{2++}$ currents in an adrenocortical cell line. EMBO J. 7: 619–624, 1988.
HESLOP, J. P., IRVINE, R. F., TASHJIAN, A. H., AND BERRIDGE, M. J.: Inositol tetrakis- and pentakisphophates in $GH_4$ cells. J. Exp. Biol. 119: 395–401, 1985.
HOKIN, M. R., AND HOKIN, L. E.: Enzyme secretion and the incorporation of $^{32}P$ intoO phospholipidsof pancreas slices. J. Biol. Chem. 203: 967–970, 1953.
HORNSBY, P. J., AND O'HARE, M. J.: The role of potassium and corticosteroids in determining the pattern of metabolism of $^3H$-deoxycorticosterone by monolayer cultures of rat adenal glomerulosa cells. Endocrinology 101: 997–1005, 1977.
HOTH, M., AND PENNER, R.: Depletion of intracellular calcium stores activates a calcium current in mast cells. Nature (Lond.) 355: 353–356, 1992.
HSU, W. H., XIANG, H., RAJAN, A. S., KENZE, D. L., AND BOYD, A. E.: Somatostatin inhibits insulin secretion by a G-protein mediated decrease in $Ca^{2+}$ entry through voltage-dependent $Ca^{2+}$ channels in the beta cells. J. Biol. Chem. 266: 837–847, 1991.
HUCKLE, W. R., DY, R. C., AND EARP, S.: Calcium-dependent increase in tyrosine kinase activity stimulated by angiotensin II. Proc. Natl. Acad. Sci. USA 89: 8837–8841, 1992.
HUNYADY, L., BAUKAL, A. J., BOR, M., ELY, J. A., AND CATT, K. J.: Regulation of 1,2-diacylglycerol production by angiotensin-II in bovine adrenal glomerulosa cells. Endocrinology 126: 1001–1008, 1990.
HUNYADY, L., KAYSER, S., CRAGOE, E. J., BALLA, I., BALLA, T., AND SPAT, A.: $Na^+$-$H^+$ and $Na^+$-$Ca^+$ exchange in glomerulosa cells: possible role in control of aldosterone production. Am. J. Physiol. 254: C744–C750, 1988.
HUNYADY, L., MERELLI, F., BAUKAL, A. J., BALLA, T., AND CATT, K. J.: Agonist-induced endocytosis and signal generation in adrenal glomerulosa cells. J. Biol. Chem. 266: 2783–2788, 1991.
HYATT, P. J., BELL, J. B. G., BHATT, F., CHU, J. F., TAIT, J. F., AND TAIT, S. A. S.: Effect of α-melanocyte stimulating hormone on the cyclic AMP and phospholipid metabolism of rat adrenocortical cells. J. Endocrinol. 110; 405–416, 1986.
IIDA, S., WIDAMAIER, E. P., AND HALL, P. F.: The phosphatidylinositide-$Ca^{2+}$ hypothesis does not apply to the steroidogenesis action of corticotropin. Biochem. J. 236: 53–59, 1986.
IINO, M., AND ENDO, M.: Calcium-dependent immediate feedback control of inositol 1,4,5-trisphosphate-induced $Ca^{2+}$ release. Nature (Lond.) 360: 76–78, 1992.
ILVESMAKI, V., AND VOUTILAINEN, R.: Interaction of phorbol ester and adrenocorticotropin in the regulation of steroidogenic P450 genes in human fetal and adult adrenal cell cultures. Endocrinology 128: 1450–1458, 1991.
INAGAKI, M., HAGIWARA, M., SAITOH, M., AND HIDAKA, H.: Protein kinase C negatively modulated by phorbol ester. FEBS Lett. 202: 277–281, 1986.
IRVINE, R. F., AND MOOR, R. M.: Micro-injection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external $Ca^{2+}$. Biochem. J. 240: 917–920, 1986.
ISHIZUKA, T., MIURA, K., NAGAO, S., AND NOZAWA, Y.: Differential redistribution of protein kinase C in human aldosteronoma cells and adjacent adrenal cells stimulated with ACTH and angiotensin II. Biophys. Biochem. Res. Commun. 155: 643–649, 1988.
JOHNSON, E. J. M., CAPPONI, A. M., AND VALLOTTON, M. B.: Cytosolic free calcium oscillates in single bovine adrenal glomerulosa cells in response to angiotensin II stimulation. J. Endocrinol. 122: 391–402, 1989.
KACZMAREK, L. K.: The role of protein kinase C in the regulation of ion channels and neurotransmitter release. Trends Neurosci. 10: 30–34, 1987.
KELLY, L. A., AND KORITZ, S. B.: Bovine adrenal cortical adenyl cyclase and its stimulation by adrenocorticotropic hormone and NaF. Biochim. Biophys. Acta 237: 141–155, 1971.
KENNEDY, M. B., AND GREENGARD, P.: Calcium/calmodulin-dependent protein kinases, which are highly concentrated in brain, phosphorylated protein I at distinct sites. Proc. Natl. Acad. Sci. USA 78: 1293–1297, 1981.
KENYON, C. J., YOUNG, J., AND FRASER, R.: Potassium fluxes in bovine adrenal cells during adrenocorticotropin stimulation. Endocrinology 116: 2279–2285, 1985.
KIGOSHI, T., UCHIDA, K., AND MORIMOTO, S.: Elongation factor 2 as the major substrate for $Ca^{2+}$/calmodulin-dependent protein kinase in rat adrenal glomerulosa cells. J. Steroid Biochem. 32: 381–385, 1989.
KIMURA, T.: Transduction of ACTH signal from plasma membrane to mitochondria in adrenocortical steroidogenesis: effects of peptide, phospholipid, and calcium. J. Steroid Biochem. 25: 711–716, 1986.
KITABCHI, A. E., AND SHARMA, R. K.: Corticosteroidogenesis in isolated adrenal cells of rats. I. Effect of corticotropin and 3′,5′-cyclic nucleotides on corticosterone production. Endocrinology 88: 1109–1116, 1971.
KOBAYASHI, N., RUSSELL, J., LETTIERI, D., AND SHERWOOD, L. M.: Regulation of protein kinase C by extracellular calcium in bovine parathyroid cells. Proc. Acad. Sci. USA 85: 4857–4860, 1988.
KOCH, G. L.: The endoplasmic reticulum and calcium storage. Bioessays 12: 527–531, 1990.
KOJIMA, I. KOJIMA, K., KREUTTER, D., AND RASMUSSEN, H.: Temporal integration of the aldosterone secretory response to angiotensin occurs via two intracellular pathways. J. Biol. Chem. 259: 14448–14457, 1984a.
KOJIMA, K., KOJIMA, I., AND RASMUSSEN, H.: Dihydropyridine calcium agonist and antagonist effects on aldosterone secretion. Am. J. Physiol. 247: E645–E650, 1984b.
KOJIMA, I., KOJIMA, K., AND RASMUSSEN, H.: Role of calcium and cAMP in the action of adrenocorticotropin on aldosterone secretion. J. Biol. Chem. 260: 4248–4256, 1985a.
KOJIMA, I., KOJIMA, K., AND RASMUSSEN, H.: Characteristics of angiotensin II-, $K^+$- and ACTH-induced calcium influx in adrenal glomerulosa cells. Evidence that angiotensin II, $K^+$, and ACTH may operate common calcium channel. J. Biol. Chem. 260: 9171–9176, 1985b.
KOJIMA, I., KOJIMA, K., AND RASMUSSEN, H.: Role of calcium fluxes in the sustained phase of angiotensin II-mediated aldosterone secretion from adrenal glomerulosa cells. J. Biol. Chem. 260: 9177–9184, 1985c.
KOJIMA, I., KOJIMA, K., AND RASMUSSEN, H.: Intracellular calcium and adenosine 3′,5′-cyclic monophosphate as mediators of potassium-induced aldosterone secretion. Biochem. J. 228: 69–76, 1985d.
KOJIMA, I., KOJIMA, K., AND RASMUSSEN, H.: Possible role of phospholipase $A_2$ action and arachidonic acid metabolism in angiotensin II-mediated aldosterone secretion. Endocrinology 117: 1057–1066, 1985e.
KOJIMA, I., KOJIMA, K., AND RASMUSSEN, H.: The effect of angiotensin II and $K^+$ on calcium efflux and aldosterone production in adrenal glomerulosa cells. Am. J. Physiol. 248: E36–E43, 1985f.
KOJIMA, K., LIPPES, H., KOJIMA, K., AND RASMUSSEN H.: Aldosterone secretion: effect of phorbol ester and A23187. Biochem. Biophys. Res. Commun. 116: 555–563, 1983.
KOJIMA, I,. AND OGATA, E.: Direct demonstration of adrenocorticotropin induced changes in cytoplasmic free calcium with aequorin in adrenal glomerulosa cell. J. Biol. Chem. 261: 9632–9638, 1986.
KOJIMA, I., SHIBATA, H., AND OGATA, E.: Phorbol ester inhibits angiotensin-

induced activation of phospholipase C in adrenal glomerulosa cells. Its implication in the sustained action of angiotensin. Biochem. J. **237:** 253–258, 1986a.

KOJIMA, I., SHIBATA, H., AND OGATA, E.: Pertussis toxin blocks II-induced calcium influx but not inositol trisphosphate production in adrenal glomerulosa cell. FEBS Lett. **204:** 347–351, 1986b.

KOJIMA, I., SHIBATA, H., AND OGATA, E.: Time-dependent restoration of the trigger pool of calcium after termination of angiotensin II action in adrenal glomerulosa cells. J. Biol. Chem. **262:** 4557–4563, 1987.

KOLETSKY, R. J., BROWN, E. M., AND WILLIAMS, G. H.: Calmodulin-like activity and calcium-dependent phosphodiesterase in purified cells of the rat zona glomerulosa and zona fasciculata cells. Endocrinology **113:** 485–490, 1983.

KRAMER, R. E., GALLANT, S., AND BROWNIE, A. C.: Actions of angiotensin II on aldosterone biosynthesis in the rat adrenal cortex. Effects on cytochrome P-450 enzymes of the early and late pathway. J. Biol. Chem. **255:** 3442–3447, 1980.

KRAMER, R. E.: Angiotensin II causes sustained elevations in cytosolic calcium in glomerulosa cells. Am. J. Physiol. **255:** E338–E346, 1988a.

KRAMER, R. E.: Angiotensin II-stimulated changes in calcium metabolism in cultured glomerulosa cells. Mol. Cell. Endocrinol. **60:** 199–210, 1988b.

KRAMER, R. E.: Evidence that angiotensin II decreases mitochondrial calcium in the glomerulosa cell. Mol. Cell. Endocrinol. **74:** 87–100, 1990.

KRAUSE, K.-H., PITTET, D., VOLPE, P., POZZAN, T., MELDOLESI, J., AND LEW, D. P.: Calciosome, a sarcoplasmic reticulum-like organelle involved in intracellular $Ca^{2+}$-handling by non-muscle cells: studies in human neutrophils and HL-60 cells. Cell Calcium **10:** 351–361, 1989.

KREBS, E. G.: The phosphorylation of proteins a major mechanism for biological regulation. Biochem. Soc. Trans. **13:** 813–820, 1985.

KRUEGER, R. J., AND ORME-JOHNSON, N. B.: Acute adrenocorticotropic hormone stimulation of adrenal corticosteroidogenesis. Discovery of a rapidly induced protein. J. Biol. Chem. **258:** 10159–10169, 1983.

KUBO, M., AND STROTT, C. A.: Calcium-dependent protein kinase activity and protein phosphorylation in zones of the adrenal cortex. J. Steroid Biochem. **29:** 407–413, 1988.

KUKULJAN, M., ROJA, E., CATT, K. J., AND STJILKOVIC, S.: Membrane potential regulates inositol 1,4,5-trisphophate-controlled cytoplasmic oscillations in pituitary gonadotrophs. J. Biol. Chem. **269:** 4860–4865, 1994.

KUNO, N., AND GARDNER, P.: Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. Nature (Lond.) **326:** 301–304, 1987.

LANG, U., AND VALLOTTON, M. B.: Angiotensin II but not potassium induces subcellular redistribution of protein kinase C in bovine adrenal glomerulosa cells. J. Biol. Chem. **262:** 8047–8050, 1987.

LANGLOIS, D., BEGEOT, M., BERTHELON, M.-C., JAILLARD, C., AND SAEZ, J. M.: Angiotensin II potentiates agonist-induced 3′,5′-cyclic adenosine monophosphate product by cultured bovine adrenal cells through protein kinase C and calmodulin pathways. Endocrinology **130:** 2189–2195, 1992.

LASSEGUE, B., ALEXANDER, R. W., CLARK, M., AND GRIENDLING, K. K.: Angiotensin II-induced phosphatidylcholine hydrolysis in cultured vascular smooth-muscle cells. Biochem. J. **276:** 19–25, 1991.

LAUBER, M., BONI-SCHMETZLER, M., MULLER, J.: Potassium raises cytochrome $P\text{-}450_{11\beta}$ mRNA level in zona glomerulosa of rat adrenals. Mol. Cell. Endocrinol. **72:** 159–166, 1990.

LAYCHOCK, S. G., AND HARDMAN, J. G.: Effects of sodium nitroprusside and ascorbic acid in rat adrenocortical cell cGMP levels and steroidogenesis. J. Cyclic Nucleotide Res. **5:** 335–344, 1978.

LAYCHOCK, S. G., AND RUBIN, R. P.: ACTH-induced prostaglandin biosynthesis from $^3$H-arachidonic acid by adrenocortical cells. Prostaglandins **10:** 529–540, 1975.

LEACH K. L., RUFF, V. A., WRIGHT, T. M., PESSIN, M. S., AND RABEN, D. M.: Dissociation of protein kinase C activation and *sn*-1,2-diacylglycerol formation. J. Biol. Chem. **266:** 3215–3219, 1991.

LEHOUX, J.-G., BIRD, I. M., RAINEY, W. E., TREMBLAY, A., AND DUCHARME, L.: Both low sodium and high potassium intake increase the level of adrenal angiotensin-II receptor type 1, but not that of adrenocorticotropin receptor. Endocrinology **134:** 776–782, 1994.

LEIER, D. J., AND JUNGMANN, R. A.: Adrenotropic hormone and dibutyrl adenosine cyclic monophosphate-mediated $Ca^{++}$ uptake by rat adrenal glands. Biochim. Biophys. Acta **329:** 196–210, 1973.

LIHRMANN, I., DELARUE, C., HOMO-DELARCHE, F., FEUILLOLEY, M., BELANGER, A., AND VAUDRY, H.: Effects of TMB-8 and dantrolene on ACTH- and angiotensin-induced steroidogenesis by free interrenal gland: evidence for a role of intracellular calcium in angiotensin action. Cell Calcium **8:** 269–282, 1987.

LITOSCH, I., AND FAIN, J. N.: Regulation of phosphoinositides breakdown by guanine nucleotides. Life Sci. **39:** 187–194, 1986.

LITVIN, Y., LEIER, M., FLEISHER, N., AND ERLICHMAN, J.: Somatostatin inhibits corticotropin-releasing factor stimulated adrenocorticotropin release, adenylate cyclase, and activation of adenosine 3′, 5′-monophosphate-dependent protein kinase isoenzymes in AtT20 cells. Endocrinology **119:** 7737–7745, 1986.

LLEDO, P-N., LEGENDRE, P., ISRAEL, J-M., AND VINCENT, J-D.: Dopamine inhibits two characterised voltage-dependent calcium currents in identified rat lactotroph cell. Endocrinology **127:** 990–1001, 1990.

LOBO, M. V., AND MARUSIC, E. T.: Effect of angiotensin II, ATP and ionophore A23187 on potassium efflux in adrenal glomerulosa cells. Am. J. Physiol. **250:** E125–E130, 1986.

LOGIN, I. S., JUDD, A. M., AND MACLEOD, R. M.: Dopamine inhibits calcium flux in the 7315a prolactin-secreting pituitary tumor. Cell Calcium **9:** 27–31, 1988.

LONG, J. A.: Zonation of the mammalian adrenal corex. *In* Adrenal Gland, Handbook of Physiology, ed. by R. O. Greep, and E. B. Astwood, vol. 6, pp. 13–24, Washington, D. C., American Physiological Society, 1975.

LOWIK, W. G. M., VAN LEEUWEN, J. P. T. M., VAN DER MEER, J.-M., VAN ZEELAND, J. K., SEEVEN, B. A. A., AND HERMANN-ERLEE, M. P. M.: A two-receptor model for the action of parathyroid hormone on osteoblasts: a role for intracellular free calcium and cAMP. Cell Calcium **6:** 311–326, 1985.

LUCKHOFF, A., AND CLAPHAM, D. E.: Inositol 1,3,4,5-tetrakisphosphate activates an endothelial $Ca^{2+}$-permeable channel. Nature (Lond.) **355:** 356–358, 1992.

LUINI, A., LEWIS, D., GUILD, S., SCHOFIELD, G., AND WEIGHT, F.: Somatostatin, an inhibitor of ACTH secretion, decreases cytosolic free calcium and voltage-dependent calcium current in a pituitary cell line. J. Neurosci. **6:** 3128–3132, 1986.

LYNCH, D. R., BRAAS, K. M., AND SNYDER, S. H.: Atrial natriuretic factor receptors in rat, kidney, adrenal gland and brain: autoradiographic localization and fluid balance dependent changes. Proc. Natl. Acad. Sci. USA **83:** 3357–3361, 1986.

MAACK, T. M., SUZUKI, F. A., ALMEIDA, D., NUSSENZVEIG, R. M., SCARBOROUGH, G. A., MCINROE, G. A., AND LEWICKI, J. A.: Physiological role of silent receptors of atrial natriuretic factor. Science (Wash. DC) **238:** 675–678, 1985.

MACFARLAND, R. T., ZELUS, B. D., AND BEAVO, J. A.: High concentrations of a cGMP-stimulated phosphodiesterase mediate ANP-induced decrease in cAMP and steroidogenesis in adrenal glomerulosa cells. J. Biol. Chem. **266:** 136–142, 1991.

MACKIE, C-M., SIMPSON, E. R., MEE, M. S. R., TAIT, S. A. S., AND TAIT, J. F.: Intracellular potassium and steroidogenesis of isolated rat adrenal cells: effect of potassium and angiotensin II on purified zona glomerulosa cells. Clin. Sci. Mol. Med. **53:** 289–296, 1977.

MACKIE, C., RICHARDSON, M. E., AND SCHULSTER, D.: Kinetics and dose-response characteristics of adenosine 3′,5′-monophosphate production by isolated rat adrenal cells stimulated with adrenocorticotrophic hormone. FEBS Lett. **23:** 345–348, 1972.

MACKIE, C., WARREN, R. L., AND SIMPSON, E. R.: Investigations into the role of calcium ions in the control of steroid production by isolated adrenal zona glomerulosa cells of the rat. J. Endocrinol. **77:** 119–127, 1978.

MACNEIL, S. M., LAKEY, T., AND TOMLINSON, S.: Calmodulin regulation of adenylate cyclase activity. Cell Calcium **6:** 213–226, 1985.

MAHAFFEE, D., AND NEY, R. L.: Effects of nucleotides possessing a 3′,5′-cyclic monophosphate on adrenal steroidogenesis. Metabolism **19:** 1104–1108, 1970.

MAHAFFEE, D. D., AND ONTJES, J. A.: The role of calcium in the control of adrenal adenylate cyclase. Enhancement of enzyme activation by guanyl-5′-YL imidodiphosphate. J. Biol. Chem. **255:** 1565–1571, 1980.

MAJERUS, P. W., CONNOLY, T. M., BANSAL, V. S., INHORN, R. C., ROSS, T. S., AND LIPS, D. L.: Inositol phosphate synthesis and degradation. J. Biol. Chem. **263:** 3051–3054, 1988.

MAJERUS, P. W., CONNOLY, T. M., DECKMYN, H., ROSS, T. S., BROSS, T. E., ISHI, H., BANSAL, V. S., WILSON, D. B.: The metabolism of phosphoinositide-derived messenger molecules. Science (Wash. DC) **234:** 1519–1526, 1986.

MALGAROLI, A., VALLAR, L., ELAHI, F. R., POZZAN, T., SPADA, A., AND MELDOLESI, J.: Dopamine inhibits cytosolic $Ca^{2+}$ increase in rat lactotroph cells. Evidence of a dual mechanism of action. J. Biol. Chem. **262:** 13920–13927, 1987.

MARIE, J., AND JARD, S.: Angiotensin II inhibits adenylate cyclase from adrenal cortex of glomerulosa zone. FEBS Lett. **159:** 97–101, 1983.

MATSUKAWA, N., NONAKA, Y., YING, Z., HIGAKI, J., OGIHARA, T., AND OKAMOTO, M.: molecular cloning and expression of cDNAS encoding rat aldosterone synthase: variants of cytochrome $P\text{-}450_{11\beta}$. Biochem. Biophys. Res. Commun. **169:** 245–252, 1990.

MATSUNAGA, H., YAMASHITA, N., MARUYAMA, Y., KOJIMA, I., AND KUROKAWA, K.: Evidence for two distinct voltage-gated calcium channel currents in bovine adrenal glomerulosa cells. Biochem. Biophys. Res. Commun. **149:** 1049–1054, 1987.

MATSUOAKA, H., ISHI, M., SUGIMOTO, T., HIRATA, Y., SUGIMOTO, T., KANAGAWA, K., AND MATSUO, H.: Inhibition of aldosterone production by alpha-human atrial natriuretic polypeptide is associated with an increase in cGMP production. Biochem. Biophys. Res. Commun. **127:** 1052–1056, 1985.

MAUGER, J.-P., POGGIOLI, J., GUESDON, F., AND CLARET, M.: Noradrenaline, vasopressin and angiotensin increase $Ca^{2+}$ influx by opening a common pool of $Ca^{2+}$ channels in isolated rat liver cells. Biochem. J. **221:** 121–127, 1984.

MAURIER, R., AND REUBI, J. C.: Somatostatin receptors in the adrenal. Mol. Cell. Endocrinol. **45:** 81–90, 1986.

MCCARTHY, R. T., ISALES, C. M., BOLLAG, W. B., RASMUSSEN, H., AND BARRETT, P. Q.: Atrial natriuretic peptide differentially modulates T- and L-type calcium channels. Am. J. Physiol. **258:** F473–F478, 1990.

MCCORMACK, J. G., AND DENTON, R. M.: $Ca^{2+}$ as a second messenger within mitochondria. Trends Biochem. Sci. **11:** 258–262, 1986.

MCKENNA, T. J., ISLAND, D. P., AND NICHOLSON, W. E.: Dopamine inhibits angiotensin-stimulated aldosterone biosynthesis in bovine adrenal cells. J. Clin. Invest. **64:** 287–291, 1979.

MCMILLAN, B. H., NEY, R. I., AND SCHORR, I.: Guanyl cyclase activity in normal

adrenals and a corticosterone-producing adrenal cancer of the rat. Endocrinology 89: 281–283, 1971.
McPHAIL, L-C., CLAYTON, C-C., AND SNYDERMAN, R.: A potential second messenger role for arachidonic acid: activation of $Ca^{2+}$-dependent protein kinase. Trans. Assoc. Am. Physicians 97: 222–231, 1984.
McPHERSON, P. S., AND CAMPBELL, K. P.: The ryanodine receptor/$Ca^{2+}$ release channel. J. Biol. Chem. 268: 13765–13768, 1993.
MEANS, A. R.: Calmodulin: properties, intracellular localization, and multiple roles in cell regulation. Recent Prog. Horm. Res. 37: 333–362, 1981.
MEANS, A. R., AND DEDMAN, J. R.: Calmodulin—an intracellular calcium receptor. Nature (Lond.) 285: 73–77, 1980.
MEISHERI, K. D., TAYLOR, C. J., AND SANEII, H.: Synthetic atrial peptide inhibits intracellular calcium release in smooth muscle. Am. J. Physiol. 250: C171–C174. 1986.
MELDOLESI, J., MADDEDU, L., AND POZZAN, T.: Intracellular $Ca^{2+}$ storage organelles in non-muscle cells: heterogeneity and functional assignment. Biochim. Biophys. Acta 1055: 130–140, 1990.
MENNITI, F. S., OLIVER, K. G., PUTNEY, J. W., AND STEARS, S. B.: Inositol phosphates and cell signaling: new views of InsP5 and InsP6. Trends Biochem. Sci. 18: 53–56, 1993.
MEULI, C., AND MULLER, J.: A potassium-induced mitochondrial protein related to aldosterone biosynthesis. Am. J. Physiol. 245: E449–E456, 1983.
MICHELL, R. H.: Inositol phospholipids and cell surface receptor function. Biochim. Biophys. Acta 415: 81–147, 1975.
MILANI, D., MALGAROLI, A., GUIDOLIN, D., FASOLATO, C., SKAPER, S. D., MELDOLESI, J., AND POZZAN, T.: $Ca^{2+}$ channels and intracellular $Ca^{2+}$ stores in neuronal and neuroendocrine cells. Cell Calcium 11: 191–199, 1990.
MILLER, R. J.: Voltage-sensitive $Ca^{2+}$ channels. J. Biol. Chem. 267: 1403–1406,1992.
MILNAR, B., BIAGI, B. B., AND ENYEART, J. J.: A novel $K^+$ current inhibited by adrenocorticotropin hormone and angiotensin II in adrenal cortical cells. J. Biol. Chem. 268: 8640–8644, 1993.
MISSALE, C., LOMBARDI, C., DE LOTIS, R., MEMO, M., CARRUBA, M. O., AND SPANO, P. F.: Dopaminergic receptor mechanism modulating the renin-angiotensin system and aldosterone secretion: an overview. J. Cardiovasc. Pharmacol. 14(Suppl. 8): S29–S39, 1989.
MOLLOY, C. J., TAYLOR, D. S., AND WEBER, A.: Angiotensin II stimulation of rapid protein tyrosine phosphorylation and protein kinase activation in rat aortic smooth muscle cells. J. Biol. Chem. 268: 7338–7335, 1993.
MORGAN, J. P., AND MORGAN, K. G.: Vascular smooth muscle: the first recorded $Ca^{2+}$ transients. Pflugers Arch. 395: 75–77, 1982.
MORRA, M., LEBOULENGER, F., DESBUES, L., TONON, M. S., AND VAUDRY, H.: Dopamine inhibits inositol phosphate production, arachidonic acid formation and corticosteroid release by frog adrenal gland through a pertussis toxin-sensitive G protein. Endocrinology 128: 2625–2632, 1991.
MOYLE, W. R., KONG, Y. C., AND RAMACHANDRAN, J.: Steroidogenesis and cyclic adenosine 3′,5′-monophosphate accumulation in rat adrenal cells. J. Biol. Chem. 248: 2409–2417, 1973.
MULLER, J.: Regulation of Aldosterone Biosynthesis, Springer-Verlag, Berlin, 1971.
NADLER, J. L., NATARAJAN, R., AND STERN, N.: Specific action of the lipoxygenase pathway in mediating angiotensin II-induced aldosterone synthesis in isolated adrenal glomerulosa cells. J. Clin. Invest. 80: 1763–1769, 1987.
NAKANISHI, S., CATT, K. J., AND BALLA, T.: Inhibition of agonist-stimulated inositol 1,4,5-trisphosphate production and calcium signaling by the myosin light chain kinase inhibitor, wortmannin. J. Biol. Chem. 269: 6528–6535, 1994.
NAKANO, S., CARVALLO, P., POCCO, S., AND AGUILERA, G.: Role of protein kinase C on the steroidogenic effect of angiotensin II in the rat adrenal glomerulosa cell. Endocrinology 126: 125–133, 1990.
NASSERUDIN, S. A., AND HORNSBY, P. J.: Regulation of 11$\beta$- and 117$\alpha$-hydroxylases in cultured bovine adrenocortical cells: 3′,5′-cyclic adenosine monophosphate, insulin-like growth factor I, and activators of protein kinase C. Endocrinology 127: 1673–1681, 1990.
NATARAJAN, R., DUNN, W. D., STERN, N., AND NADLER, J.: Key role of diacylglycerol-mediated 12-lipoxygenase product formation in angiotensin II-induced aldosterone synthesis. Mol. Cell. Endocrinol. 72: 73–80, 1990.
NATHANSON, M. H., FALLON, M. B., PADFIELD, P. J., AND MARANTO, A. R.: Localization of the type 3 inositol 1,4,5-trisphosphate receptor in the $Ca^{2+}$ wave trigger zone of pancreatic acinar cells. J. Biol. Chem. 269: 4693–4696, 1994.
NATKE, E., AND KABELA, E.: Electrical responses in cat adrenal cortex: possible relation to aldosterone secretion. Am. J. Physiol. 237: E158–E162, 1979.
NISHIZUKA, Y.: Studies and perspectives of protein kinase C. Science (Wash. DC) 233: 305–312, 1986.
NISHIZUKA, Y.: The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature (Lond.) 661–665, 1988.
NISHIZUKA, Y., TAKAI, Y., KISHIMOTO, A., KIKKAWA, U., AND KAIBUCHI, K.: Phospholipid turnover in hormone action. Recent Prog. Horm. Res. 40: 301–339, 1984.
NOWYCKY, M-C., FOX, A. P., AND TSIEN, R. W.: Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature (Lond.) 316: 440–443, 1985.
NUSSINOVITCH, I.: Somatostatin inhibits two types of voltage-activated calcium currents in rat growth-hormone secreting cells. Brain Res. 504: 136–138, 1989.
OHNISHI J., ISHIDO, M., SHIBATA, T., INAGAMI, T., MURAKAMI, K., AND MIYAZAKI, H.: The rat angiotensin II $AT_{1A}$ receptor couples with three different signal transduction pathways. Biochem. Biophys. Res. Commun. 186: 1094–1100, 1992.
OSPENKO, O. N., VARNAI, P., MIKE, A., AND VIZI, E. S.: Dopamine blocks T-type calcium channels in cultured rat adrenal glomerulosa cells. Endocrinology 134: 511–514, 1994.
PANDEY, K. N., INAGAMI, T., GIRARD, P. R., KUO, J. F., AND MISONO, K. S.: New signal transduction mechanisms of atrial natriuretic factor inhibition of phosphorylation of protein kinase C and a 240 kDn in adrenal cortical plasma membrane by cGMP dependent mechanisms. Biochem. Biophys. Res. Commun. 148: 589–595, 1987.
PAYET, M. D., BENABDARRAK, M., AND GALLO-PAYET, N.: Excitation-secretion coupling: ionic currents in glomerulosa cells: effects of adrenocorticotropin and $K^+$ channel blockers. Endocrinology 121: 875–882, 1987.
PEDERSON, R. C., AND BROWNIE, A. C.: Cholesterol side-chain cleavage in the rat adrenal cortex: isolation of a cycloheximide-sensitive activator peptide. Proc. Natl. Acad. Sci. USA 80: 1882–1886, 1983.
PERCHELLET, J.-P., SHANKER, G., AND SHARMA, R. K.: Regulatory role of guanosine 3′,5′-monophosphate in adrenocorticotropin hormone-induced steroidogenesis. Science (Wash. DC) 198: 311–312, 1978.
PERCHELLET, J. P., AND SHARMA, R. K.: Stimulation of guanosine 3′,5′-monophosphate-phosphodiesterase activity by adrenocorticotropic hormone-activated increase of guanosine 3′,5′-monophosphate in isolated adrenocortical carcinoma cells. Endocrinology 105: 879–883, 1979.
PEREZ-REYES, E., WEI, X., CASTELLANO, A., AND BIRNBAUMER, L.: Molecular diversity of L-type calcium channels. Evidence for alternate splicing of the transcripts of three non-allelic genes. J. Biol. Chem. 265: 20430–20436, 1990.
PETYREMAN, A., NICHOLSON, W. E., BROWN, R. D., LIDDLE, G. W., AND HARMAN, J. G.: Comparative effects of angiotensin and ACTH on cyclic AMP and steroidogenesis in isolated bovine adrenal cells. J. Clin. Invest. 52: 835–842, 1973.
PODESTA, E. J., MILANI, A., STEPHEN, H., AND NEHER, R.: Steroidogenic action of calcium ions in isolated adrenocortical cells. Biochem. J. 186: 391–397, 1980.
PRALONG, W.-F., HUNYADY, L., VARNA, P., WOLLHEIM, C. B., AND SPAT, A.: Pyridine nucleotide redox state parallels production of aldosterone in potassium-stimulated adrenal glomerulosa cells. Proc. Natl. Acad. Sci. USA 89: 132–136, 1992.
PRATT, J. H., ROTHROCK, J. K., AND DOMINGUEZ, J. H.: Evidence that angiotensin II and potassium collaborate to increase cytosolic calcium and stimulate the secretion of aldosterone. Endocrinology 125: 2463–2469, 1989.
PUTNEY, J. W.: A model for receptor-regulated calcium entry. Cell Calcium 7: 1–12, 1986.
PUTNEY, J. W.: Capacitance calcium entry revisited. Cell Calcium 11: 611–624, 1990.
QUINN, S. J., CORNWALL, M. C., AND WILLIAMS, G. H.: Electrical properties of isolated rat adrenal glomerulosa and fasciculata cells. Endocrinology 120: 903–914, 1987.
QUINN, S. J., ENYEDI, P., TILLOTSON, AND WILLIAMS, G. H.: Kinetics of cytosolic calcium and aldosterone responses in rat adrenal glomerulosa cells. Endocrinology 129: 2431–2441, 1991.
QUINN, S. J., WILLIAMS, G. H., AND TILLOTSON, D. L.: Calcium oscillations in single adrenal glomerulosa cells stimulated by angiotensin II. Proc. Natl. Acad. Sci. USA 85: 5754–5758, 1988a.
QUINN, S. J., WILLIAMS, G. H., AND TILLOTSON, D. L.: Calcium response of single adrenal glomerulosa cells to external potassium. Am. J. Physiol. 255: E488–E495, 1988b.
RAE, P. A., GUTMANN, N. S., TSAO, I., AND SCHIMMER, B. P.: Mutations in cyclic AMP-dependent protein kinase and corticotropin (ACTH)-sensitive adenylate cyclase affect adrenal steroidogenesis. Proc. Natl. Acad. Sci. USA 76: 1896–1900, 1979.
RHEE, S. G., AND CHOI, K. D.: Regulation of inositol phospholipid-specific phospholipase C isoenzymes. J. Biol. Chem. 267: 12393–12396, 1992.
RHEE, S. G., SUH, P-G., RYU, S.-H., AND LEE, S. Y.: Study of inositol phospholipid-specific phospholipase C. Science (Wash. DC) 244: 546–550, 1989.
RICHARDSON, M. C., AND SCHULSTER, D.: The role of protein kinase activation in the control of steroidogenesis by adrenocorticotrophic hormone in the adrenal cortex. Biochem. J. 136: 993–998, 1973.
ROBERTSON, L. M., KEITH, L. D., AND KENDALL, J. W.: Potassium modulation of corticosterone secretion from perifused mouse adrenal cells. Metabolism 33: 703–709, 1984.
ROSENTHAL, W., HESCHLER, J., TRAUTWEIN, W., AND SCHULTZ, G.: Control of voltage-dependent $Ca^{2+}$ channels by G protein-coupled receptors. FASEB J. 2: 2784–2790, 1988.
ROSS, C. A., MELDOLESI, J., MILNER, T. A., SATOH, T., SUPATTAPONE, S., AND SNYDER, S. H.: Inositol 1,4,5-trisphosphate receptor localized to endoplasmic reticulum in cerebellar neurons. Nature (Lond.) 339: 468–470, 1989.
ROSSIER, M. F., CAPPONI, A. M., AND VALLOTTON, M. B.: Inositol trisphosphate isomers in angiotensin II-stimulated adrenal glomerulosa cells. Mol. Cell. Endocrinol. 57: 163–168, 1988.
ROSSIER, M. F., CAPPONI, A. M., AND VALLOTTON, M. B.: The inositol 1,4,5-trisphosphate binding site in adrenal cortical cells is distinct from the endoplasmic reticulum. J. Biol. Chem. 264: 14078–14084, 1989.
ROSSIER, M. F., DENTAND, I. A., CAPPONI, A. M., AND VALLOTTON, M. B.: Interconversion of inositol(1,4,5)-trisphosphate to (1,3,4,5)-tetrakisphosphate

and (1,3,4)-trisphosphate in permeabilized adrenal glomerulosa cells is calcium-sensitive and ATP-dependent. Biochem. Biophys. Res. Commun. 139: 259–265, 1986.

ROSSIER, M. F., KRAUSE, K. H., LEW, P. D., CAPPONI, A. M., AND VALLOTTON, M. B.: Control of cytosolic free calcium by intracellular organelles in bovine adrenal glomerulosa cells. Effects of sodium and inositol 1,4,5-trisphosphate. J. Biol. Chem. 262: 4053–4058, 1987.

ROSSIER, M. F., AND PUTNEY, J. W.: The identity of the calcium-storing, inositol 1,4,5-trisphosphate-sensitive organelle in non-muscle cells: calcisome, endoplasmic reticulum or both? Trends Neurosci. 14: 310–314, 1991.

RUBIN, R. P., LAYCHOCK, S. G., AND END, D. W.: On the role of cyclic AMP and cyclic GMP in steroid production by bovine cortical cells. Biochem. Biophys. Acta 496: 329–338, 1977.

SAEZ, J. N., EVAIN, D., AND GALLET, D.: Role of cyclic AMP and protein kinase on the steroidogenic action of ACTH, prostaglandin E, and dibutyrl cyclic AMP in normal adrenal cells and adrenal tumor cells from human. J. Cyclic Nucleotide Res. 4: 311–321, 1978.

SARUTA, T., COOK, R., AND KAPLAN, N. M.: Adrenocortical steroidogenesis. Studies on the mechanism of action of angiotensin and electrolytes. J. Clin. Invest. 51: 2239–2245, 1972.

SCHIERINGER, R. J., BRALEY, L. M., MENACHERY, A., AND WILLIAMS, G. H.: Calcium a 'third messenger' of cAMP stimulated adrenal steroid secretion. Am. J. Physiol. 248: E89–E94, 1985.

SCHIERINGER, R. J., BRALEY, L. M., MENACHERY, A., AND WILLIAMS, G. H.: Unique calcium dependency of the activating mechanism of the early and late aldosterone biosynthetic pathways in the rat. J. Endocrinol. 110: 315–325, 1986.

SCHIFFRIN, E. L., LIS, M., GUTKOWSKA, J., AND GENEST, J.: Role of $Ca^{2+}$ in response of adrenal glomerulosa cells to angiotensin II, ACTH, $K^+$ and ouabain. Am. J. Physiol. 241: E42–E46, 1981.

SEELIG, S., AND SAYERS, G.: Isolated adrenal cells: ACTH agonists, partial agonists, antagonists; cyclic AMP and corticosterone production. Arch. Biochem. Biophys. 154: 230–239, 1973.

SHEARMAN, M. S., SEKIGUCHI, K., AND NISHIZUKA, Y.: Modulation of ion channel activity: a key function of the protein kinase C enzyme family. Pharmacol. Rev. 41: 211–237, 1989.

SHEPHERD, R. M., FRASER, R., NICHOLS, D. J., AND KENYON, C. J.: Efflux of potassium ions in angiotensin stimulated bovine adrenocortical cells. J. Endocrinol. 128: 297–304, 1991.

SHIBATA, H., AND KOJIMA, I.: Involvement of protein kinase C in angiotensin II-mediated release of 12-hydroxyeicosatetranoic acid in bovine adrenal glomerulosa cells. Endocrinol. Jpn. 38: 611–617, 1991.

SHIBATA, H., OGISHIMA, T., MITANI, F., SUZUKI, H., MURAKAMI, M., SARUTA, T., AND ISHIMURA, Y.: Regulation of aldosterone synthase cytochrome P-450 in rat adrenals by angiotensin II and potassium. Endocrinology 128: 2534–2539, 1991.

SHIMA, S., KAWASHIMA, Y., AND HIRAI, M.: Studies on cyclic nucleotides in the adrenal gland. VIII. Effects of angiotensin on adenosine 3′,5′-monophosphate and steroidogenesis in the adrenal cortex. Endocrinology 103: 1361–1367, 1978.

SHIMA, S., KAWASHIMA, Y., AND HIRAI, M.: Study on cyclic nucleotides in the adrenal gland. IX. Effects of ACTH on cyclic AMP and steroid production by the zona fasciculata/reticularis of the adrenal cortex. Acta Endocrinol. 90: 139–146, 1979.

SIMPSON, S. A., TAIT, J. F., AND BUSH, I. E.: Secretion of a salt-retaining hormone by the mammalian adrenal cortex. Lancet 2: 226–227, 1952.

SMRCKA, A. V., HEPLER, J. R., BROWN, K. O., AND STERNWEIS, A. C.: Regulation of polyphosphoinositide-specific phospholipase C activity by purified Gq. Science (Wash. DC) 251: 804–807, 1991.

SPAT, A., BALLA, I., BALLA, T., CRAGOE, E. J., HAJNOCZKY, G., AND HUNYADY, L.: Angiotensin II and potassium activates different calcium entry mechanisms in rat adrenal glomerulosa cells. J. Endocrinol. 122: 361–370, 1989.

SPEDDING, M., AND PAOLETTI, R.: Classification of calcium channels and the sites of action of drugs modifying channel function. Pharmacol. Rev. 44: 363–376, 1992.

SPIEGEL, A. M.: Signal transduction by guanine nucleotide binding proteins. Mol. Cell. Endocrinol. 49: 1–16, 1987.

STEPHENS, L. R., HUGHES, K. T., AND IRVINE, R. F. F.: Pathway of phosphatidylinositol (3,4,5)-trisphosphate synthesis in activated neutrophils. Nature (Lond.) 351: 33–39, 1991.

SUTHERLAND, E. W., AND RALL, T. W.: Fractionation and characterization of a cyclic adenosine ribonucleotide formed by tissue particles. J. Biol. Chem. 232: 1077–1091, 1958.

TAKAGI, M., TAKAGI, M., FRANCO-SAENZ, R., MULROW, P. J., AND REIMAN, E. M.: Effects of a dibutyrl adenosine 3′,5′-monophosphate, angiotensin II, and atrial natriuretic factor on phosphorylation of a 17,600-dalton protein in adrenal glomerulosa cells. Endocrinology 125: 2419–2423, 1988a.

TAKAGI, M., TAKAGI, M., FRANCO-SAENZ, R., SHIER, D., AND MULROW, P. J.: Effect of atrial natriuretic factor in calcium fluxes in adrenal glomerulosa cells. Hypertension 11: 433–439, 1988b.

TAKANO, K., ADSANO, S., AND YAMASHITA, N.: Activation of G protein-coupled $K^+$ channels by dopamine in human GH-producing cells. Am. J. Physiol. 256: E318–E325, 1994.

TAKAYANANGI, R., INAGAMI, T., SNAJDAR, R. M., IMADA, T., TAMURA, M., AND MISONO, K. S.: Two distinct forms of receptors for atrial natriuretic factor in bovine adrenocortical cells. J. Biol. Chem. 262: 12104–12113, 1987.

TAKEMURA, H., HUGHES, A. R., THASTRUP, O., AND PUTNEY, J. W.: Activation of calcium entry by the tumor promoter thapsigargin in parotid acinar cells. Evidence that an intracellular calcium pool, and not an inositol phosphate, regulates calcium fluxes at the plasma membrane. J. Biol. Chem. 264: 12666–12671, 1989.

THASTRUP, O., CULLEN, P. J., DROBAK, B. K., HANLEY, M-R. AND DAWSON, A.: Thapsigargin, a tumor promoter, discharges intracellular $Ca^{2+}$ stores by specific inhibition of the endoplasmic reticulum Ca-ATPase. Proc. Natl. Acad. Sci. USA 87: 2466–2470, 1990.

TREMBLAY, J., GERZER, R., PANG, S. C., CANTIN, M., GENEST, J., AND HAMET, P.: ANF stimulation of detergent-dispersed particulate guanylate cyclase from bovine adrenal cortex. FEBS Lett. 194: 210–214, 1986.

TREMBLAY, E., PAYET, M. D., AND GALLO-PAYET, N.: Effects of ACTH and angiotensin II on cytosolic calcium cultured adrenal glomerulosa cells. Role of cAMP production in the ACTH effect. Cell Calcium 12: 655–673, 1991.

TSIEN, R. Y., POZZAN, T., AND RINK, T. J.: Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored with a new, intracellularly trapped fluorescent indicator. J. Cell Biol. 94: 325–334, 1982.

UNDERWOOD, R. H., BRADWIN, G., AND CONLIN, P. R.: Diacylglycerol molecular species in adrenal glomerulosa cells: origins and effect of stimulation with angiotensin II (abstract). Program of the 75th Endocrine Society Annual Meeting, Las Vegas, 1993. Abstract no. 773.

UNDERWOOD, R. H., GREELY, R., GLENNON, E. T., MENACHERY, A. I., BRALEY, L. M., AND WILLIAMS, G. H.: Mass determination of polyphosphoinositides and inositol trisphosphate in rat adrenal glomerulosa cells with a microspectrophotometric method. Endocrinology 123: 211–219, 1988.

VAN DER BENT, V., DEMOLE, C., JOHNSON, E. I. M., ROSSIER, M. F., PYTHON, C. P., VALLOTTON, M. B., AND CAPPONI, A. M.: Angiotensin-II induces changes in the cytosolic sodium concentration in bovine adrenal glomerulosa cells: involvement in the activation of aldosterone biosynthesis. Endocrinology 133: 1213–1220, 1993.

VIARD, I., RAINEY, W. E., CAPPONI, A. M., AND SAEZ, J. M.: Ovine adrenal fasciculata cells contain angiotensin-II receptors coupled to intracellular effectors but are resistant to the steroidogenic effects of this hormone. Endocrinology 127: 2071–2078, 1990.

VILLA, A., PODINI, P., CLEGG, D. O., POZZAN, T., AND MELDOLESI, J.: Intracellular $Ca^{2+}$ stores in chicken purkinje neurons: differential distribution of the low affinity-high capacity $Ca^{2+}$ binding protein, calsequestrin, of $Ca^{2+}$ ATPase and of the ER lumenal protein, Bip. J. Cell Biol. 113: 779–791, 1991.

VILGRAIN, I., COCHET, C., AND CHAMBAZ, E. M.: Hormonal regulation of a calcium-activated, phospholipid-dependent protein kinase in bovine adrenal cortex. J. Biol. Chem. 259: 3403–3406, 1984.

VIVAUDOU, M. B., CLAPP, L. H., WALSH, J. V., AND SINGER, J. J.: Regulation of $Ca^{2+}$ current in smooth muscle cells by diacyglycerol and acetylcholine. FASEB J. 2: 2497–2504, 1988.

VOLPE, P., KRAUSE, K-L., HASHIMOTO, S., ZORZATO, F., POZZAN, T., MELDOLESI, J., AND LEW, D. L.: "Calciosome," a cytoplasmic organelle: the inositol 1,4,5-trisphosphate-sensitive $Ca^{2+}$ store of nonmuscle cells? Proc. Natl. Acad. Sci. USA 85: 1091–1095, 1988.

WAKELAM, M. J. O., MURPHY, G. J., HRUBY, V. J., AND HOUSLEY, M. D.: Activation of two signal-transduction systems in hepatocytes by glucagon. Nature (Lond.) 323: 68–71, 1986.

WALDMAN, S. A., AND MURAD, F.: Cyclic GMP synthesis and function. Pharmacol. Rev. 39: 163–196, 1987.

WALKER, S. W., STRACHAN, M. W. J., NICOL, M., WILLIAMS, B. C., AND BIRD, I. M.: Dose-dependent effects of angiotensin II, acetylcholine and vasopressin on the cytosolic concentration of $Ca^{2+}$ in suspension of primary cultures of zona fasciculata/reticularis cells from bovine adrenal cortex. J. Mol. Endocrinol. 6: 197–208, 1991.

WATERMAN, M. R., AND SIMPSON, E. R.: Regulation of steroid hydroxylase gene expression is multifactorial in nature. Recent Prog. Horm. Res. 45: 533–563, 1989.

WHITEBREAD, S., MELE, M., KAMBER, B., AND DE GASPARO, M.: Preliminary biochemical characterization of two angiotensin II receptor subtypes. Biochem. Biophys. Res. Commun. 163: 284–291, 1989.

WHITLEY, G. S. J., BELL, J. B. G., CHU, F. W., TAIT, J. F., AND TAIT, S. A. S.: The effects of ACTH, serotonin, $K^+$ and angiotensin analogues on $^{32}P$ incorporation into phospholipids of the rat adrenal cortex: basis for an assay method using zona glomerulosa cells. Proc. R. Soc. Lond. (Biol.) 222: 273–294, 1984.

WIDEMAIER, E. P., AND HALL, P. F.: Protein kinase C in adrenal cells possible role in regulation of steroid synthesis. Mol. Cell. Endocrinol. 45: 381–388, 1985.

WILLIAMS, B. C., MCDOUGALL, J. G., TAIT, J. F., AND TAIT, S. A. S.: Calcium efflux and steroid output from superfused rat adrenal cells: effects of potassium, adrenocorticotropin hormone, 5-hydroxytryptamine, adenosine 3′,5′-cyclic monophosphate and angiotensin II and III. Clin. Sci. (Lond.) 61: 541–551, 1981.

WILSON, J. K., AGUILERA, G., AND CATT, K. J.: Inhibitory actions of calmodulin antagonists on steroidogenesis in zona glomerulosa cells. Endocrinology 115: 1357–1363, 1984.

WONG, M., O'BRIEN, D., AND SCHIMMER, B. P.: The roles of cAMP and cAMP-dependent protein kinase in the regulation of adrenocortical function: analysis using DNA-mediated gene transfer. Biochem. Cell Biol. 64: 1066–1071, 1986.

WOODCOCK, E. A.: Adrenocorticotropic hormone inhibits angiotensin II-stimulated inositol phosphate accumulation in rat adrenal glomerulosa cells. Mol. Cell. Endocrinol. **63**: 247–253, 1989.

WOODCOCK, E. A., JOHNSTON, C. I.: Inhibition of adenylate cyclase in rat adrenal glomerulosa cells by angiotensin II. Endocrinology **115**: 337–341, 1984.

WOODCOCK, E. A., AND MCLEOD, J. F.: Adenylate cyclase inhibition is not involved in the adrenal steroidogenic response to angiotensin II. Endocrinology **119**: 1697–1702, 1986.

WOODCOCK, E. A., SMITH, A. I., AND WHITE, C. B. S.: Angiotensin II-stimulated phosphatidylinositol turnover in rat adrenal glomerulosa cells has a complex dependence on calcium. Endocrinolgy **122**: 1053–1059, 1988.

WORLEY, P. F., BARABAN, J. M., SUPATTAPONE, S., WILSON, V. S., AND SNYDER, S. H.: Characterization of inositol trisphosphate receptor binding in brain. Regulation by pH and calcium. J. Biol. Chem. **262**: 12132–12136, 1987.

YANAGIBASHI, K.: Calcium as "second messenger" in corticoidogenic action of ACTH. Endocrinol. Jpn. **26**: 227–232, 1979.

YANAGIBASHI, K., KAWAMURA, M., AND HALL. P. F.: Voltage-dependent $Ca^{2+}$ channels are involved in regulating steroid synthesis by bovine not rat fasciculata cells. Endocrinology **126**: 311–318, 1990.

YANAGIBASHI, K., OHNO, Y., KAWAMURA, M., AND HALL, P. F.: The regulation of intracellular transport of cholesterol in bovine adrenal cells: purification of a novel protein. Endocrinology **123**: 2075–2082, 1988.

YOSHIDA, A., NISHIKAWA, T., TAMURA, Y., AND YOSHIDA, S.: ACTH-induced inhibition of the action of angiotensin II in bovine zona glomerulosa cells. J. Biol. Chem. **266**: 4288–4294, 1991.