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COMMENTARY

MOLECULAR ACTIONS OF ANGIOTENSIN

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Between 1954 and 1957 angiotensin II was isolated, the amino acid sequence was determined, and the chemical synthesis of this peptide hormone was achieved. This octapeptide has been a subject of great interest, and the list of biochemical, physiological, pathological, and pharmacological actions is extensive and still growing. Angiotensin II acts directly on smooth and cardiac muscle to induce contraction and is recognized as an important factor in the regulation of peripheral vascular resistance. The peptide stimulates centers in the central nervous system which initiate an increase in sympathetic nervous system activity. Increased thirst, salt appetite, and release of antidiuretic hormone also represent central nervous system responses to angiotensin. Autonomic ganglia, the adrenal medulla and peripheral adrenergic neurons have been shown to respond to angiotensin. Electroneutral or electrogenic sodium transport is enhanced by this peptide in a variety of epithelial tissues. Angiotensin is a dominant factor in the control of aldosterone biosynthesis and secretion by the adrenal cortex. The hormone induces protein synthesis in the adrenal zona glomerulosa, heart and liver. Angiotensin stimulates phospholipases, tyrosine hydroxylase, and cyclic AMP *independent* protein kinase(s) [1]. Much is known about the molecular actions of angiotensin in certain target tissues and these will be considered in further detail.

In cultured chromaffin, cardiac, and smooth muscle cells, angiotensin has been shown to induce a slow rising action potential (slow response), which precedes or is coincident with secretion or contraction [2]. The slow inward current, which is generated in response to the peptide in the myocardium and adrenal, is carried by Ca^{2+} (Fig. 1). Removal of Ca^{2+} from the extracellular fluid or blockade of the slow channels with Mn^{2+}, La^{3+} or verapamil inhibits these contractile/secretory responses to the hormone. However, certain smooth muscle preparations maintain large intracellular Ca^{2+} stores and, in these tissues, prevention of Ca^{2+} influx only attenuates contraction in response to angiotensin. Thus, in secretion- or contraction-coupling, angiotensin stimulates the entry of Ca^{2+} into cells and translocates Ca^{2+} from intracellular organelles. The peptide-induced electrophysiological response in some types of smooth muscle may be mediated by an influx of Na^+ and/or Ca^{2+} via slow ion membrane channels.

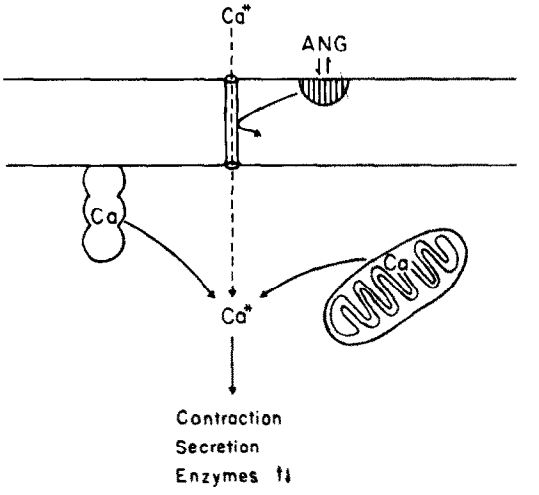

Fig. 1. Stimulation of calcium influx and translocation from cellular organelles by angiotensin. The increase in the intracellular concentration of ionic Ca^{2+} mediates a variety of tissue responses to the peptide.

Other Ca^{2+}-dependent responses to this hormone include facilitation of norepinephrine release from peripheral adrenergic neurons and the release of ADH from the posterior pituitary.

Additional, angiotensin-mediated physiological responses have been shown to require Ca^{2+}. Aldosterone biosynthesis and release by the adrenal cortex induced by angiotensin are blocked by incubation in buffer with Ca^{2+} deleted or by pretreatment with La^{3+} or verapamil. It has been suggested that the inhibition of renin release from the juxtaglomerular cells of the kidney is a Ca^{2+}-mediated response to the peptide. Finally, angiotensin-induced activation of protein kinase(s), tyrosine hydroxylase and phospholipase(s) may represent specific Ca^{2+}-dependent responses.

In liver and in some types of smooth muscle, angiotensin promotes the translocation of calcium from intracellular organelles. Recent studies in hepatocytes have demonstrated clearly that the Ca^{2+} translocated by angiotensin is mitochondrial in origin (Fig. 1) and, in the presence of low extracellular Ca^{2+} (10^{-5}M), a prominent Ca^{2+} efflux from the liver cell can be demonstrated. Release of mitochondrial Ca^{2+} appears to be dependent on the presence of some critical extracellular Ca^{2+} concentration or labile cellular Ca^{2+} pool since Ca^{2+} efflux and enzymatic responses are not induced when the divalent ion is deleted and chelator (EGTA)* is added. These

*Abbreviations: EGTA, ethyleneglycolbes (mono-ethyl-ethene) tetra-acetate; PG, prostaglandin; SDS, sodium dodecylsulfate; and DNA, deoxyrubonucleic acid.

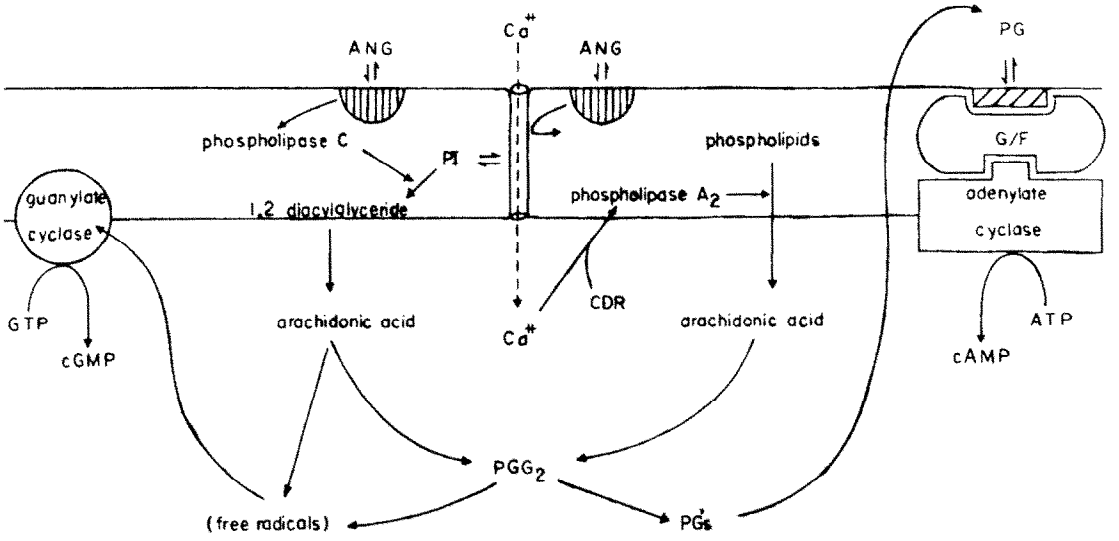

Fig. 2. Angiotensin-induced activation of phospholipases and release of arachidonic acid from membrane phospholipids (PI = phosphatidylinositol). Stimulation of PI turnover has been postulated as an important event in the gating of membrane slow ion channels. Arachidonate and endoperoxides (PGG_2) provide a free radical environment which activates guanylate cyclase and the accumulation of cyclic GMP by the cell. Prostaglandins synthesized from arachidonate can stimulate adenylate cyclase and increase the production of cyclic AMP.

data are consistent with the suggestion that a Ca^{2+} influx (trigger) may be required for the induction of Ca^{2+} translocation from intracellular organelles. The precise intracellular Ca^{2+} pool with which angiotensin interacts in smooth muscle has not been established.

Angiotensin has been shown to stimulate phospholipase C activity which results in an increase in the turnover of phosphotidylinositol in hepatocytes [3]. The stimulation of phosphatidylinositol degradation may be involved in modification of membrane slow ion channels which mediate the angiotensin-induced increase in Ca^{2+} permeability (Fig. 2). Altered membrane phospholipid composition subsequent to phosphatidylinositol turnover has been postulated as a mechanism for recruiting Ca^{2+} channels or for the gating of these channels. Since Ca^{2+}-dependent responses to angiotensin are evident in so many end organs, it is possible that altered membrane phospholipid composition may lead to the opening of additional slow channels by the hormone or that available ion channels remain open for a longer period of time. Stimulation of phosphatidylinositol degradation via phospholipase C activation by angiotensin also results in the subsequent release of arachidonic acid from 1,2-diacylglyceride. This is particularly interesting since the peptide is a well-known stimulus of prostaglandin synthesis in numerous tissues.

In addition to stimulation of phospholipase C, angiotensin may activate phospholipase A_2 (an enzyme which may be regulated by Ca–calmodulin) to release arachidonic acid (Fig. 2). However, the effects of Ca–calmodulin inhibitors such as chlorpromazine or trifluperazine on angiotensin-induced phospholipase activity are not known. In fact, at the present time, no angiotensin–calmodulin-dependent responses have been demonstrated convincingly in any tissue. After release of arachidonic acid from membrane phospholipids via activation of phospholipase (either C or A_2), the fatty acid is converted by the cyclooxygenase to PGG_2. This prostaglandin endoperoxide (PGG_2) and arachidonic acid create a free radical environment which is autocatalytic for the cyclooxygenase. In tissues in which the peptide is known to stimulate the release of prostaglandins or prostacyclin (i.e endothelial and smooth muscle cells), angiotensin has been reported to increase cellular cyclic GMP levels [4, 5]. Guanylate cyclase also is activated by free radicals. Thus, it is likely that angiotensin-induced changes in tissue cyclic GMP concentrations are mediated via the free radical environment provided for guanylate cyclase by arachidonic acid release and the subsequent conversion of the fatty acid to endoperoxides in the prostaglandin biosynthetic pathways (Fig. 2). The increase in phospholipase(s) activity produced by angiotensin initiates the formation and release of prostaglandins and prostacyclin, and these autacoids are well-known stimuli of adenylate cyclase (Fig. 2). We have shown recently that pretreatment with a cyclooxygenase inhibitor, indomethacin, blocks angiotensin-induced cyclic AMP accumulation by rat jejunum. These data and comparable observations with cultured endothelial cells suggest that activation of adenylate cyclase following administration of angiotensin is mediated by metabolites of arachidonic acid. The prostaglandin may be released from one cell type and act on another or act directly on adenylate cyclase in the cell in which angiotensin stimulated phospholipase(s). In any tissue in which the peptide causes an increase in cyclic AMP levels, prostaglandin-mediated stimulation of adenylate cyclase represents a clear alternative to any direct effect of angiotensin on the cyclase. However, the measurement of cyclic AMP or cyclic GMP concentrations may represent a sensitive monitor of phospholipase activation by angiotensin.

Radioligand studies have identified binding sites for angiotensin in adrenal cortex, adrenal medulla, aorta, bladder, brain, liver, mesenteric artery, myocardium, renal glomeruli and tubules, and uterus. Usually, the receptor is quite sensitive to limited proteolysis with trypsin. In adrenal, kidney tubules, liver and myocardium, at least two binding sites are demonstrable (a high affinity site with a $K_d \leq 1$ nM and a low affinity binding site with a K_d of ≥ 10 nM). Other tissues have a single class of high affinity sites with K_d values between 0.1 and 1.0 nM. Interaction of a guanyl nucleotide binding protein (G/F) with a high affinity receptor appears to convert the site to a low affinity site for peptide binding. In the early 1970's, guanyl nucleotides were found to affect the binding of radiolabeled ligands to plasma membranes. By the mid 1970's, the action of GTP on hormone–receptor binding had been extended to include angiotensin binding with adrenal cortical membranes. These early observations correlated the GTP effect with agents which activated adenylate cyclase. GTP exerts a dramatic effect on the dissociation rates for radioligands which are agonists but the nucleotide has little effect on the binding kinetics of antagonists. GTP interacts with a specific plasma membrane protein which is a regulatory macromolecule for the catalytic activity of adenylate cyclase [6]. Activation of adenylate cyclase by F^- and cholera toxin is mediated by the guanyl nucleotide binding protein (G/F). Recent studies have shown, however, that the binding of several agonists which do not increase adenylate cyclase activity was affected by GTP. These agonists, α_2-adrenoceptor agonists, muscarinic agonists, adenosine, and the enkephalins, inhibit the stimulation of adenylate cyclase by glucagon, histamine, isoproterenol and prostaglandins. It is not clear whether there is a single GTP binding protein or multiple proteins which can activate as well as inhibit the stimulation of adenylate cyclase. Additional studies have shown a GTP effect on angiotensin binding to plasma membranes of adrenal, liver, and vascular smooth muscle. In liver membranes, the marked GTP effect on binding is agonist specific since little GTP-induced alteration of dissociation rates for the antagonist, [^{125}I]-saralasin, was observed. In contrast, saralasin is a full agonist (stimulates Na^+ transport) in renal tubules and jejunum of rats. In preliminary studies with plasma membranes from renal tubules, the dissociation rates for [^{125}I]-saralasin and angiotensin were equally increased by GTP.

In addition to the prominent role of Ca^{2+} as a mediator of angiotensin responses, the interaction of the peptide with plasma membrane receptors is dependent on the ionic environment. In membrane preparations from vascular smooth muscle, the binding of the peptide is markedly dependent on the Ca^{2+} concentration. The peptide receptor in liver plasma membranes shows a similar requirement for divalent ions, and Ca^{2+}, Mg^{2+}, and Mn^{2+} cause a dramatic increase in maximum saturable binding [7]. Angiotensin receptors in the adrenal zona glomerulosa, myocardium, uterus, and brain also have specific ionic requirements for hormone binding. The unusual divalent ion order of Ca^{2+}, Mg^{2+} and Mn^{2+} that promotes binding of peptide to receptors in some target tissues is similar to the ionic requirements for G/F and β-adrenoceptor binding. Consequently, one can speculate that the Mg^{2+}/Mn^{2+} requisites for the binding of angiotensin may reflect the ionic requirements for the interaction of G/F with the peptide receptor. Other ionic requirements for binding may indicate the presence of different angiotensin receptors or dissimilar mechanisms of action for the hormone. In contrast, the binding of the antagonist, [^{125}I]-saralasin, to liver plasma membranes is not stimulated by the divalent cationic composition of the media used for the radioligand study. No partial agonists such as [Phe^4, Tyr^8] angiotensin II have been studied for ionic or GTP interactions and binding.

Few studies with this peptide and intact cells have been pursued along the lines defining the ionic requirements for receptor binding. Isolated cells have been used to supply a response to angiotensin for comparison with data obtained from binding studies. Experimental conditions have been established on the basis of the biological response. Angiotensin responses in some intact tissues are known to be modified by the addition of various cations. It is possible that the change in tissue responses to the hormone is caused by an alteration in angiotensin–receptor kinetics which is induced by changes in the concentration of a specific ion rather than reflecting a role for that specific ion as a mediator of end organ responses.

Angiotensin receptors are present early in fetal development, and the concentration of sites increases with maturation. In cells from the embryonic chick heart, a receptor for the peptide is evident as early as day 4 and is coupled to the slow ion channel and mechanical activity. The initial cardiocyte receptor is a low affinity receptor and does not discriminate between angiotensin I and II for either binding or positive inotropic response. At about day 17 *in ovum*, the embryonic chick ventricle develops high affinity receptors which are coupled and recognizes angiotensin II from I; however, the low affinity sites remain. The precise ontogeny of angiotensin receptor specificity and coupling for response is, however, unknown for other tissues and/or species. Direct responses to angiotensin I have been reported in the nervous system, smooth muscle, and kidney and, therefore, could be mediated by the low affinity receptor.

An ^{125}I-labeled, photoaffinity angiotensin analog has been synthesized and used to covalently trace label peptide receptors in membranes of adrenal cortex and uterus [8]. The plasma membrane fraction was solubilized with Triton X-100, and ^{125}I-labeled receptors were isolated by gel filtration and density gradient centrifugation. Two labeled macromolecules with molecular weights of 126,000 and 65,000 were isolated from each tissue. SDS-gel electrophoresis of the proteins yielded a single peak with a molecular weight between 60,000 and 70,000. These data suggest that angiotensin receptors in the adrenal cortex and uterus are similar and imply that the receptor may be a dimer composed of subunits with comparable physical behavior and size.

The modulation by GTP of the high affinity receptor for angiotensin in membranes (adrenal, liver,

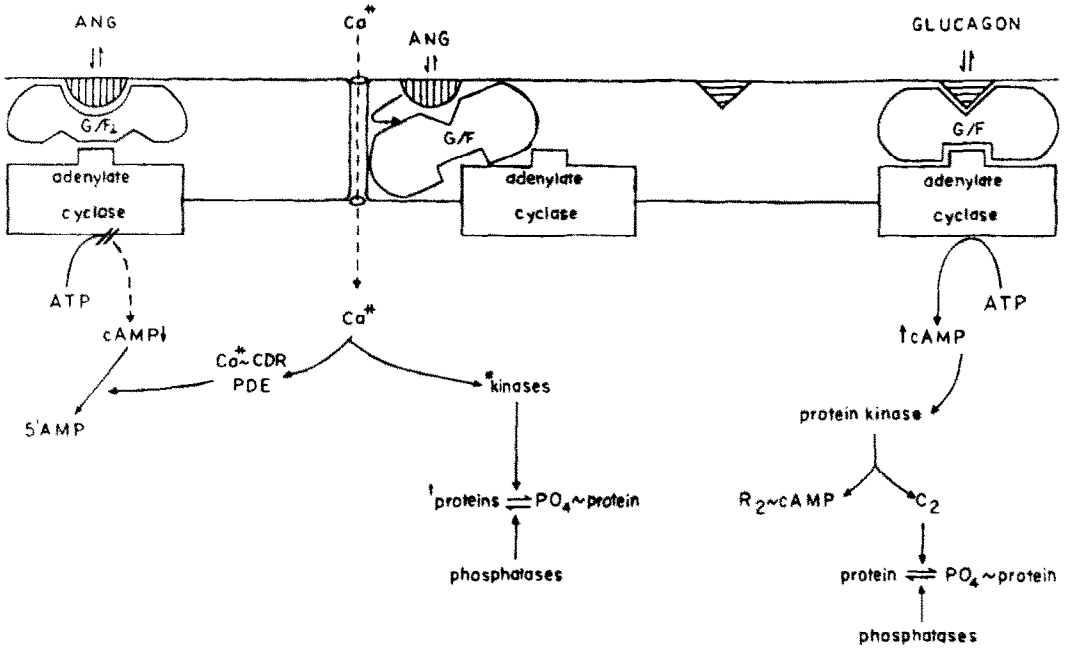

Fig. 3. Model of the effects of angiotensin based on studies in the rat hepatocyte. Angiotensin activates kinases (phosphorylase kinase, C kinase) which catalyze the phosphorylation of proteins (glycogen phosphorylase, glycogen synthase, pyruvate kinase) to modulate enzymatic activity. These kinases appear to be Ca^{2+} sensitive and *independent* of cyclic AMP. In smooth muscle, the myosin light chain kinase would be a likely candidate for stimulation by angiotensin. The binding of angiotensin to liver plasma membrane is dramatically altered by GTP, indicative of a receptor ~ guanyl nucleotide binding protein (G/F) interaction. Glucagon stimulation of adenylate cyclase is blocked by angiotensin which suggests either that G/F cannot associate with cyclase when bound to the angiotensin receptor or that cyclase is associated with an inhibitor G/F_i. Angiotensin-induced blockade of the cyclic AMP response to glucagon is not prevented by inhibitors of cyclic nucleotide phosphodiesterase (PDE).

artery) suggests that this receptor may be coupled with adenylate cyclase (Fig. 3). Although angiotensin does not stimulate adenylate cyclase in these tissues, the peptide could decrease cyclase activity or inhibit the enzymatic responses to known stimuli. As discussed previously, receptor–G/F interactions occur with agonists that stimulate adenylate cyclase or agonists that inhibit activation of the enzyme. In the hepatocyte, angiotensin attenuates the accumulation of cyclic AMP induced by glucagon and [Sar^1, Ile^8]Arg reverses the inhibitory effect of angiotensin on the glucagon response. Since angiotensin stimulates the entry of Ca^{2+} into the hepatocyte, any decrease in cyclic AMP levels might be due to Ca–calmodulin activation of phosphodiesterase. However, treatment with phosphodiesterase inhibitors (RO1 20-17243 and methylisobutylaxanthine) does not prevent angiotensin-induced blockade of glucagon-stimulated cyclic AMP concentrations.

The inhibition of adenylate cyclase and the stimulation of phospholipases by angiotensin are responses which can be monitored in isolated membranes. Any membrane response would assist in the characterization of binding sites as putative receptors which mediate responses in intact tissue. In liver, the GTP effect is on the high affinity angiotensin receptor and it is possible that the high affinity site mediates the modulation of adenylate cyclase activity. However, low affinity sites are present in many tissues including liver and, although largely ignored, low affinity receptors are coupled in at least two tissues—kidney tubules (^{22}Na flux) and embryonic chick heart (contraction). Perhaps both high and low affinity receptors mediate specific end organ responses. Also, if angiotensin modulates adenylate cyclase activation, the peptide may attenuate the stimulation of cyclase induced by prostaglandins.

In adrenal cortical plasma membranes, it is unknown whether the GTP action on binding of angiotensin is agonist specific or whether angiotensin can also attenuate ACTH-induced stimulation of adrenal adenylate cyclase. In fact, the adrenal cortex is one of the few tissues in which several investigators have reported that angiotensin increases cyclic AMP levels. The effects of angiotensin on adrenal adenylate cyclase activity, ACTH responses, and phosphodiesterase activity will require additional study, but, based on the studies of angiotensin–cyclase interactions in liver, certainly are worthy of pursuit.

In cardiac tissue, angiotensin does not appear to alter basal cyclase activity or the stimulation of cyclic AMP accumulation induced by isoproterenol. The effects of GTP on the binding of angiotensin to cardiac plasma membranes have not been determined. It is of interest that cholinergic, muscarinic agonists do show a GTP-dependent interaction with cardiac membranes and that acetylcholine blocks the adenylate cyclase and positive inotropic response to isoproterenol in ventricle. Furthermore, it recently has been reported that adenosine attenuates

histamine- and isoproterenol-induced cyclase activity and cardiac contractility. The positive inotropic action of angiotensin in cardiac muscle is additive with β-adrenergic agonists and is not inhibited by acetylcholine. These observations provide additional evidence that the effects of the peptide on the heart are independent of cyclic AMP accumulation. Since GTP modulates the binding of angiotensin to the receptor in arterial membranes, studies on the interactions of angiotensin with adenylate cyclase in vascular tissues are warranted.

In 1971, two groups of investigators presented evidence of the entrance of angiotensin into cells. [^{3}H]Angiotensin was administered into the left ventricle of rats and the myocardium was fixed immediately with glutaraldehyde. Autoradiography demonstrated perinuclear rings of radioactivity in aortic and coronary endothelial cells as well as cardiac and smooth muscle cells. The accumulation of ^{3}H by these tissues was blocked by angiotensin analogs with both agonist (angiotensin III) and antagonist ([Sar^1, Ile^8] angiotensin II) activities [9]. Other workers showed that angiotensin II coupled to macromolecules, such as horseradish peroxidase or cytochrome A, was internalized in vesicles by aortic endothelial cells. Recent studies in vascular smooth muscle also have shown rapid internalization of [^{3}H]angiotensin, and that the internalization correlated with a decrease in receptor concentration. It is attractive to speculate that tachyphylaxis to angiotensin may result from receptor internalization (Fig. 4). The dimeric form of the angiotensin receptor, which was identified using photoaffinity probes in uterine and adrenal membranes is consistent with the hypothesis that peptide-mediated receptor aggregation precedes internalization. Since internalization of [^{3}H]angiotensin is blocked by the antagonists saralasin and [Sar^1, Ile^8] angiotensin II, it is possible that competitive antagonist analogs of angiotensin do not initiate aggregation and/or internalization of receptors. It would be interesting to determine if [Sar^1, Leu^8] and [Sar^1, Cys(Me)^8] angiotensin II, analogs of the peptide which have been reported to be non-competitive antagonists in some tissues, promote receptor internalization. Alternatively, phosphotidylinoisitol turnover may be involved in tachyphylaxis to angiotensin. In the blowfly salivary gland, 5-hydroxytryptamine stimulates the turnover of phosphotidylinositol, and the gland displays desensitization to the biogenic amine. Refractoriness to 5-hydroxytryptamine is reversed by treatment of the gland with phosphatidylinositol. Since angiotensin activates phospholipase C, depletion of membrane phosphatidylinositol could occur and result in attenuation of angiotensin-induced Ca^{2+} influx. Alternatively, depletion of membrane phosphati-

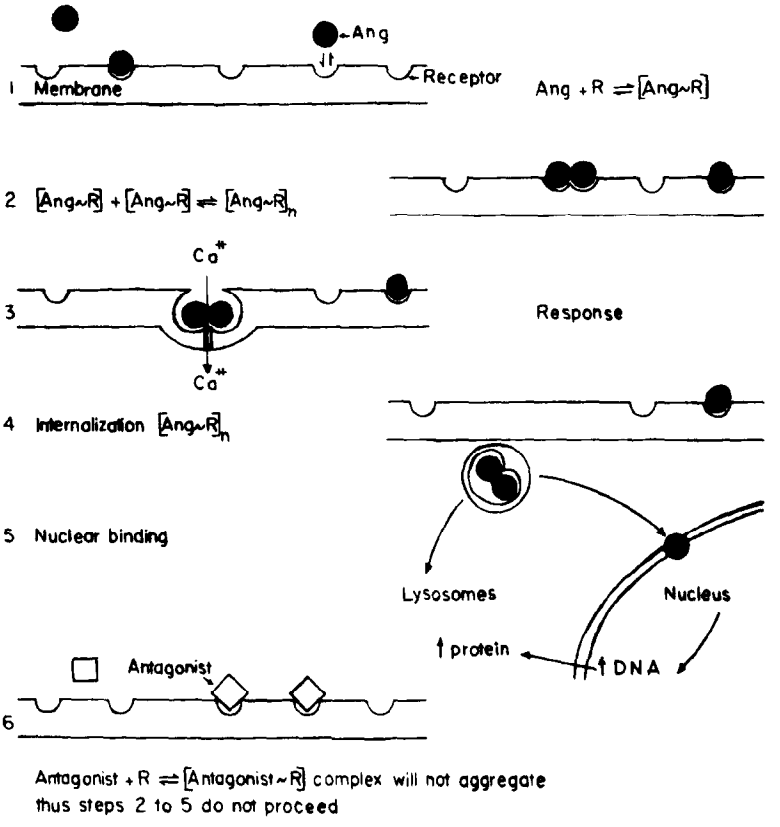

Fig. 4. Hypothetical model of angiotensin receptor binding that results in receptor aggregation and internalization of the aggregate. Steps 1 through 5 occur in sequence. Stimulation of phospholipid metabolism may reflect part of the internalization process and both events offer explanations of angiotensin tachyphylaxis.

dylinositol may accelerate receptor aggregation and/or internalization resulting in a decrease in angiotensin receptor concentration.

The intracellular localization of labeled angiotensin prompted a search for some nuclear response to the peptide. The first nuclear response that was demonstrated clearly was cardiac ventricular hypertrophy. More recently, hypertrophy of the adrenal zona glomerulosa has been demonstrated with angiotensin. In cultured fetal adrenal cells, angiotensin stimulates [^{3}H]thymidine incorporation into DNA, [^{3}H]leucine incorporation in protein, and cellular proliferation [10]. This hyperplastic response of cultured adrenal cells to angiotensin was blocked by [Sar^1, Ile^8] angiotensin II while the responses to other growth factors were not altered. The inhibitory effect of this analog on nuclear responses to angiotensin is consistent with the postulated blockade of receptor aggregation—internalization and perinuclear localization of angiotensin (Fig. 4). Another protein synthetic response to angiotensin occurs in liver. Renin substrate (angiotensinogen) synthesis and release are induced by angiotensin in isolated hepatocytes. In other studies, epithelial Na^+ transport and steroidogenic responses to the peptide are blocked by cycloheximide and puromycin. These observations have been the basis for a postulated role of protein synthesis in the mediation of the actions of angiotensin on Na^+ transport and steroidogenesis. However, the rapid onset and reversal of the blockade of angiotensin achieved with these inhibitors of the translational stage of protein synthesis suggest some other mechanism of action. Cycloheximide is known to block mitochondrial Ca^{2+} release (at least in liver) and consequently would inhibit intracellular Ca^{2+} translocation by angiotensin. However, the effect of cycloheximide on the hepatic responses to the peptide has not been determined.

In the early 1970's, angiotensin was reported to stimulate the activity of glycogen phosphorylase in strips of rabbit thoracic aorta. This activation of phosphorylase was dose-dependent and correlated very well with the magnitude of the contractions induced by the peptide. Later studies carried out in liver and cardiac tissue indicated that angiotensin stimulated a protein kinase. In 1975, we postulated that angiotensin activated a cyclic AMP-*independent* protein kinase(s) or inhibited phosphatase(s) which is responsible for the regulation of endogenous substrates such as glycogen phosphorylase. The hypothesis that angiotensin stimulates phosphorylase kinase was particularly attractive since this enzyme was known to be stimulated by Ca^{2+} as well as cyclic AMP-dependent protein kinase. The calcium ion certainly is an appropriate substance with which to link angiotensin to phosphorylase kinase and the mechanism responsible for the activation of glycogen phosphorylase (Fig. 3).

Angiotensin has been shown to increase the phosphorylated state of eleven or twelve cytosolic proteins in rat hepatocytes [11]. The kinase(s) or phosphatase(s) which mediates hepatic responses to angiotensin was not activated in Ca-free EGTA-treated cells. Three of the intracellular substrates that showed increased phosphorylation and altered enzymatic activities in the presence of the peptide have been identified clearly as glycogen phosphorylase, glycogen synthase and pyruvate kinase. It appears that the hepatocyte contains multiple Ca^{2+}-sensitive protein kinases and/or phosphatases which are activated or inhibited, respectively, by angiotensin. Inhibitors of Ca–calmodulin do not block these enzymatic responses to angiotensin in liver and this suggests that the peptide does not act via calmodulin-regulated enzymes. These changes in hepatic enzymatic activities may very well reflect the pathways for activation of metabolic responses necessary to meet energy demands of angiotensin responses in a variety of tissues. Insulin is known to block the modulation of hepatic enzyme activities by (glucagon) cyclic AMP-dependent protein kinase. Perhaps the Ca^{2+}-dependent protein kinases represent insulin insensitive mechanisms for regulation of these enzymes for glycogenolysis, lipolysis and gluconeogenesis.

In smooth muscle, in addition to phosphorylase kinase, numerous agents which stimulate contraction are activators of myosin light chain kinase. The phosphorylation of myosin light chain is involved in actin and myosin cross-bridge formation which mediates the mechanical event of muscle contraction. Myosin light chain kinase is thought to be stimulated by Ca–calmodulin and can be inhibited by the substituted phenothiazines (trifluperazine), compounds which are known to block Ca–calmodulin–enzyme interactions [12]. Recent studies in cultured smooth muscle cells from rat mesenteric artery have suggested that angiotensin stimulates the phosphorylation of the myosin light chain, and this response was blocked by calmodulin inhibitors. Although most contractile substances in rabbit aorta are inhibited by 50 μM trifluperazine, angiotensin-induced contractions are not affected. In contrast to the cells from rat mesenteric artery, perhaps angiotensin stimulates the phosphorylation of myosin light chain and contraction by a mechanism which is independent of calmodulin in rabbit thoracic aorta.

Assuming that cells contain cyclic GMP-dependent protein kinase and that this kinase has intracellular substrates, some of the phosphorylated membrane and cytosolic proteins obtained following treatment with angiotensin could be dependent on the activity of this enzyme. Peptide-induced prostaglandin synthesis and subsequent activation of cyclic AMP-dependent protein kinase also could influence phosphoprotein profiles. However, current data are consistent with the hypothesis that angiotensin activates Ca^{2+}-dependent protein kinase(s) or phosphatase(s). Some of these phosphoproteins induced by angiotensin could be macromolecules which are involved in the gating of Ca^{2+}, or which mediate ion transport in intestine, renal tubules and erythrocytes, or which regulate Ca^{2+} sequestration. In cardiac and smooth muscle, it has been suggested that cyclic AMP mediates Ca^{2+} uptake by intracellular organelles. The inhibition of activators of adenylate cyclase by angiotensin would retard Ca^{2+} uptake and potentiate the effects of Ca^{2+} influx and Ca^{2+} released from subcellular organelles. In spite of the fact that the peptide has not been shown to alter basal or stimulated adenylate cyclase activity in these

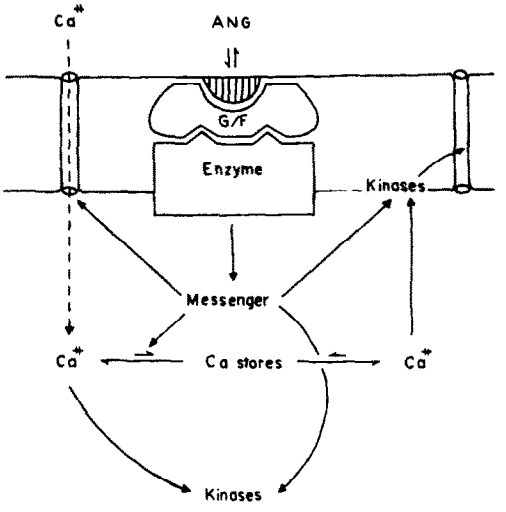

Fig. 5. Angiotensin activation of a G/F regulated unidentified membrane enzyme which results in the production of a second messenger. The messenger would mediate the intracellular response described for angiotensin (such as depicted in Figs. 2 and 3).

contractile tissues, it is attractive to suggest that a vasoconstrictor may regulate the synthesis of a vasodilator. In adrenal and liver, however, angiotensin, and hormones which act via activation of adenylate cyclase, stimulate comparable steroidogenic and metabolic responses. Therefore, it is difficult to attach physiological importance to angiotensin-induced inhibition of activators of adenylate cyclase in these tissues.

Angiotensin also could stimulate some other G/F regulated membrane enzyme to produce some as yet unknown second messenger (Fig. 5). The hypothetical messenger may mediate the peptide-induced Ca^{2+} influx or Ca^{2+} translocation and/or activation of protein kinase(s). Since little is known about guanyl nucleotide binding protein(s) other than modulation of the catalytic activity of adenylate cyclase, it seems reasonable to postulate other regulatory actions for these macromolecule(s). For instance, in the presence of GTP only low affinity receptors for angiotensin are observed in binding assays with membranes. If the high affinity receptor is coupled to gating of Ca^{2+} channels, G/F's could modulate biological responses to the peptide. One could speculate that the monomeric and dimeric forms of the angiotensin receptor have different affinities for G/F and peptide or mediate different responses. However, it is not clear which angiotensin receptors mediate what responses. Furthermore, several angiotensin peptides have biological activity (angiotensins I, II, III and [des-Asp^1, des-Arg^2] angiotensin II) and many of the actions discussed throughout this article may be shared by or may be unique to particular peptides. Certainly further comparative studies of responses to these various peptides are warranted. It must be emphasized that many of the conclusions developed in the preceding pages frequently depend heavily on data obtained from studies in a specific cell type or tissue. Care should be exercised with such generalized extrapolations since different mechanisms of action for angiotensin should be expected from tissue to tissue and be evident from species to species.

Although a great deal is known about the molecular actions of angiotensin, there are numerous effects that remain unresolved and await additional study. Much of the preceding discussion is based on data generated since 1975 and clearly reflects the enormous progress made in recent years. The next five years should be even more exciting as we continue to pursue the mechanism(s) of action of this peptide.

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