α_1-ADRENORECEPTORS AND ANGIOTENSIN RECEPTORS

Jagdish Kakadiya

Dharmaj Degree Pharmacy College, Sanskruti Suraksha Charitable Trust, Dharmaj, Tal: Petlad, Dist: Anand.
Gujarat, INDIA.

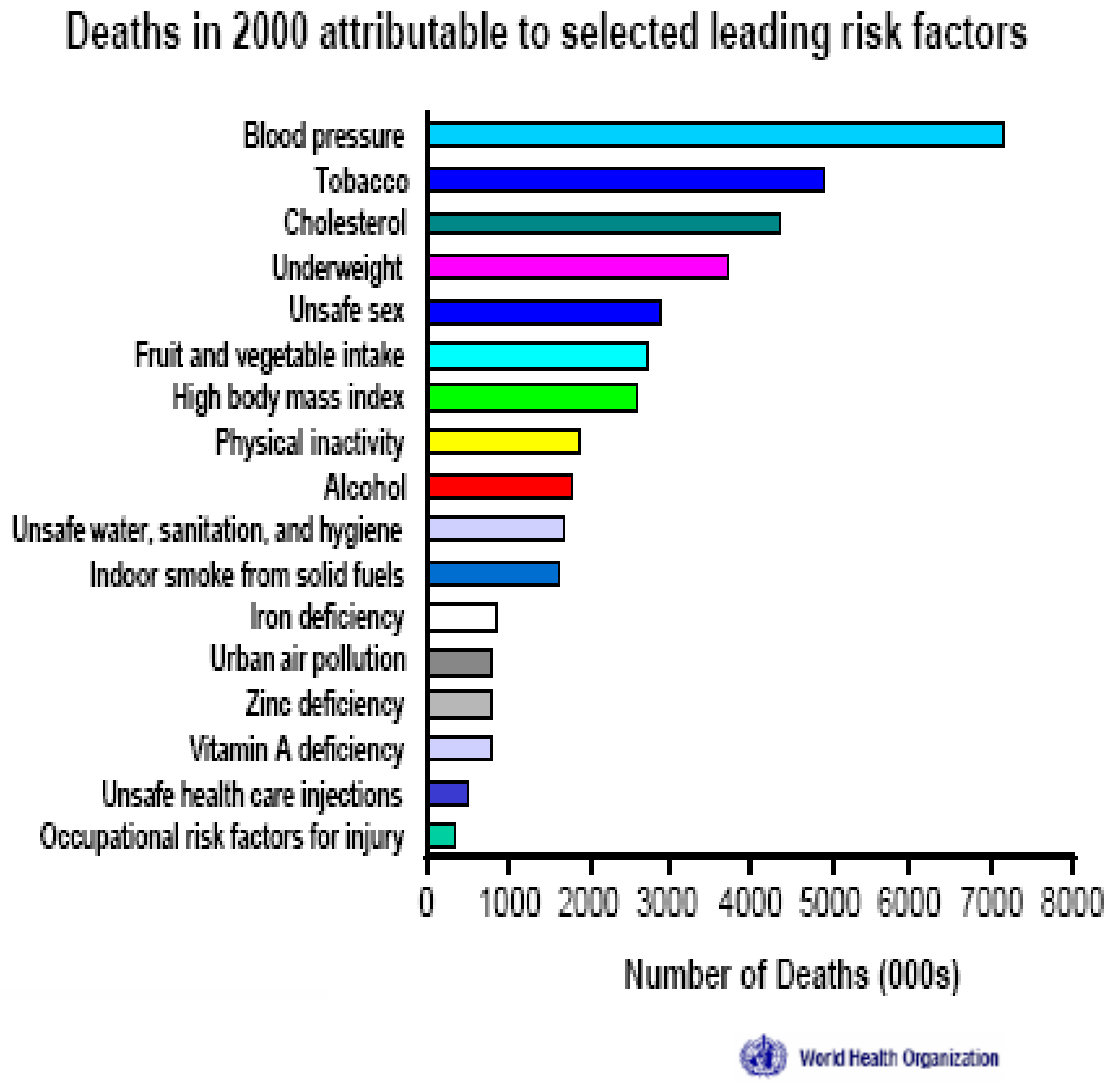
ADDRESS FOR CORRESPONDENCE

Mr. Jagdish L. Kakadiya
Dharmaj Degree Pharmacy College,
Sanskruti Suraksha Charitable Trust,
Dharmaj, Tal: Petlad,
Dist: Anand.
Gujarat, INDIA.
Phone 09825882922
jagdishkakadiya@yahoo.co.in

According to WHO Hypertension is defined as a systolic blood pressure (SBP) above 140 mmHg and/or a diastolic blood pressure (DBP) above 90 mmHg. High blood pressure (hypertension) is one of the most important preventable causes of premature death worldwide.

A survey (Figure 1) conducted by the World health organization (WHO) clearly depicts the main risk factor behind the cause of increased mortality rate is high blood pressure (Hypertension). It is almost double than any other risk factor in the list, clearly defining the importance of cardiovascular diseases.

FIGURE: 1



Joint National Committee on Prevention, Detection, Classification, Evaluation and Treatment of High Blood Pressure Classifies High blood Pressure in adults as in table:1.

TABLE: 1 Classification of Blood Pressure in Adults: (45)

Blood Pressure classification	SBP mmHg	DBP mmHg
Normal	<120	and <80
Prehypertension	120-139	or 80-89
Stage-1 Hypertension	140-159	or 90-99
Stage-2 Hypertension	>160	>100

Hypertension is associated with many risk factors, one leading to other. Concurrent occurrence of these risk factors increases morbidity rate as high as up to 700%.

TABLE 2 : Recent Indian hypertension prevalence studies (BP ≥ 140/90) (1)

Age group	Place	Sample size		Prevalence	
		Men	Women	Men	Women
20-75	Jaipur	1415	797	29.5	33.5
18-60	Mumbai	40067	59522	43.8	44.5
20-89	Trivandrum	76	130	31.0	41.2
30-60	Mumbai	1521	141	34.1	-
20-70	Chennai	518	657	14.0	-
20-75	Jaipur	550	573	36.4	37.5
20-75	Rajasthan	1982	1166	23.7	16.9
16-70	Haryana	2559	-	3.0	-

The market survey has proposed that the demand for hypertension will increase together with other cardiovascular and metabolic diseases.

ROLE OF HYPERTENSION IN TARGET ORGAN DAMAGE ; (2)

A. Cardiac

1. Left ventricular hypertrophy

2. Systolic and diastolic dysfunction
3. Congestive heart failure
4. Coronary artery disease

B. Cerebrovascular

1. strokes (acute and chronic stroke)
2. Carotid stenosis
3. Dementia

C. Renal disease

1. Acute renal disease
2. Chronic renal disease, diabetic and nondiabetic
3. Progression of renal damage

D. Other vascular diseases

1. Atherosclerotic: aneurysms, dissection embolization
2. Fibromuscular dysplasia
3. Vasospastic and inflammatory
4. Peripheral arterial disease

E. Others

1. Retinopathy
2. Sexual dysfunction

Blood pressure and Cardiovascular risk

Data from observational studies involving more than 1 million individuals have indicated that death from both IHD and stroke increases progressively and linearly from levels as low as 115 mmHg SBP and 75 mmHg DBP upward. The increased risks are present in individuals ranging from 40 to 89 years of age. For every 20 mmHg systolic or 10 mmHg diastolic increase in BP, there is a doubling of mortality from both IHD and stroke. In addition, longitudinal data obtained from the Framingham Heart Study have indicated that BP values between 130–139/85–89 mmHg are associated with a more than twofold increase in relative risk from cardiovascular disease (CVD) as compared with those with BP levels below 120/80 mmHg.

IMPORTANCE OF SYSTOLIC BLOOD PRESSURE

SBP as a major risk factor for CVDs. Changing patterns of BP occur with increasing age. The rise in SBP continues throughout life in contrast to DBP, which rises until approximately age 50, tends to level off over the next decade, and may remain the same or fall later in life. Diastolic hypertension predominates before age 50, either alone or in combination with SBP elevation. The prevalence of systolic hypertension increases with age, and above 50 years of age, systolic hypertension represents the most common form of hypertension. DBP is a more potent cardiovascular risk factor than SBP until age 50; thereafter, SBP is more important.

1.3) TREATMENT OF HYPERTENSION (overview);

From the above evidences of prevalence of hypertension in India & global, the proposed increase in the market for hypertension and the organ damage brought about by hypertension, it is clear that the importance to this cardiovascular complication is going to be one of the major challenge for man kind.

In hypertension, because there are many subcategories based on etiology (origin), risk factors, and the constitution of the individual for hypertension (elevated blood pressure), there is no single treatment. Also a single class of drug is not effective, so requires a combinational therapy. (3). One of the widely used combinations is α_1- adrenoreceptor and AT_1 receptor antagonists.

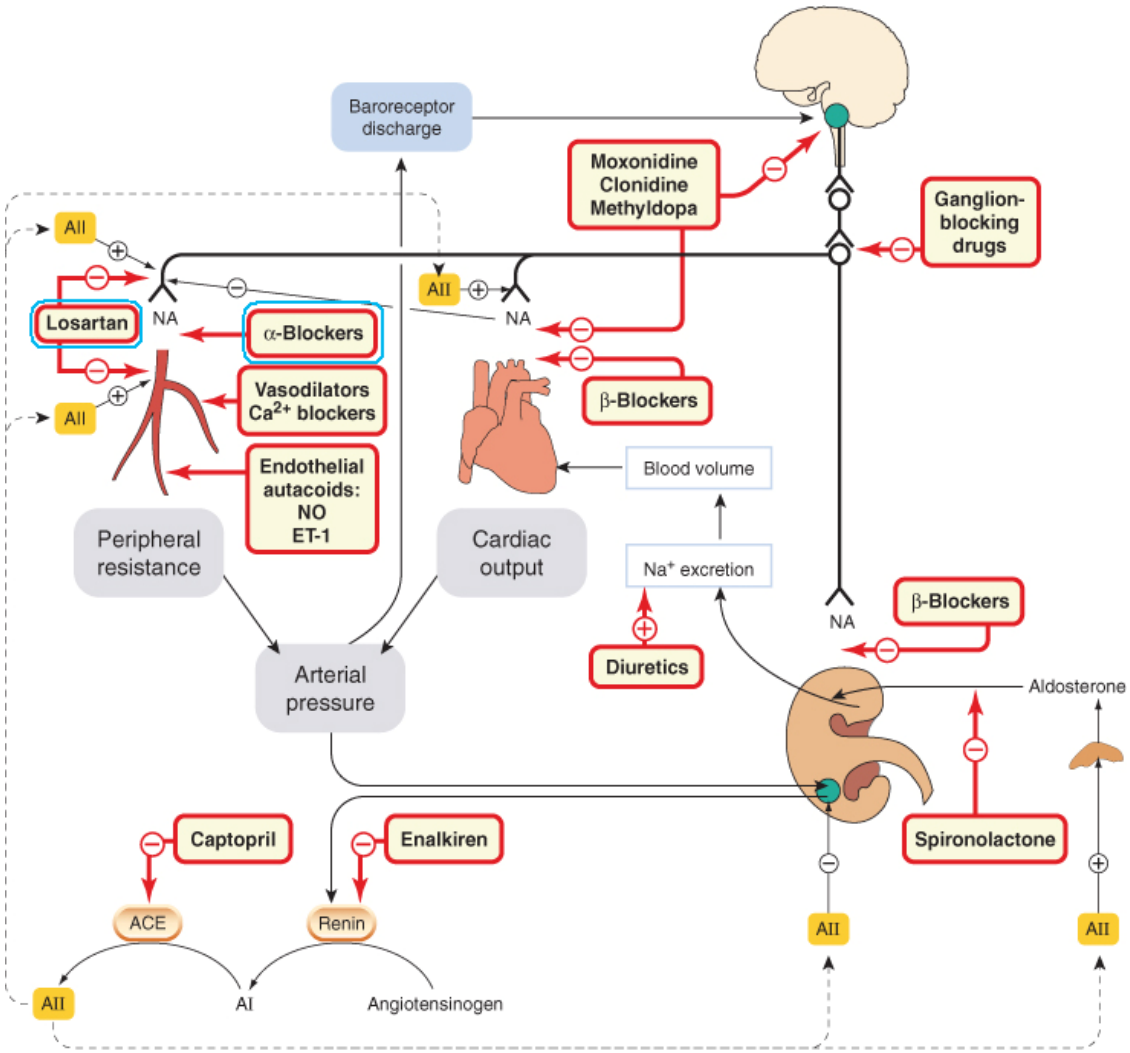
α_1-ADRENOCEPTORS:

History of Adrenergic Receptors;

It has been known for close to a century that Norepinephrine is one of many catecholamines with the ability to either elicit or inhibit smooth muscle contraction, depending on the site and concentration chosen. For instance, peripheral noradrenergic transmission will increase cardiac blood flow while reducing splanchnic, renal and hepatic blood flow. The first step leading to the discovery of the adrenoceptors was made in the cardiovascular system—the observation by Dale (1905) that the pressor effect of adrenaline was reversed by ergotoxine into a depressor effect. An explanation for this phenomenon was not apparent until 43 years later! In 1948, Ahlquist (4) performed a careful study of the responses of sympathetically innervated organs to the application of a variety of catecholamines.

He noted that although norepinephrine is a potent excitatory catecholamine, it has low inhibitory activity on smooth muscle cells (depending on the site of action) while epinephrine is equally potent as both an excitor and an inhibitor of smooth muscle. These observations led Ahlquist to divide adrenergic receptors into two classes, alpha and beta, which mediate excitatory and inhibitory responses respectively. Additionally, he suggested that these receptors have a differential affinity for the various catecholamines examined. For instance, while epinephrine would be equipotent at both alpha and beta receptors, norepinephrine would be more potent at sites where sympathetic neurotransmission (mediated through alpha receptors) is excitatory, than at sites where beta-mediated inhibitory transmission takes place.

Figure 2: Treatment of hypertension. (www.hypertensiononline.org)



The conventional treatment for the hypertension includes various drugs from the categories of Diuretics, β- Blockers, Calcium channel blockers (CCB), ACE Inhibitors, Type 1 angiotensin II receptor blockers, alpha 1 adrenoceptor blockers, Vasodilators etc. used either alone or in combinations.

Types and Subtypes of Adrenergic Receptors;

Ahlquist (1948) was the first to propose the existence of more than one adrenergic receptor, based on a study of the abilities of adrenaline, noradrenaline and other agonists which regulated various physiological processes. He proposed the designations α and β for these receptors. The receptor termed β was mainly inhibitory except in heart and the receptor termed α was mainly excitatory except in intestine.

The division was further substantiated with the identification and, in some cases, clinical use of type-selective antagonists (e.g., Phenoxybenzamine and Phentolamine for α receptors; Dichloroisoproterenol and Prapranolol for β receptors). The discovery that certain agonists and antagonists could be used to distinguish β-adrenergic responses among tissues such as cardiac muscle and bronchial smooth muscle implied the existence of subtypes of β-adrenergic receptors (β_1 and β_2) (5). A third β-adrenoreceptor (designed β_3) has been recently isolated which is encoded by a human gene. This β_3-adrenoreceptor is about 10-fold more sensitive to noradrenaline than to adrenaline and is relatively resistant to blockade by propralnolol. This β_3 – receptor may mediate responses atypical to catecholamines like lipolysis.

Later, the existence and differential tissue localization of α-adrenergic receptors were discovered and defined (6). Because norepinephrine appeared to be somewhat more potent at α_1- than at α_2-adrenergic receptors and substantially more potent at β_1 - than at β_2 - adrenergic receptors, it was inferred that α_1 and β_1 receptors were located at postsynaptic sympathetic neuroeffector junctions, where they mediate physiological responses on sympathetic –nerve activation. In contrast, α_2- and β_2-adrenergic receptors were thought to be more responsive to circulating catecholamines. Thus, they were thought to be found at sites outside neuroeffector junctions or to be “autoreceptors”-receptors located on sympathetic nerves that participate in an auto feedback loop regulating the synaptic release of norepinephrine (7).

Further advances in our understanding of α-adrenoceptors have come from the development of new pharmacological methodologies for the study of receptors. The first of these was the techniques of the radioligand binding assay, which began in the mid-1980's, demonstrated that there were subtypes of both α_1-adrenoreceptors and α_2-adrenoreceptor (6). The study of α-adrenoceptors was revolutionized by the technique of molecular biology. Six genes for α-adrenoceptors have now been identified and sequenced (α_{1A}, α_{1B}, $\alpha_{1D,}$ α_{1L} α_{2A}, $\alpha_{2B,}$ α_{2C}).

TABLE: 3 Types and Subtypes of Adrenergic Receptors.

Receptor type	Year defined	Methods of identification
Adrenoceptor	Early 1900s	Tissue response
α, β	1948	Tissue response
β_1, β_2	Late 1960	Tissue response Second-messenger analysis
α_1, α_2	Mid to late 1970s	Tissue response Second-messenger analysis Radioligand binding
α_{1A}, α_{1B},$\alpha_{2A,}$ α_{2B}	Mid to late 1980s	Radioligand binding Tissue response Second-messenger analysis
α_{1A}, α_{1B}, α_{1D},$\alpha_{2A,}$ $\alpha_{2B,}$ $\alpha_{2C,}$ β_1, $\beta_{2,}$ β_3	Late 1980s to early 1990s	Molecular cloning

Structure of Adrenergic Receptor; (8)

The adrenergic receptors constitute a family of closely related proteins. They also are related both structurally and functionally to receptors for a wide variety of other hormones and neurotransmitters that are coupled to G proteins.

This wider family of receptors includes receptors not only for catecholamines and other small molecules (such as acetylcholine, dopamine, histamine, and prostaglandins) but also for peptides (such as vasopressin, oxytocin, and angiotensin), proteins (such as glucagon, follicle-stimulating hormone, luteinizing hormone, and thyrotropin), odorants, light, and taste molecules. Thus, adrenergic receptors probably evolved from a common ancestor along with the receptors that recognize other hormones and neurotransmitters or environmental stimuli.

Figure 3: Proposed Arrangement of an Adrenergic Receptor the Plasma Membrane.

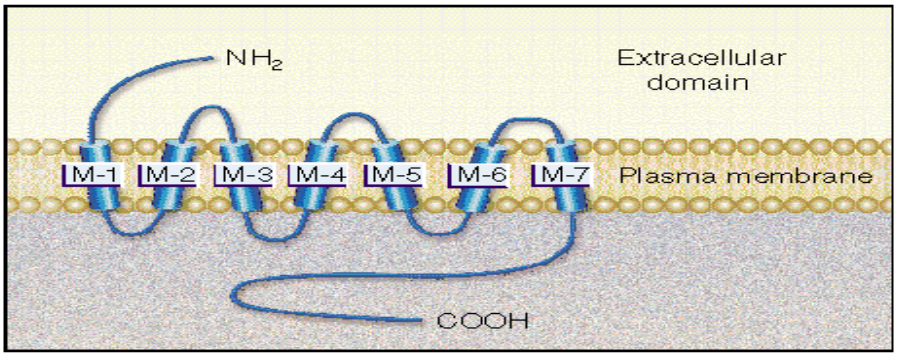
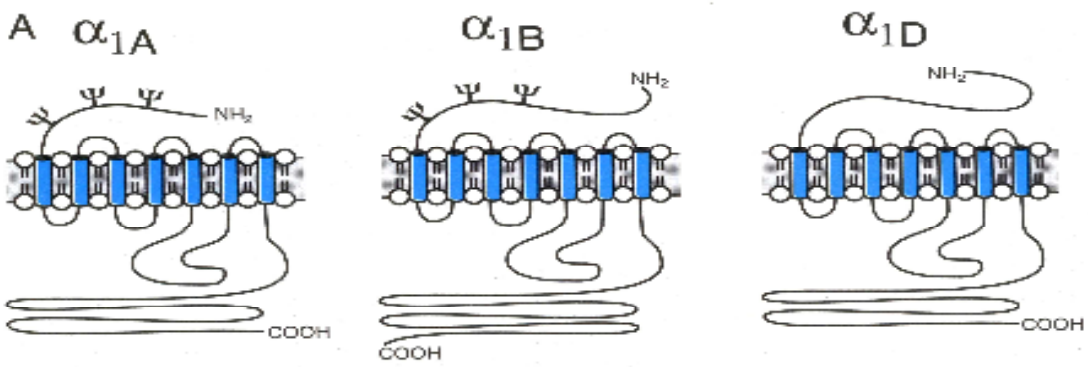


Figure 4: Subtypes of α_1-adrenergic receptor;



All G-protein–coupled receptors (Figure 2) share the following structural features: extracellular amino terminals with sites for N-linked glycosylation, seven α-helical domains that are each thought to span the plasma membrane, and intracellular carboxy terminals containing amino acid sequences that indicate probable sites of phosphorylation by one or more protein kinases. M-1 through M-7 denotes the seven α-helical membrane-spanning regions that create three intracellular and three extracellular loop domains. This arrangement is proposed for adrenergic receptors and other types of G-protein–linked receptors that span plasma membranes. Like other member of this gene family, α_1ARS are single polypeptide chains, ranging from 429 to 561 amino acids in length. There is no evidence that the polypeptide chain is posttranslationally processed, although it is posttranslationally modified by the attachment of oligosaccharides (and probably fatty acids also) as well as phosphorylated. There is no evidence for clearly defined leader sequence, so membrane insertion most likely involves the use of cryptic signal sequences.

Each receptor contains seven stretches of 20 to 28 hydrophobic amino acids that likely represent membrane-spanning regions. In several instances, these hydrophobic stretches are interrupted by charged residues that are functionally important for ligand binding and signaling.

The amino termini of α_1ARS are located extracellularly and contain several consensus sites for modification by N-linked glycosylation. The amino termini vary considerably in length, with the terminus for the α_{1D}AR being much longer (90 amino acids) than the terminus for the $\alpha_{1A/c}$AR (25 amino acids) or the α_{1B} AR (42 amino acids). This longer amino terminus of the α_{1D} AR may limit efficient translation or membrane insertion, since this subtype is more poorly expressed in the plasma membrane than the other two subtypes.

The carboxy termini are located intracellularly and contain consensus sites for phosphorylation by serine/threonine protein kinases, and modification of the proteins at these sites is involved in receptor desensitization. The carboxy terminal regions show little homology among the subtypes.

The transmembrane-spanning regions are linked by three intracellular and three extracellular loops. These loops, although variable, are each very similar in length among the subtypes. The first and second extracellular loops each contain a single cysteine residue, and analogous cysteines are highly conserved in all GPCRs. In the β-AR and in rhodopsin, these cysteine residues are essential for the correct folding of the proteins, for maturational glycosylation, and for expression in the plasma membrane. This is due to the involvement of these cysteines in a disulfide bond(s). The α_{1B} AR also has been shown to contain an essential disulfide bond, which is solvent inaccessible. Like other member of the GPCR family, the second and third intracellular loops are likely to be involved in signaling through an interaction with receptor-coupled G proteins.

Molecular Biology Of Adrenergic Receptors (ARs)

The G proteins to which adrenergic receptors link are heterotrimeric proteins with α, β, and γ subunits. The α, β, and γ subunits of G proteins have subsequently been isolated and cloned. Each subunit is part of a family consisting of multiple members: approximately 20 α subunits (which have been divided into four subfamilies —α_s, α_i, α_q, and α_{12}), at least 5 β subunits (β_{1-5}), and at least 6 γ subunits (γ_{1-6}). Although several hundred different subunit combinations

(heterotrimers) are theoretically possible, the repertoire of G proteins used by a particular receptor system is limited (9).

Each type of adrenergic receptor (Table 5) preferentially couples to a different major subfamily of G_α proteins: β-adrenergic receptors to $G_{\alpha s}$, α_1-adrenergic receptors to $G_{\alpha q}$, and α_2-adrenergic receptors to $G_{\alpha i}$. In turn, each of these G_α proteins can link to numerous effector molecules, although most target cells have preferred linkages. Thus, β-adrenergic receptors are preferentially coupled by G_s to the activation of adenylyl cyclase (and calcium-ion channels in some tissues), α_1-adrenergic receptors by G_q to the activation of phospholipases, especially phospholipase C^β, and α_2-adrenergic receptors by G_i to the inhibition of adenylyl cyclase and in some tissues to the regulation of potassium and calcium channels. Each of these linkages leads to changes in intracellular concentrations of second messengers such as cyclic AMP, calcium ion, diacylglycerol, and inositol 1,4,5-triphosphate. These second messengers modulate cellular events, regulating the phosphorylated states of cell proteins by changing the activity of a variety of protein kinases. For example, cyclic AMP activates protein kinase A, diacylglycerol and calcium ions activate protein kinase C, and calcium ions and calmodulin activate calmodulin-dependent kinases.

Catecholamines, which are hydrophilic, do not bind to the highly charged extracellular domains of the receptors as might be expected but bind instead in the more hydrophobic membrane-spanning domains (10). Occupancy by an agonist appears to produce conformational changes within the receptor, causing certain regions, in particular the third intracellular loop, to interact with G protein. Under basal conditions, the G proteins are inactive, and the guanine-nucleotide–binding site on the G_α subunit is occupied by the inactive nucleotide guanosine diphosphate. When agonist binds to the receptor, cellular guanosine triphosphate replaces guanosine diphosphate on the G_α subunit. This, in turn, promotes a conformational change in the G_α subunit, facilitating its dissociation from the β and γ subunits, which are tightly bound and appear to function as a dimer. Both the G_α subunit and the $G^{\beta\gamma}$subunit dimer can regulate the activity of effector molecules and the formation of second messengers (11). The G protein is activated until guanosine triphosphate is hydrolyzed to form guanosine diphosphate, which facilitates the reassociation of the subunits. Thus, G proteins are in a sense molecular light switch

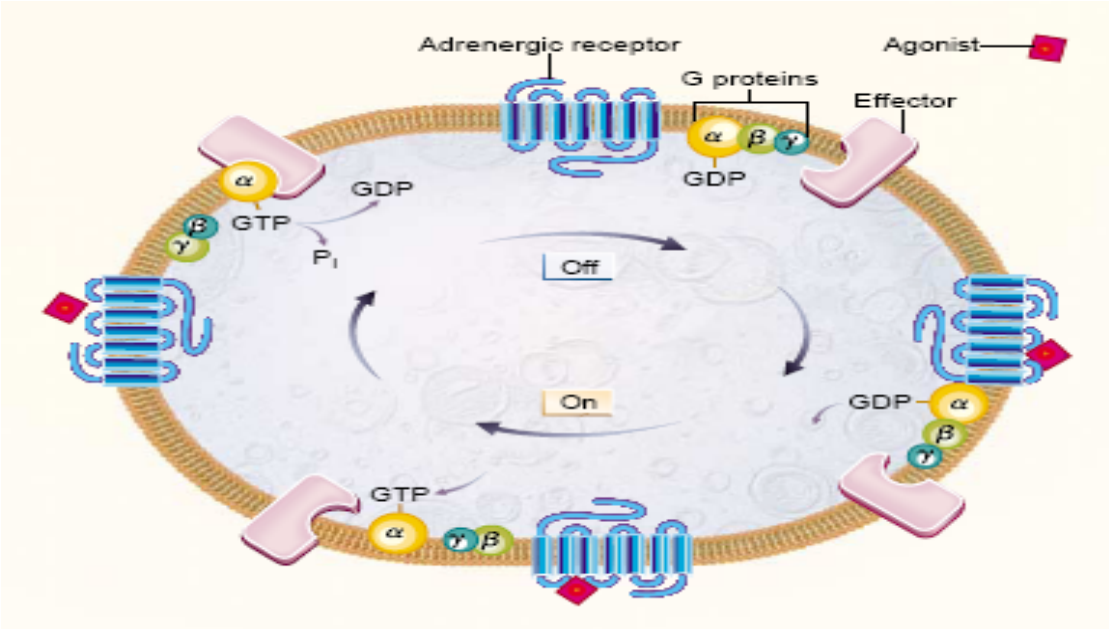
that cycle between "on" (bound to guanosine triphosphate) and "off" (bound to guanosine diphosphate) when agonist binds to receptor (8).

Information provided by the cloning of adrenergic receptors and G proteins has provided new insights into the way agonists promote the formation of second messengers (Figure 7).

Table 4: Preferred Linkage of Adrenergic Receptors to G-Protein Families and Effectors.

RECEPTOR TYPE	G PROTEIN	EFFECTORS
α_1	G_q	phospholipase C^{β} ? Other phospholipases
α_2	G_i	adenylyl cyclase calcium channels potassium channels
β	G_s	adenylyl cyclase calcium channels

Figure 5: G-Protein–Mediated Signal Transduction Promoted by Adrenergic Receptors.



An adrenergic receptor is shown as it binds an agonist and then associates with a G protein consisting of three heterogeneous subunits (α, β, and γ). The agonist promotes the exchange of guanine triphosphate (GTP) for guanine diphosphate (GDP) (the "on" reaction) in the presence of magnesium, thereby promoting the dissociation of the receptor from the G

protein as well as the dissociation of the G_α subunit from the $G^{\beta\gamma}$ subunits and the agonist from the receptor. The dissociated G_α subunit and, in some cases, $G^{\beta\gamma}$ subunits activate effector molecules. Intrinsic GTPase activity of G_α hydrolyzes GTP to GDP (the "off" reaction), thereby releasing inorganic phosphate (P_i) and facilitating the reassociation of G_α with $G^{\beta\gamma}$ to form $G_\alpha{}^{\beta\gamma}$.

α_1-Adrenoceptor;

Since their original classification of adrenergic receptors into stimulatory and inhibitory receptors and subdivision into α_1- and α_2-ARs, it became apparent that there was heterogeneity in α_1-ARs. Indeed, prior to the cloning of any receptor subtypes, numerous reports provided functional evidence of α_1-AR heterogeneity. McGrath was the first to suggest subdividing the α_1-ARs into α_{1A}- and α_{1B}-ARs. Morrow and Creese noted that the inhibition curves for a series of agonists and antagonists to displace [3H]prazosin were biphasic. Since these initial studies, the α_{1A}-subtype was pharmacologically classified to have higher binding affinity for agonists, such as methoxamine and oxymetazoline, and antagonists, such as 5-methylurapidil, niguldipine, and WB4101. In contrast, the α_{1B}-AR subtype had lower binding affinity for the above ligands (8).

After these initial pharmacological studies, the first cDNA cloned was the hamster α_{1B}-AR. This cDNA had all of the pharmacological properties of the tissue-characterized α_{1B}-AR and has never been questioned in its classification. The next receptor cloned was called the α_{1C}-AR and was thought to represent a novel subtype. However, it was later reclassified to be the tissue-type α_{1A}-AR. The confusion was centered on its inability to localize its mRNA to tissues known to express the α_{1A}-AR. The next cDNA cloned was initially termed the α_{1A}-AR but also later was reclassified to a novel subtype called the α_{1D}-AR. In this case, the confusion was due to an incomplete pharmacological profile. With the discovery of the α_{1D}-AR, its binding and functional properties were compared with the previously known tissue subtypes. The α_{1D}-AR has a binding profile much like the α_{1B}-AR. Recently an α_{1D}-AR-selective drug has become available.

Angiotensin II Receptors

Historical Background; (12)

Blood pressure was measured for the first time in 1733 by Stephen Hales, in a dramatic experiment on a horse, by inserting a brass pipe into the carotid artery. The technique of modern blood pressure measurement was introduced in 1905 by Nicolai Korotkov using the stethoscope invented by Laennec in 1815 and the relatively recently devised wraparound inflatable rubber cuff. The latter was first described by Riva-Rocci in 1896 and was improved by von Recklinghausen in 1901 (12). The first insight into the regulation of blood pressure came from the discovery of a pressor principle by Tigerstedt and Bergman in 1897. They called this factor "renin" because it was extracted from the kidney. This pioneering work led to the description of reno-vascular hypertension in animals and in humans (13). However, it was not until 1940 that a vasoconstrictor substance was isolated from renal venous blood from the ischemic kidney of a Goldblatt hypertensive dog. A similar finding was made simultaneously and independently by Page and Helmer (1940) after the injection of renin into an intact animal. This group also isolated a so-called "renin activator" that later proved to be angiotensinogen. The pressor substance was named "hypertensin" in Argentina and "angiotonin" in the United States and was laterisolated and shown to be an octapeptide (3, 14). There were differences between laboratories concerning interpretations and nomenclature but in fact hypertensin and angiotonin were the same substance. In 1958, Braun-Mene´ndez and Page agreed on the hybrid term angiotensin for the highly potent pressor octapeptide. This proved to be an appropriate choice, given the later recognition of angiotensin's numerous actions in addition to its hypertensive effects. The sequence of angiotensin II is Asp-Arg-Val-Tyr-Ile-His-Pro-Phe in the human, horse, and pig. In bovine angiotensin II, the isoleucine residue in position 5 is replaced by valine. Following this major discovery, the various components of the cascade leading to the formation of angiotensin II were characterized, including angiotensinogen, angiotensin converting enzyme (ACE), and angiotensins I, II, and III. The synthesis of the peptide angiotensin II by Bumpus et al. (1957) and by Rittel et al. (1957) was followed by a continuing series of investigations into the structure-activity relationship of angiotensin analogs, mainly in the hope of finding a peptide antagonist.

In 1987, a committee of the International Society for Hypertension, The American Heart Association, and the World Health Organization proposed abbreviating angiotensin to Ang using the decapeptide angiotensin I as the reference for numbering the amino acids of all angiotensin peptides (15).

Angiotensin II plays a key role in the regulation of cardiovascular homeostasis. Acting on both the "content" and the "container", Ang II regulates blood volume and vascular resistance. The wide spectrum of Ang II target tissues includes the adrenals, kidney, brain, pituitary gland, vascular smooth muscle, and the sympathetic nervous system. Angiotensin is not only a bloodborne hormone that is produced and acts in the circulation but is also formed in many tissues such as brain, kidney, heart, and blood vessels. This has led to the suggestion that Ang II may also function as a paracrine and autocrine hormone, which induces cell growth and proliferation and controls extracellular matrix formation (16). Other angiotensin-derived metabolites such as angiotensin 2–8 (Ang III), angiotensin 1–7, or angiotensin 3–8 (Ang IV) have all been shown to have biological activities (17).

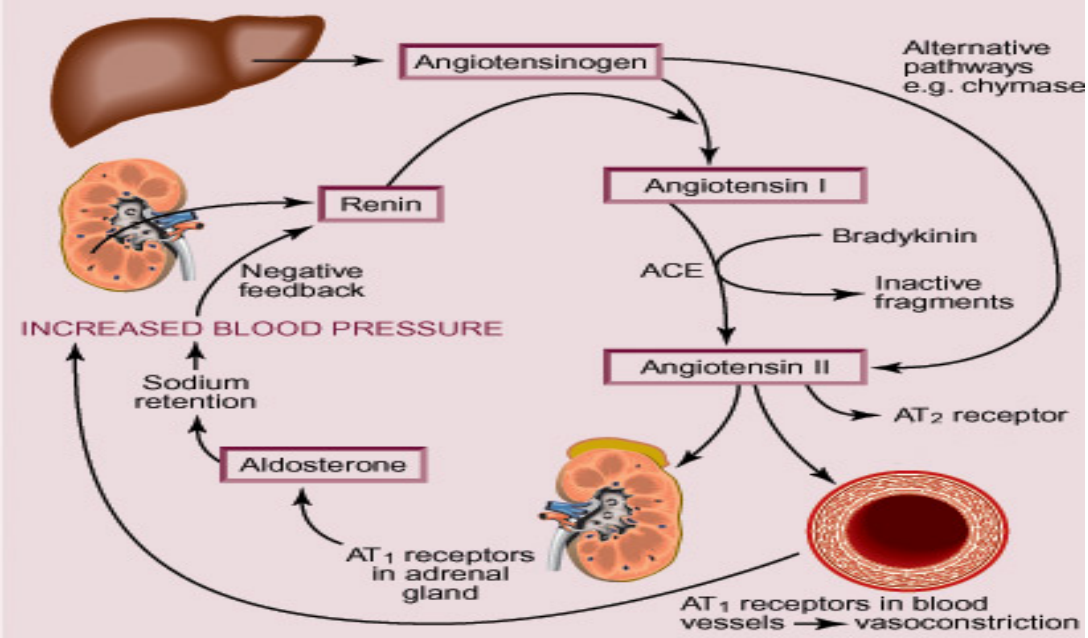
TABLE 5: Amino acid sequences of Ang II precursors and metabolites

	1 2 3 4 5 6 7 8 9 10 11 12 13 14
Angiotensinogen	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser
Ang I	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu
Ang II	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
Ang III	Arg-Val-Tyr-Ile-His-Pro-Phe
Ang IV	Val-Tyr-Ile-His-Pro-Phe
Angiotensin 1–7	Asp-Arg-Val-Tyr-Ile-His-Pro

Angiotensin II is derived from angiotensinogen in the circulation via the sequential actions of renin and angiotensin-converting enzyme (ACE) and in tissues by renin and ACE as well as several other enzymes, including chymase and cathepsin G. Angiotensin II produces biologic effects that are important in cardiovascular pathophysiology as a result of binding to and activating the angiotensin type 1 (AT1) receptor. Currently available angiotehnsin II receptor blockers (ARBs) block this interaction, and thereby prevent these angiotensin II effects.

Angiotensin II also binds to the angiotensin type 2 (AT2) receptor to produce effects that balance those elicited via the AT1 receptor. The ARBs do not block the interaction of angiotensin II with the AT2 receptor. TPA 5 tissue plasminogen activator. (18)

FIGURE 6: Angiotensin II is the main effector hormone of the renin–angiotensin system.



As for other peptide hormones, Ang II was postulated to act on a receptor located on the plasma membrane of its target cells. This receptor should possess the dual functions of specific recognition of the ligand and stimulation of the characteristic cellular response. Comparison of changes in steroidogenesis in the adrenal cortex, adrenal catecholamine release, and developed tension in aortic strips in response to Ang I, Ang II, and Ang III clearly indicated different affinities of these target organs for the three peptides (17). These pharmacological experiments showed that effector organs responded to Ang I, II, andIII with 2 to 3 log differences in potency from tissue to tissue. Based on these studies, Ang II receptor selectivity for the agonists was proposed to be structure-activity related. Comparison of Ang II and a large number of synthetic agonists and antagonists formed by substituting various amino acids of Ang II indicated marked dissimilarities between the analogs in each of the preparations, suggesting differences in the structure of the receptor sites (19).

Angiotensin Receptors:

Once angiotensin II is formed, it binds to and activates specific angiotensin receptors to produce its myriad of biologic effects (18). In humans, angiotensin II binds to two distinct angiotensin receptors, termed AT1 and AT2, which share 34% sequence homology. Both subtypes possess seven transmembrane domains that are characteristic of the G-protein– coupled receptor superfamily, and both have the same high affinity for angiotensin II (12). Nevertheless, these subtypes differ in their affinity for various angiotensin peptide fragments, and in addition, these receptors are distinguished by their selectivity of nonpeptidic antagonists. The currently available ARBs selectively bind to the AT1 receptor, whereas some experimental compounds, such as PD 123319, are selective for the AT2 receptor. In adults, the majority of angiotensin receptors are of AT1 subtype, whereas the AT2 subtype appears more involved in fetal development. Although the AT2 subtype is detected in only a small number of organs and at very low levels of expression, it appears to be up-regulated after tissue injury. (12).

The AT1 Receptor:

Angiotensin II binding to the AT1 receptor leads to activation of several classic second messenger systems (Figure 2) (12). First, AT1 receptor stimulation leads to activation of several phospholipases, including phospholipase C which hydrolyzes phosphatidyl 4,5-bisphosphate to form inositol 1,4,5-trisphosphate and diacylglycerol (20). Inositol 1,4,5-trisphosphate stimulates intracellular calcium release to activate smooth muscle contraction, whereas diacylglycerol increases protein kinase C activity. Second, AT1 receptor stimulation inhibits adenylate cyclase and leads to a reduction in cyclic adenosine monophosphate. Because cyclic adenosine monophosphate is a vasodilator, its reduction after AT1 receptor activation likely contributes to the observed angiotensin II-induced vasoconstriction. By blocking the AT1 receptor, cyclic adenosine monophosphate levels are preserved and vasoconstriction prevented. Third, AT1 receptor activation leads to opening of calcium channels and an influx of calcium into the cell. This mechanism is believed to contribute to the angiotensin II-mediated stimulation of aldosterone production andsecretion. By blocking the AT1 receptor, calcium concentrations in adrenal cells, for example, are lowered, and as a result, aldosterone production is reduced.

AT1 receptor stimulation also leads to activation of phospholipases A2 and D, which are responsible for generating prostaglandins. The influence of angiotensin II on prostaglandin E2 generation is illustrated by a study in which rats were sodium depleted for 5 days and renal interstitial prostaglandin E2 production measured by microdialysis (21, 22). In comparison to normal sodium intake, sodium depletion produced an increase in renin–angiotensin system activity, and this was associated with a marked increase in renal interstitial prostaglandin E2 production. When losartan, an AT1 receptor antagonist, was administered to sodium-depleted animals, prostaglandin E2 production was lowered. In contrast, administration of the specific AT2 receptor antagonist, PD-123319, resulted in additional prostaglandin E2 production (22). These results indicate that stimulation of the AT1 receptor is responsible for the generation of prostaglandin E2, which possesses proinflammatory properties. In patients with atherosclerosis or after myocardial infarction, some necrosis and inflammation may be related to prostaglandin generation, and therefore, it follows that blocking the AT1 receptor may ameliorate prostaglandin-mediated inflammation. Moreover, these results imply that the 2 angiotensin receptor subtypes may have opposing actions. Whereas the AT1 receptor mediates an increase in prostaglandin E2 production, the AT2 receptor may have an inhibitory effect on prostaglandin E2. By blocking the AT2 receptor with PD-123319, the inhibitory influence is removed and angiotensin II can more completely stimulate prostaglandin E2 production via the AT1 receptor.

In addition to the classic signaling pathways, activation of the AT1 receptor also stimulates protein tyrosine phosphorylation and activates mitogen-activated protein kinase, the Janus kinases, and the signal transducers and activators of transcription proteins (20).

These protein phosphorylation pathways result in activation of the early growth response genes, which control cell proliferation and hypertrophy. The amount of protein tyrosine phosphorylation that occurs after administration of angiotensin II in vivo is correlated with the duration of angiotensin II administration and the dose administered. Administration of losartan blocks angiotensin II-mediated protein tyrosine phosphorylation, confirming that this is an AT1 receptor–mediated event. Moreover, angiotensin II increases [3H]thymidine incorporation, a marker for cell hypertrophy, and this also is reduced by losartan administration (23). 22 Interestingly, an AT2 receptor has not been identified in smooth muscle cells. Administration of PD-123319 (AT2 receptor blocker) does not influence the effect of angiotensin II on protein

tyrosine phosphorylation or thymidine incorporation (24). However, in cells that do have an AT2 receptor, PD- 123319 produces a further increase in angiotensin II-induced thymidine incorporation, which suggests that the two receptor subtypes have distinct mechanisms of action. The AT1 subtype can be considered to be a "phosphorylating" receptor, whereas the AT2 subtype can be thought of as a "dephosphorylating" receptor.

Some angiotensin II actions are believed to be mediated through the release of a variety of growth factors, such as transforming growth factor-b1, which have a major impact on protein matrix in the heart, kidney, and vasculature. Angiotensin II-induced growth factor production may be important in the pathogenesis of left ventricular hypertrophy, congestive heart failure, diabetic nephropathy, and other conditions in which tissue remodeling is involved. In a myocardial infarction model in rats, for example, transforming growth factor-b1 was found to correlate with angiotensin II levels at the infarct site and at remote sites of tissue repair (25). However, in rats treated with losartan, transforming growth factor-b1 expression was attenuated, indicating that the production of transforming growth factor-b1 was mediated via the AT1 receptor.

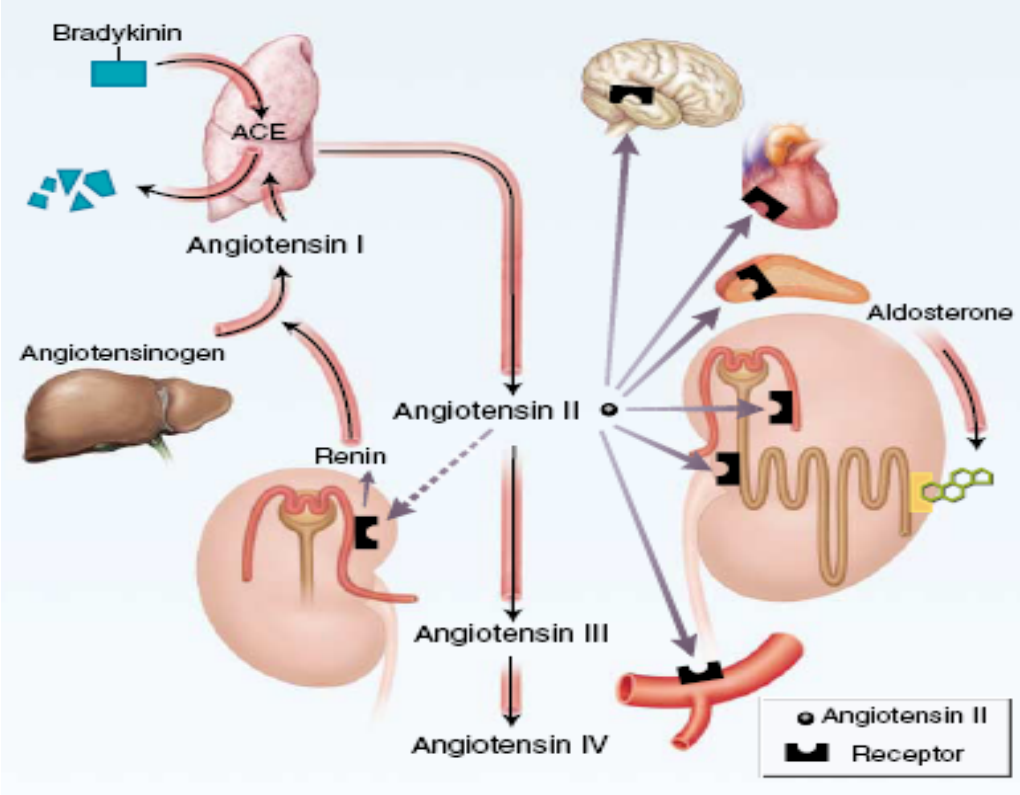
AT1 receptor function depends on its anatomic location. In blood vessels, for example, stimulation of AT1 receptors leads to vasoconstriction, and with longer-term exposure to angiotensin II, it can lead to blood vessel growth and proliferation resulting in the thickening and stiffening of the vessel wall. In endocrine glands, AT1 receptor stimulation leads to release of several hormones—aldosterone and catecholamine from the adrenals and vasopressin from the pituitary. In the proximal tubules of the kidney, AT1 receptor activation leads to stimulation of sodium reabsorption. This direct effect enhances the stimulation of sodium reabsorption that occurs indirectly via aldosterone in the distal and collecting tubules. In the heart, AT1 receptor stimulation provides an inotropic effect; it stimulates the release of catecholamines from presynaptic nerve endings; and it causes cardiac myocyte hypertrophy. In fact, the angiotensin II receptor is implicated to play a major role in the development of left ventricular hypertrophy through the AT1 receptor, because of its actions on cardiac myocyte hypertrophy and its ability to alter deposition of protein and fibrous tissue matrix.

An AT1 receptor has been identified in macula dense cells of the kidney (26), its stimulation by angiotensin II inhibits rennin release and prevents further activation of the rennin–angiotensin system. Thus, AT1 receptor stimulation eventually leads to inhibition of angiotensin II formation. By blocking this AT1 receptor with Abs, rennin release is increased, which in turn leads to greater angiotensin II production. This has opened a new avenue for the study of mechanisms related to angiotensin receptors, notably the role of the AT2 receptor subtype. Because AT1 receptors are expressed in much greater quantities than AT2 receptors in adults, the effect of AT1 receptor stimulation overwhelms activity at the AT2 receptor. Unless the AT1 receptor is blocked, it is very difficult to investigate the effect of angiotensin II at the AT2 receptor.

The AT2 Receptor:

AT2 receptor stimulation leads to dephosphorylation of regulatory cell proteins (27). This general mechanism is believed to be important in causing the anti-proliferation, apoptosis, differentiation, and vasodilation that is attributed to activation of AT2 receptors (Figure 3) (28). The mechanism by which angiotensin II stimulates AT2-mediated vasodilation was explored further in the sodium-depleted conscious rat model. The results suggest that the increase in cyclic guanosine monophosphate is an AT2-mediated event. Under these low sodium conditions, the increase in cyclic guanosine monophosphate occurred secondary to the release of nitric oxide, inasmuch as the effect was blocked by administration of the nitric oxide synthase inhibitor, nitro-L-arginine-methyl ester (LNAME) (22). During normal sodium intake, angiotensin II increased cyclic guanosine monophosphate by 2-fold, and again, PD-123319 and L-NAME but not losartan blocked the effect (22). Although circulating levels were unchanged, tissue bradykinin levels were increased after sodium depletion, an effect that was blocked by PD-123319 but not by losartan (21, 29). Thus, angiotensin II-induced AT2 receptor stimulation leads to bradykinin production and subsequent release of nitric oxide followed by elevation of cyclic guanosine monophosphate.

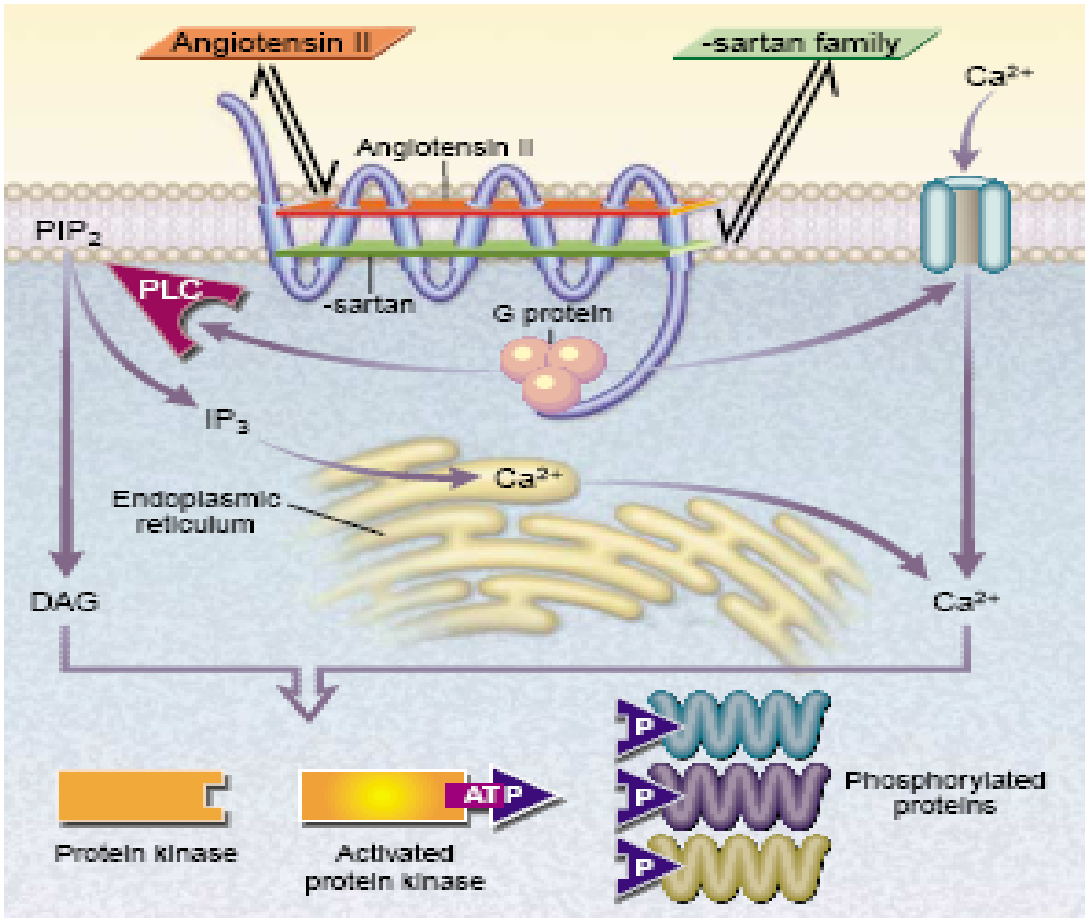
Figure 7: Formation of Angiotensins and Organs Affected by Their Actions. (30)



The left-hand side of the figure shows the classic pathway of biosynthesis that generates angiotensins in the bloodstream. Angiotensinogen is synthesized by the liver and released into the blood (arrows highlighted in red), where it is cleaved to form angiotensin I by renin secreted from juxtaglomerular cells in the kidneys. Angiotensin- converting enzyme (ACE) in the lung catalyzes the formation of angiotensin II from angiotensin I, and the same enzyme destroys bradykinin. Further proteolytic cleavage generates angiotensins III and IV. The pathways for the synthesis of angi-otensin (1–7) and the biosynthesis of angiotensins in tissue are not shown.

The right-hand side of the figure shows the angiotensin receptors in the principal organs affected by the action of angiotensin. Two renal actions are shown - the stimulation of proximal tubular function and the vasoconstriction of efferent arterioles. Also shown at left (dashed arrow) is the inhibition by angiotensin II of the release of renin by the kidney. All the receptors shown are of the AT 1 subtype except certain receptors in the brain.

Figure 8: Signal Transduction by Angiotensin AT1 Receptors. (30)



An angiotensin receptor of the AT1 subtype is shown as a linear polypeptide chain spanning the cell membrane seven times. Two regions of the receptor where angiotensin II and nonpeptide receptor antagonists interact are shown (orange and green planes). The amino terminal and other parts of the receptor protein close to the cell surface participate in binding angiotensin II. Deeper amino acid residues contribute to the binding of angiotensin antagonists, all of which contain the suffix "-sartan" in their generic names. The carboxy terminal and the three intracellular loops interact with G proteins inside the cell. G proteins activated by the hormone–receptor complex stimulate phospholipase C (PLC) and open calcium channels. Phospholipase C cleaves phosphoinositide (PIP2) to inositol trisphosphate (IP3) and diacylglycerol (DAG). Inositol triphosphate releases calcium from the endoplasmic reticulum.

DAG and calcium activate enzymes, including protein kinase C and calcium–calmodulin protein kinases.

Most of the known effects of Ang II are mediated through the AT1 receptor, e.g., vasoconstriction, aldosterone and vasopressin release, salt and water retention, and sympathetic activation without neglecting the important autocrine and paracrine effects of Ang II on cell proliferation and migration and on extracellular matrix formation. The function of the AT2 receptor has become unraveled over the last few years owing to various sophisticated approaches including gene transfection and deletion. Accumulated published data suggests that the AT2 receptor counterbalances the effect of the AT1 receptor in vitro as well as in vivo. There is an inactivation of MAPK, antiproliferation, promotion of apoptosis, differentiation and regeneration, opening of delayed-rectifier K1 channels and closing of T-type Ca21 channels. The re-expression of the AT2 receptor in various diseases suggests a role of this receptor in pathophysiology. The AT4 receptor appears to be involved in memory acquisition and recall. Like the AT2 receptor, it may also oppose the effect of the AT1 receptor as it regulates renal blood flow, inhibits tubular sodium reabsorption and affects cardiac hypertrophy. Cloning of the described angiotensin receptors and the ability to express these clones in mammalian cells will allow exhaustive structure/function studies. Further pharmacological and molecular studies will allow for a better and more complete understanding of the role of the renin-angiotensin system in pathology. (12).

RELATIONSHIP BETWEEN TYPE 1 ANG II RECEPTORS AND α_1-ADRENORECEPTORS;

The Renin-Angiotensin System and the sympathetic nervous system have each been implicated in the primary causes of certain forms of clinical hypertension and congestive heart failure. Of the effects on cardiovascular tissues, these two systems influence vascular tone and growth of smooth muscle cells (31-33). The effects of the pressor substances of these two systems, angiotensin II (ANG II) and norepinephrine (NE), are triggered by their interaction with specific receptors on the vascular wall. It has been demonstrated that the alpha1-adrenergic receptor mediates sympathetic vasoconstriction of the blood vessel (34), whereas most vascular ANG II

receptors in all species studied to date are mainly of the type 1ANG II receptor (AT1) (35) that mediates contractile and growth effects of ANG II in vascular smooth muscle (36-38).

It is well known that there are multiple interactions between the renin-angiotensin system and the sympathetic nervous system. For example, ANG II facilitates sympathetic neurotransmission at several sites, including the central nervous system (39), adrenal medulla, sympathetic ganglia (40), and presynaptic noradrenergic nerve terminals (41). On the other hand, stimulation of the sympathetic nervous system leads to renin secretion and ANG II generation (42). It is conceivable that these two systems interact at or beyond the receptor levels. Indeed, it has been shown that NE negatively regulates ANG II receptors in cultured brain neurons through its interaction with alpha 1 -adrenergic receptors (43).

It is evidence (44) that NE regulates the vascular AT1 receptor through a negative feedback mechanism both in vivo and in vitro. The mechanisms underlying this regulation are through the alpha 1 -adrenoreceptor. These studies suggest that, in vasculature, AT1 expression is regulated by ambient NE levels. Reciprocal regulation between the renin-angiotensin system and sympathetic nervous systems may play an important role in the control of blood pressure hemostasis. By extension, it seems reasonable to assume that lack of negative feedback on AT1 receptor by ANG II and/or NE may exist in hypertensive rats.

Further the observation it seems that one or two chemical moieties of the pharamcophores of type 1 ANG II receptors antagonists and α_1-adrenoreceptor antagonists look little similar.

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