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COMMENTARY

ANGIOTENSIN RECEPTORS

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Angiotensin II is an octapeptide hormone generated by the consecutive reactions of renin on renin substrate and converting-enzyme on the decapeptide product. Peripherally, angiotensin II has a potent constricting effect on vascular and non-vascular smooth muscle, stimulates the release of the mineralocorticoid aldosterone from the adrenal cortex and potentiates the activity of sympathetic nerve endings. Centrally, angiotensin II has a potent dipsogenic effect and a pressor action which are mediated by a direct interaction with specific cerebral structures. Angiotensin II appears to participate in the control of blood pressure and extracellular volume and to be involved in the pathogenesis of certain hypertensions secondary to renal alterations [1, 2].

The first step of angiotensin action in its various target-organs is, as for all peptidic hormones hitherto investigated, a specific recognition of the ligand by receptor sites located in the cell membrane, by virtue of the strict complementary conformational requirements of the receptor molecules. In smooth muscle, angiotensin–receptor interaction leads to changes in membrane permeability to ions; an increase in free cytosolic calcium concentration is responsible for the contraction of actomyosin. In other target-tissues, the biochemical events immediately distal to the hormone–receptor interaction are poorly defined. Contrary to the majority of peptidic hormones, the participation of cyclic nucleotides is unlikely.

This review summarises current understanding of the first event, i.e., the angiotensin II-receptor interaction. As for many other hormones and neurotransmitters studies in this area have benefited from the availability of radioactive angiotensin II. The initial investigation was performed with the use of radioactive iodinated angiotensin II [3]. Most of the studies have been subsequently conducted with ^{14}C or [^{3}H]angiotensin II, as these compounds, contrary to the iodinated hormone, retain a full biological activity [4]. Before the interaction of a radioactive hormone with a cellular binding site can be considered to correspond to a receptor interaction, it should exhibit characteristics of specificity, high affinity, reversibility and saturability. In addition, the kinetic characteristics of the binding should correlate with those of the hormonal effect or, ideally with those of the first biochemical event in the series culminating in the biological response [5].

A pattern of angiotensin-binding-sites reaction, compatible with that of a hormone–receptor interaction has been demonstrated in three target-organs, rabbit [6, 7] and guinea pig [8] aorta, rat uterus [9,10] and rat [11,12], rabbit [13] and bovine [12, 14] adrenal cortices. The review is divided into three parts: (i) a general review of angiotensin II–receptor interaction in different target-organs; (ii) the evidence for an angiotensin III adrenocortical receptor; (iii) the modulation of the angiotensin receptor.

1. *Angiotensin–receptor interaction*

The demonstration that angiotensin–receptors are located on the plasma membrane, which is strongly suggested by the characteristics of angiotensin action, was hampered initially by some investigations reporting that the hormone could have a direct action on isolated mitochondria [15] and could enter the nucleus [16]. In fact, localisation of angiotensin II receptors to the cell membrane has been unequivocally demonstrated on subcellular fractions enriched in plasma membranes extracted from the different target-organs [7, 8, 10–12, 14]. However the possibility that angiotensin II, or more likely some of its fragments, may enter the cell remains possible. In the various target-organs, angiotensin II binds to two classes of binding sites, which appear specific in the sense that they specifically recognize the octapeptide and have a limited capacity, but which differ by their respective affinities for the hormone. The similarity of the binding parameters of the high-affinity class of sites and the features of the hormonal response suggests that they may be the receptors involved in the hormonal response. The significance of the low-affinity sites, which have been observed with many other peptide hormones, is unknown. They should at present be classified as "acceptor sites", as they seem unrelated to the hormonal effect. The binding parameters of angiotensin receptors are indicated in Table 1. In the different target-tissues, the angiotensin–receptor interaction proceeds rapidly and reversibly with a defined number of receptors. In smooth muscle, the K_d value of angiotensin II binding to the high-affinity binding sites is in agreement with the ED_{50} of the angiotensin-induced contraction, and no species or organ difference can be detected. However, in the adrenal cortex, the K_d value exceeds the ED_{50} of angiotensin-elicited aldosterone release by 1–2 orders of magnitude. This discrepancy could reflect a large excess of "spare" receptors, an alteration of receptor structures due to mechanical or chemical treatments of target-tissues, or non-optimal conditions of binding. However, it may represent a feature peculiar to the adrenal cortex where it has been shown that the 2–8 heptapeptide

Table 1. Binding parameters for angiotensin II receptors

Target tissue	Radioactive label	High affinity site k_1 (10^5 M^{-1} sec^{-1})	High affinity site k_{-1} (10^{-4} sec^{-1})	High affinity site K_d (10^{-9} M)	low affinity site K_d (10^{-9} M)	Ref.
Aorta:						
Rabbit						
plasma membranes	^3H	1.3	8	6	–	[17]
solubilised material	^3H	2.5	40	20		[7]
Guinea pig						
plasma membranes	^{14}C	–	–	22–47	–	[8]
Uterus:						
Rat						
plasma membranes	^3H	1.6	13	8–20		[9, 10]
Adrenal:						
Bovine cortex						
particulate fraction	^3H	2.4	5	2	83	[12]
	^{125}I	–		2	17–100	[12]
plasma membranes	^3H	–	–	4		[14]
Rat						
particulate fraction	^{125}I	–	–	0.2–0.5	6–7	[12, 36]
Rabbit						
isolated zona	^3H	6.6	22	3–5	25	[11]
glomerulosa cells	^3H	2.4	69	29	–	[13]

(angiotensin III) is a steroidogenic agent at least as potent as angiotensin II. These observation led us to study angiotensin III binding in the adrenal cortex, reviewed in Section 2. The nature of angiotensin receptors, which were solubilised from rabbit aorta smooth muscle cell membranes, is unknown. However, angiotensin receptors in this target-tissue seem to be integral membrane proteins (in the sense proposed by Singer and Nicholson) as they resist moderate proteolysis; in addition, according to their sensitivity to neuraminidase, angiotensin II receptors appear to be glycoproteins, the sialic acid of which is directly implicated in the formation of the angiotensin–receptor complex [17].

In the case of many peptide hormones it has been shown that variations in ionic and in nucleotide concentrations of the incubation medium can affect the hormone–receptor interaction. We observed in rabbit aorta smooth muscle cell membranes that variations in sodium concentration were without effect whereas increasing concentrations of divalent cations had an inhibitory effect on angiotensin II binding. A similar inhibitory effect of calcium ions has been observed in uterine receptors [18]. At high concentrations, adenosine triphosphate, and to a lesser extent guanosine triphosphate, inhibited angiotensin II binding. This effect is correlated with phosphorylation since the presence of energy generating system phosphoenol pyruvate–pyruvate kinase, also prevents the binding of angiotensin II. It is therefore conceivable that changes in phosphorylation are implicated in the receptor activation–inactivation process. However, it is not possible to exclude the interpretation that variations in ionic or nucleotide composition, affect membrane structure and accordingly the accessibility of receptor sites, rather than directly changing the angiotensin–receptor interaction. In corticoadrenals, high affinity binding sites have been reported to be observable only in the presence of high sodium or potassium concentrations [19].

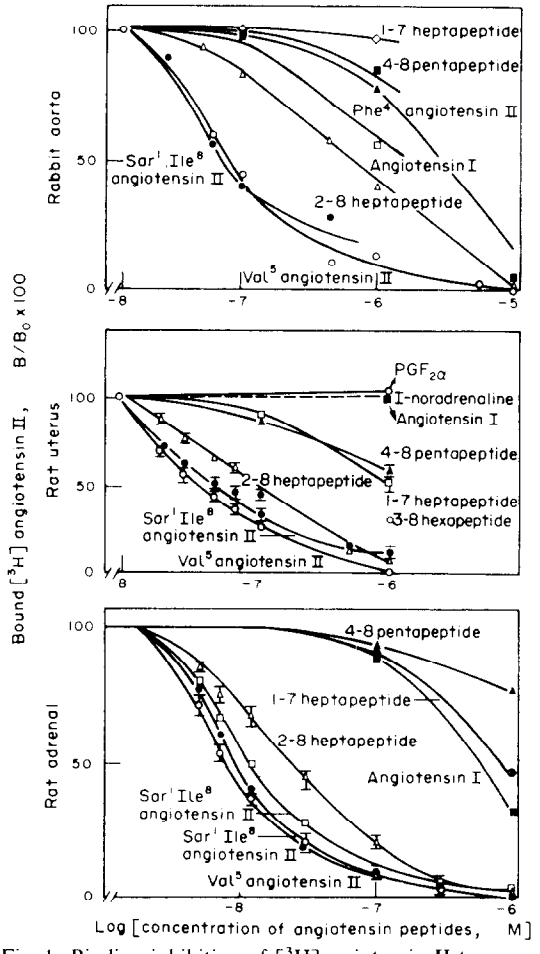

Fig. 1. Binding inhibition of [^{3}H]angiotensin II to receptors in membrane and particulate fractions of three angiotensin sensitive tissues studied with angiotensin, analogs and fragments. (a) rabbit aorta, (b) rat uterus, (c) rat adrenals. For explanation see text.

Table 2. Binding parameters for angiotensin III high affinity receptors in rat adrenal gland

Association rate constant	k_1 (10^6 M^{-1} sec^{-1})	
	From association studies	7.9
Dissociation rate constant	k_{-1} (10^{-4} sec^{-1})	
	From association studies	7.6
	From dissociation studies	4.0
Equilibrium dissociation constant	(10^{-10} M)	
	From k_{-1}/k_1	0.5–1
	From Scatchard plots	1.5

The specificity of angiotensin II receptors has been evaluated by their affinity for angiotensin fragments and analogues. The specificity of smooth muscle and adrenal receptors is very similar. The inhibitory effect of various angiotensin-derivatives on [^{3}H]angiotensin binding is shown in Fig. 1. The Sar^1 Ile^8 or Sar^1 Ala^8 substituted derivatives, which are potent competitive antagonists on the three target-tissues, are efficient competitors for these receptors. Both the N- and C-terminal of angiotensin II are required for an optimal interaction with receptors: the affinity of the 2–8 heptapeptide is reduced and that of the 1–7 heptapeptide is almost nil. The low affinity of the 2-8 heptapeptide is in agreement with its low agonistic activity effect on smooth muscle and the 1-7 heptapeptide which does not bind to receptors, is devoid of effect on all target-organs. The 4-8 pentapeptide exhibits a very low, but undisputable affinity, thus supporting the predominance of the C-terminal structure in the hormone–receptor interaction. In smooth muscle the Phe^4 analog has a low affinity for receptor, which corresponds to its low contractile effect and demonstrates that the aromatic ring of the tyrosine residue in position 4, also plays a key-role for a perfect hormone receptor interaction. The comparison between binding studies and physicochemical studies performed with the use of nuclear magnetic resonance and circular dichroism, suggests that the angiotensin-conformation ensuring a perfect adjustment to the receptor site is a beta type [20].

In both smooth muscle and adrenals, the decapeptide angiotensin I, precursor of angiotensin II, has a low affinity for angiotensin II receptors. This result emphazises again the importance of angiotensin II C-terminal structure in the binding, agrees with the low intrinsic activity of the decapeptide, and confirms a low level of smooth muscle and adrenal converting enzyme.

2. *Angiotensin III receptors in corticoadrenals*

Angiotensin III, the 2-8 heptapeptide derivative of angiotensin II, circulates in the plasma of several species [21, 22]. It was considered as a metabolite of angiotensin II, resulting from the action of angiotensinase A on the octapeptide; it has recently been suggested that it may result from converting enzyme activity on des-Asp^1-angiotensin I [23–25]. Angiotensin III has been shown to be in many species, including rat, as potent as angiotensin II in stimulating aldosterone release [26–28]. The finding that angiotensin III had a only moderate affinity for angiotensin II receptors contrasting with its high intrinsic activity led us to investigate the binding of [^{3}H]angiotensin II to the rat adrenal cortex [29, 30]. The binding parameters of the radioactive peptide are indicated in Table 2. [^{3}H]angiotensin III appeared to bind to two classes of sites. The high affinity sites had an apparent $K_{d29°C}$ of 1.5×10^{-10} M and the low affinity sites had an apparent K_d of 4.6×10^{-9} M and a larger binding capacity. The specificity of these two classes of sites was not identical. As represented in the Fig. 2, the specificity of the former was maximal for a 2-8 heptapeptide structure, whereas that of the latter was maximal for the octapeptide structure. The similarity of the binding parameters of the low affinity heptapeptide binding sites with those of angiotensin II binding sites, suggests that they may be identical structures. This is further substantiated by their preferential affinity for the octapeptide. The demonstration of specific binding sites with high affinity for angiotensin III is of great physiological significance. The concentration of angiotensin II in plasma varies between 10^{-11} M and 10^{-10} M. Given a K_d value of 10^{-9} M, the occupancy of angiotensin II receptors would be

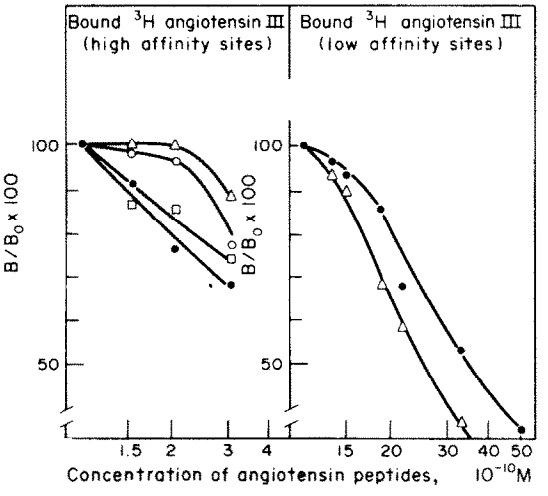

Fig. 2. Binding inhibition studies of [^{3}H]angiotensin III performed separately on the two classes of binding sites for this heptapeptide with 2-8 heptapeptide and octapeptide angiotensins. ●: angiotensin III; □: des-Asp^1-angiotensin II; △: angiotensin II; ○: Sar^1-Ile^8 angiotensin II. The 2-8 heptapeptide conformation demonstrates more effective inhibition on the high affinity [^{3}H]angiotensin III binding sites than the octapeptide conformation, while on the low affinity sites this order is reversed. The relative affinities of angiotensin II and angiotensin III for the low affinity sites is comparable with that observed when [^{3}H]angiotensin II is used as the label (see Fig. 1).

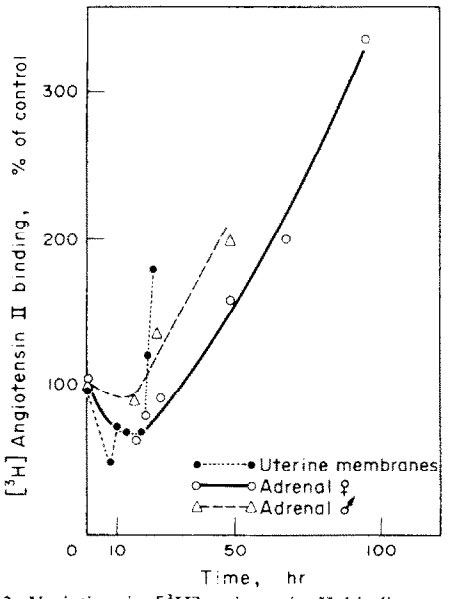

Fig. 3. Variation in [^{3}H]angiotensin II binding capacity of angiotensin II receptors on plasma membrane enriched fractions of rat uterus and adrenals following bilateral nephrectomy. In the hours immediately following nephrectomy a transient decrease in capacity was seen in all tissues. After 12–14 hr, a progressive augmentation in the number of receptors was observed which exceeded that in control tissues after 18–40 hr depending on tissue and sex. This augmentation was a function of the time elapsed.

minimal. Conversely, the interaction of angiotensin III which circulates at a concentration twice that of angiotensin II, with its high affinity of specific binding sites would appear more efficient as the K_d value of these sites is 1.5×10^{-10} M. This observation leads to the suggestion that angiotensin III actually represents the component of the renin–angiotensin system ultimately responsible for aldosterone release. This proposition is also supported by the effect of various angiotensin inhibitors which have been used as probes to conceive the molecular events occurring at level of the receptor site. It has been shown that the angiotensin II competitive inhibitors Sar^1 Ile^8 and Sar^1 Ala^8 angiotensin II which are relatively resistant to aminopeptidase activity [31], are potent antagonists of its pressor effect, but are less active on angiotensin II-induced steroidogenesis and even less effective against angiotensin III steroidogenic effects. Conversely, the des-Asp^1-Ile^8 heptapeptide was found to be a potent competitive inhibitor of both angiotensin II and angiotensin III aldosterone-releasing effect, supporting the concept that the active structure at the level of the adrenal cortex is the heptapeptide [32–34].

3. *Angiotensin II receptor modulation*

Bilateral nephrectomy, which results in the suppression of the renal renin and hence in that of major proportion (if not all) of the circulating angiotensin is followed by an increase in the number of angiotensin receptors. This observation has been made on rat uterus [10, 11] and rat adrenals [12]. A lag period of at least 18 hr separates nephrectomy and the beginning of the phenomenon, which then increases with time. A 2-fold increase in angiotensin II binding sites is clearly observed in adrenals between 45–60 hr after nephrectomy. In female rats, which are relatively tolerant to the anephric state, the post-nephrectomy phenomena are somewhat more delayed than in males (Fig. 3). The increase in angiotensin II receptors was not accompanied by a similar augmentation of other membrane-constituents (adenylcyclase, 5′ Nucleotidase, Mg-ATPase) and could not be attributed to changes in number or size of target-cells. The augmented receptors after nephrectomy did not exhibit changes in their apparent affinity or in their specificity for angiotensin II. The lag period after nephrectomy and the constancy of the K_d value demonstrates that the augmentation of receptor number is not simply the result of receptor freeing secondary to the disappearance of endogenous hormone. In addition, chronic administration (but not acute injections) of angiotensin II prevented the augmentation in number of binding sites in a dose-dependent manner. A similar limitation was obtained with the injection of an angiotensin II competitive inhibitor, suggesting that variations of angiotensin II binding sites may result primarily from their occupancy by a ligand, independant of its biological effect. An inverse relationship between circulating concentrations of an hormone and the number of receptors has been observed with many other hormones and transmitters and designated as "self-regulation of receptors" [35]. The long period necessary for the appearance of the phenomena suggests that it proceeds from complex molecular mechanisms such as variations in the rate of synthesis or degradation or from cellular translocation.

One may conceive that the increase in the receptor number may determine an increased sensitivity to the hormone. A specific supersensitivity to angiotensin II has indeed been observed on rat uterus after removal the kidneys. Increased vasopressor action of angiotensin II has been long reported, but no binding studies have been performed so far on vascular smooth muscle. It is not known at the present time if the steroidogenic effect of angiotensin in the rat is potentiated after nephrectomy, but preliminary studies from our laboratory have shown that angiotensin III receptors follow the same variations in the anephric state.

It is likely that chronic variations in circulating angiotensin are not the only factor capable of modulating angiotensin receptors. Studies from our own laboratory and others [36] have shown that in the state of positive sodium balance, where the circulating level of angiotensin is low, the number of adreno-cortical receptors appears to be decreased; conversely this number is increased by sodium restriction.

These various results, although not understood in molecular terms, represent an additional argument in favour of the complexity of an hormonal effect which not only depends on hormone secretion but also on the variations of receptors in target cells.

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