Recent Advances in Angiotensin Research

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The complexity of the renin–angiotensin system (RAS) has increased considerably in the past 2 decades. From a hormonal system occurring exclusively in the circulation, it moved into the direction of a local system existing in virtually every organ of the body. In both cases, the active end product still was angiotensin (Ang) II, acting via its classical type 1 (AT$_1$) receptor. Next, multiple novel angiotensin metabolites and receptors were discovered, all having effects of their own and often opposing those of the Ang II–AT$_1$ receptor pathway, for instance the Ang III–Ang II type 2 (AT$_2$) pathway and the Ang (1–7)–Mas receptor pathway. As a consequence, RAS stimulation and RAS blockade may have wide-ranging effects, depending on what angiotensin metabolites/receptors are up- or downregulated and often occurring in a disease- and tissue-specific manner. This review critically addresses angiotensin-related work published in *Hypertension* over the past 2 years, focusing on the brain, pregnancy, sex-related aspects, and the kidney.

Brain

All major angiotensin receptors (AT$_1$, AT$_2$, and Mas) are expressed in the brain. An important question is what angiotensin these receptors actually see: blood derived or locally synthesized Ang II, Ang III, and Ang-(1–7)? And in case of local synthesis, does a so-called brain RAS, acting independently of the circulating RAS, truly exist? An independent brain RAS would require the presence of renin, angiotensinogen, and angiotensin-converting enzyme (ACE) at levels that allow a meaningful interaction, that is, that results in angiotensin concentrations that are sufficiently high to stimulate these receptors. For instance, at the reported angiotensinogen levels in cerebrospinal fluid, corresponding with only 10% to 15% of the plasma levels,[1] one would require brain renin levels that are roughly 10-fold those in plasma to at least yield the same levels of angiotensins as in blood plasma. At renin and angiotensinogen levels that are both only 10% to 15% of those in plasma, angiotensin generation would be substantially diminished when compared with plasma. In fact, brain renin levels, if anything, are believed to be even lower than the above 10% to 15%, and, therefore, recently the idea has come up that in the brain it is prorenin, the inactive precursor of renin, which generates angiotensins locally, for instance, after its binding to the so-called (pro)renin receptor [(P)RR]. Here, we should keep in mind that (P)RR–prorenin interaction requires prorenin levels in the high nanomolar range, that is, several orders of magnitude above its plasma levels.[2] Whether such high prorenin levels occur in the brain needs to be confirmed. A final possibility is the local generation of angiotensins by a truncated variant of prorenin, which exclusively acts intracellularly (Figure 1). Although this intracellular renin was thought to be specifically expressed in the brain, its knockout resulted in angiotensin-dependent hypertension,[3] that is, the opposite of what one would expect. This therefore strongly argues against a role for intracellular renin as an angiotensin-generating enzyme in the brain.

To study the role of the brain RAS, the following procedures have been applied to selectively elevate brain angiotensin levels: human renin+human angiotensinogen were expressed in the mouse brain,[4] Ang II was administered via intracerebroventricular infusion (40 ng/min),[5] by microinjection in the paraventricular nucleus (0.2 nmol)[6] or subcutaneously (120 ng/kg per minute),[7] or Ang (1–7) was infused intracerebroventricular (200 ng/h).[8] One study administered the ACE2 activator diminazene (0.75–15 mg/kg) via intraperitoneal injection.[9] Because ACE2 generates Ang (1–7), this approach should theoretically increase brain Ang (1–7) levels, although a recent investigation denied this effect.[10] From the above approaches, it was concluded that Ang II in the brain induces thirst in a protein kinase C-dependent manner[4] and increases sympathetic outflow and blood pressure via Rho kinase activation,[5] the latter involving AT$_1$ receptors on paraventricular nucleus astrocytes.[6] Leptin upregulated the brain RAS (ACE and AT$_1$ receptors), thereby sensitizing the body to Ang II–induced hypertension during a high-fat diet.[7] Conversely, Ang (1–7) in the brain normalized blood pressure (but not leptin levels) in fructose-fed rats displaying the metabolic syndrome[8] and was suggested, on the basis of diminazene application, to protect against stroke.[9] To what degree these approaches truly increased brain Ang II or Ang (1–7) was not investigated, nor whether the alterations were in the physiological range. Thus, at this stage, all we can conclude is that, in the brain, artificial elevation of Ang II is detrimental, whereas elevation of Ang(1–7) is beneficial. Where brain Ang II and Ang (1–7) normally originate cannot be deduced from these studies.

One condition where endogenous brain RAS activation is thought to occur is neurogenic hypertension, induced by deoxycorticosterone acetate (DOCA)–salt treatment. Interestingly,
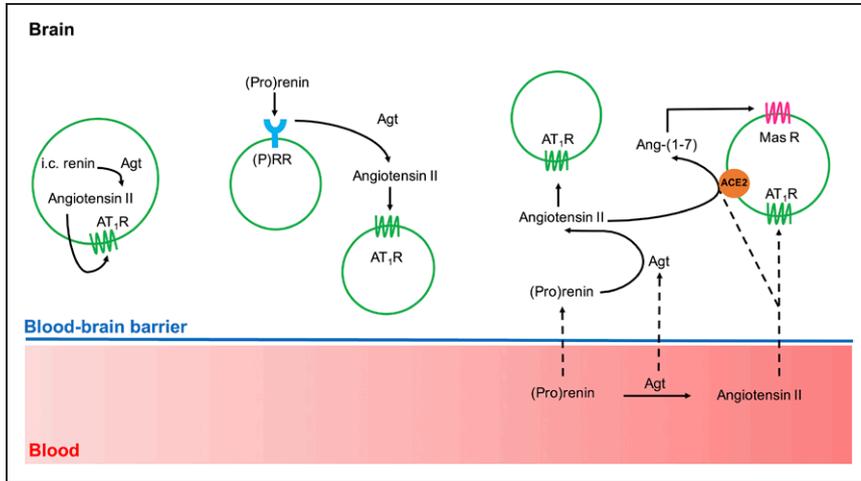
Figure 1. Scheme depicting 3 proposed mechanisms leading to activation of the brain renin–angiotensin system (RAS). From left to right, brain angiotensin II is generated intracellularly (IC) by truncated prorenin and then activates angiotensin type 1 receptors (AT_1R) in an autocrine manner; angiotensin II is generated from local angiotensinogen (Agt) by (pro)renin, after its binding to the (pro)renin receptor ((P)RR); and finally, brain angiotensin II depends on the circulating RAS, with either plasma pro(renin) and angiotensinogen crossing the blood–brain barrier and then reacting locally or plasma angiotensin II itself crossing this barrier. Angiotensin II, independent of its source, might be converted to angiotensin (1–7) by brain angiotensin-converting enzyme (ACE) 2, which might then act on Mas receptors (Mas R).

brain ACE2 overexpression attenuated such hypertension, by preventing cyclooxygenase upregulation in the paraventricular nucleus.[11] Brain (P)RR knockout also blocked it, and the authors speculated that this was because of the fact that, without the (P)RR, prorenin could no longer generate Ang I.[12] Yet, recently, we were unable to detect a rise in brain renin after DOCA-salt, whereas brain prorenin was virtually undetectable, both with and without DOCA-salt.[13] In fact, brain renin levels closely followed plasma renin levels and thus decreased after DOCA-salt. Therefore, the presumed DOCA-salt–induced brain RAS induction is questionable, and ACE2 upregulation and (P)RR knockout are more likely to exert their blood pressure-lowering effects via non-RAS mechanisms.

In the absence of evidence of angiotensin generation at brain tissue sites, we need to consider the possibility that brain angiotensin is plasma derived (Figure 1). Indeed, Biancardi et al[14] observed circulating Ang II to extravasate to multiple brain regions. Moreover, Ang II disrupted blood–brain barrier (BBB) integrity, thus facilitating its own access to brain regions that are normally inaccessible to angiotensins because of the presence of the BBB. In addition, Ang II will obviously accumulate at sites outside the BBB, such as the subfornical organ and area postrema. Potentially, from there, information might be conveyed to centers within the BBB, for instance, the centers that contribute to neurogenic hypertension, such as the paraventricular nucleus, rostral ventrolateral medulla, and the nucleus tractus solitarii in the hypothalamus and brain stem. Data demonstrating that the brain/plasma Ang II ratio decreased by >90% after AT_1 receptor blockade suggest that the accumulation of plasma Ang II in the brain occurs in an AT_1 receptor–dependent manner.[13] A unifying concept might therefore be that circulating Ang II gains access to the brain, particularly when the BBB is disturbed (eg, after DOCA-salt), and that intracerebroventricular Ang II infusion or brain renin+angiotensinogen overexpression mimics this situation. To what degree this is also valid for other angiotensin metabolites, such as Ang III and Ang (1–7), remains to be evaluated, particularly because, for instance, AT_2 receptors do not internalize. A logical consequence of ACE2 overexpression or activation might be that Ang II sequestered from the circulation is degraded more rapidly, thus explaining the beneficial effects of these approaches.

Pregnancy

Ang II responsiveness is greatly enhanced in preeclampsia. The mechanism behind this is still unknown. Women with preeclampsia are believed to display elevated levels of AT_1
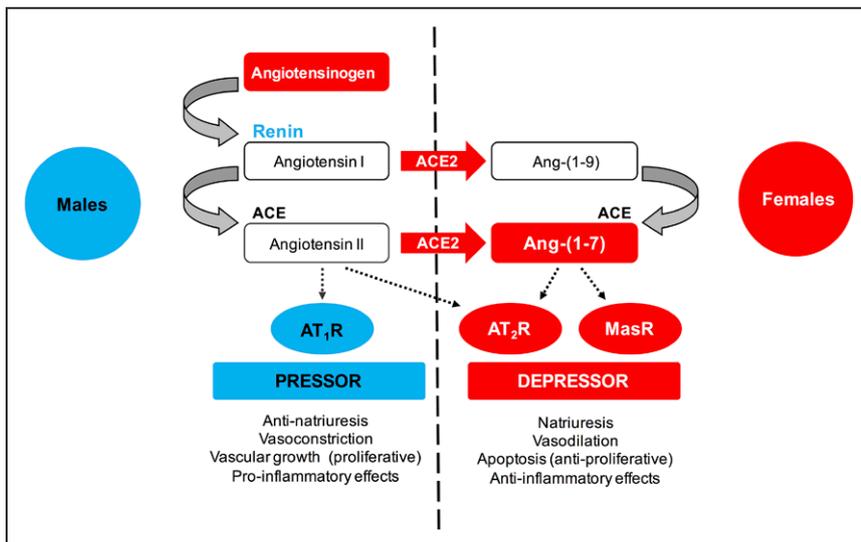

Figure 2. Sex differences in the renin–angiotensin system (RAS). Sex hormones and chromosomes differentially enhance the expression of various angiotensin fragments and receptors, with the pressor pathway and classical effects of RAS stimulation enhanced in males (blue) and the nonclassical or so-called protective arm of the RAS enhanced in females (red). ACE, angiotensin-converting enzyme; AT_1R, angiotensin II type 1 receptor; AT_2R, angiotensin II type 2 receptor; and MasR, Mas receptor.

receptor autoantibodies, and 1 possibility is that these autoantibodies, by binding to the second extracellular loop of the AT$_1$ receptor, increase Ang II sensitivity. In support of this concept, Cunningham et al,[15] after administering Ang II, AT$_1$ receptor autoantibodies, or both to pregnant rats, observed the largest decrease in renal blood flow and glomerular filtration rate during combined infusion. Yet, blood pressure rises were identical under all conditions, raising the question why the consequences of this interaction were only seen in the kidney. Verdonk et al,[16] based on studies in abdominal subcutaneous arteries from both healthy and preeclamptic women, suggested that the enhanced response might relate to the occurrence of vasoconstrictor AT$_2$ receptors. However, AT$_2$ receptors also display protective effects in pregnancy, by facilitating the decrease in blood pressure and by suppressing immune system activation in midgestation in mice.[17] Moreover, Chinnathambi et al[18] observed that testosterone decreased vascular AT$_2$ receptor density in pregnant rats and still enhanced the contractile response to Ang II. Thus, at least in healthy pregnant rats and mice, a phenotypic change of AT$_2$ receptors from relaxant to constrictor does not seem to underlie the increase in Ang II constrictor sensitivity. Of interest, Pulgar et al[19] were able to diminish the increased Ang II contraction in isolated uterine arteries by stimulating cannabinoid type 1 receptors with anandamide. However, this approach did not affect Ang II sensitivity, and thus most likely it represented physiological antagonism, that is, it would also have diminished the effect of other contractile agents. Finally, Liu et al[20] induced preeclampsia in mice by injecting AT$_1$ receptor autoantibodies from preeclamptic women. They observed that these autoantibodies, by activating tissue transglutaminase (which modifies proteins by forming isopeptide bonds), increased AT$_1$ receptor stabilization. Although this might have translated into increased Ang II responsiveness, the authors did not investigate this aspect.

An important question remains whether AT$_1$ receptor autoantibodies can function as a marker of preeclampsia. If such autoantibodies stimulate AT$_1$ receptors, one would expect preeclamptic women to display increased aldosterone levels and decreased renin levels, and thus a greatly decreased aldosterone/renin ratio. Remarkably, this is not the case.[16] To explain this discrepancy, one has to assume that either the autoantibodies selectively stimulate AT$_1$ receptors (eg, only in the vessel wall, but not in the kidney or adrenal), or that they are nonfunctional biomarkers. In addition, until now the autoantibody quantification has not occurred uniformly. Both indirect assays (quantifying signaling responses in AT$_1$ receptor–expressing cells and chronotropic responses in neonatal rat cardiomyocytes) and direct assays (ELISA) have been applied.[19,20] Ideally, before concluding that AT$_1$ receptor autoantibodies are preeclampsia markers, the various assays should be rigorously compared, and we need to understand why the antibodies do not always act as AT$_1$ receptor agonists.

Sex

Sex differences exist in response to RAS stimulation (Figure 2). We, and others, have shown that estrogen enhances AT$_2$ receptor expression and that the AT$_2$ receptor plays a greater role in the regulation of blood pressure and renal function in adult females when compared with age-matched males.[21,22] Kemp et al[22] confirmed this view with regard to AT$_2$ receptor–induced natriuresis during concomitant AT$_1$ receptor blockade but nevertheless observed identical C21-induced natriuretic effects in male and female rats without such blockade. Recently, Liu et al[23] demonstrated that the AT$_2$ receptor contributes to sex differences in the autonomic regulation of blood pressure. Moreover, in adult spontaneously hypertensive rats, Hilliard et al[24] have shown that direct AT$_2$ receptor stimulation elicits natriuretic effects in females but not males, suggesting that AT$_2$ receptor agonists may be a beneficial therapeutic approach in premenopausal women with hypertension and associated renal disease. Thus, overwhelming evidence suggests that the AT$_2$ receptor contributes to the cardiovascular protection in premenopausal women. Consequently, after the onset of menopause, reduced AT$_2$ receptor expression and activation may contribute to the increase in cardiovascular risk in women as they age. Although there is little evidence concerning age-related changes in AT$_2$ receptor expression and function in humans, it has been shown that deficits in renal AT$_2$ receptor expression contribute to a rightward shift of the chronic pressure–natriuresis relationship and enhanced pressor responsiveness to Ang II in postmenopausal mice when compared with their adult counterparts.[21]

Whether the cardiovascular protective effects of the AT$_2$ receptor can be maintained in aging females is yet to be investigated. Pessôa et al[25] demonstrated that AT$_2$ receptor–induced relaxation of mouse iliac arteries requires not only female sex hormones but also the XX sex chromosome complement. This finding suggests that estrogen replacement may restore the protective effects of the AT$_2$ receptor in postmenopausal females. Given the controversies surrounding the effect of hormone replacement therapy on cardiovascular risk, we speculate that a superior alternative to estrogen replacement may be an AT$_2$ receptor agonist. Unfortunately, the degree of AT$_2$ receptor stimulation that might occur during treatment with an AT$_1$ receptor antagonist is of no significance because there currently is no evidence that the clinical effect of AT$_1$ receptor antagonists differs from that of ACE inhibitors.[26] However, this may be because of studies being underpowered to detect sex differences. In a retrospective study of heart failure patients, ACE inhibitors were reported to be more efficacious in men, whereas AT$_1$ receptor blockers were superior in women.[27] Moreover, in postmenopausal women, AT$_1$ receptor antagonism induces a vasodilatory response to Ang II which is blocked by AT$_2$ receptor antagonism.[28]

Given that T lymphocytes express angiotensin receptors and that Ang II stimulates T-cell proliferation,[29] sex differences in response to RAS stimulation may, in part, be mediated by the adaptive immune system. In males, T cells play a key role in the development of Ang II–induced hypertension,[30,31] and T cells with intact AT$_1$ receptors are required to elicit this effect.[32] Two studies, published almost simultaneously, used Rag1$^{-/-}$ mice which lack T and B cells and have a blunted pressor response to Ang II, to investigate whether the sex of the T cell influences the ability of that T cell to restore the pressor response to Ang II.[33,34] These studies suggest that adoptively transferring female T cells protects against Ang II–induced hypertension and reduces T-cell infiltration into vascular and

renal tissues. However, these studies did not investigate the molecular mechanisms underlying how the female T cells were eliciting these effects (eg, via differences in AT_2/AT_1 receptor expression or alterations in signaling pathways). Therefore, detailed mechanistic studies are required to determine whether the immune system contributes in a meaningful way to the sex differences observed in response to RAS stimulation.

Kidney

The bulk of the filtered sodium is reabsorbed within the proximal tubule, whereas the distal tubule is responsible for the fine tuning of sodium reabsorption and arterial pressure. Using region-specific AT_1 receptor–deficient mice, the Coffman laboratory has demonstrated that selective deletion of the AT_1 receptor from the principal cells of the collecting duct decreases epithelial sodium channel activation and attenuates the initial phase of the pressor response to Ang II,[35] whereas proximal tubule AT_1 receptors are obligatory for Ang II–induced hypertension. Under physiological conditions, the dopamine D4 receptor inhibits AT_1 receptor–mediated sodium reabsorption via $Na^+–K^+$ ATPase in the proximal tubule.[36] However, during pathological situations, this pathway is dysfunctional, suggesting that deficits in the dopaminergic system contribute to AT_1 receptor–mediated antinatriuresis. Moreover, fructose, which is implicated in the development of cardiometabolic disease, has been shown to stimulate the sodium–hydrogen exchanger type 3 sodium transporter within the proximal tubule and sensitizes the proximal tubule to Ang II.[37] These findings further highlight the importance of proximal tubule AT_1 receptors in the pathogenesis of hypertension.

Interleukin (IL)-17A promotes hypertension and associated end-organ damage, and recent studies suggest that IL-17A contributes to the antinatriuretic effects of Ang II.[38–41] After a 2-week infusion of Ang II, Kamat et al[39] demonstrated that IL-17A–deficient mice were less sensitive to the pressor effects of Ang II and excreted more sodium after a saline load than their wild-type counterparts. This was associated with a reduction in sodium–hydrogen exchanger type 3 and sodium-dependent phosphate transporter 2 abundance within the proximal tubule. Conversely, after a 4-week infusion of Ang II, the attenuated pressor response to Ang II in IL-17A–deficient mice was associated with a reduction in the abundance and phosphorylation of the Na–Cl cotransporter and cleavage (activation) of the γ-subunit of epithelial sodium channel within the distal tubule.[40] These findings indicate a biphasic effect of IL-17A on renal sodium handling. The role of the AT_1 receptor and changes in activation of the angiotensin receptors, which could be upstream or downstream of IL-17A, is yet to be investigated in this model.

Furthermore, of pertinence to study of RAS stimulation on renal sodium handling, Veiras et al[42] have demonstrated that Ang II–dependent hypertension induces kaliuresis which in turn alters sodium transporter activity in the distal nephron. In male Sprague Dawley rats, the accumulation and phosphorylation of Na–Cl cotransporter during Ang II–induced hypertension was secondary to K^+ deficiency driven by epithelial sodium channel stimulation as doubling dietary K^+ intake normalized K^+ excretion which prevented changes in Na–Cl cotransporter activity. This finding raises important questions about whether the changes reported in distal nephron sodium transporters during Ang II–dependent hypertension are because of Ang II or are a consequence of potassium deficiency. Potassium status is likely to be an important methodological consideration for future studies investigating RAS stimulation and renal sodium handling.

The determination of RAS components in urine is currently gaining attention as a simple approach to obtain information on intrarenal RAS activity.[43] It is possible that renal RAS activity differs from extrarenal RAS activity, for instance, during treatment with renin inhibitors because of their selective accumulation in the kidney.[44,45] Michel et al[46] described that urinary angiotensinogen excretion associates with blood pressure, independently of the systemic RAS, in a salt-sensitive, low-renin population of African ancestry. Unfortunately, these authors did not correct their data for urinary albumin excretion. Yet, in their population with a high prevalence of diabetes mellitus and hypertension, albuminuria might be expected in a considerable proportion of their subjects. Given the comparable molecular sizes of albumin and angiotensinogen, identical amounts of both proteins will be filtered across the glomerular membrane. Indeed, in diabetic patients, the urine/plasma concentration ratios of albumin and angiotensinogen are highly correlated, and thus, urinary angiotensinogen, like albumin, is foremost a marker of glomerular permeability.[47] Under conditions where urinary angiotensinogen excretion does not follow albumin excretion, an additional concept that needs to be considered is that angiotensinogen is reabsorbed differently in the proximal tubule when compared with albumin. Such reabsorption has been reported to be megalin dependent.[48] For instance, in patients with Dent's disease or Lowe syndrome, disorders characterized by defective proximal tubular reabsorption, urinary angiotensinogen excretion increased 20- to 40-fold.[49] Moreover, similar increases in urinary renin and prorenin excretion were observed in these patients, yielding urinary renin and prorenin levels that were almost as high as their plasma levels. Yet, even when rising ≤40-fold urinary angiotensinogen still corresponded to only 1% to 2% of its levels in plasma. This is because of much larger molecular size of angiotensinogen when compared with renin and prorenin, causing its glomerular sieving coefficient to be far below that of renin and prorenin.[49,50] Taken together, before concluding that urinary RAS components truly reflect renal RAS activity in an independent manner, we need to be certain that the variation of their urinary levels does not simply reflect differences in glomerular sieving and tubular reuptake of plasma RAS components. Here, it should also be taken into consideration that Ang II itself suppresses the protein expression of megalin.[51] Consequently, under conditions of intrarenal RAS activation, such as in diabetes mellitus, urinary renin and angiotensinogen levels might selectively increase, whereas RAS blockade should normalize this phenomenon.[52]

Finally, Giani et al[53] addressed the question whether the salt sensitivity occurring after chronic L-NAME (N^ω-nitro-L-arginine methyl ester HCl) exposure (inducing renal injury) depends on renal ACE. To this end, they made use of mice with genetically reduced renal ACE activity, resulting in >90% lower renal ACE levels. Such reductions also occur during ACE inhibition in humans and are well known to be

overcome (Ang II escape) by massive rises in renin. Indeed, in humans, renin rises of >300-fold have been reported, capable of overcoming >99.5% ACE inhibition.[44] The unaltered renal Ang II levels at baseline in the low-ACE mice in Giani et al[53] study support this concept. L-NAME pretreatment identically increased blood pressure in wild-type and low-ACE mice, apparently in a renal ACE-independent manner. Subsequently, exposing the mice to a high-salt diet resulted in hypertension in wild-type mice only, leading the authors to suggest that L-NAME–induced renal RAS activation is responsible for blunting the appropriate natriuretic response to a sodium load. Surprisingly, the authors attributed the renal RAS activation to ≈2-fold rises in renal angiotensinogen and ACE, despite the fact that rises in renin are the true determinant of this phenomenon. In fact, the low angiotensinogen levels in the low-ACE mice most likely reflected high renin levels in these animals, apparently consuming all available angiotensinogen to keep renal angiotensin in the normal range. An alternative explanation of the data would therefore be that low-ACE mice can no longer upregulate renin. This is an inherent consequence of the model. Knowledge on the renin levels in the various experimental low-ACE set-ups is anxiously awaited to solve this issue. In addition, uptake of Ang II from blood plasma should be considered, although under normal circumstances this is a negligible contributor to renal Ang II levels.[54]

Summary and Conclusions

Our knowledge of the RAS continues to evolve. In this brief review, we have highlighted recent advances in angiotensin research published in *Hypertension* that have provided novel insight into the physiological and pathophysiological mechanisms of RAS stimulation and the important questions these studies raise. Understanding the mechanisms that contribute to RAS activation and the response to RAS stimulation is vital to the development of novel targets for the treatment of cardiovascular and renal diseases.

Sources of Funding

Disclosures

None.

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