Introduction

Angiotensin II (Ang II) is the primary effector molecule of the renin-angiotensin system (RAS). It is produced both systemically and locally in various tissues, including the heart and blood vessel walls. Ang II binds to two high-affinity receptors, designated the Ang II type-1 receptor (AT1) and the Ang II type-2 receptor (AT2). Signalling through the AT1 receptor results in vasoconstriction, stimulation of growth, and activation of fibroblasts and myocytes. Signalling through the AT2 receptor results in vasodilatation and antiproliferative responses, as well as an increase in apoptosis. It appears therefore that most of the damaging effects of Ang II are mediated by the AT1 receptor ***(Klahr and Morrissey, 2000).***

The synthesis of Ang II is dependent on angiotensinogen (AGT) production, which is converted by renin to angiotensin I (Ang I) and then by angiotensin-converting enzyme (ACE) to Ang II ***(Parsa et al., 2002).***

Ang II is a vasoactive peptide and growth factor that contributes to vascular reactivity, tissue remodeling and fibrosis ***(Parsa et al., 2002).*** It has also the autocrine and paracrine pro-inflammatory properties and acts not only as a cytokine but also activates the transcription nuclear factor κB (NF-κB).The subsequent production of pro-inflammatory cytokines, chemokines and adhesion molecules, recruits inflammatory

cells into the tissue. These cells in turn activate the RAS and increase the generation of Ang II locally, thus creating a cycle of tissue injury ***(Dabiri et al., 2007).***

These inflammatory pathways are critical in maintenance of disease in rheumatoid arthritis (RA). Furthermore, the wide distribution of all components of RAS system in RA indicates that locally formed Ang II in inflamed joints has a pathophysiological and therapeutic implication in RA ***(Dabiri et al., 2007).***

Ang II is also a pleiotropic molecule and strong candidate as a mediator of the development and progression of renal disease in systemic lupus erythematosus (SLE). It is also a potent pro-inflammatory modulator with the ability to augment and perpetuate immune responses in renal and non-renal tissues ***(Parsa et al., 2002).***

Aim of the Work

This work aimed to measure serum angiotensin II among a group of Egyptian children with pediatric systemic lupus erythematosus and juvenile rheumatoid arthritis. Its relationship with disease status and characteristics in addition to the different therapeutic modalities in both groups was fully studied.

Serum Angiotensin II

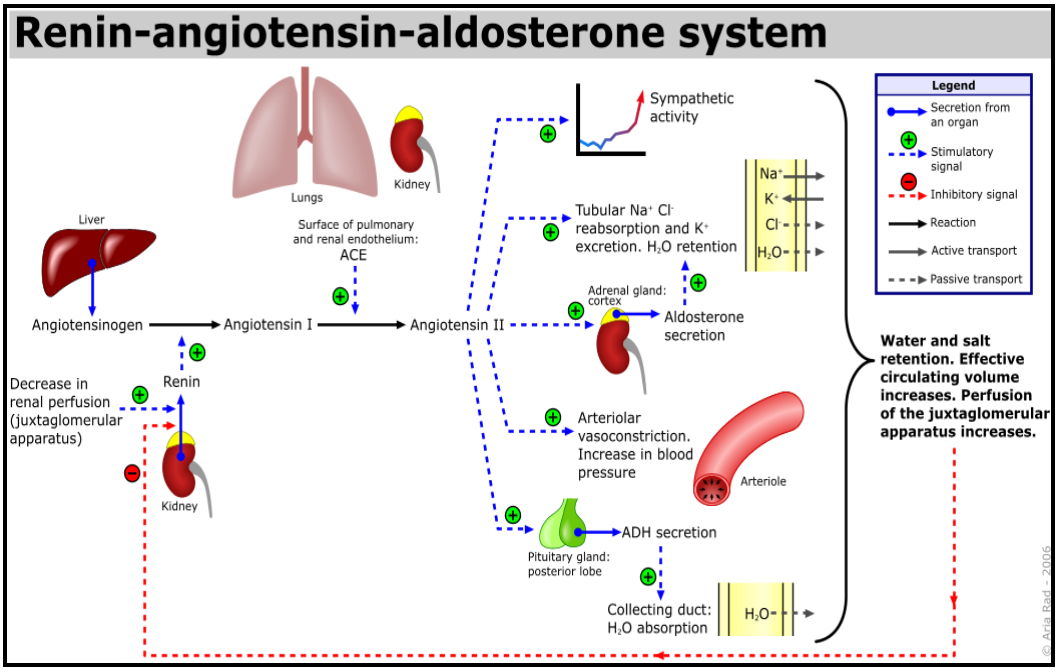
The renin-angiotensin system (RAS) plays a vital role in regulating the physiological processes of many systems. Not only does it function as an endocrine system, but it also serves local paracrine and autocrine functions in tissues and organs. The primary effector molecule of this system, angiotensin II (Ang II), has emerged as a critical hormone that affects the functions of virtually all organs, including heart, kidney, vasculature, and brain, and it has both beneficial and pathological effects (Figure 1) ***(Mehta and Griendling, 2007)***.

Ang II is an oligopeptide of eight amino acids, formed from its original precursor, angiotensinogen (AGT), by a series of two enzymatic cleavages ***(Smith and Morgan, 2012).***

First renin, a proteolytic enzyme produced by the kidney, is released into the circulation and acts on AGT, a circulating protein (alpha 2-globulin) produced by the liver. Renin cleaves AGT to produce angiotensin I (Ang I) a small fragment of only 10 amino acids ***(Wang et al., 2000).*** Ang I in turn is cleaved by angiotensin converting enzyme (ACE), to produce the octapeptide Ang II ***(Smith and Morgan, 2012).***

The concentration of ACE is highest in the lung and it had been thought that most Ang II formation occurred in the pulmonary circulation ***(Smith and Morgan, 2012).*** As all blood leaving the kidneys and liver eventually flows through the lung, the pulmonary vascular endothelium plays a major role in the

rapid conversion of Ang I into Ang II. Finally, Ang II will bind to specific cell surface Ang receptors to elicit multiple actions ***(Wang et al., 2000).***



ACE: Angiotensin converting enzyme; **ADH:** Anti-diuretic hormone

Figure (1): The renin angiotensin system ***(Quoted from Basso and Terragno, 2001).***

ACE is produced in the vascular endothelium of many tissues, thus Ang II can be synthesized at a variety of sites, including the kidney, vascular endothelium, adrenal gland, and brain. There are enzymes other than ACE could also perform the conversion of Ang I into Ang II but to a limited extent, such as tissue plasminogen activator, cathepsin G and tonin ***(Siragy, 2006).*** Also human heart chymase may be one of such enzymes but its clinical significance remains uncertain ***(Smith and Morgan, 2012).***

Similarly, Ang I and Ang II can be generated by alternate enzymatic pathways other than renin, such as tonin and cathepsin D, which can promote the formation of Ang I by cleavage of AGT. However, the contribution of these alternative pathways in Ang II production in humans is still unclear ***(Ren et al., 2002).***

Ang II is the most powerful biologically active product of the RAS, although there are other bioactive Ang peptides, including Ang III and Ang IV ***(Paul et al., 2006).***

In this regard, the kidneys, as well as the adrenal glands, are unique in terms of the tissue concentrations of Ang II, which are much greater than can be explained by the concentrations delivered by the arterial blood flow ***(Ingert et al., 2002).*** There is substantial evidence that the major fraction of Ang II present in renal tissues is generated locally from AGT delivered to the kidney as well as from AGT locally produced by proximal tubular cells. Ang I delivered to the kidney can also be converted to Ang II ***(Rosivall and Navar, 1983; Komlosi et al., 2003).***

ACE can promote the degradation of bradykinin, substance P and other small peptides. Although the physiological role of this enzymatic conversion is unclear, pharmacological blockade of ACE with specific inhibitors leads to an accumulation of bradykinin and substance P, which may be responsible for some of the beneficial effects (antihypertensive), but also some of the adverse effects (angioedema, cough) of ACE inhibitors (ACEIs) ***(Ortiz et al., 2001).***

On the other hand, a related enzyme to ACE has been identified and called angiotensin converting enzyme 2 (ACE2). ACE2 is predominantly expressed in the endothelium of the coronary and intrarenal vessels and the epithelium of the renal tubules ***(Rose and Post, 2012).***

ACE2, cleaves one amino acid from either Ang I or Ang II, decreasing Ang II levels and increasing the metabolite Ang 1–7. Thus the balance between ACE and ACE2 is an important factor controlling Ang II levels ***(Mehta and Griendling, 2007)***. Similarly ACE2, cleaves only a single amino acid from the C terminals of Ang I to form the nonapeptide Ang 1–9, whereas ACE2 does not convert Ang 1–9 to Ang II ***(Shaltout et al., 2007).*** Moreover, the enzymes that control generation of Ang III and Ang IV are aminopeptidases (Figure 2) ***(Watanabe et al., 2005).***

The physiologic importance of ACE2 is not completely understood. Disruption of the ACE2 gene in mice results in markedly reduced cardiac contractility and upregulation of hypoxia-induced genes in the heart, suggesting a role in the response to cardiac ischemia. But disruption of ACE2 did not interfere with blood pressure regulation. In contrast to ACE, ACE2 is not inhibited by ACEIs and does not metabolize bradykinin ***(Rose and Post, 2012).***

Ang 1-7 is a metabolite of Ang II. Considerable interest in Ang 1-7 and its receptor aroused in the last few years since it became apparent that it can counterbalance most of Ang II effects. Thus Ang 1-7 has vasodilator and hypotensive effects as well as

antiarrhythmic and cardioprotective roles ***(Santos et al., 2008).*** As Ang 1-7 stimulates the production of nitric oxide (NO), and improves endothelial function and vasodilator prostaglandins (PGs). In addition, in the kidney Ang 1-7 affects sodium and water flux at both the proximal and distal tubules ***(Siragy, 2006).***

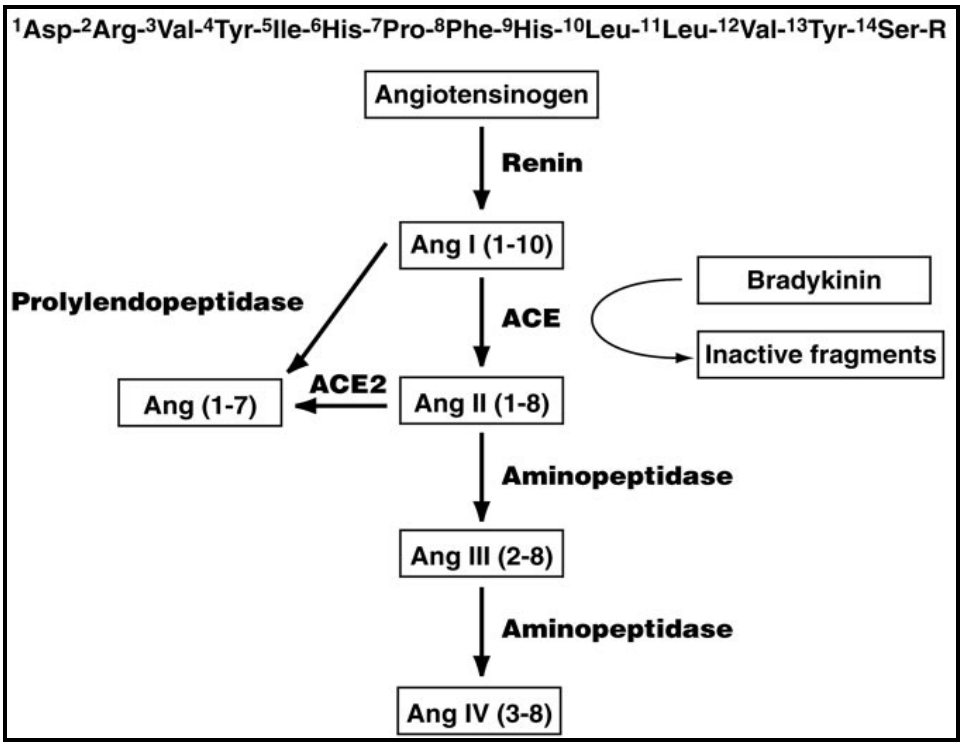


Figure (2): Metabolism of angiotensinogen to generate the peptide hormones of the renin-angiotensin system. **Ang:** angiotensin, **ACE:** angiotensin converting enzyme ***(Quoted from Watanabe et al., 2005).***

- **Ang II receptors:-**

There are two well-described subtypes of Ang II receptors, designated Ang II type 1 receptor (AT1) and Ang II type 2 receptor (AT2), both of which have a high affinity for Ang II ***(Smith and Morgan, 2012).*** They are important in the RAS as they are responsible for the signal transduction of the vasoconstricting stimulus of Ang II ***(Higuchi et al., 2007).***

The Ang receptors are a class of G protein-coupled receptors with Ang II as their ligands (Figure 3). The activated receptor activates phospholipase C and increases the cytosolic Ca^{2+} concentrations, which in turn triggers cellular responses such as stimulation of protein kinase C. Activated receptor also inhibits adenylate cyclase and activates various tyrosine kinases ***(De Gasparo et al., 2000).***

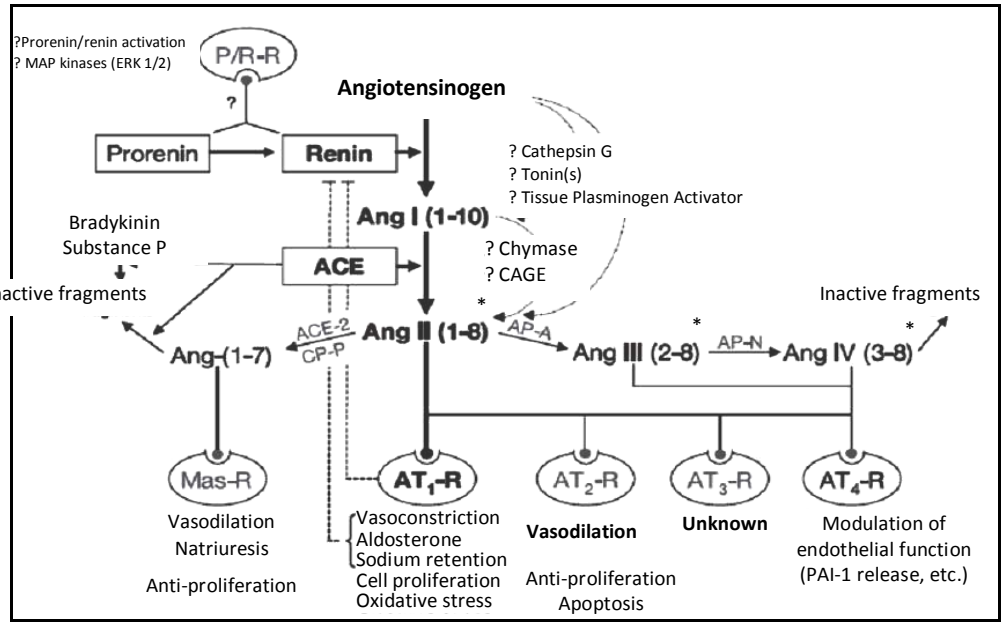


Figure (3): Pivotal role of the renin/prorenin receptor in Ang II production and cellular responses to renin ***(Quoted from Nguyen et al., 2002)***

The classical RAS pathway is highlighted in boldface type. Renin, normally secreted in response to underperfusion of the kidneys (not shown), cleaves the decapeptide Ang I from angiotensinogen, and Ang I is converted to Ang II by ACE. The dashed lines indicate feedback inhibition of renin secretion, which occurs both via a direct AT1 receptor mediated action of Ang II ("short loop") and via AT1-mediated restoration of blood pressure and volume ("long loop"). Other pathways that are speculative or of unproven physiological significance in vivo are depicted in light text. Ang II can be converted to Ang 1-7 by ACE 2 or other carboxypeptidases. Ang II can also be cleaved by aminopeptidases to form Ang III and Ang IV. These peptides exert their biological effects by binding to various subtypes of Ang receptors. In addition, Ang 1-7 can be formed directly from Ang I by the action of endopeptidases (not shown), and further metabolism of peptides to inactive fragments involves several amino-, carboxy-, and endopeptidases. A number of other proteolytic enzymes are shown that potentially can contribute to Ang I or Ang II synthesis. Lastly, both renin and prorenin may exert direct cellular actions by binding to specific pronin/renin receptor. **ACE** = angiotensin-converting enzyme; **Ang** = angiotensin; **AP-A/AP-N** = aminopeptidase A/aminopeptidase N; **AT-R** = Ang receptor subtype; **CAGE** = chymostatin-sensitive Ang II-generating enzyme; **CP-P** = carboxypeptidase P**; MAP** = mitogen-activated protein**; Mas-R** = Mas receptor; **P/R-R** = prorenin/renin receptor; **PAI-1** = plasminogen activator inhibitor-1; ***** = number of amino acids.

- **AT1 receptors:**

Most of the known physiological effects of Ang II are mediated by AT1 receptors, which are widely distributed in all organs, including liver, adrenals, brain, lung, kidney, heart, and vasculature ***(Mehta and Griendling, 2007)***.

Most of the Ang II hypertensinogenic actions are generally attributed to the AT1 receptors ***(Ito et al., 1995).*** AT1 receptor transcript has been localized to proximal tubules, the thick ascending limb of the loop of Henle, glomeruli, arterial vasculature, vasa recta, arcuate arteries, and juxtaglomerular cells ***(Tufro-McReddie et al., 1993).*** In rodents, there are two AT1 receptor subtypes, with type 1a being the predominant subtype in all nephron segments, whereas type 1b is more abundant than type 1a only in the glomerulus ***(Bouby et al., 1997).*** In mature kidneys, type 1a receptors have been localized to the luminal and basolateral membranes of several segments of the nephron, as well as on the renal microvasculature in both cortex and medulla: smooth muscle cells of afferent and efferent arterioles, epithelial cells of the thick ascending limb of Henle, proximal tubular apical and basolateral membranes, mesangial cells, distal tubules, collecting ducts, and macula densa cells ***(Paxton et al., 1993; Harrison-Bernard et al., 1997; Miyata et al., 1999).*** This evidence is consistent with the localization of the transcript for the AT1 receptor subtypes in all of the renal tubular and vascular segments ***(Miyata et al., 1999).*** Nevertheless, renal microvascular functional studies

obtained from mice lacking the type 1a receptor gene have shown that the afferent arteriole has both type 1a and type 1b receptors, whereas the efferent arteriole only expresses type 1a receptors ***(Harrison-Bernard et al., 2003).*** The distinction of AT1 is, however, not relevant to humans, in which a single AT1 receptor type is found ***(Reckelhoff, 2001).***

In the kidney, most of the Ang II is localized in the interstitium and renal tubules. This compartmentalization of Ang II is related to the fact that AGT messenger RNA and protein are localized in the proximal tubules, and act as substrate for both tubular and interstitial Ang II formation ***(Siragy, 2006).***

AT1 receptors are responsible for most of the pathophysiological actions of Ang II. By promoting proliferation, inflammation and fibrosis, Ang II contributes to chronic diseases, such as hypertension, atherosclerosis, cardiac hypertrophy and renal injury ***(Esteban et al., 2006).***

The Ang 1-7 Mas receptor is the recently identified receptor of the biologically active heptapeptide Ang 1-7. The Ang 1-7 Mas receptor is expressed in several organs including heart, kidney, blood vessels, testis and brain. Studies with Ang 1-7 Mas receptor knockout mice have demonstrated the key role of this receptor in cardiovascular regulation as well as in the regulation of learning and memory ***(Alenina et al., 2008).*** It was reported that the G protein-coupled Mas receptor is a physiological antagonist of the Ang II AT1 receptor, and could

be of great importance as a target for pharmacological intervention in cardiovascular diseases ***(Siragy, 2006).***

- **AT2 receptors:**

Although most of the vasoactive effects of Ang II occur via AT1 receptors, AT2 receptors have been shown to exert anti-proliferative and pro-apoptotic changes in vascular smooth muscle cells (VSMCs), mainly by antagonizing AT1 receptors ***(Mehta and Griendling, 2007)***.

AT2 receptor is highly expressed in fetal tissue, including fetal aorta, gastrointestinal mesenchyme, connective tissue, skeletal system, brain, and adrenal medulla. AT2 expression declines after birth, suggesting that it may play an important role in fetal development, and can be induced later in adult life under pathological conditions ***(Shanmugam et al., 1996)***.

Similarly, AT2 receptor is expressed in kidney located in the vasculature, glomeruli, juxtaglomerular apparatus, tubules, afferent arterioles and renal capsule ***(Siragy, 2006).*** AT2 receptors are also expressed in lung and liver, but their exact role in carrying out the functions of Ang II remains undetermined ***(Mehta and Griendling, 2007)***.

Effects mediated by the AT2 receptor are suggested to include inhibition of cell growth, fetal tissue development, modulation of extracellular matrix (ECM), neuronal regeneration, apoptosis, cellular differentiation, and maybe vasodilation and left ventricular hypertrophy ***(D'Amore et al., 2005).***

Moreover, Ang II stimulation of the AT2 receptor could influence blood pressure through multiple mechanisms, including increased NO production, sodium excretion, and the inhibition of renin production ***(Siragy, 2006).***

- **Other types of Ang receptors:**

Other poorly characterized subtypes include the AT3 and AT4 receptors. The AT4 receptor is activated by the Ang II metabolite which is Ang IV, and may play a role in regulation of the central nervous system ECM, as well as modulation of oxytocin release ***(Benoist et al., 2011).***

➢ Physiological effects of Ang II:

- **Renal effects**

The majority of renally produced Ang II functions as a paracrine hormone. The concept of the renal paracrine function of Ang II is supported by the fact that Ang receptors are present in the renal vasculature, glomeruli and tubules, in close proximity to the site of Ang II production ***(Siragy, 2004).*** The renal tubules and interstitial compartments contain significantly higher levels (1000-fold) of Ang II compared with plasma. This renally produced Ang II can amplify its levels through the stimulation of renal AGT production ***(Kobori et al., 2002).***

The intrarenal RAS may explain the primary role of Ang II as a paracrine substance in the control of renal functions. The direct intrarenal actions of Ang II include renal vasoconstriction, tubular sodium reabsorption, sensitivity of

tubuloglomerular feedback, modulation of pressure-natriuresis, and promotion of renal tissue growth ***(Carey and Siragy, 2003a).***

All of the understood clinical effects of Ang II are mediated at the AT1 receptor sites. Ang II binds to the zona glomerulosa, stimulating secretion of aldosterone ***(Timmermans et al., 1993a).*** Aldosterone then plays a major role in regulating sodium excretion and systemic blood pressure. Also the local renal aldosterone system is upregulated by Ang II through AT1 receptor stimulation ***(Siragy, 2006).***

Moreover, Ang II contributes to the regulation of renal sodium handling through vascular and tubular mechanisms. As Ang II causes a reduction in renal blood flow, glomerular filtration rate (GFR) and vasoconstriction of the afferent and efferent arterioles, leading to a decrease in the amount of filtered sodium. Additionally, Ang II enhances sodium reabsorption in the loop of Henle by reducing medullary blood flow. And the Ang II tubular effects are mainly related to its ability to enhance the proximal and distal sodium /hydrogen ion exchanger (NHE), the basolateral membrane sodium/bicarbonate ion co-transporter, sodium/potassium ion ATPase activities and the distal tubular epithelial sodium channel ***(Nagami, 2004).*** Whereas the AT1 receptor stimulates sodium reabsorption, the AT2 receptor enhances sodium excretion. This effect could be related to the modulation of renal tubular NHE activities ***(Siragy, 2006).***

Ang II stimulates the expression of type IIa sodium-phosphate co-transporter, leading to the increased reabsorption of phosphate in the renal proximal tubule ***(Siragy, 2006).*** There are studies have also demonstrated that Ang II stimulates ammonia production and secretion in proximal tubule segments in the presence of acidosis ***(Nagami, 2004).***

On the other hand, in conditions characterized by severe impairment of renal perfusion, such as renal artery stenosis, the afferent circulation, which is dilated as a result of autoregulation, is relatively refractory to the constrictive actions of Ang II, and the predominant constriction of efferent arterioles by Ang II plays a major role in maintaining glomerular perfusion pressure and, thus, GFR ***(Paul et al., 2006).***

The regulation of intrarenal Ang II receptors in hypertensive conditions is complex because vascular and tubular receptors respond differently during high Ang II states ***(Navar et al., 2002).*** In general, high Ang II levels decrease glomerular AT1 receptor expression but increase tubular AT1 receptor levels ***(Cheng et al., 1995).*** But the activity of the AT2 receptor varies according to changes in blood pressure. Pressure overload increases vascular AT2 receptor expression and cGMP production ***(Siragy, 2006).***

- **Extra renal effects**

Some of the responses to AT1 stimulation by Ang II are coronary, efferent arteriole, and cerebral vasoconstriction. Ang II also binds to AT1 receptor sites in the heart, mounting