Effects of Olmesartan on Arterial Blood Pressure in Salt-Loaded Rabbits Receiving Infusions of Angiotensin II

Dler Qader Gallaly

PhD (Lecturer) of Medical Physiology, Physiology department, College of Medicine, Hawler Medical University- Erbil.

Salah Al-Din M.A. Al-Merani

PhD (Professor) of Medical Physiology, Physiology department, College of Medicine, Hawler Medical University- Erbil.

Abstract

Background and Objectives: Olmesartan proved to be quite effective in lowering blood pressure (BP) in rabbits received intracarotid (IC) infusion of angiotensin II (AngII) by blocking angiotensin type 1 receptors (AT1) competitively in brain sites outside the blood brain barrier accessible to AngII and also in the periphery. Until now, no studies have been done to examine this effect of Olmesartan in salt-loaded rabbits to intravenous (IV) and IC infusions of Ang II, therefore this study is performed.

Methods: After two weeks of salt-loading, local domestic rabbits were given IV or IC infusions of AngII (10ng/kg/min).The effects of AngII were first investigated alone. When the pressor effect was clearly apparent, Olmesartan was injected intravenously as a single dose of 0.6mg/Kg. The second dose of Olmesartan was injected after recovery when the BP recording was reasonably stable. The BP and heart rate (HR) were evaluated.

Results: Both IV and IC infusions of Ang II induced moderate but highly significant increases in arterial BP. Intravenous injection of Olmesartan produced essentially no change in BP, whereas the same dose induced clear-cut hypotension during IC infusion of Ang II. The pressor response to AngII was totally prevented by Olmesartan. The hypotensive effect of Olmesartan has disappeared after 30-60min indicating a relatively short elimination half-life of the drug in rabbits. The HR remained unchanged.

Conclusions: The rapid inhibition of the pressor response of Ang II by Olmesartan confirms a direct pressor effect of Ang II and reduces the role of other endogenous pressor substances.

Keywords: Olmesartan, Blood Pressure, salt-loading, Angiotensin II, Hypertention.

Introduction

Hypertension is known to be a major risk factor for cardiovascular, cerebrovascular and renal diseases including stroke, sudden cardiac death, and coronary artery diseases. The control of blood pressure (BP) in hypertensive patients can markedly reduce morbidity and mortality resulted from these lethal complications and save lives.[1,2,3] The number of adults with hypertension in 2025 is predicted to increase by about 60% to a total of 1.56 billion.[4]

Angiotensin II (Ang II) which is a potent vasoconstrictor that increases total peripheral resistance, is involved in the pathogenesis of essential hypertension, congestive heart failure, renovascular hypertension and renal diseases associated with hypertension.[5,6] These conditions have been treated with renin-angiotensin system blockers like Omlesartan which competitively antagonize the angiotensin II receptors and produce BP lowering effects.[5]

Ang II has been shown to be 10 times more active than noradrenaline in increasing the BP[7] and directly increases sodium reabsorption in the proximal tubule. It also alters renal hemodynamics and causes the release of aldosterone from the adrenal cortex.[8,9] Several mechanisms are involved in the pressor response to Ang including direct vasoconstriction, enhancement of both sympathetic discharge and catecholamine release.[10,11] These biological actions of Ang II are mediated by cell surface receptors, Ang receptors, type 1 (AT1) and type 2 (AT2). AT1 receptors which Olmesartan binds to, have been localized in the kidney, heart, vascular smooth muscle cells, brain, adrenal gland, platelets, adipocytes, and placenta.[12]

The antihypertensive effects of Olmesartan were demonstrated in seven placebo-controlled studies in a total of 2,693 patients with essential hypertension at daily doses ranging from 2.5 to 80 mg during short-term treatment of 6 to 12 weeks, each showing statistically significant reductions in peak and trough BP.[13,14] In addition to the periphery's role of Ang II in the control of BP, it plays an important role within the brain in the pathogenesis of hypertension and other cardiovascular disorders. Some studies reveal that the circulating Ang II is unable to cross the blood brain barrier (BBB) except in some disease when BBB is disrupted.[15] However, all components of the peripheral renin-angiotensin system (RAS) are also found in the brain but the role of endogenous RAS involving in cardiovascular disorders are still not fully understood.[16]

Additionally, Ang II in the brain regulates numerous physiological responses through its central actions in the brain, where it functions as a neurotransmitter or neuromodulator to influence BP, drinking behavior, salt appetite, and several neuroendocrine processes. Some of these responses are induced by the actions of circulating Ang II at the circumventricular organs (CVOs) and other

specialized regions, and others are influenced by locally formed Ang II generated within the brain itself. Although circulating hormones are effectively excluded from most parts of the brain by the BBB, neurons in the CVOs are accessible to many circulating ligands via the fenestrated endothelial cells of their dense capillary circulation. The pressor response to circulating Ang II is mediated partially by the area postrema and the subfornical organ (SFO), highly vascularized organ in the brain which does not have a BBB.[16,17,18]

Many studies have shown that the fall in blood pressure with salt reduction is significantly related to the degree of activation of the RAS, i.e. the greater the rise in plasma renin activity (PRA), and therefore Ang II and aldosterone, the smaller the fall in blood pressure.[19-25] When the RAS is blocked by an Angiotensin Converting Enzyme Inhibitor (ACE-I), BP becomes much more dependent on sodium and water balance, and changes in salt intake have much larger effects on BP.[26]

The aim of this study is to evaluate the effect of the angiotensin type 1 (AT1) receptor blocker (Olmesartan) on blood pressure and heart rate responses to Ang II given intacarotidly (IC) to salt-loaded rabbits.

Methods

Study design

The local domestic rabbits (Oryctolagus cuniculus) of both sexes weighted (1.2-2.0 kg) were used in this study. The animals were kept in the animal house of the college of medicine with a room temperature of 18-25 ^{o}C and maintained on normal available food (barley and vegetables) and considered to be salt loaded after giving them 0.9% saline as drinking water for at least 2 weeks. The body temperature was kept at 37-37.5 °C. The rabbits were deprived of food for 15-18 hours before experimental use and then prepared for experimental procedures under an anesthetic regimen consisting of a combination of phenobarbitone (100 mg/kg body weight) and urethane (1 gm/kg body weight) intraperitoneally.[27,28] Surgical anesthesia was reached in about 15 minutes after urethane administration. Thereafter, supplementary small doses of urethane were injected intravenously (IV) as required to maintain deep and prolonged anesthesia because the administrated dose of urethane represented 1% of the recommended anesthetizing dose usually used in rabbits[28]. A tracheostomy was performed to allow free ventilation and for avoiding tracheal obstruction in animal with long-term anesthesia. In addition, the mucus and other secretions were regularly removed by suction using a syringe connected to a polythene tube.

Preparation of animals for experiments

For BP measurement, the carotid artery of the rabbit was cannulated as shown in figure 1. The arterial blood pressure was recorded by connecting the carotid artery cannula to a BP transducer. The

arterial cannula connection was through a 3-way stop-cock attached to a syringe containing heparinized isotonic saline. The blood pressure transducer was in turn connected to a two channel oscillograph and to a mercury manometer for calibration. The process of calibration allows the determination of the range of BP in which the blood pressure of the animal is recorded.

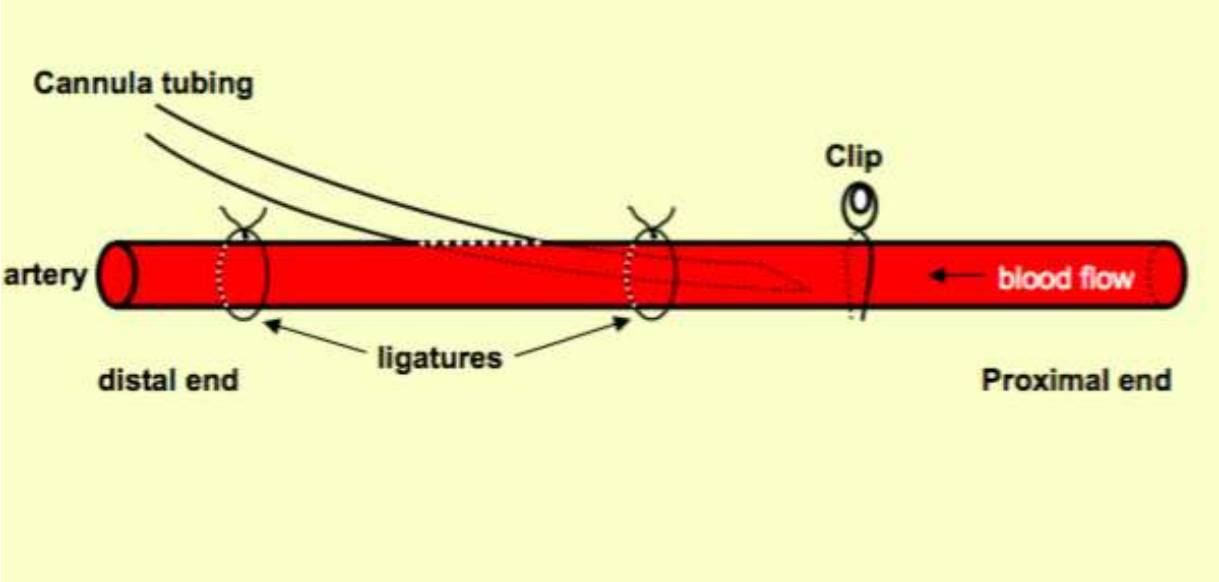

Figure 1: A diagram showing cannulation of the carotid artery.

The heart rate was determined in the rabbits by palpitation based on the method described by Dizaye in 1998 [27] in which, the heart rate is recorded from the BP trace. Each pulse in the artery is transmitted to the physiological recorder as an upward deflection representing the systolic pressure, followed by a downward deflection representing the diastolic pressure (see Figure 2). A cardiac cycle, on the recording paper consists of an upward deflection and a downward deflection. The number of cardiac cycles (beats) per unit of time can be determined from the speed at which the paper of the recorder is running.

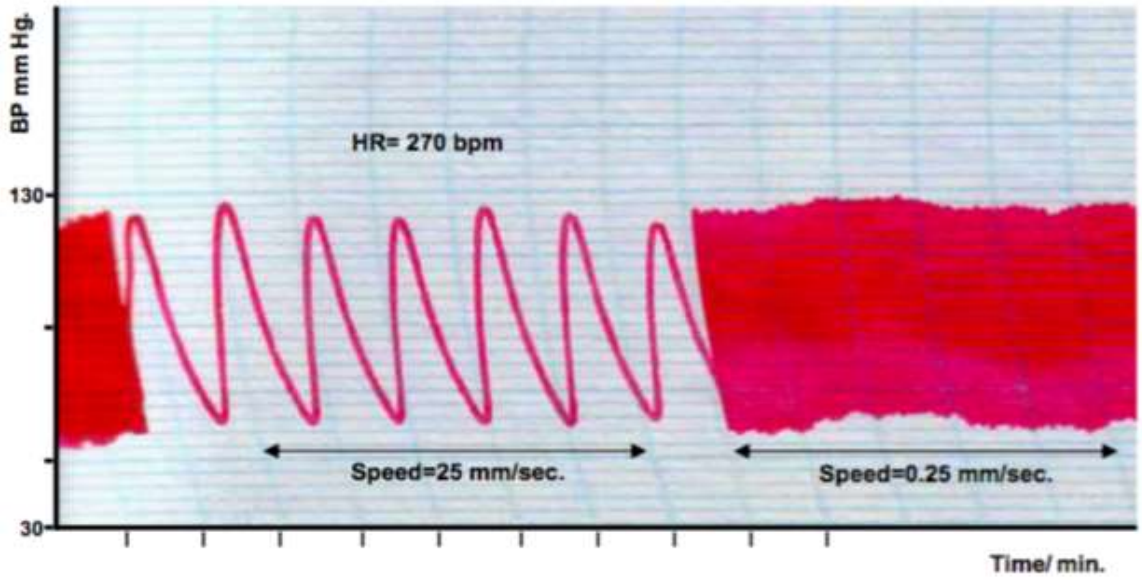

Figure 2: A trace of arterial BP recording showing cardiac cycles per second.

Ang II in a dose of 10 μg/ml was infused intracarotidly in a rate of 10 ng/kg/min. This rate of the hormone produced a moderate rise in BP of about 10-15 mm Hg. The IC infusion was performed depending on a method employed previously in the rabbits by Hamad in 1995.[29] A 27-gauge dental needle attached to pp 25 polythene tubing was inserted into the carotid artery in the direction of blood flow. The advantages of this method is that it allows IC infusion or injection without blocking the normal blood flow through the artery and also, the arterial puncture made by the needle is so small that no bleeding occurs even upon withdrawal of the needle at the end of each experiment.

A single dose of 0.6 mg/kg Olmesartan (IV) was administrated to the animals. This dose was chosen following many trails and it is within the range of effective antihypertensive daily doses used in humans. During the course of each experiment, Olmesartan injection was freshly prepared. Two tablets of Olmesartan were dissolved properly in 50 ml of sterile normal saline and filtered before injection.

The effects of IC infusion of Ang II were first investigated alone. When the pressor effect was clearly apparent, Olmesartan was injected intravenously as a single dose of 0.6 mg/Kg. After allowing 30–60 minutes of recovery as a control period, and when the BP recording was reasonably stable, a second similar dose of Olmesartan was injected. The HR was measured as previously described.

Statistical analysis of results

The results were evaluated statistically by using the Statistical Package for the Social Sciences (SPSS) computer program. All results are quoted as mean ± the standard error of the mean. In the experimental designs used, both control and experimental treatments were given to the same animal. The experimental results were evaluated mostly by t-test for paired samples. Changes were considered statistically significant when P value was less than 0.05; where P is the probability that an observed change or difference might have occurred by chance.

Results

The effects IC infusions of Ang II on arterial BP in salt-loaded rabbits:

The angiotensin infusion intracarotidly induced a significant increase in arterial BP in a range of 11-15 mm Hg (see Figures 3).

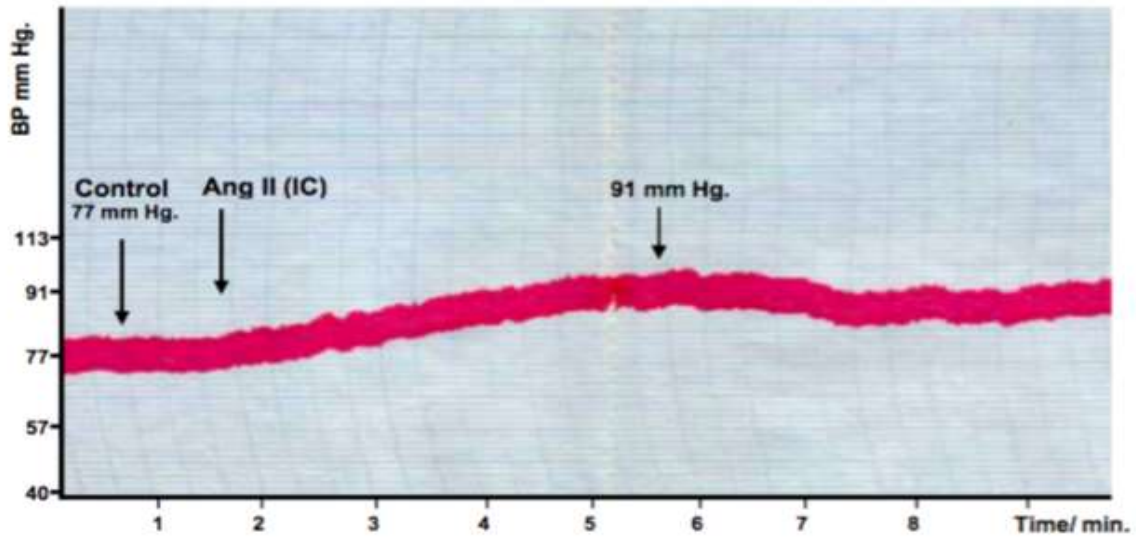

Figure 3: Blood pressure traces showing the pressor responses to Ang infusion (10 ng/kg/min) IC

The effects of Olmesartan on arterial BP and heart rate in salt- loaded rabbits receiving IC infusion of Ang II:

Intracarotid infusion of Ang II at a rate of 10 ng/kg/min produced a consistent and highly significant increase in arterial BP (Table 1). During the pressor response to Ang II, the IV injection of Olmesartan (0.6 mg/kg) clearly and rapidly reduced the arterial BP (Table 1 and Figure 4). This hypotensive effect of Olmesartan lasted about 30 minutes. Thereafter, the effect gradually faded out and complete recovery occurred approximately after one hour from the injection. There was almost no change in HR during these experimental treatments (Table 2).

Table 1: The effects of Ang II (10 ng/kg/min) infusion intracarotidly, alone and in combination with IV injection of Olmesartan (0.6 mg/kg) on arterial BP in salt-loaded rabbits.

Experiment No.	Control (A)	Ang II, IC (B)	Ang II+Olmesartan (C)
1	83	95	70.5
2	84	100	60
3	54	65	46
4	114	135	84
5	67	80	53
6	68	81	42
Mean±SE mmHg.	78.33±8.47	92.66±9.85	59.25±6.45
Statistical evaluation, students t-test for paired samples (P)			
A vs B	P < 0.0005		
A vs C	P < 0.003		
B vs C	P < 0.001		

Table (2): The effects of IC infusion of Ang II (10 ng/kg/min.), Ang II plus IV injection of Olmesartan (0.6 mg/kg) on HR in salt-loaded rabbits.

Treatments	**Control n=10**	**Ang II, IC n=6**	**Ang II+Olmesartan n=7**
Mean±SE (bpm)	**277.6 ±10.1**	**272.3 ±15.3**	**273.1 ±13.8**

Statistical evaluation by comparison between the means showed no significant differences.

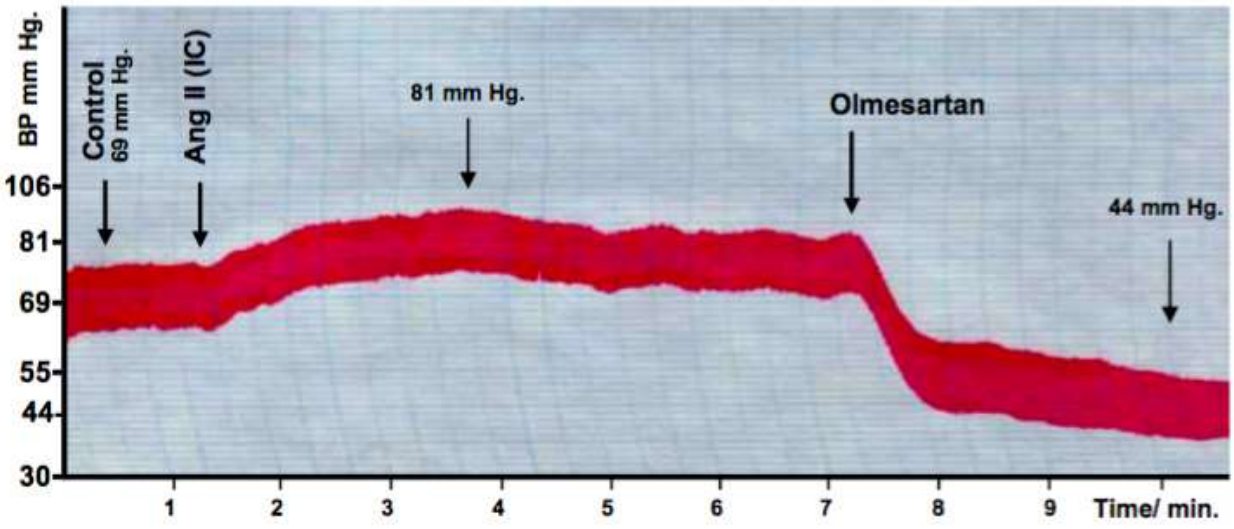

Figure 4: The effects of Olmesartan (0.6 mg/kg, IV) on the pressor response to IC infusion of Ang II (10 ng/kg/min). The recording shows a typical hypotensive response to Olmesartan in one rabbit.

The effect of Olmesartan injection on arterial BP in salt-loaded rabbits:

When the blood pressure recording was continuous and reasonably stable during control measurements, the dose of 0.6 mg/kg of Olmesartan injected intravenously produced no detectable change in arterial BP (Table 3).

Table 3: The effect of Olmesartan injection (0.6 mg/kg, IV) on BP in salt-loaded rabbits.

Experiment No.	Control	Olmesrtan
1	**65**	**59**
2	**53**	**58**
3	**62**	**62**
4	**77**	**73**
5	**94**	**95**
6	**77**	**78**
Mean±SE mmHg.	**71.3 ±5.89**	**70.8 ±5.83**

Statistical evaluation by student's t. test for paired samples showed no significant change.

Discussion

The use of relevant animal models to study human cardiovascular diseases gives useful information to understanding the causes and the potential treatments. Different animal models have been used in induction of experimental hypertension and these animals are also tools in the studying the pathophysiology of hypertension and its complication.[30]

In the present study, the animals were salt loaded at the start of the experiment with an aim to cause inhibition of renin secretion and to reduce the subsequent formation of Ang II. This led to reduced renin activity experimentally and the effects of administrated Ang II exogenously can be easily detected.

Referring to values of arterial BP during control periods in tables 1 and 3, the mean is equal to 74.8±7.2 mm Hg. This value lies within the normal range of blood pressure (73-104 mm Hg) reported by Weisbroth et al. in 1974.[31] The arterial BP significantly increased in response to IC infusion of Ang II (Figure 3). A proportion of the Ang II that is infused into the carotid artery is expected to react with Ang receptors in central areas outside the BBB, and the rest flows into peripheral circulation. Therefore, the plasma concentration of Ang in the peripheral blood would be lower than that will be produced by IV infusion and hence a smaller rise in arterial BP results from the direct vasoconstrictor effect of angiotensin.

Intravenous injection of Olmesartan in a dose of 0.6 mg/kg produced essentially no change in arterial BP (Table 3). Whereas the same dose proved to be quite effective in producing hypotension after the animals received IC infusion of Ang II (Table 1, Fig. 4). In control periods, the salt-loaded rabbits received a relatively high infusion of isotonic saline, conditions that reduce the circulating levels of Ang II and upregulates Ang receptors.[32] In such conditions when Olmesartan is given, it binds to AT1 receptors but without affecting the baseline BP levels because of the minimal concentrations of plasma angiotensin. However, when Ang is infused, the arterial BP is increased. This pressor response is totally prevented and the BP is further reduced by Olmesartan competitively blocking AT1 receptors in brain sites accessible to Ang and also in the periphery. The significant reduction of arterial BP below the control baseline value may be due to Ang II activating AT2 receptors resulting in vasodilation.[12,33]

The fact that the pressor response to IC infusion of Ang was rapidly inhibited within seconds following Olmesartan injection, confirms the direct effect of Ang in this pressor response. In other words, an indirect role of other hormonal factors like vasopressin release may be excluded. However, a central effect of Ang II resulting in rapid excitation of sympathetic activity cannot be ruled out. The hypotensive effect of Olmesartan has disappeared after 30-60 min. indicating a relatively short elimination half-life of the drug in rabbits compared to 10-18 hours in man.

Conclusion

Olmesartan proved to be quite effective in lowering blood pressure in animals received IC infusion of Ang II. This effect is due to blocking AT1 receptors competitively in brain sites accessible to Ang II and also in the periphery. The rapid inhibition of the pressor response to IC infusion of Ang II by Olmesartan confirms a direct pressor effect of Ang II and reduces the role of other endogenous pressor substances.

Conflicts of Interest

The author reports no conflicts of interest.

References

1. Kaplan NM. Kaplan's Clinical Hypertension (9th edition). Philadelphia, PA: Lippincott, Williams and Wilkins (2006).
2. He FJ, Jenner KH, MacGregor GA. WASH-world action on salt and health. Kidney Int 2010; 78:745–53.
3. Badyal DK, Lata H, Dadhich AP. Animal models of hypertension and effect of drugs. Indian Journal of pharmacology. 2003;35(6):349-62.
4. Henry JP, Liu YY, Nadra WE, Qian CG, Mormede P, Lemaire V, Ely D, Hendley ED. Psychosocial stress can induce chronic hypertension in normotensive strains of rats. Hypertension. 1993;21(5):714-23.
5. Burnier M, Brunner HR. Angiotensin II receptor antagonists. The Lancet. 2000;355(9204):637-45.
6. Barreras A, Gurk-Turner C. Angiotensin II receptor blockers. InBaylor University Medical Center Proceedings 2003;16(1):123-126. Taylor & Francis.
7. Gross F, Turrian H. Pharmacology of hypertension and synthetic analogues. Polypeptides which effect smooth muscles and blood vessels. Edited by M. Schachter, Pergamon Press, Inc., New York 1960;137-51.
8. Gross FH, burner HR, Ziegler M. Renin-angiotensin system, aldosterone, and sodium balance. Recent progress in hormone research 1965;21:119-67.
9. Guyton AC, Hall JE. Textbook of Medical Physiology. Tenth edition, W.B. Saunders Company; 2000, USA.
10. Feldberg W, Lewis GP. The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. The journal of Physiology 1964;171:98-108.
11. Liebeu H, Distler A, Wolff HP. The noradrenaline releasing effect of angiotensin II in isolated blood vessels. Acta endocrinologyica 1965;49(3 Suppl):138.
12. Timmermans PB, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ et al. Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacol Rev 1993;45(2):205–51.
13. Puchler K, Nussberger J, Laeis P, Witte PU, Brunner HR. Blood pressure and endocrine effects of single doses of CS-866, a novel angiotensin II antagonist, in salt-restricted hypertensive patients. J Hypertens 1997;15(12):1809-12.
14. Neutel JM. Clinical studies of CS-866, the newest angiotensin II receptor antagonist. Am J Cardiol 2001;87(8):37-43.
15. Yao, ST, May, CN. Intra-carotid angiotensin II activates tyrosine hydroxylase-expressing

rostral ventrolateral medulla neurons following blood–brain barrier disruption in rats. Neuroscience 2013;245:148-56.

16. Huber, G, Schuster, F, Raasch, W. Brain renin-angiotensin system in the pathophysiology of cardiovascular diseases. Pharmacological Research 2017;125:72-90.
17. McKinley MJ, Oldfield BJ. The brain as an endocrine target for peptide hormones. Trends in Endocrinology and Metabolism. 1998;9(9):349-54.
18. De Gasparo M, Catt KJ, Inagami T, Wright JW, Unger TH. International union of pharmacology. XXIII. The angiotensin II receptors. Pharmacological reviews. 2000;52(3):415-72.
19. Parfrey PS, Markandu ND, Roulston JE, Jones BE, Jones JC, MacGregor GA. Relation between arterial pressure, dietary sodium intake, and renin system in essential hypertension. BMJ 1981;283:94-97.
20. Weinberger MH, Miller JZ, Luft FC, Grim CE, Fineberg NS. Definitions and characteristics of sodium sensitivity and blood pressure resistance. Hypertension 1986;8(suppl II):127-134.
21. Overlack A, Ruppert M, Kolloch R, Göbel B, Kraft K, Diehl JR, et al. Divergent hemodynamic and hormonal responses to varying salt intake in normotensive subjects. Hypertension 1993;22(3):331-8.
22. Van Paassen P, de Zeeuw D, Navis G, de Jong PE. Does the renin-angiotensin system determine the renal and systemic hemodynamic response to sodium in patients with essential hypertension? Hypertension 1996;27(2):202-8.
23. Krekels MME, Schaper NC, de Leeuw PW. Sensitivity of blood pressure and renin activation during sodium restriction. Hypertension 1996;30:1216-22.
24. He FJ, Markandu ND, Sagnella GA, MacGregor GA. Importance of the renin system in determining blood pressure fall with salt restriction in black and white hypertensives. Hypertension 1998;32:820-824.
25. He FJ, Markandu ND, MacGregor GA. Importance of the renin system for determining blood pressure fall with acute salt restriction in hypertensive and normotensive Whites. Hypertension 2001;38:321-5.
26. MacGregor GA, Markandu ND, Singer DRJ, Cappuccio FP, Shore AC, Sagnella GA. Moderate sodium restriction with angiotensin converting enzyme inhibitor in essential hypertension: a double blind study. BMJ 1987;294:531-4.
27. Dizaye, KFH. The effects of clonidine on centrally induced renal and vascular responses in the rabbit. MSc. thesis. Salahaddin University. Iraq; 1998.
28. Laber-Laird K, Swindle MM, Fleckneell P. Rodent and Rabbit medicine. BPC Wheatons Ltd.,

Exeter U.K. 1996; First edition.

29. Hamad KR. The role of antidiuretic hormone and natriuretic factors in the regulation of sodium and water balance in the rabbit. MSc. thesis. University of Salahaddin. Iraq; 1995.
30. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. The lancet 2005;365(9455):217–23.
31. Weisbroth SH, Flatt RE, Kraus AL. The biology of the laboratory rabbit. Academic press Inc. 1974.
32. Tortora GJ, Grabowski SR. Principles of Anatomy and Physiology, John Wiley 2000.
33. Unger T. The role of the renin-angiotensin system in the development of cardiovascular disease. American Journal of Cardiology 2002;89:2321-9.