Membrane Stabilizing Effects of Calcium in Salt-induced Hypertensive Pregnancy

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Abstract

Though Calcium is involved in the mechanisms of increase contractility, it has been shown that at high concentration, calcium ions may cause membrane stabilization in which case the smooth muscle responsiveness decreases. Theoretically, a decrease in membrane permeability to calcium ions caused by increased extracellular calcium concentration stabilizes membranes. Apparently, administration of high level of calcium ion may become useful either by way of preventing or diminishing pregnancy induced hypertension (preeclampsia). The goal of this study was to examine the effect of calcium ion on the sensitivity of blood vessels during pregnancy, especially in salt induced hypertensive pregnancy. The isolated aorta of Forty adult Sprague Dawley rats [four groups of ten rats each; with Group 1=non pregnant fed with normal rat chow, Group 2=normal rat chow + 5% $CaCl_2$ prior to and during 6 weeks feeding on 1.6% NaCl, Group 3=normal rat chow + 8% NaCl for 6 weeks, and Group 4=pregnant rats (350-380) fed on normal rat chow] was cut into 2 mm ring segments and each segment was suspended between two L-shaped holders. The lower holder was fixed to the base of 20 ml organ baths containing physiological salt solution, while the upper holder was connected to isometric transducer coupled to Ugo Bassile recorder. The presence of functional endothelium was ascertained before the start of the experiment by the observation of at least, 42% relaxation to 10-7 M acetylcholine in blood vessels contracted with 10^{-7} M nor-adrenaline. Concentration response tests to phenylephrine, KCl, and $CaCl_2$ were done and result compared. Results show that the maximum contraction to phenylephrine of rings from pregnant rats fed on calcium chloride diets were insignificantly different from those of rats fed only with sodium chloride diet. Their sensitivities were however significantly ($p<0.05$) different. This observation suggests that the effect of Ca^{2+} feeding may be limited to the sensitivity, rather than the maximum contraction. Prior calcium feeding along with simultaneous high salt (sodium chloride) intake appears to interfere with the enhancement of vascular contractility associated with high salt intake. Ca^{2+} would therefore be relevant in the prevention and treatment of salt-induced hypertension in pregnancy.

Keywords Pregnancy; Calcium; Contraction; Membrane stabilization; Preeclampsia

Introduction

It is generally accepted that a rise in intracellular free calcium concentration mediates the contractile responses of all muscle types [1-3]. The activating calcium necessary for this contraction is of dual origin: release from intracellular stores or influx from the extracellular médium [1,3].

Intracellular free calcium is the final trigger in the regulation of vascular smooth muscle contractility. The sources of the activator calcium are influx from the extracellular medium through either the receptor operated calcium channel (ROC), activated by agonist such as phenylephrine [4,5] or voltage operated calcium channels (VOC), activated by membrane depolarization [6-9] and release of calcium ions from the internal stores accounts for phasic contractions [10] while tonic contractors are supported by Ca^{2+} ion influx from the extracellular medium. Furthermore, there is evidence of reduced contractility to stretch stimulus, which is known to activate the voltage operated calcium channels [11] and increased potassium induced relaxation of isolated vascular smooth muscle [12]. These changes could be due to the direct effect of pregnancy on the vascular smooth

muscle or indirect effect through blood borne agent [13]. The plasma concentration of several hormones like progesterone, estradiol, and prolactin are increased in normal pregnancy. Also respiratory alkalosis due to a fall in arterial partial pressure of oxygen, and a rise in pH to about 7.4 are features of physiological changes.

Physiological changes occur during pregnancy [14-16] and almost all systems in the body are affected by pregnancy to varying degrees. Some of these changes include, increased body weight [17-20], increased cardiac output in the first trimester13, and reduced peripheral resistance which results from general vasodilation due to the release of prostacyclin (PG12) and nitric oxide. There is also increase in blood volume, increase in the activity of the angiotensin-aldosterone system and a decrease in plasma osmolality in normal pregnancy [17,18]. There is increase renal blood flow due to increased cardiac output and reduced total peripheral resistance in the 2nd trimester, as well as a fall in blood pressure even in a previously hypertensive subject. Also there is a decrease in vascular reactivity and moderate decrease in blood pressure. It is also known that in pregnancy- induced hypertension, the vascular reactivity is reversed, and there is increased responsiveness to vasoactive agents. This increase in responsiveness has been shown to be responsible for the hypertension that occurs in this condition. However, calcium is involved in the mechanism for increase contractility and at high

concentration, may cause membrane stabilization [1,2], leading to a decrease in smooth muscle responsiveness.

A high-salt diet is one of the major risk factors in the development and maintenance of hypertension. Long-term high salt diet causes hypertension and decreases renal expression of vascular endothelial growth factor in Sprague-Dawley rats. The effects of a high-salt diet are related to the function of the renin-angiotensin system, which is normally suppressed by a high-salt diet. Endothelial dysfunction probably plays an important role in the influence of high sodium intake on blood pressure, although the exact mechanisms remain elusive.

An increase in blood pressure in pregnancy is abnormal and this is called pregnancy induced hypertension. Salt loading is known to cause hypertension in human and experimental animal. It has also been shown that high salt intake causes an increased blood pressure in pregnant and non-pregnant states. The mechanism involved in the raised blood pressure response, especially in pregnancy has not been well defined. Some studies have suggested the involvement of atrial natriuretic factor in the salt induced hypertension in human; but this is not supported by other reports [19-21]. Other proposed mechanisms include the impairment of renal handling of sodium and inhibition of erythrocyte membrane Na-K-ATPase pump [22].

In the view of these ill-defined mechanisms of raised blood pressure response in pregnancy, there is need for further examination of the role of salt intake during pregnancy, and the possibility of using $CaCl_2$ in the prevention and management of pregnancies complicated by hypertension.

Materials and Methods

Animals

Forty adult Sprague Dawley rats were used for the study. They were kept in well ventilated building and grouped accordingly:

Group 1: ten non pregnant adult virgin rats (weighing 230 g to 300 g) fed normal rat chow.

Group 2: ten pregnant (350-375 g) rats fed for 3 weeks with normal rat chow mixed with 5% $CaCl_2$ prior to and during 6 weeks feeding on 1.6% NaCl.

Group 3: ten pregnant rats (350-375) fed with normal rat chow mixed with 8% NaCl for 6 weeks.

Group 4: ten pregnant rats (350-380) fed on normal rat chow.

The rats were fed, ad libitum, on the respective diets and had free access to clean drinking water. In the experiment which involved the use of pregnant rats, pregnancy was achieved by mixing a male and a female rats for three consecutive days after which the male rats were separated from the female rats. The female rats were monitored for weight change, and palpated for presence of fetus(es) which were suggestive of pregnancy. The female rats were mixed with male rats at the end of the 3rd week of salt feeding, or salt and $CaCl_2$ feeding. Pregnancy was confirmed by the presence of fetus(es) in the rats when opened upon the 19th day of withdrawal of the male rats. Any such female rat would have been pregnant for at least 19 days out of the 21 days gestation period.

Tissue preparation

The rats were killed by stunning and decapitation. The tissues were prepared using standard methods for the study of isolated blood vessels as used by several workers [16]. The descending aorta were quickly removed, put in a petri dish containing physiological salt solution (PSS) and cleared of all adhering connective tissues. The aorta was then cut into 2mm ring segments and each segment was suspended between two L-shaped holders. The lower holder was fixed to the base of 20 ml organ baths containing physiological salt solution (PSS) while the upper holder was connected to check transducer coupled to Ugo Bassile recorder.

The aortic rings were studied under standard organ bath condition of temperature at 37°C, and pH 7.4 and the PSS was bubbled with 95% O_2, 5% CO_2 gas mixture. In some experiments the endothelium of the blood vessels were removed mechanically by gently rubbing the intimal aspects of the blood vessel rings with a roughened needle. The presence of functional endothelium was ascertained by the observation of at least 42% relaxation to 10^{-7} M acetylcholine. A passive resting tension of 1gm was applied to each ring, this was the tension at which maximum responses to 10^{-5} M phenylephrine was obtained (Figure 1). An equilibrium period of 90 minutes was allowed before the commencement of the measurements.

During the equilibration period, the aortic rings were rinsed at about 30 min interval. During this time the rings were stimulated with 10^{-7} M phenylephrine.

Concentration responses test to KCI

The experiment was performed on aortic rings from pregnant rats fed on normal rats chow (control), and those on high salt diet. The rings from the rats were exposed to increasing concentration of KCI 10-100 mM), a higher concentration of KCI was applied to the bath when the response to the immediate earlier application had remained steady. The contractile responses of the rings of rats in the two different groups were compared.

Concentration response test to $CaCl_2$

The experiment assessed the entry of calcium into the rat aortic smooth muscle cells. The procedure involved depletion of aortic smooth muscle cells of intracellular calcium. This was followed by the readmission of Ca^{2+} into the smooth muscle cells. The precise protocol is as follows. The blood vessels (aortic rings) from the pregnant rats were stimulated with 10^{-5} M phenylephrine. This produced a sustained contraction, but the rings were rinsed as soon as contractions became stable with Ca-free solution containing 1mM EGTA for 15 minutes. This was to deplete the bathing medium of Ca^{2+}, and it caused relaxation of the aortic rings. Fifteen minutes later, 10^{-5} M phenylephrine was applied to the bath. It induced transient phase contractions, which were presumed to reflect the mobilization of Ca^{2+} from intracellular stores. One minute after this application of phenylephrine to the bath, the tissues were again rinsed with Ca-free physiological salt solution containing EGTA: further stimulation with phenylephrine was ineffective, suggesting depletion of phenylephrine sensitive intracellular calcium stores. Fifteen minutes later, the Ca-free EGTA solution was replaced with Ca-free PSS without EGTA, but contained 10^{-5} M phenylephrine. The rings remained in this solution for another 10 minutes. At the end of this period, $CaCl_2$ was applied into the bath by cumulatively increasing the concentration from 2.5 ×

10^{-5} to 2.5×10^{-2} M. Concentration response curve for rings from both pregnant and non-pregnant rats were compared.

The above procedure was repeated on intact aortic ring from pregnant rats on the following diet:

- Control rats fed on normal rats chow
- Rats fed salt high diet for 6 weeks
- Rats fed high $CaCl_2$ diet for three weeks prior to, and during six weeks period of high salt feeding.
- Rats fed on three weeks $CaCl_2$ prior to six weeks of high salt feeding.

Phenylephrine induced concentration dependent contractions in rings from pregnant rats, which were fed on the following diets.

- High salt diet for 6 weeks
- High $CaCl_2$ diet for three weeks prior to, and during six weeks period of high salt diet.
- High $CaCl_2$ diet for three weeks prior to high salt diet.
- Normal rat chow (control)

The concentration response curve for phenylephrine of rings from pregnant rats fed on diet 2 and 3 were not significantly different, that is rats fed on diet with Ca^{2+} prior to salt feeding and those fed on Ca^{2+} prior to, and during salt feeding.

Preparation of solution

Normal physiological salt solution

The composition of normal physiological salt solution was: NaCl, 119.0; KCl, 4.7 KH_2PO_4, 1.2; $NaHCO_3$, 24.9 $CaCl_2$, 1.6; glucose, 11.5; $CaNa_2EGTA$ 0.03.

Calcium free Solutions

Calcium-free solution, with or without 1.0 mMol ethylene glycol Bis (β-amino ethylene) N, N' tetra-acetic acid (EGTA) was prepared by omission of $CaCl_2$ in the preparation of the PSS.

The high K^+-Calcium free solution was prepared like Ca-free PSS with equimolar replacement of NaCl by KCl. The high K^+-calcium free solutions contained 10^{-5} M Phentolamine to block the release of adrenaline from adrenergic nerve endings in the blood vessel (Vonhountte and Webb, 1979).

Drugs

The drugs used in this study were phenylephrine hydrochloride (Sigma chemical Co. St Louis, M.O. USA); Phentolamine Mesylate (Rigitine Ciba); All the drugs were prepared fresh using distilled water on each day of the experiment and kept on Ice. Concentrations were expressed as final molar concentration (Mol/L) in the organ bath.

Statistical analysis

Values were expressed as mean ± standard error of the mean (SEM). The data were analyzed using ANOVA and students. P-values less than 0.05 were considered significant.

Results

These curves were different from those of rings from pregnant rats on only high salt diet. The curves show that the maximum contractions of the rings from the three types of pregnant rats were not significantly different (Figure 2). However, the sensitivities of rings from rats on the salt alone diet, as represented by the EC_{50} values, were significantly different from those that received Ca^{2+} diets (Table 2). Also the sensitivities of rings of rats, which received Ca^{2+} diets, were not significantly different from the sensitivities of rings from pregnant rats that were fed on normal rat chow (control) (Table 2 and Figure 2). High salt diet (diet) significantly increased both the maximal contractions and the sensitivity (as indicated by the EC_{50} values) to phenylephrine of the ring from pregnant rats (Tables 1-5 and Figure 2).

S/N	250 mg stretch	500 mg stretch	750 mg Stretch	1000 mg stretch	1250 mg stretch	1500 mg stretch
1	880 mg	1200 mg	1580 mg	1600 mg	1200 mg	1360 mg
2	600 mg	800 mg	800 mg	800 mg	680 mg	420 mg
3	340 mg	700 mg	760 mg	760 mg	600 mg	600 mg
4	980 mg	1220 mg	1300 mg	1480 mg	1880 mg	1260 mg
5	520 mg	920 mg	1200 mg	1200 mg	780 mg	820 mg
6	300 mg	1020 mg	1200 mg	1200 mg	700 mg	600 mg
	278.1 ± 114.0	976.7 ± 86.3	1140 ± 127.8	1173.3 ± 140.5	973.3 ± 201.7	843.3 ± 157.5

Table 1: Showing Contraction Response of rat aorta to 10^{-5} M Phenylephrine following different levels of stretch.

Rats	EC_{50}	Max. Contraction
Pregnant rat (n=10) Diet 1 (high salt diet alone)	$2.7(\pm 0.9) \times 10^{-8*}$	1610.0 ± 122.6

Pregnant rat (n=10) Diet 2 (Ca^{2+} prior to and during salt feeding	4.4(± 1.08) × 10^{-7}	1564.0 ± 105.0
Pregnant rat (n=10) Diet 3 (Ca^{2+} prior to salt feeding)	2.9(± 0.02) × 10^{-7}	1632.0 ± 172.6
4 Control rats (n=10)	2.47(± 0.31) ×10^{-7}	1139.0 ± 103.4+
*P<0.05; compared with the other EC$_{50}$ values.		
+P<0.05; compared with the other maximal contraction values		

Table 2: EC$_{50}$ values for the response, and maximal contraction (mg) to phenylephrine of aortic rings from pregnant rats fed on diet 1, 2, 3, and 4.

EC$_{50}$=2.7 (± 0.9) × 10^{-8} Max. Contraction=1610 ± 122.6	EC$_{50}$=4.4 (± 1.08) × 10^{-7*} Max. Contraction=1564.0 ± 105.0*	EC$_{50}$=2.9 (± 0.02) × 10^{-7} Max. Contraction=1632.0 ± 172.6	EC$_{50}$=2.47 (± 0.31) × 10^{-7} Max. Contraction=1139.0 ± 103.4
213.0 ± 33.0	4.0 ± 4.0	0	4.0 ± 2.7
550.0 ± 34.9	44.0 ± 17.2	24.0 ± 7.5	56 ± 16.2
1096.7 ± 124.5	420.0 ± 105.1	456 ± 122.1	477 ± 110.6
1460.0 ± 113.3	1108 ± 68.1	1208 ± 171.8	1007 ± 143.8
1610.0 ± 122.6	1544 ± 78.8	1612 ± 184.7	1139 ± 103.4
1500 ± 101.7	1564 ± 105.0	1632 ± 172.6	1111 ± 170.5

Table 3: EC$_{50}$ values for the response, and maximal contraction (mg) to phenylephrine of aortic rings.

Rats	EC$_{50}$	Max. Contraction
Pregnant (n=10) (Normal rat chow)	25.3 ± 1.7	1859.8 ± 47.9
Pregnant (n=10) High salt diet	22.3 ± 3.6	2031.7 ± 179.2

Table 4: EC$_{50}$ values of responses and maximal contractions (mg) to KCl of aortic rings from pregnant rats fed on normal rat chow and those fed on high salt diet.

EC$_{50}$ = 25.38 ± 1.67 Max. Contraction=1859.0 ± 48.7	EC$_{50}$ = 22.55 ± 3.6 Max. Contraction=2031.7 ± 179.2
46.8 ± 17.7	215.0 ± 53.9
553.6 ± 90.3	816.7 ± 164.4
1193.6 ± 99.6	1376.7 ± 158.7
1530.9 ± 75.7	1610.0 ± 133.5
1685.4 ± 69.1	1743.3 ± 140.1
1777.2 ± 61.2	1886.7 ± 186.1
1829.0 ± 51.2	1976.7 ± 191.0
1854.5 ± 48.7	2018.3 ± 184.6
1859.0 ± 47.9	2028.3 ± 180.5
1859.0 ± 48.7	2031.7 ± 179.2

Table 5: Dose response curves to KCl of aortic rings from pregnant rats fed normal and high-salt diet.
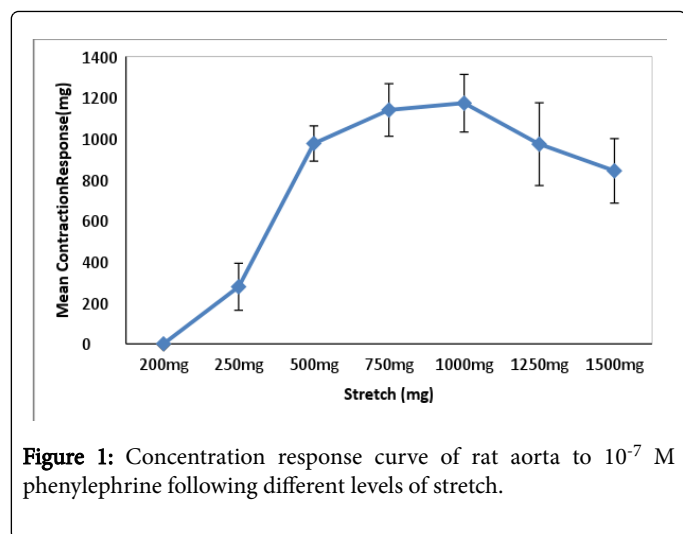
Figure 1: Concentration response curve of rat aorta to 10^{-7} M phenylephrine following different levels of stretch.
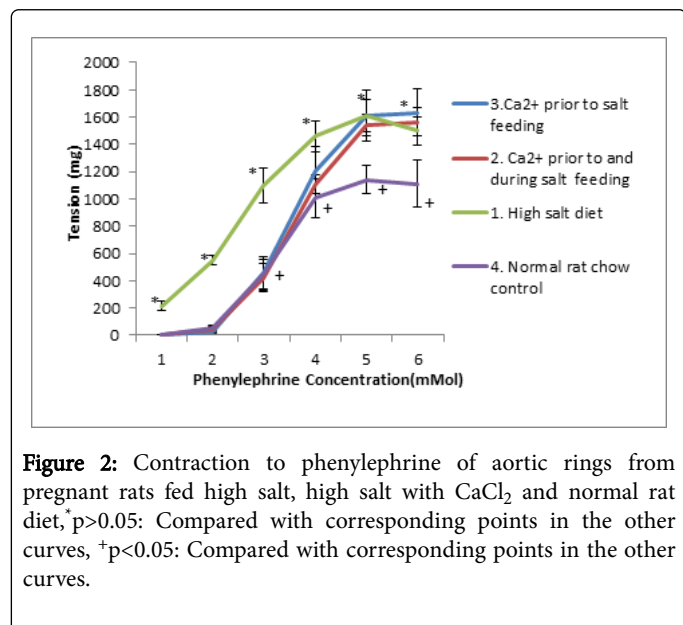

Figure 2: Contraction to phenylephrine of aortic rings from pregnant rats fed high salt, high salt with $CaCl_2$ and normal rat diet, *p>0.05: Compared with corresponding points in the other curves, +p<0.05: Compared with corresponding points in the other curves.
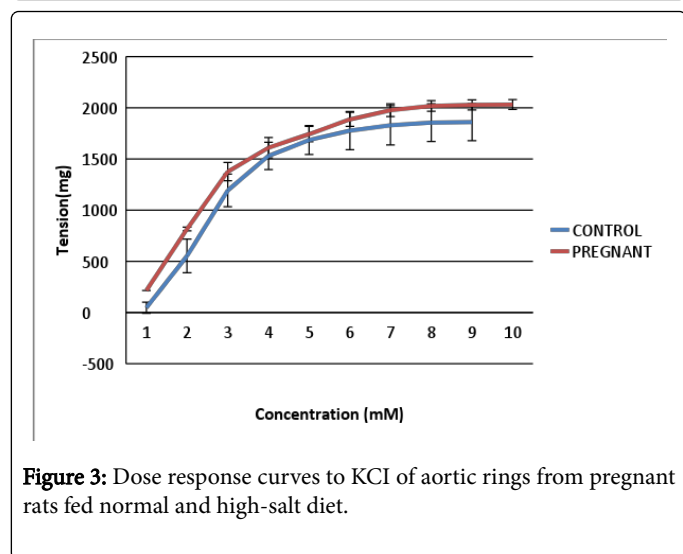

Figure 3: Dose response curves to KCl of aortic rings from pregnant rats fed normal and high-salt diet.

Discussion

High salt intake has been severally shown to induce hypertension in experimental rats [23] and the hypertension has, at least partly been attributed to enhanced sensitivity and contractility of vascular smooth muscles to contractile agents. The results of the present study have accordingly shown that both the maximal contraction and the sensitivity to phenylephrine of the aortic smooth muscle studied were increased by high salt intake. The mechanism by which salt enhanced the sensitivity and contractility of the smooth muscles seems to be related to the alteration of Ca^{2+} influx through the ROC (and not through VOC) as the concentration response curves for KCl of rings from the control rats and the salt fed rats were not significantly different. Similar conclusion had been reached on the report that Nifedipine (a voltaje-operated calcium entry blocker) failed to reduce salt induced elevation of blood pressure in rats [24].

However, some other results of the study showed that calcium feeding prior to and during high salt intake inhibits the effect of salt intake on the sensitivity of the aortic smooth muscle to phenylephrine but has no effect on the maximal contractions. The maximal contractions remained unaltered. This observation tends to suggest that $CaCl_2$ could be used in conjunction with other drugs in the prevention and management of hypertension especially during pregnancy. This would appear to remain the case as prior treatment to $CaCl_2$ for 3 weeks also decrease the sensitivity of the blood vessels during subsequent high salt feeding.

The use of Ca^{2+} for the prevention and, or management of hypertension had long been suggested but this suggestion has not really been backed with much scientific evidence. Though the precise mechanism by which $CaCl_2$ interferes with the sensitivity of blood vessels was not specifically studied, it has been suggested, and demonstrated that an increase in the extracellular concentration of Ca^{2+} stabilized the membrane of vascular smooth muscle cells, and therefore induced relaxation of the muscle1 and 2. It is presumed that Ca^{2+} ion binding to specific loci on the cell membrane will reduce the permeability to monovalent ions. Thus, by altering membrane potentials, Ca^{2+} also influences its own permeability and intracellular calcium release (Hurwitz, 1965). It is therefore a possibility that high $CaCl_2$ intake by the rats prior to high salt intake as well as during the high salt diet could have elevated the extracellular Ca^{2+} concentration and thereby stabilize the membrane of the aortic smooth muscle cells.

Conclusion

Within the ambient of vulnerability to possible errors, this study has found that the use of Ca^{2+} for the prevention and, or management of hypertension is possible. It has been suggested and demonstrated that an increase in the extracellular concentration of Ca^{2+} stabilizes the membrane of vascular smooth muscle cells, and therefore induces relaxation of the muscle leading vasodilation.

Societal benefit of study

The possible use of Ca^{2+} supplement as prophylactic medication in pregnancy, especially in situation of overt sensitivity to salt.

Recommendations

Further work is suggested by way of extending the study to other forms of hypertension.

References

1. Bohr DF (1973) Vascular smooth muscle updates. Circulate Res 32: 665-672.

2. Bohr DF (1963) Vascular smooth muscles: Daul effect of calcium. Science 139: 597-599.

3. Ebashi S, Endo M (1968) Calcium ion and muscle contraction. Prog Bioph Mol Bio 18: 123-183.

4. Gees M, Colsoul B, Nilius B (2010) The role of transient receptor potential cation channels in Ca2+ signaling. Cold Spring Harb Perspect Biol 2: a003962.

5. Albert AP (2011) Gating mechanisms of canonical transient receptor potential channel proteins: Role of phosphoinositols and diacylglycerol. Adv Exp Med Biol 704: 391-411.

6. Bolton TB (1979) Mechanisms of action of transmitters and other substances on smooth muscle. Physiol Rev 59: 606-718.

7. Yamakage M, Namiki A (2002) Calcium channels-basic aspects of their structure, function and gene encoding; anesthetic action on the channels-A review. Can J Anaesth. 49: 151-164.

8. Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J (2005) International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. Pharmacol Rev. 57: 411-425.

9. Hall, John E (2011). Guyton and Hall Textbook of Medical Physiology with Student Consult Online Access, 12th edn. Philadelphia: Elsevier Saunders. p. 64.

10. Fabiato OA, Fabiato F (1977) Calcium release from the sarcoplasmic reticulum. Circulat Res 40: 119-129.

11. Mcaughlin MK, Keve TM (1986) Pregnancy induced changed in resistance blood vessels. Am J Obstet Gynecol 155: 1296-1299.

12. Ebeigbe AB, Aloamaka (1987) Role of endothelium in magnesium-induced relaxation of rat aorta. Res Exp Med 187: 25-31.

13. Tuienko T, Schneider J, Floro C, Sicilia M (1987) The in vitro effect on arterial wall function of serum from patients with pregnancy induced hypertension. Am J Obstet Gynecol 156: 817-823.

14. Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A (2016) Physiological changes in pregnancy. Cardiovasc J Afr 27: 89-94.

15. Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, Soltani H (2012) Anatomical, physiological and metabolic changes with gestational age during normal pregnancy: A database for parameters required in physiologically based pharmacokinetic modelling. Clin Pharmacokinet 151: 365-396.

16. Tanentsapf I, Heitmann BL, Adegboye AR (2011) Systematic review of clinical trials on dietary interventions to prevent excessive weight gain during pregnancy among normal weight, overweight and obese women. BMC Pregnancy Childbirth 11: 81.

17. Hytten F (1985). Blood volume changes in normal pregnancy. In Haematolgoical disorders in pregnancy. Clin Haematol 14: 601-612.

18. Hytten FE, Leitch I (1971) The physiology of human pregnancy, 2nd edn. Oxford : Blackwell Scientific.

19. Nohr EA, Vaeth M, Baker JL, Sorensen TI, Olsen J, et al. (2008) Combined associations of prepregnancy body mass index and gestational weight gain with the outcome of pregnancy. Am J Clin Nutr 87: 1750-1759.

20. Siega-Riz AM, Viswanathan M, Moos MK, Deierlein A, Mumford S, et al. (2009) A systematic review of outcomes of maternal weight gain according to the Institute of Medicine recommendations: birthweight, fetal growth, and postpartum weight retention. Am J Obstet Gynecol 201: e1-14.

21. Fujii K, Ishimatsu T, Kuriyama H (1986). Mechanism of vasodilation induced by alpha-human atrial natriuretic polypeptide in rabbit and guinea pig renal arteries. J Physiol 377: 315-332.

22. Poston L (1987) Endogenous sodium pump inhibitors: a role in essential hypentension. Clin Sci 72: 647-655.

23. Drenjancevic-Peric I, Jelakovic B, Lombard JH, Kunert MP, Kibel A, et al. (2010). High-salt diet and hypertension: Focus on the renin-angiotensin system. Kidney Blood Press Res 34: 1-11.

24. Nwaigue C, Sofola OA (1989) Potassium but not nifedipine reduceds hypertension in anaesthetized salt-loaded rats. Med Sci Res 17: 7678-768.