ORIGINAL ARTICLE

EFFECT OF FEEDING WITH DIFFERENT SALT CONCENTRATIONS IN PRENATAL PERIOD ON THE VASCULAR RESPONSE TO EPINEPHRINE AND ACETYLCHOLINE

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Background: Determine the effect of prenatal exposure to different salt concentrations on the third month's blood pressure and vascular response to epinephrine and acetylcholine. **Methods:** Eight groups of rat, six in each group, one male and five female were exposed to different salt concentrations, Isfahan tap water and distilled water during pre-pregnancy, pregnancy and lactation period. After blood pressure measurement in infants, in one sub-group 1% body weight of blood was substituted by the same volume of NaCl 0.9% containing 1 µg/ml epinephrine and in the other group by 0.1 µg/ml acetylcholine. **Results:** Salt concentrations higher than 1% increased third month's blood pressure and vascular response to epinephrine and decreased vascular response to acetylcholine while lower salt concentrations had no significant influence. **Conclusion:** Level of dietary salt during intrauterine development can permanently alter the mechanisms that regulate cardiovascular function, blood pressure and vascular response to epinephrine and acetylcholine.

Keywords: Prenatal care, Sodium Chloride, Blood Pressure, Acetylcholine, Epinephrine

INTRODUCTION

Blood pressure is determined by three primary factors: heart rate, stroke volume, and arteriolar caliber.[1] Vascular tone is under the control of sympathetic and parasympathetic nerves as well as non-adrenergic, non-cholinergic nerves, including sensory afferent nerves that release peptides peripherally. Stimulation of these nerve terminals may lead to release of various neurotransmitters such as nor epinephrine, epinephrine, adenosine 5´-triphosphate (ATP), acetylcholine (ACh), nitric oxide (NO), vasoactive intestinal peptide (VIP), and calcitonin gene-related peptide (CGRP).[2]

The vascular endothelium plays an important role in the control of vascular tone by releasing various endothelium-derived vasoactive substances. Recently, it was shown that endothelium-dependent coronary and forearm vasodilation in humans evoked by acetylcholine is defective in several pathophysiological conditions, including hypertension.[3] Abnormal vascular reactivity is implicated in the symptomatology and pathogenesis of many disease states, both chronic diabetes mellitus, hypertension, and acute sepsis.[4]

Vasodilatation in response to acetylcholine is considered to represent normal endothelial function, and constriction or reduced dilation to be a sign of dysfunctional endothelium. However, the vasodilation in response to acetylcholine may not be entirely due to release of endothelium-derived nitric oxide. For example, acetylcholine is known to release endothelium-derived hyperpolarizing factor and prostacyclin in some species.[5]

The sympathetic nervous system not only plays an important role in the regulation of blood pressure but is probably involved in the pathogenesis of human essential hypertension. Several lines of evidence indicate that hypertensive individuals have an elevated sympathetic neural outflow.[6] There is increasing evidence suggesting that the sympathetic nervous system plays an important role in the development and maintenance of hypertension in spontaneously hypertensive rats (SHRs).[7] Secretion of adrenal catecholamines may be partially involved in the pathogenesis of hypertension too. A vasoconstrictor hyperresponsiveness to sympathetic activation has been observed in the isolated organs of SHRs, which is thought to be due to enhanced norepinephrine release from the sympathetic adrenergic nerve terminals.[6] The sympathetic nervous system appeared phylogenetically later than the parasympathetic one and developed in close relation with the vascular system.[8] Indeed, hypertensive individuals tend to have increased sympathetic nerve activity and a functional change in the sensitivity of the baroreceptor reflex.[9]

High salt intake is an important environmental factor in the exacerbation of hypertension.[10] A number of studies conducted in humans and in animals have observed that events occurring early in life are associated with the development of diseases in adulthood. Salt overload and restriction during pregnancy and lactation are responsible for functional (haemodynamic and hormonal) and structural alterations in adult offspring.[11] In a few studies the effect of exposure to different salt concentrations during prenatal period has been noticed. The aim of this study was to determine the effect of prenatal exposure to different salt concentrations on the third month's blood pressure and vascular response to epinephrine and acetylcholine in Wistar rat.

MATERIAL AND METHODS

This study was conducted at the Department of Physiology, Faculty of Medicine, Isfahan University of Medical Science, Isfahan, Iran. Prior to the initiation of experiment, study protocols were reviewed and approved by the animal research committee of Isfahan University of Medical Sciences. All works involving experimental animals were performed in full compliance with NIH Guidelines for the Care and Use of Laboratory Animals.

Male and female Wistar Rats were received at 12 weeks of age and were quarantined for one week prior to initiation of experiment. Before administration of salt concentrations, healthy animals were selected following physical examinations and 48 rats were allocated randomly to 8 groups. Each group consisting of 5 non-pregnant female rats and 1 male rat, weighing 200±20 g, animals were housed at 24 °C on a 12 h–12 h light-dark cycle with free access to Isfahan tap water and standard pellet chow containing 0.5% salt. Isfahan city potable water was delivered via a manual bottle watering system and other living conditions for all groups were similar.

Chemicals including Ketamine hydrochloride of Alfasan Co. (Woerden, Holland) and NaCl of (Sepid Daneh, Iran), Normal saline and Epinephrine (Darupakhsh, Iran) and Acetylcholine (Sigma-Aldrich, UK) were purchased. Six concentrations of NaCl (0.5, 1, 1.4, 1.6, 1.8 and 2 percent salt concentrations in distilled water) were prepared. These salt concentrations contained about 75, 150, 210, 240, 270 and 300 mmol/L Na and Cl respectively and their osmolarity was about 150, 300, 420, 480, 540 and 600 mmol/L respectively. Test groups were exposed to 0.5, 1, 1.4, 1.6, 1.8 and 2 percent of salt concentrations during pre-pregnancy, pregnancy and lactation period, another test group consumed distilled water and control group used Isfahan tap water, other living conditions for all groups were similar.

At the end of breeding period (in 90 days), 21 alive and healthy infants were required for this experiment. As the maternal mortality rate in groups consuming concentrated salt concentrations were high and the number of healthy infants was low, breeding task was performed in more cages. Consuming salt concentrations was continued by infant's mother during lactation period. After lactation period, each group's infants were kept in different cages. During breeding period, all groups consumed Isfahan potable water as the only source of water intake, with similar living conditions up to end of breeding period (90 days). At the 90^{th} day, BP was measured directly under anaesthesia condition. On the basis of previous studies, ketamine hydrochloride was selected as anaesthetic drug at an anaesthetic dosage of 125 mg/kg intra-peritoneally.[12] Rat's weight was exactly measured and recorded, the ketamine dosage for each rat was calculated accurately at 125 mg/kg and the drug was administrated via IP route. After anaesthesia induction and skin and muscle dissection, an intra artery cannula was inserted in the carotid or femoral artery, blood pressure was measured directly by a Harvard model physiograph. Epinephrine and Acetylcholine were added to NaCl 0.9%, and solutions of 1 µg/ml epinephrine and 0.1 µg/ml acetylcholine were prepared. After blood pressure measurement, blood samples were taken as the 1% of body weight at the end of the experiment via carotid artery, and in one sub-group (Seven rats) 1% body weight of blood was substituted by the same volume of NaCl 0.9% containing 1 µg/ml epinephrine and in another sub-group 1% (7 rats) body weight of blood was substituted by the same volume of NaCl 0.9% containing 0.1 µg/ml acetylcholine. After serum injection, BP was measured again for some minutes and fluctuations in BP were considered as the vascular response to epinephrine and acetylcholine.

The data were presented as Mean±SD. Statistical analysis of collected data (Third month's Blood Pressure and vascular response to epinephrine and acetylcholine) was performed by different groups ANOVA test using SPSS-11.5. A minimum significance level of $p<0.05$ was used for all comparisons.

RESULTS

The 0.5 and 1% salt concentrations and distilled water were tolerated by pregnant rat without significant problems. Consumption of salt concentrations higher than 1% were associated with some problems in pregnant rat and infants, salt concentrations higher than 1% increased the maternal and infant mortality rates. The 0.5 and 1% salt did not affect infant's SBP and DBP significantly. Salt concentrations higher than 1% increased infant's SBP and DBP, which these increases were significant in some concentrations. The mean infant's SBP and DBP of 1.8 and 2% salt concentrations groups were 112.4±17.8, 75.24±26.48 and 112.4±15.6, 77.62±17.07 respectively in 90 days. Table-1 shows mean SBP±SD and DBP±SD previous of epinephrine and acetylcholine injection.

Injection of 1% body weight normal saline containing 1 µg/ml epinephrine increased SBP±SD and mean DBP±SD. The mean infant's SBP and DBP of 1.8 and 2% salt concentrations groups were 207.86±14.10, 129.29±11.34, and 220.71±12.39, 135±9.67 respectively. Increased vascular response to epinephrine was observed in these concentrations. Table-2 show mean SBP±SD and DBP±SD after injection 1% body weight of normal saline containing 1 µg/ml epinephrine in different groups.

Feeding with salt concentrations higher than 1% during prenatal period increased vascular response to epinephrine and decreased vascular response to acetylcholine, which these changes were significant in salt concentrations higher than 1.6 percent. Injection of 1% body weight normal saline containing 0.1 µg/ml acetylcholine decreased SBP±SD and mean DBP±SD. The mean infant's SBP and DBP of 1.8 and 2% salt concentrations groups were 76.43±14.35, 52.14±21.38 and 89±23.81, 53.57±15.47 respectively. Decreased vascular response to acetylcholine was observed in these concentrations Table-3 show mean SBP±SD and DBP±SD after injection 1% body weight of normal saline containing 0.1 µg/ml acetylcholine in different groups.

Table-1: SBP and DBP before epinephrine and acetylcholine injection (Mean±SD)

	SBP			DBP		
	Mean±SD	**Median**	***p*-value**	**Mean±SD**	**Median**	***p*-value**
TW	100.7±17.4	90	-	67.86±17.51	60	-
DW	104.3±19.4	100	0.4565	68.57±19.18	65	1
0.5%	100.7±18.6	95	0.8701	64.76±19.90	65	0.7153
1%	96.7±25.5	90	0.7059	60.24±18.81	60	0.1995
1.4%	109.3±16.8	105	0.0543	74.29±16.15	70	0.1866
1.6%	110±11.6	110	0.0305	74.76±8.29	70	0.0969
1.8%	112.4±17.8	95	0.1627	75.24±26.48	60	0.5212
2%	112.4±15.6	100	0.0142	77.62±17.07	80	0.0872

Table-2: SBP and DBP after injection 1% body weight of normal saline containing 1 µg/ml epinephrine in different groups (Mean±SD)

	SBP			DBP		
	Mean±SD	**Median**	***p*-value**	**Mean±SD**	**Median**	***p*-value**
TW	173.57±23.4	180	-	110±19.79	105	-
DW	179.3±42.2	195	0.7015	105.71±23.17	120	1
0.5%	225±13.84	225	0.0033	127.86±10.75	125	0.0553
1%	206.43±15.74	210	0.0181	118.57±16.76	120	0.3711
1.4%	197.14±19.55	190	0.1417	129.29±17.42	125	0.0845
1.6%	197.86±15.24	210	0.0639	123.57±11.8	120	0.1599
1.8%	207.86±14.10	210	0.0127	129.29±11.34	125	0.0553
2%	220.71±12.39	215	0.0022	135±9.67	125	0.0298

Table-3: SBP and DBP after injection 1% body weight of normal saline containing 0.1 µg/ml acetylcholine in different groups (Mean±SD)

	SBP			DBP		
	Mean±SD	**Median**	***p*-value**	**Mean±SD**	**Median**	***p*-value**
TW	57.86±9.51	60	-	32.14±11.13	30	-
DW	60.71±7.87	60	0.5229	30±4.08	30	0.8983
0.5%	70.71±7.87	70	0.0215	39.29±9.32	35	0.16
1%	67.71±14.39	65	0.1417	35±12.91	30	0.65
1.4%	60.71±6.07	65	0.3379	36.43±3.78	35	0.25
1.6%	57.14±8.09	60	1	39.29±3.45	40	0.17
1.8%	76.43±14.35	75	0.0298	52.14±21.38	45	0.047
2%	89±23.81	80	0.0088	53.57±15.47	50	0.01

DISCUSSION

This study presented several key findings in relation to the effect of prenatal exposure to different salt concentrations on the third month's blood pressure and vascular response to epinephrine and acetylcholine in Wister rat. The findings are as follow:

First, infant's SBP and DBP did not influence significantly by distilled water, 0.5 and 1% salt concentrations. Sodium requirement increases during pregnancy, innate tendency to consume excessive amount of salt during pregnancy was shown in most species of animals. Although, a low sodium diet prevents hypertension in non-pregnant individuals, but there is no need to introduce a salt restricted diet in prenatal care, a balanced diet in sodium during pregnancy is recommended.

Second, vascular response to epinephrine and acetylcholine did not influence by distilled water, 0.5 and 1% salt concentrations significantly. Normal vascular response is related to normal vascular structure and function, normal vasoconstriction to epinephrine and normal vasodilatation in response to acetylcholine is considered to represent normal endothelial function, and constriction or reduced dilation to be a sign of dysfunctional endothelium.

Third, salt concentrations higher than 1 percent increased SBP and DBP; these increases in some concentrations were significant. Several studies suggest

that salt overload during pregnancy and/or lactation has long-term effects on offspring's body weight and blood pressure. Dietary salt overload present an increased oxidative stress and prenatal stress and malnutrition associated with low birth weights and high blood pressure. Fourth, vascular response to epinephrine and acetylcholine were influenced by salt concentrations higher than 1%, these fluctuations in some concentrations were significant. Abnormal vascular responses are responsible for functional (haemodynamic and hormonal) and structural alterations. Structural abnormalities include hypertrophy and hyperplasia of vascular smooth muscle and accumulation of collagen in the perivascular space, leading to a decrease in lumen size, these structural alterations influenced vasoconstrictor and/or vasodilator responses.[3]

Prenatal salt loading or restriction exerts considerable effects on BP development in normotensive and genetically hypertensive rats.[13] Indeed, a number of studies, both in humans and laboratory animals, demonstrate that such prenatal changes can be considered to have been 'imprinted' or 'programmed'. By modifying brain properties and organ structure, such imprinting can bring about permanent alterations in the regulatory mechanisms of the offspring.[14] High dietary salt ingestion has been associated with elevated blood pressure, especially in experimental animals such as the spontaneously hypertensive rat (SHR), Dahl salt-sensitive (DS) rat, normal Sprague-Dawley (SD) rats, Wistar-Kyoto rat, and dogs. The mechanisms that can contribute to the rise in blood pressure, among others, include enhanced responsiveness to constrictor agonists such as noradrenaline (NA), as well as, in some cases, reduced relaxation responses to endothelium-dependent vasodilators such as acetylcholine (ACh).[15]

Hainsworth *et al* in 2003 reported that mesenteric veins from dogs fed a high salt diet constrict more powerfully in response to agonists, which could contribute to the hypertensive effects of high intakes of dietary salt.[16] There is also evidence for an increase of plasma sodium concentration during a high-sodium diet (2 to 5 mmol/L) that may be sufficient to stimulate vascular reactivity and growth. An increase in transmembrane sodium gradient of vascular muscle and increased affinity of receptors for agonists may be the underlying mechanisms.[17]

Vasodilation to acetylcholine is reduced in salt-sensitive hypertensive patients even on restricted sodium diets. This may contribute to blood pressure elevation when sodium intake is increased.[18] The NO-mediated vasodilation induced by acetylcholine is attenuated during chronic NO synthase inhibition, both in vivo and in vitro. The blunted hypotensive response to acetylcholine can be demonstrated only if blood pressure of control rats is acutely increased to hypertensive levels.[19] High-salt diet is known to result in attenuation of endothelium-dependent relaxation in rat aorta. High-salt diet leads to attenuation of hyperpolarisation of rat aortic endothelial cells in response to acetylcholine, that may mediate inhibition of NO synthesis and endothelium-dependent relaxation.[20] A high-salt diet in rats has been shown to result in enhanced vasoconstrictor and/or reduced vasodilator responses of isolated arteries to agonists.[15] Recent studies indicate that a high-salt (HS) diet can impair vessel responses to endothelium-dependent vasodilator stimuli, including acetylcholine (ACh). Under normal conditions, ACh-induced dilation may involve the release of several different vasoactive compounds including nitric oxide (NO), endothelium-derived hyperpolarising factor (EDHF) and/or cyclooxygenase metabolites of arachidonic acid, depending on the specific vessel studied.[21] A high salt intake suppresses the activity of the sympathetic nervous system as well as the renin system but increases cardiovascular responsiveness to pressor hormones. A high dietary salt intake may contribute to elevated concentrations of plasma epinephrine and to its cardiovascular effects in borderline essential hypertension.[22] Previous work has established that a high dietary salt intake results in enhanced arterial vasoconstrictor responses to stimulation with agonists.[16] The obtained results in this study coincide with similar findings released by other researchers. In agreement with some earlier studies our study revealed that high salt diet during the prenatal period increased vascular response to epinephrine and decreased vascular response to acetylcholine. Previous work has established that prescribing a sodium-restricted diet or a diet rich in sodium prenatal period associated with a lot of undesirable effect. Therefore, a balanced diet in sodium during prenatal period is recommended.

CONCLUSION

Level of dietary salt during intrauterine development can permanently alter the mechanisms that regulate cardiovascular function, blood pressure and vascular response to epinephrine and acetylcholine.

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DECLARATION OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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