Experimental Hypertension in Young and Adult Animals

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SUMMARY The susceptibility of immature and adult animals to various environmental factors often differs because the response of the young organism can only involve those regulatory mechanisms that are available at the particular stage of development. Increased sensitivity to certain (e.g., hypertensive) stimuli may be limited to a relatively short age period that is usually characterized by the maturation of some important physiological functions. High salt intake seems to influence the animals especially during the weaning period and prepuberty, in the course of which profound developmental changes of circulation, electrolyte metabolism, and neurohumoral regulation have been demonstrated. Indeed, salt-dependent forms of experimental hypertension are more severe when they are induced in immature animals. Moreover, substantial differences in hemodynamics, distribution of body fluids, and involvement of pressor and natriuretic agents indicate that the mechanisms of salt hypertension need not be the same in immature and adult animals. For this reason, increased attention should be paid to developmental factors in the study of induced forms of experimental hypertension. (Hypertension 8: 1096–1104, 1986)

KEY WORDS • ontogenesis • age-dependent susceptibility • salt hypertension • electrolyte metabolism • body fluids • natriuretic factors • vasoactive systems • hemodynamics

FOLKOW proposed that hypertension results from the interaction of multiple genetic and environmental factors causing the adaptation of various systems that adjust blood pressure (BP) to actual living conditions. Numerous observations indicate that the age of experimental animals is one of the important factors in the pathogenesis of experimental hypertension.

In this review, special attention is paid to the effects of a sodium chloride overload in young rats with an immature atrial natriuretic system. We propose that a high salt intake might activate different natriuretic systems in young and adult rats according to their maturity at particular ontogenetic stages. Long-term activation of endogenous Na⁺-K⁺ pump inhibitors would explain the important role of these humoral agents in salt-induced hypertension in young rats.

Developmental Factors in Hypertension Research

Many species of laboratory animals have been used in hypertension research. The majority of genetic and induced forms of experimental hypertension have been studied in rats. The main advantage of this species seems to be the availability of strains with different genetic predispositions to the occurrence or induction of experimental hypertension. Rats are used for strain selections and back-cross experiments, as well as for the development of congenic and recombinant inbred strains, because BP changes appear early and several generations can be obtained each year. Such an approach helps to reveal the contribution of genetic factors to the pathogenesis of hypertension. The developmental approach to the study of genetic hypertension results from the early appearance of BP elevation in predisposed rats. The expression of altered genetic information occurs at some early developmental stage of the cardiovascular system and/or its regulation. Gray therefore suggested looking for primary abnormalities in very young or even prenatal spontaneously hypertensive rats (SHR).

There is a distinct contrast between the increasing number of developmental studies of SHR and the low
interest in the developmental approach to the research of induced forms of experimental hypertension. Actually, hypertensive stimuli are applied to rats of various ages ranging from prematurely weaned animals (aged 20–21 days) up to very old ones. It is evident that the response to these stimuli might involve only those mechanisms that are available at a particular stage of development in which the stimulus is applied. Moreover, certain hypertensive stimuli represent different loads for the immature and the adult organism. Therefore, different reactions to these stimuli could be expected in young and in adult animals.

Distinct postnatal age periods can be specified in rats according to essential changes in the mother-offspring relationship, nutrition and digestion, water and electrolyte metabolism, gonadal activity, and so on. The most important age periods in the postnatal life of the laboratory rat are the perinatal period (around birth), the suckling period (before Day 15 of age), the weaning period (until the age of 28 days), puberty (up to the age of 40–45 days), the period of sexual maturation (until the age of 60–70 days), and adulthood. This periodization of the development is important for the interpretation of available data since some environmental stimuli have different effects when applied at various age periods. The long-term consequences of selected stimuli that could influence the organism during short, critical periods of its early postnatal development have already been reported. Available examples range from steroid imprinting of perinatal hypothalamus to the process of socialization. The alterations of receptors or of the enzyme pattern in the target tissues represent the probable mechanisms by which further development of the immature organism is affected. The changes in postnatal development induced by environmental stimuli lead to a number of permanent alterations of various physiological parameters that often become apparent in late adulthood.

The premature presence of hormones or receptor ligands in the perinatal period can alter the adult response to endogenous hormones because of modified receptor maturation. Thus, for example, perinatal vasopressin treatment lowers the antidiuretic efficiency of vasopressin in the rat because the number of vasopressin binding sites is reduced, while it enhances the vasocostrictor response to vasopressin and norepinephrine in the aorta of adult rats. In 1961, Koldovský et al. reported that a high fat diet increased plasma cholesterol levels only in those rats that had been weaned prematurely. Hypercholesterolemia was prevented if prematurely weaned rats were fed a high fat diet in the weaning period (i.e., at the age of 16–30 days). Thus, the pattern of early nutrition seems to be very important for the enzymatic control of lipid metabolism in adult cholesterol-loaded animals.

Critical periods of development should be considered not only for the study of induced forms of experimental hypertension but also in the research of genetic hypertension. The primary abnormality (e.g., membrane defect) might be an early stimulus for abnormal development of the cardiovascular system. Experimental procedures normalizing cardiovascular development in early ontogenesis should be used for the prevention of genetic hypertension.

**Age-Dependent Susceptibility to Experimental Hypertension**

The response to many environmental factors (including some hypertensive stimuli) depends on the developmental stage at which they influence the organism. Weanling and pubertal rats are more susceptible to various forms of experimental salt hypertension than are adult animals (Figure 1). Young rats are also more prone to coarctation hypertension induced by aortic constriction between renal arteries (J. Kuneš and J. Jelínek, unpublished observations, 1983). The magnitude of the hypertensive response diminishes progressively with the advancing age from which the hypertensive stimuli begins to influence the organism. Although most information about the age dependence of experimental hypertension was obtained in rats, an increased BP response to high salt intake also has been observed in immature baboons (Papio hamadryas) as compared with adult monkeys.

Although several mechanisms have been proposed as contributing to the augmented hypertensive response of young, salt-loaded animals, the following questions should be answered before discussing these mechanisms. What is the physiological level of salt intake in the developing rat? Does the early rise in salt intake have permanent consequences? At which developmental stage are the animals most sensitive?
Laboratory rats require only 0.05% sodium chloride in the diet for normal growth, development, and reproduction. The early increase of salt intake above this level seems to modify further development of the organism, because long-term alterations of electrolyte and water metabolism have been observed in animals that were subjected to salt loading in youth. These changes include increased urinary concentration and better tolerance of hypertonic saline, more efficient excretion of a salt load, as well as alterations in volume regulation, fluid turnover, sodium conservation, plasma sodium maintenance, and salt preference.

The level of salt intake in young rats seems to be a decisive factor for the susceptibility of adult animals to salt hypertension. The BP response to renal mass reduction is increased in adult rats drinking saline from prepuberty. On the other hand, Iwai et al. found that severe salt deprivation in young Dahl salt-sensitive rats (DS) did not abolish the sensitivity of adult animals to the hypertensive effects of a high salt diet. In this experiment, however, salt deprivation was started 3 weeks after weaning, so that rats were not protected during the most sensitive period. Unfortunately, Tobian et al. treated DS with thiazides in prepuberty, did not examine the later BP response to a high salt intake in the absence of diuretics.

Dene and Rapp have reported that a high salt intake in pregnant DS or in suckling and weanling animals does not influence the development of salt hypertension in prepubertal DS. This finding seems to be in good agreement with Jelínek's suggestion that "prepuberty might be a critical period for the induction of experimental hypertension in the rat." Of course, this is particularly true for severe, self-sustaining forms of hypertension, because mild to moderate hypertension can be induced even in adult animals (see Figure 1).

Inadequate salt intake seems to be capable of inducing some long-term alterations in the developing organism. Such permanent modifications are usually consequences of the interaction with active developmental processes. Therefore, sensitivity to environmental factors is often changed during the transition to the next developmental period (i.e., when the relationship of the organism to its environment undergoes characteristic changes).

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Development of Circulatory Homeostasis in Normotensive Rats

The structural and functional maturation of the cardiovascular system is synchronized with pronounced age-dependent changes in body fluids, renal function, neurohumoral regulation, and so on. Some of these changes may be important for the different BP responses of young and adult rats to hypertensive stimuli.

The characteristic decrease of relative total body water content is greatest during the suckling period and terminates during sexual maturation. This decrease is due to the diminishing extracellular fluid volume and is accompanied by a moderate enlargement of the intracellular compartment (in relation to body weight). During the suckling period, the extracellular fluid volume decreases more rapidly than the plasma volume, but afterward they decline in parallel. These data indicate that maturation of the distribution of extracellular fluid between intravascular and interstitial compartments is long-term (Figure 2).

Blood pressure rises rapidly during the first postnatal month. There is a further slow BP increase during prepuberty and puberty that is associated with a pronounced decline of cardiac output compensated by a concomitant rise in systemic resistance. Indeed, minimal vascular hindquarter resistance does not reach adult values before the age of 9 to 11 weeks. There is also extensive maturation of the heart and great arteries. Mature arterial properties are attained relatively late in ontogenesis. Collagen biosynthesis in the rat aorta decreases to adult values at the age of 7 to 9 weeks, while the mechanical properties become stabilized in animals aged 10 to 12 weeks. Matura of the baroreceptor reflex also occurs until the age of 12 to 15 weeks.

Hemodynamic readjustments during ontogenesis are related to the changing pattern of the activity of various vasoactive systems. Although the development of the sympathetic nervous system is most pronounced in suckling and weanling rats, further maturation of sympathetic nervous activity and the sympathetic neuroeffector system can be observed in prepuberty and puberty. The activity of the renin-angiotensin system is high at birth and declines in suckling rats. There are also age-dependent changes in prostaglandin metabolism. Levels of prostaglandins \( E_2 \) and \( F_2 \alpha \) in renal venous blood and urine are higher in prepubertal than in adult rats, but vascular prostaglandin synthesis is lower in prepubertal than in adult animals.

The osmoregulatory response to dehydration or saline loading develops in parallel with the maturation of the vasopressinergic hypothalamo-neurohypophyseal system in suckling and weanling rats. The development of vasopressin-sensitive adenylate cyclase in the rat kidney extends to prepuberty. The response to the acute expansion of extracellular fluid volume or blood volume becomes mature in prepubertal rats between 30 and 45 days of age. Despite the immaturity of the weanling kidney, the deficient diuretic and natriuretic response should be ascribed to the absence of a circulating natriuretic agent in weanling rats. An atrial natriuretic factor is found in the atria of weanling rats either in lower amounts or in a less active form. The immature rat kidney, however, responds to atrial natriuretic factor in extracts from adult rat atria by adequate diuresis and natriuresis. A less efficient natriuretic response of weanling rats might also be related to their pronounced sodium and water conservation, which ensures water and electrolyte balance at this stage of development. At the age of about 40 days, prepubertal rats begin to retain potassium instead of sodium and they increase their water and sodium excretion to the adult level.
These sudden changes coincide with the maturation of the tissue ionic content. 106

The present knowledge of the maturation of circulatory homeostasis lacks data concerning the development of ion transport in vascular smooth muscle or other cells. The importance of such developmental studies is emphasized by the fact that severe alterations of cellular ion transport, 107-108 cell membrane calcium binding, 109, 110 and the structure and composition of the cellular membrane 111-114 have been reported in the erythrocytes of young prehypertensive SHR. There is also incomplete evidence regarding the role of particular regulatory mechanisms during ontogenesis and also about the participation of principal pressor and depressor systems in hemodynamic regulation. In this regard, developmental studies in normotensive animals clearly provide useful information for the research of experimental hypertension.

Mechanisms of Salt-Dependent Hypertension in Young and Adult Rats

The animals with hypertension induced by high salt intake in youth and in adulthood differ in many respects. The expansion of plasma and blood volume as well as of total body water is greater in prepubertal rats treated with deoxycorticosterone acetate (DOCA) and salt than in those treated in adulthood. 115 The plasma volume is also increased in young salt hypertensive rats with reduced renal mass but not in adult animals, although extracellular fluid volume is expanded in both age groups. 38 This age-dependent distribution of extracellular fluid is also observed in rats with DOCA-salt hypertension. 116

More pronounced expansion in young DOCA-salt hypertensive rats 115 or rats with reduced renal mass 38 can be related to the greater degree of renal damage 117 caused by an early BP elevation in the immature kidney. 118 The activity of the renin-angiotensin system is more suppressed in the kidneys of young rats than in adult rats with DOCA-salt or adrenal-regeneration hypertension. 119, 120 The inactivation of the renal renin-angiotensin system is accompanied by a decreased resistance in afferent arterioles. 121 Increased glomerular capillary pressure in DOCA-salt hypertensive rats results in glomerular damage. 122 This alteration seems to be more severe in immature than in adult kidneys. 43

Increased salt intake also exerts age-dependent hemodynamic effects. The more pronounced hypertensive response of young rats to salt or DOCA-salt treatment is due to a greater rise in systemic resistance as compared with adult animals. 123, 124

The systolic pressure of young hypertensive rats is further increased by changes in arterial compliance that augment pulse pressure. Increased arterial rigidity is found only in those rats that were subjected to high salt intake or DOCA-salt treatment from prepuberty. No significant changes were observed if these stimuli were applied in adult animals. 123, 125 This finding could explain the conflicting data on arterial compliance reported by Cox 123, 126 in younger and older rats with DOCA-salt hypertension.

Currently, there is little information regarding the participation of individual pressor systems in the maintenance of elevated BP in young and adult rats with salt-dependent hypertension. Acute converting enzyme blockade with captopril causes a mild BP decrease in adult DOCA-salt hypertensive rats but no BP changes in young hypertensive animals. 125 This finding is in concert with the finding that renin-angiotensin system activity is more diminished in young than in adult rats with experimental salt hypertension. 119, 120

The pressor effects of vasopressin are also more important for the maintenance of BP in adult than in young DOCA-salt hypertensive rats. 121 Moreover, vasopressin is essential for the induction of DOCA-salt hypertension in adult but not in young rats. 127 On the other hand, α₁-adrenergic blockade by prazosin sug-
gests a more effective participation of this pressor system in young than in adult DOCA-salt hypertensive rats.\(^{124}\) However, neonatal guanethidine-induced sympathectomy lowers BP in both young and adult salt hypertensive Brattleboro rats without abolishing the age-dependent BP difference (J. Křtěček and J. Zicha, unpublished observations, 1982). These data indicate that individual pressor systems play a different role in young and adult rats with salt hypertension.

It was recently demonstrated that the acute administration of antidigoxin serum lowered BP only in young DOCA-salt hypertensive rats\(^{128-130}\); it did not influence the BP of hypertensive rats treated in the same manner in adulthood.\(^{129, 130}\) A similar blockade of the circulating digoxin-like factor also lowered BP in young but not in adult one-kidney, one clip Goldblatt hypertensive rats, although the adult rats were more hypertensive than the young animals.\(^{124}\) The BP of SHR was independent of the digoxin-like factor except in 32-week-old animals,\(^{131, 132}\) in which suppressed activity of renal Na\(^+\), K\(^-\)-ATPase was found.\(^{133}\) Further experiments demonstrated that the acute blockade of the digoxin-like factor decreased systemic resistance in young DOCA-salt hypertensive rats,\(^{131, 134}\) in which elevated levels of circulating digoxin-like factor were found.\(^{135}\) No similar hemodynamic changes were observed in adult DOCA-salt hypertensive rats.\(^{134}\) The digoxin-like factor also participated in the maintenance of systemic resistance in normotensive rats that drank a 1.6\% saline solution from the 25th day of age (J. Zicha, unpublished observation, 1986).

These data provide a new explanation of the contradictory findings about the occurrence of endogenous inhibitors of the Na\(^+\)-K\(^-\) pump in salt-loaded rats. The onset and duration of high salt intake in basic experiments that provided supporting or opposing evidence for the participation of these humoral factors are presented in Figure 3. Supportive findings were usually obtained in rats in which the salt intake was increased in youth,\(^{128, 130, 134, 140}\) On the other hand, most of the studies that failed to confirm the role of endogenous inhibitors of the Na\(^+\)-K\(^-\) pump were performed in rats exposed to a high salt intake only in adulthood.\(^{129, 130, 134, 144, 146, 148, 149}\)

Two pioneers in salt hypertension research — Dr. G.R. Meneely and Dr. L.K. Dahl — usually placed their rats on a high salt diet at the age of 3 to 5 weeks.\(^ {144, 149}\) This procedure was not always repeated by scientists subsequently employing the same model. Thus, the original proposal by Dahl et al.\(^ {148}\) about the pathogenetic role of a "salt-excreting hormone" was based on parabiotic experiments performed in rats fed a high salt diet from the age of 4 weeks. Later studies, in which inhibition of the Na\(^+\)-K\(^-\) pump was not detected in vascular smooth muscle, used hypertensive DS that began to consume a high salt diet in adulthood.\(^ {144, 146, 149}\) When very young (3- or 5-week-old) DS were placed on a high salt diet, the activity of the Na\(^+\)-K\(^-\) pump in erythrocytes was nearly abolished\(^ {179}\) and the rise of ouabain-sensitive synaptosomal norepinephrine uptake was prevented.\(^ {153}\) These findings are in good agreement with the data on the differing participation of the digoxin-like factor in young and adult DOCA-salt hypertensive rats.\(^ {129-132, 154}\) Similarly, the rise of Na\(^+\)-K\(^-\) pump inhibitors was detected only in the brains of young rats,\(^ {42}\) not in brains of older salt-loaded rats.\(^ {148}\) Of course, there is some evidence for the presence of these humoral factors in rats that were not exposed to the salt overload until adulthood.\(^ {145, 147, 151}\) However, none of these studies permits a quantitative comparison with young animals.

The increase of salt intake activates natriuretic mechanisms that might differ according to the age of the experimental animals. The lower natriuretic response of weanling rats to volume expansion\(^ {93, 95, 100, 101}\) is accompanied by lower natriuretic activity of their atrial extracts.\(^ {102, 103}\) The natriuretic activity is elevated in extracts from atria of rats that are subjected to high salt intake after sexual maturation.\(^ {152-155}\) On the other hand, there is no increase of natriuretic activity in atria of rats that have been salt-loaded since weaning.\(^ {153, 156, 157}\)
Therefore, it appears feasible to propose that young salt-loaded rats with an immature vasodilating atrial natriuretic system must activate natriuretic systems different from those in adult animals. Moreover, a high salt intake appears to be a greater stimulus for young than for adult rats because of their immature natriuretic capability (see Figure 3). Long-term activation of the vasoconstrictor natriuretic system (Na\(^+-\)K\(^+\) pump inhibitors) triggered by a high salt intake in early life would explain 1) the occurrence of severe salt hypertension in young rats\(^{29-38}\) and 2) the permissive effects of early salt intake on the induction of salt hypertension in adult rats.\(^{41,50}\) The maturation of the acute natriuretic response to blood volume expansion is associated with a diminishing magnitude of the hypertensive response to the salt overload (Figure 3). Thus, the progressive rise in the efficiency of the atrial natriuretic system during ontogenesis might be reflected in the diminishing participation of Na\(^+-\)K\(^+\) pump inhibitors in response to various hypertensive stimuli, including chronic salt overload or chronic volume expansion.

Conclusions

The development of both genetic and induced forms of experimental hypertension should be studied with respect to the maturation of the organism. There is considerable evidence for important ontogenetic changes of structure and function of the cardiovascular and renal systems, including their neurohumoral regulations. This maturation process could be severely impaired if inadequate stimuli influenced the susceptible organism at sensitive periods of development. Thus, the effects of hypertensive stimuli differ depending on whether they are imposed on immature or adult organisms. Therefore, different regulatory mechanisms are found in animals influenced by selected hypertensive stimuli during early or late phases of ontogenesis. Prepuberty and the weaning period are probably very important for inducing severe forms of experimental salt hypertension, while earlier ontogenetic stages might be essential for the further development of genetic hypertension. The prevention of the development of both genetic and induced forms of experimental hypertension would require the elimination of the influence of endogenous or exogenous hypertensive factors particularly during these developmental periods.

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