Salt and Hypertension
Lessons From Animal Models That Relate to Human Hypertension
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A high NaCl diet can raise blood pressure in both susceptible people and in susceptible animals, and the mechanisms are probably quite similar for both humans and animals. The possibly harmful effects of a high NaCl diet are not entirely dissimilar since both prehistoric man and mammals evolved in a low NaCl world. Evolutionary forces molded mammals to adapt well to a low sodium intake; the modern high NaCl intake goes "against the grain" of this adaptation. The high NaCl diet can cause premature mortality by raising blood pressure in susceptible people. We have new evidence that in a hypertensive setting, a high NaCl diet can increase mortality even though it does not cause a further rise of blood pressure. Multiple small cerebral infarcts are a partial cause of this excess mortality. Recent evidence also indicates that a high potassium diet reduces the rise of blood pressure caused by a high NaCl diet, whereas a low normal potassium intake encourages an NaCl-induced rise of blood pressure. It is the combination of kidneys that tends to retain NaCl together with a high NaCl intake that produces a rise in blood pressure. This combination tends to cause NaCl retention, which can trigger a rise in blood pressure in susceptible humans and animals. Such a rise in blood pressure can augment renal NaCl excretion and regain the previous NaCl balance. In the Dahl salt-sensitive (DS) rat, there are several renal abnormalities that would tend to encourage sodium retention. By analogy, renal "abnormalities" are probably present in people susceptible to hypertension. When renal dysfunction plus a high NaCl diet tend to cause NaCl retention, how is this NaCl signal perceived? Evidence indicates that one signal is probably an excess of extracellular fluid volume registering in the brain. Ablation of central nervous system structures around the third brain ventricle influences NaCl hypertension. A hydrocephalus of the third and lateral brain ventricles prevents 60% of an NaCl-induced rise in blood pressure in DS rats and reduces mortality by 90%. Such observations suggest that structures near the third brain ventricle are important for receiving the excess NaCl signal. (Hypertension 1991;17[suppl I]:I-52–I-58)

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uman essential hypertension appears to be a salt-related disease. Low salt societies have no hypertension at all. Yet if such people migrate to a high salt society, about 30% will show a significant rise in blood pressure. Similarly, there are certain strains of rat that also develop a significant rise in blood pressure when placed on a high salt diet. Dogs with reduced renal mass are also susceptible to salt-induced hypertension. It is very likely that the mechanisms that cause a high NaCl intake to raise blood pressure in animals would be the same mechanisms that cause a high NaCl diet to raise blood pressure in susceptible humans. These animal models may teach us much about human hypertension. Some facets of this will be covered in this article.

For instance, humans have been on earth for 3½ million years; for the first 99.8% of that time, every human on the earth, unless he lived by the sea, was on a low sodium diet and also incidentally on a low fat and high potassium diet. If one believes in the principles of Darwin, the body has been fashioned by evolution to function most efficiently with this low salt, low fat, high potassium diet.

Our ancestors consumed about 690 mg sodium/day, whereas we average about 4,000 mg/day (equivalent to 10 g NaCl). There is also a big difference in potassium intake, 284 meq/day then versus 64 meq/day now. Many studies show that certain kinds of animals will show a rise in blood pressure on a high NaCl diet. In a recent study we found that Dahl salt-sensitive (DS) rats on a low NaCl diet have a
small rise in blood pressure with age, but if these rats eat a 2% NaCl diet they get a greater rise in pressure (159 mm Hg versus 177 mm Hg mean pressure). Americans consuming 10 g NaCl/day have about 2% NaCl in their diet. Thus, just the amount of NaCl that is common in an American diet is enough to raise the blood pressure in these NaCl-sensitive animals.

Can NaCl Increase Mortality in a Hypertensive Setting Without Raising Blood Pressure?

From working with stroke-prone hypertensive rats and considering the high stroke rate and hypertension prevalence in northern Japan where a high level of NaCl is consumed, we hypothesized that NaCl could possibly damage arteries even if it did not raise blood pressure. Once hypertension is established in humans, it is clear that some hypertensive persons will have a significant rise in blood pressure when switching from a low salt to a high salt diet, whereas other hypertensive persons will have only a minimal change in blood pressure. These latter hypertensive individuals have been termed “salt-resistant,” and it has been suggested that they would gain little from restricting NaCl in their diet. We have always been somewhat skeptical of this proposition. This skepticism was greatly strengthened when we observed that stroke-prone spontaneously hypertensive rats (SHRSP) developed very few strokes on a low NaCl diet, but when given a high NaCl diet, they developed a high incidence of strokes. However, these observations were not conclusive since the high NaCl diets also considerably raised the blood pressure of these SHRSP. To test the proposition that a high salt diet causes strokes without increasing blood pressure, we needed a hypertensive model that did not have a rise in blood pressure when consuming a high NaCl diet. We believed that the Dahl salt-resistant (DR) rat might be just right for such a study. These rats have been specifically bred to be highly resistant to NaCl-induced hypertension.

One hundred DR rats had one kidney removed and were allowed to drink only a solution containing 1% NaCl and 0.2% KCl for 6 weeks. At the time of the nephrectomy each rat received a subcutaneous silicone implant, which contained 250 mg desoxycorticosterone acetate (DOCA)/kg body wt. DOCA administration and the high NaCl drink were continued for the next 6 weeks. Then the silicone implant under the skin, and the drink was switched from 1% NaCl and 0.2% KCl for 6 weeks. At the time of the nephrectomy each rat received a subcutaneous silicone implant, which contained 250 mg desoxycorticosterone acetate (DOCA)/kg body wt. DOCA administration and the high NaCl drink were continued for the next 6 weeks. Then the silicone implant containing the DOCA was completely removed from under the skin, and the drink was switched from 1% NaCl to tap water. The diet was also changed from, regular Purina rat chow to a low NaCl chow, which contains only 0.3% NaCl. The rats were given this low NaCl diet for 4 weeks as they recovered from the effects of the DOCA and high NaCl regimen. At the end of this 4-week recovery period, every DR rat in the study underwent an intra-arterial measurement of mean blood pressure under light ether anesthesia. Without the DOCA, we found that all DR rats had virtually no rise in blood pressure when eating a high NaCl diet.

On the basis of this initial blood pressure measurement, the entire pool of rats was divided into two matched groups of about 50 rats each with precisely equal average blood pressures. The average mean intra-arterial blood pressure was 160 mm Hg for both groups (Figure 1). At that point one group of rats continued to eat the 0.3% low NaCl Purina diet, and the other group was switched to an 8% high NaCl Purina diet. At the end of 5 weeks on these diets, the mean intra-arterial blood pressure of each rat was again ascertained under light ether anesthesia.

Because all of these rats were DR rats, we were not surprised that the 8% high NaCl diet did not raise the average blood pressure at all. At the beginning of the feeding period, the average blood pressure was 160 mm Hg in both the high and the low NaCl groups, whereas at the end of 5 weeks, the blood pressures averaged 158 mm Hg in both the high and the low NaCl groups, with virtually no change of blood pressure in either group. This amounts to a mild degree of hypertension in both groups.

At the end of 8 weeks on these two dietary regimens, 26 of 49 rats eating the 8% high NaCl diet had died. This amounts to a 53% mortality rate, whereas during this same period of time not a single rat (0 of 51) eating the low NaCl diet had died (p < 0.000001). After another 7 weeks of the 8% high NaCl diet, all the rats in the 8% NaCl group had died (49 of 49). After 15 weeks of the 0.3% low NaCl diet, 44 of 50 rats, or 88%, were still alive. Thus, we had a high mortality rate in these mildly hypertensive rats on the high NaCl diet, whereas we had a very low mortality rate in rats with the same degree of hypertension but consuming the low NaCl diet.

Uremia was not the cause of death in the high NaCl group. In the DR rats eating the high NaCl diet, not a single rat had an obvious cerebral hemorrhage when the brain was grossly examined. Moreover, among the rats eating the high NaCl diet, there
was no evidence of mesenteric arteritis with local mesenteric bleeding in any of the rats. Early in the diet regimens, both groups gained weight equally.

We weighed the rats on a weekly basis, and most of the rats eating the high NaCl diet underwent a marked loss of weight just before their death. Sagittal sections of the brain hemispheres in such rats showed many small brain infarcts. Even if these rats had other causes of premature death, these small brain infarcts would also have to be considered as a partial cause of death.

Of the 49 DR rats with post-DOCA hypertension on the high NaCl diet, 86% (42 of 49) showed this pattern of weight loss. Thus, from both a "clinical" and a microscopic standpoint, it appeared that most of the rats on the high NaCl diet died as a result of cerebral vascular disease with brain infarction and possible brain edema.4

We believe that the relative rarity of cerebral hemorrhage was related to the fact that these DR rats had only a mild form of hypertension, whereas the NaCl-fed SHRSP rats generally had severely elevated levels of blood pressure, usually above 200 mm Hg.3 Whatever the form of cardiovascular disease, the high NaCl feeding caused it to be severe enough to bring about the premature death of all the NaCl-fed rats, even though their blood pressures were in the mildly hypertensive range.

Thus, rats with this degree of mild hypertension will develop a lethal amount of vascular disease when fed a high NaCl diet and will suffer almost no premature deaths if they eat a low NaCl diet.4 Moreover, because the DR rat was used for this study, we could document that the high NaCl diet caused no rise in average blood pressure whatsoever. Nevertheless, the high NaCl intake was associated with enough increase in vascular disease to cause the death of all 49 rats within 15 weeks of starting the high NaCl diet.4 Based on these findings, salt's infancy seems to go beyond blood pressure. Reid et al made the same observation in Sprague-Dawley rats with active DOCA hypertension. The high salt diet increased strokes and mortality rate even though it did not raise the blood pressure above that found in rats on the low NaCl diet.

This dangerous effect of a high NaCl diet is apparently only observed in a hypertensive setting. In DR rats that have not received DOCA, a high NaCl diet causes neither excess mortality nor a rise in blood pressure. However, in other strains of rats this same high NaCl diet will cause a rise of blood pressure and an increase in mortality rate.5

The same mechanisms may well be operating in hypertensive patients who are "NaCl-resistant." It is quite possible that such patients might be spared some vascular injury if they consumed a moderately low NaCl diet and might show aggravated vascular lesions if they consumed a high NaCl diet. It is certainly true that the northern Japanese, who in the past have almost uniformly consumed a high NaCl diet, had a high incidence of death from stroke when consuming a high NaCl diet.2 In years past this has been the leading cause of death in Japan. However, there is also a high prevalence of hypertension in this region, so it is quite possible that salt as well as a high blood pressure both contribute to the high incidence of strokes.

**Effect of Potassium on NaCl Sensitivity**

The Charles River--Kingston strain of spontaneously hypertensive rats (SHR) are considered NaCl-resistant. We confirmed that when these animals are on a high potassium diet, they are relatively NaCl-resistant after 4 weeks of a high NaCl diet (Figure 2).6 When these Charles River SHR are on a low normal potassium diet of 0.5% potassium, which is just about the same potassium level as is found in the diet of the average American, the blood pressure increases strikingly when the rats are switched from a low salt to a high salt diet (Figure 2). After 2 weeks on the high NaCl diet a much greater increase of blood pressure, 17 mm Hg, is found in the rats on a normal potassium diet versus a 7 mm Hg increase in those rats on the high potassium diet. After 4 weeks on this diet, an even greater (36 mm Hg) increase in blood pressure occurred in those rats on a normal potassium diet versus a 9 mm Hg rise in blood pressure in the rats eating a high potassium diet. In these Charles River--Kingston SHR rats, there was a 90% mortality rate after 14 weeks on the high NaCl, normal potassium diet versus a 5% mortality rate on the high NaCl, high potassium diet.6 Thus, in this strain of rats, the level of potassium in the diet has a marked influence on NaCl sensitivity.
blood pressures in both groups are 128 versus 146 mm Hg, respectively, and the pressure natriuresis curve (inflow pressure versus NaCl output) of the isolated kidney from the normotensive DS rat is shifted to the right (Figure 3). It requires more pressure to excrete a milliequivalent of sodium in the DS kidney; at equal inflow pressures, the DS kidneys excrete half as much sodium as the DR kidneys. However, a rise in blood pressure to 160 mm Hg cures the natriuretic defect, which may indicate one reason why these rats become hypertensive. Even when DS and DR rats are matched for blood pressures, there is still a large significant difference in the pressure natriuresis curves.

Why does the DS kidney tend to retain NaCl? In the prehypertensive DS rat there are several demonstrated abnormalities that could lead to an increased tendency for sodium retention. We have recently shown that in the prehypertensive state, the DS rat shows no increase in glomerular filtration rate (GFR) after an infusion of amino acids, indicating that there is no reserve capacity for increasing glomerular filtration. With this defect in glomerular filtration, maximum hemodynamic adaptations are required to barely achieve a normal GFR. Conversely, the DR rat increased its GFR by 81% after the amino acid infusion, indicating that it has a great reserve capacity for increasing GFR. The isolated kidneys from prehypertensive DS rats have a 33% reduction in GFR compared with DR rats when both types of kidneys are perfused with blood at 100 mm Hg inflow pressure. Lithium clearance is also diminished in the DS rat, suggesting a reduced rate of sodium reabsorption from the proximal convoluted tubule.

Prostaglandin E\textsubscript{2} levels are diminished in the cortex and in the outer and inner medullas of quick-frozen kidneys from prehypertensive DS rats. This reduction in prostaglandin E\textsubscript{2} would lead to excessive sodium reabsorption from the ascending thick limb of Henle's loop as well as from the cortical collecting tubule and the inner medullary collecting duct. All of these actions would encourage sodium retention. Moreover, in the quick-frozen outer medulla the DS rat has about a third reduction in the concentrations of prostaglandins E\textsubscript{2}, I\textsubscript{2}, and D\textsubscript{2}, all of which are vasodilator prostaglandins, and a 50% increase in the concentration of thromboxane, which is a vasoconstrictor prostaglandin. The deficit in vasodilator prostaglandins and the excess of a vasoconstrictor prostaglandin would tend to cause vasoconstriction in the descending vasa recta, which should lead to a diminished papillary plasma flow. In fact, in the prehypertensive DS rat on a low NaCl diet we have measured a 25% reduction in plasma flow to the renal papilla. The vasoconstriction of the descending vasa recta along with the diminished papillary plasma flow would both encourage sodium retention.

There is also evidence that the DS rat has humoral agents that reduce sodium excretion in isolated kidneys. At the start of a high NaCl diet, Iwai and our laboratory independently could find no evidence of sodium retention in DS rats, presumably because of a quick recruitment of natriuretic mechanisms, one of which is the rise of blood pressure. Other investigators have made similar observations. It is not clear how many of these renal alterations occur in humans genetically susceptible to essential hypertension. However, humans do have a reduced lithium clearance similar to that in DS rats.

When a DS rat begins eating a 4% high NaCl diet, a change occurs in the kidney that further compromises GFR. As seen in isolated kidneys perfused with blood at normotensive pressures, 20 days of a high NaCl diet will reduce the "intrinsic" GFR by 42%, whereas only 5 days of the high NaCl diet causes almost no reduction. These changes brought about by 20 days of a high NaCl diet in a susceptible species could be partially irreversible, with the kidney and the blood pressure never completely recovering even when a very low NaCl diet is reinstituted.

**How Is the NaCl Signal Transmitted?**

When the combination of a high NaCl diet plus a kidney with sluggish sodium excretion brings on hypertension in a susceptible human or rat, it is still a mystery as to how the NaCl signal is perceived. In certain people this NaCl signal may not be perceived at all. We see signs of this in individuals who gradually go into renal failure with no hypertension whatsoever and in whom a large expansion of extracellular fluid volume for 4 weeks under dialysis conditions leads to no rise of blood pressure. This lack of reception of the NaCl signal may be present in as many as 20% of the population.

One possible NaCl receptor would be the juxtaglomerular cells in the walls of the renal afferent arterioles. An increased extracellular volume as well as an increased concentration of NaCl in plasma both diminish renin secretion.
It is also possible that the signal for excess NaCl in the body could be perceived in the central nervous system (CNS). Various lesions in the CNS of the DS rat can greatly attenuate NaCl-induced hypertension. 6-Hydroxydopamine injected into the lateral brain ventricle destroys many catecholamine-containing neurons and reduces NaCl hypertension by 50%.21 Bilateral lesions of the paraventricular nuclei will also reduce NaCl hypertension by 50%.22 A thermal lesion at the anterior end of the third brain ventricle (AV3V area) will reduce NaCl hypertension by 60%.23 Moreover, a bilateral lesion of the suprachiasmatic nuclei, which are at the bottom of the third brain ventricle, will actually increase NaCl hypertension by 15 mm Hg and heart weight/body weight ratios by 15%.21 Thus, it is essential to have certain CNS systems intact to get the full expression of NaCl-induced hypertension. This raises the possibility that the NaCl signal is somehow received in the brain.

If such is the case it would be helpful to know just how the NaCl signal is received. It is well-known that hypertonic NaCl introduced into the lateral brain ventricle will induce a pressor response, and such pressor responses are greatly exaggerated in the prehypertensive DS rat.24 When an excessive amount of NaCl is incorporated in food, a meal would transiently increase the NaCl concentration and toxicity of extracellular fluid and the signal could be perceived in this way. To investigate this, we have recently prepared a high NaCl liquid diet to be fed to DS rats for 12 weeks with no additional water offered. These liquid diets contain 8 g NaCl/100 g soluble nutrients. These components were dissolved either in a minimal amount of water to produce a hypertonic liquid diet (1.4% NaCl) or these same components could be dissolved in a much greater volume of water to produce a hypotonic (0.45% NaCl) diet. Eleven DS rats were given the hypertonic 1.4% NaCl diet and 12 DS rats were given the hypotonic 0.45% NaCl diet. At the end of 12 weeks on either of the diets, the average intra-arterial mean blood pressure was 195 mm Hg for both the hypertonic and the hypotonic groups. Because just as much of a rise of blood pressure occurred when the NaCl was introduced in a hypertonic fashion, it is quite unlikely that a high NaCl concentration is the signal that brings about a rise in blood pressure.

If a rise in NaCl concentration is not the signal, the most likely alternate signal would be a rise in extracellular fluid (ECF) volume in some specialized receptor area. Many control systems involving body water are located in nuclei surrounding the third brain ventricle, which is a vertical, slitlike structure (Figure 4). It is conceivable that a high NaCl diet could bring on some excessive ECF volume in the local tissues on either side of the slit that constitutes the third brain ventricle. This localized ECF swelling of tissue on either side of the slit could cause the ependymal cells and nerve fibers in the walls of the slit to touch one another, which could give off a neurogenic or humoral signal indicating an increased ECF volume. We tested this hypothesis by blocking the aqueduct of Sylvius stereotaxically with an inert silicone material in various DS rats. Such a block creates hydrocephalus with a fourfold widening of the third brain ventricle in formalin-fixed brains, thereby partially preventing ependymal cells or nerve fibers from touching one another in response to a high NaCl diet. Twenty DS rats on a 0.23% low NaCl diet had a verified block of the aqueduct as described above, and 26 DS rats on the same low NaCl diet had a sham aqueduct block. After 6 weeks on these diets, both groups had an average intra-arterial mean blood pressure of 130 mm Hg. Thus, the block of the aqueduct had no influence on the blood pressure of...
DS rats as long as they were on a very low NaCl diet. Thirty-four other DS rats underwent a sham aqueduct block and then began consuming a 6% high NaCl diet. After 6 weeks, the intra-arterial mean blood pressure of these rats averaged 177 mm Hg, indicating a 47 mm Hg rise in blood pressure due to the high NaCl diet. In contrast to this, 17 other DS rats underwent a subsequently verified true aqueduct block and then were given the 6% high NaCl diet. After 6 weeks, the average blood pressure of this group was 149 mm Hg, indicating a 19 mm Hg increase in blood pressure due to the high NaCl diet. Thus, the true aqueduct block abolished 60% of the NaCl-induced rise in blood pressure ($p<0.001$).

These results appeared to support the hypothesis that the touching of ependymal and nerve cells lining the third ventricle produces the signal that ultimately raises the blood pressure in a susceptible species. After 12 weeks on the 6% high NaCl diet, the mortality rate for the 34 DS rats with the sham aqueduct block was 64%, whereas the mortality rate for the 17 DS rats with the true aqueduct block was only 6%.$^{25}$ Thus, the block of the aqueduct resulted in a 90% reduction in mortality rate, $p<0.001$. There were no deaths among either group of DS rats on the 0.23% low NaCl diet. Cumulative survival curves in Figure 5 indicate the striking increase in survival among the DS rats with the verified true aqueduct block. At the 11th week after surgery, none of the high NaCl rats with the true aqueduct block had died, whereas 50% of those with the sham aqueduct block had already perished.$^{25}$ The albumin in the 24-hour urine was also reduced 54% in the high NaCl DS rats with the true aqueduct block compared with those with the sham aqueduct block ($p<0.001$).

When the aqueduct is blocked with silicone, it is quite possible that some of the periaqueductal fibers of passage and nuclei might be destroyed by the pressure of the silicone, and this possible periaqueductal lesion could bring about the large reduction of blood pressure and mortality rate in the DS rats on the high NaCl diet. To examine this possibility, we made discrete thermal lesions stereotaxically in the periaqueductal structures of other DS rats just before they began the 6% high NaCl diet for 6 weeks. These rats were compared with another group of DS rats on the same diet who underwent sham thermal lesions. Twenty-five rats with the true thermal lesion and 13 rats with the sham thermal lesion were examined, and their respective average mean intra-arterial blood pressures were 176 and 166 mm Hg. With the thermal lesion, the blood pressure actually tended to be higher than with the sham lesion ($p<0.16$). Thus, a thermal lesion of periaqueductal fibers of passage certainly did not reduce the degree of NaCl-induced hypertension in the DS rats, which strengthens the notion that it is the hydrocephalus of the third brain ventricle that is reducing the hypertension and mortality in these NaCl-fed DS rats.

There are alternate theories to explain how an aqueduct block protects DS rats from a rise in blood pressure and a high mortality rate. However, it appears that the block of the aqueduct with subsequent hydrocephalus produces some change in the CNS, which greatly reduces NaCl hypertension and the death rate that results from it.

References


KEY WORDS • blood pressure • sodium-dependent hypertension • salt • circumventricular nuclei
Salt and hypertension. Lessons from animal models that relate to human hypertension.

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Hypertension. 1991;17:152
doi: 10.1161/01.HYP.17.1_Suppl.I52

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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