This is to certify that the

thesis entitled

Modification of Gustatory Nerve Activity and NaCl Preferences with Ingestion of a High Sodium Diet

presented by

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has been accepted towards fulfillment
of the requirements for

MS degree in Biological Sciences

Major professor

Date Aug 9, 1985

MODIFICATION OF GUSTATORY NERVE ACTIVITY AND NaCl PREFERENCES WITH INGESTION OF A HIGH SODIUM DIET

By

Timothy William Priehs

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Interdepartmental Program in Biological Science

1985

ABSTRACT

MODIFICATION OF GUSTATORY NERVE ACTIVITY AND NaCl PREFERENCES WITH INGESTION OF A HIGH SODIUM DIET

By

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The increased salt appetite of rats fed a sodium deficient diet and that of adrenalectomized rats is accompanied by a specific decrease in neural responsiveness to salt stimulation. We chose a set of experiments to determine whether the decreased salt preference of rats on a high salt diet was characterized by increased neural responses to salt. Neural recordings were made from the chorda tympani nerve of 8 experimental and 8 control rats following 14 days of NaCl preference tests, preceded by 10 days of adaptation to the test diet. The 24-hr 2-bottle choice method was used to determine preferences and intakes. The NaCl solutions were presented in ascending order of concentration in one-half log steps from .001M to 1.0M, with each concentration tested for two days using a restricted-random schedule. Rats were tested in staggered groups of four (2 experimentals & 2 controls) so that nerve recordings could be performed after the same relative amount of time had elapsed on diet and preference tests for all groups. Both groups received a diet which differed only in NaCl concentration--6% for high salt and 1% for normal. The experimental group exhibited a lower salt preference at all concentrations, but a paradoxical increase in saline intake below the .3M level. Nerve responses were calculated using a reference standard of .001M NaCl to measure relative differences. The nerve responses of the high salt group were significantly greater than control at all concentrations above .003M. This was also true for KCl, which was not tested behaviorally.

This thesis is dedicated to my parents for their unfailing patience and love for myself and my brothers.

ACKNOWLEDGMENTS

Suffice it to say that this undertaking would never have occurred without the encouragement, assistance, patience, and guidance of my major professor, Dr. Rudy A. Bernard. Rudy, in addition to being my mentor, also became a best friend whom I could confide in and turn to in times of need. I will always be grateful for the years we spent together and look forward to our continued friendship.

I also wish to acknowledge my other committee members, Dr. William Frantz and Dr. Frank Peabody, for their valued assistance in the preparation of this thesis. Special recognition is to be awarded my friends and colleagues, Ken J. Price and Jahan S. Eftekhar, for the assistance they so graciously gave when needed. Karen Mooney and Roger Shammas deserve a special thanks for attending to the welfare of my animals and providing technical assistance. Andrew Bernard supplied us with his computer wizardry, for which I am sincerely grateful.

I wish them all the best of luck in their future endeavors.

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INTRODUCTION

The intentions of this study were primarily two-fold. First, we examined the relationship between a high-salt intake and its effects on sodium appetite. This was accomplished by subjecting the animals to NaCl preference tests while they were ingesting a diet very high in sodium . Secondly, we obtained a direct measure of what actually occurs to the gustatory peripheral input when there is an alteration in sodium appetite. Contreras (1977) has previously shown that the high salt appetite which occurs on a sodium deficient diet is accompanied by a decreased neural responsiveness to salt stimulation. It was our desire to find whether this phenomenon of modification of salt taste reception would also occur in rats fed a high-salt diet.

The underlying hypothesis of this study is that taste is not a modality which is unchanging and fixed, but instead is a plastic, modifiable system which is capable of changing according to the current environment of the animal. Furthermore, this adaption process plays a regulatory role in informing the animal of current body requirements and also acts to help identify those elements which are not vitally needed. This is not a new idea, for it has been examined for many years. For example there are many animals which, when depleted of specific minerals or vitamins, will eat their own or other animals' feces to help replenish the stores of the material in shortage. Ordinarily the consumption of fecal material would not be considered normal behavior, but the body is able to stimulate the

consumption of what is needed by modifying the sense of taste. What was previously unpalatable becomes palatable.

We subjected our rats to a high sodium diet, and determined whether NaCl solutions which are known to be highly desirable by the rat in certain concentrations, would become undesirable as a result. Pfaffmann (1957) was one of the first to note that a decreased preference ($VOL_{saline}/VOL_{saline+water}$) for sodium solution accompanied the administration of a high-salt diet. He also found that this decreased preference extended to several concentrations of sodium chloride. Our intentions were to measure this aversion over a wide concentration range of NaCl solutions to note whether there are specific changes to the preference curve or whether it is a general adaptive phenomenon to sodium; i.e. does the animal dislike only the lower concentrations, higher concentrations, or both?

After determining how the high-salt rats reacted to an additional source of NaCl in their diet, we then examined the role which taste played in relating this increase in dietary sodium to an expected decrease in the desire for NaCl. How might this increased intake of dietary sodium affect the way rats respond to the taste of the salt solutions?

A surgical preparation exists for measuring salt taste from the tongue by accessing the chorda tympani nerve before it enters the cranium. This nerve conveys sensory information from the anterior two-thirds of the tongue to the brain. In the rat, it primarily carries the taste of salt, but to lesser degrees it also acts to carry bitter, sweet, and acidic sensory information. By measuring the neural activity from the chorda tympani in

response to stimulating the tongue with NaCl solutions, we can then quantify the variations in salt reception between those rats maintained on a high-salt diet and those on a normal diet.

If we do find that there is a significant change in the chorda tympani responses between normal rats and high-sodium rats, how might this alter the animals' behavior? Does an altered sensory input necessarily modify the normal amounts ingested? These are some of the questions which we hoped to shed light on in the pursuit of this study. In addition, if the neural response to NaCl can be changed by dietary intake, does this alteration in gustatory sense apply only to NaCl or to other substances which also taste salty. How discriminating is the sense of taste in identifing a specific ion? To help discover the answer to this question, we also tested a wide range of KCl solutions on the chorda tympani preparation. If there is a similar change in the neural responses to KCl as well as to NaCl, then it would appear that the body treats the taste of salt as a continuum and not restricted to the sodium components.

REVIEW OF THE LITERATURE

Salt Appetite

There is a large body of literature dealing with the ability of rats to adjust their salt intakes in relation to their bodily needs (Richter 1936, 1939; Nachman, 1962; Nachman and Pfaffmann, 1963; Falk, 1965; Contreras, 1977; Contreras and Frank, 1979; Denton, 1982). Much of this work was done concerning the effects of sodium deprivation on the phenomenon of salt appetite. The usual method taken when inducing this appetite is the replacement of the normal diet (1% NaCl) for one which is low in sodium, usually .1% or less. The development of a salt-deficient rat involves some amount of time in order for the aminal to deplete its body stores of sodium and for the appetite to develop. Normally, 10 days is a sufficient period of time for this to occur.

Other experimental methods have been employed for induction of an acute increase in salt appetite in the rat. These include: (a) adrenalectomy (Richter, 1936, 1939; Nachman, 1962; Wolf and Stricker, 1967); (b) the administration of mineralocorticoids (Rice and Richter, 1943; Wolf, 1965; Zheng et al., 1984); (c) administration of diuretics (Cruz, et al., 1977; Wolf et al., 1984); and (d) the injection of formalin or hyperoncotic polyethylene glycol subcutaneously to cause localized edema (Stricker and Wolf, 1966; Stricker and Jalowiec, 1970). These endeavors have the same goal as dietary sodium depletion, to cause an increase in salt appetite, but at an accelerated rate. The induction of a salt appetite is measured by the increased consumption of saline solution.

The degree of sodium appetite is normally assessed by preference tests in which rats are offered a choice between saline and water to drink. An important determinant in these preference tests is the total sodium load of the body (Wolf, 1984). Sodium deficiency induced by a salt-deficient diet or by adrenalectomy results in increases in saline intake, even at previously aversive concentrations (Richter, 1956). Furthermore, this increase in saline consumption normally extends to the point where the animal is receiving sufficient sodium that it is no longer sodium deficient. It will not normally ingest sodium beyond what is physiologically required.

Many studies have examined the role of mineralocorticoids upon sodium appetite. If the rat has been previously adrenalectomized, which causes the development of a large sodium appetite, the administration of low doses of DOCA lowers the intake and preference for NaCl solutions due to a restoration of sodium-retaining ability (Wolf, 1964). This has aptly been termed "replacement therapy". If however the administration of DOCA is increased beyond the "replacement" dosage, there is an increase in sodium appetite. The mechanism of this increase in saline consumption with high levels of mineralocorticoids is not known.

It should be recognized that the induction of a salt appetite by feeding animals a sodium-deficient diet is in part due to an elevation in mineralocorticoid levels. Cruz et al. (1977) point out that under natural conditions in which the food is deficient in sodium, the development of a body sodium deficiency is accompanied by an increase in aldosterone. Aldosterone acts to maintain homeostasis by reducing sodium losses in bodily excretions and possibly by potentiating the salt appetite. According

to Cruz et al., there are at least two independent natrorexigenic conditions, body sodium deficiency and increased mineralocorticoid levels. Both act to stimulate sodium appetite by activating receptors sensing sodium depletion.

Zhang et al. (1984) believe that the increase in sodium appetite is due to a synergism between mineralocorticoids and angiotensin. These hormones are ordinarily released in conjunction with angiotensin II causing the release of aldosterone from the adrenal gland. Administered separately in low dosage, DOCA (a precursor to aldosterone) and angiotensin have no effect upon salt appetite. If, however, rats are treated with both hormones, there is a marked increase in consumption of 3% NaCl solution. The authors believe that it is a synergy of these two hormones of salt conservation that informs the brain of the need for salt and generates the appetite for the taste of salt.

The induction of a sodium appetite by the use of Na-deficient diets may share a similar etiology. Contreras (1979) proposes that there are two separate and independent mechanisms for the induction of salt appetite, one adrenally mediated and the other a specific hunger for the sodium ion. With the loss of sodium in the diet, the natural response is increased release of renin, causing elevated levels of aldosterone and angiotensin in the blood (Davis and Freeman, 1976). It may be that it is the action of these two hormones on the brain which causes the salt appetite (Zhang et al., 1984). This also lends credence to the idea that it is a hormonal mechanism which alters the neural response to sodium in salt-deficient rats.

Effects of a High Salt Diet

Much less attention has been directed to the behavior of rats maintained on a high-salt diet (Binet et al., 1955; Pfaffmann, 1957; Fregly et al., 1965). Although it has been shown that a sodium deficiency will result in an increased saline intake and increased preferences, there is conflicting evidence whether saline intake, and preferences, decrease with a high-salt diet. Fregly et al. (1965) demonstrated that in male rats fed a diet of either 2%, 4% or 6% NaCl for five days, saline intake per 100 g body weight was constant for all three diets. In other words, as the diet became increasingly salty, the rats did not correspondingly decrease their saline intakes. These findings must be prefaced by the knowledge that the investigators examined the effects on only three concentrations of saline (.15M, .2M, and .25M NaCl), all in a very narrow range, and all hypertonic. As the concentration of the saline solutions increased, the rats ingested correspondingly less solution so that the milliequivalent intake of sodium remained constant (1.3 - 1.5 mEq/100 g). Fregly et al. suggest that these results show that salt intake is "regulated" by the concentration of NaCl in solution and not by the concentration in food. They believe that the constancy of intake of NaCl solution demonstrates that rats "regulate" their intake of sodium and that "regulation" occurs by way of NaCl in solution and not in food. This necessitates that in order for sodium appetite to be regulated, the NaCl must be tasted.

This point of view is not shared by all investigators. Wolf et al.

(1984) believe that the notion that taste input is the sole mechanism for satiation implies an essential difference between salt appetite and other appetites, i.e. for food and water. These drives can be reduced without taste stimulation by gastric loading. They also point out that regulatory systems of the body typically involve multiple control mechanisms. If taste were the only mechanism that could satiate salt appetite, it would be atypical for this reason.

Pfaffmann (1957) and Richter (1956) found that rats do decrease their intake of NaCl in solution when dietary intake of sodium is increased, in contrast to the findings of Fregly et al. (1965). Pfaffmann found that when NaCl concentrations in food are increased from 5% to 10%, there is a reduction in saline preference. Unfortunately, no mention is made of how long the rats were maintained on this diet. What occured in this study was that the rats were transferred from a high-salt diet to one which was extremely salty. The 10% NaCl diet was the greatest concentration of dietary NaCl found in the literature. Administered in the diet, concentrations above 8% NaCl often result in severe health problems for the animals involved (information provided by TEKLAD®), so it is not likely these rats were continued on this diet for a long period of time.

Richter also studied the role of a high dietary intake of salt on saline consumption. He also found that the intake of salt solutions declines when dietary salt is increased. He used NaCl concentrations which were much more moderate, increasing from 1% (standard diet concentration) to 4% NaCl. Unfortunately, the time spent on the high-salt diet in this study was also omitted. The usual length of time employed on a specific diet (high- or

low-salt) before the initiation of preference tests is 10 days. It is therefore difficult to address the discrepencies between Fregly and Richter/Pfaffmann if the duration of the diets is not known.

Alteration in Gustatory Nerve Activity

Another aspect of sodium deficiency which has been investigated is the change in salt taste reception. Contreras (1979, 1984) has demonstrated that in a rat made sodium deficient, by way of adrenalectomy or diet, electrophysiological recordings from the chorda tympani nerve show that neural responses to NaCl solutions are reduced. This reduction is evident at all levels of NaCl, but is most evident at the higher concentrations. A plausible consequence of this decrease in neural response is a general decrease in the ability of the rat to taste salt (Contreras, 1978). In effect, salt would taste less salty and therefore more NaCl would be ingested to satisfy the desire for salt. This phenomenon has been recorded from both whole nerve preparations and single units of the chorda tympani (Contreras, 1977; Contreras and Frank, 1979).

There is conflicting evidence concerning the effect of sodium deprivation on afferent nerve activity from the tongue. Contreras has performed extensive studies detailing the change in taste reception for NaCl solutions in salt-deprived animals (Contreras and Hatton, 1975; Contreras, 1977; Contreras and Frank, 1979; Contreras et al., 1984). Nachman and

Pfaffmann (1963) found that in whole nerve chorda tympani recordings from sodium deficient rats, there was no effect on the afferent gustatory signal when the tongue was stimulated with NaCl solutions. Both groups of investigators found an increase in saline consumption with sodium deficiency, but had opposite results concerning the neural data. Nachman and Pfaffmann employed whole nerve preparations in their recordings whereas Contreras utilized a single unit preparation originally, but he later repeated his findings with whole nerve preparations. Contreras (1979) states that he believes the major reason for the discrepency between his and Nachman/Pfaffman's results is because the latter calculated response ratios utilizing .01M NaCl as their reference. According to Contreras, this would effectively mask any absolute change in responsivity to NaCl brought on by sodium deprivation, and in fact when he recalculated his responses relative to .1M he could find no differences between the sodium-deficient and control animals. At the .01M concentration, there is already a significant change in the neural responses between low-salt and sodium replete animals. In order for Nachman and Pfaffmann to have shown this they would hav had to use a reference standard of lower concentration. Contreras used the baseline, or response to the water rinse, to calculate the response ratios.

Contreras has found that sodium depleting rats by either a sodium-deficient diet (1976, 1979) or by adrenalectomy (1984) resulted in a marked reduction in chorda tympani activity when the tongue was stimulated with NaCl solutions. He found, in fact, that in order to obtain the same neural response in sodium deficient animals as found in control animals, it was

necessary to increase the saline concentration 10-fold. Contreras and his colleagues believe that this decreased neural activity is a result of an altered taste sensitivity for salt. This altered receptor sensitivity may be an adaptive mechanism to influence salt consumption. Also because repeated stimulation of the tongue with NaCl solutions results in increased chorda tympani responsivity to NaCl, it may be that salt receptor sensivity is altered simply through disuse of the receptors, as would occur on a Na deficient diet.

No similar studies have been done to date exploring the relationship between a high-salt diet and neural responses. Based on Contreras' data that a sodium deficiency will result in supression of nerve responses, a high-sodium diet might be expected to result in enhanced nerve responses to NaCl on the tongue. This is the premise, and basis, upon which we launched this study.

MATERIALS AND METHODS

Subjects

The animals employed in this study were 16 male Sprague Dawley rats, 8 experimentals and 8 controls, varying in weight from 340 to 456 grams at the time of their surgeries. All of the rats had the same date of birth, but since the surgeries were done on staggered groups, they were not all of the same age at the time of the neural recordings. Each was individually housed in a plexiglass cage (32 x 35 x 16 cm) with a detachable metal top. All food, water, and flavor (NaCl) solutions were available ad libitum. Cages were changed weekly, at which time animal weights were obtained. The animals were housed in an isolated room with the temperature maintained at 70°-72° F and a 12-12 hour light/dark cycle in effect.

The animals were given one of two diets; control rats were fed a purified rat chow diet purchased from TEKLAD® (P.O. Box 4220, Madison, WI) containing .97% NaCl, experimental animals received an identical diet except that it contained 6% NaCl. The diets were pelletized into hard oblong pieces which were the exact size of standard rat chow and a pale yellow color. Both groups began receiving their respective diet 10 days prior to the initiation of preference tests (see below) and then for the 14 days of the preference tests. At the conclusion of this time span, the animals were anesthetized for neural recordings and subsequently sacrificed. Therefore, total time of exposure to the diet for each animal before the neural recordings began was 24 days. Additionally, the rats were tested in staggered groups of two experimentals and two controls, so that nerve

recordings could be performed after the same relative amount of time on the diet and preference tests.

The preference tests were of the 24-hour 2-bottle choice method, in which one bottle contains distilled water and the other a taste solution. The rat is then allowed to drink freely from both bottles, during which time it usually drinks more from one bottle than the other so that a preference for the taste solution can be determined. Preference is calculated by dividing the amount of taste solution drunk in a 24 hour period by the total fluid consumption from both bottles. This will result in a percentage which is expressed as the preference for that concentration of taste solution. For example, if a rat drinks 53 ml of .01M NaCl and 34 ml of distilled water in a 24 hour period, his preference would be calculated by: 53 ÷ 87 = .61 x 100 = 61%. Therefore, for a given NaCl solution intake, preference is a function of water intake. The more water consumed in relation to the salt solution, the lower the preference and vice versa.

Another method of expressing saline intakes is by milliequivalents of sodium consumed. When presented as quantity of solution drunk (ml), saline intakes do not accurately portray the amount of NaCl ingested. The highest concentrations of saline result in the least amount of solution being drunk, but conversely the greatest amount of NaCl is ingested at these concentrations. This is because the molarity of the solutions is so great that very small amounts of solution account for great amounts of sodium.

A potential problem with this measure of NaCl intake is that a small error in measuring the quantity of solution consumed at high concentrations can result in a proportionately greater error in the mEq of NaCl consumed.

Spillage of even one ml of a 1M solution will account for 1 mEq of NaCl, which may be as much as 20% of the total intake. This source of error is most important when the molarity of the solutions is high, which unfortunately is also when the rats consume the least amount of solution, compounding the potential source of error. This factor was recognized and great care taken when changing cages, bottle weighing, etc. to minimize inadvertant bottle spillage.

Preferences and saline and water intakes were calculated by daily computerized weighings of the bottles. The NaCl solutions were presented to the rats in ascending order of concentration, in one-half log steps from .001M to 1.0M. Each concentration was tested for two days with the position of the NaCl bottle changed on the second day to prevent positional preferences from biasing the intakes.

Neural Surgery

Nerve recordings were achieved by monitoring activity of the chorda tympani nerve, which provides gustatory innervation to the anterior two-thirds of the tongue. The animals were anesthetized with a combination of Xylazine (Rompun®), 7.5 mg/kg body weight, and Ketamine Hydrochloride (Vetalar®), 37.5 mg/kg body weight, injected intramuscularly. Supplementary doses of the above drugs were sometimes necessary to maintain a sastifactory level of anesthesia, but dosages were reduced by

one-half. A tracheostomy was performed during all surgeries to facilitate respiration. Body temperature was monitored via a rectal temperature probe, and maintained at 36°-37° C. The lower incisors were severed to allow greater access to the tongue, to which a suture was affixed through the ventral epithelium. The tongue could then be drawn out of the mouth and held in position by fastening the suture to the operating board.

The animal was secured to the operating board ventral surface down and the head turned so that the left side of the face and cranium pointed upward. From this position the right chorda tympanic branch of the seventh cranial nerve was dissected free from overlying bones, blood vessels and muscle. This procedure required the use of a binocular operating microscope customarily set between 6X and 16X magnification. After isolation, the chorda tympani was cut where it exits from the bulla and cleared of connective fascia until approximately 5-7mm were available for placement on the platinum electrode. Before placement, the neural sheath was peeled back using fine microdissection forceps. Once this was accomplished, the entire nerve was placed on the electrode and the electrode, nerve, and cavity surrounding the surgery site was covered with a 50/50 mixture of petroleum jelly and paraffin oil. This mixture was pre-mixed and warmed to 37° C before application to the surgery site. We had previously found that utilizing this viscous concoction greatly increased the viability of the nerve preparation by preventing nerve dehydration[1].

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[1] Thanks are due to Dr. Fay Ferrell, Dept. of Nutrition, Univ. of California-Davis, for sharing this information with us.

Neural Recording

Upon completion of the surgery and nerve placement, the tongue was drawn from the mouth and fastened so as to remain outside the mouth with a minimum amount of tension. An inert plastic tube which conveyed various solutions to the tongue was then placed in close proximity to the dorsal surface of the tongue. Through this tube, via a gravity flow system, flowed the stimulus solutions and the subsequent distilled water rinses. Flow rates through this system measured approximately 10-20 ml per minute.

Once flow was initiated, the tongue was continuously bathed in either the stimulus solution or distilled water rinse. The stimulus solutions reached the tongue from a common reservoir which was filled from one liter bottles by a compressed air positive pressure system. Because the stimulus solutions were presented in ascending order, it was not necessary to rinse the reservoir with water each and every time. However, when changing from one type solution to another (NaCl to KCl for example), the reservoir was thoroughly rinsed to prevent contamination. The distilled rinse had its own separate reservoir.

Changing between the stimulus and rinse solutions bathing the tongue was accomplished with a pneumatic valve which was controlled by a programmable, electronic timer. Both stimulus and rinse times could be individually set. The stimulus period lasted for 20 seconds and rinse period usually for one minute, or until nerve activity returned to baseline levels. It

was sometimes necessary to extend the length of the rinse period after stimulating the tongue with high molarity solutions.

Nerve activity was continuously monitored by oscilloscope tracings, and quantified by an electronic integrator whose output was displayed on a polygraph recorder. In addition, the integrator output was routed via an analog-to-digital converter to an Apple II Plus® computer where baseline, peak voltage, and total response magnitudes were calculated, displayed, printed, and stored on a magnetic disk.

It is necessary in this type of study to have a normalization procedure by which voltage differences in individual rats can be prevented from biasing the results. By using a reference standard against which all other responses are measured, and then expressing the difference between the two as a ratio, it is possible to calculate a response relative to a common denominator. In this manner, we were able to compare differences in the two groups' neural responsiveness without concern for individual voltage differences between preparations. One animal often will respond with a different voltage to a particular stimulus than another animal in the same group. Also deterioration of the preparation or other changes with time were compensated for by the pre- and post-stimulus period application of the reference standard. Within limits, as a stimulus response decreases with time, so does the reference standard, so that the ratio between the two remains the same as it would be at the beginning of the experiment. For these reasons, it was necessary to use a standard against which all stimuli were compared and a ratio between the two determined.

The reference standard employed in this experiment was .001M NaCl.

This concentration was used because it produced absolute responses (mean ± SD) in both groups which were not significantly different (570 ± 148mV vs. 695 ± 238mV), thereby allowing us to make across the board comparisons for each stimulus above this concentration.

A characteristic sequence would be:

(1) The application of the reference standard followed by a rinse period.

(2) The application of three separate stimulus solutions, all of the same concentration, each followed by a distilled water rinse.

(3) The application of the reference standard followed by a rinse period.

The neural response for each stimulus strength was expressed as the ratio of the test response to the reference response. This was accomplished by dividing the median value of the three test peak responses by the mean of the two reference responses. Since all stimulus responses were equated to .001M NaCl, as long as we could obtain a detectable and repeatable .001M response we could formulate a ratio of reference standard to stimulus concentration for each solution tested. Curves plotting nerve response vs. concentration were thus generated for each animal and for the group means as well.

Although neural data was collected from all of the rats for all concentrations of the NaCl solutions, data was not collected from three rats

when measuring KCl nerve responses. This was due to the fact that KCl stimuli were applied after NaCl stimuli and in three instances (two control and one experimental) the condition of the nerve had deteriorated to a point where the experimenter could not ensure the validity of the data. The viability of the nerve preparation was regularly assessed by stimulating with .1M NaCl, since normally it would give a significant and repeatable peak response. The deterioration of these three preparations could be attributed to no specific cause and instead probably was due to a general decrease in the status of the rat under anesthesia. These animals were then sacrificed without further collection of data.

RESULTS

Diets

All animals remained healthy for the duration of the experiment. There was some concern that the high-salt diet would cause those rats receiving it to possibly consume less food than their control counterparts, due to the decreased palatability of the diet. This, however, proved to be an unfounded concern. The experimental rats gained an average of 34 ± 7 grams over the course of the experiment (figure 1). The control rats gained slightly more, 44 ± 10 grams, but not enough to be statistically significant. None of the animals exhibited a weight loss while on either the experimental or control diets.

Preference Tests

Only sodium chloride solutions were tested in this part of the experiment. One of the intriguing aspects of this study was to determine whether saturating an unavoidable source of sodium (the food) would result in a marked rejection of other sources of sodium which were freely available to the rat. In this case the dependent variable would be the solutions of NaCl, which varied in concentration, and the observed effect would be whether this diet altered the normal preference curve of the rat.

The experimental rats did exhibit lower preferences than the

Fig. 1.-- Weight gain of rats on high-salt diet (Exp) and normal (Ctr) diet. Day 1 represents initiation of the particular diet which was maintained for 24 days, at which time neural recordings were begun. There is no significant difference in growth rates.

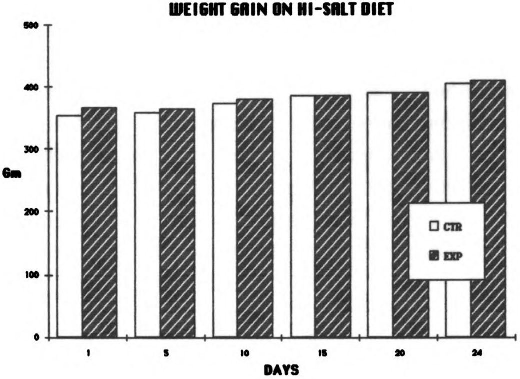
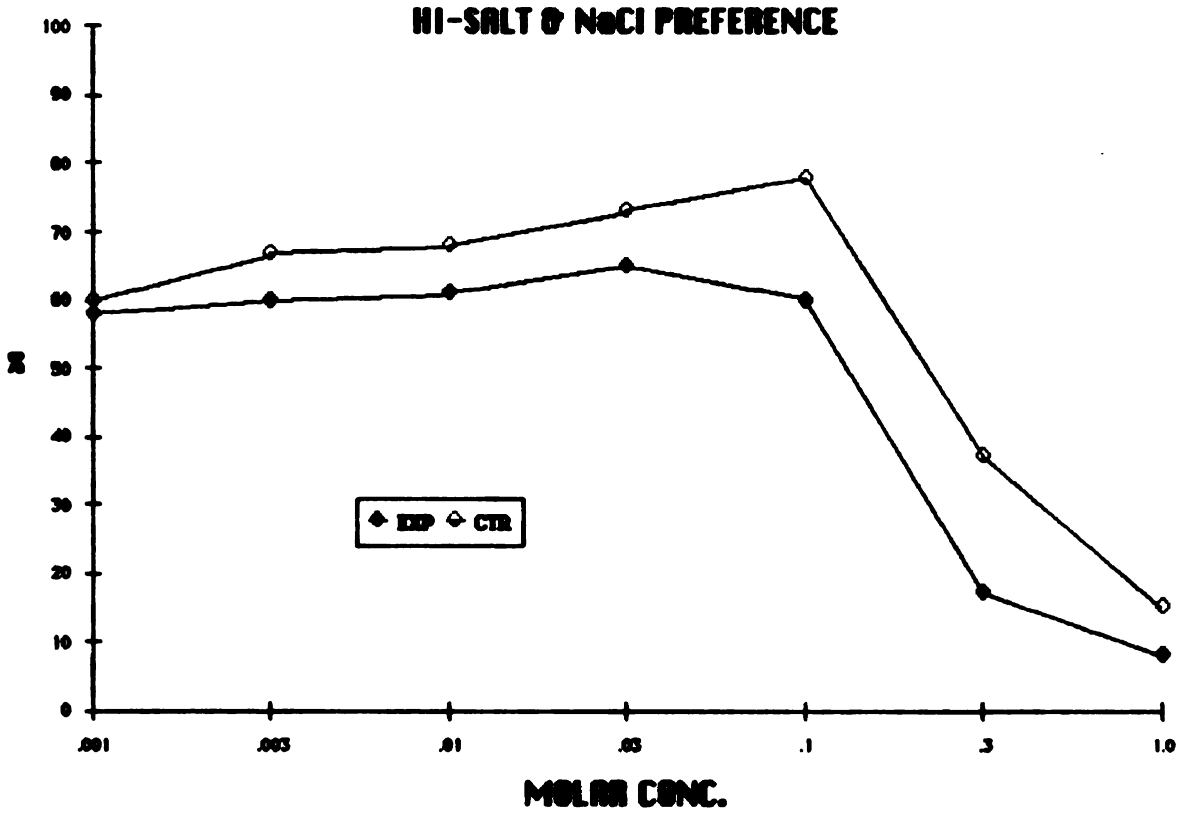

WEIGHT GAIN ON HI-SALT DIET
500
400
300
200
100
0
Gm
1
5
10
15
20
24
DAYS
☐ CTR
▨ EXP

Fig. 2.-- NaCl preferences by high-salt (Exp) and normal (Ctr) rats. NaCl concentrations were presented in ascending order of concentration in half-log steps from .001M to 1.0M. Differences between the two groups were statistically significant ($p<.025$) from .03M to .3M, although the experimental group had lower values than the controls at all concentrations.

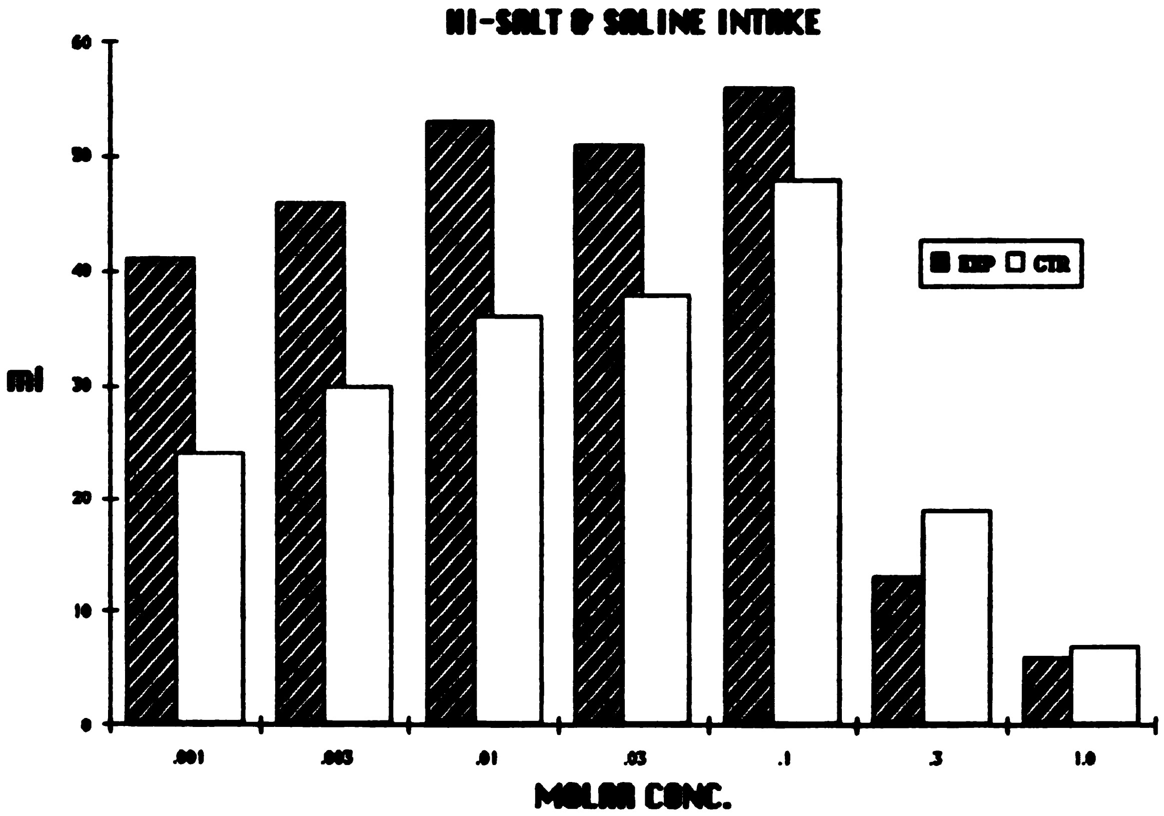

HI-SALT & NaCl PREFERENCE
100
90
80
70
60
50
40
30
20
10
0
%
◆ EXP ◇ CTR
.001
.003
.01
.03
.1
.3
1.0
MOLAR CONC.

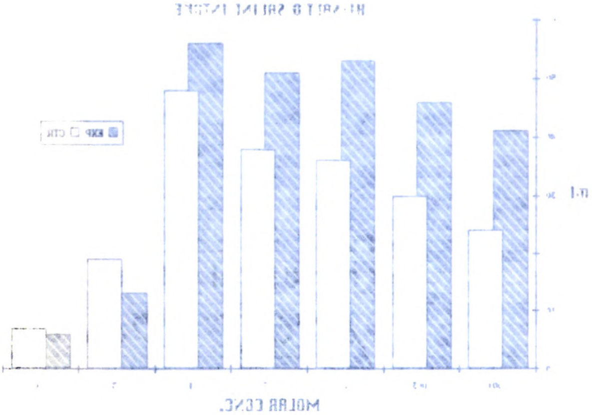
control rats for the entire range of NaCl concentrations (figure 2). For the initial concentration tested, 001M, both groups showed approximately the same percent preference, 60% for control vs. 58% for experimental. This was the nearest the two groups came in their preference values. The greatest discrepancy occurred at .1M, normally the peak preference point of the curve, which it was for the controls. The experimentals, however, displayed a minor decrease in preference at the .1M level, from 65% to 60%, with their peak preference occuring at the .03M level. Both group preferences fell precipitously after .1M.

One important feature to be distinguished about the preference curves is that the preferences of experimental rats never rose above 65% at any point along the concentration series. In contrast, the control rats showed a marked and steady increase in preference until they reached a peak of 78% at the .1M level. The rats on the high-salt diet, therefore, did not ingest much of the low to middle concentrations of saline in contrast to their normal diet counterparts. There appeared to be relative indifference to these solutions by the experimental rats until the highest concentrations were reached, when they exhibited a strong aversion for these solutions.

An unexpected finding of the preference tests was the fact that although the experimental rats exhibited lower preferences than the controls for the salt solutions, they displayed higher absolute intakes of the solutions at almost every concentration. Figure 3 illustrates the marked dissociation between preference for and intake of NaCl in the experimental animals. Although they preferred the saline solutions less

Fig. 3.-- Intake of NaCl solutions by high-salt (Exp) and control (Ctr) rat groups in the preference tests shown in fig. 3. The experimental group consumed greater amounts at all but the highest concentrations of saline, with the greatest discrepancies in intake occuring at the lowest concentrations.


HI-SALT & SALINE INTAKE
ml
0
10
20
30
40
50
60
EXP
CTR
.001
.003
.01
.03
.1
.3
1.0
MOLAR CONC.

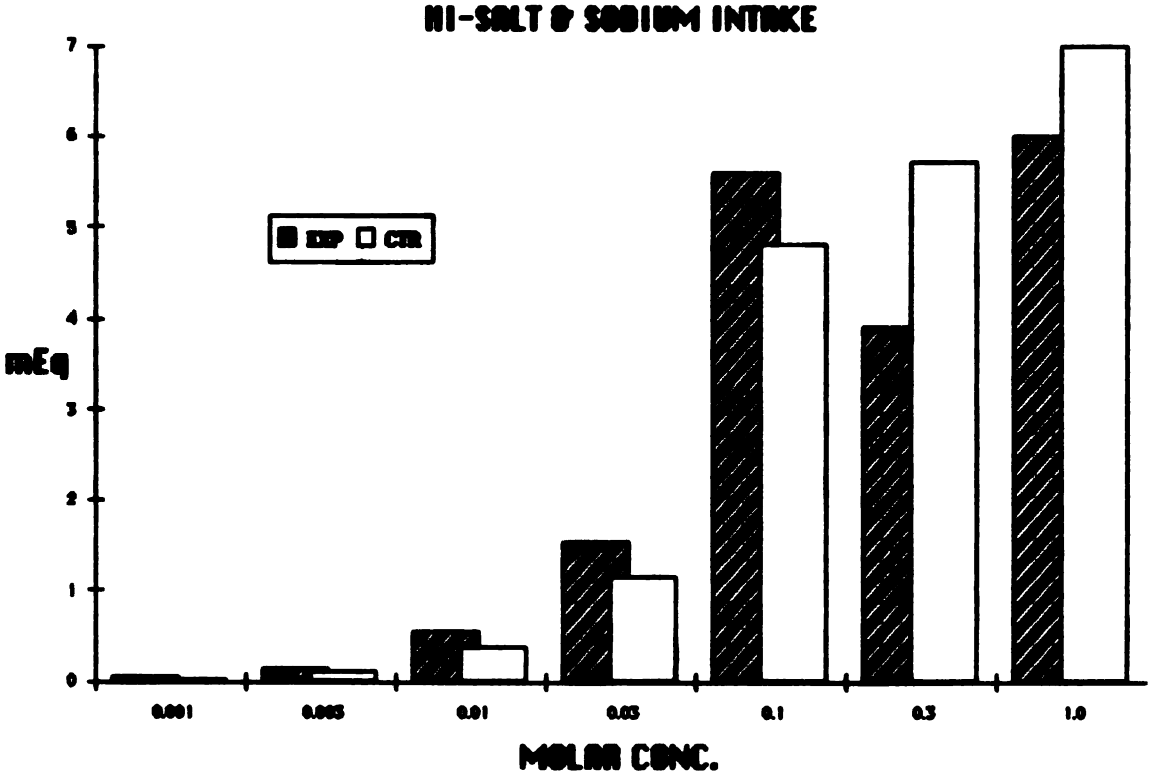

HI-SALT & SALINE INTAKE
EXP
CTR
ml
MOLAR CONC.

than did the control rats, they drank more of them. Between .001M and .1M, for example, the high-salt diet rats consumed much greater quantities of the solutions than did the control rats. The greatest difference occurred at the lowest concentration, with the experimental rats drinking almost twice as much .001M (41 ml) as did the control rats (24 ml). This difference progressively narrowed as the concentration increased, until intakes became essentially equal at 1.0M.

Figure 4 shows the milliequivalents of sodium ingested via the NaCl solutions. In contrast to the volume intake, the amount of sodium ingested increased with each successive NaCl concentration (except for one point). This is because volume intake does not take into account the fact that although the rats may be ingesting much less of the .3M and 1.0M NaCl solutions, the concentration of these solutions is sufficiently greater to account for an overall net increase in sodium intake. Thus, even though the experimental group only drank 6 ml of the 1.0M solution compared to 55 ml of the .03M solution, the actual quantities of sodium ingested were 1.5 mEq at .03M and 6 mEq at 1.0M. The highest amounts of sodium intake in terms of milliequivalents occured at 1.0M, but so did the lowest NaCl intakes for either group in terms of volume of solution consumed.

Water intakes, of course, need no such transformation. As figure 5 shows, the experimentals displayed a relatively steady water intake from .001M to .1M, which was approximately twice that of the controls, (33 ml ± 3 vs. 15 ml ± 2). When exposed to NaCl concentrations above .1M, the experimental group maintained its relative position of consuming

Fig. 4.-- Intake of sodium in milliequivalents calculated from the amount of solution drunk in the preference tests shown in figs. 2 and 3. Both high-salt (Exp) and control (Ctr) groups increased their sodium intakes throughout exposure to the saline solutions even though quantities of solution ingested diminished with increased concentration.


HI-SALT & SODIUM INTAKE
EXP
CTR
mEq
7
6
5
4
3
2
1
0
0.001
0.005
0.01
0.03
0.1
0.3
1.0
MOLAR CONC.

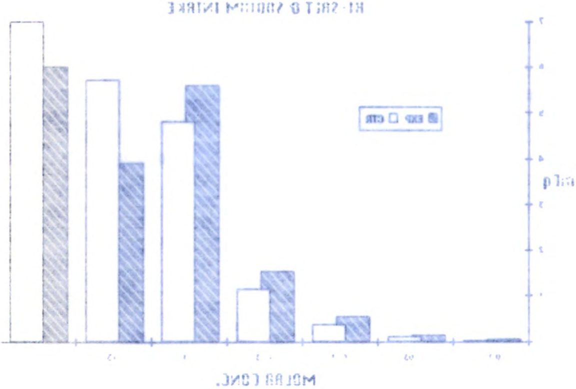
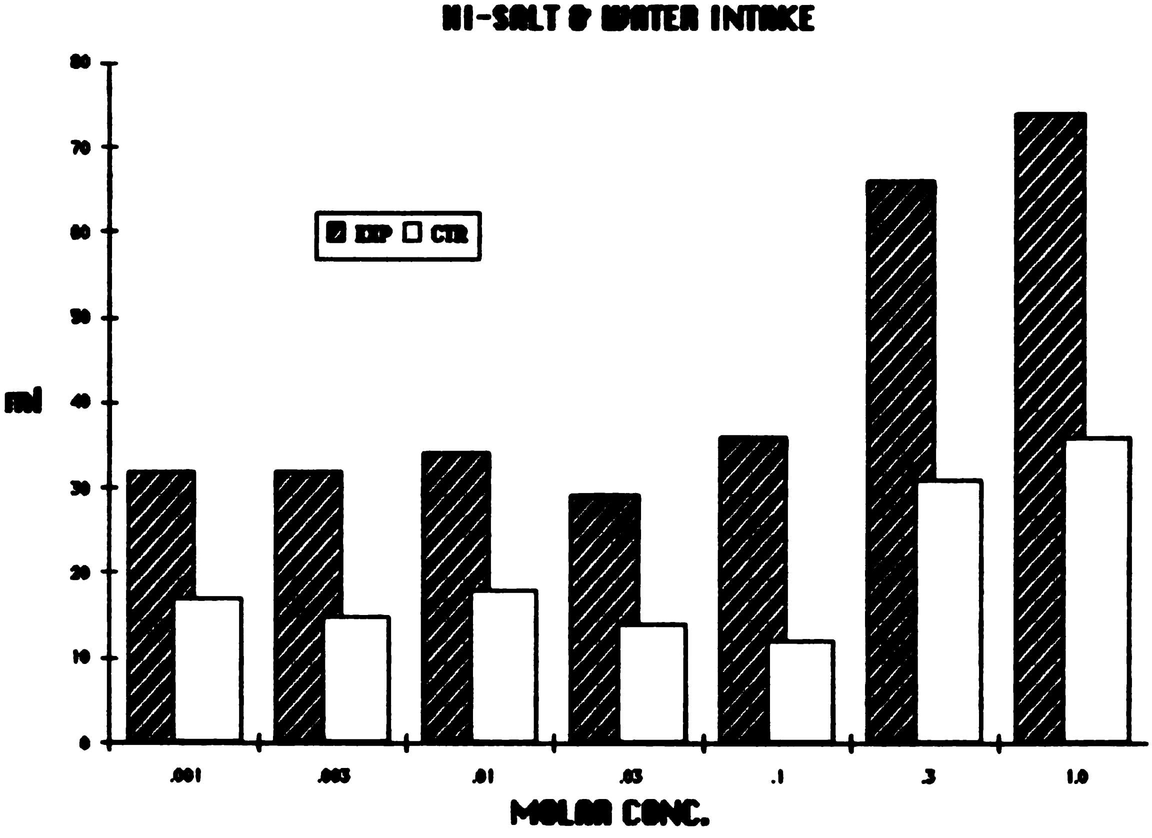

KI-SALT @ SODIUM INTAKE
EXP
CTR
mEq
MOLAR CONC.

Fig. 5.-- Water intakes for the high-salt (Exp) and control (Ctr) groups. The experimental group drank significantly greater quantities of water than did the controls. Both groups dramatically increased their water consumption when exposed to saline concentrations greater than .1M.


HI-SALT & WATER INTAKE
80
70
60
50
40
30
20
10
0
ml
▨ EXP ☐ CTR
.001
.003
.01
.03
.1
.3
1.0
MOLAR CONC.

twice as much water, but intakes of water increased dramatically for both groups. For example, at the 1.0M level, the experimentals consumed 74 ml of water as opposed to 36 ml for the controls.

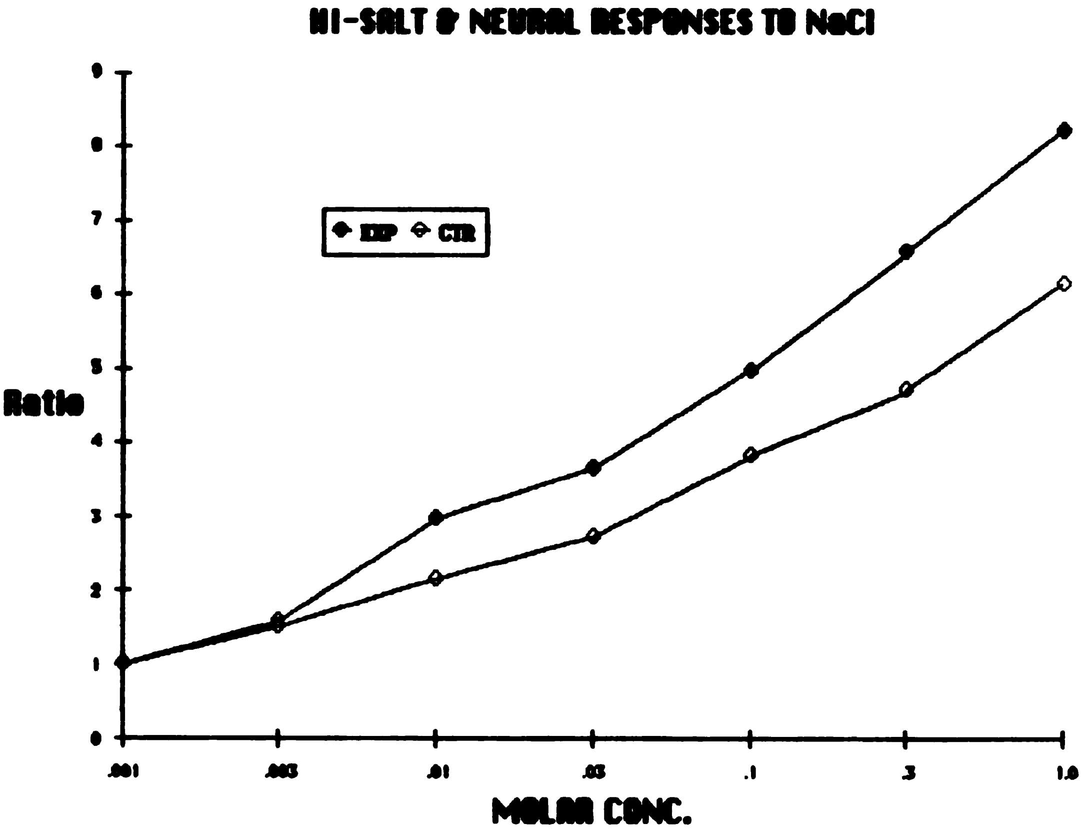
Neural Recordings

The experimental rats had larger neural responses than the controls to almost all the concentrations of NaCl and KCl tested (figures 6 and 7).There was no significant difference in the absolute response of the two groups to .001M NaCl. Thus this response could serve as a connom reference for normalizing and quantifying the responses to all subsequent stimuli, which are expressed as ratios. It is important to note that the differences between the neural responsiveness of the two groups increased with increasing concentration of solution. The neural response curves begin at a common point and progressively diverge as the concentration increases, with the greatest difference at the 1.M levels.

Comparison of the response ratios within each animal group, shows that the NaCl series produced greater response ratios at each concentration of stimulus tested than did the corresponding KCl series. Although the NaCl and KCl responses were roughly equivalent at the lower concentrations, the ratios at the higher NaCl concentrations were significantly greater than the corresponding KCl response ratios.

The reasons for using potassium chloride in the neural recording phase of the study, even though it was not used in the preference tests, will be discussed below.

Fig. 6.-- Median chorda tympani nerve responses of high-salt (Exp) and control (Ctr) groups when exposed to the same NaCl concentrations as were used in the preference tests. Experimental values were significantly greater than control values ($p<.005$) beginning at .01M. Responses are expressed in relative units with the response to .001M NaCl serving as the basis for the calculated ratios.


HI-SALT & NEURAL RESPONSES TO NaCl
◆ EXP ◇ CTR
Ratio
0
1
2
3
4
5
6
7
8
9
.001
.003
.01
.03
.1
.3
1.0
MOLAR CONC.

[illegible]

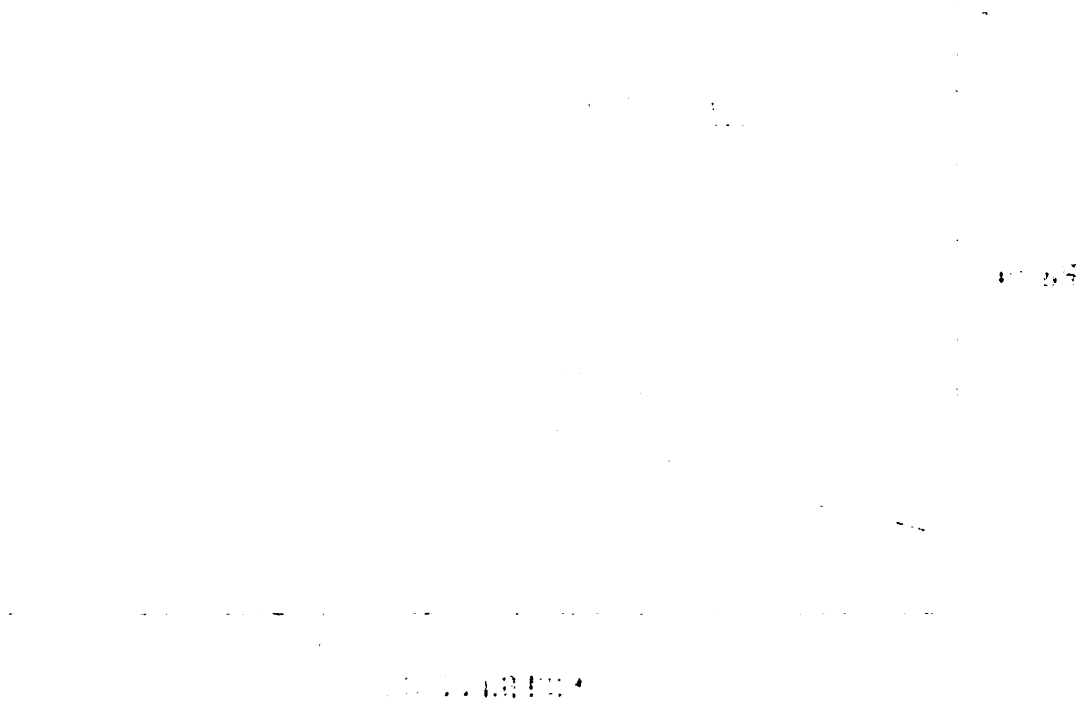
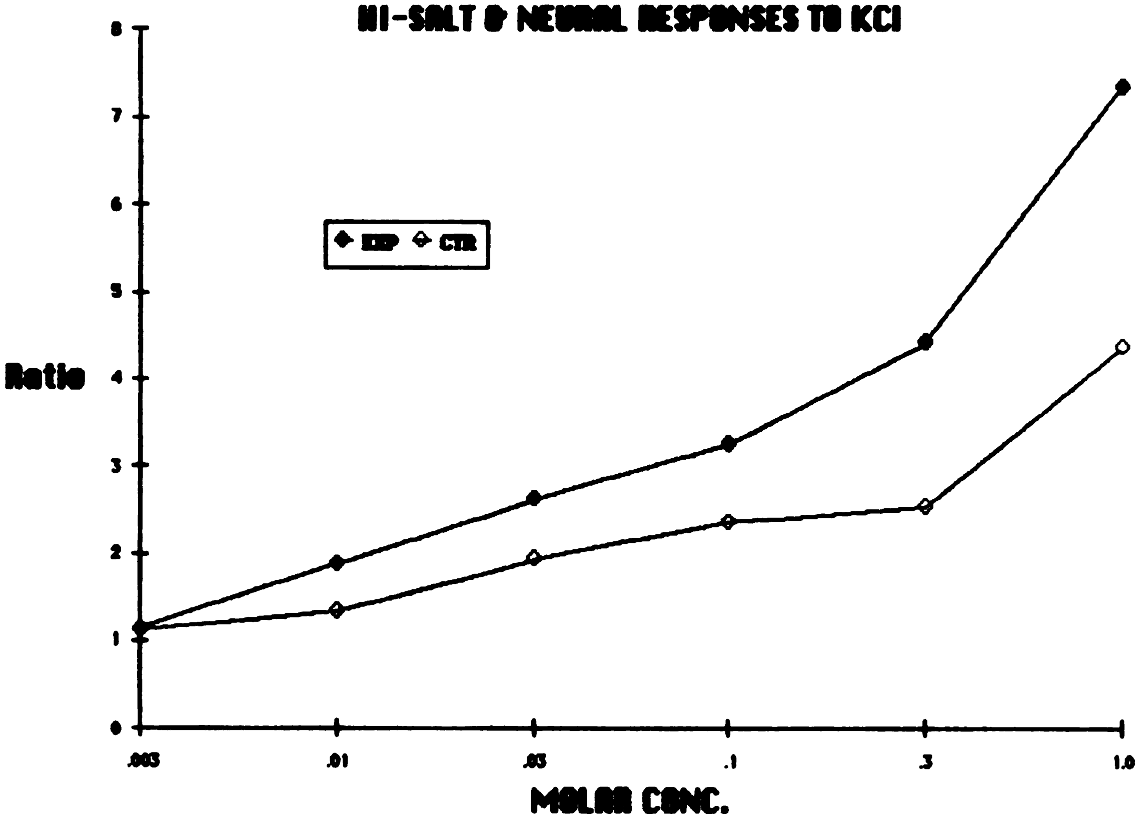


Fig. 7.-- Median chorda tympani nerve responses of high-salt (Exp) and control (Ctr) groups when exposed to KCl concentrations similar to those of the NaCl series (.001M KCl not tested). The experimental group showed significantly greater neural responses beginning at .01M. Ratios were calculated with respect to the response to .001M NaCl.


HI-SALT & NEURAL RESPONSES TO KCl
◆ EXP ◇ CTR
Ratio
0
1
2
3
4
5
6
7
8
.003
.01
.03
.1
.3
1.0
MOLAR CONC.

DISCUSSION

Preference tests

Our laboratory has had extensive experience in mapping the taste preferences of rats in various experimental settings. We have repeatedly observed a 50% preference for NaCl beginning at approximately .001M and gradually increasing to a peak at .1M solution. At this concentration, in a normal rat, the preference may be as high as 85%. Above .1M, the preferences drop rapidly reaching a low of approximately 10% at 1.0M. These were approximately the preferences shown by the control rats in this study. (figure 2). The experimental rats fed the high-salt diet, however, exhibited a marked decrease in preference for NaCl when compared to the controls (figure 2).

It is important to point out the distinctions between the three parameters that have been used in this study to portray differences in salt appetite. NaCl intake in milliliters (figure 3) is simply a measure of how much saline was drunk at a particular concentration by an animal or animal group. This is usually large in the lesser concentrations and then drops greatly as the solutions become hypertonic, above .1M, where they appear to be avoided. NaCl preference is a measure of how much saline is drunk as compared to water during the same period of time. It may be viewed as a measure of the rats' like or dislike for a particular concentration of saline, although there is some controversy on this point. Some investigators believe

that other factors, such as tonicity, are the overriding determinants limiting intake at higher concentrations. If the rat finds the saline solution sufficiently agreeable, more saline will be consumed than water and therefore the preference will be greater than 50%. Preference is always a measure of the amount of saline consumed compared to water and is thus not directly related to absolute volume intake when two different groups of animals are compared.

Both of the above parameters give useful and valuable information, but neither of them give an actual measure of how much NaCl in solution was consumed. Volume intakes give a measure of how much saline was drunk by the rats, but since the molarity of the solutions changes it does not tell us how much salt is actually ingested. For this we must convert the volume intake to milliequivalents of NaCl (amount drunk multiplied by the molarity of the solution). This gives an accurate reflection of how much NaCl in solution was actually ingested by the rats (figure 4). Each of these measures of salt appetite serves a necessary purpose in deciphering how rats react to a particular saline solution, but it is also important to bear in mind their respective limitations.

One factor that must be taken into account, therefore, when one looks at the lower preferences of the high-salt rats, is the fact that these rats consumed much greater quantities of water than did the controls (figure 5). This is necessary for these rats to maintain normal body osmolarity. However, since preferences are calculated as the amount of saline ingested divided by the total amount of fluid drunk (both saline and water), the

increased water consumption resulted in a greater total fluid intake and consequently a decreased preference. This could be one reason why NaCl preferences were depressed in the experimental group. Upon closer observation, though, it is clear that although the rats on the high-salt diet did consume greater amounts of water, they also consumed greater amounts of saline. These two factors probably act to counterbalance one another so that the decreased preferences seen are a real phenomenon and not an artifact.

Why the rats on the high-salt diet increased their intake of NaCl solutions is difficult to answer. One explanation in light of the recent evidence of Atrial Natriuretic Factor (ANF), is that the high-salt diet triggers the endogenous release of ANF, which then causes the excretion of large amounts of NaCl. This could result in the body reacting to prevent development of hyponatremia and thereby causing an increased sodium consumption. Although we did not house these particular animals in metabolic cages, a previous group of rats maintained on an identical excess-salt diet were monitored for their urine sodium and potassium concentrations. Even though the high-salt rats had a slightly higher urinary sodium level than did the controls, the urine measurements were still within the normal ranges. This does not exclude the possibility of the rats on the sodium-excess diet from excreting greater quantities of sodium, though, since they also had a much increased urinary output. So the cause of the elevated salt appetite may be due to an excessive sodium loss.

It should also be pointed out that although potassium chloride was utilized for the neural recordings, it was not used in the preference tests. The inclusion of KCl in these tests would have been desirable, but was omitted for several reasons. One, we did not wish to maintain the rats on a high-salt diet for the additional two weeks that would be required for the conclusion of the KCl preferences. Even though there were no signs of ill health among the high-salt rats during the course of this study, extending the duration of the diet could have had adverse consequences. In conversations with the diet supplier, TEKLAD®, I had been informed that 6% NaCl was the upper limit of the range for sodium supplementation. Concentrations above 6% NaCl could result in premature death for the animals involved; at 8% NaCl, for example, the rats ingesting this diet could be expected to die within 30 days. Another reason for the exclusion of KCl from the preference tests was that we wished to correlate the time our rats spent on the experimental diet to the time spent on a sodium deficient diet by previous investigators. It is easier to make direct comparisons between sodium replete and sodium deficient rats if the experimental protocols are approximately equal.

Neural Recordings

The results of the neural recordings were not unexpected in light of experiments performed with sodium deprived rats. Contreras (1977, 1979) had shown previously that rats placed on a sodium deficient diet responded by decreasing their neural responsiveness to NaCl. Hence our expectation was that rats placed on a high-salt diet would exhibit the opposite effect, i.e. an increased neural responsiveness when stimulated with NaCl, which is in fact what occured (figure 6). Not only was there an elevation in the neural response to NaCl stimuli, but to KCl stimuli as well (figure 7), suggesting that this is not a response to a specific ion, but to a particular taste, i.e. anything which tastes salty.

What might be the biological advantage of altering the taste reception of NaCl? It could be that by changing the "taste" of NaCl, the animal can thereby change its intake of the mineral. If the dietary intake of sodium is so great that the ingestion of more sodium would be detrimental to the health of the subject, then, perhaps, taste may be the way the body prevents further consumption. Since the neural data from the high-salt rats shows that for a given concentration of NaCl there is a heightened response, it may be that any given level of NaCl tastes "saltier" to the experimental rat than it does to a normal rat. In this manner, the experimental rat will decline concentrations of NaCl which taste too salty to him (as if a higher concentration than it really is) but not to a normal rat.

Although this is a possible explanation, it fails to take into account the fact that the high-salt rats actually ingested greater quantities of saline than did the controls. If any given concentration of NaCl tastes "saltier" to an experimental rat than to a normal rat, why would the experimental rat continue to ingest quantities above and beyond what the control rat does? Especially in light of the fact that the experimental rats had a dietary intake of sodium greatly exceeding that of the controls. It is true that the experimental rats had a peak preference at a lower concentration than did the control rats, albeit a marginal change, indicating that the high-salt animals began to display an aversion to NaCl before the control animals. But even with this earlier drop in preference, the experimentals still drank more saline than did the controls at the middle concentrations of NaCl. Only with the highest concentrations did the controls exceed the experimental group intakes.

This paradoxical increase in saline consumption in the face of lowered preferences for the same solutions is very puzzling and remains unanswered. It could be that with the increased consumption of water that naturally occurs with a high-salt diet, there is a concomitant increase in saline intake so that the preference values will remain fairly close to normal. This raises the possibility that although the body has a large sodium load, it does not overwhelm the natural attraction that the rat has for some concentrations of saline. The body recognizes the fact that more water is needed to counteract the dietary sodium intake, but this does not necessarily affect the pleasurable taste of salt at the lower concentrations.

It may also be a hybrid of these two ideas. That is, the body recognizes that it has too much of a NaCl load and attempts to limit ingestion of further amounts by enhancing the neural reception of salt. But it is an incomplete block, the rat still enjoys the taste of salt. The body is trying to decrease the amount that would normally be ingested but the taste

of salt is such a pleasant stimulus that the rat continues to drink saline. The result is a reduction in the preferences but an increase in the consumption precisely because there is so much water being drunk.

We have demonstrated in this study that there is an alteration of peripheral neural input in the rat when a high-salt diet is consumed. This is similar to what Contreras found with salt-deficient rats (1977, 1979). But whereas he found a decrease in nerve response with sodium depletion, we found an increase with a high-salt diet. Whether this increase in peripheral neural response is accompanied by an alteration in the central nervous system is not known, and lies beyond the scope of this investigation. It may be that there is a central "resetting" of salt taste so that NaCl tastes more salty when large amounts of sodium are consumed. The method by which this alteration in peripheral nerve response occurs is not known, although there are several possibilities.

DeSimone (DeSimone et al.,1984; Heck et al., 1984) presents evidence that gustatory transduction with respect to salt taste is dependent upon the passage of sodium ions through specific transport pathways in the taste bud. Consequently, agents which alter the passage of sodium ions through these channels can alter the response of the taste bud to NaCl, as has been demonstrated with amiloride, a sodium channel blocker. Amiloride acts to attenuate the response of NaCl upon the tongue. It may be that endogenous humoral substances released in response to a high-salt or low-salt diet act upon these channels to modify the response to NaCl. This could be the mechanism by which the peripheral neural input from the tongue to the brain

is increased.

A candidate for this hormonal agent, in the high-salt rat, is the recently discovered atrial natriuretic factor (ANF). ANF is released in response to increased blood volume and promotes natriuresis. It may also affect sodium transport across cell membranes. It is therefore conceivable that increased blood levels of ANF act upon the lingual epithelium to increase sodium transport, enhancing the neural responses to NaCl, and causing the increase in peripheral neural activity. The examination of this hypothesis is presently being undertaken by our laboratory.

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