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The Effects of Reproductive Status on Sodium Chloride Intake and Preference Curves in Rats

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A Thesis

in

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of

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ABSTRACT

The Effects of Reproductive Status on Sodium Chloride Intake and Preference Curves in Rats.

Laura A. Schleifer

The purpose of the following studies was to examine the nature of the changing salt appetite associated with various reproductive states. Five sodium solutions (6.13 mM, 80 mM, 154 mM , 200 mM and 400 mM) were presented in ascending order during consecutive 24 hour periods, and absolute intake as well as preference for those solutions were measured. In experiment 1, pregnant animals (n=8) displayed a significantly greater intake of all but 1 of the 5 solutions (200 mM) as compared to cycling females (n=8) and a significantly higher preference for 3 of the 5 solutions (6.13 mM, 80mM, 400mM). In experiment 2, using a similar procedure, males (n=8) and nonlactating females (n=11) do not differ significantly in their sodium appetite and lactating animals (n=12) displayed significantly greater overall intake of sodium solutions as compared to nonlactating controls (n=19). A comparison of the results of experiments 1 and 2 suggests that the taste preferences and/or needs of pregnant and lactating animals are not necessarily identical, as reflected in varying height and skewness of the intake and preference curves for these two groups. In experiment 3, the sodium appetites of 4 groups were compared: 10 sham-operated lactating, 9 galactophore cut, 10 impregnated with litters removed on day 1 postpartum and 10 nonreproducing animals. The results of this study demonstrate that the experience of pregnancy and parturition alone, without the hormonal changes associated with pup stimulation, is not sufficient to produce the changes in sodium appetite characteristic of postparturient animals, while milk delivery is not a necessary precondition for its expression. The final experiment assessed the role of naturally

occurring fluctuations in gonadal hormones associated with the estrous cycle using a single solution (100mM) presented for 8 consecutive days (2 estrous cycles) to 21 animals. There was no effect of phase of estrous cycle on sodium appetite, given the solution used.

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General Introduction

Pregnancy and lactation represent great challenges to the reproducing mammal in that she must create a suitable environment, both internal and external, in which pups can grow and develop normally without depleting her own resources or reducing the likelihood of future successful reproductive episodes (Galef, 1983). In the case of the rat, pups are altricial, immobile and essentially helpless for the first several days postpartum (Numan, 1988). The dam therefore is responsible for the maintenance of proper thermoregulatory balance, protection against predation, and for the elimination of pup waste via anogenital licking. None of these behaviors, however, entails as great an energetic demand as that imposed by the nutritional requirements of pregnancy and the even more rigorous nutritional demands of lactation (Brody, 1945; Redman and Sweney, 1976).

Neonatal rats depend solely on maternal milk for nutrients and water throughout the first two weeks postpartum (Babicky, 1970). Individual offspring may grow to more than one-third their adult weight while still dependent on their dam for nutrition (Paterson, 1967 in Galef, 1983), and litters of suckling young may approach twice the weight of their dam before they wean (Kaczmarski, 1966). The calories provided in milk are sufficient to meet the energetic demands of the growing young during that time, that is, 35-40 kcal/day/100g body weight (Brody, 1945). Reported caloric values of milk for a female with a litter of eight range from 10-15 kcal on day 1 to 65-80 kcal on day 15 to 80-115 kcal on day 21 postpartum (Brody, 1945), resulting in a total of 500-1400 kcal of milk produced over the first three weeks of lactation. Milk production reaches a maximum on days 15-16 postpartum, then gradually declines and

terminates by days 28-30 (Babicky et al, 1970).

To provide for the growth of young, reproducing rodents require from 166% to 234% of the calories needed by reproductively inactive controls (Millar, 1979). The requirements of lactating rats for the majority of essential dietary components are from 2 to 10 times the amounts needed by nonlactating animals (Nelson and Evans, 1961) and this has consequences for both dam and pups. For example, the demand for calcium during lactation is high and if it is unavailable, the lactating dam is forced to compensate by the resorbtion of calcium from the maternal skeleton. Lactating rats contribute the equivalent of up to 50% of the calcium in their skeletons to their offspring (de Winter et al, 1975). In addition, during pregnancy and lactation, protein is needed to sustain the growth of the offspring as well as the mother's own growth (Leshner, 1972), and fat is necessary for the storage of energy (Richter and Barelare, 1938).

Sodium for the tissues of the developing foetus and adnexa and for the milk, is also crucial to the efficiency of the reproductive process. During pregnancy, maternal tissue fluid and blood volume are expanded. The changes in blood volume and electrolyte concentration in the body influence the synthesis and secretion of renin, which in turn, regulates the secretion of the sodium-retaining hormone, aldosterone (Vander, 1967). In order to maintain plasma and tissue sodium concentrations in the expanded volume, sodium requirement is increased (Pike and Yao,1971). Striking effects of a sodium-deficient diet concurrent with pregnancy have been clearly demonstrated. For example, pregnant animals maintained on a low salt diet display evidence of marked adrenal hypertrophy and significant reduction in the sodium content of maternal adrenal glands, muscle, brain, bone and plasma, as compared to pregnant animals maintained on a sodium sufficent diet or nonreproducing females maintained on a sodium deficient diet

(Kirksey et al, 1962; Kirksey and Pike, 1962; Pike et al, 1962; Pike and Yao, 1971). In addition, there is evidence to suggest that pregnant animals fed low sodium diets gain less weight, eat less and display general languor and debility (Kirksey and Pike, 1962; Ganguli et al, 1969). The evidence with respect to reproductive outcome indicates that restriction of dietary sodium (.01%) results in reduced litter size, litter weight, and has deleterious effects on overall pup viability (Ganguli et al, 1969). Slightly higher levels of dietary sodium (.025% - .03%) results in a striking diminuation of these negative outcomes (Kirksey and Pike, 1962; Ganguli et al, 1969).

In addition to creating new nutritional requirements, milk secretion dramatically alters the water balance of lactating females. The amount of water transferred to the young can be considerable. For example, at day 10 postpartum, a female with a litter of eight pups produces 42-48 ml. of milk (Friedman and Bruno, 1976) which contains 31 35 ml. of water. This water loss represents 30%-35% of the females total daily water intake (Friedman, Bruno and Alberts, 1981) and imposes a severe drain on the females body fluid balance.

These increased demands for a variety of nutrients, both during pregnancy and during lactation, create further obstacles to the already difficult task of maintaining homeostatic control over various metabolic functions. Homeostatic mechanisms were first defined by Cannon (1932) as "The coordinated physiological processes which maintain most of the steady states in the organism". More recently, it has become apparent that homeostatic control of a subset of functions is achieved through behavioral as well as physiological mechanisms. For example, Richter and his colleagues have demonstrated the ability of rats to compensate behaviorally for the experimental removal of physiological regulators under several circumstances: parathyroidectomy,

by increasing ingestion of a calcium solution (Richter and Eckert, 1937); pancreotomy, by ingesting large amounts of water, presumably to assist in eliminating the unoxidized glucose (Richter and Schmidt, 1941); pancreotomy with marked diabetes by choosing large amounts of fat and protein and rejecting carbohydrate (Richter and Schmidt, 1941); removal of the posterior lobe of the pituitary, one of the chief regulators of water balance, by increasing water intake (Richter, 1935); adrenalectomy, by increasing intake of sodium solutions (Richter, 1936).

Animals need not be in a state of deficiency, as in the Richter studies described above, however, to select nutrients that are conducive to their continued health and growth. In their natural environment, animals are faced with the challenge of selecting a balanced diet. This entails gaining an adequate number of calories from among available dietary items which may differ widely in caloric density and nutritional composition (Hill et al, 1980). An illustration of the ability of animals to successfully self-select appropriate nutritive substances was provided by Richter, Holt and Barelare in 1938. They demonstrated the ability of animals to select, among a choice of several purified substances, a diet that maintained growth rate and activity level at a level consistent with that of animals fed standard lab diet. That the rats actually made very efficient choices is shown by the fact that although they grew at the same rate, their total food intake as measured in grams was 36 % less than that of controls.

Animals are also capable of adjusting their self-selection of nutrients to reflect fluctuating nutritional requirements, as during periods of activity and quiessence. For example, it has been demonstrated that animals allowed access to a running wheel will self-select a diet lower in protein and higher in carbohydrate than will inactive animals (Collier et al, 1969).

Given the fluctuations in nutritional requirements created by the demand for nutrients that the developing fetuses and suckling young make on their dam, it is perhaps not surprising that reproduction is accompanied by both behavioral and physiological adaptations to assist in meeting these demands. For example, physiological changes include alterations in the size, weight and absorptive capacity of the digestive tract (Cripps and Williams, 1975). In addition, there are several strategies that can be employed by the reproducing female to ensure adequate provision of nutrients to the young: Firstly, the dam can accrue stores prior to and during pregnancy followed by a depletion of these stores during lactation; secondly, the dam can catabolize her own tissue and go into debt until the reproductive episode is complete; and finally, the dam can behaviorally compensate for the increased demand by increasing food intake (Woodside et al, 1981). The reproducing rat probably employs some element of all three of these strategies, but the latter strategy, increasing intake of needed substances, is most prevalent. For example, water intake increases during lactation by 100%-200% (Nelson and Evans, 1961; Richter and Barelare, 1938). During pregnancy, food intake increases by 60% while during lactation it is increased to 180%-250% of that of nonreproducing females (Cripps and Williams, 1975; Ota and Yokoyama, 1967). Average daily food intake may increase from 15 g to 60 g over the first 25 days of lactation (Brody, 1945; Redman and Sweney, 1976). As a result, lactating females may consume up to 250 kcal/day over the first three weeks postpartum, depending on the caloric value of their diet (Brody, 1945).

Along with increases in overall food intake, it has been repeatedly demonstrated that intake of various nutrients changes drastically during pregnancy and lactation in the rat. Thus, the increased demand for sodium, calcium, fat and protein is reflected in an

increased preference for these substances during pregnancy and lactation. This was first demonstrated in Richter and Barelare's (1938) classic study on self-selection of purified nutrients by reproducing females. Attempts at direct replication of these findings have proven difficult , however, probably due to problems of palatability associated with different nutrients (eg. Tribe, 1955). Later investigations, using altered methodologies, have confirmed many of Richter's initial results. For example, with respect to protein, several researchers have demonstrated selective increases in intake during both pregnancy and lactation (Cohen and Woodside, 1989; Leshner, 1972). The same has been shown to be true for calcium intake during lactation (Woodside and Millilere, 1987; Millilere and Woodside, 1989). In addition, several authors have reported increased sodium intake during pregnancy and lactation. For example, Scott, Smith and Verney (1948), using a methodology very similar to that of Richter and Barelare (1938) found that only sodium intake increased during pregnancy. They did not note any increases in fat or protein intake. Similarly, Pike and Yao (1971) found that pregnant animals maintained on a sodium deficient diet had an increased appetite for sodium choride (as evidenced by greater intake of an additional source of NaCl solution) as compared to pregnant animals maintained on a sodium sufficient diet or non-pregnant animals maintained on a sodium deficent diet. With respect to lactation, Alberts (1983) has shown that it is associated with an increase in appetite for a hypotonic sodium chloride solution when utilizing a long-term testing paradigm.

Behavioral compensation for specific nutrient needs is not the sole strategy utilized by reproducing animals. For example, despite adequate calcium intake during lactation, animals lose calcium stores and thus, as described earlier, go into calcium "debt" (de Winter et al, 1975). The maintenance of sodium balance, however, is more difficult in

that there are no stores upon which the reproducing female can draw. There is no sodium reservoir and as such the animals cannot go into debt. Beyond the effects on pup and fetus viability and dam health, some authors suggest that variations in dietary salt during pregnancy may result in an external environment for the developing fetus that differs from typical conditions in its water, electrolyte and hormonal composition (Bird and Contreras, 1986) and that sodium intake during both pregnancy and lactation may have consequences for the developing nervous system, in particular its gustatory and regulatory components which mediate salt preference behavior and thirst in adulthood (Bird and Contreras, 1986; Hill et al, 1986; Mouw et al, 1978; Contreras and Costen, 1983).

Given the importance of sodium to the success of the reproductive episode, and to the health of both dam and pups, these studies were designed to compare the sodium appetite of dams during various reproductive states to assess whether the fluctuations in need states is associated with concommitant fluctuations in compensatory behavior that would serve to complement physiological mechanisms.

The physiological control of sodium balance is comparatively well established and has been shown to involve the interaction of several hormones. Sodium excretion is controlled by aldosterone, a steroid hormone secreted by the adrenal cortex. Aldosterone directly stimulates the sodium pumps in the walls of the renal tubules. Two factors stimulate the secretion of aldosterone: increased activity of the sympathetic axons to the kidneys and a falling blood flow through the kidneys. These events cause the juxtoglomerular cells of the kidney to secrete renin which catalyzes the conversion of angiotensinogen into angiotensin I and II which in turn stimulates the adrenal cortex to produce aldosterone. Water excretion is controlled by antidiuretic hormone, which

serves to increase the permeability of the renal tubules and collecting ducts to water. (see Fitzsimons, 1979, for review).

Sodium appetite is a particularly interesting phenomenon for, although many of the specific hungers are learned (eg.thiamine, see Rozin and Kalat, 1971), many agree that certain basic components of the sodium appetite system are innately organized (Rozin and Kalat, 1971; Rodgers, 1966; Wolf, 1969; Quartermain et al, 1967; Nachman, 1962; Richter,1956; Epstein and Stellar, 1955). That is, sodium appetite emerges spontaneously with the animals first experience with deficit. It does not depend on past experience or the association of sodium with positive postingestional consequences.

Evidence for this hypothesis includes the results of studies by Nachman (1962), who has demonstrated that adrenalectomized rats will ingest sodium salts but reject nonsodium salts (except lithium) soon after the initial taste and before any significant alteration of body sodium can occur. Epstein and Stellar (1955) have further demonstrated that the gradually increasing preference for sodium typical of adrenalectomized animals is not the result of post-operative experience with sodium, but rather is the result of a gradually increasing physiological need for the sodium ion. That is, when sodium-deprived adrenalectomized animals are presented with sodium solution several days post-operatively, they will immediately begin consuming amounts equivalent to non-deprived adrenalectomized animals, indicating that need, rather than experience, is critical. In addition, Quartermain, Miller and Wolf (1967) have demonstrated that the ingestion of sodium by a sodium-deplete animal is not simply a reflexive act but is in fact a "voluntary motivated behavior". That is, that there is an innately determined relationship between the degree of deficiency and the strength of motivation to attain sodium, as reflected in willingness to bar press to obtain it.

Further evidence was presented by Richter (1956), who demonstrated that when sodium is removed from the diet of intact animals, they will begin drinking salt solution freely beginning the first day of deprivation and they will continue to drink a constant amount which closely approximates the amount which had been removed from the diet. Given that animals raised on standard lab chow, which contains an excess of sodium chloride, have never experienced sodium deficiency or had the opportunity to taste sodium independently of other taste stimuli, the only means through which the rat could specifically associate sodium with the reduction of the need is by some innate mechanism which makes it especially sensitive to the taste of sodium during deficiency (Wolf, 1969).

Behavioral and internal sodium regulatory functions appear closely integrated and probably share common receptor, endocrine, and neural mechanisms (Epstein, 1982). For example, it has been shown that sodium appetite can be induced in sodium replete animals by administration of physiological doses of angiotensin in combination with aldosterone or its precursor, deoxycorticosterone (DOCA), (Fluherty and Epstein, 1983) and interference with the actions of these hormones completely suppresses the salt appetite of the deficient rat (Weiss, Moe and Epstein, 1986; Sakai et al, 1986).

In addition to regulatory factors, gustatory factors are also involved in the motivation to ingest sodium. The sense of taste seems critically important to the identification of sodium and the maintenance of appropriate intake levels (Wolf, McGovern and Dicara, 1974). Richter (1956) has suggested that mammals have an instinctive liking for salt and that the genetic ability in mammals to taste salt is consistent with the fact that an ability to taste nutritious substances confers a survival advantage. The influence of gustatory factors, independent of deficit, is demonstrated by

the fact that rats ingest excessive amounts of sodium when it is in a palatable form (eg. a weak sodium chloride solution) even though they are receiving abundant sodium elsewhere in their diet and their body fluids are in normal equilibrium (Fregly et al, 1965; Young, 1949). Using classic psychophysical techniques, it has also been demonstrated that preference for sodium over water begins at concentrations too low to have any physiological value. This is an indication that the preference is dependent on taste alone, as opposed to the post-ingestional consequences of intake or some regulatory process (Richter and Campbell, unpublished data, cited in Richter, 1942-43).

In addition, sodium intake resulting from a deficit of body sodium or from the hormonal conditions (increased mineralcorticoid levels) normally accompanying a deficit of body sodium is likely mediated by changes in taste sensitivity (Richter, 1956). It has been suggested that adrenalectomized rats have a lower salt taste threshold than normal rats, perhaps as much as 15 times lower than control animals (Richter, 1939A). However, the results from these types of inquiries have not been consistent (eg. Brosvic, 1989). Another experimental method utilized to ascertain the role of gustation in deficit-induced salt intake is the surgical elimination or reduction of gustatory stimulation. For example, Richter (1939B) found that after combined section of the taste nerves, adrenalectomized rats, that lose a great deal of sodium in their urine, are unable to increase their salt intake, while intact adrenalectomized rats are able to maintain sodium levels at appropriate levels. Similarly, rats that receive electrolytic lesions centered in the gustatory subnucleus are impaired in their ability to increase sodium chloride after experimental depletion of body sodium (Wolf and Dicara, 1974).

Thus regulatory and gustatory factors interact to maintain sodium balance during situations of deficit. Epstein and Stellar (1955) have investigated the interaction of

these two factors by measuring preference aversion functions under differing conditions of salt intake. They compared normal control animals, adrenalectomized animals given 3% (513 mM) sodium solution daily during a maintenance period, and adrenalectomized animals only allowed access to sodium during the one hour test period. They demonstrated that, in control animals, the maximal preference point for sodium solution was approximately .8% (137 mM) and the aversion point approximately 1.5% (256 mM). In the adrenalectomized animals allowed access to sodium during the maintenance period, the curve was elevated except for the lowest concentrations as compared to normal controls and the maximal preference point was shifted towards higher concentrations. In animals allowed access to sodium only during the test period, they noted a dramatic elevation in the motivation to drink all solutions, particularly the weak concentrations. They concluded that although differential taste stimulation plays a role in the adrenalectomized rat's salt-drinking behavior, the level of the physiological need for the sodium ion is also extremely important. The level of salt need determines the over-all height of the preference aversion-function as well as it's shape.

The question remains as to whether the salt appetite of pregnant and lactating animals is governed by similar mechanisms to those of nonreproducing sodium deficient animals, or whether the hormones of pregnancy and lactation exert an influence in a manner unique to the reproductive episode. That is, does the increased demand for sodium during pregnancy and lactation constitute a deficit that produces a shift in taste preference and/or sensitivity similar to those seen following adrenalectomy (Richter, 1939A, Epstein and Stellar, 1955), and if so, what internal mechanisms are acting to produce these changes.

Previous research has already established that, along with changes in dietary needs,

reproduction is associated with changes in taste preference for various salient (nonnutritive) substances. Wade and Zucker (1969, 1970), for example, have demonstrated that responses to both a palatable saccharin solution and an aversive quinine solution are diminished during pregnancy and lactation. That is, reproducing females ingest less of a palatable fluid, and more of an aversive fluid, than do nonreproducing females or males. Wade postulates, based on these data, that there may be some utility in the lower taste sensitivities of reproducing females as compared to cycling females, since the dietary requirements of reproducing females are altered. He proposes that lowered saccharin intake during lactation is a reflection of some process that limits the intake of sweet substances (eg.carbohydrates) during the time when the organisms requirements for less palatable food (eg.proteins) are greatest. This conclusion is consistent with the findings that reproducing animals do selectively increase their intake of a less palatable protein substance (Leshner, 1972; Richter and Barelare, 1938; Cohen and Woodside, 1989) and it would not be unreasonable to postulate that a similar mechanism might be acting on the sodium appetite of reproducing animals.

Previous investigations of sodium appetite have been plagued by methodological inconsistencies resulting from a lack of clarity in the definition of sodium appetite. What many authors refer to as sodium appetite (eg. Epstein and Stellar, 1955; Pike and Yao, 1971; Barelare and Richter, 1938 etc.) is in fact absolute sodium intake, as opposed to preference in a choice situation. While these studies are revealing, it is becoming increasingly apparent that the measurement of sodium appetite by the absolute intake of a single solution is not adequate to present a complete picture. In addition, long term intake of a given solution will reveal different information than that revealed

during short-term preference testing, especially with respect to fluctuations in taste sensitivity independent of regulatory requirements. Therefore, the purpose of the following studies was to elucidate the nature of the changing salt appetite during various reproductive states, and therefore perhaps the role of the hormones of reproduction on sodium appetite, using 24-hour 2-choice preference tests. It will be assumed that sodium appetite is reflected in both absolute intake of a given solution and preference for that solution (preference being defined as the amount of sodium solution ingested divided by total fluid intake as described by Deems and Friedman in 1988). This method obviates the confounding effects of overall increases in fluid intake that might be present during various reproductive states. In addition, by looking at preference and intake curves rather than simply analysing appetite for a single solution, it will be possible to draw conclusions regarding possible shifts in taste sensitivity along with changing needs for sodium and assess how these two function together to produce the behavioral expression of sodium appetite. In addition, the role of suckling and milk delivery in the increased sodium appetite seen in lactation will be investigated using the same methodology. Finally, the nature of sodium appetite over the estrous cycle will be examined, to assess the possibility that the naturally occuring fluctuations in gonadal hormones that are associated with the phases of the estrous cycle might be adequate to influence sodium appetite.

Experiment 1: Effects of Pregnancy on Sodium Chloride Intake and Preference Curves in Rats

Introduction

Although the huge energetic cost of lactation is not present during pregnancy, the dam is none-the-less required to supply a suitable environment, both internal and external, in which the fetuses can grow and develop normally. One way in which this is accomplished, as discussed earlier, is through the ingestion of both quantitatively and qualitatively appropriate nutritive substances.

For example, there is evidence that the appetite for sodium chloride is altered substantially during pregnancy in the rat. Several researchers have found an increase in sodium chloride intake throughout the gestational period (Richter and Barelare, 1938; Scott, Smith and Verney, 1948; Pike and Yao, 1971). For example, Richter and Barelare (1938) have demonstrated that rats ingest twice as much of a highly hypertonic sodium solution during the first ten days of gestation, and three times as much during the second half of gestation, as compared to the ten days preceding impregnation. This increase in intake frequently begins as early as day 3 of gestation.

Further evidence that pregnancy represents a state of salt deficiency was presented by Steinberg and Bindra (1962) in an examination of the relationship between genital licking during pregnancy and salt intake. It had been suggested that increased genital licking at parturition was a result of an increased preference for sodium chloride brought about by salt deficit in the body (Bindra, 1959). They reasoned that if genital licking during pregnancy was a result of salt deficit, then presentation of external sources of sodium chloride should result in a decrease of genital licking. The results

were as predicted and they concluded that the external source of sodium had compensated for the deficit, thus reducing the animals need to seek sodium in their vaginal secretions.

The first experiment examined more closely the NaCl appetite of pregnant rats during the third week of gestation, when sodium appetite has been shown to be greatest (Richter and Barelare, 1938). In order to take into account the definitional problems inherent in the concept of salt appetite (see General Introduction), NaCl appetite was assessed by both intake and preference measures, using solutions of varying concentration.

Method

Subjects:

Subjects were 16 virgin female wistar rats obtained from the Charles River Breeding Farm (St. Constant, Quebec). Animals weighed 220-240 grams at the beginning of the experiment. In order to be included in the study, animals were required to display normal 4-day estrous cycles as demonstrated by daily vaginal smears.

Housing and Adaptation:

Animals were housed in individual plastic cages with beta chip bedding, 38x33x17 cm. in dimension. They were kept on a 12 hour light/dark cycle (lights on 0900 hr., lights off 2100 hr.) in a temperature controlled room maintained at 20±2 C. During an adaptation period that lasted at least 4 days, animals had free access to Agway rat chow in powdered form and one 300 ml. glass bottle of demineralized water equipped with a rubber stopper and a metal spout. In order to avoid the development of a side bias, the position of the water bottle was alternated daily.

Impregnation Procedure:

Following the adaptation period, animals were randomly assigned to one of 2 groups, impregnated or nonimpregnated. Animals assigned to the impregnated group were identified using color coded tail markings and placed in group mating cages with a stud male on the evening of proestrus. Vaginal smears were taken the next day, and the

presence of spermatozoa confirmed impregnation.

Procedure:

Beginning on day 15 of gestation, both demineralized water and 1 of 5 concentrations of sodium solution (6.13 mM, 80 mM, 154 mM (isotonic), 200 mM and 400 mM) were presented to all animals in 300 ml. glass bottles equipped with rubber stoppers and metal spouts. Solutions were presented in ascending order during consecutive 24 hour periods. Nonimpregnated controls were tested for an equivalent time period.

Statistics:

For sodium intake and preference, two-way split-plot anovas were performed, with solution as the within group factor and reproductive status as the between group factor. Post Hoc analyses were carried out using one-tailed t-tests with multistage bonferroni corrections where appropriate. Critical values of t for each stage are indicated in brackets following each analysis.

Daily food and water intake were recorded and averaged over a period beginning day 12 of gestation and continuing until day 20 of gestation (or an equivalent period for nonlactating animals). Weight gain was recorded as the difference between day 6 and day 19 of gestation (or an equivalent period for nonlactating animals). One-tailed t-tests were employed for statistical analyses of these measures. For source tables, see appendix A.

Results

Sodium Intake:

Fig. 1 indicates that pregnant animals ingest more sodium solution then do cycling animals (significant effect of reproductive status, $F(1,14)=11.649$, $p=.0042$). As well, all animals clearly display greater intake of select solutions (significant effect of solution, $F(4,56)=29.565$, $p=.0001$). The significant interaction of reproductive status X solution concentration indicates that intake of a given solution varies as a function of reproductive status ($F(4,56)=4.85$, $p=.002$).

Post hoc analyses revealed that the two groups differed significantly for the 6.13 mM solution ($t(14)=4.708$, $p=.0002$), the 80 mM solution ($t(14)=3.463$, $p=.0019$), the 154 mM solution ($t(14)=2.366$, $p=.0165$) and the 400 mM solution ($t(14)=2.956$, $p=.0052$).

(t_{crit} stage 1=.05/4=.0125, t_{crit} stage 2=.05/1=.05)

Sodium Preference:

Fig. 2 reveals that pregnant animals display a greater preference for all solutions with the exception of the 200 mM solution. The main effect for group did not quite reach significance however, due to the cross over at the 200 mM solution (effect of reproductive status, $F(1,14)=3.861$, $p=.0696$). This was reflected in a significant interaction of reproductive status X solution concentration (interaction effect, $F(4,56)=2.934$, $p=.0284$). Both groups displayed greater preferences for select solutions

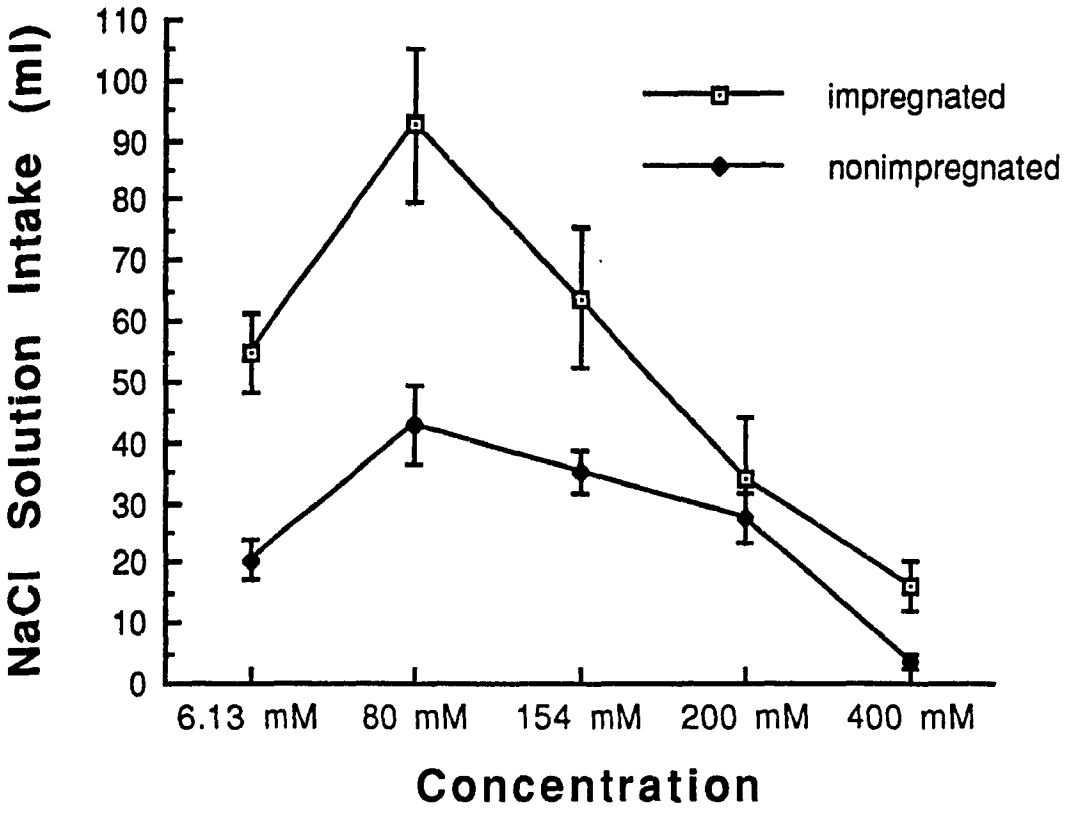

Figure 1: Comparison of NaCl solution intake curves for impregnated and nonimpregnated rats.

(significant effect of solution, $F(4,56)=47.633$, $p=.0001$).

Post Hoc analyses revealed that the greatest difference in preference between the two groups occurred for the 6.13 mM solution ($t(14)=2.545$, $p=.0117$), the 80 mM solution ($t(14)=2.195$, $p=.0228$) and the 400 mM solution ($t(14)=2.932$, $p=.0054$). The relationship breaks down for the solutions lying at the centre of the curve.

(t_{crit} stage 1=.05/3=.0167, t_{crit} stage 2=.05/1=.05)

Food intake:

Pregnant animals ingested significantly more food than did cycling control animals ($t(14)=1.847$, $p=.043$). On average, daily food intake for pregnant animals was 28.57 ± 1.11 grams. For cycling animals average daily food intake was 25.53 ± 1.21 grams.

Weight Gain:

Average weight gain for impregnated animals during the last two weeks of gestation was 97.35 ± 2.2 grams. Average weight gain for nonimpregnated animals for an equivalent time period was 13.43 ± 3.06 grams. This difference was significant ($t(14)=22.247$, $p=.0001$).

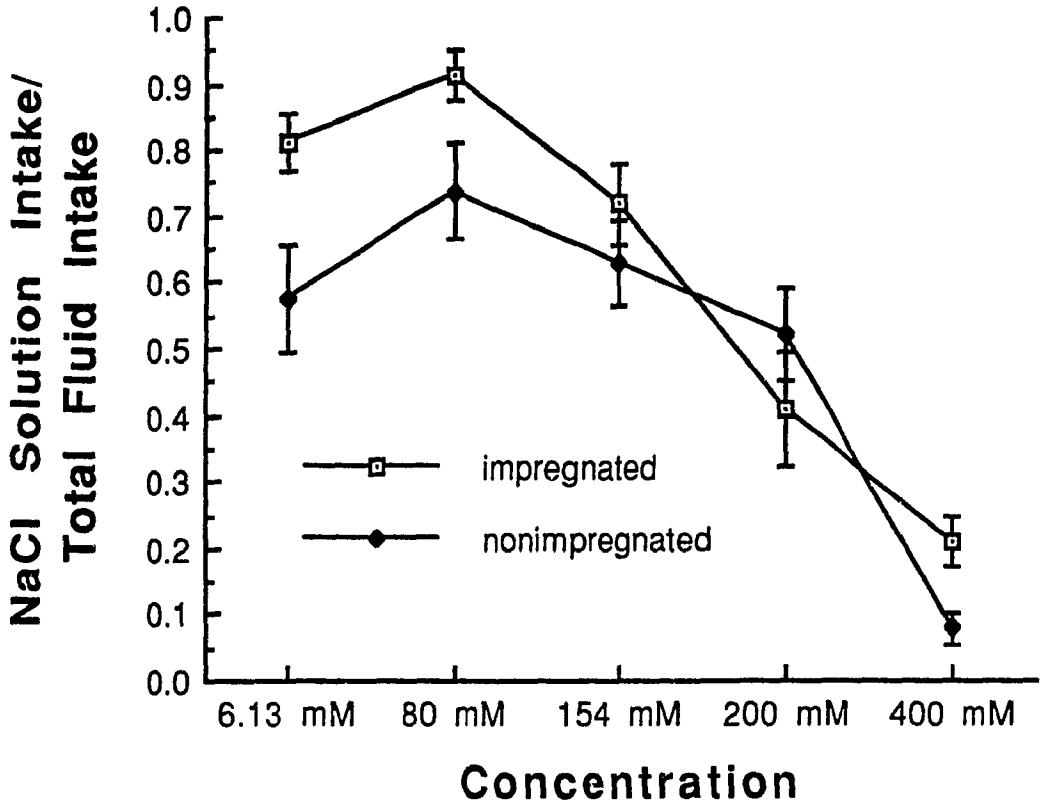

Figure 2: Comparison of NaCl solution preference curves for impregnated and nonimpregnated rats.

Water intake:

Average daily water intake for the impregnated animals during the last week of gestation (including the test period) was significantly greater than that of cycling controls for an equivalent time period ($t(14)=2.546$, $p=.0117$). Impregnated animals ingested an average of 35.31 ± 1.97 ml. of water, while nonimpregnated animals ingested an average of 28.9 ± 1.57 ml.

Reproductive Outcome measures:

Parturition for all impregnated animals occured on day 22 of gestation. Average litter size was $12\pm.41$ pups, weighing, on average, 69.87 ± 2.43 grams. Average weight of litters culled to 8 in number was $46.6\pm.58$ grams.

Discussion

These data indicate that pregnant animals consume a markedly greater amount of sodium solutions than do cycling females. This difference is significant for all but one of the 5 solutions (200 mM). Their preference for 3 out 5 of the solutions (6.13 mM, 80 mM, 400 mM) was also significantly greater than that of cycling animals .

In comparing the curves of pregnant and cycling animals, we see that there are some interesting differences and similarities. Firstly, the intake curves of pregnant and cycling animals are similar in terms of shape and peak preference point (80 mM). The most obvious difference between these two groups is with regard to the height of the curves, with pregnant animals displaying an exaggerated intake of the most preferred solutions.

A similar situation exists for the preference curves. The shape of the preference curve for cycling animals is similar to that of the pregnant animals, with the major difference being height. Worthy of note too is the fact that for both groups, the preference curves mirror the intake curves to a large extent. This may be an indication that for both pregnant and cycling animals, either measure is a relatively accurate index of sodium appetite.

Experiment 2 : Effects of sex and lactation on Sodium Chloride Intake and preference curves.

Introduction

As indicated above, lactation represents a state of high energetic demand to the reproducing female. For some time after birth, the mother's milk is the only source of water and electrolytes for the developing young. Thus, the production of milk is an important feature in the water and salt regulation of mothers and young. It is not surprising, therefore, that the sodium intake of the lactating female has been shown to differ substantially from that of a nonreproducing female. For example, Barelare and Richter (1938) found that while intake of a highly concentrated hypertonic (3%=513 mM) sodium chloride solution is only slightly higher during the first ten days of lactation, after that time sodium appetite increases significantly, reaching a peak just before weaning, at which time there is an immediate and drastic drop off.

In an extension of this work, Alberts (1983) presented lactating and nonreproducing female rats with a choice of a dilute, hypotonic (80 mM) sodium solution and water throughout the course of lactation. Lactating dams displayed a significantly greater preference for this hypotonic solution beginning on day 2 postpartum as compared to nonlactating controls.

In addition to evidence regarding the effects of reproductive status on sodium appetite, recently there has also been evidence of the existence of sex differences in sodium appetite . For example, it has been demonstrated that female rats drink more of a 3% sodium solution than do males. Further, these differences can be eliminated by neonatal androgenization of females or neonatal castration of males (Krecek et al, 1972;

Krecek, 1973). Similarly, Epstein and Sakai (1987) have demonstrated that the consequences of one episode of sodium depletion differ for males and females. That is, prior experience with sodium depletion (or the hormonal correlates of sodium depletion) results in an enhanced response to sodium during subsequent depletions in both male and female rats, but this effect is greater in females than in males.

The purpose of the second experiment, therefore, was two-fold; firstly, to investigate the shape of the NaCl intake and preference curves for lactating animals using hypo and hypertonic sodium solutions, and secondly, to investigate possible sex differences in sodium appetite in sodium replete animals with no history of sodium deficit.

Method

Subjects:

Subjects were 23 adult virgin female and 8 adult male wistar rats obtained from the Charles River Breeding Farms (St. Constant, Quebec). The animals weighed 220-240 grams at the beginning of the experiment. In order to be included in this study, female animals were required to display normal 4-day estrous cycles as demonstrated by daily vaginal smears.

Housing and Adaptation:

Housing and adaptation procedures were identical to those described in the methods of experiment 1.

Impregnation Procedure:

Female animals were assigned randomly to 1 of 2 groups, lactating (N=12) or non-lactating (N=11), at the beginning of the experiment. The impregnation procedure was identical to that described in the method of experiment 1. Animals were left undisturbed throughout the course of pregnancy, with ad lib access to powdered food and demineralized water. On the day following parturition (day 1 postpartum, all litters were culled to 8 in number. Postparturient animals were included in the study only if maternal behavior was evident and if litter size and weight were within normal ranges.

Procedure:

The procedure for this experiment was identical to that described for the first experiment, with the exception that testing began on day 6 postpartum.

Statistics:

For sodium intake and preference, two-way split plot anovas were performed, with solution as the within group factor and reproductive status/sex as the between group factor.

Daily food and water intake were recorded and averaged over a period beginning day 1 postpartum and continuing until day 11 postpartum (or an equivalent period for nonlactating animals). Weight gain was recorded as the difference between day 1 postpartum and day 11 postpartum (or an equivalent time period for nonlactating animals). One-way between groups anovas were performed on all these measures.

Post Hoc analyses were carried out using one-tailed, unpaired t-tests. Multistage bonferroni corrections were employed where appropriate. Critical values of t for each stage are indicated in brackets following each analysis. For source tables, see appendix B.

Results

Effect of Sex on Sodium Intake and Preference:

No significant overall sex differences were demonstrated between the males and the nonlactating controls for sodium intake or preference (effect of reproductive status, $F(1,17)=1.21$, $p=.2866$, effect of reproductive status, $F(1,17)=.816$, $p=.3791$ respectively). In addition, no significant interactions of reproductive status X solution were demonstrated for either intake or preference (interaction effect, $F(4,68)=1.657$, $p=.1703$, interaction effect, $F(4\ 68)=\ .762$, $p=.5535$ respectively) (see figures 3 and 4).

Effect of reproductive status on sodium intake:

As we found no significant differences between the males and the nonlactating females in terms of sodium intake or preference, we combined the data for these two groups to create a single nonlactating animal control group.

Fig. 5 demonstrates that lactating animals consume consistently greater quantities of sodium solution than nonlactating animals (significant effect of reproductive status, $F(1,29)=48.274$, $p=.0001$). The significance of the repeated measure effect indicates that the solutions are differentially attractive to the animals (significant effect of solution, $F(4,116)=44.282$, $p=.0001$) and the significant interaction of reproductive status X solution indicates that intake of a given solution varies as a function of group membership (significant interaction effect, $F(4,116)=9.223$, $p=.0001$).

Post Hoc analyses revealed that lactating animals ingest significantly greater

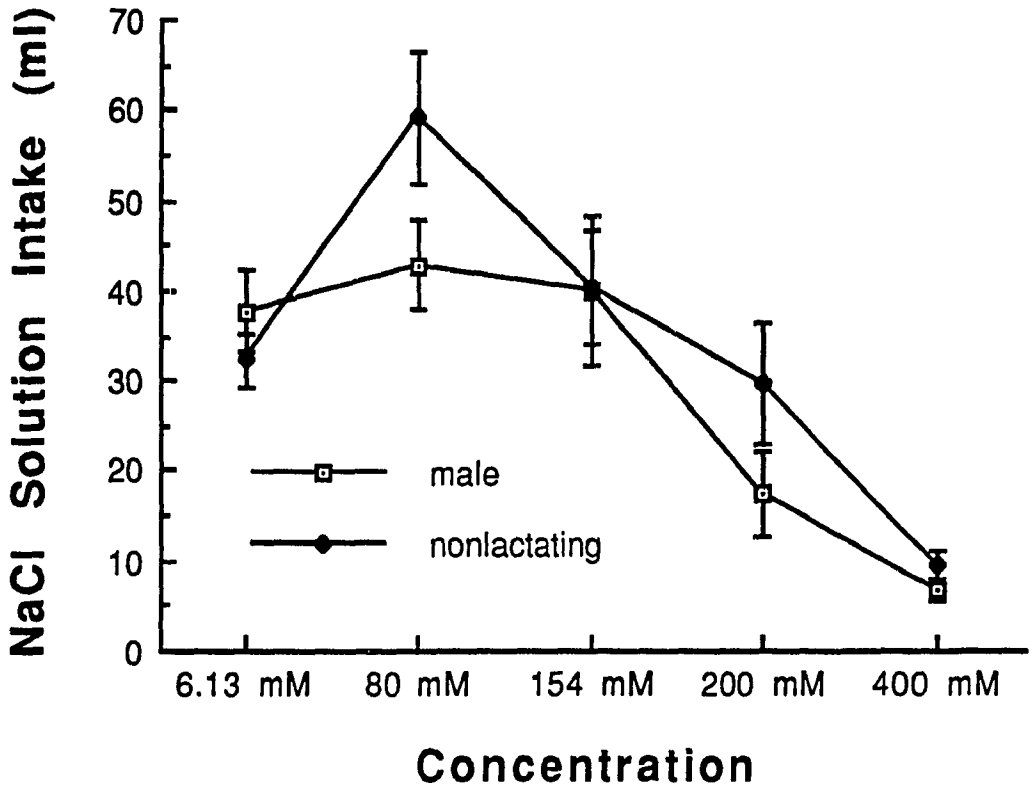

Figure 3: Comparison of NaCl solution intake curves for male and nonlactating female rats.

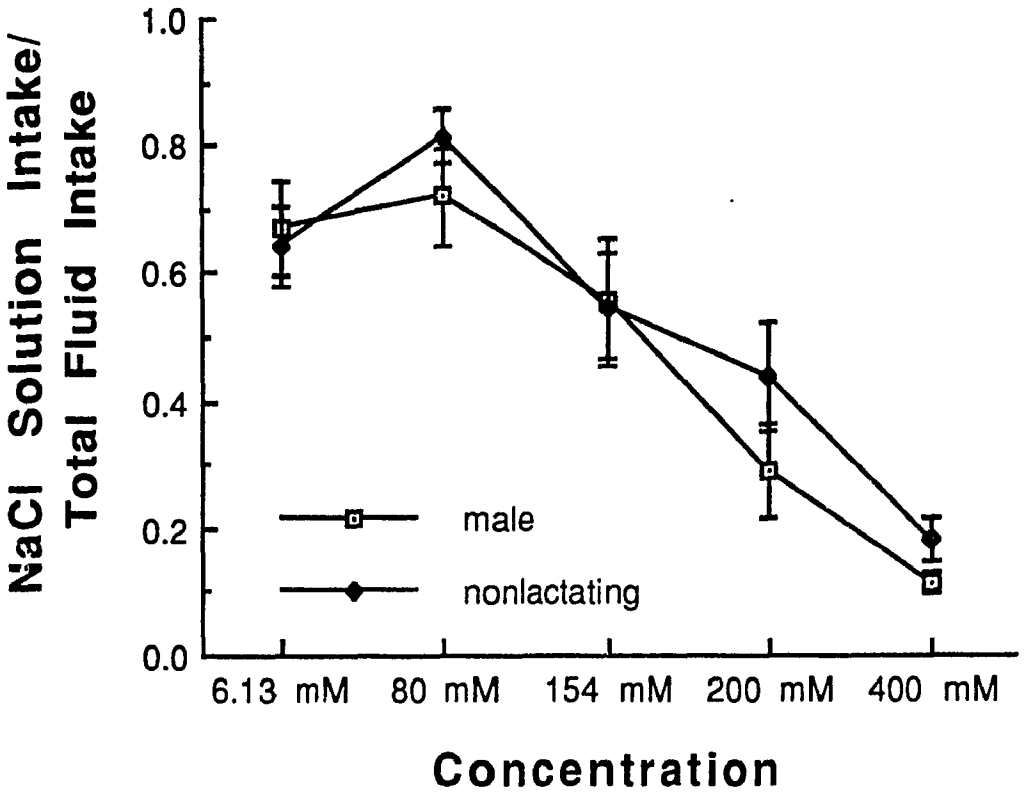

Figure 4: Comparison of NaCl solution preference curves for male and nonlactating female rats.

amounts of the 80 mM, the 154 mM, the 200 mM and the 400 mM solutions ($t(29)=4.259$, $p=.0001$; $t(29)=6.572$, $p=.0001$; $t(29)=4.074$, $p=.0002$; $t(29)=2.855$, $p=.0039$ respectively).

(t_{crit} stage 1=.05/4=.0125)

Effect of reproductive status on sodium preference:

Fig. 6 reveals that while lactating animals appear to show greater preference for sodium solutions, this effect did not reach statistical significance (effect of reproductive status, $F(1,29)=3.052$, $p=.0912$). There was however a significant interaction of reproductive status X solution ($F(4,116)=3.827$, $p=.0059$).

Post Hoc analyses revealed that lactating animals showed a statistically significant preference for the 154 mM solution ($t(29)=2.783$, $p=.0047$).

(t_{crit} stage 1=.05/1=.05)

Food Intake

Average daily food intake for lactating animals was 37.77 ± 1.035 grams as compared to 22.19 ± 1.14 grams for nonlactating animals and 31.89 ± 2.05 grams for males. These differences were statistically significant ($F(2,29)= 17.924$, $p=.0001$). Post Hoc analysis revealed that lactating animals ate significantly more than both nonlactating and male animals ($t(21)=10.138$, $p=.0001$; $t(18)=2.82$, $p=.0057$ respectively) and males ate significantly more than nonlactating females ($t(17)=-4.429$, $p=.0002$).

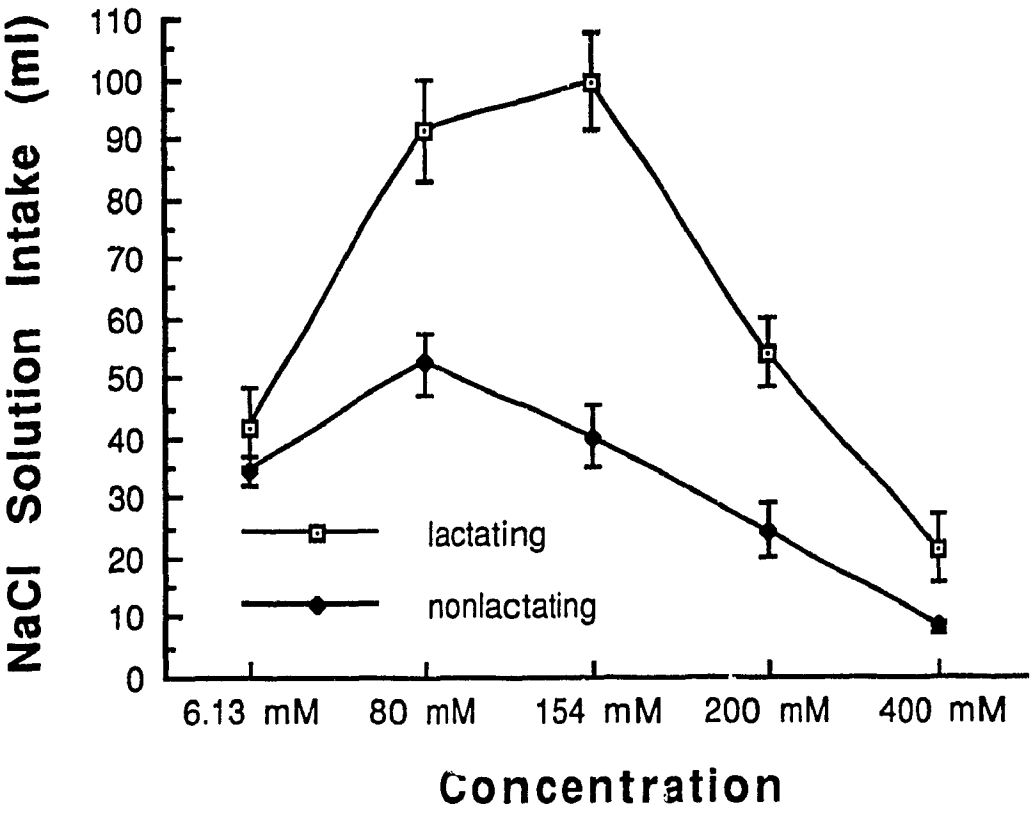

Figure 5: Comparison of NaCl solution intake curves for lactating and nonlactating rats.

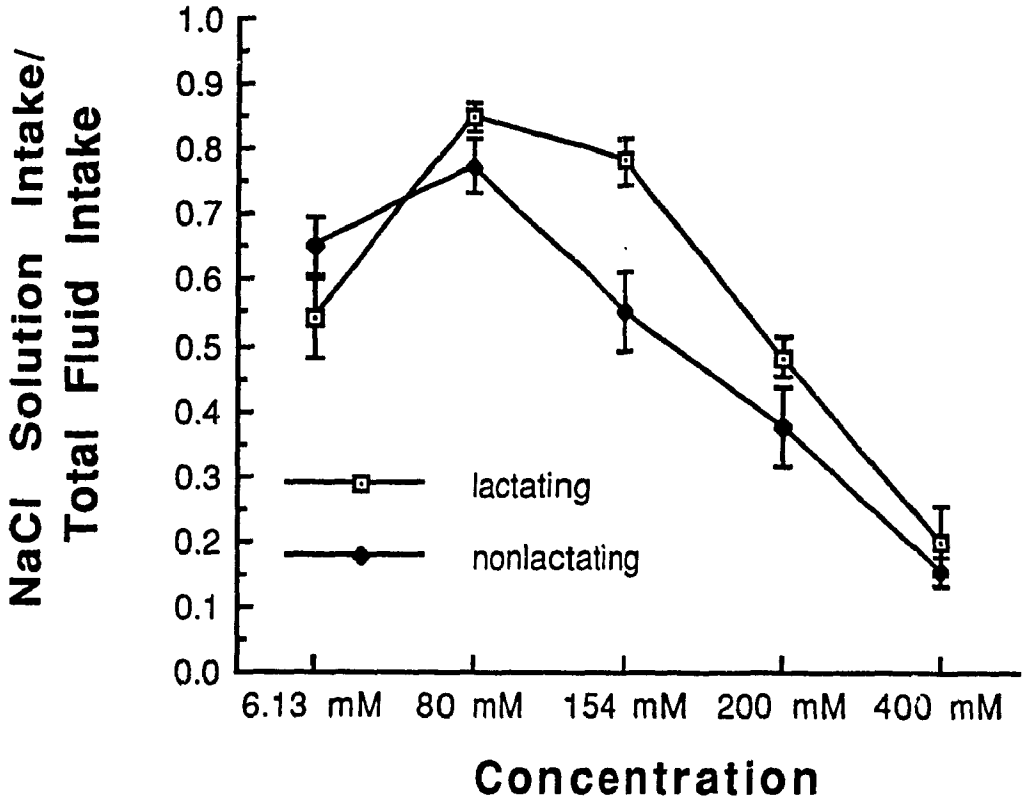

Figure 6: Comparison of NaCl solution preference curves for lactating and nonlactating rats.

(t_{crit} stage 1=.05/3=.0167)

Weight Gain:

Weight gain differed significantly among the three groups (effect of reproductive status, $F(2,28)=58.46$, $p=.0001$) with the males gaining an average of 64.2 ± 2.4 grams, the lactating females gaining an average of 19.84 ± 3.37 grams and the nonlactating females gaining an average of 16.81 ± 3.27 grams. Post Hoc analyses revealed that the males were significantly different in their weight gain from both the lactating and nonlactating groups ($t(18)=-9.669$, $p=.0001$; $t(17)=-10.859$, $p=.0001$ respectively).

(t_{crit} stage 1=.05/2=.025)

Water intake:

Water intake differed significantly among the three groups (significant effect of reproductive status, $F(2,28)=11.191$, $p=.0003$), with lactating animals drinking an average of 46.53 ± 2.13 ml. daily, nonlactating animals drinking an average of 33.52 ± 2.0 ml. daily and males drinking an average of 36.7 ± 2.22 ml. daily. Post Hoc analyses revealed that the lactating animals drank significantly more than the nonlactating and male animals ($t(21)=4.434$, $p=.0001$; $t(18)=3.097$, $p=.0031$ respectively).

(t_{crit} stage 1=.05/2=.025)

Reproductive Outcome Measures:

Average litter size of the lactating animals was 12.92±.71. Average weight of litters culled to 8 in number on day 1 postpartum was 55.59±1.07 grams. Average weight gain of culled litters over the course of the experiment was 134.55±6.1 grams.

Discussion

Lactating animals, like pregnant animals, display a markedly greater overall intake of sodium solutions than do nonlactating animals. This difference is statistically significant for all but one of the 5 solutions (6.13 mM). The preference curve, however, gives us a rather different picture. Unlike pregnant animals, the only solution to produce a significantly greater preference in lactating animals was the isotonic saline solution (154 mM).

That we were unable to show a significant difference in overall preference is not at all surprising, given the great demand on lactating animals to maintain water balance in the face of the huge metabolic cost of milk production and delivery. The large amount of water lost in the milk as well as the dramatic increase in food consumption, seen in this study and many others (eg. Millar, 1979; Ota and Yokoyama, 1967; Redman and Sweeney, 1976), throughout the course of lactation necessitates a concommitant increase in water intake to maintain extracellular fluid balance.

The 6.13 mM solution was the only one to which lactating animals remained essentially indifferent, in terms of both intake and preference. Conversely, for nonlactating animals, preference for this solution was quite close to peak levels. This may be an indication of a shift in taste sensitivity during lactation, perhaps making highly dilute sodium solutions less salient.

The 80 mM solution, while clearly palatable to lactating animals, was also highly palatable to nonlactating animals. Thus, a significant difference in intake was shown, but there was no significant difference in preference. This finding is inconsistent with that of Alberts (1983), who demonstrated that lactating animals display a clear

preference for this solution throughout the course of lactation as compared to nonlactating controls. These inconsistencies may be a function of the nature of the two testing situations. Whereas in Alberts' study, animals had constant access to this solution throughout lactation, in the present experiment, exposure to the solution was limited to a discrete 24 hour period.

If intake alone is used as the index, lactating animals may have a greater appetite for the 200 mM and 400 mM solutions as compared to their nonlactating counterparts. In this respect, these data are consistent with those of Richter and Barelare (1938). There were, however, no significantly greater preferences for these solutions, perhaps due to the compensatory increases in water intake required of lactating animals to maintain water balance when ingesting a hypertonic sodium solution.

Close inspection and comparison of the curves of lactating, pregnant and cycling animals reveals some interesting differences and similarities. For example, the intake curve of the lactating animals was markedly different from those of the pregnant and cycling animals in terms of height, shape and maximal intake point (154 mM for lactating animals, as opposed to 80 mM for pregnant and nonreproductive animals), while the latter two groups differed only in terms of height. A similar situation exists for the preference curves. In addition, while pregnant and nonreproductive animals express their sodium appetite consistently across the two measures (intake and preference), there are some striking discrepencies between the intake and preference curves of lactating animals.

In summary, these data demonstrate that overall, lactating animals have a greater sodium appetite than nonlactating controls. It seems plausible, then, that the hormones of lactation have an influence on both sodium intake and sodium preference. No

significant sex differences were found, however, thus the notion that sodium appetite is influenced by naturally occurring sex differences in gonadal hormones was not supported by these data. This is somewhat surprising given the findings by other researchers that males and cycling females demonstrate differences in taste preferences for palatable saccharin solutions (Wade and Zucker, 1969a - see experiment 4). It is also inconsistent with the findings by Krecek and his colleagues that sex differences in salt preference do exist, but this may be explained in terms of the differences in methodology (long-term sodium intake vs. short-term sodium intake and preference testing).

Experiment 3: Effects of Milk Delivery and Suckling Stimulation on the Sodium Chloride Intake and Preference Curves of Lactating Rats

Introduction

The results of experiment 2 indicate that there is an increased sodium appetite associated with lactation, as expressed by greater intake of 4 out of 5 solutions presented, and increased preference for an isotonic saline solution (154 mM). It is impossible to determine, based on these data, whether lactating animals are responding solely to the increased metabolic demand of milk production and delivery, per se, or if the suckling induced changes in the hormonal status of lactating animals are mediating, at least partially, these changes in appetite.

The hormonal pattern present during lactation is greatly different from that observed during pregnancy. After the postpartum estrus, estrous cyclicity is suspended, prolactin levels are high, moderate levels of progesterone secretion are maintained, and estradiol levels are very low (Rosenblatt et al, 1979). Suckling stimulation from the young causes the release of a complex of hormones necessary for lactation and milk ejection, which includes the release of prolactin, growth hormone, and adrenocorticotropic hormone (ACTH) from the anterior pituitary, increased corticosterone release from the adrenals and the release of oxytocin from the neurohypophysis (Blake, 1974; Saunders et al, 1976; Higuchi et al, 1985; Voogt et al, 1969). Suckling stimulation also inhibits the release of FSH and LH from the anterior pituitary, possibly the result of a depressed pituitary sensitivity to GnRH (Battin et al, 1985; Van Der Shoot et al, 1978, Hansen et al, 1983; Fox et al, 1984). In addition, it has been demonstrated that endogenous opiates, specifically B-endorphin-like

immunoreactivity, increase rapidly and are maintained at high levels during suckling (Riskind et al, 1984).

Research investigating the role of suckling and it's hormonal sequelae on lactational hyperphagia and weight gain has indicated that suckling does indeed play a role in both (Cotes and Cross, 1954 ; Ota and Yokoyama, 1967). For example, it has been demonstrated that elimination of the suckling stimulus through removal of litters immediately following parturition (Cotes and Cross, 1954) or on day 12 postpartum (Ota and Yokoyama, 1967) results in a significant depression of food intake (that begins 24 hours after pup removal in the latter case) and a decreased growth rate. Further, resumption of suckling on day 17 results in an increase in both body weight and food intake, even before restoration of milk secretion, indicating a direct stimulatory effect of suckling on appetite (Ota and Yokoyama, 1967).

Conversely, suckling stimulation in the absence of milk yield (a condition produced through galactophore ligation) maintains lactational hyperphagia although at a level somewhat lower than that of normal lactating controls (Cotes and Cross, 1954). These data suggest that the suckling stimulus and the associated hormonal changes, rather than the metabolic condition associated with milk secretion, is the important factor in the extra growth and food intake of suckled animals.

Along with being an important factor mediating increased food intake, suckling also has an effect on the changes in appetite for specific nutrients seen during lactation. For example, galactophore ligated animals demonstrate calcium intake levels similar to normally lactating animals during the first week postpartum, indicating that calcium appetite is not dependent on milk delivery (Millelire and Woodside, 1989). In addition, it has been demonstrated that animals with galactophore ligations display a significantly

greater intake and preference for a hypertonic (3%) sodium solution throughout the course of lactation (Woodside, unpublished data). These data suggest that the self-selection of calcium and sodium in postparturient rats is not dependent on milk delivery and may be a function of the suckling stimulus itself and/or the hormonal changes it induces.

The third experiment was designed to investigate the mechanisms responsible for the changing patterns of sodium intake and preference seen in lactating animals. More specifically, to assess the role of milk delivery and suckling in the changing patterns of sodium appetite during lactation. By eliminating the metabolic cost of lactation (eg. milk delivery) through galactophore ligation, while maintaining the suckling stimulation and the consequent hormonal environment, it would be possible to determine if milk production and delivery are necessary for the expression of the specific pattern of increased sodium appetite associated with postparturient animals. In addition, by removing the litters of one group of animals on day 1 postpartum we will examine the possibility that simply undergoing the experience of pregnancy and parturition is adequate to alter sodium appetite.

Method

Subjects:

Subjects were 39 virgin female wistar rats obtained from the Charles River Breeding Farms in St. Constant Quebec. Animals were randomly assigned to one of 4 groups; nonlactating (N) (n=10), impregnated litter removed (LR) (n=10), galactophore cut (GC) (n=9), or sham operated (Sh-O) (n=10). In order to be included in this study, female animals were required to display normal 4-day estrous cycles as demonstrated by daily vaginal smears.

Housing and adaptation:

All animals were housed in group cages during a two week adaptation period prior to surgery, during the postoperative recuperation period, and during mating. All other housing and adaptation procedures were identical to those described in the methods of experiment 1.

Surgical Procedures:

Galactophore cuts were carried out under metafane anesthesia. Two midline incisions were made exposing the galactophores of all mammary glands. All galactophores were cut and the midline incisions closed. The success of the operations was verified by weighing litters before and after suckling and by postmortem examination of the mammary glands immediately following the last day of testing.

Identical procedures were followed for sham operated animals, but galactophores were left intact. Animals were then returned to group cages where they were checked periodically for infection and progression of healing.

Impregnation procedure:

The impregnation procedure was identical to that described in the methods of experiment 2 and took place approximately 2 1/2 weeks after surgery. All animals were recovered and healthy at that time.

Procedure:

In order to ensure an equal suckling stimulus to the Sh-O and GC animals, and to maintain pup health, each normally lactating rat was paired with a galactophore ligated rat and the litters were exchanged at the end of each 12 hour period throughout the experiment. Litters were removed from the LR animals on day 1 postpartum.

All other procedural details were identical to those described in the method of experiment 2.

Statistics:

For sodium intake and preference, two-way split plot anovas were performed, with solution concentration as the within group factor and reproductive status as the between group factor.

In order to assess the changing nature of food intake over the course of lactation, food intake data was averaged over 5 day blocks (days 1 through 5 postpartum and days 6 through 10 postpartum) and a two-way split plot anova was performed, with reproductive status as the between group factor and block as the within group factor.

Daily water intake was recorded and averaged over a period beginning day 1 postpartum and continuing until the end of the test period (or an equivalent period for nonlactating animals). Weight gain was recorded as the difference between day 1 postpartum and day 11 postpartum (or an equivalent time period for nonlactating animals). One-way between groups anovas were performed on all these measures.

Post Hoc analyses were carried out using one-tailed, unpaired t-tests, with multistage bonferroni corrections where appropriate. Critical values of t for each stage are indicated in brackets following the analyses. For source tables, see appendix C.

Results

Sodium Intake and preference: N vs. LR

No significant overall differences were demonstrated between the the two nonlactating groups (N and LR) for sodium intake or preference (effect of reproductive status, $F(1,18)=.229$, $p=.6379$; effect of reproductive status, $F(1,18)=.096$, $p=.7603$ respectively). In addition, no signifcant interactions of reproductive status X solution was demonstrated for either intake or preference (interaction effect, $F(4,72)=.468$, $p=.7589$; interaction effect, $F(4,72)=.037$, $p=.9974$ respectively) (see figures 7 and 8).

Sodium intake: Effect of reproductive status

As we found no significant differences between the N and LR groups in terms of sodium intake or preference, the data for these two groups was combined to create a single nonlactating animal control group (n=20).

Figure 9 demonstrates that lactating animals with intact galactophores consume greater quantities of sodium solution than do galactophore ligated animals or nonlactating controls (significant effect of reproductive status, $F(2, 36)=8.967$, $p=.0007$). The significance of the repeated measure effect indicates that the solutions are differentially attractive to the animals (significant effect of solution , $F(4, 144)=.45.447$, $p=.0001$) and the significant interaction of reproductive status X solution indicates that intake of a given solution varies as a function of group membership (significant interaction effect,

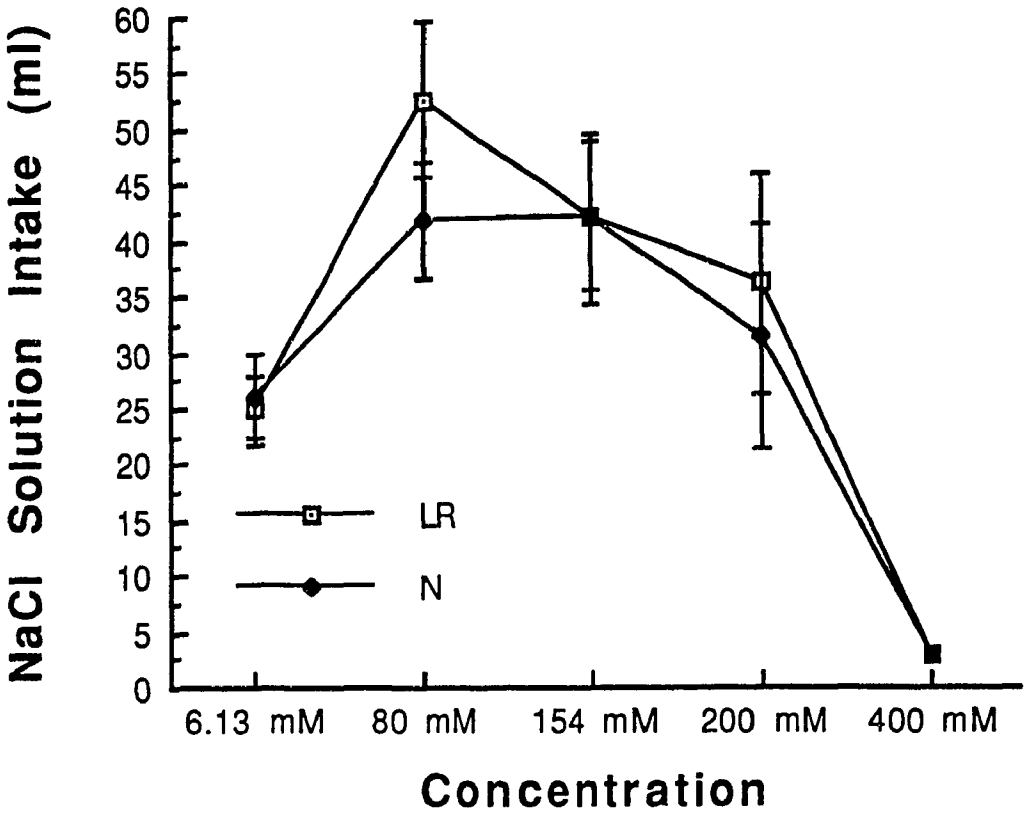

Figure 7: Comparison of NaCl solution intake curves for nonlactating (N) and impregnated litter-removed (LR) rats.

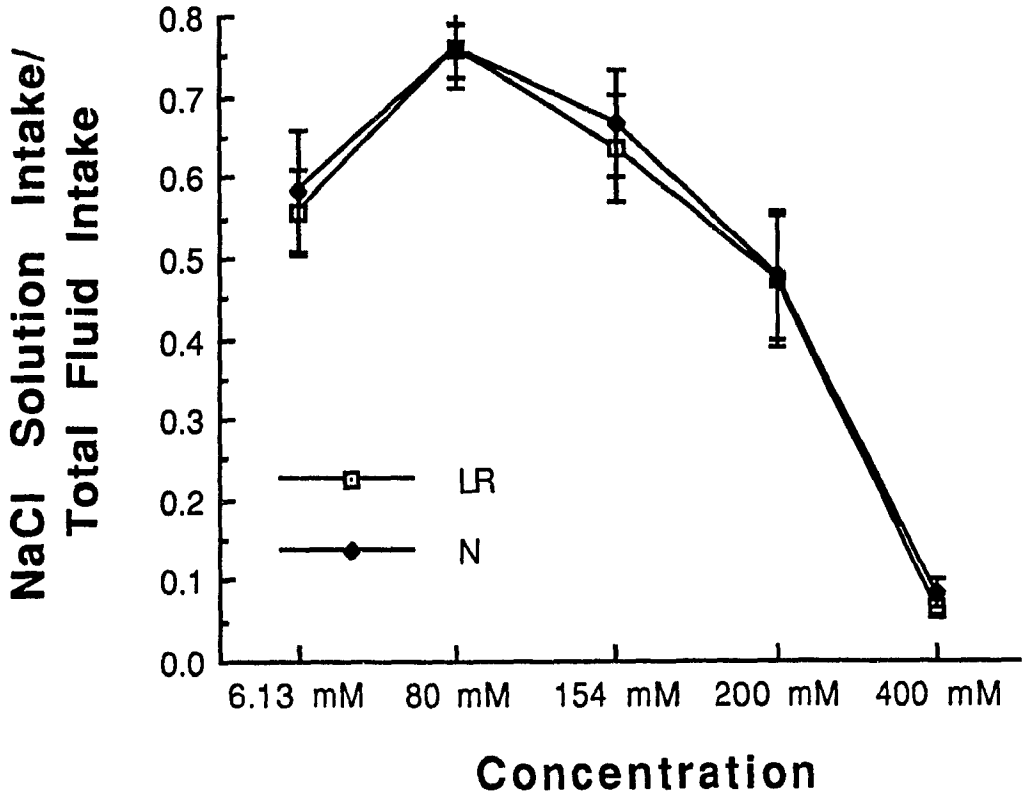

Figure 8: Comparison of NaCl solution preference curves for nonlactating (N) and impregnated litter-removed (LR) rats.

F(8,144)=3.668, p=.0006).

Post Hoc analyses revealed that Sh-O animals ingested significantly greater amounts of the 6.13 mM , the 80 mM and the 154 mM solutions as compared to the galactophore cut animals (t(17)=-4.095, p=.0004; t(17)=-2.553, p=.0103; t(17)=-2.293, p=.0174 respectively) and significantly greater amounts of the 6.13 mM , the 80 mM, the 154 mM and the 400 mM solutions as compared to nonlactating animals (t(28)= -5.077, p=.0001; t(28)=-3.588, p=.0007; t(28)=-4.388, p=.0001; t(28)= -4.555, p=.0001respectively). GC animals drank a significantly greater amount of the 400 mM solution compared to N and Sh-O animals (t(27)=-5.981, p=.0001; t(17)=2.278, p=.0179 respectively).

(t_{crit} stage 1=.05/9=.0056, t_{crit} stage 2=.05/3=.0167, t_{crit} stage 3= .05/2=.025)

Sodium preference: Effect of reproductive status

In terms of preference, there was no significant overall effect for reproductive status (F(2,36)=1.017, p=.3719), but there was a significant effect for solution (F(4, 144)=96.34, p=.0001) and a significant interaction of reproductive status X solution (F(8, 144)=3.525, p=.0009) (see figure 10).

Post Hoc analyses revealed that GC animals demonstrate a significantly greater preference for the 200 mM and 400 mM solutions as compared to both the Sh-O animals (t(17)=2.744, p=.0069; t(17)=4.478, p=.0002 respectively) and the N animals (t(27)=-1.86[1], p=.0368; t(27)=-6.84, p= .0001 respectively).

(t_{crit} stage 1=.05/4=.0125, t_{crit} stage 2 =.05/1=.05)

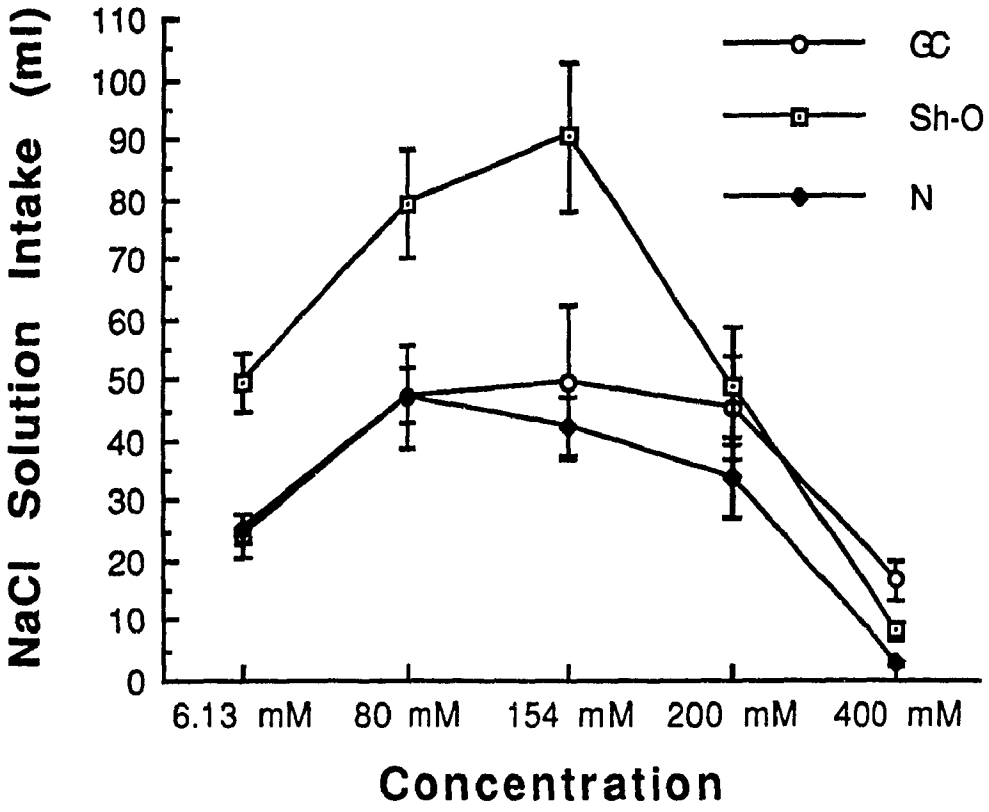

Figure 9: Comparison of NaCl solution intake curves for galactophore cut (GC), sham-operated lactating (Sh-O) and nonlactating (N) rats.

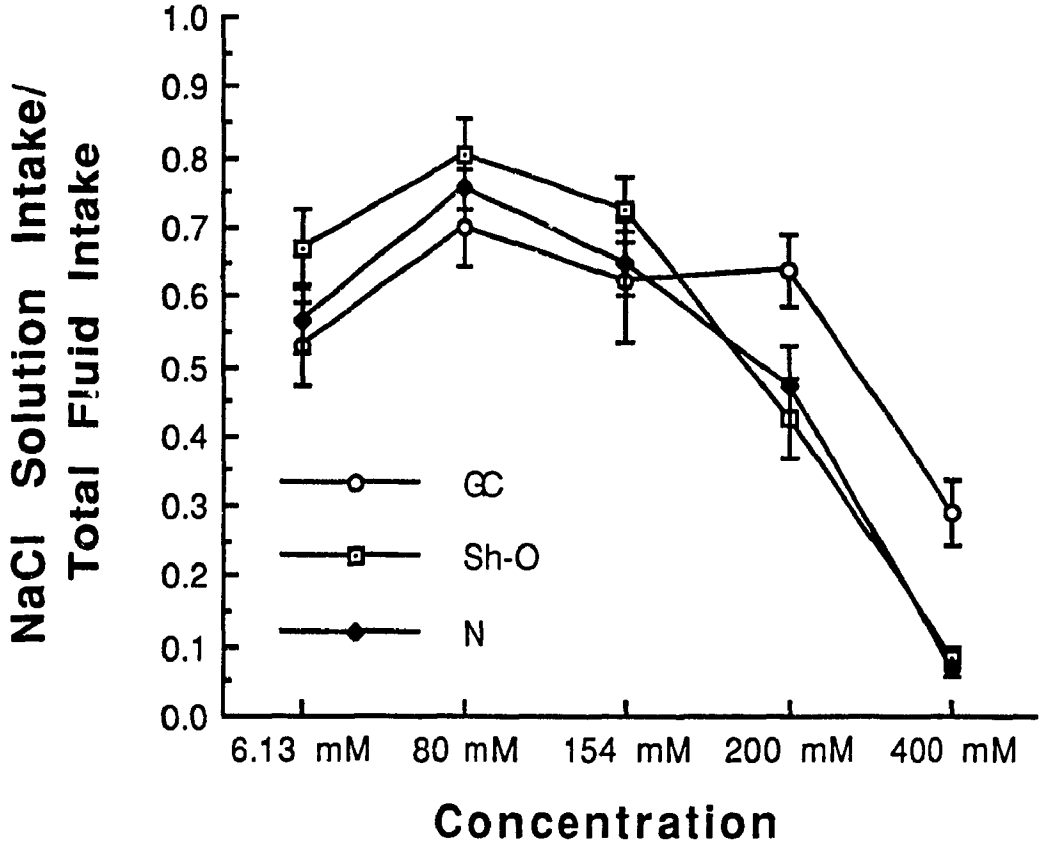

Figure 10: Comparison of NaCl solution preference curves for galactophore cut (GC), sham-operated lactating (Sh-O) and nonlactating (N) rats.

Food Intake:

As no significant differences were demonstrated between the LR and the N animals in terms of overall food intake (unpaired, two-tailed t(18)= -1.251, p=.2268), for the following analyses these two groups were combined to form one nonlactating control group (n=20).

Overall food intake differed significantly among the three groups (significant effect of reproductive status, F(2, 36)=116.4, p=.0001), with Sh-O animals ingesting an average of 46.943±1.277 grams daily over the two blocks, as compared to 25.677±1.107 grams daily for GC animals and 24.085±.949 grams daily for N animals. In addition, there was a significant effect for block, indicating that food intake changed significantly over time (F(1, 36)=132.429, p=.0001). The significance of the interaction effect indicates that food intake differed for each block of time as a function of reproductive status (significant interaction of reproductive status X block, F(2, 36)=90.247, p=.0001)(see figures 11A and 11B).

During block 1 (days 1 through 5 postpartum) Sh-O animals ingested an average of 36.692±.816 grams daily as compared to an average of 21.882±.865 grams daily for GC animals and an average of 23.938±1.126 grams daily for N animals. Post Hoc analyses revealed that Sh-O animals ingested significantly more food than both the GC and N animals during block 1 (t(17)=-12.456, p=.0001; t(28)=7.489, p=.0001 respectively).

During block 2 (days 5 through 10 postpartum) Sh-O animals ingested an average of 57.194±1.964 grams daily as compared to 29.471±1.6 grams daily for GC animals and 24.232±.889 grams daily for N animals. Post Hoc analyses presented a somewhat

different picture than that described for block 1. While the Sh-O animals maintained their significantly greater food intake over GC and N animals (t(17)=-10.791, p=.0001; t(28)=17.7, p=.0001 respectively), the GC animals increased their intake during this period such that they ate significantly more than N animals (t(27)=3.082, p=.0023).

(t_{crit} stage 1=.05/5=.01)

Weight Gain

Sh-O animals gained an average of 29.8±3.855 grams over the experimental period, as compared to 22.033±6.853 grams for GC animals, 9.79±2.43 grams for N animals and -5.36±3.626 grams for LR animals. These differences were statistically significant (F(3,35)=12.697, p=.0001). Post Hoc analyses revealed that the Sh-O animals gained significantly more weight than both the N and the LR animals (t(18)=4.391, p=.0002; t(18)=6.643, p=.0001 respectively) as did the GC animals (t(17)=1.757, p=.0485; t(17)=3.638, p=.001 respectively). In addition, the N animals gained significantly more weight than did the LR animals (t(18)=-3.471, p=.0014).

(t_{crit} stage 1=.05/5=.01, t_{crit} stage 2=.05/1=.05)

Water Intake:

Average daily water intake differed significantly between the 4 groups (F(3,35)= 30.91, p=.0001), with Sh-O animals ingesting an average of 49.9±1.977 ml. daily as

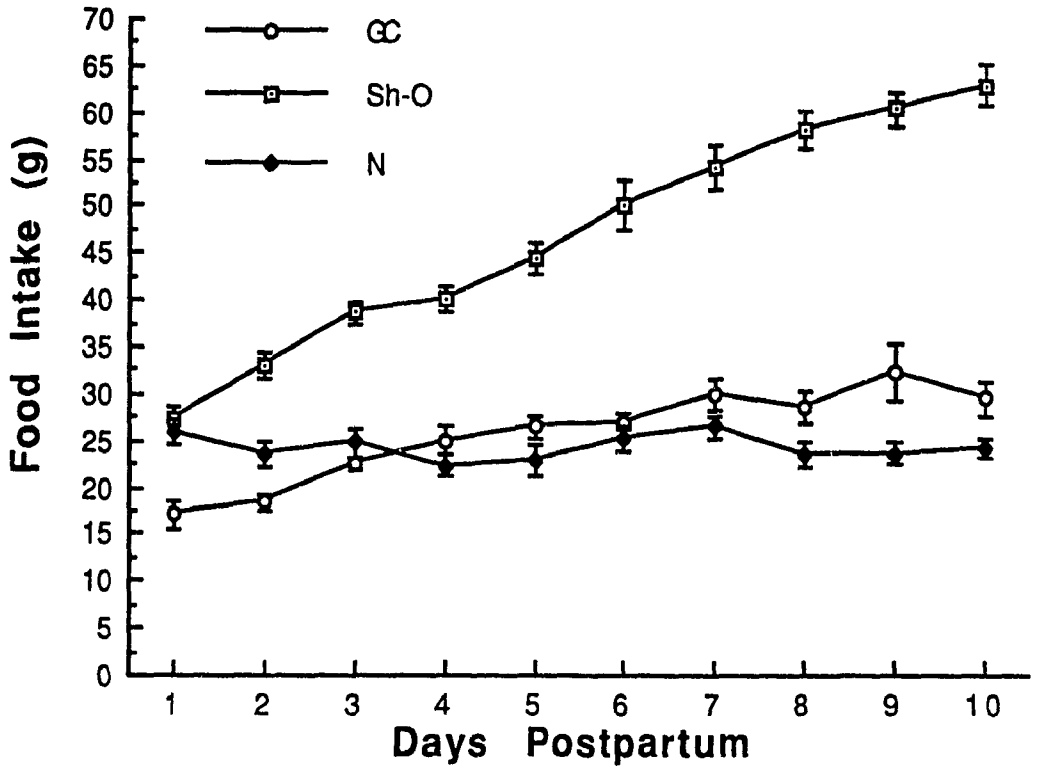

Figure 11a: Change in food intake during the first 10 days postpartum in galactophore cut (GC) and sham-operated lactating (Sh-O) rats as compared to nonlactating (N) controls.

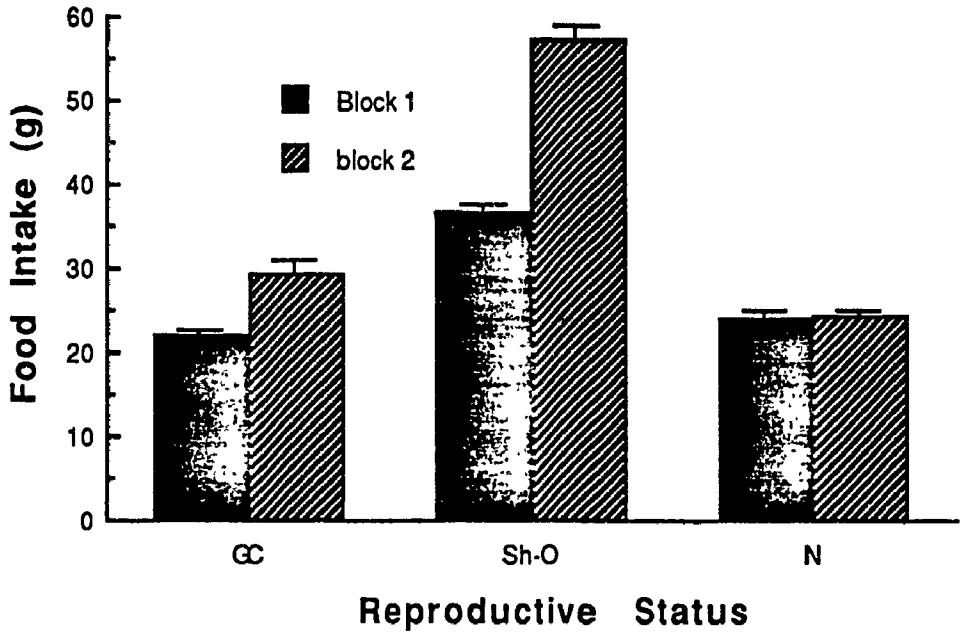

Figure 11b: Change in food intake from block 1 (days 1 through 5 postpartum) to block 2 (days 6 through 10 postpartum) for galactophore cut (GC), sham-operated lactating (Sh-O) and nonlactating (N) rats.

compared to 31.774±2.043 ml. daily for GC animals, 28.34±1.641 ml. daily for N animals and 33.77±1.227 ml. daily for LR animals. Post Hoc analyses revealed that the Sh-O animals drank significantly more water than the GC, LR and N animals ($t(17)=-6.369$, $p=.0001$; $t(18)=6.932$, $p=.0001$; $t(18)=8.932$, $p=.0001$ respectively). In addition, the LR animals drank significantly more water than did the N animals ($t(18)=2.65$, $p=.0082$) (see figure 12).

(t_{crit} stage 1=.05/4=.0125)

Reproductive Outcome Measures:

Average litter size of the three reproducing groups was not different (Sh-O group; mean = 12.7±.578 pups, GC group; mean = 12.11±.676 pups and LR group; mean = 11.5±1.213 pups). This is also true of litter weight on day 1 postpartum. (Sh-O group; mean = 80.71±3.518 grams, GC group; mean = 71.682±2.943 grams and LR group; mean = 74.12±6.297 grams. Average weight of experimental litters culled to 8 in number was 49.749±1.184 grams on day 1 postpartum, and 125.73±3.92 on day 11 postpartum.

Surgical Procedure Outcome Measure:

Bidaily pup weighings, utilized as an indirect measure of milk delivery, revealed that litters gained an average of 11.708±.477 grams during the 12 hour period they were housed with a Sh-O dam while they lost an average of 5.027±.328 grams during the 12 hour period they were housed with a GC dam.

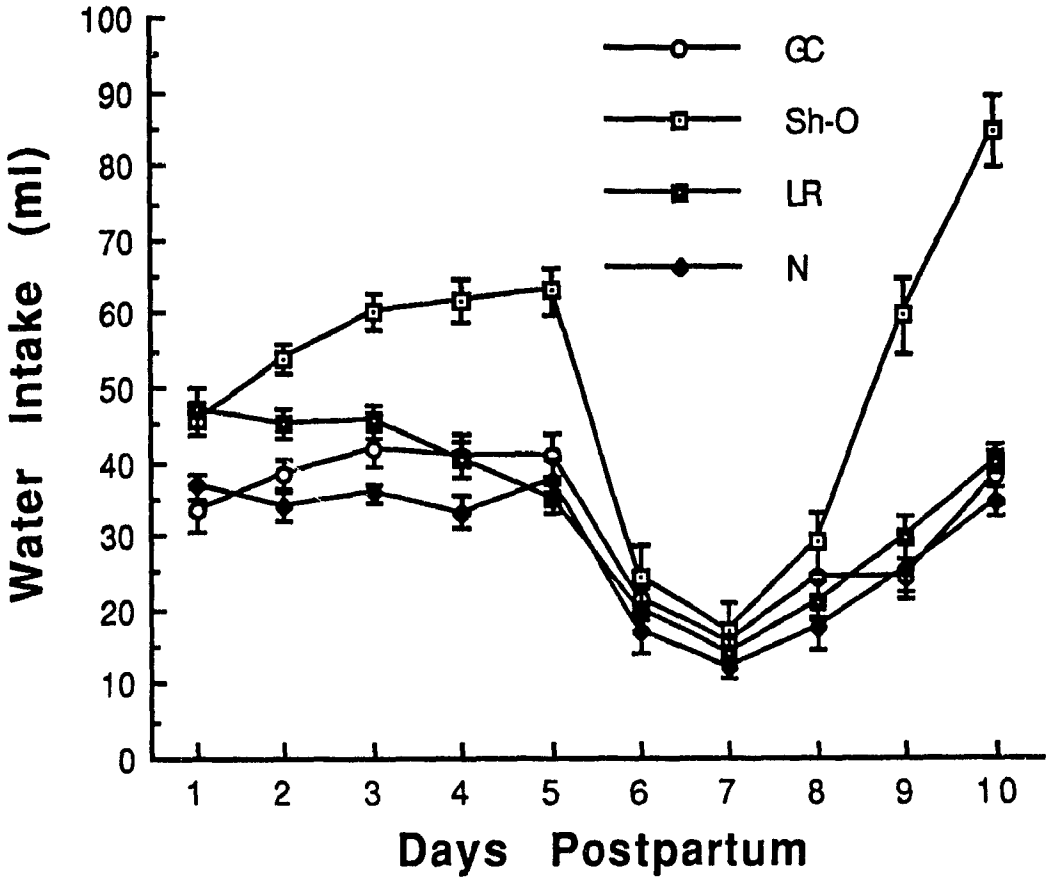

Figure 12: Change in water intake in galactophore cut (GC), sham operated (Sh-O), impregnated litter-removed (LR) and nonlactating (N) rats.

Discussion

In this study, galactophore cut animals displayed an increased sodium appetite compared to both lactating and nonlactating animals.

In terms of intake, this appetite was expressed as a significantly greater intake of the most concentrated solution presented (400 mM). GC animals ingested, on average, nearly twice as much of this solution than did normally lactating animals, and more than 4 times as much as nonlactating animals. Given that this level of tonicity is usually considered aversive, this finding is a clear demonstration of the strength of the sodium appetite in suckled animals. The shape of the intake curves for the three groups revealed some interesting differences as well. GC animals displayed a far lower intake of the two hypotonic solutions and the isotonic solution as compared to normally lactating animals, and in fact the shape of the intake curve until that point is far more similar to that of nonlactating animals. At the 200 mM concentration, Sh-O animals' intake drops off rapidly, while GC animals maintain the same level of intake, resulting in these two groups ingesting essentially equivalent amounts of this solution. Sh-O intake continues to decrease sharply for the 400 mM solution, while GC animals decrease far more gradually, resulting in a significantly greater intake of this solution by GC animals.

The increased sodium appetite of GC animals was also expressed as a significantly greater preference for the two hypertonic solutions presented (200 mM and 400 mM). The preference curve for the GC animals is quite similar to that of the Sh-O animals, although consistently lower, until hypertonicity. At this point, GC animals maintain their preference, and in fact increase it for the 200 mM solution and only begin to gradually decrease their preference at the 400 mM level. Sh-O animals sodium

preference decreases sharply after isotonicity resulting in the significantly greater preference of GC animals for both hypertonic solutions.

The finding that there are no differences between nonreproducing females and impregnated females with litters removed after parturition in terms of sodium appetite indicate that the experience of pregnancy and parturition alone, without the hormonal changes associated with pup stimulation, is not adequate to produce changes in sodium appetite.

These data indicate, therefore, that the elimination of milk delivery does not abolish the increased sodium appetite associated with lactation, and in fact functions to alter the expression of sodium appettite such that preference is maintained at higher concentrations when compared to normally lactating animals. These findings are consistent with previous investigations of the role of milk delivery on sodium appetite (Woodside, unpublished data), and extends them to short term preference testing situations.

Milk production and delivery necessitates a large energetic expenditure on the part of the lactating rat and presents a stress on the animals ability to maintain water balance. Galactophore ligation removes, to a large extent, the challenges to water balance created by increased food intake and water loss through milk delivery, while maintaining the suckling stimulus. In addition, GC animals enjoy the benefits of ingesting considerable amounts of hypotonic pup urine. For example, over a 24 hour period, a litter of 8 ten day old pups produces 21 ml. of urine, nearly all of which is consumed by the mother (Friedman and Bruno, 1976; Friedman et al, 1981). This combination of circumstances considerably reduces the GC animals' challenge to maintain water and sodium balance and may therefore allow for a more accurate representation of

sodium taste preference in suckled animals. That is, the large quantities of water ingested by normally lactating animals may serve to mask otherwise strong preferences.

These data did not replicate the finding from experiment two that lactating animals display a significantly greater preference for an isotonic sodium solution as compared to nonlactating controls may be due to fluctuations in environmental conditions that altered overall fluid needs (eg. level of moisture in the powdered diet). Given that lactating animals were consistent in the shape of their preference curve over the two experiments, the change in significance is probably largely due to the variability of the nonlactating controls. This once again highlights the difficulties inherent in demonstrating preference in intact lactating animals, and emphasizes the need for multi - measure assessments of sodium appetite.

In summary, these data indicate that milk delivery is not necessary for the expression of an increased sodium appetite in postparturient animals, while the experience of pregnancy and parturition are not sufficient. The critical factor appears to be the suckling stimulus and the associated hormonal status of the suckled dam.

Experiment 4: Effect of Phase of Estrous Cycle on Sodium Chloride Intake and Preference in Rats

Introduction

Given the results of the above experiments, it seems plausible to hypothesize that gonadal hormones are acting to influence the sodium appetite of reproducing females.

Previous research has established that gonadal hormones exert an influence on the behavioral and physiological regulation of body weight in the female rat. For example, ovariectomy of adult female rats results in an increase in body weight and food intake that returns to baseline with the administration of exogenous estrogen (Tarrtelin and Gorski, 1973). Conversely, administration of exogenous progesterone results in an increase in body weight in intact animals, but is ineffective in ovariectomized animals (Galetti and Kopper, 1964).

In addition to the findings involving pharmacological manipulations, naturally occuring fluctuations in the circulating titers of ovarian hormones associated with the phases of the estrous cycle have also been shown to be adequate for producing changes in body weight and food intake . More specifically, it has been shown that the rat displays a temporary hyperactivity coincident with a depression in food intake following the proestrus peak in plasma estradiol (Brobeck et al, 1947; Ota and Yokoyama, 1967; Wurtman and Baum, 1980) .

Based on these converging lines of evidence, Wade (1972) concluded that estradiol is the principal ovarian steroid regulating body weight in the female rat, and that it may act centrally to inhibit food intake and stimulate locomotor activity. Progesterone was

thought to play a secondary role in the regulation of energy balance, primarily through the inhibition of the central actions of estradiol.

As well as cyclic variations in over-all food intake and energy balance, fluctuations in the intake of specific macro-nutrients have been demonstrated . For example, when presented with a choice between a high and low protein diet in a self-selection paradigm, rats selectively decrease their intake of the low protein diet during the estrous phase of the cycle, thus increasing the proportion of protein in their diet while decreasing carbohydrate and overall food intake (Wurtman and Baum, 1980).

While much attention has been given to the study of the effects of ovarian hormones on nutritional self-regulation, or energy balance, much less research has examined the relationship of ovarian hormones to changes in gustatory sensitivity that may guide these choices.

Sex differences in taste preferences in rats, however, have been clearly demonstrated. Female rats demonstrate greater preference for sweet solutions than do male rats (Wade and Zucker, 1969a). Ovariectomy of adult females substantially diminishes this preference (Zucker, 1969). Ovariectomy also serves to diminish responsiveness to quinine solutions, implicating the activational actions of ovarian hormones in responsiveness to both palatable and aversive tastes (Zucker, 1969; Wade and Zucker, 1970). In addition, there appears to be an organizational component to the influence of sex hormones on these differences. Administration of exogenous testosterone to female rats during the first week of life also significantly reduces their saccharin preference at adulthood (Wade and Zucker, 1969b).

Although gonadal hormones do exert an influence on saccharin preference, fluctuations in these hormones corresponding to the phases of the estrous cycle do not

effect taste preferences for this substance (Wade, unpublished data, cited in Wade, 1976). Therefore, although activational effects of ovarian hormones have been demonstrated (Zucker, 1969), they do not appear to operate within the bounds of the hormonal fluctuations associated with the estrous cycle.

There is some evidence indicating an association between sodium intake and phase of the estrous cycle. For example, Antunes-Rodrigues and Covian, (1963) have noted cyclic fluctuations in NaCl intake corresponding to phase of cycle, but these data are hard to interpret due to an absence of statistical analysis and evidence of irregular cyclicity in the subject pool. Similarly, Thornborough and Passo (1975) have suggested that the intake of sodium and potassium fluctuates over the estrus cycle, with highest intake ocurring during the metestrous and diestrous phases of the cycle, and lowest during the estrous phase of the cycle. In this study, however, the nutrients were presented in a composite diet so that changes in nutrient intake were neccessarily confounded with changes in food intake.

This experiment, therefore, was designed to determine if there are reliable cyclic variations in sodium appetite in normally cycling females given free access to a palatable hypotonic sodium solution.

Method

Subjects:

Subjects were 21 adult virgin female Wistar rats obtained from the Charles River Breeding Farms (St. Constant, Quebec). The animals weighed 220-240 g at the beginning of the experiment. In order to be included in this study, animals were required to display normal 4-day estrus cycles as demonstrated by daily vaginal smears.

Housing and Adaptation:

Housing and adaptation procedures were identical to those described in experiment one.

Procedure:

For a period of eight days (two complete estrous cycles) following adaptation, animals were presented with a choice between demineralized water and one NaCl solution (100 mM) in 100 ml. graduated cyclinders equipped with rubber stoppers and metal spouts. This solution was chosen due to it's proven palatability in males (Deems and Friedman, 1988) and it's proximity to a solution shown to be palatable in females (Alberts, 1983).

All other procedural details were identical to those described in experiment one.

Statistics:

All statistics were calculated based on data averaged over two estrous cycles. One-way repeated measures anovas were performed for all measures, with phase of cycle as the within group factor. Post Hoc analyses were carried out using one-tailed, paired t-tests, with multistage bonferroni corrections where appropriate. Critical values of t for each stage are indicated in brackets following each analysis. All source tables are presented in appendix D.

Results

Sodium intake and preference:

Neither sodium intake nor sodium preference fluctuated significantly as a function of phase of cycle as can be seen in figures 13 and 14 ($F(3,60)=1.104$, $p=.3544$; $F(3,60)=.465$, $p=.7081$ respectively).

Food intake:

Fig.15 shows the mean food intake of all animals as a function of phase of estrus cycle. Food intake varied significantly as a function of phase of cycle (significant main effect for phase of cycle, $F(3,60)=3.24$, $p=.0282$). Post Hoc analysis revealed that intake at the estrous phase of the cycle was significantly reduced as compared to the metestrous phase, the diestrous phase and the proestrous phase ($t(20)=-1.886$, $p=.0369$; $t(20)=-2.55$, $p=.0096$; $t(20)=-2.218$, $p=.0191$ respectively).
(t_{crit} stage 1=.05/3=.0167, t_{crit} stage 2=.05/2=.025, t_{crit} stage 3=.05/1=.05)

Weight gain:

As can be seen in figure 16, weight gain was shown to vary significantly as a function of phase of cycle (significant main effect of phase of cycle, $F(3,60)=6.113$, $p=.0011$). Weight gain was significantly reduced at the estrous phase of the cycle as compared to the metestrous and diestrous phases ($t(20)=-2.796$, $p=.0056$; $t(20)=-2.809$, $p=.0054$ respectively). A similar pattern was seen at the proestrous phase of

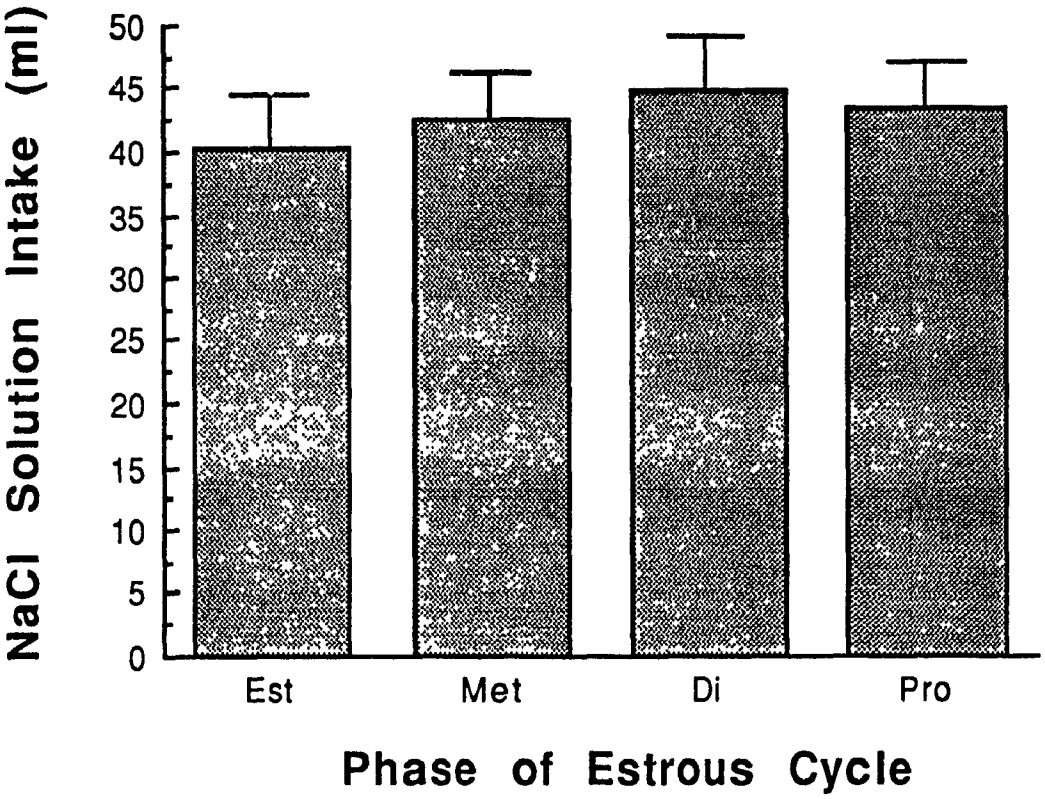

Figure 13: Intake of 100 mM NaCl solution as a function of phase of estrous cycle, averaged over two cycles.

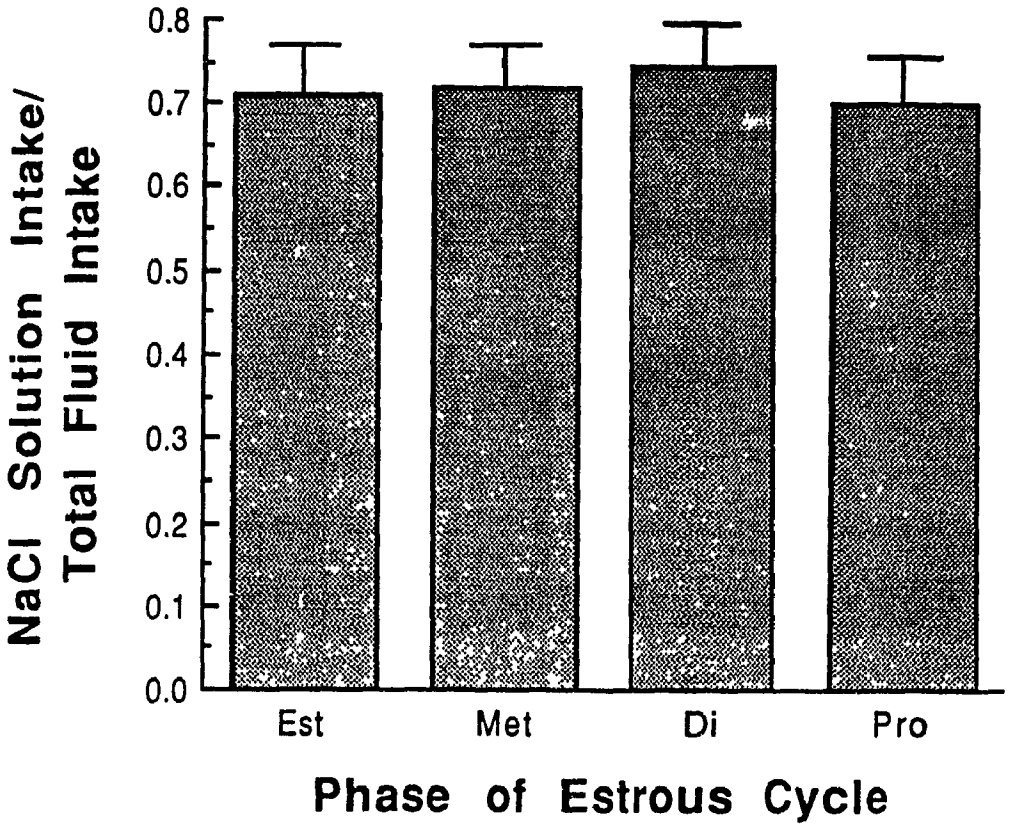

Figure 14: Preference for 100 mM NaCl solution as a function of phase of estrous cycle, averaged over two cycles.

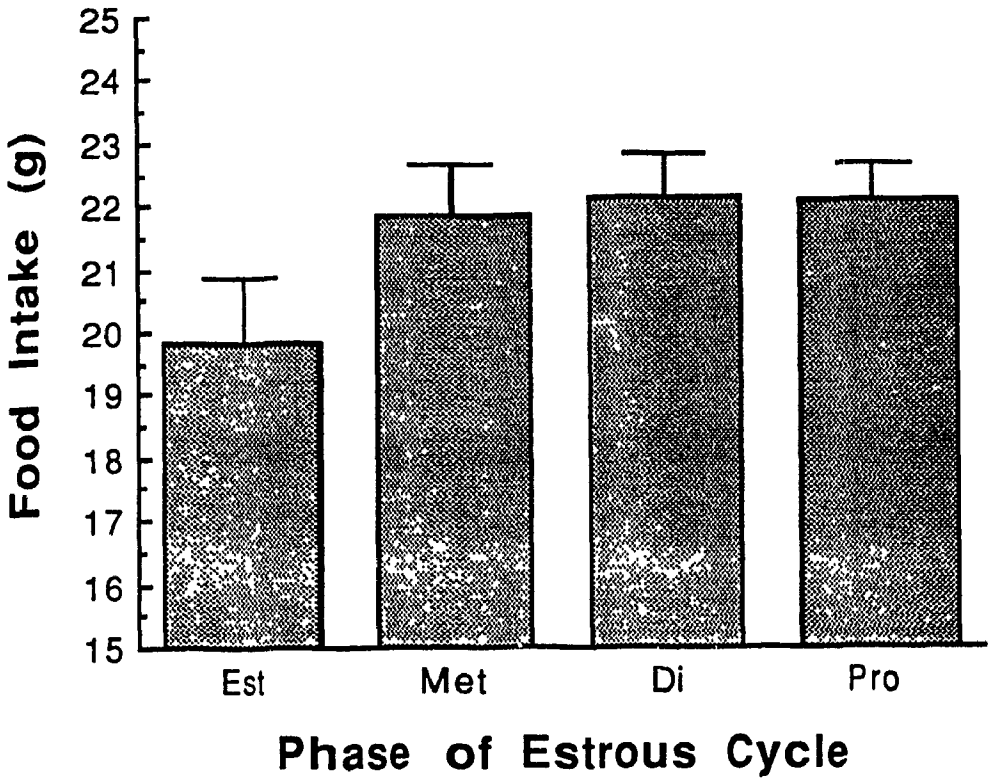

Figure 15: Food intake as a function of phase of estrous cycle, averaged over two cycles.

the cycle, where weight gain was significantly less than at the metestrous and diestrous phases ($t(20)=-3.065$, $p=.0031$; $t(20)=-2.909$, $p=.0044$ respectively). (t_{crit} stage $1=.05/4=.0125$)

Water Intake:

No effect of phase of estrous cycle was demonstrated for water intake ($F(3,60)=.905$, $p=.4442$) (see figure 17).

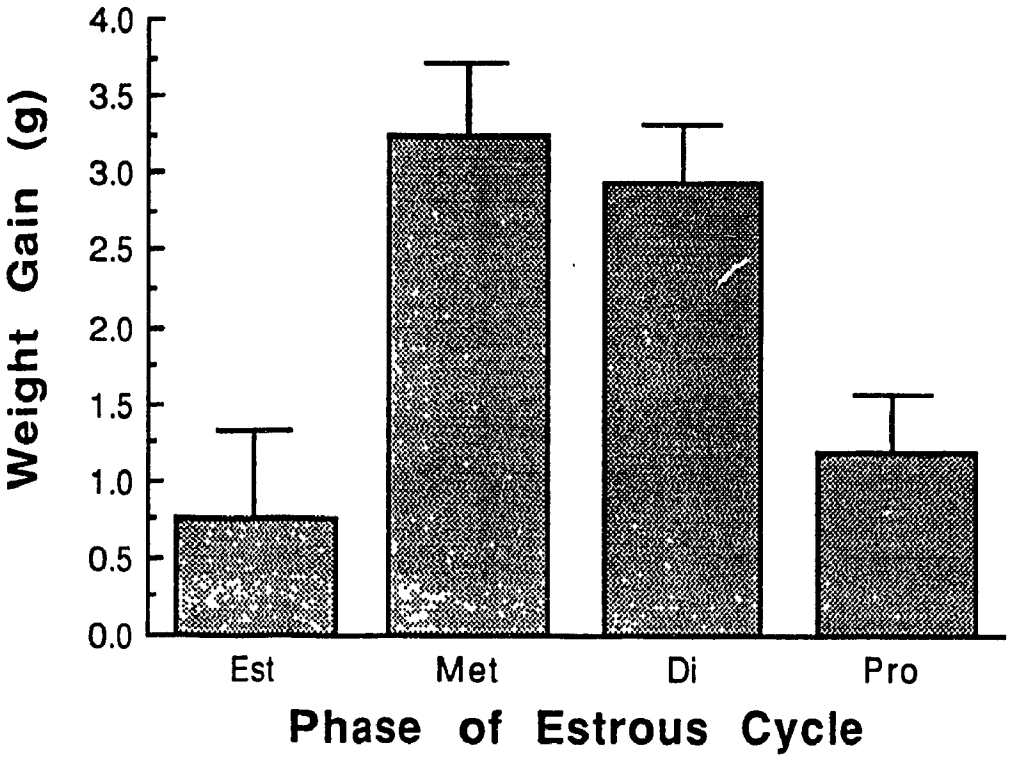

Figure 16: Weight gain as a function of phase of estrous cycle, averaged over two cycles.

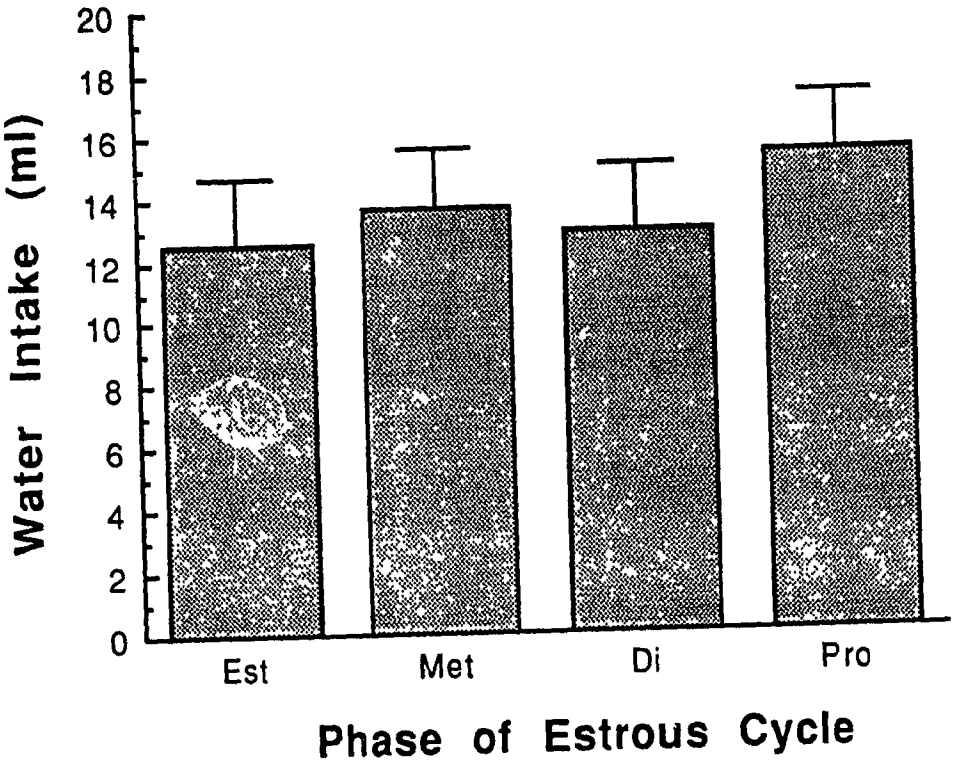

Figure 17: Water intake as a function of phase of estrous cycle, averaged over two cycles.

Discussion

In this experiment, sodium intake and sodium preference were not significantly affected by fluctuations in naturally occurring gonadal hormones corresponding to phases of the estrus cycle. It seems, therefore, that ovarian hormones do not have an influence on NaCl intake within the bounds of the naturally occurring fluctuations associated with the estrous cycle, at least given the concentration of sodium solution used. This is consistent with the finding of Wade (see Wade, 1976) that taste preferences for saccharin do not fluctuate over the estrous cycle. These data are not consistent, however, with those of Antunes-Rodrigues and Covian (1963) or Thornborough and Passo (1975). This is probably a function of the methodological differences described in detail in the introduction to this experiment.

In this study, the finding first reported by Brobeck et al (1947) that food intake and weight gain fluctuate significantly as a function of estrus cyclicity, with the lowest levels of both occuring at the estrus phase of the cycle, was replicated. Sodium intake does not appear to follow this pattern. Thus, while estrogen appears to be critical to the regulation of food intake and body weight (Brobeck et al, 1947; Wade, 1972), it does not appear critical to the selection of specific nutrients, such as NaCl, in cycling animals.

GENERAL DISCUSSION

It is vident from these data that reproduction in rats is associated with an alteration in behavioral sodium regulation in addition to the alterations in physiological mechanisms previously established. These data establish that along with changes in overall sodium chloride intake, the various stages of the reproductive episode are associated with identifable patterns of both intake and preference as the reproductive episode progesses and nutritional requirements fluctuate, as reflected in shifting height and skewness of the preference and intake curves. It was further demonstrated that milk delivery is not a necessary precondition to the increase in sodium appetite observed during lactation and that the experience of pregnancy and parturition are not sufficient, in and of themselves, to produce the sodium appetite characteristic of postparturient animals.

Thus, prior to impregnation, when the sodium/water regulatory system is not challenged, sodium intake and preference remains relatively constant. During the third week of gestation, consequent to the changing sodium requirements imposed by the physiological changes associated with pregnancy and/or the nutritional demands of the developing fetuses, sodium appetite is altered such that overall sodium intake is increased as compared to non-impregnated controls, as is preference for 3 out of 5 sodium solutions presented (6.13 mM, 80 mM, 400 mM). Similarly, lactating animals display a significantly greater overall intake of sodium solutions compared to nonlactating controls, but display only inconsistent increases in preference for an isotonic saline solution (154 mM).

Thus, while pregnancy and lactation are both associated with changes in sodium

intake and preference, these changes are specific to the particular stage of the reproductive episode.

Pregnant animals appear to display a greater intake and preference for hypotonic solutions. Lactating animals display a more consistent increase in intake and tend to be shifted on the curve towards more concentrated solutions, perhaps indicating a shift in taste sensitivity. However, perhaps due to the huge metabolic cost of lactation, preference for sodium solutions is difficult to quantify in lactating animals. It has been demonstrated that sodium intake is associated with increased water intake in pregnant animals (Bird and Contreras, 1986). If the same holds true for lactating animals, when water balance is even more difficult to maintain due to fluid loss through milk delivery and high food intake, then one would expect that the extremely high sodium intake typical of lactating animals would necessarily be accompanied by large increases in water intake, especially for hypertonic solutions. Thus while pregnant animals intake may not be as consistently high as lactating animals, they are not required to compensate for the water loss imposed by lactation and thus appear to have a greater preference for sodium solutions than do lactating animals.

The results of experiment 3, however, suggests that if relieved of the metabolic cost of milk delivery, suckled animals display a marked preference for very concentrated sodium solutions that is not otherwise observable. Thus, not only is milk delivery unnecessary for the expression of increased sodium appetite in post parturient animals, it may also serve to mask it.

Assessing these data according to the model proposed by Epstein and Stellar (1955), we might propose that pregnant animals have similar taste sensitivity to cycling animals, with higher need reflected in the greater height of both intake and

preference curves. Lactating animals, however, appear to exhibit changes in both taste sensitivity and need, as reflected in the changing height and shape of the curves.

The next question, then, is what is mediating these changes and are the same mechanisms operating during both pregnancy and lactation. If the sodium appetites of pregnancy and lactation are the same, but only appear different due to the metabolic expense of milk delivery, then you would expect the sodium intake and preference curves of galactophore cut animals to be similar to those of pregnant animals. As we have seen, this is not the case. GC animals, by removing the "need" aspect of lactation, appear more similar to cycling animals in their ingestion of hypo and isotonic solutions. After isotonicity, however, GC animals display a strong and apparently "need-free" appetite for hypertonic solutions not seen in either pregnant or intact lactating animals. This may be taken as an indication that lactation is associated with a shift in taste preference and/or sensitvity which is masked by the metabolic demands of lactation.

In order to assess the validity of this hypothesis, future research might more directly assess the taste sensitivities of lactating and pregnant animals using, for example, the gustometry technique developed by Brosvic and his associates (1986). Using this technique, they have demonstrated that adrenalectomized animals have an unaltered taste sensitivity for sodium chloride (Brosvic et al, 1989) If the mechanisms responsible for the changing sodium appetites of pregnant and lactating animals are different, we might then find that pregnant animals are similar to adrenalectomized animals in that their taste sensitivities have not changed, while lactating animals may demonstrate shifts in sensitivity. Based on the above data it is impossible to predict the exact nature of these shifts. One possibility may be that lactating animals would display a lowered taste threshold in order to facilitate the

identification of potential sources of sodium. It is equally possible, however, that a higher threshold would be present in order to facilitate the ingestion of highly concentrated sodium sources that would otherwise be aversive.

Along with the possibility of changes in taste sensitivity, sodium appetite may also be altered in such a way as to effect actual preference. Thus, while taste threshold may or may not be altered, the hedonic properties of sodium may change such that all or certain sodium solutions are more intrinsically appealing. There is evidence that this is the case under certain circumstances, for example, the litters of dams exposed to a high sodium diet during pregnancy and lactation demonstrate altered preference for sodium without altered taste sensitivity as adults (Bird and Contreras, 1987).

If it is true that the sodium appetites of pregnant and lactating animals are different, then it is possible that the hormonal mechanisms underlying these appetites are different as well. The most obvious hormonal difference between pregnant and lactating animals (including GC animals) is with respect to circulating levels of prolactin. The contention that prolactin somehow contributes to sodium appetite is not unreasonable given it's known osmoregulatory function in teleostats (see Denton, 1983 for review) and evidence for the existence of prolactin receptors in mammalian kidneys (Horrobin, 1980). It is known that there exist two separate renin-angiotensin systems (one renal, wich acts to conserve sodium and one cerebral which acts to induce appetite) (Epstein, 1982). It is conceivable, then, that while prolactin acts on the kidney to inhibit sodium secretion, it may also have a central action that produces behavioral sodium appetite. Thus the kidney may be only one of many loci of action for prolactin. High prolactin may be an impetus to both behavioral and physiological upgrading of sodium retention and ingestion during lactation. One way to assess the

function of prolactin in this capacity is through the use of prolactin inhibitors (eg. bromocryptine) during lactation in intact and galactophore cut animals. Conversely, one might administer prolactin centrally and/or peripherally in nonreproductive animals.

The next step in understanding the hormonal mechanisms of sodium appetite during reproduction is to assess the effects of manipulations of the aldosterone/ angiotensin system directly. For example, both peripheral and intracerebroventricular administration of captopril, a competive inhibitor of angiotensin-converting enzyme which blocks the conversion of angiotensin I to the active angiotensin II, has been shown to suppress depletion induced sodium appetite in rats (Moe, Weiss and Epstein, 1984; Weiss, Moe and Epstein, 1986). The use of these types of techiques during pregnancy and lactation would allow for assertions concerning the generalizability of hormonally mediated sodium appetite. As there is evidence for increased ACTH release during lactation, a potential role for mineralcorticoids at this time is suggested.

Finally, hormonal sensitization procedures, such as those described by Bridges in 1984 (eg. the adminstration of estradial and progesterone to ovariectomized virgin rats), would be useful in ascertaining the role of the fetus and the products of conception on the sodium appetite of pregnant animals, as opposed to mother-mediated hormonal influences. In addition, the sensitization technique described by Siegal and Rosenblatt (1975), which involve the simulation of the hormonal changes present during the third trimester of pregnancy through the use of pregnancy termination by hysterectomy, would reveal useful information as to the importance of timing in studies of this nature.

To conclude, the use of the techniques described above in future investigations would allow not only for the delineation of the role of reproductive status on sodium appetite, but would also contribute to a greater understanding of the hormonal basis of sodium

appetite more generally.

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APPENDIX A:

Source Tables of Analyses of Variance (Experiment 1).

Table 1

Difference in Sodium Intake between impregnated and nonimpregnated rats.

Source	SS	df	MS	F	P
Reproductive Status	13912.81	1	13912.81	11.649	.0042
Subjects w. groups	16720.58	14	1194.33		
Repeated measure (B)	29551.93	4	7387.98	29.565	.0001
AB	4847.88	4	1211.969	4.85	.002
B X subjects w. groups	13993.8	56	249.889		

Table 2

Difference in Sodium preference between impregnated and nonimpregnated rats.

Source	SS	df	MS	F	P
Reproductive Status	.217	1	.217	3.861	.0696
Subjects w. groups	.788	14	.056		
Repeated measure (B)	4.557	4	1.139	47.633	.0001
AB	.281	4	.07	2.934	.0284
B X subjects w. groups	1.1339	56	.024		

APPENDIX B:

Source Tables of Analyses of Variance (Experiment 2).

Table 3

Difference in NaCl intake between male and nonlactating female rats.

Source	SS	df	MS	F	P
Reproductive Status	658.34	1	658.34	1.21	.2866
Subjects w. groups	9247.28	17	543.96		
Repeated measure (B)	21112.15	4	5278.04	23.854	.0001
AB	1466.13	4	366.53	1.657	.1703
B X subjects w. groups	15045.72	68	221.26		

Table 4

Difference in NaCl preference between male and nonlactating female rats.

Source	SS	df	MS	F	P
Reproductive Status	.071	1	.071	.816	.3791
Subjects w. groups	1.482	17	.087		
Repeated measure (B)	4.5	4	1.125	33.31	.0001
AB	.1031	4	.026	.762	.5535
B X subjects w. groups	2.297	68	.034		

Table 5

Difference in NaCl intake between lactating and nonlactating rats.

Source	SS	df	MS	F	P
Reproductive Status	32176.17	1	32176.17	48.274	.0001
Subjects w. groups	19329.40	29	666.53		
Repeated measure (B)	60866.65	4	15216.6	44.282	.0001
AB	12677.07	4	3169.27	9.223	.0001
B X subjects w. groups	39861.49	116	343.63		

Table 6

Difference in NaCl preference between lactating and nonlactating rats.

Source	SS	df	MS	F	P
Reproductive Status	.185	1	.185	3.052	.0912
Subjects w. groups	1.758	29	.061		
Repeated measure (B)	7.297	4	1.824	62.378	.0001
AB	.448	4	.112	3.827	.0059
B X subjects w. groups	3.393	116	.029		

Table 7

Difference in food intake between lactating, nonlactating and male rats.

Source	SS	df	MS	F	P
Between groups	1407.721	2	703.86	37.973	.0001
Within groups	518.996	28	18.536		
Total	1926.717	30			

Table 8

Difference in weight gain between lactating, nonlactating and male rats.

Source	SS	df	MS	F	P
Between groups	12508.02	2	6254.01	58.46	.0001
Within groups	2995.438	28	106.98		
Total	15503.46	30			

Table 9

Difference in water intake between lactating, nonlactating and male rats.

Source	SS	df	MS	F	P
Between groups	1049.47	2	524.74	11.191	.0003
Within groups	1312.94	28	46.89		
Total	2362.41	30			

APPENDIX C:

Source Tables of Analyses of Variance (Experiment 3).

Table 10

Difference in NaCl intake between impregnated litter-removed and nonlactating females rats.

Source	SS	df	MS	F	P
Reproductive Status	222.01	1	222.01	.229	.6379
Subjects w. groups	17432.18	18	968.454		
Repeated measure (B)	24295.44	4	6073.86	23.736	.0001
AB	479.24	4	119.81	.468	.7589
B X subjects w. groups	18424.52	72	255.896		

Table 11

Difference in NaCl preference between impregnated litter-removed and nonlactating female rats.

Source	SS	df	MS	F	P
Reproductive Status	.007	1	.007	.096	.7603
Subjects w. groups	1.323	18	.074		
Repeated measure (B)	5.527	4	1.382	54.882	.0001
AB	.004	4	.001	.037	.9974
B X subjects w. groups	1.813	72	.025		

Table 12

Difference in NaCl intake between galactophore cut, sham-operated lactating and nonlactating rats.

Source	SS	df	MS	F	P
Reproductive Status	20982.68	2	10491.34	8.967	.0007
Subjects w. groups	42117.91	36	1169.942		
Repeated measure (B)	63056.95	4	15764.24	45.447	.0001
AB	10179.32	8	1272.415	3.668	.0006
B X subjects w. groups	49949.72	144	346.873		

Table 13

Difference in NaCl preference between galactophore cut, sham-operated lactating and nonlactating rats.

Source	SS	df	MS	F	P
Reproductive Status	.118	2	.059	1.017	.3719
Subjects w. groups	2.094	36	.058		
Repeated measure (B)	9.217	4	2.304	96.34	.0001
AB	.674	8	.084	3.525	.0009
B X subjects w. groups	3.444	144	.024		

Table 14

Difference in food intake between galactophore cut, sham-operated lactating, and nonlactating rats.

Source	SS	df	MS	F	P
Reproductive Status	7469.453	2	3734.727	116.4	.0001
Subjects w. groups	1155.073	36	32.085		
Repeated measure (B)	999.464	1	999.464	132.429	.0001
AB	1362.223	2	681.112	90.247	.0001
B X subjects w. groups	271.698	36	7.547		

Table 15

Difference in weight gain between galactophore cut, sham-operated lactating, impregnated litter-removed and nonlactating rats.

Source	SS	df	MS	F	P
Between groups	7001.799	3	2333.93	12.697	.0001
Within groups	6433.413	35	183.81		
Total	13435.21	38			

Table 16

Difference in water intake between galactophore cut, sham-operated lactating, impregnated litter-removed and nonlactating rats.

Source	SS	df	MS	F	P
Between groups	2729.17	3	909.724	30.91	.0001
Within groups	1030.11	35	29.432		
Total	3759.28	38			

APPENDIX D:

Source Tables of Analyses of Variance (Experiment 4).

Table 17

Change in NaCl intake over the estrous cycle.

Source	SS	df	MS	F	P
Between subjects	23246.31	20	1162.315	16.47	.0001
Within subjects	4445.938	63	70.57		
treatments	232.676	3	77.559	1.104	.3544
residual	4213.262	60	70.221		
Total	27692.25	83			

Table 18

Change in NaCl preference over the estrous cycle.

Source	SS	df	MS	F	P
Between subjects	4.272	20	.214	16.35	.0001
Within subjects	.823	63	.013		
treatments	.019	3	.006	.465	.7081
residual	.804	60	.013		
Total	5.095	83			

Table 19

Change in food intake over the estrous cycle.

Source	SS	df	MS	F	P
Between subjects	555.216	20	27.761	3.224	.0002
Within subjects	542.509	63	8.611		
treatments	75.628	3	25.209	3.24	.0282
residual	466.881	60	7.781		
Total	1097.726	83			

Table 20

Change in weight gain over the estrous cycle.

Source	SS	df	MS	F	P
Between subjects	30.118	20	1.506	.23	.9997
Within subjects	412.52	63	6.548		
treatments	96.569	3	32.19	6.113	.0011
residual	315.95	60	5.266		
Total	442.64	83			

Table 21

Change in water intake over the estrous cycle.

Source	SS	df	MS	F	P
Between subjects	5075.56	20	253.78	6.982	.0001
Within subjects	2289.75	63	36.345		
treatments	99.095	3	33.032	.905	.4442
residual	2190.66	60	36.511		
Total	7365.31	83			