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NaCl preference increases during pregnancy and lactation: assessment using brief access tests

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Abstract

Pregnancy and lactation are characterized by increases in NaCl intake, as determined by long-term consumption tests, which cannot examine the relative contribution of taste and postingestive factors to this phenomenon. Consequently, in this study, changes in NaCl preference during pregnancy and lactation were studied in nulliparous Long–Evans rats using a brief access test (lickometer). In Experiment 1, rats were maintained on a Na^+-adequate diet (0.03% Na^+), habituated to lickometer testing, and subsequently assessed during pregnancy and lactation with three 30-s exposures to each of seven taste solutions: 0.075 M sucrose (base), 0.089 M NaCl in base, 0.158 M NaCl in base, 0.281 M NaCl in base, 0.5 M NaCl in base, 0.158 M NaCl and 0.281 M NaCl. Results indicated higher lick rates to the 0.5 M NaCl in base, 0.158 M NaCl and 0.281 M NaCl solutions during late pregnancy and late lactation (Day 13 and beyond). In Experiment 2, a comparison of two diets differing in sodium content (0.03% vs. 0.3% Na^+) determined that these changes in NaCl preference during pregnancy and lactation were unrelated to dietary sodium. Thus, the apparent increase in NaCl preference during pregnancy and lactation, independent of dietary sodium, suggests that this change in preference is not in response to physiological sodium need.

Keywords: NaCl preference; Pregnancy; Lactation; Rats

1. Introduction

The reproductive episode (pregnancy and lactation) is characterized by increased sodium need and sodium retention to ensure viability of the pregnancy, optimal fetal growth and development, and to compensate for maternal loss of sodium during lactation (Churchill et al., 1980, 1981). In the rat, as in other species, this sodium need, which is associated with consistent increases in the voluntary consumption of NaCl solutions, has been observed early in gestation (Day 3 or 4) and persists until the weaning of the young (Denton and Nelson, 1971; Frankmann et al., 1991; Moore and Lux, 1998; Richter and Barelare, 1938; Robb et al., 1970; Wintour et al., 1976). These effects on maternal salt intake may be mediated by changes in the regulatory mechanisms underlying salt and water balance and/or reproduction.

In the absence of physiological need, most strains of rats prefer NaCl solutions within the isotonic range, with 0.15 M NaCl being maximally preferred (Breslin et al., 1993; Rowland and Fregly, 1988; Young and Falk, 1949). Yet, during reproduction, pregnant rats appear to differ from nonpregnant rats not only in their increased intake of preferred concentrations of NaCl but also in their voluntary consumption of NaCl concentrations that are typically rejected by nonpregnant rats (Frankmann et al., 1991). For example, Frankmann et al. (1991) reported that Long–Evans rats exhibited an increased consumption of a hypertonic 0.3 M NaCl solution, a typically aversive concentration. This response persisted for an extended period, up to a month following the weaning of a second litter.

Interestingly, the increase in sodium intake reported during pregnancy and lactation has been observed in animals maintained on diets that provide more than the necessary amounts of sodium, even for pregnancy (Brot et al., 2000; Frankmann et al., 1991; McBurnie et al., 1988, 1999). While rats require only 0.03% dietary sodium for optimal physiological functioning (Brensilver

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et al., 1985; Ganguli et al., 1969; Kirksey & Pike, 1962; Kirksey et al., 1962) commercial rodent diets contain about 10 times as much, that is, 0.3% sodium. Thus, rats consuming sodium-rich diets that exceed dietary sodium requirement display the increased salt intake observed during reproductive episodes. It remains unclear, however, whether the intake observed during pregnancy and lactation is regulated at all by dietary sodium levels.

The investigation of salt preference has typically relied on intake as a measure of this phenomenon. In the standard intake paradigm, a taste stimulus is presented either alone or in conjunction with water. The relative level of consumption of the taste stimulus is then interpreted as evidence of preference for or aversion to that taste quality. Intake measures, however, can be influenced by preingestive and postingestive factors (Breslin et al., 1992), which is problematic in studies assessing NaCl preference during pregnancy and lactation. Long-term testing makes it difficult to separate the role of taste from postingestive consequences that may influence intake. To circumvent such problems, the present study employed a brief access paradigm with lick rate analysis to assess salt preference during pregnancy and lactation. This test permits short access to taste solutions, thus, preference can be assessed within the first 30 s of exposure to the taste solution without postingestive influence (Breslin et al., 1993).

Pregnancy and lactation are characterized by an increase in salt intake, even in rats maintained on diets with sodium contents in excess of what is required physiologically (Brot et al., 2000; Friedman et al., 1981; Moore and Lux, 1998; Pike and Yao, 1971; Thiels et al., 1990). Thus, the observed increases in salt intake would not appear to be a response to outright sodium deficiency. Nonetheless, this preference change could be modulated by the pregnant animals' sodium status such that lower, but adequate, dietary sodium levels would be associated with an earlier or more vigorous increase in NaCl preference. Therefore, the present studies used lick frequency analysis in brief access tests to investigate changes in salt preference during pregnancy and lactation, and then to examine the influence of dietary sodium levels on these changes.

2. Materials and methods

2.1. Subjects

Subjects were nulliparous, adult female Long–Evans rats (weighing 250–275 g at the beginning of the study) bred at the University of Washington colony. During experiments, rats were housed individually in a temperature-controlled colony room on a 12:12 h light–dark cycle. Each rat was given unrestricted access to water and pelleted chow (Teklad No. 8604; Na^+ content 0.29%).

2.2. Apparatus

The Davis MS160 lick detection (lickometer) system (Dilog Instruments, Tallahassee, FL) was used to present several taste solutions varying in sodium concentration, to the rats. The lickometer consisted of a transparent, Plexiglas cage (29 cm long × 14.5 cm wide × 23 cm high) with a metal grid floor, and a front metal wall containing a small portal covered by a shutter that could be raised or lowered electronically. This portal allowed access to one of up to 16 drinking tubes mounted on a sliding rack located on the front of the Plexiglas cage. A microcomputer controlled the position of the shutter and the sliding rack to control stimulus access, stimulus duration and intertrial interval. Each contact made with the rat's tongue and the drinking tubes (1-ms resolution) completed an electrical circuit (<60 nA of current). The signal was amplified and sent to the computer that recorded and stored all lick data.

2.3. Habituation

One week after arrival in the lab, the rats were habituated to testing. Initially, this involved being placed in the lickometer for 60 min a day for 3 days while fluid-replete. After this rats were fluid-deprived for approximately 23.5 h daily, and placed on a regimen whereby they received 30 min access to a palatable 0.15 M sucrose solution while in the lickometer. This regimen continued until rats were readily licking the sucrose solution within the prescribed interval. At this time, deprivation was discontinued while the rats continued to receive 30 min access to the 0.15-M sucrose solution in the lickometer. Over time, the length of exposure to the sucrose taste solution was decreased from 30 to 10 min. Once rats were readily licking the sucrose taste solution within this short interval, the regimen was altered. Rats now received 30-s exposures to each of the four sucrose solutions — 0.03, 0.15, 0.3 and 0.5 M sucrose — with each solution presented twice in random order. There was a 20-s interval between presentations, and subjects were allowed 40 s to initiate licking. If licking did not occur during that time, the shutter closed until the next solution was presented 20 s later. This regimen continued until rats readily responded to the brief presentations of the taste solutions.

2.4. Diet

Once the rats exhibited a high rate of licking with only a brief access to solutions, they were placed on one of two pelleted diets (ICN Biochemicals No. 902902; custom-made diet with different amounts of Na^+). The first diet was a Na^+-adequate diet, which contained 0.03% Na^+, an amount considered sufficient for normal functioning in the

adult rat (Brensilver et al., 1985). The second diet was a Na^+-regular diet, which contained 0.3% Na^+, the amount found in commercial rodent chow.

2.5. Taste solutions

About 1 week after being placed on the new diet, the seven test solutions were introduced. The test solutions were: 0.075 M sucrose (base), 0.089 M NaCl in base, 0.158 M NaCl in base, 0.281 M NaCl in base, 0.5 M NaCl in base, 0.158 M NaCl and 0.281 M NaCl. The use of NaCl–sucrose mixtures was based on previous research in our laboratory (see Brot et al., 2000). Each of the seven solutions was presented three times in random order, for a total of 21 presentations. Rats had 30-s access to each solution, with 20-s intervals between presentations. They were allowed 40 s to initiate licking, and if licking did not occur during that time, the shutter closed until the next solution was presented 20 s later. Prepregnancy (baseline) lick rates to the seven taste solutions were based on 4 days of testing.

2.6. Mating

Once baseline measures to each test solution had been obtained, each female was placed overnight in a cage with a male rat, on three consecutive nights. Evidence of mating (i.e., sperm plugs) or lack thereof led to the classification of females as pregnant or nonpregnant. Day 1 of pregnancy was the day on which any evidence of mating was found.

2.7. Experiment 1 — evidence of salt preference during pregnancy and lactation

Fourteen female rats were assigned to two groups based on reproductive status: pregnant ($n=6$) and nonpregnant ($n=8$). To maximize the likelihood of detecting changes in NaCl preference during pregnancy and lactation, these animals were maintained on a Na^+-adequate diet consisting of approximately 0.03% Na^+, an amount that is sufficient for normal functioning, including reproductive performance, but which is 1/10th of the amount of sodium found in most commercial rodent diets. NaCl preference was assessed during pregnancy (or the equivalent period for nonpregnant controls) on Days 1, 4, 7, 10, 13, 16 and 19; and during lactation, on Days 13, 16 and 19. Solutions were presented using the same schedule as during the prepregnancy assessment.

2.8. Experiment 2 — salt preference during pregnancy: diet comparison

To determine whether the increased salt preference displayed during pregnancy is responsive to the sodium level of the diet, dietary sodium levels were varied in this experiment. Animals were maintained on one of two diets throughout the experiment: Na^+-adequate (0.03% Na^+) and Na^+-regular (0.3% Na^+; the amount typical of commercial rodent diets). Thus, the rats used in this study were assigned to four groups based on reproductive status and diet: pregnant/Na^+-adequate diet, $n=5$; nonpregnant/Na^+-adequate diet, $n=8$; pregnant/Na^+-regular diet, $n=3$; nonpregnant/Na^+-regular diet, $n=8$. Measurements of NaCl preference were obtained as previously described.

2.9. Data analysis

Results were analyzed separately for each day of testing by calculating the mean number of licks made during all three presentations of each taste solution. A minimum criterion of three licks was set for inclusion of data. For Experiment 1, lick counts were analyzed with a 2 (State) × 7 (Taste Solutions) factorial analysis of variance (ANOVA). For Experiment 2, lick counts were analyzed with a 2 (Diet) × 2 (State) × 7 (Taste Solutions) factorial ANOVA. Paired comparisons were performed using Fisher's least significant difference procedure.

3. Results

3.1. Experiment 1

3.1.1. Prepregnancy

Fig. 1 illustrates prepregnancy (baseline) lick rates for the seven taste solutions. There were no reliable differences in the baseline lick rates of rats that subsequently did or did not become pregnant after the mating opportunity, $P>.05$. In contrast, as expected, there was a significant main effect of taste solution evidenced by a higher lick rate to the 0.075 M Sucrose solution relative to the 0.5 M NaCl in base, 0.158 M NaCl and 0.281 M NaCl solutions, $F(6, 84)=18.209$, $P<.001$.

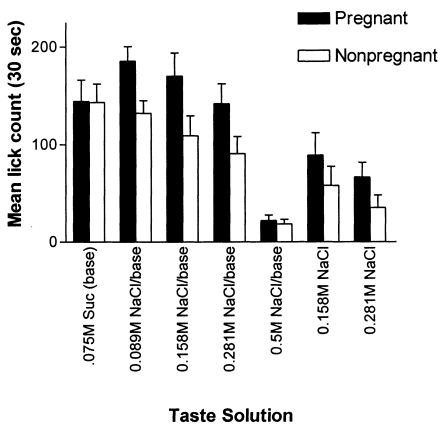

Fig. 1. The mean prepregnancy (baseline) number of licks displayed following 30-s exposure to each of the seven taste solutions. Error bars represent S.E.

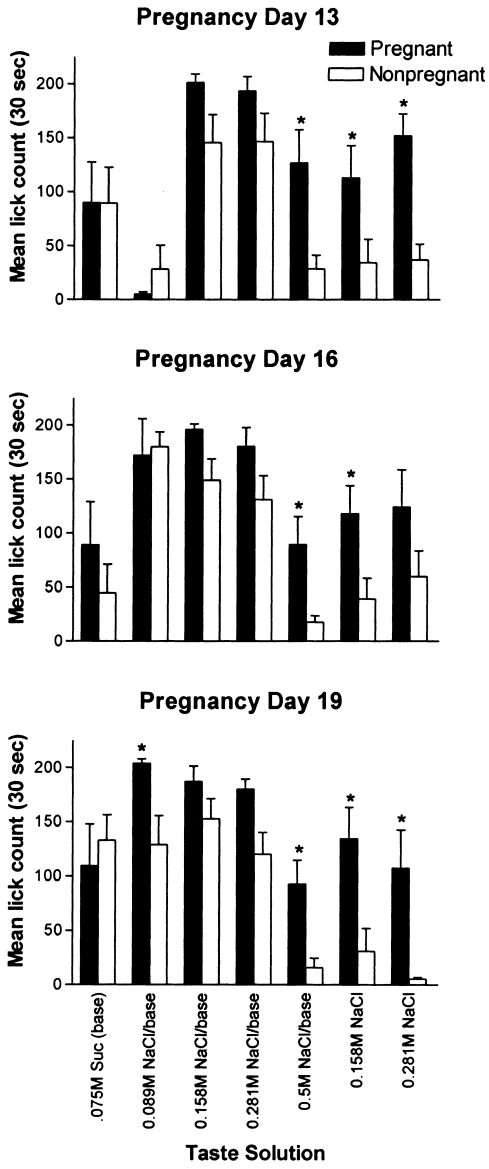

Fig. 2. The mean number of licks displayed to each taste solution by pregnant and nonpregnant rats on Day 13 (A), Day 16 (B) and Day 19 (C) of pregnancy (* $P \leq .05$ relative to nonpregnant animals). Error bars represent S.E.

3.1.2. Pregnancy

There were no significant differences in the lick rate responses displayed by pregnant and nonpregnant rats in the first half of gestation (or the equivalent period for controls) (data not shown). In contrast, during the second half of gestation (Days 13–19), pregnant rats displayed higher lick responses overall than did nonpregnant rats [Day 13: $F(1, 84) = 17.15$, $P < .001$; Day 16: $F(1, 84) = 15.5$, $P < .001$; Day 19: $F(1, 84)$ 27.77, $P < .05$]. Furthermore, on Day 13, there was a significant State × Taste Solution interaction characterized by pregnant rats displaying higher lick rates than nonpregnant rats to three of the solutions presented: 0.5 M NaCl in base, 0.158 M NaCl and 0.281 M NaCl [$F(6, 84) = 2.197$, $P < .05$]. A similar pattern of results was observed on Day 16 and Day 19 as supported by the Fisher post hoc analysis (Fig. 2).

3.1.3. Lactation

Since some of the dams seemed initially reluctant to take care of their pups, we considered it prudent not to disturb the nests. Thus, our assessment of lick rate responses during lactation did not commence until Day 13 of lactation. Generally, results indicated that the effect of reproductive status on NaCl preference observed during pregnancy continued into lactation (Fig. 3). Overall, lactating rats had higher lick rates than did nonlactating rats for each of the days assessed [Day 13: $F(1, 70) = 36.36$, $P < .001$; Day 16: $F(1, 70) = 97.05$, $P < .001$; Day 19: $F(1, 70) = 77.95$, $P < .001$]. Further analyses revealed that

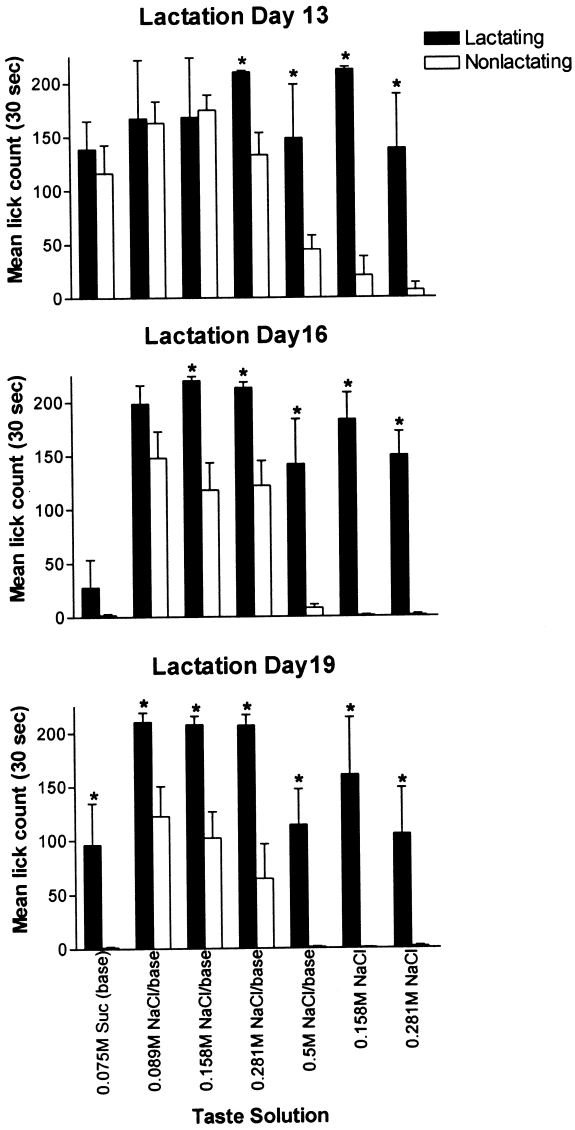

Fig. 3. The mean number of licks displayed to each taste solution by lactating and nonlactating rats on Day 13 (A), Day 16 (B) and Day 19 (C) (* $P \leq .05$ relative to nonpregnant animals). Error bars represent S.E.

on each of these days, lactating rats exhibited significantly higher lick rate responses to specific solutions in comparison to nonlactating rats (P's $<.05$) (Fig. 3). On Day 13, lactating rats exhibited higher lick rates to four of the solutions presented: 0.281 M NaCl in base, 0.5 M NaCl in base, 0.158 M NaCl and 0.281 M NaCl. By Day 16, lactating rats showed elevated responses to these tastants, as well as the 0.158 M NaCl in base solution. On Day 19, lactating rats had significantly higher lick rates to all of the solutions presented in comparison to the lick rates of the nonlactating controls.

Collectively, these results indicate that rats maintained on a Na^+-adequate diet exhibit increased NaCl preference during pregnancy and lactation, detectable using a brief access paradigm such as the lickometer.

3.1.4. Reproductive capability

To verify that the nonpregnant control rats of Experiment 1 were indeed able to reproduce these females were placed overnight with males until there was evidence of mating. Subsequently, this experiment was conducted a second time with the previous control animals as the pregnant rats. The results obtained in this miniexperiment (data not presented) confirmed the observations made in Experiment 1.

3.2. Experiment 2

Experiment 1 revealed an increase in preference for NaCl solutions both in late pregnancy and late lactation. These results were observed in animals maintained on a diet with adequate, but not excessive, amounts of sodium. To determine whether these effects were modulated by dietary Na^+ level, the present study compared the lick rate responses of animals tested on a Na^+-adequate diet (0.03% Na^+) to those on a Na^+-regular diet (0.3% Na^+).

3.2.1. Prepregnancy

The baseline lick rate response to the presentation of the seven test solutions in this experiment, paralleled observations made in the first experiment (Fig. 4). First, there was no reliable difference in the baseline lick rates of the rats that subsequently did or did not become pregnant maintained on the Na^+-adequate diet or the Na^+-regular diet, $P>.05$ (Fig. 4A,B). In contrast, there was a reliable effect of taste solution with the 0.5M NaCl in base, 0.158 M NaCl and 0.281 M NaCl solutions eliciting lower lick rates relative to the 0.089 M NaCl in base, 0.158 M NaCl in base and 0.281 M NaCl in base solutions [$F(6, 133)=4.19$, $P<.001$] (Fig. 4C). Notably, there were no statistically reliable differences between the rates of licking displayed by rats maintained on the two diets despite a 10-fold difference in sodium content.

3.2.2. Pregnancy

As seen in Experiment 1, there were no significant differences in the lick responses displayed in the first half

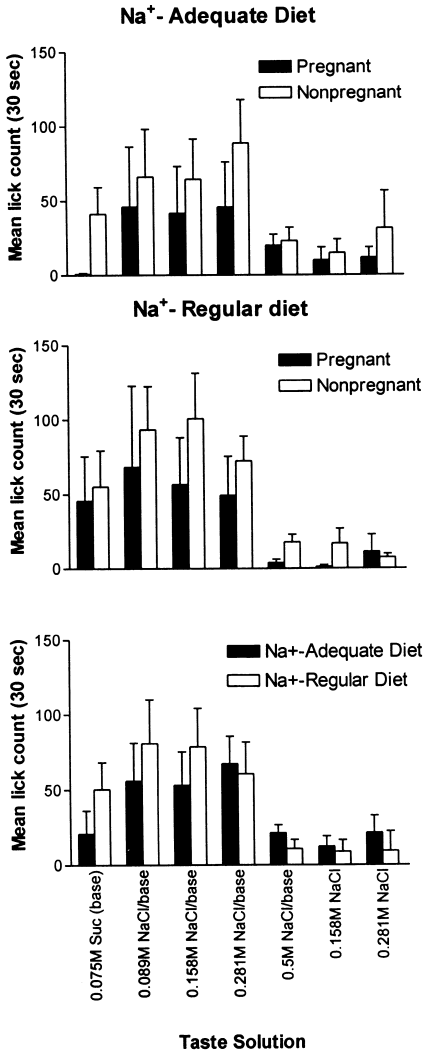

Fig. 4. The mean prepregnancy number of licks displayed by rats maintained on the Na^+-adequate diet and the Na^+-regular diet. Error bars represent S.E.

of gestation (Days 1–10) (P's>.05; data not shown). The second half of gestation, Days 13, 16 and 19, was characterized by differences in the lick responses with pregnant rats displaying significantly higher rates of licking overall, relative to their nonpregnant counterparts [Day 13: $F(1, 133)=19.44$, $P<.001$; Day 16: $F(1, 133)=33.76$, $P<.001$; Day 19: $F(1, 133)=67.17$, $P<.001$] (Fig. 5). In addition, on Day 13, there was a significant State × Diet interaction [$F(1, 133)=6.44$, $P<.05$] evidenced by a greater disparity between the lick counts of pregnant and nonpregnant rats on the Na^+-regular diet vs. rats on the Na^+-adequate diet. As depicted in Fig. 5A,D, pregnant rats on the Na^+-regular diet had higher lick count than did the other groups assessed, to several of the taste solutions: sucrose (base), 0.89 M NaCl in base, 0.158 M NaCl in base, 0.281 M NaCl in base and 0.281 M NaCl. Similarly, on Day 16, there was a significant main effect of diet

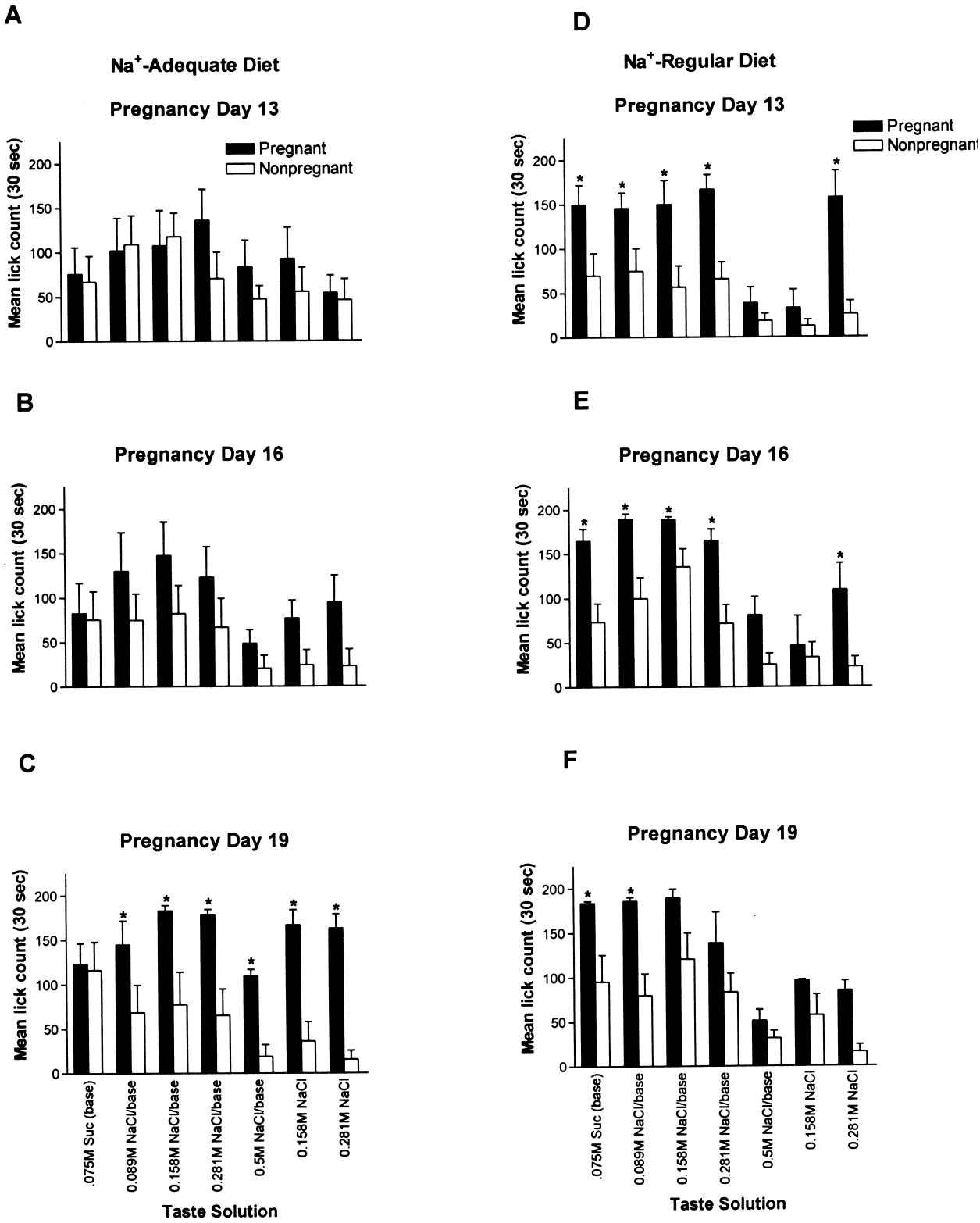

Fig. 5. The mean number of licks displayed to each taste solution by pregnant and nonpregnant rats maintained on the Na^+-adequate diet or on the Na^+-regular diet on Day 13 (A, D), Day 16 (B, E) and Day 19 (C, F) of pregnancy ($*P \leq .05$ relative to nonpregnant animals). Error bars represent S.E.

characterized paradoxically by rats on the Na^+-regular diet (0.3% Na^+) having higher lick counts than rats on the Na^+-adequate diet [$F(1, 133) = 4.27$, $P < .05$]. As on Day 13, pregnant rats on the Na^+-regular diet exhibited higher

lick rates to specific taste solutions relative to their nonpregnant counterparts. Finally, on Day 19, pregnant rats on each diet exhibited higher lick rates than did nonpregnant rats to several different solutions (Fig. 5C,F). Pregnant rats on the Na^+-adequate diet showed higher lick rates than their nonpregnant controls to the 0.89 M NaCl in base, 0.281 M NaCl in base, 0.5 M NaCl in base, 0.158 M NaCl and the 0.281 M NaCl taste solutions. Pregnant rats on the Na^+-regular diet exhibited higher lick rates to the sucrose and 0.89 M NaCl in base solutions than did their nonpregnant controls.

3.2.3. Lactation

Of the eight rats that delivered pups, four had litters that failed to survive postdelivery. Of these litters, three were born to dams maintained on the Na^+-regular diet and one to a dam on the Na^+-adequate diet. Thus, since higher pup mortality was not seen in the group on the Na^+-adequate diet, we do not believe that this mortality was due to maternal dietary NaCl insufficiency. Several other factors may have contributed to this problem including the inexperience of the dams. Nevertheless, the loss of litters eliminates the ability to conduct a systematic analysis of the effect of diet on salt preference during lactation. Statistical analysis of the effect of lactation on salt preference, however, collapsed across diet, did confirm that lactating rats had higher lick rates overall than nonlactating rats for all of the days assessed, P's < 0.05 (data not shown).

4. Discussion

Pregnancy and lactation are characterized by an increase in salt intake, even in animals maintained on diets with sodium contents far in excess of what is required physiologically (Frankmann et al., 1991; McBurnie et al., 1999). This enhanced consumption of salt, in the absence of physiological need, suggests that reproduction precipitates a change in the preference for salt directly. Therefore, the goal of the present studies was to use lick frequency analysis to characterize the changes in salt preference, which occur in the course of pregnancy and lactation. Taste solutions varying in sodium concentration were presented to rats during pregnancy and lactation, and lick rates to these taste solutions were analyzed.

Collectively, our findings indicate significant elevations in salt preference during the second half of pregnancy (Days 13, 16 and 19), as well as in lactation (Days 7, 10, 13, 16 and 19). Both pregnant and lactating animals on either the Na^+-adequate or Na^+-regular diets displayed increased licking to the solutions presented including the NaCl only taste solutions, which stimulated very little licking in nonpregnant rats. Interestingly, the magnitude and timing of these effects did not appear to be influenced entirely by dietary sodium. It is this particular observation that supports the contention that sodium intake associated with reproduction is not a response to any actual sodium deficiency. In this sense, it does not appear to be a regulatory response. Rather, it appears to be a response that is adaptive since it would avert any sodium deficiency during a time when sodium is important to fetal viability. Of course, it is quite possible that the influence of dietary sodium levels on salt preference may be more apparent with the use of diets higher or lower in Na^+ content. However, it should be noted that in this study, dietary sodium differed 10-fold, not a trivial difference.

Overall, the lickometer analysis of NaCl preference used here was a particularly effective method of assessing changes in salt preference during pregnancy. This paradigm allowed us to test preference for NaCl, both in mixtures and alone, in the absence of dehydration since that would be a poor experimental strategy for studying pregnant and lactating animals. In addition, because it assesses NaCl preference without significant consumption of sodium, it can probe preference over an extended time of testing, without the testing itself having an impact on sodium status. This averts the problem inherent in experimental approaches that vary dietary sodium, but then allow continuous access to NaCl solutions. The response to the solutions alters sodium status, and for longitudinal testing, confuses any assessment of the influence of physiological sodium status on subsequent preference (e.g., Frankmann et al., 1991; McBurnie et al., 1999).

It has been hypothesized that the increase in salt intake during the reproductive cycle may be mediated by changes in the regulatory mechanisms underlying salt and water balance and/or reproduction. Aldosterone and angiotensin II, the principal hormones involved in the regulation of salt intake, both increase during pregnancy (Churchill et al., 1981; McBurnie et al., 1988; Taylor and Martin, 1997). Thus far, however, there is no convincing evidence that the increase in salt intake during pregnancy and lactation can be accounted for by changes in the levels of aldosterone and angiotensin II. For example, Churchill et al. (1981) report that, while aldosterone levels peaked at midpregnancy and then declined, elevated sodium intake and retention continued until parturition. More recently, Rowland et al. (1999) reported that varying the levels of dietary NaCl during pregnancy lead to striking differences in plasma aldosterone levels. Their basal NaCl diet, which was similar in sodium content to our Na^+-adequate diet, was associated with aldosterone levels that were significantly higher than those of rats maintained on a mid-NaCl diet, which was similar to our Na^+-regular diet. Therefore, if we assume similar effects on plasma aldosterone in our study, the lack of a difference between diet groups would not appear to support a key role for aldosterone in the elevated NaCl preference of pregnancy. The role of angiotensin II in the elevated NaCl preference of pregnancy remains to be examined.

Several of the reproductive hormones are able to precipitate significant increases in the intake of salt,

similar to levels of sodium intake displayed during pregnancy and lactation. The repeated administration of progesterone and estradiol to nonpregnant and pseudopregnant female rabbits produced reliable increases in the intake of NaCl solution (Denton, 1982). Similarly, the administration of prolactin, oxytocin and adrenocorticotrophic hormone (ACTH) caused the salt intake of nonpregnant female and male rabbits to increase to about 9 mmol/day, approximately 50% of the levels typically seen during lactation. Increases in salt intake were also observed in rats administered an oral contraceptive containing estrogen and progesterone (Denton and Nelson, 1971, 1978; Fregly and Newsome, 1980). Normal variations in levels of these reproductive hormones have also been reported to affect NaCl preference. For example, studies of humans indicate that salt preference increases around the time of ovulation, and decreases during menstruation when estrogen and progesterone levels are basal (Frye and Demolar, 1993). In animal studies, female rats tend to exhibit greater preference for certain salty solutions than do male rats (Krecek, 1973). Our observations of increases in salt preference both during late pregnancy, when estrogen and progesterone are elevated, as well as in lactation when oxytocin and prolactin are elevated, suggests an endocrine basis for changes in salt preference during reproduction.

Using a brief access lick rate analysis, we were able to identify changes in salt preference during pregnancy and lactation. In so doing, we were able to circumvent some of the problems associated with long-term access tests, not the least of which is the tendency to vary dietary NaCl but still allow continuous access to NaCl solutions. Under these circumstances, the response to the solutions alters sodium status, and for longitudinal testing, confuses any assessment of the influence of physiological sodium status on subsequent preference. In light of our findings using short-access, lick rate analysis, the potential is there not only to examine changes in salt preference during pregnancy and lactation, but also to evaluate the role of potential hormonal mediators of these changes.

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