Failure of Loop Diuretics to Induce Nursing Sickness in Mink at Weaning

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ABSTRACT

Nursing sickness in mink is thought to be precipitated by inadequate salt intake, whether this is due to inadequate salt levels in the diet or inadequate total dietary intake. To test this hypothesis, lactating females raising large litters were given 2 daily intramuscular injections of the loop diuretic furosemide (Lasix, 4 + 4 mg/kg/day) for 2.5 d during the normal weaning period 6 wk after parturition or served as untreated controls. Following the same protocol, barren mink (i.e. unsuccessfully mated females) were treated similarly. Dams were carefully inspected for clinical signs of nursing sickness during and after the treatment. Urinary osmolality and concentrations of sodium, potassium, chloride, creatinine and carbamide (urea) were measured prior to treatment (day 1) and on day 3, immediately before and 4 h after the final diuretic treatment. Plasma concentrations of aldosterone and cortisol were determined by radioimmunassay 4 h after the last injection with furosemide on day 3. Biochemical changes in urine (a low osmolality, low concentrations of carbamide and creatinine, and extremely low sodium concentrations) and in plasma (aldosteronism) similar to those found in nursing sickness were elicited in the nursing dams. Nevertheless, none of the dams developed overt clinical signs of nursing sickness. It is concluded that the biochemical signs of volume and salt depletion associated with nursing sickness are sequelae rather than etiological factors of this disorder.

RÉSUMÉ

Chez le vison, la maladie de l'allaitement est considérée comme étant déclenchée par une consommation inadéquate de sel, due à des concentrations insuffisantes de sel dans la ration ou à une consommation alimentaire déficiente. Afin de vérifier cette hypothèse, des femelles en lactation avec des portées nombreuses ont reçu, lors de la période du sevrage environ six semaines après la parturition, pendant 2.5 j deux injections IM quotidiennes de furosémide (Lasix, 4 + 4 mg/kg/j) ou ont servi de témoins non-traités. Un troisième groupe constitué de femelles accouplées mais non-gestantes a reçu le même traitement. Les femelles étaient examinées attentivement pour détecter tous signes cliniques de maladie de l'allaitement durant ou après le traitement. L'osmolarité urinaire ainsi que les concentrations urinaires de sodium, potassium, chlorure, créatinine et carbamide (urée) ont été mesurées au jour 1 et au jour 3, immédiatement avant et 4 h après le dernier traitement. Les concentrations plasmatiques d'aldostérone et de cortisol ont été déterminées par radio-immuno-essai au jour 3, 4 h après la dernière injection de furosémide. Des changements biochimiques urinaires (osmolarité faible, faible concentration de carbamide et de créatinine, et concentration très faible de sodium) et plasmatiques (aldostéronisme) similaires à ceux observés lors de maladie de l'allaitement ont pu être reproduits chez les femelles en lactation. Toutefois, aucune femelle n'a présenté de signes cliniques évidents de maladie de l'allaitement. Il semble donc que les changements biochimiques de réduction de volume et de sel associés à la maladie de l'allaitement soient des séquelles plutôt que les facteurs étiologiques de cette condition.

(Traduit par docteur Serge Messier)

INTRODUCTION

Nursing sickness is an important disease in domestic mink dams which occurs during the weaning period, approximately 6 wk after parturition. The disease is characterized by inanition, emaciation, severe extracellular volume and electrolyte depletion, and a high mortality rate. The biochemical abnormalities include hyponatremia, hyperkalemia, aldosteronism, and in the terminal stage of the disease uremia, azotemic acidosis, and hyperglycemia (1–4). Biochemical changes compatible with latent nursing sickness, e.g. low urinary osmolality and a low urinary concentration of sodium, are regularly found in apparently healthy dams who are weaning large litters (5,6). The etiology of nursing sickness remains unclear. The syndrome is, however, often thought to be precipitated by inadequate salt intake, whether this is due to inadequate salt levels in the diet, or inadequate total dietary intake. Inanition of the dam is easily overlooked at the stage when the kits and dam compete for the food. In animals with latent salt deficiency, overt clinical signs might be precipitated by increasing urinary sodium excretion. In the present study this hypothesis was examined by giving mink dams a loop diuretic during the weaning period succeeded by inspection for development

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This study was supported by Aarhus Universitets Forskningsfond (Grant no. F-1993-SUN-144) and the Novo Nordisk Foundation.

Submitted April 5, 1995.

Table I. Design of experiment with and without furosemide treatment

Treatment	Group	Number of mated female mink
Controls	1	5 dams raising 7.6 ± 1.1* kits
	3	5 barren
Furosemide**	2	10 dams raising 7.0 ± 1.2* kits
treated	4	4 barren

* SD

** Lactating dams and barren females received intramuscular injections of furosemide (Lasix, 2 × 4 mg/kg) for 2.5 d

of nursing sickness and measurement of pertinent parameters in blood and urine.

MATERIALS AND METHODS

In the 1993 breeding season at the Danish Fur Research Farm "West" 24 female mink of either wild or pastel colour type were used for the experiment: 15 dams raising litters of ≥ 5 kits and 9 barren (i.e. unsuccessfully mated) females. Lactating dams and barren females were randomly distributed into groups as outlined in Table 1. Five dams (group 1) with an average litter size of 7.6 ± 1.1 (*s*) at day 38–40 after parturition and 5 barren females (group 3) served as untreated controls. The experimental animals, 10 lactating dams (group 2) with an average litter size of 7.0 ± 1.2 (*s*) at day 38–40 after parturition and 4 barren females (group 4) received intramuscular injections of furosemide (Lasix, Hoechst A/G, FRG), 4 mg per kg body weight twice daily in the morning and the afternoon for 2½ d. In the morning on day 3 the final injection was given 4 h before bleeding by venipuncture for hormone analysis. A larger group of nursing dams receiving furosemide was included in order to increase the probability for experimentally induced nursing sickness.

Housing and management of the animals were as previously described (1). They were inspected at least twice a day and clinical observations were registered individually in separate files of the data recording system (SAS Institute Incorporated, Cary, North Carolina) used at the research farm. They had free access to food and once daily throughout the gestation and lactation period the mink were fed a fresh, commercial mixture based upon industrial fish (23%), fish offal (49%) and chicken offal (12%). The content of metabolizable energy was 5.15 MJ/kg (= 1230 kcal/kg) and the protein:fat:carbohydrate energy distribution ratio was 57:32:11. The feed mixture was supplemented with NaCl to a final content of 1.00 g NaCl/MJ (0.42 g NaCl/100 kcal). The animals were given free access to tap water by means of an automatic system.

During handling mink often spontaneously void urine; urine was collected in this way on day 1 before first injection and on day 3 immediately before and 4 h after the last injection of furosemide. Samples were analyzed for Na^+, K^+, osmolality, urea (carbamide) and creatinine as previously described (2). Animals did not always void urine during handling and thus the number of analyses on urine (Figs. 1 & 2) happened to be fewer than those on blood (Fig. 3).

Blood samples were obtained from v. cephalica antebrachii 4 h after last injection of furosemide, stabilized with sodium heparin and the plasma separated by centrifugation for hormone analysis. Plasma concentrations of aldosterone and cortisol were determined using Coat-A-Count 125-I-labelled RIA kits (Diagnostic Products Corporation, USA). Pooled plasma obtained from healthy barren mink in 1992 was used as an internal standard during the 2 seasons. The accuracy using different batches of the RIA kit and the precision of the method in our hands is illustrated by the following values (mean ± SEM) obtained in 1992 and 1993, respectively: aldosterone 90.5 ± 8.5 (n = 6) and 90.4 ± 5.3 (n = 8) pg/mL; cortisol 104.9 ± 8.9 (n = 3) and 94.5 ± 1.4 (n = 4) nmol/L.

All management procedures including confinement, feeding and handling were carried out according to the guidelines approved by the member States of the Council of Europe for the protection of vertebrate animals used for experimental and other scientific purposes.

STATISTICAL ANALYSIS

Prior to analysis of any difference between the mean values obtained in the furosemide treated and the control group of nursing dams or barren females a comparison of the calculated variances was carried out. If the test quotient exceeded the significance level for F-distribution (P = 0.05) values for combined SEM and for the degree of freedom were calculated. The significance level was finally determined by comparison of the calculated t-mean value with Student's two-tailed distribution.

RESULTS

Urinary Na^+, K^+, osmolality, carbamide and creatinine are illustrated in Figs. 1 & 2. On day 1 before treatment with the loop diuretic furosemide no significant differences were found between the 2 groups of nursing dams or between the 2 groups of barren females. On day 3, before the last injection of furosemide, urinary osmolality and concentration of carbamide were significantly lower in furosemide treated dams than in control dams. Four hours after the last injection urinary K^+ and creatinine were also significantly different between the 2 nursing groups.

In the control groups of nursing dams and barren females mean plasma concentrations (± SEM) of aldosterone were 190 ± 58 (n = 5) and 108 ± 33 (n = 5) pg/mL, respectively. When compared to the untreated control groups a nearly 10-fold and highly significant increase in plasma aldosterone was seen after furosemide treatment both in nursing mink and in barren females (Fig. 3). Moreover, plasma aldosterone was also significantly higher in nursing dams than in barren females during diuretic treatment as seen from the right hand part of Figure 3. No significant changes or differences in plasma cortisol were found (Fig. 3).

None of the dams in the experimental group or in the control group in this study developed clinical signs of nursing sickness.

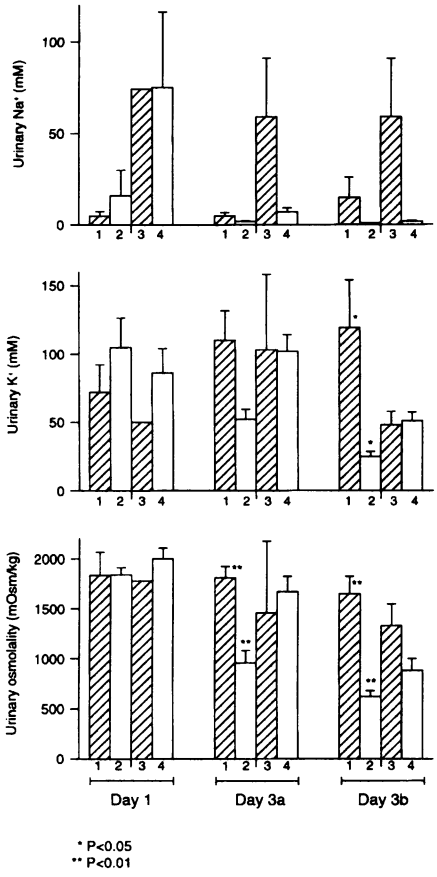

Figure 1. Urinary Na^+ and K^+ concentrations and urinary osmolality before (day 1) and after 2½ days of furosemide treatment (day 3). Samples were obtained before (day 3a) and 4 h after (day 3b) last injection of furosemide. Group 1 (n = 3–4) and 2 (n = 5–8) are nursing dams and group 3 (n = 1–4) and 4 (n = 3–5) are barren females. Hatched columns (groups 1 and 3) are the untreated controls and blank columns (groups 2 and 4) are the furosemide-treated individuals. Bars indicate SEM. Asterisk indicates values in furosemide treated females significantly different from untreated controls.

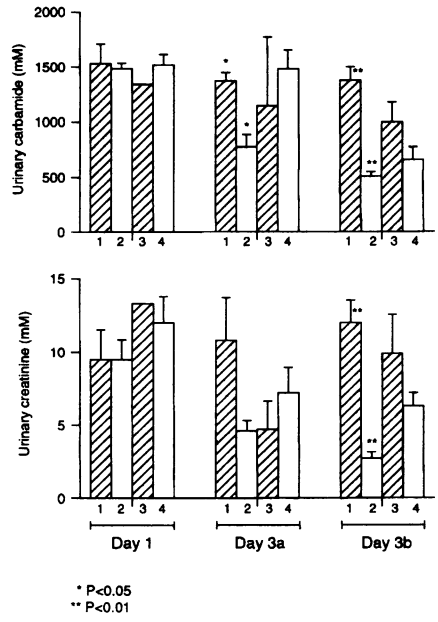

Figure 2. Urinary carbamide and creatinine concentrations before (day 1) and after 2½ days of furosemide treatment (day 3). Samples were obtained before (day 3a) and 4 h after (day 3b) last injection of furosemide. Group 1 (n = 3–4) and 2 (n = 5–8) are nursing dams and group 3 (n = 1–4) and 4 (n = 3–5) are barren females. Hatched columns (groups 1 and 3) are the untreated controls and blank columns (groups 2 and 4) are the furosemide-treated individuals. Bars indicate SEM. Asterisk indicates values in furosemide treated females significantly different from untreated controls.

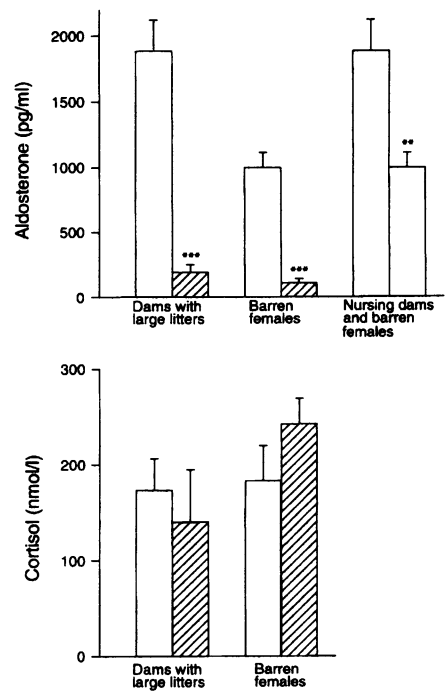

Figure 3. Plasma aldosterone and plasma cortisol in nursing mink (n = 9) and in barren females (n = 4) 4 h after last injection of furosemide compared with nursing (n = 5) and barren (n = 5) controls. Hatched columns (groups 1 and 3) are the untreated controls and blank columns (groups 2 and 4) are the furosemide-treated individuals. Bars indicate SEM. Asterisk indicates values in furosemide treated females significantly different from untreated controls or furosemide treated dams significantly different from furosemide treated barren females.

DISCUSSION

Among the prominent biochemical changes of nursing sickness are reduced urinary osmolality, aldosteronism, and extremely low concentrations of urinary Na^+. Thus, the concentrating ability of the kidney is on one hand reduced, whereas the ability of avid Na^+ reabsorption on the other hand is preserved. An increase in Na,K-ATPase activity of the kidney, i.e. an adaptation of the capacity for Na^+ reabsorption, has been observed in dams with nursing sickness (7). Trends towards the urinary deviations characteristic of this ailment, especially a low urinary Na^+ concentration, are, however, often noticed in apparently healthy dams during the last weeks of the lactation period. If the latent symptoms of the dams during the weaning period arise from a borderline salt deficiency and if the clinical syndrome represents a breakdown of the homeostatic mechanisms for salt preservation, one would expect a sudden collapse under conditions of additional salt and water losses.

Before furosemide treatment, urinary data were as previously recorded in healthy nursing dams during the weaning period as well as in barren females (2,3,6). The osmolality, preferentially due to carbamide (urea), was high indicating a rather low water intake and for that reason antidiuresis and a normal concentrating ability of the kidneys. No significant deviations point to latent nursing sickness, probably due to the supplementation of the feed mixture with NaCl. In spite of a reasonably high Na^+ content of the diet, urinary Na^+ seems low in the nursing groups. The difference between them and barren females, however, is not significant in the present study.

Though solely based upon analysis of spot urine, an induced polyuria seems clearly documented from urinary data obtained on day 3. While being offered the very same feed mixture, urinary osmolality and the main determinant of this, carbamide, were significantly reduced during furosemide treatment compared to controls. Moreover, the urinary concentrations of potassium and creatinine were significantly lowered after the last injection with furosemide. The urinary Na^+ concentration approached zero in nursing dams as well as barren

females after furosemide treatment. Significant differences between them and untreated controls, however, were not attained for this parameter.

Mink dams raising large litters, and thus disposed for development of nursing sickness at weaning, were successfully inflicted with symptoms identical to those seen in this disorder: extracellular volume depletion and dehydration as indicated by aldosteronism and extremely low urinary Na^+ concentrations and in spite of this, urinary hypo-osmolality. Nevertheless, no clinical signs of nursing sickness were elicited in any of the dams.

The experiment thus supports the hypothesis that the biochemical signs of volume depletion and salt deficiency associated with nursing sickness are sequelae rather than etiological factors of this disorder. The real problem in the weaning period that has to be identified seems to be a loss of appetite and the onset of inanition which easily leads to salt deficiency. If feed consumption is continued any borderline deficiency of Na^+ is apparently not exceeded. In case of partial inanition, however, supplementation of the feed ration with NaCl may prevent outbreak of clinical nursing sickness by preventing the start of a vicious circle. In the present study diuretic treatment apparently did not elicit loss of appetite.

ACKNOWLEDGMENTS

The authors are grateful to the staff of Fur Research Farm "West" for excellent cooperation throughout this study. The skilled technical assistance by Lis Horne, Lis Hygom, Annette Linde and Toke Nørby is gratefully acknowledged. Thanks are due to Dr. Adrian Harrison, PhD, Cambridge, United Kingdom, for linguistic guidance.

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