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Hypertonic NaCl infusions affect milk composition in goats

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The capability of goats to maintain milk production during water deprivation is remarkable and not yet fully understood. The aim of the present study was to investigate whether intravenous infusions of hypertonic NaCl cause release of both vasopressin and oxytocin and whether the peptides, in combination with the hyperosmolality, affect milk flow and milk composition. Six Swedish domestic landrace goats in their first to third lactation were milked every 30 min during experiments. Hypertonic NaCl (HNaCl) or isotonic NaCl (IsoNaCl) were infused for 90 min. Goats were not allowed to drink during infusions. Plasma vasopressin concentration increased during HNaCl infusions, and did not change in response to IsoNaCl infusions. Plasma oxytocin concentration did not change during either infusion. Milk flow was maintained during the infusions. Milk fat concentration decreased in the three samples taken before onset of the infusions, but then increased gradually during HNaCl infusions, while it continued to fall during the IsoNaCl infusions. Milk osmolality followed the rise in plasma osmolality during the HNaCl infusions and did not change in IsoNaCl experiments. Milk lactose concentration increased throughout both series of experiments, the concentration being higher during HNaCl infusions. Milk protein concentration did not change during HNaCl infusions, but fell in the IsoNaCl experiments. It is concluded that the hyperosmolality in combination with elevated plasma vasopressin levels did not disturb the secretory activity of the mammary cells, but rather facilitated emptying of the alveolar milk. Such a mechanism may help to explain the sustained milk production in water deprived goats.

Keywords: Goat, lactation, milk, hypertonic NaCl, oxytocin, vasopressin.

Whether vasopressin has a role in milk secretion is not clear. For a long time it has been accepted that vasopressin at physiological plasma concentrations does not affect milk production (Peaker & Linzell, 1973). At high doses, vasopressin injections decreased milk flow in goats (Konar & Thomas, 1970), but increased it in sheep (Bruckmaier et al. 1997). Recently, it was shown that intravenous infusions of vasopressin at physiological plasma concentrations increased milk flow in goats. The effect was short-lasting, accompanied by increased fat concentration, and was similar to that seen with corresponding infusions of oxytocin (Olsson et al. 2003). Vasopressin may act on the oxytocin receptors, although with less efficiency (Zingg, 1996; Akerlund et al. 1999) and it was suggested that vasopressin stimulated the oxytocin receptors and emptied the mammary gland of alveolar milk during the infusions.

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During water deprivation, plasma vasopressin levels increase in response to elevated plasma osmolality. Milk osmolality follows that of plasma, but it is well known that vasopressin cannot concentrate milk. Instead, milk production continues at almost normal levels as long as the goats continue to eat (Maltz & Shkolnik, 1980; Dahlborn, 1987).

In dogs and rats, water deprivation or a load of hypertonic NaCl cause release of both vasopressin and oxytocin (Weitzman et al. 1978; Negoro et al. 1988; Mezey & Kiss, 1991; Windle et al. 1993). In rats, infusions of hypertonic NaCl have been shown to increase the intramammary pressure during milk ejection (Negoro et al. 1983). In goats, Andersson (1951) demonstrated that intracarotid infusions of hypertonic NaCl caused milk ejection without suckling. Plasma oxytocin and vasopressin concentrations were not measured, but in both reports it was implied that the effect was due to elevated oxytocin levels. However, in goats, Mengistu et al. (2004) showed that the vasopressin, but not

the oxytocin, concentration increased during water deprivation.

To investigate further the effects of vasopressin and oxytocin on milk secretion, we have increased plasma osmolality by infusions of hypertonic NaCl intravenously in goats. The animals were in normal water balance before the experiments started and were not allowed to drink during the infusions. We hypothesized that the infusions would increase plasma vasopressin but not oxytocin levels and that vasopressin would facilitate emptying of the alveolar milk.

Material and Methods

Animals

The studies were done 4–12 weeks after parturition in six goats belonging to the Swedish domestic landrace (*Capra hircus*). The goats weighed 43·5 ± 2·5 kg and were in their first to third lactation. Between experiments, all goats were kept together with their kids in a large pen. Room temperature was kept at 17 ± 1 °C. The animals were fed hay and oats/concentrates at 07·00 and 15·00. Hay was distributed in several feeding buckets in the pens in amounts corresponding to 0·8 kg per goat and feeding occasion. Goats were fed about 0·3 kg of oats/concentrates twice daily. The oats/concentrates were a mixture of 60% oats (10·7 MJ/kg DM, CP 80 g/kg DM) and 40% concentrates (11·8 MJ/kg DM; CP 240 g/kg DM, Komet 130) (AB Johan Hansson, Uppsala, Sweden). Goats had free access to water and mineral licks in the pen. The Local Ethical Committee in Uppsala, Sweden approved the care of the animals and the experimental design.

Experimental procedure

At 8·00 on an experimental day, two of the goats were taken from the pen and walked to metabolism cages in an adjacent room. Hay was available, but no water until 30 min after cessation of the infusions, when the goats were given water in a bucket placed in front of them. The goats were hand milked immediately upon arrival in the metabolism cage and the milk was discarded. Thereafter the goats were hand milked every 30 min by the same well-trained animal technician, who was not familiar with the hypothesis of the experiment. The milk was weighed and a sample taken for analysis. After shaving and cleaning the neck, a local anaesthetic was applied to the skin covering the jugular veins (lidocain ointment, Emla®, AstraZeneca, Sweden). About 45 min later, a catheter (Secalon T®, Ohmeda, Swindon, UK) was inserted into each jugular vein. The first blood sample was taken about 30 min after the catheters had been inserted. The catheters were removed immediately after cessation of an experiment. Fifteen ml of blood was collected at each sampling time and the catheter filled with 0·2 ml sodium citrate to prevent coagulation. About 0·5 ml of blood was first taken and discarded to avoid dilution with sodium citrate before a sample was collected. The blood was distributed into one tube containing Li-heparin and one ice-chilled tube containing K_3-ethylenediaminetetra-acetic acid (EDTA). The tubes were centrifuged at 4 °C for 20 min at 1500 ***g***, the plasma removed and stored at −70 °C until assayed.

After control samples had been taken, an intravenous infusion was given of either HNaCl or isotonic NaCl (IsoNaCl) for 90 min. The infusion rate was 1·0 ml/min. The concentration of the hypertonic solution was adjusted according to body weight (0·18 g NaCl per kg) in order to give the goats the same load of NaCl. The experiments were randomized with at least a 1-week interval.

Analyses

Milk fat, protein, and lactose concentrations were determined by an infrared technique (Milkoscan 104, Shields Ltd, York, UK). Sodium and potassium concentrations were analysed by flame photometry (IL 943, Instrumentation Laboratory, Milan, Italy). Osmolality was measured on a LABEX (Helsingborg, Sweden) freezing-point osmometer.

Hormone analysis

Blood samples were analysed for oxytocin and vasopressin by radioimmunoassay after extraction as previously described for oxytocin (Schams, 1983) and vasopressin (Olsson et al. 2003).

Statistical analysis

Values are presented as means ± SEM. Data were analysed using the SAS software (SAS Institute, 2005). The repeated measurement analysis of variance (procedure MIXED) was applied to the respective parameters. The statistical model included the fixed effect of blood plasma or milk sample, respectively, and the random effect of goat. Pairwise comparisons against the sample at time zero and of values between treatments were tested for significance. The significance level was set at $P < 0{\cdot}05$.

Results

Milk flow and milk fat concentration

Milk flow tended to increase during the experiments, but the HNaCl infusions did not cause a significant change (Fig. 1). Milk flow at the start of the HNaCl infusions (time 0) was 1·1 ± 0·2 g/min. It was 1·4 ± 0·2 g/min at 90 min and 1·5 ± 0·3 g/min at 120 min (Fig. 1). Milk flow at the start of the IsoNaCl infusions was 0·8 ± 0·1 g/min, 1·1 ± 0·1 g/min at 90 min (NS), and 1·6 g/min at 120 min ($P < 0{\cdot}05$) (Fig. 1). Comparisons between treatments revealed no differences

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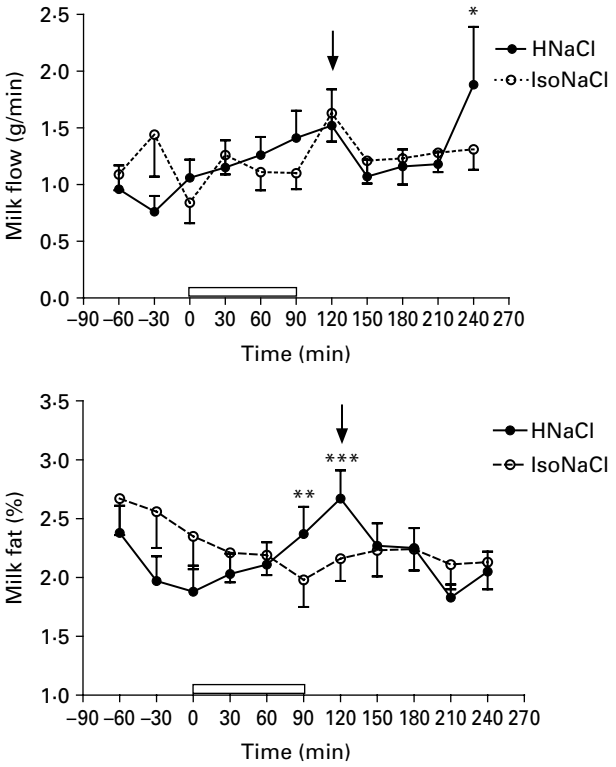

Fig. 1. Milk flow and milk fat concentration during infusions of hypertonic NaCl (HNaCl) and isotonic NaCl (IsoNaCl) in 6 goats. The bar shows the time of infusion. The goats were given access to water at 120 min (arrow). Values are means ± SEM. *$P<0·05$, **$P<0·01$ and ***$P<0·001$ *v.* the value at time 0 during HNaCl infusions (time 0).

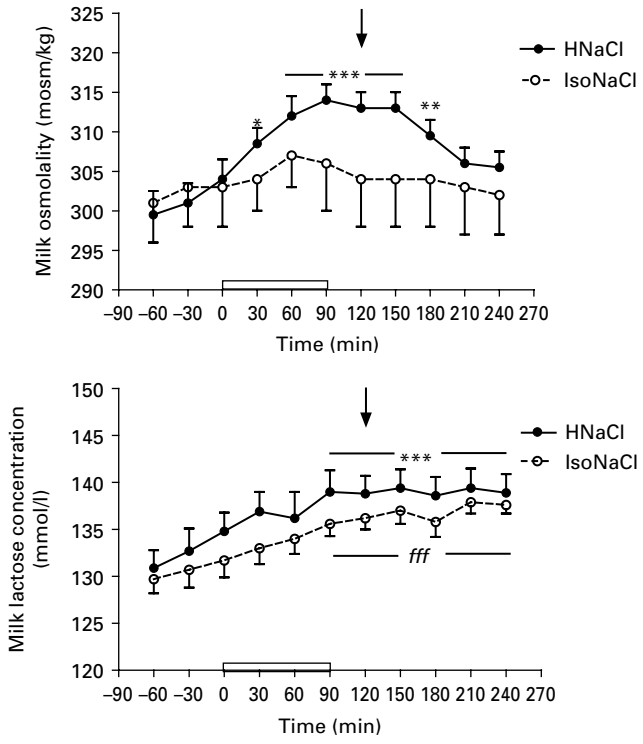

Fig. 2. Milk osmolality and milk lactose concentration during infusions of hypertonic NaCl (HNaCl) and isotonic NaCl (IsoNaCl) in 6 goats. *fff* $P<0·001$ *v.* the value at time 0 during the IsoNaCl infusions. For explanations of abbreviations and other symbols, see Fig. 1.

in response to the infusions. In total the goats milked 402 ± 37 g (HNaCl) and 402 ± 53 g (IsoNaCl) during the 5·5 h the experiments lasted.

Milk fat concentration initially decreased during the control period, but started to increase during HNaCl infusions (Fig. 1). In HNaCl experiments, it was 1·88 ± 0·22% at time 0 and increased to 2·37 ± 0·23% at 90 min ($P<0·05$) and at 120 min to 2·67 ± 0·24% ($P<0·01$). It then declined to pre-infusion values. In the IsoNaCl experiments the fat concentration was 2·35 ± 0·28% at the onset of the infusions (Fig. 1) and 1·98 ± 0·23% (NS) at 90 min. At 90 min it was lower than the first and second control samples ($P<0·01$). At 120 min it was 2·16 ± 0·19% (NS) at which level it remained throughout the observation time.

Milk osmolality and concentrations of lactose, sodium, potassium and protein

During HNaCl infusions, milk osmolality increased from 304 ± 3 mosm/kg at time 0 to 314 ± 2 mosm/kg at 90 min ($P<0·001$) and it remained at this level until 180 min, when it was 310 ± 2 mosm/kg ($P<0·01$). Thereafter it declined (Fig. 2). Milk osmolality was 303 ± 5 mosm/kg at the onset of IsoNaCl infusions and 306 ± 7 mosm/kg at 90 min (NS) and then remained at this level throughout the observation time (Fig. 2).

Lactose concentration increased continuously both in HNaCl and IsoNaCl experiments (Fig. 2). At the onset of HNaCl infusions milk lactose concentration was 135 ± 2 mmol/l and at time 90 min it was 139 ± 2 mmol/l ($P<0·001$), at which level it remained throughout the observation period. At the onset of IsoNaCl infusions, lactose concentration was 132 ± 2 mmol/l and at 90 min it was 136 ± 1 mmol/l ($P<0·001$). Lactose concentration was higher during HNaCl compared with IsoNaCl experiments at time 0 ($P<0·05$), at 30 min ($P<0·01$) and at 90 min ($P<0·05$).

Milk sodium concentration was 12·5 ± 0·8 mmol/l and 11·7 ± 0·8 mmol/l at time 0 during HNaCl and IsoNaCl experiments, respectively. Corresponding milk potassium concentrations were 56·7 ± 1·7 mmol/l and 54·3 ± 1·9 mmol/l, respectively. These variables did not change during the experiments.

Milk protein concentration at time 0 was 2·37 ± 0·08% (HNaCl) and 2·43 ± 0·10% at 90 min (NS). At the onset of IsoNaCl infusions, protein concentration was 2·61 ± 0·07% and at 90 min it was 2·53 ± 0·07% ($P<0·05$). It steadily

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declined after completing the IsoNaCl infusion and was 2·41±0·09% ($P<0·001$ *v.* time 0) in the last sample at 240 min. There was no significant difference between infusions.

Plasma osmolality and concentrations of sodium, potassium and protein

Plasma osmolality was 297±2 mosm/kg and 296±4 mosm/kg at the onset of HNaCl and IsoNaCl infusions, respectively. It was 309±2 mosm/kg at time 90 min in the HNaCl experiment ($P<0·001$ *v.* time 0) and 297±5 mosm/kg in the IsoNaCl experiments (NS). The difference in osmolality between infusions was significant from time 45 min until the last sample during the observation time.

Plasma sodium concentration at time 0 was 142±1 mmol/l (HNaCl) and increased to 151±1 mmol/l ($P<0·001$) during HNaCl infusions at 90 min. During IsoNaCl infusions, plasma sodium concentration was 144±2 mmol/l at time 0 and 146±3 mmol/l at 90 min. Plasma potassium concentration at time 0 was 4·16±0·10 mmol/l and 4·10±0·20 mmol/l (HNaCl) at 90 min (NS). It was 3·83±0·26 mmol/l at time 0 and 4·32±0·13 mmol/l (IsoNaCl) at 90 min ($P<0·05$).

Plasma protein concentration at time 0 was 71±2 g/l before HNaCl infusions and decreased to 68±1 g/l at 90 min ($P<0·001$). During IsoNaCl infusions the protein concentration was 69±1 g/l at time 0 and 69±2 g/l at 90 min. Comparisons between treatments showed no significant difference.

Plasma vasopressin and oxytocin concentrations

Plasma vasopressin concentration at time 0 was 0·58±0·12 pmol/l (HNaCl) and increased to 2·48±0·38 pmol/l at 90 min ($P<0·001$). Corresponding values in response to IsoNaCl were 0·47±0·10 pmol/l at time 0 and 0·90±0·29 pmol/l at 90 min (NS) (Fig. 3). Vasopressin concentration was higher in the HNaCl experiment compared with the IsoNaCl experiments from the samples at time 45 min until 195 min ($P<0·05$–$0·001$).

Plasma oxytocin concentration at time 0 was 4·0±0·4 pmol/l (HNaCl) and 4·8±0·6 pmol/l (IsoNaCl). These values did not change in response to either infusion (Fig. 3).

Water intake

Goats did not have access to water in the metabolism cage until 30 min after cessation of the infusions, when they were given free access to the water. They drank immediately and some of them took one or two more sips during the rest of the observation time. In total they drank 3·0±0·4 l (HNaCl) and 2·5±0·4 l (IsoNaCl) (NS) during the observation time.

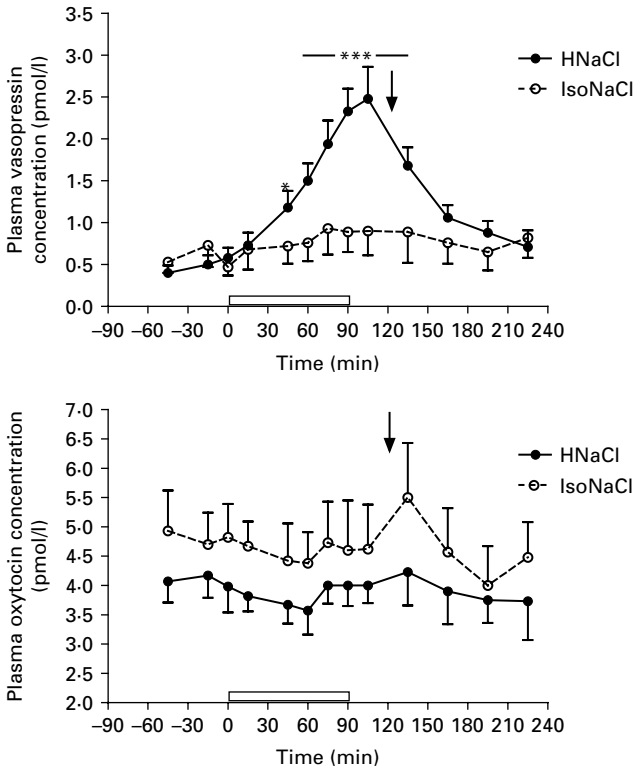

Fig. 3. Plasma vasopressin and plasma oxytocin concentrations during infusions of hypertonic NaCl (HNaCl) and isotonic NaCl (IsoNaCl) in 6 goats. For explanations of abbreviations and symbols, see Fig. 1.

Discussion

A fall in milk fat concentration was expected during the first hours of very frequent milking (Linzell, 1967; Maltz et al. 1984). This was seen in the present IsoNaCl experiments and in the first three control samples in the HNaCl experiments. However, the milk fat concentration started to increase after onset of HNaCl infusions and continued to increase until 30 min after end of the infusions. This indicates that proportionally more alveolar milk was emptied into the cisterns and excreted during the milking (Ontsouka et al. 2003). It supports findings in rats (Negoro et al. 1983) and goats (Andersson, 1951) that an osmotic stimulus affects milk ejection.

Plasma vasopressin concentration increased during HNaCl infusions, but plasma oxytocin concentration did not change. Thus lactating goats did not respond with an increased oxytocin release to osmotic stimuli, which is in agreement with previous findings in water-deprived male goats (Mengistu et al. 2004). Therefore, we suggest that it was vasopressin and not oxytocin that caused contraction of the myoepithelial cells and released more fat into the milk. This is not to say that the plasma oxytocin level did not increase in the usual manner during the milking procedure and took part in the milk ejection. Instead, it appears likely that the milk ejection was a combined action

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of increasing vasopressin levels in response to the hypertonic NaCl infusions together with oxytocin released by neuroedocrine reflex at milking.

Milk flow tended to increase during the whole observation time during both the HNaCl and the IsoNaCl experiments. Frequent milking may cause a temporary increase in milk flow and also in milk lactose and protein concentrations simultaneous with a fall in milk fat concentration (Linzell, 1967; Maltz et al. 1984). In the present experiments, milk protein concentration did not change significantly during HNaCl infusions, but started to fall after IsoNaCl infusions. Lactose concentration increased during both experiments, with a higher lactose concentration during HNaCl infusions, which is consistent with the elevation of the milk osmolality. As expected, milk sodium concentration did not change despite the load of hypertonic NaCl in the plasma and hence did not contribute to the change in milk osmolality. The principal mechanisms behind this phenomenon have been discussed by Linzell & Peaker (1971) and by Peaker (1977). They demonstrated that lactose cannot leave the milk compartment once it has been synthesized and that sodium is actively pumped out of the mammary epithelial cells to the extracellular fluid compartment.

There was no significant increase of milk flow during HNaCl infusions, whereas intravenous infusions of vasopressin to normohydrated goats increased both milk flow and milk fat concentration (Olsson et al. 2003). During water deprivation milk flow does not increase, but is maintained or slightly decreased (Maltz & Shkolnik, 1980; Dahlborn, 1987). In all three situations, vasopressin concentration is elevated, but the milieu of the mammary cells is different. Water leaves the cells during infusions of hypertonic NaCl and during water deprivation, but not when vasopressin is infused in normohydrated animals. The conditions did not appear to diminish the function of the secretory cells since neither lactose nor protein concentrations changed in normohydrated goats (Olsson et al. 2003) or increased as observed for lactose in the present experiments and during water deprivation (Dahlborn, 1987). In a recent experiment, goats subjected to the same load of hypertonic NaCl as the one used here but allowed to drink during the infusions increased both milk flow and milk fat concentration (Olsson & Stein, 2004). Taken together these experiments indicate that hyperosmolality did not interfere with the secretory activity of the mammary cells and that vasopressin facilitated emptying of alveolar milk. Such a mechanism would help to maintain milk flow during water deprivation. For physiological plasma concentrations of vasopressin to cause increased milk flow, the animals must be in normal water balance or be allowed to drink freely in response to a hypertonic load of NaCl.

Salt loading has been shown to activate hypothalamic oxytocin neurones and hence to elevate plasma oxytocin levels in rats (Brimble et al. 1978; Negoro et al. 1988) but the response was attenuated in lactating compared with virgin rats (Koehler et al. 1993). To the best of our knowledge, changes in plasma vasopressin concentrations during an osmotic stimulus have not been investigated simultaneously with changes in milk secretion in rats. Further comparisons between species therefore seem of interest.

Why is the mammary gland not concentrating milk as does the kidney to osmotic stimuli? Vasopressin receptors have been found in rat mammary plasma membranes (Soloff et al. 1989) but have not been studied in goats. However, vasopressin-immunoreactivity has been demonstrated around small blood vessels, at the basal levels of the glandular alveoli and at, or in the proximity of the myoepithelial cells in goats subjected to water deprivation or infusions of vasopressin. It was not found in goats in normal water balance (Dahlborn et al. 1990). These results agree with the present finding that vasopressin may affect milk secretion, but do not explain why the milk was not concentrated. However, Matsuzaki et al. (2005) found aquaporins 1 and 3 in the mammary glands of rats and mice, but not aquaporin 2, which is responsible for water reabsorption in response to vasopressin in the kidneys. This offers an explanation for the fact that vasopressin cannot concentrate milk, but that milk osmolality follows plasma osmolality. Aquaporin 1 was found in the mammary vessel and was suggested to play a role in water transport from blood to interstitial tissue. Aquaporin 3 was located to the basolateral membrane of the alveolar and duct epithelial cells and was suggested to participate in the transfer of water from the interstitial space to the cytoplasm. Activity of these protein channels could be of importance for the changes in milk flow during different stages of water balance in lactating animals.

It is concluded that the hyperosmolality in combination with high plasma vasopressin levels did not disturb the secretory activity of the mammary cells, but rather facilitated emptying of the alveolar milk. Such a mechanism may help to explain the sustained milk production in water deprived goats.

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