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Can vasopressin induce milk ejection in the dairy goat?☆

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ABSTRACT

Suckling increases plasma levels of both oxytocin (OT) and vasopressin (AVP) and intravenous infusions of AVP increase milk flow and milk fat concentration in goats. We hypothesized that vasopressin can cause contraction of the myoepithelial cells and thereby milk ejection. Eight goats were used in each of two series. They were kept together with their kids and were both suckled and hand milked. At experiments, one teat was first emptied by hand and after 3 min the same teat was milked again. On control days, cisternal milk (CM) was achieved at the first milking and at the second milking only a small volume of alveolar milk (AM) with modest fat content was received. On experimental days the goats were milked and immediately thereafter OT (10 mg) or AVP at the low dose 125 ng (AVPL) was injected intravenously followed by the second milking 3 min after the first (series I). In series II saline (0.9% NaCl) or vasopressin at the high dose 250 ng (AVPH) was injected. The plasma OT concentrations were unchanged after AVP injections. The CM volume did not differ between treatments within each series. Injections of AVPH or OT increased volume and fat content of AM. The proportion of AM to total milk volume was 15% in control experiments, 21% after saline and 29% after AVPL. However, after AVPH and OT the AM proportions were 56 and 57%, respectively, accompanied by an increased fat content. In conclusion, the high dose of AVP elicited a milk ejection reflex similar to that of OT.

1. Introduction

Oxytocin (OT) and arginine vasopressin (AVP) are two closely related neuropeptide hormones synthesized in the hypothalamus and stored in the posterior lobe of the pituitary gland. The two peptides can act as neurotransmitters in the brain and as hormones in the peripheral tissues, being able to join each other's receptors (Zingg, 1996). At low plasma levels AVP acts as a water saving hormone in the kidneys and at high levels it is a vasoconstrictor agent increasing blood pressure. The main functions of OT are constriction of the smooth muscles in uterus during parturition and milk let down. Moreover, OT plays an essential role in establishing the mother–offspring bonding.

In ruminants, milk is stored in two udder compartments. The cisternal milk (CM) is located in teats, gland cistern and large milk ducts. Alveolar milk (AM) is found in small ducts and alveoli, which are surrounded by myoepithelial cells having OT receptors. Fat globules (especially the largest ones) are stored in the alveolar area and do not pass freely from the alveoli to cistern (Ayadi et al., 2004). During OT actions the myoepithelial cells contract and the alveolar milk is transported to the cisternal area (Lefcourt and Akers, 1983). Milk from the gland cisterns is therefore low in fat but milk from the alveoli has a high fat

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concentration (Linzell and Peaker, 1971; McKusick et al., 2002a).

The "golden standard" to separate CM and AM is to use OT – receptor antagonists (e.g. atosiban) to block spontaneous milk ejection (Knight et al., 1994; Wellnitz et al., 1999). However, such antagonists also act on AVP receptors (Akerlund et al., 1999) and an alternative method was necessary to study effects of AVP.

Goats have large cisterns where 40–80% of the milk can be stored (Cross, 1977; Bruckmaier and Blum, 1992; Salama et al., 2004) and therefore large volumes of milk can be obtained without OT stimulation (Peris et al., 1996). In dairy ewes kept together with their lambs in a mixed suckling and milking system only CM was obtained at milkings (McKusick et al., 2002b). We have earlier shown that, when does and kids are reared jointly, suckling but not hand milking causes both OT and AVP release (Olsson and Högberg, 2008). In addition, AVP-immunoreactivity has been found in the myoepithelial cells in goats (Dahlborn et al., 1990) and intravenous infusions of AVP increased milk flow and milk fat concentration (Olsson et al., 2003).

Therefore, the first aim of this study was to investigate if it is possible to separate CM and AM without using an OT receptor antagonist in goats suckled by their kids.

In the second part of this study, this separation technique was used to investigate if AVP can cause milk ejection.

2. Materials and methods

2.1. Animals

A herd of Swedish dairy goats (*Capra hircus*) was kept indoors in a free stall system where the goats were kept together with their kids. During five weeks before the experiments the goats were accustomed to the experimental procedures. Fourteen goats (one goat had one kid, the others had twins) were used in two series of experiments ($n = 8$ in each series; two goats participated in both series) during two consecutive lactations. The experiments were performed in lactation week 6–9 and the goats were in their 1–4 lactation. Room temperature was kept at $17 \pm 1\,^{\circ}C$. The care of the animals and the experimental procedures were approved by the Local Ethical Committee, Uppsala, Sweden.

2.2. Experimental procedures

At all milkings, one teat was first emptied by hand and after 3 min the same teat was milked again. Test milkings showed that milk with a low fat content was achieved at the first milkings and only small amount of milk with higher milk fat content was obtained from the second milking. Thus, it was possible to separate cisternal milk (CM) from the alveolar milk (AM) in the present management system.

At 06.30 h on the experimental days the goats walked into a separate room with individual cages provided with hay and water. Upon arrival the udder was hand milked (both teats) by experienced technicians. About 30 min after that milking, a local anaesthetic ointment (Lidocain ointment, Emla, AstraZeneca, Sweden) was applied on shaved skin covering the jugular veins on both sides. After an additional 60 min a catheter (Secalon T®, Ohmeda, Swindon, UK) was inserted in the jugular vein (one side only). Three hours after the morning milking the experiment started with collection the first blood sample (BS 1) from the inserted catheter followed by the first milking (one teat only, CM was collected), then BS2 was taken followed by an OT or AVP injection, according to the experimental schedule (see below) in the contralateral vein. Three minutes after the first milking the second was done (AM was collected) and the experiment finished with withdrawal of BS3.

Series I: During three days before injection day, the goats were milked according to the experimental schedule and CM and AM were weighed and collected and the values used as control values. Lottery decided which four of the eight goats that were to be administered AVP (Vasopressin Euro-Diagnostica AB, Malmö, Sweden) on the first day and OT (Partoxin®, Pharmaxim, Helsingborg, Sweden) on the next. The other four goats were given OT injections first and AVP last. The injections were done with syringe and needle into the contralateral vein. Each goat was given a single low dose of AVP (AVPL, 0.08 IU = 125 ng/goat) or OT (5 IU = 10 mg/goat) dissolved in 2 ml of 0.9 NaCl %.

Series II: The same procedure as in Series I except that saline (0.9% NaCl) instead of OT was injected and a high dose of AVP was used (AVPH, 0.15 IU = 250 ng/goat).

2.3. Analyses

Milk from one udder half was weighed and 10 ml were collected. After heating to 40 °C in a water bath the milk fat content was analyzed with a mid-infrared spectroscopy method (Miris farm milkanalyser, Uppsala, Sweden).

Ten millilitres of blood was taken from the jugular vein using the catheter and syringe and transferred into ice-chilled K3-EDTA tubes (Sarstedt, Nümbrecht, Germany). The tubes were centrifuged at $1500 \times g$ at 4 °C for 20 min and stored at −80 °C until assayed.

The blood plasma was extracted with acetone petroleum benzene (Merck, Darmstadt, Germany). Vasopressin was analyzed by radioimmunoassay (Euro-Diagnostica AB, Malmö, Sweden), the minimum detection limit in series I was 0.68 pmol/l and the intraassay coefficient of variation was <10% (values between 1.45 and 60 pmol/l). Corresponding numbers from series II were 1.39 pmol/l and <10% (values between 0.93 and 60 pmol/l). Oxytocin was analyzed using an ELISA kit for OT (Electra-Box Diagnostica AB, Tyreso, Sweden) and validated for goat plasma in our laboratory (Norrby et al., 2011). The minimum detectable OT limit was 5.3 pmol/l (series I) and 6.3 pmol/l (series II), respectively. The intra-assay coefficient of variation was for OT series I <10% (values between 55 and 845 pmol/l) and for series II <10% (values between 22 and 490 pmol/l, respectively). Cortisol were analyzed by radioimmunoassay (Coat-A-Count, DPC, Los Angeles CA, USA) and the lower detection limit was 3.17 nmol/l (series I) and 5.35 nmol/l (series II). The intra-assay coefficient of variation was for series I <10% (values between 15 and 1380 nmol/l). Corresponding numbers for series II were I <10% (values between 15 and 1270 nmol/l).

2.3.1. Statistical analyses

Values are presented as means ± SD. Mixed linear models for repeated measurements (SAS software, 2011 v.9.3, SAS Institute, Inc., Cary, NC, USA) were used to analyze the data. The statistical model included the fixed effect of treatments on blood or milk samples and the random effects of goat and experimental error. Pairwise comparisons of values between treatments were tested for significance. The significance level was set at $P < 0.05$.

3. Results

3.1. Series I

There was no difference in CM between controls and before injections (Fig. 1, above, left). The AM fraction obtained during control conditions (13 ± 4 g) increased after AVPL (36 ± 6 g; $P < 0.01$) and OT (118 ± 6 g; $P < 0.001$) injections. The mean AM fat concentration was higher than that of the CM ($P < 0.01$) in both controls (+36%) and AVPL (+37%) with no difference between them (Fig. 1, above, right). After OT treatments, the AM fat concentration increased by +97% ($P < 0.001$). The proportion of AM to total milk volume was 15% in controls, 29% after AVPL and 57% after OT.

3.2. Series II

Both AM volume and fat concentration increased after AVPH injections compared to saline treatments ($P > 0.001$)