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# Effects of α- and β-adrenergic receptor stimulation and oxytocin receptor blockade on milking characteristics in dairy cows before and after removal of the teat sphincter

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Alpha (α)- and beta (β)-adrenergic receptors in the bovine mammary gland are mainly present in the teat muscles and in the region where large milk ducts reach the cisternal cavities. The aim of the study was to test the hypothesis that the region of the large mammary ducts is the most important location of α- and β-adrenergic receptor stimulation affecting milk ejection and milk removal. Effects of α- and β-adrenergic receptor stimulation and of oxytocin (OT) receptor blockade on milking characteristics were tested in six cows. Milk flow was measured before and after the distal part of one teat, including the teat canal and teat sphincter, had been partly amputated. Before the operation, milk yield and peak flow rate decreased during α-adrenergic receptor stimulation and during the OT receptor blockade, and increased during β-adrenergic stimulation. After removal of the teat tip, relations of milk yield and peak flow rates after administration of α- and β-agonists and after application of an OT receptor blocking agent were similar to those before operation. Only total milk yield had decreased in the teat-amputated quarter owing to unhindered flow of cisternal milk before cluster attachment. Since responses to α- and β-adrenergic receptor stimulation as well as to OT receptor blockade do not differ with or without the teat sphincter, it is concluded that milk flow is mainly influenced by the muscle tone of the large mammary ducts.

**Keywords:** Bovine mammary gland, adrenergic receptors, milkability.

Alpha (α)- and beta (β)-adrenergic receptors mediate effects of the adrenergic system target-specifically. They are stimulated systemically by the adrenal catecholamines, adrenaline and noradrenaline, or by the sympathetic nervous system with noradrenaline as neurotransmitter. In addition, adrenergic receptors are stimulated pharmacologically by sympathomimetic drugs, e.g. phenylephrine (PE), a selective α-adrenergic receptor agonist, or isoproterenol (ISO), a selective β-adrenergic receptor agonist (Civantos Calzada & de Artinano, 2001). Alpha- and β-adrenergic receptors are present in the bovine mammary gland (Roets et al. 1989). Densities of $\alpha_1$-, $\alpha_2$- and $\beta_2$-adrenergic receptor binding sites are high in the smooth muscles of the teat and in the region of the large mammary ducts but are much lower in the parenchyma (Hammon et al. 1994).

There are circular as well as longitudinal muscle fibres in the teat wall. In the region of the teat orifice those circular muscle fibres build the teat sphincter (Ziegler & Mosimann, 1960). Around the large mammary ducts and their entrance to the gland cistern there are circular smooth muscles in the surrounding tissue, which can have a contractile effect especially on the large ducts (Ziegler, 1941).

Total milk yield and peak flow rate decrease in response to α-adrenergic receptor stimulation (Bruckmaier et al. 1991). Contraction of the gland cistern and teat in response to α-adrenergic stimulation can be observed by ultrasonography (Bruckmaier & Blum, 1992). Teats also shorten as longitudinal smooth muscles in the teat wall contract. Peak flow rates increase in response to β-adrenergic receptor stimulation (Bernabé & Peeters, 1980; Blum et al. 1989).

Since stimulation of α- and β-adrenergic receptors causes contraction or relaxation of smooth muscles, which also form the circular fibres of the teat sphincter (Ziegler & Mosimann, 1960), it may be concluded that the sympathetic tone of the teat sphincter influences milk yield and peak flow rate. Results obtained by treatment with

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Atosiban (ATO) may serve as reference values for the effects of α-adrenergic receptor stimulation. ATO is an oxytocin (OT) receptor blocking agent that competitively binds to OT receptors without causing myoepithelial contraction and milk ejection, i.e. it inhibits milk ejection without smooth muscle contraction (Wellnitz et al. 1999a).

Reduction of milk flow in response to α-adrenergic stimulation might not only be due to contraction of smooth muscles in the teat wall and teat sphincter (Bernabé & Peeters, 1980) but also in the region of the mammary ducts, so blocking milk flow into the cistern. In this study, we tested the hypothesis that stimulation of α- and β-adrenergic receptors at the level of the mammary ducts has more important effects on milk removal than at the level of the teat sphincter.

## Materials and Methods

### *Animals*

The six cows (German Fleckvieh breed) were in their first to sixth lactation. Their daily milk yield at the start of experiments was 15·5 ± 1·3 kg/d. The cows were kept in tie-stall barns and given hay and concentrates according to their individual production levels.

### *Milking experiments*

Routine milking took place at 05.00–06.00 and at 15.00–16.00. Experimental milkings were performed in the afternoon; morning milking was routine milking only. During experimental milkings, quarter milk flow was recorded using a mobile unit (LactoCorder®, WMB AG, CH-9436 Balgach, Switzerland) as described by Wellnitz et al. (1999b).

There were four treatments on 4 consecutive days immediately before teat surgery and approximately 2 weeks afterwards, depending on wound healing. Cows were given (i.v.) 30 μg/kg BW phenylephrine (PE, Sigma Chemical Industries, St Louis, MO 63178, USA), an α-adrenergic receptor agonist, 1 μg/kg BW isoproterenol (ISO, Sigma Chemical Industries), a β-adrenergic receptor agonist, or 20 μg/kg BW Atosiban (ATO, provided by Ferring Research Institute AB, Malmö, Sweden), an OT receptor blocking agent. Normal milking without drug administration served as control. The drugs were applied via a catheter in the auricular vein 3 min before attaching the milking cluster. To ensure physiological milk ejection, 0·5 i.u. oxytocin (OT) was injected 2 min later into all cows except those that were given ATO, since OT has a higher affinity for OT receptors than has ATO and would thus diminish the effects of ATO (Melin, 1994). After a further minute, milking began. If the udder could not be completely emptied owing to the drug treatments, milking was repeated 1 h later after i.v. injection of 20 i.u. OT to induce complete evacuation of the udder.

### *Teat surgery*

Cows were operated on after the first four experimental milkings. The distal part of the teat, including the teat canal and teat sphincter, of one of the rear quarters was amputated. After casting of the cows, cleaning and disinfection of teats, infiltration anaesthesia (10 ml Procasel®, Procain) was applied and a tourniquet was fixed at the proximal end of the teats to reduce bleeding and milk flow during the operation. The amputation was performed approximately 2 cm proximally of the teat tip to ensure that the whole teat canal was removed. After haemostasis, cows were carefully milked by hand after an injection of 10 i.u. oxytocin. Finally, an antibiotic (Peracef®, Cefoperazon) was instilled into the udder to prevent mastitis. For prophylaxis, antibiotics were given every day until the end of the whole experiment. Enrofloxacin was given systemically and Cefoperazon direct into the operated quarter. There were no cases of mastitis. The wound was left open for healing. Because of the open teat cistern after operation (~1–2 mm diameter) milk could flow from the teat without obstruction. The quarter contralateral to the operated quarter served as control quarter during milk flow recording. All experimental treatments, including surgery, were reported to and permitted by the state government under the German animal welfare legislation.

### *Statistical evaluation*

Results are presented as means ± SEM. For statistical evaluations, a repeated measures analysis of variance was calculated using the MIXED procedure of the SAS program package (SAS, 8.01, 1999). The animal was the repeated subject. Drug treatment and period of milking (before or after operation) were calculated for the control and the operated quarter, respectively. Significant differences ($P<0·05$) were identified using the Bonferroni *t* test.

## Results

### *Milk yield*

Milk yields during α-adrenergic receptor stimulation were lower than for control milkings ($P<0·05$) and during β-adrenergic receptor stimulation were higher than for control milkings, both before and after operation, in the contralateral as well as in the operated quarter (Table 1). Before and after teat surgery, milk yields were lowest during OT receptor blockade. In the operated quarter, milk yields were lower after removal of the teat tip than before in all groups. Differences were significant during β-adrenergic receptor stimulation and in control milkings. Within treatment groups, milk yields of the contralateral quarter did not change significantly throughout the experiment.

**Table 1.** Effects of α- (phenylephrine) and β- (isoproterenol) adrenergic receptor stimulation, and OT-receptor blocking (Atosiban) on milking characteristics before and after amputation of the teat tip

Values are means ± SEM for $n = 6$

| Treatment | Dosage [μg/kg] | Quarter | Milk yield before operation | Milk yield after operation | Peak flow rate before operation | Peak flow rate after operation |
|---|---|---|---|---|---|---|
| Control milkings | — | operated | 1·89 ± 0·40 | 1·10 ± 0·10† | 0·58 ± 0·10 | 0·65 ± 0·08 |
| | | contralateral | 1·65 ± 0·20 | 1·70 ± 0·35 | 0·55 ± 0·08 | 0·60 ± 0·10 |
| Phenylephrine (PE) | 30 | operated | 1·02 ± 0·41‡ | 0·50 ± 0·21† | 0·48 ± 0·14 | 0·21 ± 0·07‡ |
| | | contralateral | 0·75 ± 0·26‡ | 0·70 ± 0·20‡ | 0·41 ± 0·05 | 0·37 ± 0·10‡ |
| Atosiban (ATO) | 20 | operated | 0·39 ± 0·19‡ | 0·24 ± 0·10†‡ | 0·34 ± 0·13 | 0·22 ± 0·11‡ |
| | | contralateral | 0·42 ± 0·16‡ | 0·58 ± 0·19‡ | 0·25 ± 0·07‡§ | 0·32 ± 0·07‡ |
| Isoproterenol (ISO) | 1 | operated | 2·12 ± 0·31 | 1·34 ± 0·12† | 0·77 ± 0·13 | 0·98 ± 0·15‡ |
| | | contralateral | 1·96 ± 0·31 | 1·92 ± 0·28 | 0·65 ± 0·09 | 0·71 ± 0·07 |

† For means, within a treatment, the effects of before and after operation are significant ($P < 0·05$)
‡ Corresponding means for each of the treatments PE, ATO, and ISO are significantly different from control ($P < 0·05$)
§ Corresponding means for treatments ATO and PE are significantly different ($P < 0·05$)

### *Peak flow rate*

Peak flow rates during α-adrenergic receptor stimulation and OT receptor blockade were lower than in control milkings before surgery and significantly lower after surgery ($P < 0·05$) in the contralateral as well as in the operated quarter (Table 1). During β-adrenergic receptor stimulation, peak flow rates were higher than in control milkings before operation, in the contralateral and in the operated quarter, and significantly increased after operation in the operated quarter. Peak flow rates during OT receptor blockade and PE administration were similar after operation in both quarters. In the operated quarter, during α-adrenergic receptor stimulation and OT receptor blockade, peak flow rates were lower after amputation of the teat tip than before. During β-adrenergic receptor stimulation and in control milkings, peak flow was higher after operation than before. Within treatment groups, peak flow rates of the contralateral quarter did not change significantly throughout the experiment.

## Discussion

Milk yields and peak flow rates changed during α- and β-adrenergic receptor stimulation in the contralateral quarter, as described in previous studies (Bruckmaier et al. 1991). There was an obvious decrease of milk yield after administration of the α-agonist, PE, whereas after administration of the β-agonist, ISO, milk yield scarcely changed. However, peak flow rates numerically increased in response to ISO, probably because of an accelerated milk flow through relaxed mammary ducts from the alveolar tissue to the cistern (Blum et al. 1989). Peak flow rates decreased only slightly in response to PE. Provided that enough milk is in the cistern, milk flow remains at a constant level (Bruckmaier et al. 1997). Contractions of smooth muscles around mammary ducts obviously caused interruption of milk flow during α-adrenergic stimulation. Hence the reduction of milk flow was probably due to the reduced volume of milk available for removal.

Milk yields in quarters whose teat tips had been amputated (operated quarter) were reduced after the operation for all treatments (α- and β-adrenergic receptor stimulation, OT receptor blockade, and control treatment). This reduction was due to an unhindered flow of cisternal milk before attaching the milking cluster. Significant amounts of milk do not enter the cistern until 5 h after the previous milking (Knight et al. 1994; Bruckmaier & Hilger, 2001). Consequently, in our experiment milk began to flow 5–6 h from the previous milking because there was no barrier after amputation of the distal part of the teat including teat canal and teat sphincter.

Irrespective of the amputation, treatment effects followed a similar pattern, i.e., after administration of α-adrenergic agonists milk yield decreased, whereas milk yields during β-adrenergic stimulation were slightly higher than for control milkings. This agrees only in part with earlier studies, which suggest that inhibition of the sympathetic tone in the teat enhances the efficiency of milk evacuation from the alveoli (Roets et al. 1986). Our results showed that effects of β-adrenergic stimulation did not change after removal of the teat tip.

After administration of PE, milk yield decreased after teat amputation even though milk was in the udder, teat barriers were removed, and the teat cistern was open. This leads to the conclusion that there was a partial inhibition of milk ejection during α-adrenergic receptor stimulation. Although OT was high, milk flow from the alveolar tissue to the cistern was interrupted, depending on the strength of smooth muscle contraction around the mammary ducts (Bruckmaier et al. 1997). Earlier observations led to similar conclusions about the influence of the sympathetic nervous system on mammary duct tone (Findlay & Grosvenor, 1969). Anatomical and physiological studies indicate that

the adrenergic system supplying smooth muscle in different regions of the mammary gland exerts a measure of control over the duct diameter (Grosvenor & Findlay, 1968). Another early observation affirms our results: touching a cow's injured teat having a teat fistula, with milk flowing from the teat cistern, caused immediate cessation of milk flow from the fistula while the other teats were milked normally. It was concluded that this effect was probably due to a contraction of smooth muscle between the gland and the teat cistern, activated by the sympathetic nervous system (Cochrane, 1949).

Treatment with ATO induced very low milk yields and accordingly low peak flow rates in the contralateral quarter as well as in the operated quarter, owing to inhibited milk ejection (Wellnitz et al. 1999a). Mean milk yield in the contralateral and operated quarter before operation amounted to ~20% of mean milk yield for control milkings, indicating that ATO induced removal of only the cisternal milk fraction. In comparison with milk yields during α-adrenergic receptor stimulation, it can be concluded that, after giving PE, not only the cisternal milk fraction but also the milk from the distal, very large mammary ducts was removed. This is not contradictory to our hypothesis, that contractions of large mammary ducts lead to an obvious decrease of milk yield and peak flow rate during α-adrenergic stimulation, because after administration of PE milk yield and peak flow rates were low, even after removal of the teat sphincter. Our hypothesis, however, does not agree with earlier studies that described the sphincter muscle tone as the most important characteristic determining milk flow (Naito et al. 1964).

During α-adrenergic stimulation milk flow was very low. Contractions of myoepithelial cells induced by application of high amounts of OT could not force milk through the contracted milk ducts, as discussed previously (Bruckmaier et al. 1997).

Peak flow rates in the operated quarter, in controls and during β-adrenergic stimulation, tended to be higher after surgery than before because of an enhanced milk flow through the open teat without the barriers of teat canal and teat sphincter. After administration of the α-agonist PE, however, peak flow was lower than before amputation of the teat tip. Most of the cisternal milk fraction dripped off before attaching the milking cluster, and hence there was insufficient milk to reach potential peak flow rate, before the udder was emptied.

Since densities of adrenergic binding sites in the mammary parenchyma are very low (Hammon et al. 1994) they play no role in influencing milkability in response to adrenergic receptor stimulation.

In conclusion, the results support the hypothesis that effects of α- and β-adrenergic receptor stimulation in the bovine mammary gland are mainly mediated by reactions of the large mammary ducts. Contraction and relaxation of smooth muscles in the teat wall and sphincter after administration of α- and β-agonists seem to have less influence on milkability and milk flow than reactions of smooth muscles around the large mammary ducts.

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