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Milking characteristics and their relation to adrenergic receptor mRNA expression and ligand binding in the mammary gland of dairy cows

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Abstract

Stimulation of α- and β-adrenergic receptors in the bovine mammary gland affects milking characteristics such as milk yield and peak flow rate. The aim of this study was to detect possible correlations between milkability, adrenergic receptor binding capacity and receptor expression at the mRNA level. In addition, dose–response relationships of α- and β-adrenergic receptor stimulation were evaluated after application of an α- and β-adrenergic receptor agonist, respectively in different dosages. Density and distribution of adrenergic receptor binding sites in the region around the large mammary ducts were investigated as well as adrenergic receptor mRNA expression. Milk flow of one-quarter was recorded in 10 cows without or with additional α- and β-adrenergic receptor stimulation in three dosages each. After slaughter, mammary tissue was taken from the region around the large mammary ducts in the previously investigated quarters. Protein and RNA were extracted for measuring α_1-, α_2-, and β_2-adrenergic receptor binding sites and mRNA expression levels by real-time reverse-transcription polymerase chain reaction (RT-PCR). Peak flow rate without additional adrenergic receptor stimulation was negatively correlated with α_2-adrenergic receptor binding (maximal binding capacity, B_{max}) and positively correlated with α_2-adrenergic receptor expression at the mRNA level (crossing point (CP) of the real-time PCR). During α-adrenergic receptor stimulation, there was a positive correlation between milkability and α_2-adrenergic receptor mRNA expression, whereas during β-adrenergic receptor stimulation no correlations were detected. Dose–response relationships were existing during α-adrenergic receptor stimulations, but not during β-adrenergic receptor stimulations at four dosages each including control milking. Significant changes in milk yield and peak flow rate mainly occurred after application of an α-adrenergic receptor agonist. In conclusion, high mRNA expression levels or binding capacities of adrenergic receptors do not necessarily lead to according reactions in vivo, concerning milk yield and peak flow

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rate. To influence milking characteristics, individual reactions of the cow on adrenergic stimulation have to be considered.

Keywords: Cow; Mammary gland; Adrenergic receptors; Milkability

1. Introduction

Milking characteristics such as milk yield and peak flow rate in cows are influenced by adrenergic receptor stimulation. After application of α-adrenergic agonists milk yield and peak flow rate decreased [1,2]. In contrast, during β-adrenergic receptor stimulation peak flow rates were enhanced while milk yield remained unchanged [3]. It was recently shown that the effects of α- and β-adrenergic receptor stimulation are mainly mediated by contraction or relaxation, respectively of smooth muscles around the milk duct system and not by the teat sphincter [4].

The ratio between α- and β-adrenergic receptors was shown to be a measure for milkability. Roets et al. [5] pointed out that good milkability was associated with a low ratio of β_2- to α_2-adrenergic receptor densities in teat tissue, and with a high ratio of extrajunctional β_2- to α_2-adrenergic receptor densities in blood cells.

Adrenergic receptors are pharmacologically classified into the receptor types α_1 and α_2, and β_1, β_2, and β_3. α-Adrenergic receptors can be subdivided into the receptor subtypes α_{1A} (formerly α_{1C}), α_{1B}, and α_{1D} (formerly α_{1AD}), and α_{2AD}, α_{2B}, and α_{2C}, respectively [6]. The effects of α- and β-adrenergic receptor stimulation are mediated systemically by binding of the endogenous catecholamines adrenaline and noradrenaline, by the sympathetic nervous system with noradrenaline as neurotransmitter, or by pharmacological ligands.

α_1-, α_2-, and β_2-Adrenergic receptors have been detected by radioligand binding studies in the bovine mammary gland [7–10]. Competitive binding studies indicated a majority of β_2-adrenergic receptor binding sites among the β-adrenergic receptors [7]. Densities of α_1-, α_2-, and β_2-adrenergic receptor binding sites are high in smooth muscles of the teat and in the region of large mammary ducts but much lower in the parenchyma [11]. These results correspond with other investigations finding highest concentrations of α_{1A}- and β_2-adrenergic receptor mRNA in teat tissue and around the mammary ducts and lowest in parenchyma [12].

The aim of the present work was, firstly, to detect possible dose–response relationships of α- and β-adrenergic receptor stimulation, respectively at different dosages each. Secondly, correlations between individual milking characteristics during normal milking and at different dosages of α- and β-adrenergic agonists, respectively and densities of α_1-, α_2-, and β_2-adrenergic receptor binding sites were evaluated. Thirdly, mRNA expression of the three most abundant adrenergic receptor types (α_{1A}, α_{2AD}, and β_2; [13]) was measured and also correlated to the data obtained in vivo and at the protein level. The hypothesis of this study was that there should be a positive correlation between mRNA expression and density of receptor binding sites. Correlations between receptor expression and milk flow were expected to be positive for β-adrenergic and negative for α-adrenergic receptors.

2. Materials and methods

2.1. In vivo experiments

2.1.1. Animals

The 10 lactating Brown Swiss dairy cows were in their first to fourth and late lactation. The cows were aged 3 years ($n = 2$), 4 years ($n = 4$), 5 years ($n = 1$), 6 years ($n = 1$), and 7 years ($n = 2$), respectively. Daily milk yield of cows at the start of experiments was 20.8 ± 1.2 kg per day. There were no cases of clinical or subclinical mastitis neither before nor during or after the experiment.

Cows were kept in tie stall barns and fed maize silage, hay and concentrates according to their individual production levels.

2.1.2. Milking experiments

Milking was performed during routine milking time from 05:00 to 06:00 h and from 16:00 to 17:00 h. To avoid influences of the previous treatment, experimental milkings were performed only once daily in the afternoon, while morning milking was routine milking only without any measurements and drug or vehicle applications. During experimental milkings milk flow of one-quarter was recorded by a mobile unit (LactoCorder®, WMB AG, Balgach, Switzerland) as described [14]. In each cow one of the rear quarters was chosen to serve as experimental quarter whose milk yield and peak flow rate was evaluated and whose tissue was later used for protein- and RNA-extraction. The rear quarter with highest milk yield and peak flow rate was chosen, thus it was either the right or the left one.

Seven treatments were performed on 7 consecutive days. Milk flow was recorded during normal milking without additional drug treatment to determine milkability. This recording also served as control for the additional drug treatments. Cows were i.v. administered 5, 10, and 20 μg/kg BW phenylephrine (PE), an α-adrenergic receptor agonist, and 0.2, 0.5, and 1 μg/kg BW isoproterenol (ISO), a β-adrenergic receptor agonist. The vehicle of both drugs was saline (9 g/l). During control milkings only the vehicle was administered. Chronological order of the seven treatments was different and randomly chosen for each cow to exclude effects that might be due to a specific treatment order.

The drugs were applied via a catheter in the jugular vein 3 min before cluster attachment. One minute before the start of milking, i.e. 2 min after drug administration, a 1 min manual teat stimulation was applied to induce milk ejection. When the measurement of milk flow stopped at the end of experimental milking, the cows were milked by hand to gain residual milk. This part of the milking curve was not used for the evaluation of data. If the udder could not be completely emptied owing to the treatments with PE, milking was repeated 1 h later after i.v. injection of 20 i.u. oxytocin to ensure complete udder evacuation.

All different steps of the procedure were always carried out by the same persons who the animals were used to.

PE and ISO were purchased from Sigma Chemical Industries, St. Louis, MO 63178, USA.

2.1.3. Sample collection

Cows could not be slaughtered unless the required waiting period of the drugs for meat consumption, which was 4 weeks subsequent to the last drug treatment, was over. During

that time cows were thoroughly milked to prevent mastitis. Immediately after slaughter mammary tissue samples were taken from the region around the gland cistern including the large mammary ducts in the experimental quarter of each cow. Samples were snap frozen in liquid nitrogen, and stored at −80 °C.

2.2. Receptor binding assays

2.2.1. Preparation of membranes

The frozen samples were minced into small pieces of ∼0.5 g and put into 10 volumes of ice cold Tris–HCl buffer, pH 7.4. The buffer used in our assays contained 50 mM Tris–HCl, 6 mM $MgCl_2$, and 1 mM EGTA. The material was homogenized four times for 20 s at low speed (8000 rounds/min) with an Ultra-Turrax homogenizer (T 25, Janke & Kunkel, Staufen, Germany). The homogenate was first centrifuged for 10 min at 800 × *g* at 4 °C, and the resulting supernatant for 10 min at 10,000 × *g* at 4 °C. With the following supernatant a third centrifugation step was performed for 1 h at 49,000 × *g* at 4 °C. The resulting supernatant was decanted completely and the remaining pellet was suspended in ice cold Tris–HCl buffer by a motor-driven Glass–Teflon homogenizer.

The protein concentration was determined using a kit (BCA Protein Assay Kit; Sigma–Aldrich, St. Louis, MO 63103, USA) and the membrane suspension was then adjusted to 200 μg protein/100 μl.

2.2.2. Saturation binding assays

Adrenergic receptor binding was tested with ^{3}H-prazosin, a highly α_1-selective antagonist, with ^{3}H-rauwolscine, an α_2-selective antagonist [15], and with ^{3}H-dihydroalprenolol (^{3}H-DHA), a non-selective β-adrenergic receptor antagonist [7].

Membrane suspensions of 100 μl were incubated with increasing concentrations of the ^{3}H-labeled ligand (25 μl), starting from 9 nM (7.5, 6, 4.5, 3.6, 2.4, 1.2, 0) (α_1), 18 nM (15, 12, 9, 6, 3, 1.5, 0) (α_2), or 24 nM (18, 15, 12, 9, 6, 3, 0) (β), respectively with an excess concentration of an unlabeled ligand (25 μl) to determine non-specific binding (NSB), or without the unlabelled ligand and with 25 μl buffer instead for determining total binding (TB), respectively for 15 min at 37 °C with constant shaking. For testing α_1- and α_2-adrenergic receptor binding, 10 μM phentolamine (final concentration) was used as the unlabelled ligand (competitor), whereas for evaluation of the β-adrenergic receptor binding 1 μM (±)-propranolol (final concentration) was used as competitor. All drugs were diluted in Tris–HCl buffer as described above. The binding reaction was terminated by adding 1 ml of ice cold buffer. The material was immediately passed through glass fiber filters (MN GF-3, diameter 25 mm; Macherey-Nagel, Düren, Germany), using a vacuum filtration manifold (Hölzel, Dorfen, Germany). Filters were rapidly washed twice with 2 ml saline (9 g/l) and placed into plastic vials. Scintillation cocktail (3 ml Xyloflour®, J.T. Baker Chemicals B.V., Deventer, The Netherlands) was added, and the vials were incubated for 30 min at 60 °C and afterwards shaken for 30 min at room temperature. The bound ^{3}H-activity was measured in a liquid scintillation counter (1209 RACKBETA, PerkinElmer Wallac, Freiburg, Germany).

Specific binding of the ^{3}H-ligand was defined as total binding minus non-specific binding and constituted 20–80% of the total binding depending on the mammary tissue sample and on the type of the ^{3}H-labeled ligand. The range of specific binding of ^{3}H-prazosin,

^{3}H-rauwolscine, and ^{3}H-DHA, respectively was 30.3–79.0, 19.5–50.9, and 44.0–77.7%, respectively.

Specific binding was expressed as fmol bound ^{3}H-ligand per mg of membrane protein. Maximal binding capacities (B_{max}) and dissociation constants (K_D) were calculated by a curve-fitting computer program (Graph Pad Prism, GraphPad Software Inc., San Diego, CA 92121, USA).

2.3. Drugs

^{3}H-prazosin (87 Ci/mmol), ^{3}H-rauwolscine (84 Ci/mmol), and ^{3}H-DHA (90 Ci/mmol) were purchased from Amersham Pharmacia (Little Chalfont, Buckinghamshire, UK). Phentolamine mesylate and (±)-propranolol–HCl were purchased from Sigma (St. Louis, MO 63178, USA).

Solutions were prepared freshly before each assay and substances were dissolved in Tris–HCl buffer (see above).

2.4. Measurement of mRNA expression

2.4.1. Total RNA preparation and cDNA synthesis

Total RNA of mammary tissue ($n = 10$) was isolated using TriPure Isolation Reagent (Roche Diagnostics, Mannheim, Germany) according to the manufacturers instructions for tissue. In order to quantify the extracted total RNA, optical density was determined at 260 nm.

Synthesis of first stranded complementary DNA (cDNA) was performed with 200 units reverse transcriptase (MMLV-RT, Promega, Madison, WI, USA) and 100 pmol random hexamer primers (MBI Fermentas, St. Leon-Rot, Germany). Final concentration of reversely transcribed total RNA was 25 ng/μl.

2.4.2. Quantification by real-time polymerase chain reaction

Real-time polymerase chain reaction was performed in the LightCycler (Roche Diagnostics) with 25 ng reversely transcribed total RNA. Further components for the real-time PCR reactions were 1.0 μl LightCyclerMastermix (Roche), 4 mM $MgCl_2$, 4 pmol forward primer, 4 pmol reverse primer, and sterile water up to a finale volume of 10 μl. According to the PCR results gained in previous studies [13], primer pairs of the three most abundant adrenergic receptor (sub)types were used (α_{1A}, α_{2AD}, β_2). Primer sequences for α_{1A} were GTGAACATTTCCAAGGCCAT (forward) and GGTCGATGGAGATGATGCAG (reverse), for α_{2AD} TCATCTCGGCCGTCATCTCCTT (forward) and CGCACATAGACCAGGATCATGAT (reverse), and for β_2 TCATGTCGCTTATTGTCCTGG (forward) and CACCAGAAGTTGCCAAAAGTCC (reverse). Product length was 310 (α_{1A}), 178 (α_{2AD}), and 202 (β_2) base pairs, respectively. PCR conditions were described before [13]. Real-time PCR was performed with 40 cycles.

2.5. Statistical evaluations

Data are presented as means±S.E.M. For statistical evaluations, a repeated measures analysis of variance was calculated using the MIXED procedure of the SAS program package

[16]. The animal was the repeated subject. Milk yield and peak flow rate of two drug treatments in four different dosages each (including the control which was the same for both drugs) were calculated for the investigated quarter. Significant differences ($P < 0.05$) were localized by using Bonferroni's *t*-test.

Pearson's correlation coefficients were calculated using the CORR procedure of the SAS program package [16]. Correlations between milking characteristics and adrenergic receptor expression were calculated using the data of milk yield and peak flow rate during normal milking without any additional drug treatment (Table 3). During α- and β-adrenergic receptor stimulation at different dosages each, the changes in milk yield and peak flow rate were taken as percentage of milk yield and peak flow rate during normal milking. Correlations were calculated between the slopes of the animal-specific four-point regression lines for PE- and ISO-treatment, respectively.

Distribution of the most highly expressed adrenergic receptor (sub)types within the three receptor types α_1, α_2 and β_2 at the level of mRNA expression was determined by evaluation of the crossing points (CP) in the real-time PCR. The crossing point value is represented by the intersection of a fixed fluorescence threshold line and the PCR amplification curve. The higher the receptor is expressed, the lower the CP is. For each receptor type and for the housekeeping genes Ubiquitin (UbC) and glyceraldehyde–phosphate–dehydrogenase (GAPDH) the CP value is determined. Relative mRNA expression levels are given by the arithmetic formula $2^{-\Delta\Delta CP}$ where the CP of the target gene is normalized to a housekeeping gene (mean of UbC and GAPDH) (=ΔCP) and relative to an internal calibrator (the lowest expressed receptor subtype) (=ΔΔCP), and assuming that the efficiency (*E*) of the PCR is 2 where the PCR product is completely replicated in each cycle [13].

3. Results

3.1. In vivo experiments

3.1.1. Milk yield

Milk yield during normal milking without any drug administration was 1.82 ± 0.18 kg in the investigated quarters (Table 1).

During α-adrenergic receptor stimulation milk yields were reduced. At a dosage of 5 μg/kg BW PE milk yield was not significantly lowered as compared to control milking, whereas at higher dosages of 10 and 20 μg/kg BW PE milk yields were significantly lower than during control milking and during a dosage of 5 μg/kg BW PE ($P < 0.05$). Milk yields at 20 μg/kg BW PE were significantly lower than at 10 μg/kg BW PE.

During β-adrenergic receptor stimulation milk yields were not significantly higher than during control milking but were similar at all dosages of ISO (0.2, 0.5, and 1.0 μg/kg BW).

Intraventricular injection of 20 i.u. oxytocin was necessary for four cows after application of 10 μg/kg BW PE, and for all cows after application of 20 μg/kg BW PE, respectively to induce complete udder evacuation.

3.1.2. Peak flow rate

Peak flow rate during normal milking without any drug administration was 0.70 ± 0.07 kg/min in the investigated quarters (Table 1).

Table 1
Milk yield and peak flow rate after application of three different dosages of an α-adrenergic (phenylephrine, PE) or a β-adrenergic (isoproterenol, ISO) receptor agonist in the investigated quarters

Treatment	Dosage (μg/kg BW)	Milk yield (kg)	Peak flow rate (kg/min)
Control	0	1.82 ± 0.18 a	0.70 ± 0.07 a
PE	5	1.60 ± 0.19 a	0.67 ± 0.07 a
	10	0.86 ± 0.20 b	0.43 ± 0.07 b
	20	0.30 ± 0.08 c	0.35 ± 0.07 b
ISO	0.2	1.95 ± 0.21	0.80 ± 0.08 b
	0.5	1.99 ± 0.24	0.87 ± 0.10 b
	1	1.96 ± 0.21	0.83 ± 0.07 b

Treatment means without common letters (a–c) are significantly different ($P < 0.05$). Values are means ± S.E.M. for $n = 10$ animals.

During α-adrenergic receptor stimulation peak flow rates were reduced. At a dosage of 5 μg/kg BW PE peak flow rate was not significantly lower than during control milking, whereas at higher dosages of 10 and 20 μg/kg BW PE peak flow rates were significantly lower than during control milking and during a dosage of 5 μg/kg BW PE ($P < 0.05$). Peak flow rate at 20 μg/kg BW PE was lower than at a dosage of 10 μg/kg BW PE but the difference was not significant.

During β-adrenergic receptor stimulation (0.2, 0.5, and 1.0 μg/kg BW) peak flow rates were significantly higher than during control milking ($P < 0.05$).

3.2. Binding studies

The specific binding of ^{3}H-prazosin (α_1), ^{3}H-rauwolscine (α_2), and ^{3}H-DHA (β_2) to membrane suspensions was a saturable process. Each receptor type was characterized by the K_D values which were significantly different ($P < 0.05$) (Table 2). Having the lowest K_D value the α_1-adrenergic receptor showed highest affinity for its specific ligand ^{3}H-prazosin.

Table 2
Correlations between adrenergic receptor characteristics (K_D: dissociation constant; B_{max}: maximal binding capacity) and adrenergic receptor mRNA expression (ΔCP: crossing point of RT-PCR, normalized to a housekeeping gene)

Adrenergic receptor type	K_D (nM)	B_{max} (fmol/mg protein)	ΔCP[a]	Correlations B_{max} vs. ΔCP[a]
α_1	0.42 ± 0.08 a	26.3 ± 5.0 (13.6–64.3)	6.81 ± 0.37 (4.00–7.94)	$r = -0.87$, $P = 0.001$
α_2	8.60 ± 1.97 b	23.7 ± 4.5 (3.3–39.8)	5.74 ± 0.18 (5.09–6.45)	$r = 0.02$, $P = 0.96$
β_2	2.10 ± 0.25 a	55.0 ± 8.0 (29.9–113.0)	7.13 ± 0.17 (6.18–7.88)	$r = -0.38$, $P = 0.27$

Means without common letters (a, b) are significantly different ($P < 0.05$). Values are means ± S.E.M. for $n = 10$ animals. Ranges of B_{max} and ΔCP, respectively are given in brackets; r: correlation; vs.: versus.

[a] The lower ΔCP, the higher mRNA expression levels.

Table 3
Correlations between milking characteristics (milk yield (MY): 1.82 ± 0.18 kg; peak flow rate (PFR): 0.70 ± 0.07 kg/min), receptor binding capacity (B_{max}: maximal binding capacity of α_1, α_2 and β_2, respectively) and adrenergic receptor mRNA expression (ΔCP: crossing point of RT-PCR of α_{1A}, α_{2AD} and β_2, respectively normalized to a housekeeping gene) for each adrenergic receptor type in the untreated mammary gland without adrenergic receptor stimulation

Adrenergic receptor type	Correlations			
	MY vs. B_{max}	PFR vs. B_{max}	MY vs. ΔCP[a]	PFR vs. ΔCP[a]
α_1	$r = -0.33$, $P = 0.35$	$r = -0.19$, $P = 0.59$	$r = 0.30$, $P = 0.40$	$r = 0.23$, $P = 0.53$
α_2	$r = -0.43$, $P = 0.25$	$r = -0.59$, $P = 0.09$	$r = 0.57$, $P = 0.08$	$r = 0.66$, $P = 0.04$
β_2	$r = -0.38$, $P = 0.28$	$r = -0.16$, $P = 0.66$	$r = -0.07$, $P = 0.84$	$r = -0.20$, $P = 0.58$

r: correlation; vs.: versus ($n = 10$ animals).
[a] The lower ΔCP, the higher mRNA expression levels.

Highest B_{max} values for ^{3}H-DHA binding indicated a majority of β-adrenergic receptor binding sites in the membrane suspensions, followed by α_1- and α_2-adrenergic receptor binding sites.

3.3. Measurement of mRNA expression

Eight of nine adrenergic receptor (sub)types were detectable in the mammary gland at the level of mRNA expression. Among the α_1- and α_2-adrenergic receptors, the α_{1A} and α_{2AD}, respectively were most abundant, and within the β-adrenergic receptors, the β_2 was most highly expressed [13]. As shown in Table 2, the α_{2AD}-adrenergic receptor had the lowest ΔCP value and thus showed highest mRNA expression levels followed by α_{1A} and β_2.

3.4. Correlations between milking characteristics, adrenergic receptor binding and adrenergic receptor mRNA expression

Pearson's correlations were calculated for corresponding parameters within one receptor type α_1, α_2, or β_2, respectively.

There were no correlations found between adrenergic receptor capacity (B_{max}) and adrenergic receptor mRNA level (ΔCPs) except for the α_1-adrenergic receptor type where the B_{max} value was correlated with the α_{1A}-ΔCP ($r = -0.87$, $P = 0.001$) (Table 2).

As shown in Table 3, during milkings without any adrenergic receptor stimulation there was a negative correlation between peak flow rate and the maximal binding capacity B_{max} for the α_2-receptor ($r = -0.59$, $P = 0.09$). Positive correlations were found between milk yield and peak flow rate, respectively and the α_{2AD}-ΔCP ($r = 0.57$, $P = 0.08$, and $r = 0.66$, $P = 0.04$, respectively).

During β-adrenergic receptor stimulation (data not shown) there were no correlations between relative milking parameters and adrenergic receptor capacity (α_1-, α_2- or β_2-B_{max} values) or adrenergic receptor mRNA expression (α_{1A}-, α_{2AD}-, and β_2-ΔCP), respectively.

4. Discussion

Receptor binding capacity in tissue around the large mammary ducts was highest for β-adrenergic receptors, followed by α_1- and α_2-receptors. Occurrence of α_1- and α_2-adrenergic receptor binding sites was similar. The relation between α_1-, α_2-, and β-adrenergic receptor binding sites in the membrane suspensions were similar to earlier results. Hammon et al. [11] also described the β-adrenergic receptor binding sites as most abundant in the region of the large mammary ducts. Competitive binding assays with several adrenergic agonists and antagonists inhibiting specific ^{3}H-DHA binding resulted in a majority of β_2-adrenergic receptors [11].

Mean receptor density found in teat tissue by radioligand binding studies was highest for α_2-receptors, and slightly lower for α_1-receptors [8,10,11], whereas among the β-adrenergic receptors the β_2-receptor type was most abundant but had a lower B_{max} value than α_1- and α_2-receptors [7,11].

K_D-values calculated for ^{3}H-prazosin, ^{3}H-rauwolscine and ^{3}H-DHA binding were significantly different from each other and characterized the receptor types α_1, α_2, and β_2, respectively. Having the lowest K_D value the α_1-adrenergic receptor featured highest affinity for its specific ligand ^{3}H-prazosin, followed by β_2 (^{3}H-DHA) and α_2 (^{3}H-rauwolscine).

Distribution of adrenergic receptor binding sites in the bovine mammary gland did not necessarily agree with distribution of α- and β-adrenergic receptor mRNA. There was an obvious majority of β_2-receptor binding sites in the region around the mammary ducts but at the level of mRNA the β_2-receptor was lower expressed than α_{2AD} and α_{1A}. In addition, the α_{2AD}-adrenergic receptor mRNA was by far most abundant among all adrenergic receptor (sub)types [13] but had the lowest B_{max} value in binding studies.

Among the 10 animals used in the experiment the B_{max} and the ΔCP value of the α_1-adrenergic receptor were negatively correlated during normal milkings without additional drug treatment. The negative correlations are in context with the fact, that lower ΔCP values represent higher expression levels [17]. That means in this case, the higher the ΔCP value (the less α_1-adrenergic receptor mRNA), the less α_1-adrenergic receptor binding sites were detected. It can be concluded that there was a considerable relation between α_1-adrenergic receptor expression at the mRNA-level and the functional adrenergic receptors in the membranes. There was, however, no correlation between α_1-adrenergic receptor mRNA expression or binding capacity and milking characteristics during milkings without drug treatment but between peak flow rate and mRNA expression or binding capacity, respectively of the α_2-adrenergic receptor. The lower the mRNA expression (the higher the ΔCP), and the less α_2-receptor bindings sites, the higher was the peak flow rate. It seems obvious that milkability improves with less α_2-adrenergic receptors around the mammary ducts.

In this study it was not possible to differentiate between postsynaptic and presynaptic α_2-receptors. It can be assumed that presynaptic as well as postsynaptic adrenergic mRNA as well as receptor protein was extracted by the methods used in this experiment. The α_{2AD}-receptor subtype, however, was described to be the most abundant α_2-receptor mediating postsynaptic as well as presynaptic effects in different tissues and species [6]. Stimulation of presynaptic receptors would lead to a diminished release of noradrenaline and thus to less stimulation of postsynaptic α_2-receptors and—in this case—to a higher peak

flow rate. It is therefore more likely that the detected α_2-receptors in the bovine mammary gland were mainly postsynaptic. Good milkability was described to be associated with a low ratio of β_2- to α_2-adrenergic receptor densities in teat tissue where the α_2-adrenergic receptors were located mainly presynaptically which resulted in a decline of noradrenaline release [5].

During α- and β-adrenergic receptor stimulation at three dosages each, milk yield and peak flow rate changed as expected according to earlier investigations [1]. After application of an α-adrenergic receptor agonist milk yield and peak flow rate were diminished in comparison to control milking. Looking at the significant changes between milk yield at different dosages of PE, a dose–response relationship was obvious at a dosage of 10 μg/kg BW PE and beyond. At a lower dosage (i.e. 5 μg/kg BW) milk yield as well as peak flow rate did not show significant changes as compared to control milking but were numerically decreased.

After application of a β-adrenergic receptor agonist, peak flow rates were significantly increased compared to control milking but did not show any significant changes at different dosages of ISO. It can be concluded that there was no dose–response relationship at the used range of dosages. Significant changes in milk yields were not expected as it was previously shown that β-adrenergic receptor stimulation led to a relaxation of smooth muscles in the region around mammary ducts and thus facilitated milk flow, whereas the amount of milk remained unchanged [1].

The dose–response relationship during α-adrenergic receptor stimulation results in the presumption that it may be possible to stimulate different amounts of α-adrenergic receptors leading to reactions distinct from each other. Effects of β-adrenergic receptor stimulation, however, can hardly be distinguished from each other; maybe the dosages used in this study were too high to show more precise changes in peak flow rates at different dosages. On the other hand, assuming that the peak flow rates obtained in our experiment after application of ISO are maximum values, the range between control and maximum peak flow rate seems to be very narrow.

Correlations between milkability during α-adrenergic receptor stimulation and receptor expression was only seen at the mRNA level. Milk yield as well as peak flow rate after application of PE at different dosages (calculated with the slope of the regression line) were positively correlated with the α_{2B}-ΔCP ($r = 0.56$, $P = 0.09$, 0.59, and 0.07, respectively). The more the α_{2B}-receptor subtype was expressed, the lower milk yield and peak flow rate were after application of the α-adrenergic agonist PE, i.e. the effects of an α-adrenergic receptor stimulation increased. At the mRNA level, the α_{2B}-receptor subtype was remarkably lower abundant than the α_{2AD}-adrenergic receptor [13]. Therefore it is not obvious why the α_{2B} instead of the α_{2AD}-adrenergic receptor subtype was correlated with milking characteristics during α-adrenergic stimulation.

Correlations between milkability during β-adrenergic receptor stimulation, binding capacity, and mRNA expression of the β_2-receptor were not found. On the contrary, looking at the individual cow, the one with most β_2-receptor binding sites responded to β-adrenergic receptor stimulation in vivo with milk yields and peak flow rates lower than during normal milkings without any drug treatment.

In conclusion, we found no consequent relation between adrenergic receptor expression at the mRNA level, adrenergic receptor capacities and lastly at the level of responses to

adrenergic receptor stimulation in vivo in the bovine mammary gland. High expression levels obtained in the PCR or in receptor binding studies do not necessarily result in predictable reactions in vivo. Each expression level has to be considered by its own as the protein-translation lies in between the gene and the in vivo-reaction. Down-regulation of adrenergic receptors in response to application of adrenergic receptor agonists [18] represent another difference between the protein and the mRNA level. In addition, facts like presynaptic or postsynaptic location of the receptors and thus opposite physiological effects, and the exact anatomical site of the receptors in the connective tissue around the mammary ducts—which is yet unknown—influence data obtained and the interpretation of the results.

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References

[1] Bruckmaier R, Mayer H, Schams D. Effects of α- and β-adrenergic agonists on intramammary pressure and milk flow in dairy cows. J Dairy Res 1991;58:411–9.
[2] Bruckmaier RM, Wellnitz O, Blum JW. Inhibition of milk ejection in cows by oxytocin receptor blockade α-adrenergic receptor stimulation and in unfamiliar surroundings. J Dairy Res 1997;64:315–25.
[3] Blum JW, Schams D, Bruckmaier R. Catecholamines, oxytocin and milk removal in dairy cows. J Dairy Res 1989;56:167–77.
[4] Inderwies T, Riedl J, Kiossis E, Bruckmaier RM. 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. J Dairy Res 2002.
[5] Roets E, Vandeputte-van Messom G, Burvenich C, Peeters G. Relationship between numbers of α_2- to β_2-adrenoceptors in teat tissue and blood cells and milkability of primiparous cows. J Dairy Sci 1989;72:3304–13.
[6] Civantos Calzada B, de Artinano AA. Alpha-adrenergic receptor subtypes. Pharmacol Res 2001;44:195–208.
[7] Roets E, Peeters G, Leysen JE. Identification of β-adrenoceptors in bovine teat muscles by ^{3}H-dihydroalprenolol binding. Archives Internationales de Pharmacodynamie et de Therapie 1984;270:203–14.
[8] Roets E, Peeters G. Identification and characterization of ^{3}H-prazosin binding to α_1-adrenoceptors in bovine teat muscles. Archives Internationales de Pharmacodynamie et de Therapie 1985;275:189–98.
[9] Roets E, Vauquelin G, Peeters G, Braeckman R. Homogeneity of β-adrenoceptors in bovine teat muscles. Archives Internationales de Pharmacodynamie et de Therapie 1985;276:44–9.
[10] Roets E, Peeters G. A comparison of the binding characteristics of the α_2-adrenoceptor antagonists ^{3}H-yohimbine and ^{3}H-rauwolscine in bovine teat muscles. Archives Internationales de Pharmacodynamie et de Therapie 1986;279:212–22.
[11] Hammon HM, Bruckmaier RM, Honegger UE, Blum JW. Distribution and density of α- and β-adrenergic receptor binding sites in the bovine mammary gland. J Dairy Res 1994;61:47–57.
[12] Wellnitz O, Zurbriggen A, Friis RR, Blum JW, Bruckmaier RM. α_1- and β_2-Adrenergic receptor mRNA distribution in the bovine mammary gland detected by competitive RT-PCR. J Dairy Res 2001;68:699–704.
[13] Inderwies T, Pfaffl MW, Meyer HHD, Blum JW, Bruckmaier RM. Detection and quantification of mRNA expression of α- and β-adrenergic receptor subtypes in the bovine mammary gland. Domest Anim Endocrinol 2002;24:123–35.

[14] Wellnitz O, Bruckmaier RM, Blum JW. Milk ejection and milk removal of single quarters in high yielding dairy cows. Milchwissenschaft 1999;54:303–6.
[15] Shi AG, Ahmad S, Kwan CY, Daniel EE. Characterization of α-adrenoceptor subtypes by [^{3}H]prazosin and [^{3}H]rauwolscine binding to canine venous smooth muscle membranes. Can J Physiol Pharmacol 1989;67:1067–73.
[16] SAS, 1999. SAS/STAT User's Guide, release 8.01. Cary, NC: SAS Institute.
[17] Rasmussen R. Quantification on the lightcycler. In: Meuer S, Wittwer C, Nakagawara K, editors. Rapid cycle real-time PCR, methods and applications. Heidelberg: Springer; 2001. p. 21–34.
[18] Bruckmaier RM, Blum JW. Responses of calves to treadmill exercise during β-adrenergic agonist administration. J Anim Sci 1992;70:2809–21.