The relation between milking characteristics and adrenergic receptor mRNA-expression and ligand binding in the mammary gland of dairy cows

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Abstract

Adrenergic receptor stimulation in the bovine mammary gland affects milking characteristics such as milk yield and peak flow rate.

The aim of this study was to detect correlations between milkability, 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 α- and β-adrenergic agonists, respectively. Density 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 3 dosages each (table 1). 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 RT-PCR (table 2).

Peak flow rate without additional adrenergic receptor stimulation was negatively correlated (figures) with α_2-adrenergic receptor binding (maximal binding capacity B_{max}) and positively correlated with α_2-adrenergic receptor expression at the mRNA level ($p<0.05$) (table 3). During α-adrenergic receptor stimulation, there was a negative correlation ($p<0.05$) between milkability and α_2-adrenergic receptor mRNA expression, whereas during β-adrenergic receptor stimulation no correlations were detected. Dose-response relationships existed during α-, but not during β-adrenergic receptor stimulation. Significant changes ($p<0.05$) of milk yield and peak flow rate mainly occurred after α-adrenergic receptor stimulation.

In conclusion, high mRNA expression or binding levels of adrenergic receptors are not necessarily related to milk yield and peak flow rate. To influence milking characteristics, individual reactions of the cow on adrenergic stimulation have to be considered.

Table 1:

Milk yield (MY) and peak milk flow rate (PFR) after application of three different dosages of an α-adrenergic (phenylephrine) or a β-adrenergic (isoproterenol) receptor agonist in the investigated quarters.

(A-C): treatment means without common superscript letters are significantly different ($P < 0.05$). Values are means ± SEM for n = 10 animals.

Treatment	Dosage [µg/kg BW]	Milk yield [kg]	Peak flow rate [kg/min]
Control treatment	0	1.82 ± 0.18^{A}	0.70 ± 0.07^{A}
Phenylephrine	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}
Isoproterenol	0.2	1.95 ± 0.21	0.80 ± 0.08^{B}
	0.5	1.99 ± 0.24	0.87 ± 0.10^{B}
	1.0	1.96 ± 0.21	0.83 ± 0.07^{B}

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 real-time RT-PCR, normalized to a mean housekeeping gene (HKG) expression of Ubiquitin and GAPDH). Ranges of B_{max} and ΔCP, respectively, are given in brackets.

(A-B): means without common superscript letters are significantly different ($P < 0.05$).

*: the lower ΔCP, the higher mRNA expression levels. Values are means ± SEM for n = 10 animals.

Adrenergic receptor type	K_D [nM]	B_{max} [fmol/mg protein]	ΔCP*	r
				B_{max} vs. ΔCP*
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

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 real-time RT-PCR of α_{1A}, α_{2AD} and β_2, respectively, normalized to a mean HKG expression of Ubiquitin and GAPDH) for each adrenergic receptor type in the untreated mammary gland without adrenergic receptor stimulation.

*: the lower ΔCP, the higher mRNA expression levels. n = 10 animals

	Correlations			
Adrenergic receptor type	MY vs. B_{max}	PFR vs. B_{max}	MY vs. ΔCP*	PFR vs. ΔCP*
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

Figures:

Correlations between peak milk flow rate (PFR), receptor binding capacity (B_{max} = maximal binding capacity of adrenergic receptor type α2) and adrenergic receptor mRNA expression (ΔCP = Crossing point of real-time RT-PCR of α_{2AD} normalized to a mean HKG expression of Ubiquitin and GAPDH) for adrenergic receptor type α2a in the untreated mammary gland without adrenergic receptor stimulation (n = 10 animals).