Expression of steroid receptor mRNA in the bovine gastrointestinal tract and quantification by real-time RT-PCR: the gastrointestinal tract as possible target of steroid hormone action

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INTRODUCTION

Steroid hormones, like estrogens, androgens and gestagens, play an important role in the cell and tissue differentiation as well as in the regulation of metabolic processes. The steroid hormone effect in the target cell is mediated by its cytoplasmic receptor which binds the steroid with high affinity and high selectivity. As a consequence of the steroid-hormone-receptor interaction the transcription of specific genes is being activated and led to an increased synthesis of specific proteins in the target cell.

Goal of this study was to evaluate the deviating tissue sensitivities and the influence of estrogens on the regulation of steroid receptor expression in ten compartments of the bovine gastrointestinal tract.

Following receptor types of the steroid receptor family were investigated:

- Androgen receptor (**AR**)
- Estrogen receptor alpha (**ERα**) and Estrogen receptor beta (**ERβ**)
- Progestin receptor (**PR**)

The localisation and dominant expression of ERβ in both kidney regions and in the jejunum leads to the hypothesis that ERβ plays a dominant role in the gastrointestinal tract (*Pfaffl et al., 2001*). AR and ERα was already detected earlier in the bovine gastrointestinal tract (*Sauerwein et al., 1995*). ERβ might be a major actor in absorptive processes in the gut and in patho-physiological processes like gastrointestinal cancer.

To quantify these mRNA transcripts also in low abundant tissues, like in different compartments of the gastrointestinal tract, sensitive and reliable real-time RT-PCR quantification methods were developed and validated on the LightCycler.

MATERIAL & METHODS

Eight heifers were treated over 8 weeks with multiple RALGRO pellet implantations (0x, 1x, 3x, 10x). During treatment period and after slaughtering Zeranol concentrations were measured in plasma by enzyme-immuno-assay (*Lange et al., 2001*) (Figure 1).

As estrogen treatment we used implants with an estrogen active preparation RALGRO, which contains Zeranol (1 implant = 36 mg Zeranol). Zeranol is a derivative of the mycotoxin Zearalenone and shows strong estrogenic as well as anabolic effects. It is well known that steroids lead to an increased synthesis of specific proteins and it is proposed that estradiol can stimulate its own receptor expression, at least in the uterus (*Jungblut et al., 1976*).

Four stomachs (*rumen, reticulum, omasum, abomasum*) and six different gut regions (*duodenum, jejunum, ileum, caecum, colon, rectum*) were sampled, total RNA extracted, reverse transcribed and investigated in quantitative real-time RT-PCR.

Figure 1: Plasma Zeranol concentrations of treated animals in comparison to control animals (background = 6.9 pg Zeranol/ml plasma).

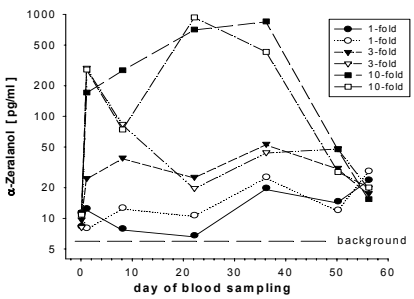


Table 1: Characterization and validation of steroid receptor real-time RT-PCR.

steroid receptors	AR	ERα	ERβ	PR
RT-PCR product length	172 bp	234 bp	262 bp	227 bp
detection limit	<12 molecules	<2 molecules	<10 molecules	<14 molecules
quantification limit	120 molecules	165 molecules	100 molecules	760 molecules
quantification range (test linearity) (*correlation*)	120 - 1.20*10^{10} molecules (*r = 0.996*)	165 - 1.65*10^{9} molecules (*r = 0.995*)	100 - 1.06*10^{10} molecules (*r = 0.997*)	760 – 7.60*10^{9} molecules (*r = 0.998*)
PCR efficiency	1.91	1.81	1.83	1.94
intra-assay variation	28.2% (n = 3)	18.7% (n = 4)	13.8% (n = 4)	5.7% (n = 4)
inter-assay variation	19.7% (n = 7)	28.6% (n = 4)	19.7% (n = 4)	25.7% (n = 4)

RESULTS

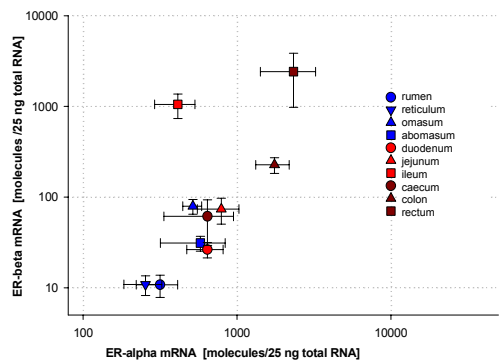
Expression results indicate the existence of AR and both ER subtypes in all ten gastrointestinal compartments. PR receptor was expressed at very low abundancy (table 2). Gastrointestinal tissues exhibit a specific ERα and ERβ expression pattern with high expression levels for both subtypes in rectum, colon and ileum (figure 2). With increasing Zeranol concentrations a significant down-regulation for ERα and ERβ was observed in jejunum ($p<0.05$). Significant up-regulations under estrogen treatment could be shown in abomasum for ERα ($p<0.05$) and in rectum for ERβ ($p<0.001$). For AR and PR mRNA expression no significant correlation with increasing Zeranol concentrations could be observed. In all stomachs and duodenum the expression ratio (R) of ERα/ERβ was high ($29 > R > 6.5$) and low ($R < 1$) in ileum and rectum, where ERβ was higher concentrated.

Table 2: Mean expression data (mRNA molecules per 25 ng total RNA) CV (in %) of AR, ERα, ERβ, PR and ERα / ERβ mRNA expression ratio.

[n.d. = mRNA not detected; # = most mRNA samples under quantification limit; * = some mRNA samples under detection limit]

	Estrogen receptors					Androgen receptor		Progestin receptor	
tissue	**Mean ERα**	**CV ERα (%)**	**Mean ERβ**	**CV ERβ (%)**	**ERα / ERβ**	**Mean AR**	**CV AR (%)**	**Mean PR**	**CV PR (%)**
rumen	316	85	11 #	78	29.3	6131	73	n.d.	82
reticulum	254	78	11 #	69	23.4	3941	51	n.d.	130
omasum	517	40	79 #	52	6.5	8465	25	23 #	93
abomasum	578	127	31 #	53	18.6	420	33	211 #	106
duodenum	641	75	26 #	54	24.3	969	49	117 #	40
jejunum	790	85	74 #	90	10.7	226	28	12 *	86
ileum	412	83	1053	85	0.4	648	36	6 *	43
caecum	642	135	61 #	148	10.5	395	45	2 *	80
colon	1753	69	227	56	7.7	242	67	8 *	108
rectum	2328	111	2418	169	0.96	577	74	12 *	87

Figure 2: Tissue specific ERα and ERβ mRNA expression cluster in ten different bovine gastrointestinal compartments.



DISCUSSION & CONCLUSION

In view of the data provided for sensitivity, linearity and reproducibility, the steroid hormone receptor RT-PCR assays developed herein allows for the absolute and accurate quantification of low abundant steroid receptors mRNA on molecule basis.

The authors conclude, that especially estrogens and the expression of their corresponding receptor subtypes may play an important role in the modulation and regulation in gastric as well as gut functions, cell proliferation and possibly in carcinoma progression, especially in ileum, colon and rectum cancer. The functionalities of the ERα and ERβ have to be demonstrated in further studies. The different expression patterns of both ER subtypes can be regarded as support of the hypothesis that the subtype proteins may have different biological functions in the gastrointestinal tract.

AR and PR seem to be not estrogen dependent and may have a minor influence in gastrointestinal tissues. However, any notations on direct physiological or patho-physiological effects of steroids on gastrointestinal tissues remain speculative.

References

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