Receptor blockers — general aspects with respect to their use in domestic animal reproduction

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Abstract

Receptor blockers compete with the respective agonist for binding to a given receptor without inducing complete signal transduction. In recent years, major interest has focused on sex-steroid hormone receptor blockers (antagonists). Indications have been obtained that inadequate changes in receptor conformation and subsequent failure of transcriptional activation are major events preventing hormonal activity. However, various subtypes and variants of receptors and receptor mutations have also been identified. Expression of antihormonal effects may vary depending on the type of receptor the blocker is bound to. Hence, receptor blockers may also have an inherent agonistic activity. Aglepristone® is the first antiprogestin registered for veterinary use with the indication “interruption or prevention of pregnancy”; similarly, these types of compounds were successfully used for induction of parturition in the dog and cat and for conservative treatment of pyometra in the dog. Moreover, application of antiprogestins has clearly demonstrated the role of progesterone as a major factor controlling overt pseudopregnancy in dogs. With respect to farm animals, parturition was induced in cows without an increased incidence of retained fetal membranes. Other than antiprogestins, antioestrogens and antiandrogens are still in a more experimental phase. In particular for use in humans, high-affinity blockers binding to the oxytocin/vasopressin receptor are in development; they exert distinct tocolytic activities. Also, the release of GnRH can be inhibited by respective antagonists; however, their use in reproduction is still hampered by the high dose requirement and the side effects observed. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The removal of endocrine organs and study of the resulting effects has been a classical approach in endocrine research. However, by such an operation in general not only one but a whole array of hormonal factors is removed. Thus the observed responses may be of multifactorial origin. In order to overcome this problem, more specific approaches were followed in the past, like active and passive immunisation against specific hormones, the development of competitive enzyme blocking agents, development of neurotransmitter-like agents blocking the release of a distinct hormone (see Concannon et al., 1987), development of competitive receptor blockers (see below) and — as the latest addition — the targeted disruption of genes encoding for mediators or the respective receptors in knockout models.

This paper deals with receptor blockers and their use in domestic animal orientated basic research, biotechnology and therapy. By taking into account data from own studies, main attention will be given to those agents blocking the activity of sex steroid hormones. However, reference will also be made to those agents blocking the activity of peptide hormones of hypothalamic origin.

2. Steroid hormone receptor blockers

Knowledge about the mechanisms of action of receptor blockers for steroid hormones is still incomplete. They exert their activity by binding to the steroid hormone receptor without inducing transcription. The understanding of this phenomenon is based on the knowledge of the processes induced by hormone–receptor interaction. Steroid hormones are small lipophilic hormones able to pass the membranes of target cells. They bind to intracellularly located receptor proteins and after decades of controversial discussion there is now overwhelming evidence that these receptors are basically located in the nucleus (Yamashita, 1998). Nuclear hormone receptors represent an evolutionary conserved class of transcription factors being present from flies to mammals (for review see Beato et al., 1995). According to the hormone they bind to, these receptors can be classified into those binding to steroids (e.g. progestins, androgens, oestrogens), steroid derivates (vitamin D3), nonsteroids (e.g. thyroid hormone) and receptors for which no ligands have been found yet (orphan receptors). Hormone binding leads to a conformational change, resulting in specific binding to palindromic DNA sequences (hormone responsive elements, HREs) as liganded receptor dimers (Gronemeyer, 1992; Beato et al., 1995; Tenbaum and Baniahmad, 1997). Thus, one role of the hormone is to induce DNA binding (see Fig. 1).

In general nuclear receptors possess a highly conserved DNA binding region separating the receptor into a variable amino (N) terminal and a higher conserved carboxy (C) terminal part. The DNA binding region is essential for sequence-specific recognition of the hormone response element of the target gene by the receptor. It consists of 66 amino acids containing two zinc fingers. The N terminals of the receptor are the least conserved parts. Length and sequence composition varies strongly among receptors.
However, a constitutionally active transcriptional activation function (AF-1) was demonstrated to be located in the N terminals for most of the analyzed receptors. The C terminals of nuclear receptors harbour multiple functions such as hormone binding (ligand binding), transcriptional activation (AF-2), transcriptional repression (silencing), nuclear translocation and dimerisation (see Fig. 2). C-terminal receptor functions are modelled upon hormone binding. Thus, in contrast to AF-1, the second transactivation function is dependent on ligand binding (Evans, 1988; Gronemeyer, 1992; Tenbaum and Baniahamad, 1997).

There is now ample evidence that a ligand may interact with various isoforms or variations of a nuclear receptor. Thus progesterone may be bound to either progesterone receptor A, B or C, most likely resulting in different transcriptional activities (Kastner et al., 1990; Gronemeyer et al., 1991; Vegeto et al., 1993; Wen et al., 1994; Wei et al., 1996; Giangrande et al., 1997). In particular for the classical oestrogen receptor (ERα) the formation of many variants and mutants has been described, mostly resulting from the deletion of one or several complete exons by alternative splicing (Hu et al., 1996; Pfeffer et al., 1996; Friend et al., 1997; Murphy et al., 1997; Okada et al., 1998). Cloning of another oestrogen receptor from a rat prostate cDNA library (Kuiper et al., 1996) led to the definition of the oestrogen receptor β, which has been demonstrated in an increasing list of mammalian (Mosselman et al., 1996; Tremblay et al., 1997; Pau et al., 1998; Rosenfeld et al., 1999) and nonmammalian species (Lakaye et al., 1998;
Tchoudakov et al., 1999). In man, the genes for the two oestrogen receptors are located on different chromosomes (Gosden et al., 1986; Enmark et al., 1997). However, homogeneity of the DNA binding domain between the two receptors is high (96%); it is distinctly less in the other domains (Enmark et al., 1997, Mosselman et al., 1996). For the oestrogen receptors α and β, a different but overlapping distribution pattern has been described (Enmark et al., 1997; Kuiper et al., 1997, 1998; Shugrue et al., 1998; Hiroi et al., 1999). Thus, in many cases they may occur simultaneously in the same target cell, possibly together with other receptor variants. In addition it has been shown that one ligand may be bound with different affinities to the various receptor variants (Kuiper et al., 1997) or may exhibit different agonist/antagonist activities (Barkhem et al., 1998). Moreover, it must be assumed that heterologous dimerisation products may form following binding of the ligand (Cowley et al., 1997; Pace et al., 1997; Pettersson et al., 1997). Thus, depending on the expression of receptors in a target tissue, different responses may be induced by the same ligand.

Also steroid hormone antagonists show a high affinity for the respective nuclear receptors with dissociation constants in the picomolar to lower nanomolar range (Capony and Rochefort, 1978; Katzenellenbogen et al., 1981; Hurd and Moudgil, 1988; Terakawa et al., 1988). As with agonists, this binding induces dimerisation, which is followed by binding to the HREs of the respective genes, however, without or only slightly inducing transcription. This latter phenomenon is still poorly understood. Recent investigations point to the role of a coactivator in the case of inducing transcription and a corepressor in case of steroid hormone antagonists (Baniahmad et al., personal communication, see Fig. 1). In addition, the steroid hormone antagonist leads to conformational changes of the receptor. Since inhibition of transcriptional activity by the steroid hormone antagonist may vary, most antihormones have a partial agonistic activity. In addition, expression of agonistic and antagonistic activity may vary between hormone dependent tissues, depending on the spectrum of locally available nuclear receptors. Based on these observations it can be explained why tamoxifen, an anticancer
drug used in human therapy, acts predominantly as a strict anti-oestrogen in the mammary gland (Gottardis et al., 1988; Jordan, 1992, 1997) while it shows clear oestrogenic activity on the uterine endometrium (Malfetano, 1990; Daniel et al., 1996).

In conclusion, the characteristics of steroid hormone antagonists may be summarised as follows.

- They bind to the ligand-binding domain of a receptor.
- Binding is competitive and reversible.
- They induce a different conformation of receptors.
- They reduce hormone dependent gene activation and/or other receptor mediated effects.

3. Observations following the use of hormone antagonists in laboratory and domestic animal species

Steroid hormone antagonists exhibiting a competitive binding to the oestrogen, androgen and progesterone receptor have been developed and are presently in use for therapy in human medicine. To the knowledge of the authors, there is so far only one registration of a sex hormone antagonist in veterinary medicine Aglepristone®, Virbac, France. However, apart from the application in laboratory animal species, there are data from experimental use in large and small animals with apparent emphasis on the application of antiprogestins. In view of the multitude of the experiments performed, this paper will restrict itself to observations in domestic animal species.

4. Antiprogestins

The chemical structure of three antiprogestins, mifepristone (RU38486), aglepristone (RU46534) and onapristone (ZK98299), is shown in Fig. 3. They exhibit a high structural similarity and it has been proposed that the hydrophobic side chain at C17 is responsible for the high-affinity receptor binding while the additional aromatic ring at C11 with a dimethyl-amino- group is responsible for the changes in receptor conformation leading to a suppression of transcription (Baulieu, 1985, 1987). As shown in Table 1 for RU38486, apart from binding to the progesterone receptor, binding to the glucocorticoid receptor and — to a lesser extent — also to the androgen receptor has been observed. Binding affinity may vary between species with no binding observed to the progesterone receptor of the chicken oviduct (Sakiz et al., 1984). No binding was observed to sex-hormone-binding globulin and transcortin in the human, monkey and chicken, confirming own observations in the dog (Gerres, 1991). Thus it must be expected that the effects observed after treatment with an antiprogestin will vary depending on receptor expression, the affinity to the receptor and the dose applied.
Moreover, effects will vary depending on the role of progesterone as an endocrine and paracrine factor at a given stage of the reproductive cycle.

Table 1
Binding of RU38486 to steroid receptors and plasma proteins in different species (from Baulieu, 1985)

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>Species</th>
<th>Binding Strength</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone receptor</td>
<td>rat/rabbit</td>
<td>(+ + +) (≈ P)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>man/monkey</td>
<td>(+ +) (≈ P)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>chicken</td>
<td>(−)</td>
<td></td>
</tr>
<tr>
<td>Glucocorticoid receptor</td>
<td>rat/mouse</td>
<td>(+ +) (≈ D)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>man/monkey</td>
<td>(+ +) (≈ D)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>chicken</td>
<td>(−)</td>
<td></td>
</tr>
<tr>
<td>Androgen receptor</td>
<td>rat (+1/4 T)</td>
<td>(+ + +) (≈ D)</td>
<td></td>
</tr>
<tr>
<td>Mineralocorticoid receptor</td>
<td>rat (−)</td>
<td>(+ + +) (≈ D)</td>
<td></td>
</tr>
<tr>
<td>Sex-hormone-binding globulin</td>
<td>man/monkey (−)</td>
<td>chicken (−)</td>
<td></td>
</tr>
<tr>
<td>Transcortin</td>
<td>man/monkey (−)</td>
<td>chicken (−)</td>
<td></td>
</tr>
</tbody>
</table>

*In comparison to the affinity of the corresponding agonist.*
5. Application of antiprogestins in the dog and cat

Different from other domestic animal species the course of the progesterone concentrations in peripheral plasma is virtually identical in pregnant and nonpregnant dogs. They begin to increase prior to ovulation, reach a maximum during early dioestrus and decrease thereafter, reaching baseline levels (below 1 ng ml\(^{-1}\) plasma) about 70–90 days after onset of dioestrus. In pregnant animals parturition is preceded by a change from the more gradual to a precipitous decline prior to parturition (length of pregnancy 63 ± 2 days). Luteolysis in the dog is independent of a uterine luteolysin (Hoffmann et al., 1992). However, depending on the dose regimen selected, application of prostaglandin F\(_2\alpha\) (PGF\(_{2\alpha}\)) during the second half of pregnancy may lead to a temporary or permanent depression of luteal function resulting in abortion (Romagnoli et al., 1993). Also in the cat, an induced ovulator, ovarian function is independent of the uterus. After sterile matings cats become pseudopregnant and the resulting corpora lutea pseudogranduates show a life span of about 40 days (Tsutsui and Stabenfeldt, 1993).

5.1. Effects of antigestagen treatment during early pregnancy and prior to parturition

Observations after treatment during early pregnancy are limited to the dog. There, implantation occurs about 14–15 days after ovulation. As was shown by Fiényi et al. (1996), parenteral treatment prior to implantation with two times 10 mg aglepiristine kg\(^{-1}\) bw 24 h apart completely blocked establishment of pregnancy. None of the 35 mated bitches became pregnant, confirming our own observations (Hoffmann et al., unpublished data).

Also during the second half of pregnancy treatment with the antigestagen interfered with pregnancy. Oral application of 20 mg RU38486 kg\(^{-1}\) bw induced abortion in two bitches; no reactions were observed in two other dogs (Linde-Forsberg et al., 1992). Concannon et al. (1990) report about abortions or resorptions in conjunction with vulval discharge after oral application of 2–5 mg RU38486 kg\(^{-1}\) bw over several days. Interestingly, in these studies luteolysis was observed after 40–45 days. Following parenteral application of two times 10 mg aglepiristine 24 h apart, Fiényi et al. (1996) report about a 97.1% success rate in inducing resorption-abortion up to day 55 post mating.

In our own experiments repeated subcutaneous injections of 6 mg RU38486 kg\(^{-1}\) bw after day 56 of pregnancy led to an onset of parturition in the presence of unchanged high progesterone concentrations. Otherwise, initial changes (drop in body temperature, onset of cervical opening, maternal behaviour) resembled a normal parturition. However, concerning the further cascade of events leading to expulsion of the fetuses, the prepartal increase of PGF\(_{2\alpha}\) was missing in the treated dogs and parturition had to be finished by cesarean section (Nohr et al., 1993; Hoffmann et al., 1996). From these observations a combined treatment with aglepiristine and PGF\(_{2\alpha}\) was developed to initiate parturition in the dog (Hoffmann et al., 1999; Riesenbeck et al., 1999). Using a similar type treatment regimen abortion could be induced in a 5-week pregnant cat suffering from fibroadenomatosis (see below). These data clearly show that in dogs and cats progesterone is the
dominating factor throughout the entire length of pregnancy controlling uterine and cervical function.

5.2. Effect of antigestagen treatment on gynaecological disorders

Pseudopregnancy is an inherent phase of the reproductive cycle of the nonpregnant bitch. Depending on the appearance of clinical symptoms, it may be classified as covered or overt (Chakraborty, 1987). Clinical symptoms associated with overt pseudopregnancy are mammary gland hyperplasia with or without secretion, increased aggressiveness, nesting behaviour and acceptance of dummy puppies. Symptoms may appear as early as 30 days after ovulation and can last 30–90 days (Arbeiter and Winding, 1977). The underlying endocrine mechanisms are still poorly understood and in order to test for the involvement of progesterone, in a controlled clinical study overtly pseudopregnant dogs were treated with the antiprogestin RU38486 (Gerres and Hoffmann, 1994). Treatments commenced on days 24, 35 and 43 after onset of prooestrous bleeding, the antiprogestin was given by subcutaneous injections in a dose of 2 mg kg⁻¹ bw in 2–3 day intervals until progesterone concentration had reached levels between 1 and 2 ng ml⁻¹ (3–6 nmol l⁻¹) plasma. Treatment with the antigestagen led to an earlier onset of overt pseudopregnancy while clinical symptoms were less. Furthermore, duration of pseudopregnancy was shortened when treatment commenced during the first and second third of dioestrus (Table 2). These results implicate a role for progesterone in the onset and maintenance of overt pseudopregnancy. The advanced onset of overt pseudopregnancy in the treatment group was interpreted as a result of the mimicked progesterone withdrawal induced by treatment with the antiprogestin. The other observations of reduced clinical symptoms and duration suggest that maintenance of pseudopregnancy at least partly depends on progesterone.

Another gynaecological problem in the dog is the development of a pyometra (endometritis purulenta). In respect to the importance of progesterone in controlling uterine and cervical function and based on the observation that over 60% of the dogs presented with pyometra are during the state of dioestrus with progesterone levels higher than 1 ng ml⁻¹ plasma (> 3.18 nmol l⁻¹), in a series of experiments the hypothesis was tested that progesterone might be an important regulatory factor involved. An initial pilot study (Blendinger et al., 1997) clearly demonstrated that treatment with RU38486 at a dose of 6 mg kg⁻¹ bw twice on day 1 of treatment and once on days 2, 3 and 4 led to a complete evacuation of uterine contents. These observations were confirmed in an open clinical study comprising 41 bitches (Lemmer, 1999). However, successful treatments were bound to an undisturbed ovarian function and progesterone levels above 1 ng ml⁻¹ plasma at onset of treatment. As far as reported, dogs regained their breeding capacity.

Fibroadenomatosis is a noncarcinogenic tumour of the mammary gland in cats occurring spontaneously during pregnancy or pseudopregnancy. The enlargement of the mammary gland is predominantly due to a gestagen-dependent proliferation of its stromal component. Common therapy is ovariohysterectomy and/or mastectomy. Treatment with the antiprogestin RU38486 given daily by subcutaneous injection over 5 days not only resulted in abortion (after additional treatment with PGF₂α, see above), but also
Table 2
Effects of the antiprogestin RU38486 on onset and duration of overt pseudopregnancy and mammary gland development; values expressed as $\bar{x} \pm SD$

<table>
<thead>
<tr>
<th>Onset of treatment (day after onset of pro-oestrous bleeding)</th>
<th>Control cycle</th>
<th>Treatment cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Onset$^a$</td>
<td>Duration (days)</td>
</tr>
<tr>
<td>24</td>
<td>48.2 ± 7.5</td>
<td>48.8 ± 10.8</td>
</tr>
<tr>
<td>35</td>
<td>62.0 ± 4.5</td>
<td>40.2 ± 21.2</td>
</tr>
<tr>
<td>43</td>
<td>62.5 ± 4.5</td>
<td>43.7 ± 25.3</td>
</tr>
</tbody>
</table>

$^a$ Day after pro-oestrous bleeding.

$^b$ Mean maximum score of mammary gland development (0–4).
in a reduction of mammary gland hyperplasia. After further treatments on days 9, 14, and 20 mammary gland enlargement was completely regressed on day 28 (Blendinger et al., 1994). These observations clearly confirm that fibroadenomatosis is a progestagen-mediated disease in the cat and that blocking of progesterone at the receptor level is an effective therapy. That was demonstrated in several clinical cases and also a male cat which developed severe fibroadenomatosis after application of a depot progestagen for hormonal castration was successfully treated with the antigestagen (Hoffmann, unpublished data).

5.3. Effect on luteal function in the dog

Following treatment with RU38486 for induction of abortion Concannon et al. (1990) and Linde-Forsberg et al. (1992) reported about an accelerated luteal regression. Similarly, in our own studies when treating dogs with pyometra, progesterone declined significantly during treatment from 18.3 ng ml⁻¹ on day 1 to 6.1 ng ml⁻¹ on day 6 (Blendinger et al., 1997) and from 16.8 nmol l⁻¹ on day 1 to 6.82 nmol l⁻¹ on day 14 and 4.9 nmol l⁻¹ on day > 21 (Lemmer, 1999). On the other hand, in a hysterectomized dog luteal function was not affected by subcutaneous applications of 2 mg RU38486 kg⁻¹ bw in 2-day intervals over the entire cycle (Gerres, 1991). This raises the question whether the observed accelerated luteal regression in ‘‘intact’’ dogs is a direct or rather indirect effect of the antiprogestin. The conclusion that it is a rather indirect effect is supported by observations of Li et al. (1991a) in gilts, where it was shown that the acute luteolytic effect of RU38486 depended on the presence of the uterus and/or conceptuses.

5.4. Other observations

Antiglucocorticoid effects of RU38486 were clearly demonstrated in the human and laboratory animals. Thus in both species prior treatments with RU38486 inhibited the activity of dexamethasone (Philibert et al., 1981, Baulieu, 1985). In the dog, Wade et al. (1988) report about significantly increased cortisol and ACTH concentrations following oral application of 20 and 50 mg RU38486 kg⁻¹ bw, respectively. However, as it was indicated by unchanged cortisol levels when treating pseudopregnant dogs (Gerres and Hoffmann, 1994) and dogs with pyometra (Lemmer, 1999) in our own studies using a different dose regimen no interference with adrenal function became obvious. The only side effects observed were labour-like pains after a dog with pyometra had been treated with a single dose of 20 mg kg⁻¹ bw.

6. Application of antiprogestins in large animals

Application of RU38486 induces premature parturition in cattle and sheep. In cattle treatment with 2 mg RU38486 kg⁻¹ bw at 8 h on days 277 and 278 resulted in parturition 55 h later, whereas in vehicle-treated control cows parturition occurred after 210 h (p < 0.01). Different from induction of parturition with glucocorticoids or PGF₂α.
Adams, 1969; Wagner et al., 1974; Johnson and Jackson, 1982), no increase in retained fetal membranes was observed (Li et al., 1991b). In sheep the interval from the first treatment to the onset of lambing was 31 ± 2 h in animals given 4 mg RU38486 kg⁻¹ bw on day 144 while it was 121 ± 27 h in the vehicle-treated controls (p < 0.01) (Gazal et al., 1993). In both animal species progesterone levels started to decline following treatment with the antiprogestin, though the prime source of progesterone in the sheep is the placenta while it is the corpus luteum in the late pregnant cow (Hoffmann, 1994). Also in pigs oral treatment with 4 mg RU38486 kg⁻¹ bw on days 111 and 112 of pregnancy resulted in parturition on day 112.7 compared to day 114.7 in the control group (p < 0.01). In treated animals progesterone decreased abruptly from a pretreatment mean of 11 to less than 0.6 ng ml⁻¹ during the second day of RU38486 treatment; preterm parturition coincided with an advanced relaxin peak with a maximum on day 112.1. Also in this study, hysterectomized gilts carrying persistent corpora lutea were submitted to treatments with RU38486. Rather than a decrease of progesterone concentration an abrupt increase of progesterone and prolactin secretion was observed leading to the conclusion that the acute luteolytic effect of RU38486 observed in pregnant pigs depends on the presence of the uterus and/or conceptuses (Li et al., 1991a). Thus, it may be concluded that luteal regression in cattle and the decrease of placental progesterone synthesis in sheep following treatment of pregnant animals with RU38486 may also be an effect depending on the presence of the uterine/fetal compartment. Gonçalves et al. (1997) studied the effect of an antiprogestin (onapristone) on in vivo and in vitro fertilization. Studies were performed in oestrus synchronised, superovulated ewes. Treatment with the antiprogestin 3 and 15 h after sponge removal had no effects on synchronization of oestrus, ovulation and oocyte maturation; however, in vivo fertilization rate decreased significantly from 41% in the control group to 2–3% in the treatment group (p < 0.001) due to sperm arrest in the cervix. Also fertilization of bovine oocytes in vitro decreased significantly from 62.6% in the control group to 48% in the treatment group. These observations clearly point to the role of progesterone in respect to control of cervico-uterine (tubal) functions and to sperm–oocyte interaction.

7. Antiandrogens and antioestrogens

The antioestrogen tamoxifen and the antiandrogen cyproterone acetate (see Fig. 4) are established compounds used in human therapy. Indication for treatment with tamoxifen is mammary cancer therapy; similarly, an indication for the application of cyproterone acetate is a nonsurgically accessible prostate carcinoma. Other indications are male hypersexuality and hyperandrogenisation in females. The phenylethylene derivatives tamoxifen or chlomifen (see Fig. 4) are mixed antagonists–agonists of oestrogen action and belong to the group of type I antioestrogens (Clark, 1994; Klein-Hitpaß et al., 1998). Type I oestrogen antagonists partially inhibit the action of agonists, but, due to their own inherent weak agonistic properties, they also induce to some extent oestrogenic responses. The degree of agonistic or antagonistic activity depends on the species, organ, tissue, or cell type that is being examined. Thus in the human tamoxifen exerts distinct antioestrogenic activities in the mammary gland while it exerts agonistic activities on the
uterus (Gottardis et al., 1988; Jordan, 1992, 1997). The underlying mechanisms are not yet fully understood; however, they may relate to the expression of cell-type-specific oestrogen receptor variants, cell-type-specific arrays of cofactors or to different ways of receptor–DNA interactions. Moreover, in the dog tamoxifen acts rather like an agonist than antagonist (Morris et al., 1992), suggesting also species specific variations with respect to the agonistic activities of type I antioestrogens.

The 7α-alkyl-amide analogues ICI 164,384 and ICI 182,780 may be considered pure antioestrogens and are classified as type II antioestrogens. They lack agonistic activity for uterine growth as shown in rats and mice and they are unable to induce the progesterone receptor in the immature rat. Similarly with type I antioestrogens, and as shown in experiments with transiently transfected mammalian cells, these type II antioestrogens also induce receptor dimerisation and DNA binding. Since there seems to be no significant reporter gene activation, they obviously prevent the activity of both AF-1 and AF-2 (for review see Klein-Hitpaß et al., 1998).
Type I antioestrogens have been applied in domestic animals to further explore the endocrine functions of estradiol-17β. Thus, Jacobs et al. (1988) could demonstrate that the oestrogen provoked preovulatory LH release in heifers is inhibited by concomitant treatment with tamoxifen. The same experiment showed that the blocking of endogenous estradiol-17β prevented the endometrial PGF₂α-release leading to luteolysis. However, Cloprostenol*-induced luteolysis was not prevented. Janowski et al. (1996) treated prepartal cows with a dose of 360 mg tamoxifen in 4-h intervals starting on day 268 of pregnancy. They observed no changes in respect to course of parturition and oestrogen synthesis, raising the question on the biological role of prepartal oestrogen production in cattle.

Cyproterone acetate (see Fig. 4) is the 1,2-cyclopropal derivative of chlormadinone acetate. It exerts antiandrogenic and gestagenic activities with an inherent androgenic activity. In laboratory animals cyproterone acetate inhibits development of accessory sexual organs but has little effect on neuro-gonadal feedback mechanisms and hence testosterone biosynthesis. Other than cyproterone acetate, cyproterone and flutamid, a nonsteroidal receptor-binding antiandrogen (see Fig. 4), inhibit the negative feedback of androgens which in consequence leads to increased LH and testosterone values (Neumann et al., 1984; Fuhrmann et al., 1998). In rats, guinea pigs or mice no inhibiting effect of cyproterone acetate on male sexual activity was observed, yet inhibition was observed in dogs, rabbits and in ewes in which a male behaviour had been induced by repeated treatment with testosterone (reviewed by Fabre-Nys, 1982). Daily treatment of boars with 200 mg cyproterone acetate completely suppressed development of boar taint (Horst and Bader, 1969). However, based on the intrinsic hormonal activities of cyproterone acetate, this effect is probably a result of the progestagenic rather than antiandrogenic activity. Also in the dog it has been shown that cyproterone acetate has an antigonadotrophic effect (van Sluijs, 1997). This inherent diversity of hormonal and antihormonal effects of cyproterone acetate makes it difficult to relate experimental observations to distinct hormonal activities, which may explain the paucity of information on the use of these type antiandrogens in domestic animal species.

8. Peptide hormone receptor blockers

In respect to reproduction GnRH and oxytocin antagonists have been developed. Briefly these two hormones comprise 10 (GnRH) and 8 (oxytocin) amino acids, their main synthesis is by hypothalmic neurons though synthesis in periphal organs like the ovary (oxytocin) and placenta (GnRH-like peptide) have been described (Döcke, 1994a,b). They bind to membrane receptors inducing second messenger mediated intracellular signal cascades. The GnRH and oxytocin receptors are members of the large family of G-protein-coupled receptors and have seven transmembrane domains (Kimura et al., 1992; Flanagan et al., 1997; Salvatore et al., 1998). They have been identified in various organs and their specific inhibition would provide further information on the biological role of these two hormones in addition to the opening of new therapeutic approaches. In conjunction with dog reproduction, Vickery et al. (1989) refer to GnRH antagonists with different relative potencies measured on the inhibition of LH release. Side effects observed concern the mast cell degranulating activity of these
compounds and the side effects to be expected from the release of histamine and other mediators. Together with the high dose requirements, this asks for the development of more potent and safer analogues. However, since similar effects may be observed by downregulation of GnRH receptors following application of a continuously high dose of GnRH, there is only a relative need for these type compounds to interfere with small animal reproduction.

The oxytocin receptor gene is expressed in the myometrium, endometrium and cervical epithelium and blocking the activity of oxytocin by an appropriate antagonist might proof to be a valuable approach to control gynaecological or obstetrical disorders (reviewed by Goodwin and Zograbyan, 1998). Concerning their application in domestic animals, Fuchs et al. (1997) report about the affinity and specificity of the antagonist [1-D(CH₂)₃, Tyr(ME)², Thr⁴, Tyr-NH₂³] ornithine vasotocin and its use in late pregnant cows. The antagonist showed a similarly high binding to endometrial and myometrial oxytocin binding sites and a 40-fold molar excess of antagonist inhibited the release of prostaglandin induced by oxytocin in the bovine endometrium prior to parturition. Similar observations in the sheep (Jenkin et al., 1994) point to the role of these compounds as tocolytic agents. Thus the oxytocin and vasopressin antagonist atosiban has reached the phase III of clinical development for human use (Bossmar, 1998).

9. Conclusions

As was demonstrated during the past 20 to 30 years hormone antagonists blocking the activity of reproductive hormones have shown to be powerful tools in endocrine research, therapy and biotechnology. Of particular interest are compounds which block agonists by competitive binding to the respective receptor, thereby inducing complete loss of receptor functions (pure antagonists). In respect to sex-steroid hormone antagonists the effects observed not only depend on the molecular structure of the antagonist but also on the structure of the respective receptor. Thus for example, for the oestrogen and progesterone receptor various subtypes, variants and mutations have been identified by adequate cloning experiments and their distribution varies between cell types, tissues and species. In spite of their highly conserved DNA-binding domain transcriptional activation seems to be regulated differently, leading to a tissue (cell)-specific expression of — for example — oestrogenic activity. Accordingly, the phenomenon of antagonists exhibiting a partial agonistic activity may at least partly be explained on interactions with different subtypes of receptors. However, in spite of recent data explaining in more detail the regulation of receptor activity, still only little is known about the receptor inactivating mechanisms of action of hormone antagonists. Further insight into these mechanisms together with a comprehensive mapping of receptors and receptor subtypes are necessary to develop more specific and selective hormone antagonists.

In spite of the still existing limitations research and also therapeutical approaches in domestic animal reproduction have benefited from the availability of hormone receptor antagonists. This particularly concerns progestagen antagonists and their use in small and large animals. There are also distinct indications for the application of androgen, oestrogen and GnRH antagonists; however, particularly in respect to therapy further research is necessary.
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