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Differences in affinities between the homologous and the heterologous rabbit prolactin–receptor interaction with respect to proliferation and differentiation activities

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ABSTRACT

Interspecies differences in PRL–receptor binding and their relationship with bioactivity deserve investigation since cross-reactivity is relevant to the design of many experiments.

We have previously shown that the lower affinity of rabbit prolactin (rbPRL) binding to its homologous receptor is due to its faster and more complete dissociation compared with that of ovine PRL (oPRL). In order to obtain sufficient amounts of rbPRL to study the functional consequences of its low affinity homologous interaction, rbPRL was expressed recombinantly in *Escherichia coli* (rec rbPRL) as insoluble inclusion bodies, refolded and purified to homogeneity, yielding electrophoretically pure, over 98% monomeric rec rbPRL. Proper renaturation of rec rbPRL was evidenced by comparison of its CD spectra, binding parameters and bioactivity with those determined for the rbPRL.

The binding potency of rec rbPRL to its receptor, expressed either endogenously in the mammary gland or recombinantly in mammalian cells is one log unit lower than that to the receptor expressed recombinantly in insect cells. This difference is probably related to differences in cell-dependent receptor densities. The proliferation potency of rbPRL or rec rbPRL was one log unit lower than that of oPRL, consistent with its lower binding affinity, but the differentiation potencies of these PRLs were similar.

Thus, the proliferation activity is sensitive to PRL–receptor affinity and dissociation kinetics, whereas the differentiation response is marginally modulated.

1. Introduction

Prolactin (PRL), a pituitary secreted hormone, has been associated with the growth hormone (GH)/placental lactogen (PL) protein family, because of their structural similarity, sequence identity and ensuing common features of their various biological activities (Ben-Jonathan et al., 2008).

In mammals, GH is usually linked to physiological processes related to growth and morphogenesis, whereas PRL is principally involved in growth and differentiation of the mammary epithelium, lactation, reproduction and immunomodulation. PL exerts PRL-like and GH-like effects at both foetal and maternal levels. The biological actions of PRL are mediated through homodimers of specific membrane receptors (PRLR), called lactogenic, which are structurally related to growth hormone receptors (GHR), both of which belong to the cytokine/hematopoietic receptor superfamily (Thoreau et al., 1991). PL probably transduces the signal through PRLR homodimers or PRLR–GHR heterodimers (Biener et al., 2003) and acts as a GHR antagonist in the absence of PRLR (Herman et al., 1999). Hence, PRL biology works through regulated cross-reactivity, because its specific receptors bind three hormones: PRL, PL and hGH. This mechanism may contribute to PRL's diversity of actions.

As expected, because of the similar folding pattern of these hormones and their receptors, the general mechanism of GH/PRL/PL-induced receptor activation follows the same sequential dimerization scheme (Voorhees and Brooks, 2010). This involves two distinct binding sites on the hormone with a lower site 2 affinity for PRLs compared to GHs and PLs. Some species-specificity determinants seem critical however. This is exemplified in contradictory results sometimes reported, concerning the binding properties and activities of members of this hormone family, depending on the

Abbreviations: PRL, prolactin; GH, growth hormone; PL, placenta lactogen; h, human; o, ovine; b, bovine; rb, rabbit; p, porcine; r, rat; m, murine; R, receptor; bp, binding protein; ECD, extracellular domain; rec, recombinant; h, hours; A, absorbance; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; DTT, dithiothreitol; CD, circular dichroism; SPR, surface plasmon resonance.

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bioassay and the experimental setting using homologous (from the same species as the hormone) or heterologous receptors.

For example, G129R-hPRL is a potent antagonist of hPRLR in a differentiation assay, but it is an agonist of the rat Nb2 receptor in a proliferation assay (Bernichtein et al., 2003).

Interaction of PRLRs with heterologous PRLs or PLs gives rise to stable 1:2 complexes, which are stronger than those with the homologous hormones. Moreover, interaction of PRLRs with heterologous PLs is stronger than with PRLs, while such a difference does not exist in the case of homologous interactions (Gertler et al., 1996). PL activates the heterologous but not the homologous GHR (Herman et al., 1999). Mutations change in different ways the activity of PL mediated through homologous (drastical reduction) *versus* heterologous (no effect) lactogenic receptors (Helman et al., 2001).

On the other hand, primary sequence homology does not always predict binding of PRL or PL to a heterologous receptor and activity. For instance, as opposed to rbPRL, oPRL and oPL both bind to rbPRLR with similar high affinity, despite the differences in sequence identity with rbPRL (83% between oPRL and rbPRL and 43% between oPL and rbPRL). Despite high sequence identity between murine and rat PRLs (84%), mPRL is a poor hPRLR agonist, less potent than rPRL and a partial hPRLR antagonist, although it exhibits high affinity receptor binding (Utama et al., 2009). In spite of the higher sequence identity between pPRL and hPRL (80%), compared to that between bPRL or oPRL and hPRL (73%), pPRL exhibits 40-fold lower potency than o/bPRLs towards hPRLR in human T47D breast epithelial cells (Utama et al., 2009).

Moreover, despite the higher sequence homology to hGH (85%) than to hPRL, hPL exerts its GH-like metabolic functions through binding to hPRLR, since its binding capacity to the hGHR is 2300-fold weaker than that of hGH (Newbern and Freemark, 2011). Conversely, bPL exhibits only 20% sequence homology with bGH but binds to bGHR with high affinity (Sakal et al., 1997).

Despite the common practice of treating cells from one species with PRL from another, studies on interspecies differences in PRL–receptor binding and activation have not been given a high priority. This deserves attention, especially because potential cross-reactivity is relevant to the design and interpretation of some experiments (xenotransplant modelling of human breast cancer in mice, study of human eye pathogenesis in rabbits).

On the other hand, given the species-specificity in PRL–receptor binding and bioactivity, the use of homologous PRL is preferable to achieve the most relevant biological effect. However, although the sequence of PRLs from more than 30 mammalian species is known (Petridou et al., 2001), to date only a few recombinant mammalian PRLs are available (human, ovine, bovine, buffalo, equine, giant panda, mouse, canine and cat).

Rabbits share characteristics, such as reproductive fecundity, short generation time, sufficient size and low cost, with rats and mice, which make them animals of choice for research. In addition, their physiology exhibits differences and are therefore suitable models for studying human health and disease (Duranthon et al., 2012; Miller et al., 2014), including the role of PRL in the reproductive system (Fischer et al., 2012), in immunomodulation, in eye pathogenesis and regulation of the lacrimal gland function (Wang et al., 2007a,b). Most of these studies were performed using oPRL instead of rbPRL. One reason for this is that it is more difficult to purify PRL from the pituitary gland of small animals such as rabbits than from that of ewes. Another reason is that homologous binding between rbPRL and its specific rbPRLR is barely detectable, because of its rapid reversibility (Petridou et al., 1997).

In order to better understand the biological actions of PRL in rabbits and the functional consequences of the low affinity homologous interaction, we have developed a procedure to prepare pure recombinant rbPRL (rec rbPRL) for proper characterization and use in physiological experimental settings. We compared the interactions of oPRL, rbPRL and rec rbPRL with rbPRLR, either endogenously expressed in rabbit mammary gland microsomes (MG) or recombinantly expressed in mammalian CHO-R (Goupille et al., 1997) or in insect SF9-R cells (Cahoreau et al., 1993). We also compared, in heterologous and homologous assays, the biological activity of the rec rbPRL to those of rbPRL and oPRL.

Our results suggest that the low affinity, rapidly reversible rbPRL–receptor binding is sensitive to cell-dependent receptor densities. The proliferation potency is improved by increasing the PRL–receptor affinity as a result of decreased dissociation kinetics, whereas the differentiation potency is marginally modulated. Results of this work have been reported (Petridou and Djiane, 1998).

2. Materials and methods

2.1. Materials

The materials used were: BioTaq DNA polymerase (Bioprobe, UK), restriction and other enzymes (Boehringer), oligonucleotides (Eurofins MWG, Les Ulis, France), culture media (Gibco-BRL, Cergy Pontoise, France) and L-[3,4,5-3H] leucine, [^{3}H] TdR, ^{125}I (Amersham, UK). Other reagents were purchased from Sigma, VWR or Merck. Reagents and instruments for surface plasmon resonance (SPR) experiments were from GE, Healthcare.

2.2. Hormones and iodination conditions

Native hormones were extracted from the pituitary gland. oPRL (NIDKK AFP-8277E for radiodination and AFP-9221A of specific activity 31 IU/mg for biological assays) and pPRL (NIAMDD AFP-5000) were provided by the National Hormone and Pituitary Programs (Bethesda, MD, USA) and rbPRL (AFP 1974C) by Dr. A.F. Parlow (Harbor-UCLA Medical Center, Torrance, CA, USA). Hormones were iodinated by the chloramine T method as described elsewhere (Petridou et al., 1997) and their specific radioactivity ranged from 62 to 125 μCi/μg. Recombinant hGH was provided by Sanofi laboratories.

2.3. Construction of rbPRL expression vector

The cDNA for rbPRL, previously cloned in reverse orientation in the EcoRI site of pUC19 (pUC19 rbPRL) and sequenced (Gabou et al., 1995), was modified by PCR to remove the signal peptide. Synthetic oligonucleotides (primers) were used to amplify a 213 bp fragment starting from the first nucleotide C of the mature rbPRL sequence of the pUC19 rbPRL template, using BioTaq DNA polymerase and 25 cycles, as follows: denaturation step at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min, for 25 cycles followed by final extension at 72 °C for 10 min. Primer extension was completed with additional incubation at 72 °C for 10 min. The forward primer 5′-GGA ATT CCA TAT GCC CAT CTG TCC CAG TGG-3′ contained a NdeI (underlined) restriction-enzyme site immediately upstream from the second aminoacid (Pro) of the mature rbPRL. The NdeI site introduces an A to C substitution in the rbPRL sequence, which changes the first aminoacid of the mature rbPRL from Leu to Met. The reverse primer 5′-GGC TTG CTC CTT GTC TTC TGG TGT AGA GAG GG-3′ is depicted 213 bp from the first nucleotide C of the mature rbPRL sequence. The PCR fragment encodes a polypeptide of 71 aminoacids beginning at Leu29 (the first aminoacid downstream from the putative signal peptide cleavage site) converted to initiator Met and extends to Ala100. The PCR fragment was cleaned and digested with NdeI and PvuII, prior to ligation to the pUC18 vector double digested with NdeI and SmaI. The sequence of the inserted