Specificity of different RIA systems for measurement of bovine pregnancy-associated glycoproteins against carbohydrates and placental hormones

A. Ayad[1], N.M. Sousa[1], J. Sulon[1], E. Clerget[1], M. Iguer-Ouada[2] and J. F. Beckers[1]

[1] Department of Physiology of Animal Reproduction, Faculty of Veterinary Medicine, University of Liege, B-4000 Liege, Belgium
[2] Department of Organisms and Populations Biology, Faculty of Nature and Life Sciences, University A. Mira, 06000, Bejaia, Algeria

Introduction

The pregnancy-associated glycoproteins (PAG) constitute a large family of glycoproteins specifically expressed in the outer epithelial cell layer of the placenta in eutherian species. They are members of the aspartic proteinase family (Green et al., 2000).

Radioimmunoassay for PAG detection in serum or plasma samples is currently used as a specific serological method for pregnancy diagnosis in cattle (Zoli et al., 1992).

Different numbers of N-glycosylation sites of asparagines have been observed in PAG sequences deduced from cDNA or obtained after N-terminal microsequencing (Xie et al., 1997a). Note that the placental glycoproteins from human (hCG) and equine origins (PMSG) have also been shown to contain several lateral sugar chains.

Aim

➢ The aim of this study was to test the specificity of five PAG-RIA systems against different carbohydrates and placental hormones.

Materials and methods

➢ The following carbohydrate preparations of commercial origin were used to test the specificity of each RIA: N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, N-acetylneuraminic acid, D(+)-lactose, D(+)-glucose, Methyl-α-D-mannopyranoside, Mannitol, and D(+)-galactose. Furthermore, the specificity was tested against the following hormones: PMSG and hCG.

➢ Each carbodrate was dissolved and diluted in Tris- BSA buffer to obtain the following concentrations: 1.0, 10^2, 10^4 and 10^6 ng/ml. For PMSG and hCG, the tested concentrations were 10^{-3}, 10^{-1}, 10^1 and 10^3 IU/ml.

➢ Polyclonal antisera were collected from rabbits immunized (R#) against different PAG preparation according to the technique of Vaitukaitis et al. (1971): R#497 was raised against boPAG67, R706 against caPAG55+62, R#780 against ocPAG57+59, R#809 against ovPAG55.

➢ These four antisera were mixed (R#497 one part; R#706 one part; R#780 two parts; and R#809 two parts) and used as an additional antiserum (Pool) (Ayad et al., 2006).

➢ The measurement were performed according to a modified method of Perényi et al. (2002b).

➢ A dilution of a product was considered as crossing-reacting if the determined B/B0 value lower than the MDL/B0 value.

Results

In RIA-809, there were weak inhibition B/B0 (%mean ± SD) by N-acetylneutraminic acid and hCG when tested in the concentration of 1 mg/mL (88.11 ± 5.34) and 1000 UI/mL (93.89 ± 0.84), respectively.

The other four RIA systems did not give any cross-reaction.

Carbohydrates tested	B/B0 binding interference at the following concentrations (ng/mL)																			
	RIA-497				RIA-706				RIA-780				RIA-809				RIA-Pool			
	1	10^2	10^4	10^6	1	10^2	10^4	10^6	1	10^2	10^4	10^6	1	10^2	10^4	10^6	1	10^2	10^4	10^6
N-acetyl-D-galatosamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N-acetyl-D-glucosamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N-acetylneuraminic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
D(+)-Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D(+)-Glucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl-α-D-mannopyranoside	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D(+)-Galactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Placental protein tested	B/B0 interference at the following concentrations (IU/mL)																			
	RIA-497				RIA-706				RIA-780				RIA-809				RIA-Pool			
	10^{-3}	10^{-1}	10^1	10^3	10^{-3}	10^{-1}	10^1	10^3	10^{-3}	10^{-1}	10^1	10^3	10^{-3}	10^{-1}	10^1	10^3	10^{-3}	10^{-1}	10^1	10^3
PMSG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
hCG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-

Conclusion

In conclusion, four RIA systems can be considered as specific for the detection of PAG concentrations in palsma or serum.

Only N-acetylneutraminic acid and hCG could decrease the binding of the radiolabelled PAG tracer to the antibodies, and were able to slightly cross-react with the antiserum 809 used.

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