

SERUM PROTEASES DO NOT CLEAVE PROLACTIN TO VASOINHIBINS AT PHYSIOLOGICAL pH

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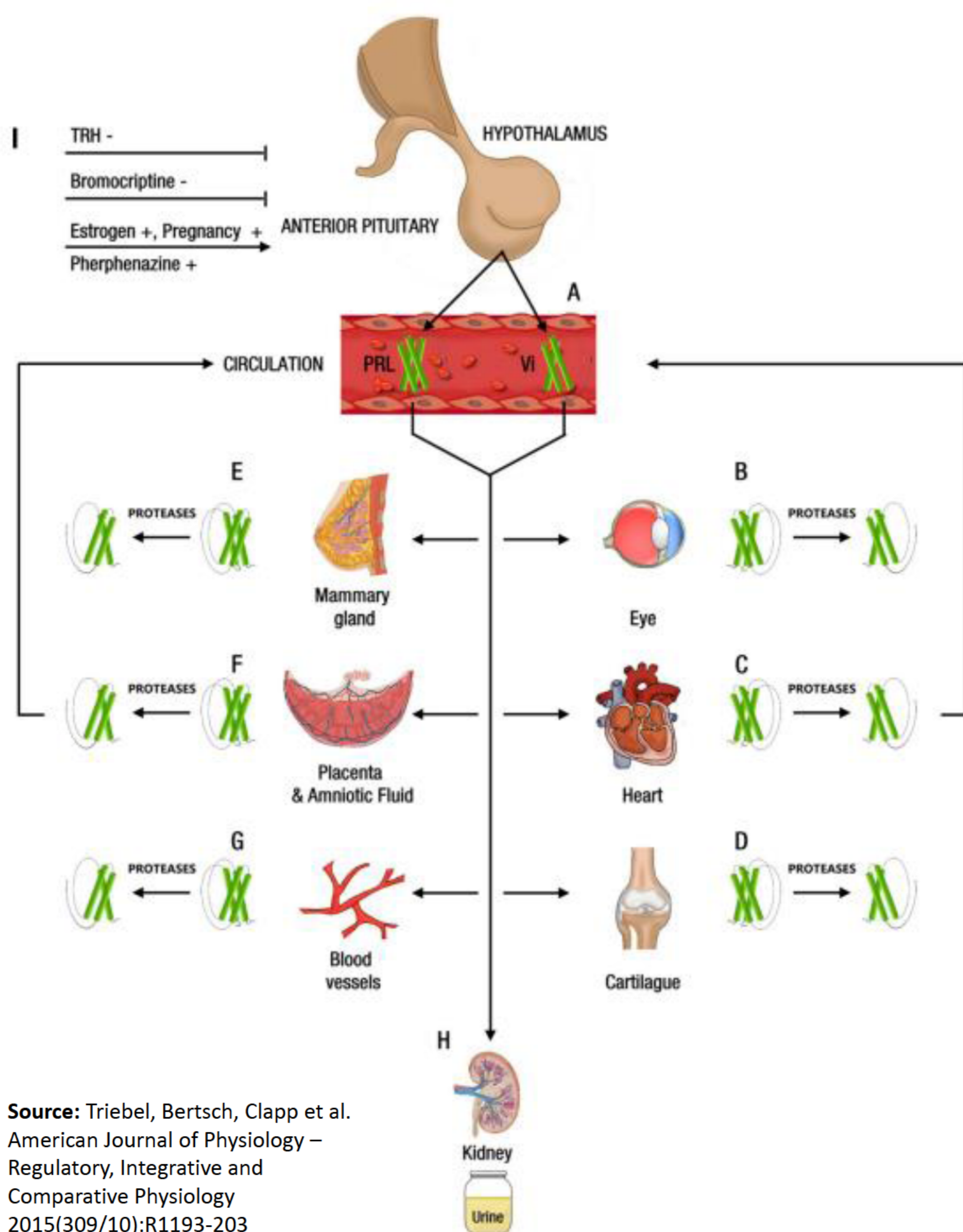
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BACKGROUND

The prolactin/vasoinhibin axis constitutes a novel endocrine axis in which the generation, secretion, and actions of the pituitary hormones prolactin and vasoinhibins are under control of the hypothalamus, the pituitary gland, and the target tissue microenvironment. Vasoinhibins are generated in the pituitary gland and in multiple target tissues through proteolytic cleavage of prolactin. **Here, we investigated whether prolactin is cleaved to vasoinhibins in the circulation.**

The prolactin/vasoinhibin axis essentials



A: Anterior pituitary. Prolactin (PRL) and vasoinhibins (Vi) are secreted from the anterior pituitary gland.
B: Eye. Hyperprolactinemia leads to vasoinhibin accumulation in the retina and inhibition of pituitary PRL secretion with the dopamine agonist bromocriptine lowers retinal vasoinhibins. A dysregulation of retinal/ocular vasoinhibins is linked to vasoproliferative retinopathies.
C: Heart. Higher circulating PRL levels lead to higher left ventricular myocardial vasoinhibin levels. Local activity of cathepsin D regulates local vasoinhibin levels. An excessive myocardial vasoinhibin synthesis is linked to peripartum cardiomyopathy. Vasoinhibins generated in the heart can enter the circulation.
D: Cartilage. Matrix metalloproteinases in cartilage generate vasoinhibins from circulating and cartilage-produced PRL.
E: Mammary gland. Local activity of cathepsin D regulates local vasoinhibin levels. Vasoinhibins participate in mammary gland involution.
F: Placenta and amniotic fluid. Local activity of bone morphogenetic protein-1 (BMP-1) and cathepsin D and upregulation of placental mRNA PRL expression regulate local vasoinhibin synthesis. An excessive, dysregulated placental vasoinhibin synthesis is linked to preeclampsia, fetal growth abnormalities, and maternal diabetes mellitus. Vasoinhibins generated in the placenta can enter the circulation.
G: Endothelium. Endothelial cells express PRL mRNA and generate vasoinhibins. The role of circulating PRL and vasoinhibins on the levels of vasoinhibins at the endothelium is unclear.
H: Kidney. Vasoinhibins appear in the urine of women with preeclampsia, pointing toward an altered renal elimination of vasoinhibins under pathophysiological conditions.
I: Central regulation. Thyrotropin releasing hormone (TRH) and bromocriptine inhibit the synthesis of vasoinhibins in the anterior pituitary gland. Estrogen, the state of pregnancy and the antipsychotic drug perphenazine stimulate the synthesis of vasoinhibins in the anterior pituitary.

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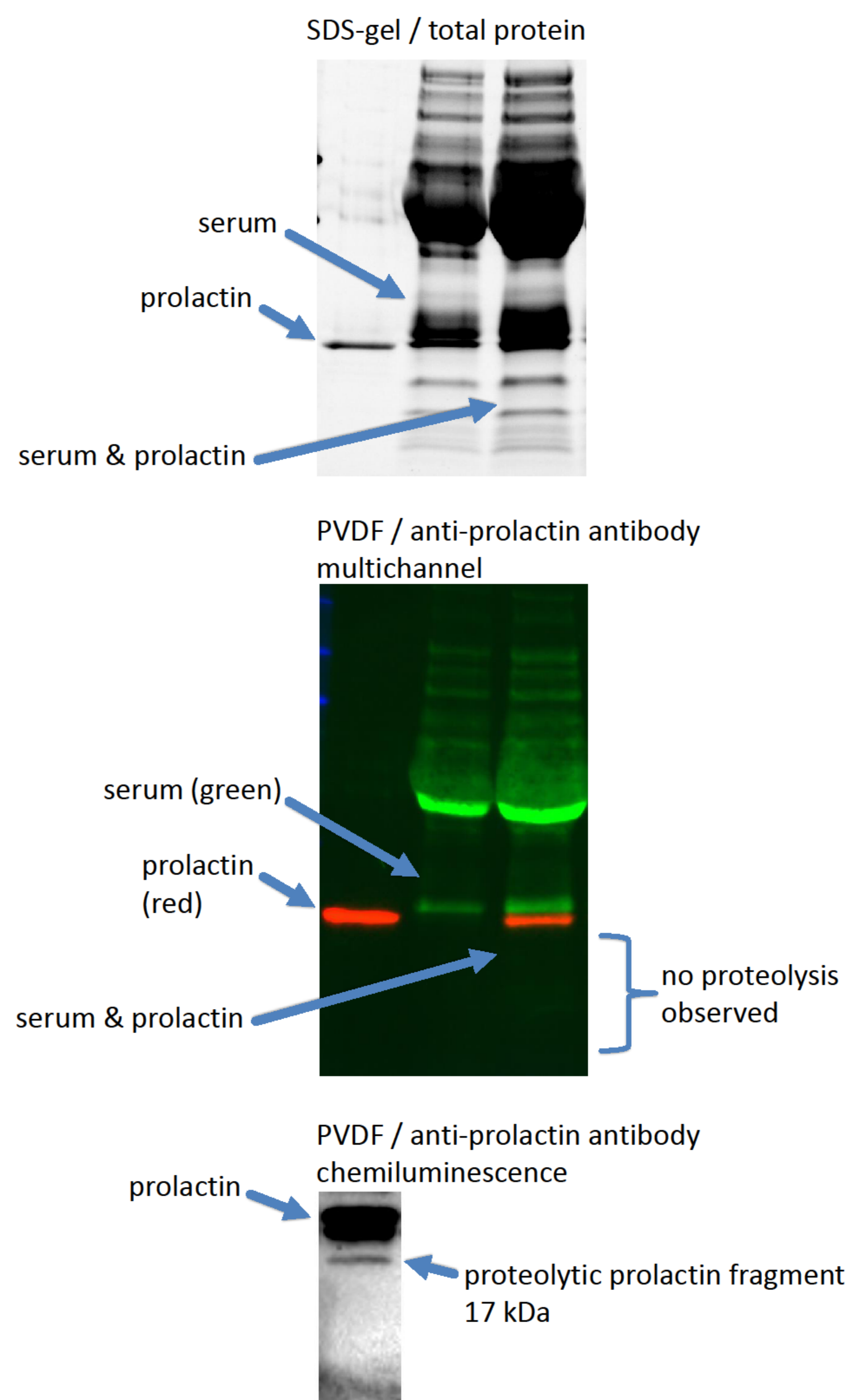
METHODS

Human serum or plasma and recombinant human prolactin were incubated at 37° C, alone and in combination, with a variation of pH, time, and buffer composition. The samples were resolved on sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western Blotting was performed using anti-prolactin and anti-vasoinhibin antibodies.

RESULTS

Sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western Blotting analyses demonstrated the presence of the recombinant prolactin-protein with an apparent molecular mass of 23 kDa. Samples incubated throughout a time period ranging from 10 minutes to 24 hours at pH 7.4 did not demonstrate immunoreactive bands lower than 23 kDa. An anti-vasoinhibin immunoreactive band featuring an apparent molecular mass of 17 kDa was intermittently observed in samples incubated at pH 3.4.

Gelelectrophoresis & Western Blotting



CONCLUSION

Vasoinhibin generation by enzymatic cleavage of prolactin does not occur in human serum under physiological conditions. The generation of vasoinhibins seems to be restricted to the pituitary gland and the target tissue level. A limited proteolysis of prolactin, resulting in the generation of a prolactin-fragment with an apparent molecular weight of 17 kDa, seems to occur at acid pH.