Tissue oxytocinase activity in diestrus mares
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Oxytocin is important in the regulation of key physiologic processes in mares such as: uterine clearance, luteal maintenance, milk ejection, passage of the fetal membranes, and maternal foal bonding, however while it is known that oxytocin has a short half-life, there is a lack of understanding of how oxytocin is metabolized. In most species the metabolism of oxytocin occurs through serum and tissue oxytocinase (OTase) often referred to as insulin regulated aminopeptidase (IRAP) or leucyl-cystinyl aminopeptidase (LNPEP). We hypothesized that OTase in mares would be similar to other species and would be found widely distributed in tissues and serum. The objective of this study was to characterize OTase activity in serum and in various tissues in diestrous mares. Jugular blood samples were obtained from five mares following the ultrasound detection of ovulation (day 0), every other day from day 3 to 15 of diestrus. Serum was separated and stored frozen at -20°C until analysis. Tissue samples (liver, kidney, myometrium, endometrium, corpus luteum, and follicle wall) from six other healthy mares, that were euthanized for another study, were collected at necropsy and stored frozen at -80°C. Examination of the ovaries in these six mares showed active luteal tissue. Tissue samples were thawed, rinsed in ice-cold 0.02 mmol/L PBS pH 7.2, and minced. Tissue samples (10 mg) were homogenized in PBS using 1.4 mm ceramic beads (Omni International, Bead Kit, Kennesaw, GA) and then centrifuged at 1500 x g for 15 minutes. The resulting supernatant was stored at -80°C and assayed at a later time. A commercial ELISA (LNPEP for horses, MyBioSource, San Diego, CA) which was validated in our laboratory, with a detection range of 6.25 – 200 U/L and an intra and interassay coefficient of variation of <15%, was used for the analysis. The diestrus serum OTase levels were variable with daily mean levels ranging from 1.3-6.4 U/L, with an overall mean diestrus serum OTase (mean ± SD) level of 5.49 ± 0.9 U/L. The mean tissue OTase activity U/L ± SD (range) per 10 mg of tissue ranked from highest to lowest activity was: myometrium 48.4 ± 3.1 (44.3-51.6), endometrium 45.6 ± 1.3 (42.6-50.8), liver 41.7 ± 3.1 (35.9-44.9), corpus luteum 24.1 ± 3.0 (20.1-33.6), and follicle 22.8 ± 2.3 (18.7-25.9). This is the first description of the presence of OTase in equine tissues. In mares both serum and tissues have detectable levels of OTase activity that likely play an important role in the metabolism of oxytocin. Further investigation is required to determine if serum and tissue OTase activity varies during the estrous cycle and pregnancy.

Keywords: Oxytocinase, mare, tissue, diestrus, serum