

Ex vivo assessment of cervical remodeling via ^{23}Na MRS

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Introduction

Preterm birth occurs in 12.5% of births in the United States. Greater understanding by which the two main processes, uterine contraction and cervical remodeling are regulated is required to reduce rates of preterm birth. One key aspect of the cervical remodeling is a change in the composition and abundance of glycosaminoglycans (GAGs) which influences the loss of tensile strength or stiffness of the cervix during labor. Understanding the changes in GAG concentration during cervical remodeling will help elucidate the normal physiological process in the cervix and may enhance our understanding of potential causes of preterm birth. At physiological pH, GAGs are negatively charged. To maintain the overall electroneutrality of the tissue, mobile ions such as Na^+ and Cl^- are distributed such that the negative charge is neutralized. Similarly to studies in connective tissue such as cartilage, and intervertebral disc¹, in the current study, ^{23}Na NMR spectroscopy is used to evaluate the changes in Na^+ concentration in the mouse cervix during pregnancy, labor and postpartum as a means of evaluating the GAG changes during each stage of cervical remodeling.

Materials and Methods

The C57BL/6 x 129SvEv mice were used in the study. Cervices at different gestation days and postpartum were collected for the NMR and Biochemistry assay studies. The total GAG concentration was measured by a hexuronic acid assay using the method described by Blumenkr. N and Grande-Allen KJ². The ^{23}Na NMR experiments on NaCl standard solution and the cervical tissue were performed back-to-back. The ^{23}Na NMR signals were integrated and calibrated by comparing with the signal of a 25 mM NaCl standard solution in water to obtain the absolute Na^+ content.

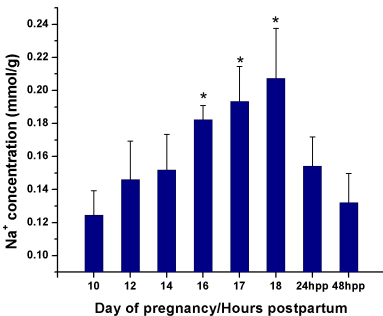


Fig. 2. Na^+ concentration (mmol/g tissue wet weight) as a function of gestation day/hour postpartum (hpp). Each group represents the mean \pm S.D. of 5 samples. (*) $P < 0.05$ when compared to day 10. Data was analyzed by One Way ANOVA.

Result and Discussion

The hexuronic acid assay study shows that similar to studies in human and animal models cervical GAGs steadily increased with progression of pregnancy in the mouse. (Fig.1) Compared to early pregnancy there was a significant increase in hexuronic acid by gestation day 15 which reached a peak 2 to 4 hours after birth and began to decline by 24 hours postpartum. ^{23}Na NMR study shows that the Na^+ concentration increased progressively starting at the softening phase and continuing through the course of cervical remodeling. A maximum is reached immediately prior to labor during cervical ripening. (Fig.2) Compared to gestation day 10, Na^+ concentration was significantly increased by day 16 consistent with the rise in hexuronic acid measured at this time point. The concentration decreased significantly and rapidly at the postpartum phase consistent with the decline in hexuronic acid measured at these time points. The Na^+ concentration increased approximately two-fold by the end of the pregnancy, which is similar with the two-fold change observed in the GAG measurement. The temporal pattern of Na^+ correlates with the GAG measurements in the hexuronic acid assay.

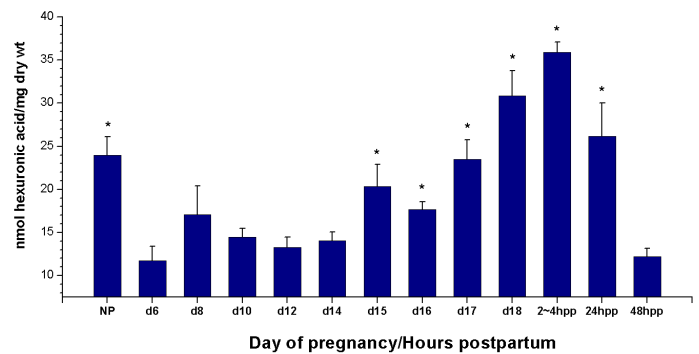


Fig. 1. Hexuronic acid levels in proestrous nonpregnant (NP), pregnant (d6-18) and cervix collected 2-4, 24, and 48 hours postpartum. (*) $P < 0.05$ when compared to d6. Data was analyzed by One Way ANOVA.

Conclusion

We present here the observation of the progressive increase in Na^+ concentration during the course of cervical remodeling using ^{23}Na NMR spectroscopy. A hexuronic acid assay showed similar trends for GAG concentration as a function of gestation day. The significant changes seen over this time course render Na^+ a potential biomarker of cervical remodeling.

References

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