The Effects of Progesterone and Estrogen on Vasoconstriction in Rats

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ABSTRACT

It has been suggested that coronary hyperactivity can be prevented by high levels of progesterone. The objective of this research study has been to investigate rat cardiac artery relaxation and contraction rates for the pre estrus stage of the cycle, where high levels of estrogen are present, versus meta-estrus, where high levels of progesterone are present, using electric field stimulation of isometric vascular muscle with force measured using IonOptix graphing software. To view the rates of contraction and relaxation, caution arteries were isolated from the tails of female rats in either pre estrus or meta-estrus stages of their reproductive cycle, and determined by PAP staining of vaginal smears. Pilot results suggest possibly that progesterone causes a faster relaxation rate but has no effect on the rate of contraction within the cardiac arteries in comparison to rates of contraction and relaxation in cardiac arteries of rats at high estrogen stages of their cycle.

INTRODUCTION

In rats, the stages of the female reproductive cycle can be tracked by following standard PAP staining procedure and viewing the cells of vaginal smears at low microscopic magnification. Typically, one rat cycle has a duration of four days consisting of pro estrus and estrus, which for the purpose of this experiment are grouped together as “pre estrus”, and also metestrus and diestrus, which here are grouped together as “meta- estrus”. During the pre estrus stage of the cycle, estrogen levels are relatively high and progesterone levels are relatively low. Inversely, during the meta-estrus stage of the cycle, estrogen levels are relatively low and progesterone levels are relatively high. It has already been experimentally determined that estrogen has no effect on arterial muscle relaxation, suggesting there is another mechanism for the protective effect of female reproductive hormones in hypertension (Packer, 2002). For this experiment, relaxation and contraction rates were used to see whether the presence of progesterone would alter rates of contraction and relaxation in rats and therefore suggest a protective effect in hypertension.

MATERIALS/METHODS

• Rat cardiac arteries were excised in pre estrus and meta-estrus stages, cut into 2 mm cylinders, and transferred to a bath containing modified ice-cold mammalian Krebs-Henseleit Buffer solution bubbled with 95% O2, 5% CO2. The arterial segments were then mounted onto a wire myograph (DMT model 120CW, Danish Myotechnology) in ice-cold bubbled Krebs solution that was warmed to 37°C over a time span of 20 minutes. The muscle was then isometrically stretched in this warmed, body temperature solution for ~90 minutes throughout the duration of the experiment.

• Isometric force was recorded with a multichannel software program (IonOptix). Changes in contractile force over time were then plotted relative to maximal contractile force. In all of the studies of vascular reactivity, only those that showed normal force generation and complete relaxation were included in the analysis. Resting tension was achieved by stretching the diameter of each artery so that the measured force of it’s tension was between 9 and 12 mN.

• Contraction of arteries was achieved by membrane depolarization with electrical field stimulation the presence and absence of [Ca2+]i.

• Contractile force changes were recorded using IonOptix multichannel software program and DMT wire myograph. These changes will be fitted to a curve using the Optimization function of Sim Physione Modeling software.

RESULTS

Figure 1. Typical Data of four day cycling rats. Halvorsen, Brooks, Johnson, 2005. “A quantitative method for assessing stages of the estrous cycle.”

Figure 2. Experimental vaginal PAP smear pictures. Top two representative of pre estrus cycle Bottom two representative of meta-estrus cycle

Figure 3. Contraction with (orange) and without (green) Calcium Pre estrus

Figure 4. Relaxation with (orange) and without (green) Calcium Pre estrus

Figure 5. Contraction with (red) and without (blue) Calcium Meta-estrus

Figure 6. Relaxation with (red) and without (blue) Calcium Meta-estrus

Figure 7. Contraction of Both (orange- Pre estrus, red-meta-estrus )

Figure 8. Relaxation of Both (orange- Pre estrus, red-meta-estrus )

FINDINGS/ FUTURE PLANS

The differing progesterone and estrogen contraction and relaxation rates suggest:
1. During the pre estrus stages of the rat reproductive cycle where high levels of estrogen are known to be present, we found that relaxation rates appeared to be slower than those that related in the meta-estrus cycle where high levels of progesterone are known to be present.
2. Contraction rates for both meta-estrus and pre estrus rats appeared to be similar, suggesting the high presence of either hormone does not affect the contraction of the artery.
3. For both pre estrus and meta-stages of the rat reproductive cycle, a calcium-free solution abolished the contractions.

In the future, we plan to study contraction and relaxation changes to a curve using the Optimization function of Sim Physione Modeling software. We also plan to test rats that have been experimentally induced to use if the differences in hormones present will have any effect on those rates. Experiments still need to be repeated and replicated to test for statistical significance of these results.

REFERENCES

Packer, C. S., Pele, A. T. C. Johnson, Gender difference in reactivity to the vasoreactive oxidant hydrogen peroxide in spontaneously hypertensive rat [S/R]. [Pulmonary Artery Branch, 2004].


ACKNOWLEDGMENTS

We would like to thank Louis Stokes Alliance for Minority Participation at Purdue University, IN.