The Effects of Progesterone on Relaxation Rates in Rat Caudal Artery

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ABSTRACT

The overall goal of this research is to investigate a potential benefit of progesterone on menopausal women. It's been suggested that menopausal women are more at risk of heart attack, possibly as a result of diminishing postmenopausal production of hormones such as progesterone. Currently, we have been investigating rat caudal artery relaxation rates for stages of the cycle where high levels of natural progesterone are present versus where low levels of progesterone are present. Rates of relaxation are measured following electrical stimulation of isometric vascular muscle with force measured using IonOptix data acquisition software. Data are fit to a curve for deriving rate constants. The general equation for relaxation of these arteries was A(x)=RT+a1*Exp((-A0*0.693)/K1) +a2*Exp((-A0*0.693)/K2). Rats are known to experience a peak and decline in natural progesterone levels every four days. Changes in cell structure visualized on PAP smears were used to indicate hormone levels. Preliminary results of these experiments suggest possibly that high progesterone levels cause a faster relaxation rate within the tail arteries in comparison to rates of relaxation in caudal arteries where rats had low levels of progesterone. More data still needs to be gathered for the comparison of these rate constants and in accurately represent the statistical significance of these results.

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 low and progesterone levels are relatively high. This has already been experimentally determined that estradiol 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 tested to see whether the presence of progesterone would alter rates of relaxation in rats and therefore suggest a protective effect in hypertension.

MATERIALS/METHODS

• Rat caudal 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 isometrically held (DMT model 1200C; Danish Mymotechnology) in ice-cold bubbled Krebs solution that was kept at 37°C over a time span of 20 minutes. The muscle was kept isometrically stretched in this warmed, body temperature solution for ~90 minutes throughout the duration of the experiment.

• Contractile force changes were 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 its tension was between 5 and 10 mN. Contraction of arteries was achieved by membrane depolarization with electrical field stimulation.

• Curves that followed the general equation of A(x)=RT+a1*Exp((-A0*0.693)/K1) were selected to be optimized. There were two relaxation constants, one slow and one fast, contributing to the overall optimized curve. These changes were fitted to a curve using the Optimization function of Sim Physiome Modeling software to find both rate constants. A(x)=RT+a1*Exp((-A0*0.693)/K1) +a2*Exp((-A0*0.693)/K2)

RESULTS

Figure 1: Typical Data of four day cycling rats. (Kikuchi, Brooks, Johnson, 2005, "A quantitative method for assessing stages of the estrous cycle")

Figure 2: Experimental PAP stained samples of rat 2A Left to right: Proestrus, Estrus, Metestrus, Diestrus

Figure 3: Experimental PAP stained samples of rat 1B Left to right: Proestrus, Estrus, Metestrus, Diestrus

Figure 4: Relaxation curve matched by JSIM optimizer for Rat 1B

Figure 5: Relaxation curve matched by JSIM optimizer for Rat 2A.

Figure 6: Comparing raw data to curves of optimized constants.

Table 1: Comparing Relaxation Rate Constants

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<thead>
<tr>
<th>Rat</th>
<th>Relaxation Rate Constant of Both Optimized Curves for RCA (K, seconds)</th>
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<tbody>
<tr>
<td>1B</td>
<td>K1 28.0300 3.3565</td>
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<tr>
<td>2A</td>
<td>K2 45.9525 4.5615</td>
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FINDINGS/ FUTURE PLANS

Our findings indicate:

1. Using rat PAP smear tests, we were able to determine which stage of the cycle the rats were at a given day and time and therefore determine what levels of hormones were present, either high estrogen or high progesterone. Using these tests we determined rats 1B and 2A were both in the stage of their cycle where high levels of progesterone were present the day of sacrifice.

2. Using Electrical Field Stimulation, we were able to contract rat caudal arteries isolated from rats 1B and 2A where they were at high progesterone levels of their cycles. Using Sim Physiome Modeling software, we were able to optimize our raw relaxation data collected from healthy multiphase software.

3. Relaxation rates calculated for rats 1B and 2A slightly differed even though both were in high progesterone. Experiments still need to be repeated and replications tested for statistical significance of these results.

In the future, we plan to fit more relaxation changes to a curve using the optimization function of Sim Physiome Modeling software. We also plan to use more rats in high estrogen and also collect more data on rats in high progesterone for better statistical data. Experiments still need to be repeated and replications tested for statistical significance of these results.

REFERENCES


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