

ALTERATION OF COREPRESSOR SMRT IN MICE

ORIGINAL RESEARCH

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ABSTRACT

Nuclear hormone receptors can repress transcription through their interaction with corepressor complexes, such as silencing mediator of retinoid acid and thyroid hormone receptor (SMRT). SMRT is a corepressor that associates with vitamin A and thyroid hormone receptors. SMRT's silencing ability can result in a condensed chromatin state that is inhibitory to transcription. Therefore, it is important in regulating thyroid hormone actions such as metabolism, development and differentiation of all cells of the human body. In this study, the effect of SMRT in neonatal mice development using genetically engineered mice whose SMRT gene had been turned off through a gene knockout was analyzed. Based on the data collected, it was found that SMRT seems to play some role in the regulation of thyroid actions, most notably the electrical activity of the heart.

HYPOTHESIS

THE EARLY DEVELOPMENT OF THE HEARTS OF KNOCKOUT NEONATAL MICE SHOULD BE SIGNIFICANTLY AFFECTED DUE TO THE OVEREXPRESSION OF THE THYROID HORMONE AS WELL AS VITAMIN A.

DISCUSSION

Results might not be enough to make a solid conclusion because of an insufficient number of trials were performed. However, results seem to indicate that removal of the SMRT gene has a significant negative effect on the development of neonatal mice's hearts.

Based on the electrocardiogram (EKG) results, the general shapes of the EKG diagrams of the Heterozygous (Het) group mice and the Wild type (WT) group mice were very similar. The averages of P wave, PR interval (from the beginning of the P wave to the beginning of the QRS complex), QRS complex, Q, R and S waves (if present) as well as the J point, and T wave from these two groups of mice displayed differed only by 1 or less for all the waves and intervals values except for P_{on} (1.421 difference), Q_{on} (2.3649 difference), J (4.523115 difference), and the QT interval (4.3517 interval). However, when compared with Knockout mice (KO), both the shapes of the EKG diagrams and the values of waves and intervals showed significant difference. KO Mouse #8 did not even have a measurable P_{on}, P_{max}, P_{off}, PR_{or} Q_{on} value, which are all supposed to be present in a normal heart beat. This indicated that the mouse has heart dysfunctions, a condition where there is abnormal electrical activity in the heart which may lead to cardiac arrest and sudden death. Although some of the averages of the waves and wave interval values from KO group of mice were relatively similar to that of the average of WT and Het group of mice, the standard deviation value in the KO mice was much bigger (range from 11 to 40 in KO group mice, and 4

to 12 and 3-13 in WT group mice and Het group mice respectively), suggesting that the waves and wave interval values in the KO group were dramatically different from mouse to mouse. For example, in the KO group, mouse #5 had an 8.5 P_{on} value which much lower than the normal average whereas mouse #12 had a 24.8 P_{on} value which is much higher than the normal average. This is perhaps due to the over corrections or compensations of insufficient repression of thyroid hormone function through other pathways. Since all 3 KO mice demonstrated abnormal electrical activity in the heart, data proposed that SMRT may play a significant role in the development and functions of the heart in neonatal mice. However, only 3 KO mice were tested, therefore the results were not sufficient to fully support the hypothesis. For the same conditions, compared to the WT group and Het group mice, KO group mice also showed lower weight, decreased glucose levels, and slightly higher lactate acid levels.

During the course of experimentation, several assumptions were made. First, it was assumed that different genetic makeup had an insignificant affect on neonatal heart development and functions in comparison to the presence or lack of the SMRT gene. Since mice from 3 different litters of mice were tested, there would have been differences in other genes, not only SMRT. Even for mice born in the same litter, there should be some differences in their genetic makeup, which could also contribute to differences in the electrical activity of the mice's hearts as well as glucose, lactate and weight. Additionally, different

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degrees of effectiveness of the SMRT corepressor gene or different degrees of response to the same amount of hormones, causing differences in the electrical activity of the heart, glucose, lactate acid levels and the body weight. If the mice's different genetic makeup caused significant differences in values, then it would mean results from different mice cannot be compared since other factors besides presence or lack of the SMRT gene also affected EKG data. Although the knockout mice have very different results from the averages from both the heterozygous and the wild type mice, it may be due to other genetic mutations and not necessarily the lack of SMRT.

A second assumption made was that differences in the amount mice were fed beforehand by their mothers did not contribute significantly to differences in electrical activities of their hearts. It is known that diet will affect EKG results as different levels of different nutrients may cause variable amounts of electrical activity in the heart. However, the mice tested were not all fed, and even those that were fed were fed differently. Neither mouse #9 and 10 were fed, implying that the mother did not feed them after giving birth to them. Similarly, mouse #11 and 12 were not fed either. However, since Mice #13-15 were all fed, and came from the same litter, it is unknown why the mother did not feed Mouse #11 and 12. Additionally, for the mice that were fed, it was assumed that the milk of their mothers had a similar composition. Otherwise, different levels of specific nutrients will also cause differences in EKG values. However it was assumed that mice would have consistent EKG results despite differences in amount and type of food consumed.

A third assumption made was that the other electrical sources present did not affect EKG values. It is known that the Genetic Expressions Laboratory contains a lot of machinery, and that the EKG machine is placed right by electrical wires. If the EKG results were significantly affected by other electrical sources, then all of the values, except the intervals, were too high. Also, since not all of the trials were performed on the

same day, different amounts of interference would have been present in EKG data, thus making averages high than they should be. Additionally, the trials performed on the KO mice were performed on two different days, so comparing their EKG values to the average EKG values from all four days for the other two genotypes would be inaccurate, since there would be a different normal range of wave and wave interval values.

Additionally, body tremors and shivering will add new electrical signals, which also will be recorded by the EKG machine. Because some mice were struggling, EKG was taken after a period of 5 minutes or more after mice were removed from the heating pad. The heating pad was about 35°C, while room temperature is about 22°C-23°C. Since there is over a 10°C difference in temperature, the mice may have been shivering which would have added new electrical signals, thus making the results differ from expected results.

Another source of error was that the PCR materials, including dNTP, primers and Taq polymerase, were stored at 4°C. Ideally, they should be stored at -20°C for maximum stability. However, a small amount was stored in the 4°C for use by the student. This could have affected PCR results as the PCR amplification was not as efficient as it could have been due to degradation of the Taq polymerase enzyme, dNTP, and primers. PCR results would be more blurry and not as clear, making it harder to determine the genotype of the mouse. Since the genotype of the mouse is determined by the location of the bands on the Agarose gel, it may be possible that some mice were given the incorrect genotype, due to the bands not being distinct enough, and possibly being confused with each other. For example, heterozygous mice are supposed to show two bands, located relatively close to each other. However, if the bands were too blurry to see both bands, they may have been given a KO or WT genotype instead.

Accuracy of genotyping is also an important improvement in technique that could be made. When electrophoresis gels were analyzed, there was always some smearing of DNA, indicating that the PCR product was not very accurate. In order to increase accuracy, better pipetting skills could be learned. Also, vortexing all materials before use would help to increase accuracy as it ensures the correct amount of the correct concentration of a solution is being used.

In addition, a possible problem that occurred during genotyping was not collecting a large enough

portion of tail or ear from the mouse, so that genotyped results did not show up clearly or at all. For future labs, cutting about 1 cm of tail works the best, while a little more than an ear punch of diameter around 0.5 cm is sufficient. Following these measurements have been shown to produce the best results when genotyping.

Additional observations made were that mice #11-12 had dark red skin color. Mice #11-12 also both displaced comparatively very high lactate values at 16.5 and 13.1 respectively. Lack of oxygen is the most probably explanation for the dark red skin color, and high lactate values. Mouse #11 had most EKG values greater than the average value for mice of the same genotype, Heterozygous, except for P_{on}, P_{max}, and P_{off} values. Mouse #12 had values that were greater by 10 or more, except for the PR interval, PR segment and QRS interval values (PR interval was greater by 8.223 compared to WT mice average, and 7.521 compared to Het mice average, PR interval was greater by 7.5923 compared to WT mice average and 8.3955 compared to Het mice average, while QRS values differed from WT and Het mice average respectively by 0.1111 and 0.06252). Because both mice that seemed to be suffering from a lack of oxygen and both had higher EKG values, there seems to be a correlation between lack of oxygen and greater electrical activity of the heart. In order to further investigate this possible correlation, EKG's can be performed on people with similar physical characteristics. By first taking the resting EKG of the group of people, and then recording EKG values after strenuous exercise, the correlation between lack of oxygen and EKG values can be investigated. If after exercise, EKG values increase, then there seems to be a negative correlation between amount of oxygen available and EKG values.

Furthermore, it is noted that when mouse #15 was dissected, its brain appeared more red in contrast to the pale pink color of the brains of the other mice. It may have been brain hemorrhaging. However, mouse #15 did not have significantly different values both for lactate and glucose level as well as electrical activity values. This implies that brain hemorrhaging took place after the EKG was performed. A brain hemorrhage would've resulted in a huge spike in adrenaline, which would've lead to a spike in electrical activity of the heart since the SINO-ATRIAL node is regulated in part by adrenaline.

In order to reconfirm results, a different species of mice could be tested. For this experiment,

only black lab mice were tested. In order to confirm results, performing the same experiment using albino mice should yield similar trends. Normal ranges of electrical activity as well as glucose and lactate levels will differ due to different genetic makeup. However, knockout mice should still display abnormal electrical activity values and glucose levels on the lower end of the normal glucose level range.

Future research that can be performed to gain additional information include dissecting the mice and examining their brain, lungs, and other vital organs. The basic physical makeup and shape of the organs should be compared. Since results are inconclusive and it is known that knock out mice typically do not survive past two weeks after birth, it is possible that SMRT is critical for the development of other vital organ(s). Thus, by examining the other organs of the knockout mice and comparing them to the organs of heterozygous and wild type mice, it can be determined whether SMRT significantly affects the development of any other organ(s).

