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The role of FGF2 in stress-induced cFos expression

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Abstract. During periods of stress, organisms' endocrine systems respond by releasing glucocorticoids which later get expressed through corticosterone, simplified as CORT. CORT is proved to express FGF2, proteins found in critical glial cells named astrocytes. Increased FGF2 expression leads to higher rate of cell proliferation - including neurons. There's been discoveries that newborn neurons, specifically those who have been exposed to FGF2, led to early neuron activation and enhanced neuronal function. What's unclear is whether FGF2 played a role in somehow newborn neurons having enhanced function and activating earlier than usual (by releasing the early activation gene cFos). An experiment has been outlined in order to test the hypothesis that FGF2 does indeed play a role in altering some neurons. Using nanoparticles containing the micro RNA of NUDT6 - a gene that suppresses FGF2 expression - to target FGF2 in adult male Sprague-Dawley rats would directly test if FGF2 played a role in enhanced neuron activation.

Keywords: Fibroblast Growth Factor-2, Corticosterone, Nanoparticles, Nudix Hydrolase 6, Acute Stress

1. Introduction

The release of glucocorticoids is a classic endocrine response to increase of stress. Increase in glucocorticoid levels could help regulate glucose levels during stress through fat breakdown stimulation, balancing glucogenesis and amino acid mobilization [1]. Glucocorticoids actions are usually expressed through GRs (glucocorticoid receptors), and corticosterone (CORT) acts as a glucocorticoid that regulates the body in multiple aspects and acts on nearly all cells in the human body. CORT is usually expressed in vast amounts during acute stress [1].

CORT has been proved to increase FGF2 (Fiberblast Growth Factor-2), a type of protein that are expressed by astrocytes, glial cells that are essential to the neural environment [2]. Immobilization, a form of acute stress, and the injection of CORT of 40 mg/kg into an adult male Sprague-Dawley rat actually increased FGF2 mRNA by 1.59 and 2.54 folds [1].

Unsurprisingly, cell proliferation of progenitor cells has also been affected by the changes in CORT and FGF2 expressions. CORT-treated astrocytic media has increased the percent of progenitor cells by a 1.52-fold, which is a huge rise from the original amounts of cell division [1]. This data indicates astrocytic FGF2 is the direct and the main factor contributing to the CORT-affected proliferation of progenitor cells. Cells tested in this experiment have been marked with a BrdU+ injection marker, which makes it easy to differentiate when investigated confocally.

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Newborn neurons in an adult male rat usually takes 2 weeks or more to physically become active and become “mature”. That suggests that the optimal time to test and measure the stress-induced neuron cells marked with BrdU+ is right after 2 weeks. However, after measures and research, it was discovered that non-stress related newborn neurons and stress-induced neurons have similar features and functions, except that the BrdU+ marked stress-induced newborn neurons have generated and expressed more amounts of the Immediate Early Gene, cfos [1,3]. This directly suggests that there has been greater activation in the stress-affected rats (immobilization and other types of acute stress), causing enhanced neural function and integration into circuits than neuron cells that has not been affected by stress. This enhanced activation has later on led to research about it having later memory benefits [4].

Research suggests that there isn’t a factor yet that has discovered the driving signal for this enhanced activation of newborn BrdU+ neurons. There are multiple neurochemicals that we could consider; CORT may have had reactions with the newborn neurons. Astrocytic FGF2 might have had effects on the BrdU+ cells during the 2-week period which is highly likely. I hypothesize that astrocytic FGF2 plays a role during the 2-week period where it has affected the newborn neurons and its enhanced integration and function.

2. Method

2.1. Design

Since the topic revolves around testing the effects of astrocytic FGF2 during the 2-week period where newborn neurons are maturing, the possible results that could help determine this is the cfos level that the newborn neurons are expressing if astrocytic FGF2 could possibly be limited. Different cfos levels show whether if newborn neurons are sending signals that they have been early activated. If FGF2 is not part of the picture anymore, the investigation is based on the cfos level that new neurons express that helps determine whether FGF2 was a direct factor in this mystery or not.

The experiment will be conducted on adult male Sprague-Dawley rats, and they are fed and regulated on a 12-hour light and dark cycle, with lights on at 7 am. This is to help mimic a natural ecosystem [5].

Some procedures that will have to be taken before the experiments begin. Culturing of hippocampal progenitor cells and culturing of astrocytes are essential, as it proves that proliferation and FGF2 expression is based purely on the cause of CORT, and not external factors that might cause FGF2 expression and progenitor cell proliferation [6].

2.2. Injections and Stressors

One of the more crucial chemicals needed in this experiment is the BrdU+ marker. This helps mark the difference between stress-induced cells and normal cells that hasn’t been affected by stress or CORT. BrdU+ (5-Bromo-2’-deoxyudrine) is dissolved in saline and will be injected into the test subjects approximately 3 hours after activating the stressors [6-8]. The stress-induced cells then could be analyzed confocally after being marked with BrdU+.

Stressors in this experiment all involve producing acute stress for the test subjects. The three stressors that this experiment will adapt is footshock, immobilization and a novel environment (does not include a controlled group of rats) [1]. Those 3 stressors all had their own respected times where it was acted upon on the test subjects. In usual cases, the test subjects were left upon and undisturbed.

Test subjects will also have a fear conditioning chamber [1]. It is the location where the test subjects experience the stressors; however, it is beneficial to return them to the chamber every day for 10 minutes without shock in order to generate acute stress without continuously providing them with vast amounts of stress.

2.3. FGF2 regulation

One chemical that has the gene to regulate FGF2 expression and its cycle is NUDT6, a gene that is actually expressed with FGF2 in C6 rat glioma cells. It has been known that the NUDT6 gene encodes a protein that somehow suppresses the expression of FGF2, and the micro-RNA of NUDT6 has been

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implicated in FGF2 suppression [2]. It was later discovered that knockdown of either FGF2 or NUDT6 would cause a significant rise in the amount of the other complementary chemical, meaning they hold an inverse relationship with each other. Reciprocal effects were also observed on a protein level, meaning the two chemicals have two transcripts that are regulatory of each other. This is why NUDT6 is an excellent choice here to serve as the chemical that limits and regulates FGF2 expression and function during the 2-week period.

The experiment will be conducted using a relatively new source of "precision" delivery system using nanoparticles. Nanoparticles (NPs) are artificially assembled particles that are usually 1-100 nanometers in diameter and have been developed medically in order to deliver therapeutics or other type of chemicals or compounds into the human body [9].

Nanoparticles has been developed to overcome biological barriers – systemic, cellular, and environmental. NPs has now entered the era of precision medicine, diving into a system where it could possibly deliver chemicals to a desired area and cell. Chemicals, compounds, microRNA, and genes could be delivered as they are "encapsulated" within the nanoparticle core, trapped inside the polymer matrix of the NP [9,10]. NUDT6 could either be chemically bonded or "conjugated" to the NP or just bound to the surface of the NP [10-12].

The reason NPs will be utilized is straightforward; there is a need to regulate and limit the expression of FGF2 in this experiment, and NPs are precise in where they deliver their chemicals – more precise than many other methods that could potentially deliver chemicals to regulate FGF2 (viral delivery and peptide-based delivery). NPs will need to carry a specific chemical / microRNA that limits the function of FGF2 and the expression of the chemical, and NP will attach NUDT6's microRNA within its matrix and deliver it to the FGF2 produced in the body by astrocytes [12].

2.4. Experiment 1

The first experiment focuses with adult male Sprague-Dawley rats as test subjects and attempts to measure the differences in the Immediate Early Gene "cfos" between two groups. The reason cfos is measured is due to the fact that it signifies if neurons had early activation, and the degree of how early it has activated than normal newborn neurons (not stress-induced). Treatment needed here includes nanoparticles with NUDT6 microRNA within the polymer matrix of the NPs or bound to the surface of the NPs. BrdU+ markers are also needed.

The experimental group here goes through a procedure with the regulation of FGF2. The control group contains 15 adult male Sprague-Dawley rats that have been regulated on a 12-hour light and dark cycle with regular food.

The test subjects in the experimental group will have to place in fear-conditioning chambers to go through 2 types of stressors to produce acute stress – footshock and immobilization. After 30 minutes (footshock) and 3 hours (immobilization), test subjects have to be injected with a BrdU+ marker to make sure that the stress-induced progenitor cells would be recognized confocally. Limiting FGF2 would be the next step, so using inhalation or IV to let the NPs (containing NUDT6) travel through the body of the test subjects targeting the astrocytic FGF2 would achieve that. The amount of NUDT6 micro-RNA would be decided upon the amount of FGF2 expressed after the test subjects experience acute stress. After FGF2 has been regulated, a 2-week wait would allow the newborn neurons to properly mature. After the 2-week wait, cfos level should be measured, as it would signify whether the neurons had early activation.

The control group goes through the same procedure with the same amount of test subjects (15 rats), except it does not need nanoparticles to help regulate FGF2. The goal for the control group is to generate a cfos level with the FGF2 playing a role.

The final product of this experiment is the difference of the cfos levels expressed after the 2-week period. That's essential, since if the cfos level where FGF2 was regulated was higher than where FGF2 wasn't regulated (if the experimental group's cfos level was higher), that would implicate FGF2 had no effects on the newborn neurons during the 2-week period where the neurons are maturing.

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2.5. Experiment 2

Experiment 2 thoroughly investigates the cfos level expressed between a control group and an experimental group, however on different test subjects. Progenitor cells in culture will now be the test subjects, as it would significantly reduce the possibility of external factors allowing FGF2 to keep expressing.

The experimental group contains progenitor cells in culture. Injecting CORT into the progenitor cells in culture would mimic an environment where there is acute stress; BrdU+ markers will still be injected as the cells would now be "stress-induced" and would later have to confocally analyzed. Nanoparticles will have to be delivered into the cells in culture containing the microRNA of NUDT6. After 2 weeks, cfos level would have to measured. The control group has similar procedures except there is no delivery of NPs, as FGF2 is not to be limited or regulated. cFos levels will have to be measured at the end of two weeks.

3. Expected results

The expected results will align with my hypothesis, being that FGF2 had a role on the newborn neurons during the 2-week period. cFos level in the experimental groups would likely be lower than the cFos levels where astrocytic FGF2 wasn't regulated. As shown on the table and on the bar chart, the control groups (con 1 and con 2) are expected to have higher cFos levels than experimental groups that allowed FGF2 regulation.

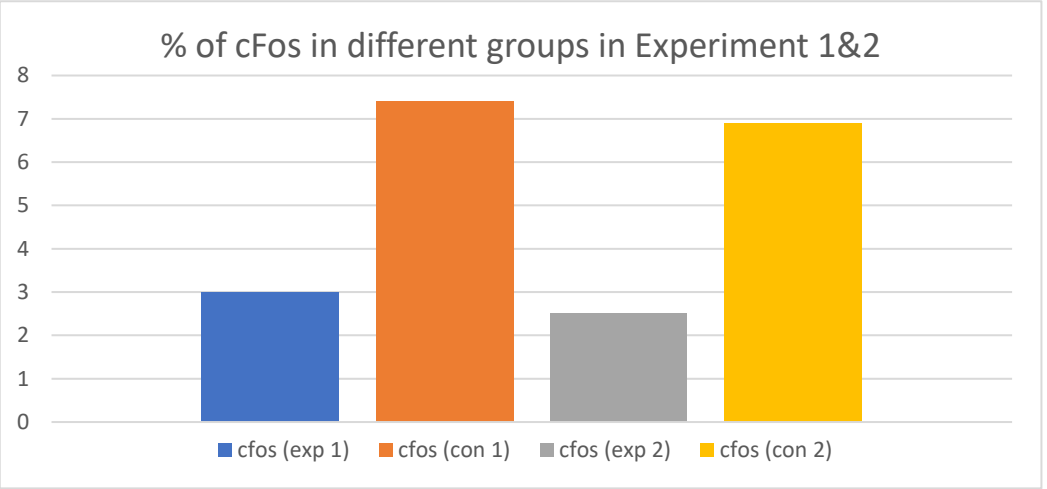

Figure 1. % of cFos in different groups in Experiment 1&2

Table 1. Estimation of cFos expression for all experiments

	Amount of NPs containing NUDT6	Cell Proliferation (BrdU+ cells per **μm^2)**	FGF2 expressed	cFos expressed
Exp 1	varies	stress-induced	decreases	low
Con 1	varies	stress-induced	natural	high
Exp 2	varies	CORT-induced	decreases	low
Con 2	varies	CORT-induced	natural	high

* "varies" is based on how many FGF2 is naturally produced due to stress

4. Discussions and implications

It was hypothesized that FGF2 played a role during the 2-week period where newborn neurons were maturing, and FGF2 was the main factor why cFos level was high after the 2 weeks. High cFos level meant that the new neurons had early activation and enhanced integration than normal neurons which did not get affected by stress. My expected results align with my hypothesis.

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Enhanced neuron integration and early activation of the newborn neurons have been researched to show memory benefits. If this fact was being used to its advantage, it would bring an impact to the medical field.

However, there are many investigations yet to be made surrounding this topic. It was brought up that since both FGF2 and NUDT6 were found in rat glioma cells, NUDT6 may already play a role in the process of enhanced cell activation. This role is unknown, but there could be experiment conducted; testing the NUDT6 level in test subjects and regulating it to see if cFos levels have been changed. It was also discovered that NUDT6 and FGF2 holds such a close biological relationship that knockdown of either one chemical could have the potential to decrease cell proliferation, which might cause further research of this topic to be extremely difficult. There are also mysteries surrounding what exactly FGF2 does during the 2-week period. After knowing it plays a role, it's difficult to research what exactly the role is and the molecular and cell science behind it.

5. Conclusion

The conclusion should be started by going over the logic of background information and how it leads to the experiment. Acute stress causes release of glucocorticoids – and glucocorticoids help release corticosterone (CORT) which has been proved to increase astrocytic FGF2 levels. Cell proliferation of NPCs (progenitor cells) has been found to become affected by FGF2 levels, therefore also being affected by CORT. Further research has been done to show that during the 2-week period where neurons are nearing maturation, the early activation gene cFos has been expressed. The question was either or not CORT-affected FGF2 (also could be seen as stress-induced FGF2) had anything to do with this early and enhanced activation of neurons. Experiments have been outlined that would adapt nanoparticles containing the FGF2-suppressing gene NUDT6 to travel in adult male Sprague-Dawley rats (under different types of stress). In the end, researchers will test and measure the amount of cfos expressed in order to determine whether stress-induced FGF2 has been a factor or not.

It has been hypothesized and predicted that cFos would indeed have a higher expression when the 2-week neuronal activation week is over. This implies that FGF2 expression is indeed the direct factor that leads to early cFos expression. The bigger picture now implicates that acute stress would in fact lead to earlier neuron activation, giving access to organisms for potential memory benefits. Experiments on whether memory benefits do exist or not due to early neuronal activation are still under further conduction and research.

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