Introduction

Alcoholism and alcohol abuse is wide spread in the United States. Like any addictive drug it has many adverse health and societal effects. Because of the wide spread effects of alcoholism it is not surprising that a large amount of research has been done to attempt to understand how alcohol affects the brain and body. In a not-yet-published meta-analysis, Dr. Michael Miles used a scoring algorithm to integrate multiple alcohol related data-sets and generate a list of genes involved in response to alcohol cross species. About 15 of the top candidates from this list, which integrated "microarray, linkage, and single gene studies from human, mice, Drosophila and C. elegans" are being inserted into viral vectors in the Miles lab. Construction of the vectors is part of a large project involving the viral infection in mice mouse brain and subsequent behavioral studies (Webb et al. 2008).

A portion of this project is interested in the genes GADD34 (growth-arrest and DNA-damage-inducible protein 34) and PERK (protein kinase RNA activated-like endoplasmic reticulum kinase). Both of these genes work to regulate the phosphorylation state of eIF2α (eukaryotic initiation factor 2α). Eukaryotic initiation factors are responsible for the initiation of mRNA translation. eIF2α is a subunit of the eIF2 complex which mediates the first step of translation initiation: the attachment of the initiator methionine-tRNA to the 40S ribosomal subunit (Lang et al. 1999). Gomez et al. demonstrated that the "inhibition of eIF2α phosphorylation in glucose-deprived cells by the overexpression of dominant-negative PERK or an N-terminal truncation mutant of GADD34 leads to a 53% increase in the rate of total protein synthesis" (Gomez et al. 2008)/

Eukaryotic initiation factors are of interest to the study of alcohol because of their apparent involvement in ethanol craving. In rodents, the ethanol deprivation effect is used to model ethanol craving. The model in mice is ethanol is provided a period of time then the alcohol bottle is removed from the cage for a period of days. Upon the return of the alcohol bottle to the cage the mice will drink more than they previously did. A microarray study of the ethanol deprivation effect in mice revealed a change in eIF expression just previous to the return of ethanol to the cage compared with non-drinking mice (unpublished data from the Miles lab). Additionally, in two papers Lang et al. demonstrates that alcohol inhibits protein translation in both the muscle and liver. Therefore, it is hypothesized that if ethanol can affect translation rate, affecting the translation rate of protein in the brain could affect drinking behaviors.

Methods

Varying the rates of protein expression in order to study behavioral changes require protein expression to be varied in vivo. This requirement usually limits scientists to the use of knockout animals, drugs or RNAi. Knockout organisms are time consuming to develop partially because the absence of some genes early in development can results in death or the absence of the gene in every cell of the organism can results in death. Therefore, the emerging practice of using viral transfections fits well with the goals of this project, as it would be difficult to engineer multiple knockout organisms to study all of the genes of interest.

Gomez et al. were kind enough to send the Miles lab their dominant negative forms of GADD34 and PERK known as GADDΔN and PERKΔC. Many preliminary steps are necessary to prepare these inserts for injection into mouse brain. First, the GADDΔN and PERKΔC inserts will be cut with restriction enzymes and separated through gel purification before being ligated into adeno-associated viral vectors. After cloning the plasmid and using a DNA miniprep to purify it, a diagnostic digest will be used to check for the presence of the insert and that it has ligated into the plasmid in the correct orientation. Once a plasmid with the insert is identified in this manner, a portion of the plasmid will be sequenced to ensure that the insert present is in fact the one of interest. The next validation step, is to transfect AAV-293 cells with the plasmid and recover the mutant protein. A Western blot will indicate whether the protein being translated is the correct size and that it is being transcribed and translated at a high rate. Immunohistochemistry will be used to stain the cells for the presence of the protein of interest.

Once these validation steps are complete, first the GADDΔN plasmid will be grown in large quantities and purified using a maxi-prep DNA purification procedure. This plasmid will be transfected into cell culture along with two other plasmids (Helper and RC) that will package the plasmid into adeno-associated virus. Once this virus can be harvested at a high enough titer and level of purity for in vivo infection, the virus containing the GADDΔN plasmid will be injected into mouse brain. After allowing time for infection, a number of behavioral studies will be run on the injected mice including drinking behavior, ethanol deprivation, anxiety, stress and inherent reward. These mice will be sacrificed, and their brains will be stained using immunohistochemistry to check expression levels for the protein of interest.

Discussion

The use of viral vectors to modulate gene expression in vivo is a relatively new procedure and isn't used in many labs. There are about four types of virus currently being used for neuronal gene transfer known as HSV (Herpes Simplex-1 Virus), AAV (Adeno-Associated Virus), Sindbis Virus, and Lentivirus (Green 182). The decision to use the Adeno-Associated Virus was based on the facts that it is "naturally replication deficient, produces no inflammation response, readily infects neurons, and produces stable transgene expression for long periods of time" (Green 183).

The obvious benefit of using viral transfection is that it is possible to do in vivo and therefore behavioral studies are possible in addition to the more usual study of the effects of overexpression in cell culture. The Miles lab is working on a larger project that involves making viral vector constructs of many genes that have been highly implicated as involved with alcohol. Hopefully testing the over expression and under expression of these genes in vivo with the use of both constitutively active and dominant negative forms of proteins will allow for in vivo assessment of what effects these genes actually have on behavior.

Conclusion

This study could allow for the development of drugs that interact with one of the proteins of interest if the protein is found to illicit a desired behavioral response. More generally, the study will further the knowledge about how alcohol effects specific behaviors. Additionally it is important that more labs begin to use viral vectors in vivo as they have great potential for behavioral studies and wide spread use will allow for the refinement of the procedures.

References

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