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**Differential Expression of Tachykinin Receptor Isoforms by Cells of the CNS**

**Background:**

Infections that occur in the central nervous system can often be life threatening or lead to severe neurological damage. Bacterial, viral, and fungal infections that make their way into the CNS can lead to medical cases such as meningitis. Bacterial meningitis alone has about 4,100 cases and 500 deaths each year due (CDC). This damage from meningitis is largely due to the inflammation that occurs in the CNS. Even in cases where patients live, they often go on to live with severe neurological damage.

The inflammation has been shown to be regulated by pro-inflammatory cytokines and immunosuppressive cytokines. These cytokines have been shown to be controlled by substance P in its binding with the neurokinin-1 receptor, and further research has also shown that treating this receptor with an antagonist reduces the amount of pro-inflammatory cytokines in the system (Marriott). However, there is both a full length form of the NK-1 receptor and a truncated version. In others’ research, it has been shown that there is a varying amount of mRNA for the truncated and the full length form in different locations of the CNS and peripheral tissues in the human body analyzed after death (Caberlotto). This demonstrates a variability in expression levels of the two isoforms. Little is known though about the function of the truncated form, but as it also reacts with substance P, it could react in numerous ways, such as producing further inflammatory responses, conducting more long term responses in the system, or acting as a decoy receptor.

**Significance:**
Information on the expression of the isoforms can provide valuable insights towards the importance of each and how they affect the sensitivity of CNS cells towards infections. This information will further the understanding of the truncated isoform in CNS cells for their specific roles after infections, and more importantly can also give a much better understanding of the overall role of each isoform in relation to the other and the system as a whole. Furthermore, with these additional understandings, future treatments can be more easily created and tested to prevent the neurological damage and death that comes with CNS inflammations.

The knowledge of the levels of expression of each form can also help with understanding the environment that any drug or chemical meant for the NK-1 receptor will potentially interact, such as differences that may occur during an infection.

Finally, any evidence found that correlates to a variable expression of the mRNA versus the actual protein for the full length of the truncated form can help in identifying regulation pathway components in future research. If there is a difference between them in certain environmental conditions, it could indicate that one is more important in the regulation than the other and give ideas of where to look towards pathways.

**Question and Outcome:**

In my research, I would like to look at the variable expression of the two isoforms of the NK-1 receptor in CNS cells over time after infections, to different levels of substance P, various bacterial challenge types, and what roles the truncated form of the NK-1 receptor has in inflammation.
At this time, it is known that the truncated NK-1 receptor isoform has a lower affinity for substance P than the full length. Because of this, it is expected that a higher amount of substance P will be required for response from the truncated NK-1 receptor. Once a high enough amount of substance P is reached, it is expected that an up-regulation of the truncated NK-1 receptor will occur. Based on past research in our lab, it is expected that an overall up-regulation of the NK-1 receptor will occur in microglia, but a large portion of that I expect to be attributable to the truncated form of the receptor. Furthermore, it is an expectation that the truncated isoform will act as an opposing action towards the full length’s inflammatory responses by either acting through opposing signals or as a decoy receptor. This is expected because of the low affinity of the truncated form for substance P, which lends the ideas that a high amount of substance P would be required to activate through this receptor and that it is likely associated with regulating the reaction from potentially overproducing the pro-inflammatory cytokines.

**Methodology:**

The main difference in the truncated NK-1R is that it is shorter on the cytoplasmic tail, but it transverses the membrane the same and has the same extracellular tail. Because it is shorter, I can use western blots with fluorescence tagging to test for the expression of the truncated NK-1R in different scenarios. In the western blotting tests, there are two antibiotics that can be used to detect the cells. One antibiotic can attach to the extracellular tail, so it can detect both isoforms. The other antibiotic only attaches to the cytoplasmic cell, so it can be used to detect the amount of full length NK-1 receptors. The difference of the two can be taken to determine the expression levels of the truncated NK-1 receptors. There is also a difference in the amino acid size of the isoforms, the full length being 407 amino acids long and the truncated
form being 311 amino acids, which will allow the visualization of two separate lines in the western blot analysis (Lai). This data can also be verified with RT-PCR, which can further quantitate the amount of mRNA that is being produced by the CNS cells for each isoform of the receptor as the length and genetic code of both forms is known.

Citations


