British Sjögren's Syndrome Association

Originally published in the Summer 2010, Volume 25, Issue 2 of Sjögren's Today magazine

Inflammation, Nitric Oxide and Sjögren's Syndrome

By John Malone, PhD student, Liverpool Dental Hospital

Everybody is familiar with the effects of inflammation, as wasps, bees or nettles have at one time or another stung us all. Following a sting, the area becomes hot, red, swells-up, and hurts. The heat and redness are caused by an increase in blood flow to the affected area. The swelling is the result of accumulation of fluid in the affected tissue, and the pain is a result of the nerve endings being stimulated. This familiar sounding scenario is acute inflammation and it is the body's most basic immune response. Once the source of the inflammation has been removed the area heals, and the inflammation disappears.

Sjögren's Syndrome is an autoimmune disorder, in which inflammation is triggered by a sufferer's own tissues. In this case, as the body cannot remove its own tissues, the inflammation does not go away. Chronic inflammation can lead to permanent damage and to the presence of additional immunologically active cells in the tissues. Some of these cells usually 'eat up' foreign cells, some secrete antibodies and some secrete chemicals that are designed to damage and destroy their targets. One of these secreted chemicals, the focus of our current research, is nitric oxide.

Nitric Oxide (NO) is a gas which is an environmental pollutant, present in car exhaust and the emissions from coal fired power plants. However, it is also produced by the human body and it is vitally important in a near endless list of processes including: communication within and between cells, regulation of blood flow, and the immune response. In fact, the 1998 Nobel Prize for Medicine was awarded to Furchgott, Ignarro and Murad for their 'discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system'.

What exactly is nitric oxide? NO is a very small gaseous molecule with the chemical formula NO, that is, it is made up of one atom of Nitrogen and one atom of Oxygen. It should not to be confused with nitrous oxide (or 'laughing gas', formula:N20), used as an anaesthetic. The fact that nitric oxide is a small gas molecule allows it to move (diffuse) through the cells and tissues of the body rapidly, which is tremendously important in its role in cell to cell communication.

But what does NO have to do with Sjögren's Syndrome? In 1999 data were published noting was an increased level of NO in the expired air of individuals with Sjögren's Syndrome. Since NO is important in the process of tissue inflammation, it seems likely that the inflammation observed in Sjögren's Syndrome could be related to the increased levels of NO. We have set out to determine whether NO and the inflammatory response might have a direct effect on salivary secretion.

The first step in understanding the reasons why, and how, salivary glands cease to function in disease is to understand how they work normally.

The cells in your glands need to be told to secrete the fluid which becomes saliva (Figure 1). These orders are transmitted from the brain down your nerves to the cells of the salivary glands. The nerve ends secrete a neurotransmitter called acetylcholine (ACh) - think of this substance as a key which, by binding to specific receptors on your cells, can unlock fluid secretion. Through ACh, your nerves start a complex series of reactions leading ultimately to an increase in the calcium concentration inside the cell. The increase in calcium causes salt (sodium and chloride) to move from the blood into the salivary glands and the salt drags water with it. This water, plus some important proteins also secreted by the glands, is released into the mouth as saliva. Anything which disrupts the capacity of the nerves to stimulate the glands, or which affects the response of the cells to stimulus will have an effect upon the amount of saliva released by the glands. Over the last few years, we have thought of several possible places where things could go wrong.

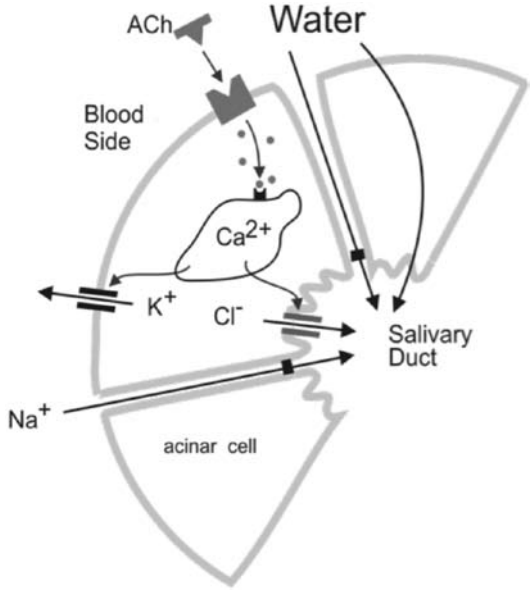

Fig. 1
Standard model of salivary secretion. Acetylcholine (ACh) released by nearby nerves binds with the surface receptors. The activated receptors in turn release secondary messengers inside the cell to release calcium (Ca2+) from the cell's stores. This increase in calcium triggers the movement of salt (NaCl) into the secretory ducts, forcing the movement of water along with it.

Effects of Nitric Oxide on Salivary Glands

Anything that affects the cell's ability to respond to a stimulus will affect the ability of a cell to secrete fluid. Nitric Oxide has the potential to do just that. While brief exposure to NO can stimulate salivary gland cells to secrete fluid, a prolonged exposure appears to be too much of a good thing and we have shown it to have a detrimental effect on the response of the cells to stimulus. We understand how NO can cause an

Continued next page

initial increase in secretion (it's complicated), but we are still investigating possible ways in which continuous exposure to NO may actually damage key players in the secretory pathway.

Two of the ideas that we have under study both involve receptors.

Receptor Internalisation (Figure 2A) : probably sounds very complicated, but it isn't. Going back to the lock and key analogy mentioned earlier for the neurotransmitter and the receptor, a key is only of use to you if there is also a keyhole. One possibility is that increased levels of nitric oxide can remove keyholes or, more precisely, cause the receptors on the outside surface of the cell to be moved inside where the neurotransmitter can't possibly reach them. If there is no activation of the receptors, there can be no secretion of saliva.

Receptor Alteration (Figure 2B): The pivotal step in fluid secretion is the release of calcium held inside stores locked away in the cell. There is a different 'lock' and a different 'key' than the ones mentioned previously, but the principle is the same. Nitric oxide has the potential to subtly alter the structure of the 'locks' that keep calcium inside the stores - much like somebody bending some of the pins inside the lock mechanism thus rendering the key useless. If there is no release of calcium inside the cell, there can be no secretion of saliva.

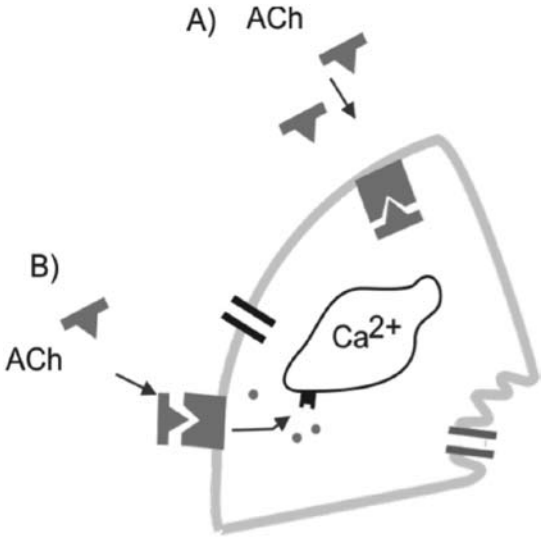

Fig. 2
A) Nitric Oxide has been seen to cause receptor internalization. Acetylcholine released by the local nerves is no longer able to reach, and therefore activate the receptor. While the receptor itself is capable of function, the signal from the nerves is unable to trigger the receptor's function.

B) Nitric Oxide may change the very shape of receptors. The receptor is still present, but the changed receptor shape prevents the messenger from activating the store's receptors, in effect blocking the message for calcium to be released from the stores.

The consequence of both of these scenarios is the lack of liberation of calcium from the cell stores, and therefore the loss of secretory function.

The BSSA funded research I am currently carrying out focuses upon the role of Nitric Oxide in lacrimal gland hypofunction, observed in individuals suffering from Sjögren's Syndrome. Why lacrimal glands? Well, both salivary and lacrimal glands are affected by Sjögren's Syndrome and the mechanisms of secretion of saliva and of tears are similar but not identical. If lacrimal gland cells respond to NO in the same way as salivary glands then we will be able to conclude that we have found a common factor in the disease and this could be an important step in developing more effective treatments for the condition. So far, the results are interesting. The effect of NO upon lacrimal glands looks very similar to that we have seen before in salivary glands - an initial increase in response, followed by a decline and eventual complete abolition of secretory response.

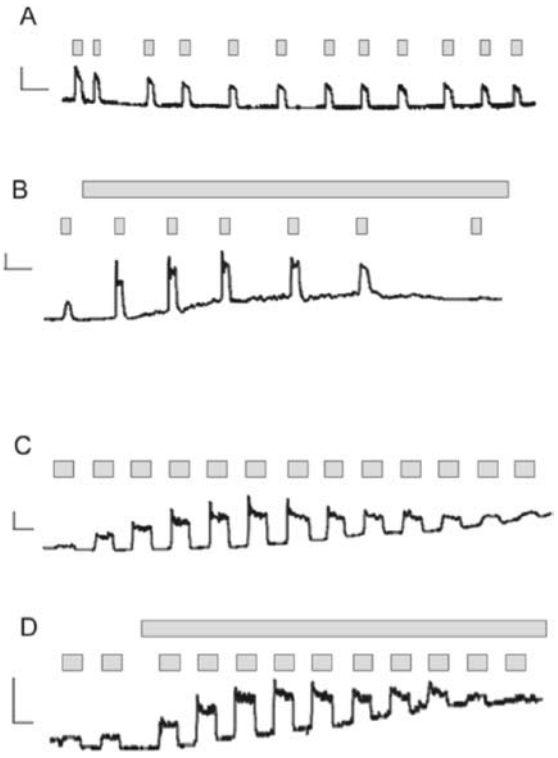

Fig3.
Data from our lab showing levels of calcium inside the cell release upon stimulation with ACh in the presence an absence of added Nitric Oxide.
A) Calcium inside mouse submandibular acinar cells, repeatedly stimulated with ACh (blocks show stimulation points).

B) Mouse submandibular acinar cells, repeatedly stimulated with ACh (blocks) in the presence of NO (solid bar). The cells initially show an increased response, followed by a rapid decline in response.

C) Mouse lacrimal acinar cells, repeatedly stimulated with CCh.

D) Mouse lacrimal acinar cells, repeatedly stimulated with CCh in the presence of NO.

Unfortunately, nothing in research is ever simple and we have also noticed that, under certain conditions, lacrimal glands may be producing their own NO. This in itself raises more questions, but the identification of the small differences between the glands may yet be an important piece of the Sjögren's Syndrome puzzle. For example, our data suggest that glandular hypofunction in Sjögren's syndrome is likely to be caused by a number of factors. Perhaps this is why some treatments work better for some people than for others. Identification of the different causes of Sjögren's Syndrome could bring about more effective, tailored treatments for sufferers. Tailored treatments should have a better chance of success and be less likely to produce unwanted side effects. While we don't have all the answers yet, and we still don't know exactly what mechanism brings about this detrimental effect upon the glands, each experiment is another piece in the puzzle that may one day provide better treatments for the dry eye and mouth experienced by Sjögren's Syndrome sufferers.

Summary
- Nitric Oxide is important in the process of inflammation.
- Increased NO levels have been observed in the exhaled breath of Sjögren's sufferers.
- We have shown that increased levels of NO can have a detrimental effect upon the functioning of salivary and lacrimal glands.
- The exact mode of NO damage is unknown at the moment, but has the potential to be a major part of the aetiology/ pathology of the disease, and a possible target of future therapies.