# Unmasking the roles of the tachykinins in the human brain

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## Abstract

The tachykinins are a family of peptides that have biological actions in the human brain. They are implicated in the control of several autonomic, affective and higher cerebral functions and in the pathophysiology of some neurogenerative and psychiatric disorders. We have discovered new members of this group of peptides that are also active in the brain. Here, we review our current understanding of the roles of the tachykinins as neuro- and immunomodulators in the brain.

## Introduction

The tachykinins are a family of bioactive peptides that includes the neuropeptides substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) (Page, 2006a). By definition, the tachykinins are classified in the same peptide family due to their common C-terminal sequence of Phe-X-Gly-Leu-Met-NH$_2$ (FXGLM-NH$_2$) where X is an aromatic or β-branched aliphatic amino acid (Page, 2006a) (Table 1). The tachykinins are believed to be predominantly expressed in the brain but can be found in the peripheral nervous system where they produce their effects by discharging from nerve endings and reacting with local tachykinin receptors (Page *et al*., 2001). The three fore mentioned tachykinins are all encoded on two preprotachykinin genes (*TAC*1 and 3). *TAC*1 encodes both SP and NKA, producing each by generating four mRNAs via alternative RNA processing. The *TAC*3 gene only encodes NKB (Page, 2004).

The recent discovery of another tachykinin gene, *TAC*4, by Zhang *et al*. in 2000 has prompted the discovery of several more tachykinin peptides and, more unexpectedly, the tachykinin gene-related peptides (Page *et al*., 2003, Page, 2004). The new tachykinins include endokinins (EK) A-B (Page *et al*., 2003) and hemokinin-1 (HK-1) (Zhang *et al*., 2000). The human *TAC*4 gene is encoded on five exons, which are alternatively spliced to give four variants, α, β, γ and, δ (Page *et al*., 2003). EKB is encoded by all four transcripts. EKA is encoded by an additional, albeit rare, splice variant at the end of exon 1 (Page, 2004). Two tachykinin gene-related peptides are also found in humans, EKC and EKD that are encoded by α- and β*TAC*4, respectively (Page *et al*., 2003; Page, 2004). EKA and EKB are classified as typical tachykinin peptides in that they have the common C-terminal motif FFGLM-NH$_2$. However

EKC and EKD's C-terminal region is different being F**Q**G**LL**-NH$_2$ which means they are not classified as tachykinins (Patacchinni *et al*., 2004; Page 2004).

| Tachykinin | Peptide sequence |
|---|---:|
| SP | RPKPQQ**FFGLM**–NH$_2$ |
| NKA | HKTDS**FVGLM**–NH$_2$ |
| NKB | DMHDF**FVGLM**–NH$_2$ |
| EKA | DGGEEQTLSTEAETWVIVALEEGAGPSIQLQLQEVKTGKASQ**FFGLM**–NH$_2$ |
| EKB | DGGEEQTLSTEAETWEGAGPSIQLQLQEVKTGKASQ**FFGLM**–NH$_2$ |
| HK–1 | SRTRQ**FYGLM**–NH$_2$ |

***Table 1:*** *Amino acid sequences of the tachykinins*

The tachykinin peptides mediate their effects within the central nervous system and the periphery via three transmembrane G-protein coupled tachykinin receptors, named NK$_1$, NK$_2$ and NK$_3$ (Page *et al*., 2001). Each tachykinin can ligand bind with each receptor, though each has different affinities for each receptor. SP has the greatest affinity for NK$_1$ and NKA for NK$_2$ whereas NKB is the preferred ligand for NK$_3$ (Patacchini & Maggi, 2001). EKA and B along with HK-1 have been found to act as full agonists at all three receptors with the greatest preference for the NK$_1$ receptor (Page *et al*., 2003; Berger & Paige, 2005). It is thought that different tachykinins binding with the same receptor can induce separate signalling pathways and therefore co-ordinate different physiological effects (Page, 2004).

## Physiological roles of the tachykinins in the brain

In the brain tackykinins have important roles as neurotransmitters, as well as having neuromodulatory and neurotrophic effects. They occur particularly in large quantities in the areas involved in the control of several autonomic and endocrine functions, of affective and emotion responses, and of higher cerebral functions (Page, 2006b). They are also often co-localise with other neurotransmitters such as dopamine, endomorphin and serotonin (Page, 2006b). SP and its preferred receptor NK$_1$ have been shown to be expressed in the amygdala, hypothalamus, hippocampus and the periaqueductal gyrus (Otsuka & Yoshioka, 1993). It should be noted that since NKA is found to be transcribed from the same preprotachykinin gene (*TAC*1) as SP (Nawa *et al*., 1984) it is generally believed that SP is co-synthesized, co-localized, and co-secreted with NKA (Page, 2006b).

Animal studies have shown that NK$_1$ receptor antagonists modulate behavioural responses due to emotional states in guinea pigs (Rupniack, 2002). Ballard *et al*. (2001) have shown that an NK$_1$ receptor antagonist is effective in reducing the foot tapping seen in gerbils that are foot shocked. Effects of both NK$_1$ agonists and antagonists have been identified in states of anxiety (Teixeira *et al*., 1996). The amygdala is involved in emotion and fear memory processing, which makes it an ideal location for NK$_1$ receptors controlling emotion, anxiety and fear. Weidenhofer *et al*. (2006) showed that the NK$_1$ receptor is widely distributed in the amygdala, with the highest density in the amygdalostriatal region. They also observed a very high relative density in the basal nucleus of the Meynert, which is immediately dorsal to

the amygdala. Weidenhofer and colleagues propose that $NK_1$ receptors in the Meynert, via connections with other brain regions including the amygdala and the prefrontal cortex, may affect emotional behaviour. Three types of neurons were observed within the amygdala to express the $NK_1$ receptor. These were pyramidal cells, large bipolar cells with long dendritic processes and small stellate cells. Weidenhofer *et al.* (2006) believe the two types of interneurons plus the pyramidal cells observed may account for two isoforms of the $NK_1$ receptor detected (Weidenhofer *et al.*, 2006). There is a long form of the $NK_1$ receptor, thought to be the primary receptor, and there is a short or truncated form, missing the cytosolic C-terminal region. Reasons for the two receptor isoforms have not been accounted for as of yet, but could represent a difference in downstream signalling and therefore a difference in physiological function for the two $NK_1$ receptors (Page, 2006a).

SP has been know for sometime to induce scratching and hyperalgesia in rats, linking it to a role in responses to pain, when administered intrathecally. A recent study by Yoshioka *et al.* (2006) looked at the effects of pain processing in rats of the endokinins EKA, EKB, EKC, and EKD compared to SP. They found that the two true tachykinins from the *TAC*4 gene, EKA and EKB, produced similar effects in rats to SP. The onset of symptoms took 30 seconds, with the scratching lasting five minutes and the hyperalgesia lasting at least 30 minutes. These observed responses lead to the conclusion that these tachykinins are capable of inducing both direct and indirect signalling pathways. These results can also be used to make comparisons and predictions as to the effects of SP and the endokinins in humans. SP works in a similar way in both rats and humans, and so rat studies have been used as models for some time. However, rats do not express the endokinins, but as they have a similar function to SP conclusions can be drawn that SP, EKA and EKB contribute to pain processing and vasodilation in humans (Yoshioka *et al.*, 2006)

In addition, SP has been linked with the regulation of dopamine release in a number of brain regions. These include the striatum, the substantia nigra where SP release is firstly dependent on calcium produced by depolarisation, and the nucleus accumbens where SP appears to modulate dopamine release via a presynaptic mechanism. In the ventral tegmented area SP infusion was shown to increase locomotion and rearing, which could then be completely blocked with dopamine antagonist administration, leading to the conclusion that SP regulates dopamine here as well (Page, 2006b). Also, NKA has been linked to an excitatory role on dopamine neurons in the substantia nigra. NKA has been found to be ten times more potent than SP in the ventral tegmented area, which suggests that this particular area may have a high concentration of $NK_2$ receptors (Page, 2006b). Changes in dopamine release have been strongly linked to neurodegenerative diseases such as Parkinson's and Alzheimer's. The tachykinins links with dopamine regulation may well mean that they play important roles in the prevention or onset of these neurodegenerative diseases.

In the peripheral nervous system, SP has been shown to be secreted by neurons in response to local tissue damage where it is capable of inducing and augmenting many inflammatory responses including plasma extravasation, leukocyte activation, endothelial cell adhesion molecule expression, cytokine production and mast cell activation (Quinlan *et al.*, 1999; Vishwanath & Mukherjee, 1996). This demonstrates SP's ability to induce a local immune response when needed by recruiting

inflammatory agents. SP is also known to up regulate secretion of interleukins (IL)-1, IL-6, IL-12 and tumour necrosis factor (TNF)-$\alpha$ by cells of myeloid linage (Marriot, 2004). The interleukins and TNF-$\alpha$ are proinflammatories, demonstrating SP's role in inducing an inflammatory and therefore an immune response. SP also causes up regulation of IFN-$\gamma$ produced by T-cells, and down regulates anti-inflammatory cytokines, including transforming growth factor (TGF)-$\beta$ (Nessler *et al.*, 2006). Therefore, SP acts not only to induce inflammatory agents but to also suppress any agent which may counteract its desired effects.

Since SP has been implicated in immune responses, as just described, and is also expressed predominantly in the brain it would be reasonable to suggest that it has a role in inflammatory responses here. Indeed, Nessler *et al.* (2006) have used a selective NK$_1$ receptor antagonist to gain further insight into the roles of SP and its receptor, NK$_1$, in neuroinflammation using an adoptive transfer experimental autoimmune encephalomyelitis (EAE) model in mice. This technique is used as an animal model to study neuro-immune and endocrine interactions. It is initiated via adoptive transfer of myelin specific T cells and causes an inflammatory response initiated by CD4+ MHC class II restricted Th1 cells. Previous to this study, Nakagawa *et al.* (1995) and Quinlan *et al.* (1999) demonstrated that SP is able to up regulate intercellular adhesion molecule (ICAM)-1 on human venular endothelial cells and vascular cell adhesion molecule (VCAM)-1 expression on human dermal endothelial cells, respectively. Furthermore, Annunziata *et al.* (2002) found that SP is secreted by rat endothelium once stimulated with TNF-$\alpha$ or IFN-$\gamma$. This indicates a positive feedback loop, with SP inducing and being induced by proinflammatories. Nessler *et al.* (2006) concluded from their experiments that administration of the selective NK$_1$ antagonist early after T cell transfer reduced the EAE incidence and disease severity significantly. They speculated that the protective effects of the NK$_1$ selective antagonist is more than likely related to the down regulation of Th1 cytokines and the stabilisation of the blood brain barrier (Nessler *et al.*, 2006). Overall, these results define an important role for SP in initiating and maintaining an inflammatory response. However, whether SP or NK$_1$ receptor antagonists can be used in the future to treat inflammation in the brain remains to be determined.

## Conclusions

The precise functions and mechanisms of action of the tachykinins and their receptors as neuro- and immunomodulators in the brain are still unravelling. Moreover, it is clear that over the years they have been implicated in playing significant roles in various states of pathogenesis in humans and in animals. These have included amongst others the neurodegenerative disorders of Parkinson's disease, Alzheimer's disease and psychiatric disorders. The establishment of the reasons behind each of the tachykinins and their ability to ligand bind to each of the three tachykinin receptors raises important questions in this area of medical research. One such question is understanding the physiological differences between the brain tachykinins SP, NKA and NKB, and the newly discovered endokinins and HK-1. Understanding why and when they are produced and how they work could lead to a better understanding of their associated disease states and the potential to develop novel strategies for

treatment. We are currently continuing our research to unmask some of these pressing medical challenges.


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