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THE ROLE OF TACHYKININS IN CENTRAL NERVOUS SYSTEM INFLAMMATORY RESPONSES

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1. ABSTRACT

While glial cells are recognized for their roles in maintaining neuronal function, there is growing appreciation of the ability of resident glial cells to initiate and/or augment inflammation following trauma or infection in the central nervous system (CNS). The tachykinin, substance P, is found throughout the CNS, with evidence for both neuronal and glial cells as being sources of this neuropeptide. Substance P is well known to augment inflammatory responses at peripheral sites, such as the gastrointestinal tract and skin, which raises the possibility that this tachykinin might serve a similar function within the brain. This review focuses on the evidence for tachykinins in regulating the immune functions of CNS glial cells. Neurokinin-1 (NK-1) receptors have a high affinity for substance P and are expressed by a number of resident CNS cell types, including astrocytes and microglia. Importantly, substance P/NK-1 receptor interactions elicit activation of signal transduction pathways in both cell types and can initiate, or augment, inflammatory responses by astrocytes and microglia. The ability of substance P to augment immune responses of glial cells has important ramifications for the development of protective host responses within the CNS or, alternatively, the progression of damaging inflammation.

2. INTRODUCTION

Tachykinins have long been known to be present within the mammalian central nervous system (CNS). Of the mammalian tachykinins, substance P is the most abundant in the brain (1) and is distributed throughout the CNS (2). The presence of substance P in the CNS mediates a variety of behavioral effects and plays a key role in the transmission of pain signals. Furthermore, tachykinins have been implicated in regulating neuronal survival and degeneration. Central receptors for substance P regulate cardiovascular and respiratory function, and are involved in activating the emetic reflex. In the spinal cord, substance P receptors are activated during synaptic transmission especially in response to noxious stimuli applied at the receptive field of primary afferent neurons. Accordingly, it has been speculated that inhibition of substance P/substance P receptor interactions could be used to produce analgesia, as antiemetics, and for treatment of certain forms of urinary incontinence due to detrusor hyperreflexia (as reviewed in 3).

In addition, substance P in the CNS has recently been implicated in depression in both animal models and human patients (4). Furthermore, peripheral blood concentrations of substance P have been shown to increase in response to psychological anxiety in humans, leading to the suggestion that substance P may serve as a mediator in stress-induced immune reactions (5). In support of such hypotheses, substance P receptor antagonists have been reported to have antidepressant-like actions in animal models (6) and to abrogate anxiety (7, 8). However, it should be noted that while recent clinical trials have confirmed the efficacy of substance P receptor antagonists in alleviating depression and chemotherapy-induced emesis, these agents have not been effective in abrogating pain (6-9).

The important roles of tachykinins, such as substance P, in neuronal transmission and behavioral responses are well recognized and have been reviewed elsewhere (3, 10-12). However, increasing evidence indicates that tachykinins provide an important component in bi-directional interactions between glial cells and neurons during health and disease. For example, the tachykinins, substance P and neurokinin A, have been shown to elicit the production of cytokines by malignant

Table 1. CNS cellular sources and stimuli that induce expression of substance P

Cell source	Expression stimulus	Reference
Neurons	Excitatory or noxious stimuli	Reviewed in 12
	Capsaicin	39
	Eosinophil major basic protein	41
	Ischemic injury	40
	Cell line differentiation	38
Astrocytes	In vitro culture	46
	Ischemic damage	47
Microglia	In vitro culture	42
Cerebral endothelial cells	TNF-alpha or IFN-gamma	48

glial cells in brain tumors that originate from astrocytes (as reviewed in 13). Importantly, this cytokine production was found to further increase the proliferation rate of these cells. Such an effect was demonstrated to be due to specific interactions between these tachykinins and their receptors by the ability of the substance P receptor antagonists, MEN 11467 and MEN 11149, to inhibit tumor growth (14). Perhaps more importantly, tachykinins may play a role in the initiation and/or progression of inflammation within the CNS following injury or infection.

Traumatic injury and infectious agents can elicit marked inflammatory responses within the CNS. While such responses may represent protective immune responses to certain pathogens, inflammation elicited by infectious agents often results in progressive damage to the CNS. A hallmark of developing immune responses is the synergistic interactions between cells and their products, which can amplify the response. Such amplification and positive feedback loops serve to recruit cells to the site of infection, while promoting activation signals which continue to expand the response. Following initiation of inflammation within the CNS, infiltrating immune cells including macrophages and dendritic cells can contribute to the production of pro-inflammatory signals such as the cytokines, IL-6 and TNF-alpha. A compelling body of evidence has accumulated to indicate that substance P plays an important role in augmenting inflammatory immune responses at peripheral sites such as the gastrointestinal tract (15). This is evidenced by diminished inflammatory responses in studies where substance P/substance P receptor interactions are inhibited *in vivo* (16-19), or in studies employing mice genetically devoid of substance P/substance P receptor interactions (20-23). Central to the ability of substance P to augment inflammation is the finding that this neuropeptide can modulate the function of myeloid cells, such as macrophages and dendritic cells, via specific substance P receptors (17, 18, 24-26). Interaction of substance P with its receptor (neurokinin-1 receptors: NK-1 receptors) activates the transcription factor, NF-kB (27), consistent with the ability of substance P to initiate the production of the proinflammatory cytokines, IL-1, IL-6, IL-12 and TNF-alpha (18, 28-30). These findings suggest that the functions of brain perivascular macrophages, or those of infiltrating immune cells, such as macrophages and dendritic cells, might be similarly affected by the presence of substance P during immune responses in the CNS.

However, while it is likely that infiltrating immune cells contribute to the development of immune responses within the brain, a growing body of evidence suggests that resident glial cells may play a major role in the initiation and progression of inflammation following CNS challenge. Potential glial contributors and/or regulators of inflammatory events within the CNS include parenchymal microglia, perivascular microglia/macrophages and astrocytes (31-34). The following review will focus on observations that suggest tachykinins, and in particular substance P, can have an important influence on the immune functions of resident CNS cell types.

3. SOURCES OF SUBSTANCE P IN THE CENTRAL NERVOUS SYSTEM

Unlike peripheral tissues, such as gastrointestinal tract or skin, where there is dense innervation by substance P-containing dorsal root ganglion neurons, the brain lacks such sensory innervation (as reviewed in 35). At the same time, substance P is found throughout the CNS (2), and is the most abundant mammalian tachykinin in the brain (1). Furthermore, this expression may be enhanced following bacterial challenge, as evidenced by the ability of systemic bacterial lipopolysaccharide (LPS) to induce levels of substance P in the spinal cord (36). It is now recognized that a variety of CNS cell types can express substance P. Interestingly, these cells include both neurons and glial cell types that may produce this neuropeptide either constitutively, or following inflammatory and/or damaging stimuli. Table 1 summarizes the cellular sources of substance P within the CNS with a focus on glial cells that have been shown to express this neuropeptide.

Substance P can be secreted by neuronal cells within the CNS, in addition to those in the peripheral nervous system (as reviewed in 12 and 37). Recently, the human neuronal cell line, NT2-N, was demonstrated to express the four isoforms of the preprotachykinin-A gene transcript (alpha, beta, gamma, and delta), which result from the differential processing of RNA transcribed from a single gene (38). This expression was found to increase concomitantly with differentiation into neurons (38). Importantly, neurons have been found to release substance P following challenge with excitatory or noxious stimuli (as reviewed in 12). For example, substance P is released by nerve terminals by the pharmacological agent, capsaicin (39), and is expressed in a population of glutamatergic pyramidal cells following ischemic injury (40). A possible link between neurons and immune cells in the release of this tachykinin has been suggested by the observation that activated eosinophils produce major basic protein which

can elicit the release of substance P by cultured dorsal root ganglion neurons (41). Such a finding reveals the tantalizing possibility that control of tachykinin release by neurons can be regulated by cells of the immune system. In addition to CNS neurons, several glial cell types have been reported to express the tachykinin, substance P. Human fetal microglia express the four mRNA isoforms of the preprotachykinin-A gene (42). The expression of substance P by these myeloid cells is consistent with the reported ability of peripheral monocytes/macrophages to produce this neuropeptide (43-45). However, microglia have been reported to constitutively produce higher levels of protein secretion (640-850 pg/10^6 cells) than do human macrophages (25-50 pg/10^6 cells) (42). In addition to microglia, astrocytes have been reported to be capable of expressing substance P. Message encoding the preprotachykinin-A gene has been detected in cultured rat astrocytes (46), and the substance P peptide has been detected in reactive astrocytes in the ischemically and retrogradely/anterogradely damaged adult forebrain nuclei (47). While the production of substance P by myeloid progenitor-derived microglia might be anticipated, the expression of this neuropeptide by glial cells that are not leukocytic in origin is wholly unexpected.

In addition to neurons and glial cells, there are other possible sources of substance P within the CNS. As noted above, peripheral macrophages may be a source of this tachykinin either constitutively, or following activation (43-35). As such, perivascular macrophages, or infiltrating monocytes/macrophages may contribute to the local production of substance P. Furthermore, one recent study suggests that endothelial cells isolated from rat cerebral vasculature express mRNA encoding substance P (48). Interestingly, this expression is upregulated following stimulation with the inflammatory cytokines, TNF-alpha and IFN-gamma (48). This observation has lead to the suggestion that such pro-inflammatory signals might elicit changes in vascular permeability of the blood-brain barrier via production of this tachykinin (48).

4. EXPRESSION OF THE SUBSTANCE P RECEPTOR IN THE CENTRAL NERVOUS SYSTEM

4.1. Neurons

Consistent with the recognized roles of substance P in the transmission of pain and in behavioral responses, authentic NK-1 receptors for substance P have been shown to be expressed by neuronal cells. NK-1 receptors have been detected in neurons from the spinal dorsal horn in rats (49), and substance P has been demonstrated to elicit calcium influx in these cells (50). Radiolabelled substance P has been found to bind to sites in the rabbit forebrain (51). Furthermore, message encoding the NK-1 receptor has been detected in the human neuronal cell line, NT2-N (38). Importantly, this expression increases with NT2 differentiation into neurons (38). In addition to the constitutive expression of substance P receptors in some neurons, injury has been found to result in increased neuronal NK-1 receptor expression. For example, no binding sites for radiolabelled substance P have been detected in normal rabbit optic nerve or tract. However, a very high density of binding sites was found in the optic nerve and tract after transection of retinal ganglion cell axons (51). In addition, message for the NK-1 receptor was only expressed in a population of glutamatergic pyramidal cells following ischemic injury (40).

4.2. Astrocytes

Astrocytes are the major glial cell type in the brain and are well known to play essential roles in the development, survival, and functioning of CNS neurons. However, it has become increasingly apparent that astrocytes may also be important contributors to inflammatory immune responses within the brain. Stimulated astrocytes have been demonstrated to express an array of inflammatory cytokines and chemokines (as reviewed in 34). The pattern of inflammatory molecule production is one that can initiate leukocyte migration through the blood-brain barrier (52) and initiate leukocyte effector functions in these infiltrating cells. Furthermore, it has been suggested that astrocytes express, or may be induced to express, antigen presenting MHC class II molecules and costimulatory molecules such as CD40 that may serve to activate infiltrating T-lymphocytes (34). However, the signals required to initiate optimal induction of the immune functions of this important glial cell type remain poorly understood.

A growing body of evidence has accumulated to suggest that astrocytes express authentic NK-1 receptors for substance P. Evidence for the presence of NK-1 receptors on astrocytes comes from the demonstration of ^{125}I-labeled Bolton-Hunter conjugated SP binding (Kd = 0.2 nM) in mixed glial cultures that contain a 9:1 ratio of astrocytes to microglia (53). That this interaction occurs with astrocytes, rather than microglia, was concluded from the observation that such binding was not observed in enriched microglial cultures (53). This conclusion was supported in C6 astrocytes where NK-1 receptors were found to be expressed as determined by radiolabelled binding studies, and confirmed by the ability of an antibody raised against an external domain of the NK-1 receptor to specifically block this binding (54). Furthermore, mRNA encoding the substance P receptor has been detected in isolated cultures of rat astrocytes (46). Interestingly, there appear to be differences in the level of expression of this receptor on astrocytes based upon their location within the CNS. Human spinal cord astrocytes have been reported to have 6 times more NK-1 receptors than brain astrocytes (55).

Further evidence for the presence of substance P receptors on astrocytes comes from the ability of substance P or other NK-1 receptor agonists to elicit activation of signal transduction pathways within these cells. Substance P treatment has been demonstrated to induce membrane potential changes in rat cortical and spinal cord astrocytes, and these responses were mimicked by the NK-1 receptor agonists, substance P-methyl ester and septide (56). Challenge of human and murine astrocytes has been reported to activate phospholipase C (57) leading to the accumulation of inositol trisphosphate (55, 58, 59) and a

Table 2. Expression of tachykinin receptors by resident CNS cell types

Cell	Receptor	Expression	References
Astrocytes	NK-1	Yes	46, 53-55
	NK-2	No	57, 105
	NK-3	No	57, 105
Microglia	NK-1	Yes	42, 78, 79
	NK-2	No	75, 77
	NK-3	No	75, 77
Oligodendrocytes	NK-1	Yes	50, 59, 86
	NK-2	Not known	
	NK-3	Not known	

subsequent elevation in the concentration of intracellular calcium (58). However, it is important to note that, while a role for elevations in intracellular calcium in the initiation of substance-P-mediated responses has been reported in a variety of leukocytic cell types (60-64), a number of studies have reported the ability of substance P to induce immune responses in the absence of such elevations (27, 65, 66). While the reason for such discrepancies is unclear, it as been reported that the NK-1 receptor may be coupled to multiple G proteins that can activate differing second messenger pathways (67). Alternatively, it is possible that amphiphilic peptides, such as substance P, may insert into the plasma membrane and directly interact with intracellular proteins (68, 69). This is particularly possible when substance P is applied at higher concentrations, and raises the possibility that substance P could elicit calcium mobilization in glial cells through indirect mechanisms or via nonclassical substance P receptors, rather than via interaction with NK-1 receptors.

Importantly, substance P has been reported to activate several transcriptional regulators that are known to play a pivotal role in the control of pro-inflammatory molecules expression. In human astrocyte cell lines, substance P has been demonstrated to elicit the activation of NF-kB (70, 71), and p38 MAP kinase (71). In addition, this neuropeptide has been reported to induce the expression of the nuclear factors, c-fos and c-jun (58). Taken together, these studies provide circumstantial evidence for the presence of functional substance P receptors on this important glial cell type and provide possible mechanisms by which this neuropeptide can augment astrocyte immune responses.

Evidence for the presence of tachykinin receptors on astrocytes is summarized in Table 2. It is important to note that, while the preponderance of evidence supports the presence of NK-1 receptors on astrocytes, at least one recent study reports the absence of such receptors on astrocytes *in vivo* either constitutively or following ischemic brain injury using *in situ* hybridization, immunohistochemistry and confocal microscopy (40). The reason for this apparent discrepancy between this finding and the studies described above is currently unclear.

4.3. Microglia

Microglia are resident immune cells of the CNS and, like macrophages and dendritic cells, are of myeloid lineage. As such, these cells are likely to play an important role in either the development of protective immune responses or the progression of damaging inflammation during CNS disease states (32, 72, 73). While the presence of receptors for substance P has been established for neurons and astrocytes, the expression of authentic NK-1 receptors on microglia has been somewhat more controversial. The presence of NK-1 receptors on this cell type has previously been inferred by substance P effects on microglial function (74-77). More recently, mRNA encoding the NK-1 receptor has been detected in human fetal microglia (42, 78). Furthermore, cell membrane substance P immunoreactivity was detected on these cells lending support for the notion that this message is translated as a cell surface protein (42). In contrast, a number of studies report the absence of such receptors on murine microglia. Luber-Narod and co-workers (53) reported that microglia exhibited no detectable ^{125}I-labeled Bolton-Hunter conjugated substance P binding sites in either resting cells or following activation with bacterial LPS. Similarly, one recent study failed to detect *in vivo* NK-1 receptor expression in rat microglia following ischemic brain injury using *in situ* hybridization, immunohistochemistry and confocal microscopy (40). Alternatively, it has been suggested that microglia express binding sites for substance P but that such binding sites represent nonclassical NK-1 receptors (74).

In a recent study we have employed methods to demonstrate both the presence of both mRNA encoding the NK-1 receptor and presence of the receptor protein in murine microglia. In this study we utilized antibodies directed against the NK-1 receptor protein that were developed by our laboratory (25, 26) to directly assess the expression of this receptor in microglia by both Western blot analysis and flow cytometry (79). Importantly, we described the presence of these receptors in primary cultures of murine microglia in addition to two transformed microglial cell lines, M4T.4 and EOC13. Furthermore, we determined the functional nature of these NK-1 receptors by demonstrating the ability of substance P to activate the transcription factor, NF-kB, in microglia as assessed by increased translocation of the RelA subunit of NF-kB to the nucleus. Evidence for the presence of tachykinin receptors on microglia is summarized in Table 2.

The presence of NK-1 receptors on microglia has important implications for the initiation and/or progression of immune responses within the CNS. Microglia respond to traumatic injury or the presence of infectious organisms by migrating to the site of challenge where they assume many

of the immune effector functions typically associated with macrophages. For example, microglia are known to be facultative phagocytes and express antigen presenting MHC class II molecules (80). Importantly, microglia can be induced to produce key pro-inflammatory cytokines such as TNF-alpha, IL-1 and IL-6 (74, 81, 82), and inflammatory prostanoids (83). As such these cells are ideally suited to detect and respond to pathogens that invade the CNS. As described elsewhere, results from diverse model systems point to a common role for substance P/substance P receptor interactions as an important determinant in optimal immune responses mediated by myeloid cells. Given the recognized role for such interactions in inflammatory responses, the demonstration of authentic NK-1 receptors on cultured microglia may indicate a role for this neuropeptide in the regulation of inflammatory responses mediated by activated microglia in the CNS.

4.4. Oligodendrocytes

Oligodendrocytes synthesize and maintain myelin in the CNS. In addition, these cells have also been suggested to be a source of neuron trophic signals and growth factors (as reviewed in 84). While it is recognized that physical damage or infection can influence oligodendrocyte function (85), the mechanisms responsible for such responses have not been resolved. Evidence for the presence of substance P receptors on oligodendrocytes comes from the ability of substance P to promote phosphoinositide accumulation in oligodendrocyte/type-2 astrocyte progenitor cells (59). Substance P increases calcium mobilization in glial cells isolated from the dorsal root ganglion identified as being oligodendrocytes due to the expression of the O4 surface antigen (50). Similarly, substance P has been shown to increase intracellular calcium in both O-2A progenitor cells and mature oligodendroglia (86). While the ability of substance P to elicit calcium mobilization is consistent with the expression of receptors for this neuropeptide on oligodendrocytes, it is presently unclear whether such a response occurs via authentic NK-1 receptors. As discussed above, the signal transduction pathways activated following interaction between substance P and NK-1 receptors remain controversial. As such, substance P may cause a rise in intracellular calcium following NK-1 receptor engagement or, alternatively may act via indirect mechanisms or nonclassical substance P receptors. Evidence for the presence of tachykinin receptors on oligodendrocytes is summarized in Table 2.

5. MODULATION OF GLIAL-MEDIATED IMMUNE RESPONSES BY SUBSTANCE P

As indicated at the beginning of this review, there is increasing awareness that resident glial cells can play a major role in the initiation and/or progression of inflammation within the CNS. Given the presence of substance P throughout the CNS, the expression of high affinity NK-1 receptors for substance P on glial cells, and the documented ability of substance P to enhance inflammatory immune responses of leukocytes in peripheral tissues such as the gastrointestinal tract and skin, the possibility exists that substance P can similarly augment the immune functions of resident CNS cells.

The notion that substance P/substance P receptor interactions may influence microglia-mediated immune responses in the CNS is supported by the observation that substance P initiates activation of the transcriptional activator, NF-kB, in microglia (79). Our laboratory has previously demonstrated that substance P signals through the NK-1 receptor to initiate the activation of NF-kB in myeloid cells, including murine macrophages and dendritic cells (27). NF-kB activation has previously been demonstrated to play a key role in the regulation of the proinflammatory cytokines, TNF-alpha (87-91), IL-1 (90, 92, 93), IL-6 (90, 91, 94) and IL-12p40 (95, 96) by myeloid cells. The potential importance of the involvement of NF-kB in the regulation of these genes is underscored by observations *in situ* that NF-kB is activated in macrophages at sites of inflammation (97, 98). As such, the finding that substance P initiates the activation of NF-kB in microglia provides a possible mechanism by which substance P/substance P receptor interactions can contribute to microglia-mediated inflammatory responses in the CNS.

Some evidence suggests that substance P is a full and sufficient stimulus to induce microglial responses. Substance P has been demonstrated to stimulate chemotaxis at nanomolar concentrations in murine microglia (77), and can elicit modest elevations in thromboxane synthesis in these cells (76). Importantly, these concentrations of substance P have been reported to directly stimulate the production of the pro-inflammatory cytokine, IL-6, in microglial cell lines (75, 99). This production could be induced by NK-1 receptor specific agonists and is inhibited by NK-1 receptor antagonists (75) suggesting that this effect is mediated by substance P/substance P receptor interactions. However, a number of studies indicate that substance P cannot elicit immune responses alone, but can augment microglial responses to other inflammatory stimuli. For example, substance P alone fails to induce IL-1 production rat brain microglia, but synergistically augments LPS-induced IL-1 production (74). In addition, stereotaxic injection of substance P into the brainstem has been reported to increase IFN-gamma-mediated MHC class II upregulation in parenchymal microglia (100). Again, such an effect was inhibited by a specific NK-1 receptor antagonist (100).

In a study recently submitted for publication, we have demonstrated that substance P synergistically augments prostanoid synthesis by murine microglia in response to suboptimal doses of a bacterial CNS pathogen (unpublished data). Specifically, we showed that substance P can augment the ability of *Borrelia burgdorferi,* the spirochete responsible for Lyme disease, to elevate expression of mRNA encoding the key inflammatory enzyme, cyclooxygenase-2. Importantly, the increase in message for this enzyme correlated with a synergistic increase in the secretion of the prostanoid, PGE_2. These effects were mediated via NK-1 receptors as evidenced by the ability of L-703,606, a specific NK-1 receptor antagonist, to abrogate these effects. Furthermore,

Table 3. Substance P augmentation of immune functions in resident CNS cell types

Cell	Stimulus	Response	Reference
Microglia	LPS	IL-1	74
	IFN-gamma	MHC class II	100
	B. burgdorferi	PGE_2	Unpublished data
Astrocytes	LPS	TNF-alpha	53
	IL-1	IL-6 and PGE_2	55
Brain endothelium	TNF-alpha or IFN-gamma	Vascular permeability MHC class II	48

substance P was not able to elicit these synergistic increases in microglia isolated from mice genetically deficient in the expression of NK-1 receptors. Interestingly, substance P also synergizes with *B. burgdorferi* to augment the expression of the two prostanoid receptors most commonly associated with pro-inflammatory effects, EP2 and EP4 receptors. In addition, we demonstrated that substance P augments activation of the transcription factor, NF-kB, in *B. burgdorferi*-stimulated microglia, suggesting a possible mechanism whereby this neuropeptide augments microglial responses to such CNS pathogens. Substance P may synergize with suboptimal numbers of the spirochete, *B. burgdorferi*, to elevate NF-kB activation in microglia which can augment transcription of the enzyme, COX-2, which can, in turn, increase PGE_2 synthesis. Furthermore, the ability of substance P to synergistically augment EP2 and EP4 prostanoid receptor expression raises the possibility that augmented PGE_2 secretion may act in an autocrine manner to further potentiate microglial function. As such, this study suggests that substance P can serve to promote robust inflammatory responses in the presence of relatively few infectious organisms. Evidence for the ability of substance P to augment microglial inflammatory responses is summarized in Table 3.

Evidence for a role of substance P in immune responses mediated by glial cells during human CNS disease comes from the detection of substance P-immunoreactive cells in postmortem white matter tissue from patients with multiple sclerosis (101). These cells were identified as being astrocytes by morphological criteria at both inflammatory and non-inflammatory lesions (101). In addition, substance P receptor antagonists have recently been demonstrated to reduce CNS inflammation due to *Trypanosoma brucei* infection (102), and reduce astrocyte activation following drug treatment of late-stage African Trypanosomiasis (103).

More direct evidence for the ability of substance P to modulate astrocyte-mediated immune functions comes from studies employing isolated cultures of these cells. Substance P can mobilize intracellular calcium in astrocytes (104) and can activate the transcription factors, NF-kB (70), and p38 MAP kinase (71), in astrocyte cell lines. Importantly, such activation elicits the production of the proinflammatory cytokines, IL-1 (104), IL-6 (71), and IL-8 (70), by this major glial cell type. The specificity of such effects has been confirmed by the ability of specific NK-1 receptor antagonists to block substance P-induced production of IL-6 and IL-8 by a human astrocyte cell line (105). In addition to proinflammatory cytokine production, substance P has been demonstrated to initiate the synthesis of the inflammatory mediators, PGE_2 and thromboxane B2, by astrocytes (106). However, it must be noted that contradictory findings have been reported that indicate that substance P does not alter basal PGE_2 synthesis, or that induced by exposure of rat astrocytes to IL-1 (107). As such, the ability of substance P to promote prostanoid synthesis in astrocytes remains in question. In addition, substance P treatment has been reported to promote the secretion of TNF-alpha by rat astrocytes. However, this induction appears to occur as a secondary effect mediated by the enhanced expression of IL-1, as evidenced by the ability of IL-1 neutralizing antibodies to antagonize this effect (108-110).

While these studies suggest that substance P can promote astrocyte immune responses in the absence of additional stimuli, some findings indicate that substance P alone fails to elicit cytokine production by these cells, but this neuropeptide can augment such responses induced by other inflammatory stimuli. For example, substance P does not induce cytokine production by astrocyte rich mixed glial cultures (53). However, nanomolar concentrations of substance P augments LPS-induced production of TNF-alpha (53). This secretion was determined to be from astrocytes, as cytokine secretion was not enhanced by substance P in enriched cultures of microglia (53). However, in agreement with the reported production of TNF-alpha by astrocytes treated with substance P alone (108-110), the augmented production of TNF-alpha by LPS-stimulated cells is mediated by IL-1 and is blocked by neutralizing antibodies to this cytokine (53). Similarly, in another study, no cytokine production was detected in human astrocytes treated with substance P, but this neuropeptide does potentiate the ability of IL-1 to induce the production of IL-6 and PGE_2 (55). Finally, as discussed earlier, astrocytes may be a source of substance P in the CNS. As such, the expression of NK-1 receptors on this cell type, and the ability of substance P to augment the immune functions of astrocytes, raises the possibility that substance P can act in an autocrine manner following astrocyte activation and further potentiate immune responses by these glial cells (46). Evidence for the ability of substance P to augment astrocyte-mediated inflammatory responses is summarized in Table 3.

It must be noted that, while this review has focused on the ability of substance P to augment glial cell immune function, the presence of substance P within the CNS is likely to have a significant effect on the responses of perivascular macrophages or infiltrating leukocytes during CNS trauma or infection. Furthermore, it is possible that other brain cell types that bear substance P receptors can

contribute to inflammatory responses within the CNS. For example, substance P interacts with NK-1 receptors on the NT2-N human neuronal cell line to initiate the expression of the chemokine, MIP-1 (38). Given the ability of this chemokine to recruit leukocytes into areas of inflammation or infection (111), the production of MIP-1 by substance P challenged neurons could represent an important component in the initiation of host responses in the CNS. Furthermore, a role for substance P in the regulation of blood-brain barrier permeability has been suggested following the observation that NK-1 receptor antagonists can inhibit IFN-gamma or TNF-alpha-mediated increases in permeability and MHC class II molecule expression in rat brain endothelial cells isolated from cerebral microvessels (48).

6. EXPRESSION OF OTHER TACHYKININS WITHIN THE CENTRAL NERVOUS SYSTEM

While substance P is the most abundant tachykinin within the CNS and has been found throughout the brain, there is evidence for the presence of other tachykinins within the CNS.

6.1. Neurokinin A

Neurokinin A is a tachykinin that interacts preferentially with neurokinin-2 (NK-2) receptors, although it has been suggested that NK-1 receptors may have a second high affinity binding site for neurokinin A (112). Neurokinin A, and the neurokinin A-like tachykinin, neuropeptide K, have been identified within the CNS and have been reported to have a distribution in the brain that is strikingly similar to that seen for substance P (1, 113, 114). This observation has lead to the suggestion that tachykinin systems may act co-operatively in the brain (114). Importantly, some studies have indicated that the expression of neurokinin A may be modulated following exposure to inflammatory stimuli. Systemic administration of bacterial LPS has been demonstrated to induce levels of this tachykinin in the spinal cord (36). Furthermore, administration of the inflammatory cytokines, IL-1 (115) and IL-6 (116), have been reported to augment neurokinin A release in the brain. However, it must be noted that IL-1 only promoted neurokinin A release in castrated animals (115), and other investigators report the inability of systemic LPS to augment neurokinin A levels in the CNS (116). As such, the ability of inflammatory stimuli to elevate CNS levels of this tachykinin remains in question.

To date, there is little evidence to suggest that neurokinin A can affect the immune functions of glial cells. Rat cortical and spinal cord astrocytes demonstrate membrane potential changes following neurokinin A agonist addition (56). However, these effects were mimicked by the NK-3 agonist, DiMe-C7, suggesting that such effects are not mediated via neurokinin A/NK-2 receptor interactions. Indeed, mouse astrocytes have been reported to be devoid of binding sites for NK-2 receptor ligands (57). In support of this notion, there is no additional effect of NK-2 receptor ligands when used in combination with substance P on phospholipase C activation (57). Accordingly, NK-2 receptor agonists have been reported to be 1000 times less effective than substance P at inducing production of IL-6 or IL-8 in a human astrocytoma cell line, and NK-2 receptor antagonists are several orders of magnitude less effective at blocking the effects of substance P (105). In contrast to substance P, NK-2 receptor agonists fail to elicit chemotaxis in murine microglia (77), and are 250-500 fold less effective in eliciting IL-6 production by this cell type (75).

6.2. Neurokinin B

In addition to substance P and neurokinin A, the tachykinin, neurokinin B, has been detected in the CNS. Message encoding this tachykinin, and the presence of the neurokinin B peptide, has been detected throughout the brain but appears to be partitioned more to forebrain than to brainstem structures (117). Neurokinin B interacts preferentially with neurokinin-3 (NK-3) receptors, but may bind with lower affinity to NK-2 receptors, and at high concentrations with NK-1 receptors. However, there is little evidence that this neuropeptide influences immune responses mediated by glial cells. While the NK-3 agonist, DiMe-C7, has been reported to elicit membrane potential changes in rat cortical and spinal cord astrocytes (56), murine astrocytes have been reported to be devoid of binding sites for NK-3 receptor ligands (57). In support of this notion, there is no additional effect of NK-3 receptor ligands when used in combination with substance P on phospholipase C activation in astrocytes (57). Accordingly, NK-3 receptor agonists have been reported to be 1000 times less effective than substance P at inducing production of IL-6 or IL-8 in a human astrocytoma cell line, and NK-3 receptor antagonists are several orders of magnitude less effective at blocking the effects of substance P (105). In contrast to substance P, NK-3 receptor agonists fail to elicit chemotaxis in murine microglia (77), and are 250-500 fold less effective in eliciting IL-6 production by this cell type (75).

6.3. Hemokinin-1

A novel endogenous tachykinin, hemokinin-1, has recently been described that shows selectivity for NK-1 receptors over NK2 and NK3 receptors (118). While hemokinin-1 was initially reported to be produced primarily in tissues outside of the CNS (118), more recent studies have described a broader distribution, with message encoding this tachykinin being present in many brain regions (119). To date, the biological functions of this molecule are not fully understood. However, hemokinin has been reported to activate mast cell degranulation and promote B-lymphocyte development (118). Importantly, direct administration of hemokinin-1 into the CNS initiates foot-tapping and scratching behaviors, effects that are blocked by NK-1 receptor antagonists (119). The expression of message encoding hemokinin in the brain, and the ability of this novel tachykinin to interact with NK-1 receptors to initiate immune and behavioral responses, raises the possibility that hemokinin could influence the immune responses of NK-1 receptor expressing glial cells.

7. PERSPECTIVE

While glial cells are best known for their important roles in maintaining neuronal growth and function, there is growing appreciation of the ability of

resident glial cells to initiate and/or augment inflammation following trauma or infection in the CNS. The tachykinin, substance P, is found throughout the CNS, with evidence for both neuronal and glial cells as being potential sources of this neuropeptide. Such expression may be constitutive, or may be elicited following injury or exposure to CNS pathogens. Substance P is well known to augment inflammatory responses of leukocytes at peripheral sites, such as the gastrointestinal tract and skin, which raises the possibility that this tachykinin might serve a similar function within the brain. Substance P may act on CNS perivascular macrophages or infiltrating leukocytes that are likely to express functional NK-1 receptors for substance P, to augment inflammatory responses mediated by these cells. This review has focused on the evidence for a role for tachykinins in regulating the immune functions of CNS glial cells. Astrocytes, the major glial cell type within the CNS, and microglia, resident immune cells of myeloid origin in the brain, express specific NK-1 receptors for substance P. Importantly, substance P/substance P receptor interactions elicit activation of signal transduction pathways in both glial cell types and can initiate, or augment, inflammatory responses by astrocytes and microglia. There is currently little evidence for other tachykinin family members in the modulation of glial immune functions.

The ability of substance P to augment inflammatory responses of CNS cells has important ramifications for the development of protective immune responses within the CNS or, alternatively, the progression of damaging inflammation. Substance P produced locally in the CNS can influence the function of brain cells that bear NK-1 receptors which have a high affinity for this tachykinin. Substance P and its receptor may be expressed constitutively by glial cells or, alternatively, may be induced following tissue damage or exposure to CNS pathogens. As such, the actions of substance P on CNS cells may be regulated both at the level of tachykinin production and at the level of expression of its receptor. While the inflammatory actions of substance P on unstimulated glial cells may be limited, this neuropeptide appears to be capable of significantly augmenting such responses in activated cells. Glial cells stimulated in this manner produce soluble inflammatory mediators that can act in an autocrine and/or paracrine manner to further potentiate glial cell functions, or can facilitate leukocyte recruitment and activation within the brain. Taken together, the possibility exists that substance P could contribute to the development of inflammatory responses in the CNS that are out of proportion with the initial stimulus. Such an effect might explain previous observations in bacterially-mediated CNS diseases, such as Lyme neuroborreliosis, in which robust inflammation occurs in the presence of relatively low numbers of spirochetes. Furthermore, the possibility exists that this neuropeptide could serve to exacerbate damaging inflammation that occurs following the onset of CNS autoimmune disorders.

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