Chemokines and chemokine receptors in the brain: implication in neuroendocrine regulation

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Abstract

Chemokines are small secreted proteins that chemoattract and activate immune and non-immune cells both in vivo and in vitro. In addition to their well-established role in the immune system, several recent reports have suggested that chemokines and their receptors may also play a role in the central nervous system (CNS). The best known central action is their ability to act as immuno-inflammatory mediators. Indeed, these proteins regulate leukocyte infiltration in the brain during inflammatory and infectious diseases. However, we and others recently demonstrated that they are expressed not only in neuroinflammatory conditions, but also constitutively by different cell types including neurons in the normal brain, suggesting that they may act as modulators of neuronal functions. The goal of this review is to highlight the role of chemokines in the control of neuroendocrine functions. First, we will focus on the expression of chemokines and their receptors in the CNS, with the main spotlight on the neuronal expression in the hypothalamo–pituitary system. Secondly, we will discuss the role – we can now suspect – of chemokines and their receptors in the regulation of neuroendocrine functions. In conclusion, we propose that chemokines can be added to the well-described neuroendocrine regulatory mechanisms, providing an additional fine modulatory tuning system in physiological conditions.

Background

The history of chemokines started in 1977, when the secreted platelet factor 4 (PF4/CXCL4) was purified without any knowledge of its function (Walz et al. 1977, Wu et al. 1977). Two decades ago, the discovery that interleukin-8 (IL-8/CXCL8) showed chemotactic activity for neutrophils established that chemokines are key elements in the control of leukocyte migration (Yoshimura et al. 1987). A second major development in chemokine research occurred during 1995–1996, with the discovery that certain chemokines function as HIV-suppressive factors in vitro by blocking viral interaction with specific chemokine receptors and that some chemokine receptors act as co-receptors for viral entry (Cocchi et al. 1995, Bleul et al. 1996, Feng et al. 1996, Oberlin et al. 1996). It has become apparent over the past years that chemokines are involved in virtually all pathologies that present an inflammatory component. This includes nervous system pathologies, such as neurodegenerative or neuroinflammatory diseases, that induce the expression of a number of chemokines and chemokine receptors in activated astrocytes and microglia, suggesting their involvement in the activation of the central nervous system defense mechanisms (Bleul et al. 1996, Oberlin et al. 1996, Thibeault et al. 2001). However, we and others have demonstrated that some chemokines and their receptors are constitutively expressed by neuronal cells in normal adult brain and may function as neuromodulators. Among the described neuromodulatory activities of chemokines, the current literature postulates that chemokines could directly interact with neuroendocrine functions. It is this latter new concept that we would like to illustrate by reviewing recent results.

Chemokine and chemokine receptor classification

Chemokines are a family of large peptides (60–100 amino acids (aa)). They are subdivided into four families based on the number and spacing of the conserved cysteine residues in the N-terminal position and are named CXC, CC, CX3C, and C, in agreement with the systematic
nomenclature. All chemokines signal through G protein-coupled receptors (GPCR). In general, several chemokines can bind to the same receptor and, conversely, a given chemokine may recognize more than one receptor. However, there are exceptions where unique ligand–receptor pairs exist. To date, more than 50 chemokines and about 20 chemokine receptors have been identified. Their classification and nomenclature can be found in reviews (Murphy 2002, Floridi et al. 2003).

Structure of chemokines

The structure of chemokines comprises three distinct domains: a highly flexible N-terminal domain, which is tamped down by the N-terminal cysteine(s), a long loop, which leads into three antiparallel β-pleated sheets, and an α-helix that overlies the sheets (Baggiolini & Loetscher 2000). The structural activities revealed that the N-terminal region is important for receptor binding and activation (Clark-Lewis et al. 1991, Proudfoot et al. 1996). Interestingly, all chemokine structures described to date more or less conform to this three-dimensional pattern, even though they may bear little homology in their primary amino acid sequence (Clark-Lewis et al. 1995, Clore & Gronenborn 1995).

Chemokine–receptor interaction

Based largely on a well-known model, it was postulated that the N terminus and the extracellular loop of the chemokine receptor are responsible for the initial binding event. This interaction induces a conformational change allowing the N-terminal region of the ligand to interact with the helices of the receptor triggering signal transduction. However, as for many GPCRs, this two-site paradigm does not appear to hold true for all chemokine–receptor pairs. Additionally, it was reported that following chemokine interaction, several chemokine receptors undergo a dimerization (heterodimerization and homodimerization) and oligomerization process with membrane receptors for classical neurotransmitters, representing all critical steps in triggering biological responses (Vila-Coro et al. 1999, 2000, Mellado et al. 2001).

Chemokine receptor-mediated signal transduction

Relating to second messenger systems modulated by chemokine receptors, one of the first pathways identified was the inhibition of adenylyl cyclase activity to reduce the intracellular cAMP levels, involving Gqα subunit of G proteins (Zheng et al. 1999, Bajetto et al. 2001). It was also established that the majority of chemokine receptors activate phospholipase C, involving Gqz proteins. The latter leads to the formation of diacylglycerol and inositol 1,4,5-triphosphate with a subsequent increase in protein kinase C activity and transient elevations of cytosolic Ca2+ levels (Jones et al. 1995, Knall et al. 1996, Wells et al. 1998). More distal signaling pathways modulated by chemokines include the activation of the mitogen-activated protein kinase (PK) cascade, especially the pathway involving extra-cellular signal-regulated kinase 1/2 activation, as well as the phosphorylation of the cytoskeletal-associated kinases (Ganju et al. 1998a,b, Wang et al. 2000, Bajetto et al. 2001). Studies of chemokine receptor activation have also reported the activation of a family of proteins known as Janus kinases and signal transducers and activators of transcriptions (Mellado et al. 1998, Wong & Fish 1998, Vila-Coro et al. 1999). Chemokine receptors also activate the signaling pathway of nuclear factor-κB.

Involvement of chemokines in neuroendocrine interactions

Some chemokines such as cytokine-induced neutrophil chemoattractant (CINC), stromal cell-derived factor (SDF-1/CXCL12), and monocyte chemoattractant protein-1 (MCP-1/CCL2) have been found to be expressed in the hypothalamus (Banisadr et al. 2005a). Such neuroanatomical localization suggests possible neuroendocrine functions (Table 1).

Chemokines and stress

The implication of chemokines in stress responses was reported in several studies. Initially, it was observed that IL-8/CXCL8 mRNA is expressed in the rat paraventricular nucleus (PVN), where corticotropin-releasing hormone is synthesized, and in the hippocampus, where negative feedback to hypothalamo–pituitary–adrenal axis is generated. Thus, IL-8/CXCL8 could be a component of a stress-related neuroendocrine system (Licinio et al. 1992). Moreover, after a noxious stimulation consecutive to a s.c. injection of bacterial lipopolysaccharides (LPS), an up-regulation of CINC and interferon γ-inducible protein (IP-10/CXCL10) mRNAs expression was found in the PVN (Sakamoto et al. 1996a, Reyes et al. 2003). In addition, it was observed that IP-10/CXCL10 expression is also up-regulated following LPS in the circumventricular organs (CVO) including the subfornical organ (SFO) and the area postrema. Other chemokines such as MCP-1/CCL2 and growth-related gene alpha (GROz/CXCL1) demonstrate a LPS responsiveness. Indeed,
an up-regulation of MCP-1/CCL2 transcription is detected in the choroid plexus, CVO, blood vessels, and meninges (Thibeault et al. 2001, Reyes et al. 2003). GROα/CXCL1 also exhibits an up-regulation of its transcription in the PVN and the choroid plexus but not in the CVO (Reyes et al. 2003).

Immobilization stress can be liable for an overexpression of chemokine expression. Indeed, it was shown that CINC mRNA expression is increased during stress immobilization in the parvocellular and magnocellular subdivisions of the PVN but not in the supraoptic nucleus (SON). Moreover, CINC immunostaining intensity is increased in the posterior pituitary parallel to an increase in serum in response to stress immobilization, suggesting that CINC, synthesized in the PVN, could be axonally transported through the median eminence and released into the peripheral blood from the hypothalamo-neurohypophysial system (Sakamoto et al. 1996b).

Finally, painful stress induced by a noxious stimulation consecutively with s.c. injection of formalin into hind footpad increases the CINC immunoreactivity in the posterior pituitary and external layers of the median eminence. The increased CINC mRNA expression is also detected in the PVN, posterior pituitary, external layers of median eminence, and SON (Sakamoto et al. 1996a,b, Matsumoto et al. 1997, Sakamoto et al. 1996b, Koike et al. 1997).

### Table 1 Neuroendocrine functions of the chemokines

<table>
<thead>
<tr>
<th>Chemokines</th>
<th>Reference</th>
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<tbody>
<tr>
<td>CINC</td>
<td>PVN, posterior pituitary, external layers of median eminence</td>
</tr>
<tr>
<td>IL8/CXCL8</td>
<td>PVN, hippocampus</td>
</tr>
<tr>
<td>IP-10/CXCL10</td>
<td>PVN, subfornical organ, area postrema</td>
</tr>
<tr>
<td>GROα/CXCL1</td>
<td>PVN, choroid plexus</td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>Choroid plexus, circumventricular organs, blood vessels, meninges</td>
</tr>
<tr>
<td>RANTES/CCL5</td>
<td>Administration in AH/POA</td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>Co-localization with AVP neurons in the SON and PVN</td>
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<tr>
<td>SDF-1/CXCL12</td>
<td>Co-localization with AVP projections in the median eminence and posterior pituitary</td>
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<tr>
<td>PF4/CXCL4</td>
<td>i.v. Administration</td>
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<tr>
<td>IL-8/CXCL8</td>
<td>i.v. Administration</td>
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<tr>
<td>IP-10/CXCL10</td>
<td>i.v. Administration</td>
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<td>SDF-1/CXCL12</td>
<td>Expression in the MCH neurons</td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>i.v. Injection</td>
</tr>
<tr>
<td>MIP-1α/CCL3</td>
<td>Expression in the MCH neurons</td>
</tr>
<tr>
<td>MIP-1β/CCL4</td>
<td>Injection into the ventromedial HT or AH/POA</td>
</tr>
<tr>
<td>RANTES/CCL5</td>
<td>i.v. injection</td>
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eminence and the CINC secretion in the peripheral blood (Matsumoto et al. 1997).

**Chemokines and body temperature**

Several studies demonstrated that chemokines are implicated in the pyrogenic responses. Indeed, injections of IL-8/CXCL8, macrophage inflammatory protein-1 alpha and beta (MIP-1α/CCL3 and MIP-1β/CCL4), and RANTES/CCL5 induce hyperthermia. It was observed that MIP-1α/CCL3 and MIP-1β/CCL4 administered intravenously in the rabbit or into the anterior hypothalamic/preoptic area (AH/POA), a region containing thermosensitive neurons in the rat, evoke a dose-dependent febrile response characteristic of endogenous pyrogen (Davatelis et al. 1989, Minano et al. 1990, 1991a). It was reported that these two chemokines present differences in their ability to induce hyperthermia. MIP-1β/CCL4 exerts a hyperthermic response with a longer latency and lower magnitude (Myers et al. 1993, Minano et al. 1996). Moreover, it was observed that the co-injection of MIP-1α/CCL3 and MIP-1β/CCL4 into the AH/POA attenuates the increase in body temperature by either chemokine injected alone (Minano et al. 1996). Effects of MIP-1α/CCL3 and MIP-1β/CCL4 on the body temperature seem to be independent of the prostaglandin pathway, since the MIP-1-produced fever is not blocked by inhibitors of prostaglandin synthesis (Davatelis et al. 1989, Minano et al. 1991b). Another chemokine, RANTES/CCL5, is also a powerful hyperthermic molecule when injected directly into the AH/POA. Indeed, the action of RANTES/CCL5 is characterized by an immediate and intense dose-related fever following injection. This effect is prevented by pretreatment with a prostaglandin synthesis inhibitor (Tavares & Minano 2000). The magnitude of the febrile response induced by RANTES/CCL5 is greater than that produced with equipotent dose of MIP-1β/CCL4. It appears that the two chemokines RANTES/CCL5 and MIP-1β/CCL4 present different pathway mechanisms to induce hyperthermia, although they share the same receptor CCR5. Indeed, it was established that microinjections of either MIP-1β/CCL4 or RANTES/CCL5 into the AH/POA produce an immediate and intense fever, but pretreatment with a specific antibody against receptor CCR5 fails to affect the fever induced by MIP-1β/CCL4, but significantly attenuates the febrile response induced by RANTES/CCL5. Therefore, it can be concluded that the receptor CCR5 is functionally involved in the modulation of body temperature by RANTES/CCL5, but not in the MIP-1β/CCL4-induced fever mechanism (Tavares & Minano 2004).

**Chemokines and feeding**

It was discovered that chemokines can also alter food intake when centrally administered. Indeed, i.c.v. administration of PF4/CXCL4, a mediator of inflammatory and allergic responses, suppresses the 2-h nighttime and total daily food intake, whereas daytime food intake does not change. This effect of PF4/CXCL4 is centrally mediated, since an i.p. administration of PF4/CXCL4 has no effect on food intake (Plata-Salaman 1988). MIP-1α/CCL3 and MIP-1β/CCL4 are also involved in the control of food intake. Indeed, when they are directly injected in the rat ventromedial hypothalamus (a structure implicated in the inhibition of feeding behavior) or into the rat AH/POA, they decrease feeding (Minano & Myers 1991, Myers et al. 1993). Moreover, it was reported in the rat that i.c.v. administration of two members of CXC chemokines, IL-8/CXCL8 and IP-10/CXCL10, and two members of CC chemokines, MCP-1/CCL2 and RANTES/CCL5, are also effective in decreasing the short-term (2 h) food intake (Plata-Salaman & Borkoski 1994). More recently, we demonstrated by immunohistochemical studies that SDF-1α/CXCL12 is constitutively expressed in melanin-concentrating hormone (MCH)-expressing neurons located in the lateral hypothalamic area (LHA), neurons which are mainly involved in the stimulation of food intake (Banisadr et al. 2003). Moreover, it was shown that the SDF-1/CXCL12 receptor, CXCR4, is also within MCH-expressing neurons confirming the hypothesis that SDF-1/CXCL12 could alter the feeding behavior by acting on its receptor expressed by MCH neurons in the rat LHA. Thus, by patch-clamp recordings of rat LHA slices, it was demonstrated that SDF-1/CXCL12 has a direct effect on voltage-dependent membrane currents of MCH neurons by decreasing or increasing peak and discharge frequency of action potentials, suggesting that SDF-1/CXCL12 must directly regulate MCH neuronal activity (Guyon et al. 2005). In addition, in the LHA, another chemokine, MCP-1/CCL2, co-localizes with MCH-expressing neurons, suggesting that MCP-1/CCL2 could also similarly act as a modulator of MCH neuronal activity (Banisadr et al. 2005b).

**Chemokines and pituitary hormones**

There is increasing evidence that the immune system-derived molecules can regulate the hormonal release directly at the pituitary level. Thus, it was shown that CINC stimulates prolactin and growth hormone secretions in a concentration-dependent manner. In the same study, it was also observed that CINC stimulates adrenocorticotropic but not thyrotrophin secretion and that CINC suppresses the basal luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretions from
normal anterior pituitary cells in a dose-dependent manner (Sawada et al. 1994). Moreover, a recent study shows that CXCR4 mRNA is expressed in the rat pituitary adenoma cell line GH4C1 (a cell line that releases growth hormone) and that SDF-1α/CXCL12 causes both proliferation and growth hormone release, suggesting that the activation of CXCR4 may represent a novel regulatory mechanism for growth hormone secretion and pituitary cell proliferation, which may contribute to pituitary adenoma development (Florio et al. 2006).

**Figure 1** Immunohistochemical localization of SDF-1/CXCL12 (in green) with AVP (in red) in the internal layer of the normal rat median eminence.

**Figure 2** Electrophysiological modulation of action potential firing of AVP neurons by 25 nM SDF-1/CXCL12 in the magnocellular part of the hypothalamic SON. In normal conditions, AVP neurons exhibit a bursting pattern characterized by periods of intense bursts and silences (A and B). Such periodic pattern is necessary for AVP release (Gouzenes et al. 1998). Following SDF-1/CXCL12 application on hypothalamic SON fragments, the firing pattern is modified. Thus, SDF-1/CXCL12 inhibits the activity of AVP neurons by increasing the silent periods (C) or stimulating the electrical neuronal activity (D). In both cases, it results in an inhibition of AVP release. Adapted from Callewaere et al. (2006).
Chemokines and water balance

An emerging concept recently arose from the observation that chemokines could be involved in water balance and expressed by arginine vasopressin (AVP) neurons in the PVN and SON of the hypothalamus. Indeed, it was previously demonstrated that the CINC is predominantly co-localized with AVP neurons in the SON (Sakamoto et al. 1996b). Moreover, after osmotic shock (induced by an i.p. injection of a hypertonic

Figure 3 Immunohistochemical localization of CXCR4 in the circumventricular organs: organ of the lamina terminalis (A) and subfornical organ (B). The circumventricular organs (CVO) located in the walls of the brain ventricular system and innervating the AVP neurons in the supraoptic and paraventricular nuclei detect changes in plasma osmolarity. It is thus possible that, similarly to what was observed in the magnocellular nuclei, SDF-1/CXCL12 in the CVO, through CXCR4, can modulate AVP release by autoregulating responses of CVO Angiotensin II neurons.
solution) that activates AVP neurons, CINC mRNA expression is rapidly up-regulated in the rat SON. A similar but much lower increase is also found in the PVN, mainly in its magnocellular subdivision of the PVN (Koike et al. 1997). In addition, our group has recently demonstrated by immunohistochemical studies that MCP-1/CCL2 is also constitutively expressed by AVP magnocellular neurons in the PVN and SON, notably in cell bodies and proximal processes, as well as in processes in the internal layer of the median eminence and in the posterior pituitary, suggesting a role of MCP-1/CCL2 in the neuroendocrine regulation of AVP-expressing neurons (Banisadr et al. 2005b).

Moreover, in another recent report, we investigated a possible neuroendocrine role of another chemokine: SDF-1/CXCL12. By means of dual fluorescent immunohistochemistry (Banisadr et al. 2003, Callewaere et al. 2006), we first demonstrated the co-localization of SDF-1/CXCL12 and its receptor CXCR4 with AVP neurons in the magnocellular neurons of the SON and PVN hypothamic nuclei and on AVP projections in the median eminence (Fig. 1) to the neurohypophysis (Callewaere et al. 2006). Electrophysiological recordings of SON neurons clearly demonstrate that SDF-1/CXCL12 through its receptor CXCR4 affects the electrical activity of AVP neurons. The effect of SDF-1/CXCL12 results in changes in the bursting pattern of AVP neurons by either increasing the silences between bursts or inducing a constant stimulation of AVP neurons. Such changes in AVP neuronal electrical activity are known to result in the inhibition of AVP release (Fig. 2). Indeed, we observed that SDF-1/CXCL12 can blunt the autoregulation of AVP release in vitro and counteract angiotensin II-induced plasma AVP release in vivo. Furthermore, a short-term physiological increase in AVP release induced by enhanced plasma osmolarity produced by 1 M NaCl i.p. administration is similarly blocked by central injection of SDF-1/CXCL12 through its receptor CXCR4.

**Figure 4** Schematic drawing representing the hypothalamic modulation of AVP release by SDF-1/CXCL12. In acute stimulation conditions, such as an increase in osmolarity or Angiotensin II, SDF-1/CXCL12 co-localized with AVP can be somatodendratically released and inhibit AVP release by an autoregulatory mechanism involving its receptor CXCR4. In conditions in which AVP is chronically stimulated, such as dehydration, SDF-1/CXCL12 is strongly released, resulting in a down-regulation of CXCR4. Such down-regulation of the receptor blocks the inhibitory effect of SDF-1/CXCL12 on AVP release, allowing a sustained increase in AVP secretion.
The CVOs such as the vascular organ of the lamina terminals or SFO located in the walls of the brain ventricular system and innervating the AVP neurons in the SON and PVN detect changes in plasma osmolarity. Interestingly, we observed that CVO contained CXCR4 (Fig. 3) and SDF-1/CXCL12 (not shown). A possible hypothesis is that SDF-1/CXCL12 in the CVO, through CXCR4, can inhibit locally stimulatory substance of AVP release such as angiotensin II, since angiotensin II receptor are known to be located in these CVOs. Finally, a change in water balance by long-term salt loading induces a decrease in both SDF-1/CXCL12 and CXCR4 parallel to that of AVP immunostaining in the SON. These data suggest that chemokines may represent a new class of neuromodulatory peptides that may be involved notably in the autocrine neuroendocrine control of AVP neurons (Fig. 4; Callewaere et al. 2006).

Concluding remarks

Very recent evidence thus suggests that chemokines may be added to the multiple peptides involved in the regulation of neuroendocrine pathways. This topic is of emerging significance. Although expressed at a much lower concentration in the brain than in other known regulatory peptides, chemokines are able to play a subtle but essential role in hormonal regulation, both in the brain and at the pituitary level. It represents a step forward in our knowledge in neuroendocrine regulation. Chemokines and their receptors, by their release such as angiotensin II, since angiotensin II receptor are known to be located in these CVOs. Finally, a change in water balance by long-term salt loading induces a decrease in both SDF-1/CXCL12 and CXCR4 parallel to that of AVP immunostaining in the SON. These data suggest that chemokines may represent a new class of neuromodulatory peptides that may be involved notably in the autocrine neuroendocrine control of AVP neurons (Fig. 4; Callewaere et al. 2006).

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