REVIEW

Multiple actions of the chemokine stromal cell-derived factor-1 α on neuronal activity

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Abstract

The chemokine SDF-1 α and its cognate receptor CXCR4 are expressed in several neuronal populations. This review focuses on our current knowledge about the actions of this chemokine on neuronal excitability, through CXCR4 or other yet unknown pathways. In various neuronal populations (CA1 neurons of the hippocampus, granular and Purkinje cells of the cerebellum, melanin-concentrating hormone neurons of the lateral hypothalamus, vasopressinergic neurons of the supraoptic and the paraventricular nucleus of the hypothalamus, and dopaminergic neurons of the substantia nigra), SDF-1 α can modulate the activity of neurons by multiple regulatory pathways including and often combining: (i) modulation of voltage-dependent channels (sodium, potassium, and calcium), (ii) activation of the G-protein-activated inward rectifier potassium current, and (iii) increase in neurotransmitter release (gamma-amino butyric acid (GABA), glutamate, and dopamine), often through Ca-dependent mechanisms. The possible mechanisms underlying these effects and their consequences in terms of modulation of neuroendocrine systems and physiopathology are discussed.

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Introduction

Chemokines are small secreted proteins with chemoattractant properties for immune cells (Luster 1998, Luther & Cyster 2001). At least 50 chemokines have been found to date and have been classified according to the number and spacing of the conserved cysteine residues at the N-terminal position (Murphy *et al.* 2000). Phylogenetic analyses showed that the large, highly redundant CXC chemokine family is a very recent phenomenon that is exclusive to higher vertebrates. Interestingly, its ancestral role might be within the central nervous system and not within the immune system (Huisin *et al.* 2003). Chemokines exert their biological effects through cell surface receptors that belong to the superfamily of seven-membrane domain G-protein-coupled receptors (GPCRs). At least 22 chemokine receptors have been characterized, which are designed following chemokine nomenclature. Most chemokines bind to several chemokine receptors and most chemokine receptors recognize several chemokines (Bacon & Harrison 2000). Besides their role in the immune system, chemokines and their receptors may play an important role in the central nervous system. For example, neurodegenerative and neuroinflammatory disorders, such as multiple

sclerosis, Alzheimer's disease, Parkinson's disease, and human immunodeficiency virus (HIV)-associated dementia are commonly associated with local chemokine release (Streit *et al.* 2001, Vila *et al.* 2001, Lee *et al.* 2002, McGeer & McGeer 2004, Cartier *et al.* 2005). However, the effects of these pro-inflammatory factors on neural activity remain elusive.

Among CXC chemokines, CXCL12/SDF-1 has attracted much attention. This chemokine was originally described as a secreted product of bone marrow stromal cell line (Tashiro *et al.* 1993). Three protein isoforms, SDF-1 α , SDF-1 β , and SDF-1 γ , which arise from alternative mRNA splicing, have been characterized (Gleichmann *et al.* 2000, Pillarisetti & Gupta 2001, Stumm *et al.* 2002); most studies have focused on SDF-1 α , which is the object of this review.

In the nervous system, *in situ* hybridization and dual immunohistochemistry revealed that SDF-1 α is constitutively expressed not only in astrocytes and microglia but also in neurons, in discrete neuroanatomical regions (Stumm *et al.* 2002, Banisadr *et al.* 2003). Indeed, neuronal expression of SDF-1 α is found mainly in cerebral cortex, substantia innominata and medial septum, globus pallidus, hippocampus, paraventricular and supraoptic hypothalamic nuclei, lateral hypothalamus, substantia nigra (SN), ventral tegmental

area, and oculomotor nuclei (Banisadr *et al.* 2003). Overall, SDF-1 α appears to be expressed in cholinergic, monoaminergic, and neuropeptide-expressing neurons, raising the possibility that SDF-1 α could act as a neuromodulator (Banisadr *et al.* 2003, 2005a).

Until very recently, CXCR4 has been thought as the sole receptor for CXCL12/SDF-1 chemokine among six CXC receptors (Bajetto *et al.* 2001a, Bonavia *et al.* 2003). Another receptor for this chemokine has recently been described in T lymphocytes and named CXCR7 (Balabanian *et al.* 2005), but to date, there is no evidence of its presence in the normal brain.

CXCR4, as its ligand SDF-1 α , is constitutively expressed by glial and neuronal cells in the CNS (Bajetto *et al.* 2001a, Bonavia *et al.* 2003). *In situ* hybridization and immunocytochemistry showed that CXCR4 neuronal expression was mainly found in cerebral cortex, globus pallidus, caudate putamen and substantia innominata (where CXCR4 immunoreactivity is co-localized with choline acetyltransferase immunoreactivity; Banisadr *et al.* 2002), supraoptic and paraventricular hypothalamic nuclei (where it is expressed in arginine-vasopressin (AVP) neurons; Banisadr *et al.* 2003), lateral hypothalamus (where CXCR4 is co-localized with neurons expressing the melanin-concentrating hormone (MCH); Guyon *et al.* 2005b), ventromedial thalamic nucleus, and SN (where CXCR4 is expressed on dopaminergic (DA) neurons of the pars compacta; Banisadr *et al.* 2002), and also on GABAergic neurons of the pars reticulata (Guyon *et al.* 2006) and in the cerebellum (where it is expressed both in the Purkinje neurons and granule cells and in the glial radial fibers; Ragozzino 2002). It is interesting to note that there is a co-distribution of CXCL12/SDF-1 and CXCR4 proteins in a number of brain regions, which strongly suggests that they could constitute together a functional receptor/ligand system in specific neuronal pathway.

CXCR4 activation by SDF-1 α activates multiple intracellular pathways (Lazarini *et al.* 2003). CXCR4 activation is coupled through pertussis toxin (PTX)-sensitive G proteins to at least two distinct signaling pathways. The first pathway, involving phosphatidylinositol-3 (PI-3) kinase and extracellular signal regulated kinase (ERK) $_{1/2}$, has been described in rodent astrocytes, neuronal progenitors, and cortical neurons (Bacon & Harrison 2000, Lazarini *et al.* 2000, Bajetto *et al.* 2001b, Bonavia *et al.* 2003). The other pathway involves the phospholipase C β whose activation leads to an increase in the intracellular calcium in astrocytes, cortical neurons, and cerebellar granule cell, as well as in primate fetal neuron and microglia (Bajetto *et al.* 1999, Klein *et al.* 1999, Zheng *et al.* 1999). The increase in calcium leads to the activation of proline-rich tyrosine kinase (PYK2), which may itself lead to ERK $_{1/2}$ activation (Bajetto *et al.* 2001b). CXCR4

stimulation can directly modulate ionic channel of the plasma membrane in neurons, particularly high-threshold calcium channels (our results and Zheng *et al.* 1999), and this could also result in the intracellular calcium increase and PYK2 activation (Lazarini *et al.* 2003). Finally, in the primary cultures of neurons, CXCR4 can also inhibit cAMP pathways through the Gi component of GPCRs (Liu *et al.* 2003).

Under ligand stimulation, CXCR4 undergoes a desensitization and internalization. Signaling and internalization of CXCR4 are regulated by receptor phosphorylation-dependent and -independent mechanisms. When independent of receptor phosphorylation, desensitization appears to be a consequence of the phosphorylation of phospholipase C β (Haribabu *et al.* 1997).

SDF-1 α has been shown to exert various functions in the brain (Lazarini *et al.* 2003). SDF-1 α has proved to be a potent chemoattractant for primary mouse microglial cells, but not for astrocytes (Tanabe *et al.* 1997). SDF-1 α does not seem directly to be a chemoattractant for neurons, but it reduces axonal responsiveness to several known repellents (Chalasani *et al.* 2003a). SDF-1 α is synthesized constitutively in the developing brain and has an obligate role in the neuronal migration during the formation of the granule-cell layer of the cerebellum (Ma *et al.* 1998, Zou *et al.* 1998, McGrath *et al.* 1999) and other brain area (Tran & Miller 2003). Indeed, mice that lack SDF-1 or CXCR4 died soon after birth and showed major defects in their vascular, hemopoietic, and central nervous system, in particular, in cortex and cerebellum development (Nagasawa *et al.* 1998, Tachibana *et al.* 1998, Stumm *et al.* 2003). Apart from this role in angiogenesis and development, CXCR4 is a receptor for the GP120 protein of the HIV, thus being a co-receptor for HIV entry into target cells and was thus also named fusin (Feng *et al.* 1996, Doranz *et al.* 1997). Neurotoxic effects of CXCR4 activation have been extensively studied in relation to the involvement of CXCR4 in HIV-associated dementia (Kaul & Lipton 2004, Khan *et al.* 2004). Massive stimulation of chemokine receptors during inflammatory processes may lead to apoptosis and neurodegeneration (Glabinski & Ransohoff 1999). On the other hand, SDF-1 α is neuroprotective in cultured hippocampal neurons when apoptosis is stimulated by gp120 treatment (Meucci *et al.* 1998). In addition, SDF-1 α has strong survival-promoting effects on cultured embryonic retinal ganglion cells through an action on CXCR4 (Chalasani *et al.* 2003b).

The aim of this review is to summarize the recent data which show that the chemokine SDF-1 α can also modulate the activity of several neuronal populations that may have a role under physiological and/or pathological conditions.

Effects on neuronal activity and neurotransmitter release

Recently, SDF-1 α was shown to act in the brain as a neuromodulator (reviewed in Lazarini *et al.* 2003, Banisadr *et al.* 2005b). The effect of chemokines on neuronal activity has been studied using electrophysiological and/or calcium-imaging techniques.

SDF-1 α modulates the activity of vasopressinergic neurons recorded in the rat supraoptic and paraventricular nucleus slices through CXCR4, resulting in changes in the AVP release (Callewaere *et al.* 2006). SDF-1 α 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 was similarly blocked by central injection of SDF-1 α through CXCR4 and a change in water balance induced a decrease in both SDF-1 α and CXCR4 parallel to that of AVP immunostaining in supraoptic nucleus (Callewaere *et al.* 2006).

CXCR4 and SDF-1 α are expressed in MCH neurons of the lateral hypothalamus (LHA), a peptide-expressing neuronal population mainly involved in feeding intake and energy storage regulation (reviewed in Nahon 2006). SDF-1 α exerts multiple effects on this neuronal system (Fig. 1A): using rat brain slices of hypothalamus in which MCH neurons were identified electrophysiologically and *a posteriori* by single-cell RT-PCR, we have shown that SDF-1 α increases spontaneously glutamate and gamma amino butyric acid (GABA) release on these neurons and activates a G-protein-activated inward rectifier potassium (GIRK) current (Guyon *et al.* 2005b). SDF-1 α also modulates the action potential discharge of these neurons and interestingly, the effects vary as a function of the concentration (Guyon *et al.* 2005b): low concentrations (0.1–1 nM) decrease the frequency of discharge (Fig. 2B1), an effect blocked by the competitive antagonist AMD 3100, thus mediated through CXCR4, although this compound also has a weak partial agonist activity (Zhang *et al.* 2002). This decrease in frequency of discharge of action potentials is mediated through a decrease of voltage-gated sodium current and delayed rectifier-potassium current (thus inducing a slowing of the repolarization of the action potential; Guyon *et al.* 2005a). At the opposite, higher concentrations increase the frequency of discharge (Fig. 2B2), but this effect is not blocked by AMD 3100, suggesting that it is not mediated through CXCR4 (Guyon *et al.* 2005b). These different effects of SDF-1 α were recently confirmed by our group using brain slices from transgenic mice expressing the green fluorescent protein (GFP) under the promotor of *MCH* gene, a transgenic mouse model kindly provided by Prof. Jeffrey Friedman's laboratory, where MCH neurons can be easily identified before electrophysiological recordings (Fig. 2A1–A7).

SDF-1 α (25 nM) can enhance the excitatory synaptic transmission in rat hippocampus (Fig. 1B). This effect was antagonized by ω -conotoxin GVIA, a N-type calcium channel antagonist, and by 12G5, a specific antibody against CXCR4, suggesting that SDF-1 α acted through a CXCR4-mediated increase in intracellular calcium through N-type Ca currents (Zheng *et al.* 1999). However, in embryonic primary cultures of rat hippocampus, SDF-1 α at higher concentrations (50–100 nM) reduced the frequency of synchronized Ca spikes among hippocampal neurons through the activation of CXCR4 by inhibiting cAMP pathways (Liu *et al.* 2003).

In the cerebellum (Fig. 1C), SDF-1 α induced calcium transient in cultured granule cells through a PTX-resistant mechanism (Limatola *et al.* 2000). Purkinje neurons recorded in cerebellar slices in patch-clamp responded to SDF-1 α applications by an increase in spontaneous GABAergic activity and a slow inward current, which was drastically reduced by ionotropic glutamate receptor blockers. This current developed fully in a medium in which synaptic transmission was inhibited, suggesting that it was mediated by extra-synaptic glutamate, possibly released by surrounding glial and/or nerve cells. This current was followed by a rise in intracellular calcium sensitive to MCPG, thus mediated through metabotropic glutamate receptors, mGluRs (Limatola *et al.* 2000).

In rat SN (Fig. 1D), the CXCR4 receptor is expressed on both DA neurons and GABA axonal processes. Using whole-cell patch-clamp recordings in DA neurons of rat SN slices, we showed (Guyon *et al.* 2006 and unpublished data) that SDF-1 α exerts multiple pre- and postsynaptic effects on DA neurons, including (1) an increase in the frequency of spontaneous and miniature GABA_A postsynaptic currents by presynaptic mechanisms, consistent with the presence of CXCR4 on GABAergic neurons of the SN, as revealed by immunocytochemistry, (2) a glutamatergic inward current resistant to tetrodotoxine (TTX), likely due to glutamate release from non-neuronal cells; this inward current is not blocked by the CXCR4 antagonist AMD 3100 (1 μ M) consistent with the lack of CXCR4 on astrocytes under basal conditions as shown by immunocytochemistry, (3) an outward GIRK current, through CXCR4 activation, TTX sensitive, and prevented by the application of the GABA_B antagonist CGP 55845A suggesting GABA spillover onto GABA_B receptors, and (4) SDF-1 α (0.1–10 nM) also increases the amplitude of total high voltage-activated calcium (HVA Ca) currents through CXCR4 activation. This effect was reversibly reduced by ω -conotoxin GVIA, suggesting that SDF-1 α acted on N-type Ca currents, known to be mainly involved in DA release. However, at 100 nM, SDF-1 α inhibits 65% of HVA Ca currents by a CXCR4-independent mechanism. These effects of SDF-1 α on dopamine neuron activity were paralleled by modulations of dopamine release by DA neurons from the rat substantia

nigra, measured in preparations of dissociated neurons of rat mesencephalon as well as *in vivo* in the striatum, a projecting site of DA neurons, when SDF-1 α was injected in the SN (unpublished data of Patrick Kitabgi's team; U732 INSERM, Paris, France). These data strongly suggest that chemokines such as SDF-1 α can act as neuromodulators of DA neuronal activity.

It is interesting to note that SDF-1 α has convergent presynaptic actions in the different brain structures where it has been tested: it increases glutamate and/or GABA synaptic activities in lateral hypothalamus (Guyon *et al.* 2005b), hippocampus (Zheng *et al.* 1999), cerebellum (Limatola *et al.* 2000), and substantia nigra (Guyon *et al.* 2006). However, the presynaptic mechanisms of action of SDF-1 α vary from one structure to the other: for example, the increase in frequency of GABA_A postsynaptic events in response to SDF-1 α occurs through an indirect mechanism involving glutamate in the cerebellum (Limatola *et al.* 2000), while the effect is direct through CXCR4 in the SN (Guyon *et al.* 2006). Similarly, the glutamate release is TTX dependent in the lateral hypothalamus (LHA) (Guyon *et al.* 2005b), while it is TTX independent in the SN (Guyon *et al.* 2006). The target effects on the postsynaptic neurons also vary depending on the structure. For example, the SDF-1 α increase in presynaptic GABA release in the LHA evokes a tonic GABA_A current in MCH-expressing neurons but does not induce GIRK current through GABA_B receptors stimulation (Guyon *et al.* 2005b). This contrasts to what we found in DA neurons where no GABA_A tonic current was induced by SDF-1 α , but a GIRK current was activated through GABA_BR stimulation through a GABA spillover (Guyon *et al.* 2006). This could be due to various subunit compositions of the GABA_A receptor expressed in the two neuronal populations, with different kinetics, and/or different subcellular localization of the GABA_{A/B}/CXCR4 receptors and GIRK channels. Interestingly, in MCH neurons, SDF-1 α also induced the activation of a GIRK current, but this happened directly through CXCR4 stimulation. Finally, CXCR4 stimulation is able to modulate various voltage-dependent channels: Na⁺ and K⁺ channels of the

action potential in MCH neurons (Guyon *et al.* 2005b) and HVA Ca channels, in particular of the N-type, in DA neurons of the SN (A Guyon, unpublished data) and in presynaptic glutamatergic terminals of the hippocampus (Zheng *et al.* 1999).

In conclusion, from one structure to another, SDF-1 α has often similar consequences on neuronal transmembrane currents, but through different mechanisms.

How can SDF-1 α have opposite effects depending on the concentration?

SDF-1 α often appears to have opposite effects on neuronal function depending on the concentration. For example, in DA neurons, at low concentrations, it acts as a neuromodulator by potentiating K⁺-induced DA secretion and HVA calcium currents, whereas at higher concentration, it decreases DA release and HVA calcium currents. This can be paralleled to what happens in MCH neurons of the lateral hypothalamus, where SDF-1 α also exerts opposite effects on the action potential discharge depending on the concentration (Guyon *et al.* 2005b). Moreover, this can also be observed in other contexts, for example, low levels of SDF-1 (<100 ng/ml) are attractive, whereas higher levels (>1 μ g/ml) are repulsive for T cells (Zlatopolskiy & Laurence 2001). Several putative mechanisms for these opposite effects, which are not mutually exclusive, are reviewed in Fig. 3.

Two affinity sites on the CXCR4 receptor?

SDF-1 α interactions with its receptor CXCR4 occur at two binding sites in amino acids 1–17 of SDF-1 α (Crump *et al.* 1997). The initial step involves a 'docking site' on SDF-1 α (amino acids 12–17) in the N-terminus of CXCR4 (amino acids 10–21). Subsequently, residues 1–9 of SDF-1 α bind to another region within CXCR4. Although the signal appears to be transduced only when this 'signaling site' is bound, the occupation of

Figure 1 Different effects of SDF-1 α in various brain structures. (A) In the LHA, SDF-1 α increases spontaneous presynaptic glutamate and GABA release. Moreover, CXCR4 stimulation activates a GIRK current in MCH neurons. SDF-1 α also modulates the action potential discharge of these neurons through a modulation of voltage-gated sodium current and delayed rectifier-potassium current (Guyon *et al.* 2005a,b). (B) SDF-1 α induces an enhancement of excitatory synaptic transmission in rat hippocampus through CXCR4 and N-type calcium channel-dependent mechanism (Zheng *et al.* 1999). (C) In granule cells of the cerebellum, CXCR4 activation by SDF-1 α induces calcium transient. In Purkinje neurons, although they express CXCR4, SDF-1 α effects are mediated through extrasynaptic glutamate, possibly released by surrounding glial and/or nerve cells. Glutamate induces a slow inward current, 6-cyano-7 nitroquinoxaline-2,3 dione (CNQX) sensitive, thus involving the non-N-methyl D aspartate (non-NMDA) ionotropic glutamate receptors. This is followed by a rise in intracellular calcium sensitive to MCPG, thus mediated through metabotropic glutamate receptors, mGluRs (Limatola *et al.* 2000). (D) In rat substantia nigra (SN), SDF-1 α increases the frequency of spontaneous and miniature GABA_A postsynaptic currents by presynaptic mechanisms. It also induces a glutamatergic inward current resistant to TTX, likely due to glutamate release from non-neuronal cells. On DA neurons, SDF-1 α activates an outward GIRK current, through CXCR4 presynaptic activation and GABA spillover onto GABA_B receptors (Guyon *et al.* 2006). SDF-1 α (0.1–10 nM) also increases the amplitude of total high voltage-activated calcium (HVA Ca) currents through CXCR4 activation. However, at 100 nM, SDF-1 α inhibits 65% of HVA Ca currents by a CXCR4-independent mechanism.

Figure 2 Opposite concentration-dependent effects of SDF-1 α on the action potential discharge of MCH neurons of transgenic MCH-GFP mice. At the top are presented reconstructed transversal 250 μ m thick hypothalamic sections obtained from a 15-day-old transgenic mouse in which GFP is under the control of MCH promoter (MCH-GFP, A1). Notice the MCH neurons detected as green fluorescence and their absence in wild-type (WT) mouse (A2). A3 shows GFP fluorescence of a MCH neuron at a higher magnification when compared with background noise in WT mouse (A4). The same neuron as in A3 is presented in A5 by infrared-differential interference contrast (IR-DIC) microscopy (arrowhead on the recording electrode tip). A6 is an immunostained section of the lateral hypothalamus at P15 obtained with an anti-MCH antibody. Note that the distribution of MCH neurons is similar to that in A1. A7 is a Nissl staining of a hypothalamic slice at the same anteroposterior level, showing the different structures: ARH, arcuate nucleus; DMH, dorsomedian hypothalamus; fx, fornix; ic, internal capsule; LHA, lateral hypothalamus; ot, optic tract; VMH, ventromedian hypothalamus; ZI, zona incerta. (B) Whole-cell patch-clamp recordings in the current-clamp mode of MCH-GFP fluorescent neurons. At a low concentration (1 nM, B1), SDF-1 α decreases the frequency of action potential discharge, whereas at a higher concentration (10 nM, B2), SDF-1 α does the opposite.

one or two sites of the CXCR4, depending on the concentration, could activate different signaling pathways. Desensitization and internalization at the highest SDF-1 α concentrations could also be involved (Fig. 3A).

Homo-heterodimerization?

Following SDF-1 α interaction, CXCR4 undergoes a dimerization which is necessary for its functionality and signaling (Mellado *et al.* 2001, Toth *et al.* 2004).

Dimerization is accompanied by receptor phosphorylation as well as changes in signal transduction processes (Rodriguez-Frade *et al.* 2001). This dimerization enables the activation of the JAK/STAT pathway, which allows the subsequent triggering of G-protein-dependent signaling events (Vila-Coro *et al.* 1999; (Fig. 3B)).

Furthermore, SDF-1 α itself can form a dimeric structure in solution at non-acidic pH. This dimerization has been shown to be stabilized by glycosaminoglycan, and heparin-mediated oligomerization may be essential for signaling (Sadir *et al.* 2001, Veldkamp *et al.* 2005). It is therefore possible that, depending on the concentration, SDF-1 α would act as a monomer or oligomer on CXCR4 monomers or homodimers, leading to different responses. Heterodimerization is known to play a role in signal transduction of other metabotropic receptors, for example, GABA_B receptors interact with metabotropic glutamate receptors (Hirono *et al.* 2001). CXCR4 could also form heterodimers with other GPCRs, which could lead to complex responses according to the chemokines/peptides/neuromediator environment present in the extracellular medium. Indeed, we have preliminary data suggesting that CXCR4 should interact with GABA_B receptors. As SDF-1 α influences presynaptic GABA release, this could be another way to explain how different SDF-1 α concentrations lead to different effects. Furthermore, CXCR4 and CCR2 (the receptor for the chemokine MCP-1) are co-expressed in DA neurons of the SN. As they have been shown to form heterodimers (Percherancier *et al.* 2005), we are currently investigating whether SDF-1 α and MCP-1 can exert synergistic effects on DA neuron activity.

Action on receptors other than CXCR4?

It is not excluded that SDF-1 α at high concentrations could act on a receptor other than CXCR4 to exert its actions. This could explain why, in MCH and DA neurons, the effects of high concentrations of SDF-1 α were not blocked by the selective CXCR4 antagonist AMD 3100 at concentrations up to 1 μ M. Indeed, the T

Figure 3 Putative mechanisms of the opposite effects of SDF-1 α at low and high concentrations. (A) Depending on the concentration, SDF-1 α could bind only to the high-affinity site or to both high- and low-affinity sites, leading to distinct responses. (B) CXCR4 could induce different responses as monomer, homodimer, or heterodimer. The formation of the various complexes could depend on SDF-1 α concentration (and on its dimerization), but also probably on other factors, such as the CXCR4 phosphorylation/internalization, the local concentration of heparane sulfate, and/or some synergistic transmitters/GPCRs. (C) SDF-1 α could bind to another receptor than CXCR4, leading to a distinct response. (D) SDF-1 α cleaved by several proteases could lead to active peptides that would induce other responses than intact SDF-1 α .

lymphocytes orphan receptor, RDC1, just described as a new receptor for SDF-1 α and named CXCR7 (Balabanian *et al.* 2005) has been reported in tumor endothelial cells of the brain (Madden *et al.* 2004), but no data are available in the normal brain (Fig. 3C).

Actions of SDF-1 α metabolites?

Peptide metabolites can be active in other systems. For example, AVP fragments have a biological activity distinct from intact peptide (Stoehr *et al.* 1992, Fujiwara *et al.* 1997). This should also be the case for SDF-1 α . SDF-1 α can be cleaved by several enzymes, leading to peptides inactive on CXCR4. For example, dipeptidyl peptidase IV (DPP IV) cleaves the peptide into SDF-1 (3–68) product (Proost *et al.* 1998, Mentlein 1999), leukocyte elastase into SDF-1 (4–67) (Valenzuela-Fernandez *et al.* 2002), matrix metalloprotease (MMP)-2 into SDF-1 (5–67) (Zhang *et al.* 2003), and cathepsin G into SDF-1 (6–67) (Delgado *et al.* 2001); all proteolyzed fragments becoming inactive on CXCR4. Interestingly, leukocyte elastase also inactivates CXCR4 (Valenzuela-Fernandez *et al.* 2002). It is worth noting that the enzymes are often carried or secreted by cells attracted by SDF-1 α . In the case of MMP-2, pro-MMP-2 is produced by macrophages and activated by neuronal MT1-MMP (Zhang *et al.* 2003). Furthermore, CXCR4 activation by SDF-1 α itself has been shown to increase the secretion of MMPs (Klier *et al.* 2001). Thus, it appears that there may be a feedback process inactivating SDF-1 α once the target cells have reached the site of infection. On the other hand, the peptide SDF-1 α can be protected from DPPs cleavage by its binding with heparane sulfate present on cell membranes, which would result in concentrating it locally (Amara *et al.* 1999, Sadir *et al.* 2004). Among the metabolites of SDF-1 α , some may have physiological effects. Indeed, SDF-1 (5–67) implanted into the basal ganglia of mice can produce neuronal death and inflammation, and its actions are mediated through a G-protein-coupled receptor, as yet unidentified (Zhang *et al.* 2003). It is therefore possible that the opposite effects observed on neuronal activity at higher SDF-1 α concentrations could come from a cleavage of SDF-1 α by enzymes when heparan sulfate sites have been saturated. The possibility that other SDF-1 metabolites could affect neuronal activity/survival will have to be investigated by enzyme blockers or by applying the cleaved peptides directly (Fig. 3D).

Physiopathological considerations

The fact that SDF-1 α and its receptor CXCR4 are expressed in the same or interrelated neuronal populations suggests that SDF-1 α could act as a

neuromodulator and exert a tonic action on neurons. For instance, low concentrations of SDF-1 α could exert a tonic inhibition on MCH neurons, which are known to have a hyperpolarized membrane potential in basal conditions, when compared with orexin neurons of the LHA, which are in an intrinsic state of membrane depolarization (Eggermann *et al.* 2003). In addition, the CXCR4 antagonist AMD 3100 has its own effects when applied alone, which suggests that a tonic activation of CXCR4 occurs, at least in slices preparations, and that low levels of SDF-1 α are secreted under basal conditions. However, the slice preparation, in which the cells have been stressed, could also be considered as an inflammatory state (Fig. 4).

Inflammation is often accompanied with anorexia, but the mechanisms underlying this phenomenon are poorly understood (Plata-Salaman 2001). One of the pathways could involve MCH neurons. Indeed, the following acute injections of lipopoly saccharide (LPS) in mice, which induce an inflammatory response, there is a decrease in the expression of MCH mRNAs (Sergeyev *et al.* 2001). A decrease in MCH release from MCH neurons of the LHA could lead to anorexia. Following inflammation, cytokines are released in the blood and they can reach the brain, the blood–brain barrier permeability being increased. Cytokine stimulation could lead to higher levels of SDF-1 α by activation of glial or endothelial cells that release chemokines (Meucci *et al.* 1998, Ohtani *et al.* 1998, Lee *et al.* 2002). The released SDF-1 α could reach MCH neurons, bind CXCR4, and induce a change in the excitability of the neurons that could induce an adaptive answer to the inflammation and anorexia. One can also imagine that a prolonged inflammation, leading to higher levels of SDF-1 α , could lead to neurotoxicity through one or several of the mechanisms described in Fig. 3, and to neurodegenerescence. In this context, chronic LPS injections lead to a decrease in the number of MCH neurons (Gerashchenko & Shiromani 2004).

Similarly, SDF-1 α could exert autocrine effects on vasopressinergic neurons, since the chemokine as well as its receptor are present in these neurons. The known autoregulation of AVP on its own neurons and its control by apelin which is co-expressed with its receptors in these cells (De Mota *et al.* 2004), could be accompanied by a third autocrine system mediated by SDF-1 α . SDF-1 α could therefore affect the neuroendocrine circuits controlling drinking as well as feeding behaviors. We have preliminary data that sustain such hypothesis in the LHA (K Palin and F Moss, personal communication). It will be interesting to determine whether this is a particularity of AVP (and MCH) neurons or if most neuroendocrine cells express modulators and their receptors.

Figure 4 Physiopathological consequences of the effects of SDF-1 α in two neuronal populations: the MCH neurons of the LHA and the DA neurons of the substantia nigra. SDF-1 α released by axon collaterals or somato-dendritic structures (particularly in the case of the substantia nigra; Ludwig & Pittman 2003) could exert a tonic effect on MCH and DA neurons in basal conditions (left). In the case of an acute infection or inflammation, following activation of glial cells and/or vascular SDF-1 α transport, the SDF-1 α concentration increases and other factors such as glutamate are secreted. By modifying the firing pattern of neurons, SDF-1 α leads to neuronal and behavioral adaptive changes: decreased MCH release and anorexia, and increased DA release and compensatory mechanisms during asymptomatic steps of Parkinson disease (middle). When the SDF-1 α concentrations reach a certain threshold, when SDF-1 α exposure is prolonged in time or when SDF-1 α is associated to other factors or cleaved in metabolites, it could have irreversible consequences, such as neurodegenerescence (right).

Parkinson's disease is one of the most prevalent neurodegenerative disorders and is characterized by the progressive loss of DA neurons in the SN. In the presymptomatic states of the disease, the remaining DA neurons can compensate for the loss of DA neurons by increasing their activity (Zigmond 1997). There is increasing evidence to suggest that the brain inflammatory response contributes to Parkinson's disease pathogenesis (Kurkowska-Jastrzebska *et al.* 1999, Cicchetti *et al.* 2002). The loss of these neurons is associated with a glial response composed mainly of activated microglial cells and, to a lesser extent, of reactive astrocytes (Liberatore *et al.* 1999, Vila *et al.* 2001). This glial response may be the source of tropic factors that can protect against reactive oxygen species and glutamate. As we proposed in our and Patrick

Kitabgi's team studies, chemokines such as SDF-1 α by increasing DA release could also participate to promote compensatory mechanisms of remaining DA neurons at the early stages of the pathology. Apart from these beneficial effects, the glial response under chronic conditions can mediate a variety of deleterious events related to the production of reactive species, and pro-inflammatory prostaglandin and cytokines (Vila *et al.* 2001). In this context, it was recently reported that SDF-1 α expression was markedly increased in the reactive astrocytes in the SN of rat treated with 6-hydroxydopamine, a neurotoxin that induces selective destruction of central DA neurons (E Apartis Communication at the 7th French Neuroscience Society meeting, Lille, France 2005). This could represent a novel pathway associated with the induction

of DA neuronal death in Parkinson's disease, possibly through enhanced release of SDF-1 α and production of SDF-1 α (5–67) in the SN. Overall, these results call for further investigations into the role of chemokines, such as SDF-1 α on the activity and survival of DA neurons under normal and pathological conditions. Agents acting on CXCR4 (Heveker *et al.* 2001) could thus represent useful agents in neurodegenerative diseases involving neuroinflammatory disorders, such as multiple sclerosis, Alzheimer's disease, Parkinson's disease, and HIV-associated dementia.

General conclusion

Convergent data suggest that SDF-1 α could act in the central nervous system as a classical neuromediator under normal conditions and could modulate the activity of several neuroendocrine networks. However, during a pathological state (altered immune response or inflammation), abnormal concentrations of SDF-1 α and/or its presence at unusual sites can be found, due to its local production by glial and/or endothelial cells and/or its diffusion and transportation through the vascular circulation. This enhanced production of SDF-1 α could affect neuronal and neuroendocrine activities and modify the functioning of the brain, leading to pathological behaviors and/or neurotoxicity.

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