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Viewing Chemokines as a Third Major System of Communication in the Brain

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ABSTRACT

There is irrefutable proof that opioids and other classes of centrally acting drugs have profound effects on the immune system. Evidence is mounting that products of the immune system, such as chemokines, can reciprocally alter the actions of these drugs and the endogenous ligands for their receptors. Chemokines are a family of small (8 to 12 kDa) proteins involved in cellular migration and intercellular communication. With a few exceptions, they act on more than one receptor. Although the chemokines and their G protein-coupled receptors are located in both glia and neurons throughout the brain, they are not uniformly distributed. They are found in such brain areas as the hypothalamus, nucleus accumbens, limbic system, hippocampus, thalamus, cortex, and cerebellum. Among the chemokines differentially localized in brain neurons and glia are CCL2/MCP-1, CXCL12/SDF-1 α , CX3CL1/fractalkine, CXCL10/IP 10, CCL3/MIP-1 α , and CCL5/RANTES. Functional roles for the chemokine system, composed of the chemokine ligands and their receptors, have been suggested in brain development and heterologous desensitization. The system can alter the actions of neurologically active pharmacological agents such as opioids and cannabinoids and interact with neurotransmitter systems. In this review, we propose that the endogenous chemokine system in the brain acts in concert with the neurotransmitter and neuropeptide systems to govern brain function. It can thus be thought of as the third major system in the brain.

KEYWORDS: opioids, cannabinoids, chemokines, brain, desensitization, neurotransmitters

NEUROACTIVE COMPOUNDS

Several groupings of compounds are used in an attempt to categorize chemicals that can affect neuronal activity. The

common thread is that these agents not only affect the neuronal activity, but they play a functional role in regulating activity of the brain and nervous system in pathological and homeostatic states. A discussion of these agents may be found in many texts in neuropharmacology, neurophysiology, and neuroscience (for example, see Cooper et al¹ and Steward²). Moreover, it often depends on the particular situation as to which of the categories applies to a particular compound at a particular time. Among the most commonly used terms are neurotransmitter, neuromodulator, neuropeptide, and neurohormone. Even within these classes, overlap exists. For example, hypothalamic releasing factors are considered to be neurohormones, but they are a type of neuropeptide. Nevertheless, it is useful to consider some of the characteristics generally assigned to each of these categories in order to determine if chemokines fit into any of these categories.

1. Neurotransmitters are chemicals that are best understood in terms of how they function in interneuronal communication and in neuron-to-effector messaging. They are endogenous chemicals that are synthesized and released presynaptically following nerve stimulation and are found differentially distributed in the brain (as well as elsewhere). They are stored in a form ready to be released, and they can potentiate or block postsynaptic responses. There are mechanisms for inactivation or for removal from the synaptic cleft. Well-known examples are serotonin, dopamine, acetylcholine, and GABA.
2. Neuromodulators, unlike neurotransmitters, have no intrinsic activity but can modify synaptic activity, modulate transmission, and involve a second messenger system. Several neuropeptides have these characteristics. Although it is generally thought of as a neurotransmitter, serotonin has neuromodulator characteristics in certain areas such as the facial motor nucleus.
3. Neurohormones have intrinsic activity, are released from both neuronal and nonneuronal cells, and can

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travel and act at a site distant from their release site (eg, β -endorphin). In certain cases, neurotransmitters can also travel to other sites (eg, dopamine in the pituitary).

4. Neuropeptides generally target specific cells in the nervous system, including neurons and glia. Precursors are synthesized in the neuronal cell body (eg, pro-opiomelanocortin for β -endorphin and ACTH). Certain neuropeptides appear to act as neurotransmitters at certain sites (eg, Substance P at sensory afferents to the dorsal horn in the spinal cord). The mechanisms for inactivation in the synaptic cleft are not yet known for all members of the group. Examples of neuropeptides are enkephalins, dynorphin, cholecystokinin, substance P, endocannabinoids, and somatostatin.

Neuroscientists generally think of the systems of chemicals classified as neurotransmitters or neuropeptides as being responsible for neuronal transmission or modulation of neuronal events. The molar concentration of neuropeptides is 2 or 3 orders of magnitude lower than neurotransmitters and their receptors respond to the lower concentrations. Neurotransmitters and neuropeptides may be colocalized in individual synaptic terminals and may be coreleased. Like the neurotransmitter and neuropeptide systems, the chemokine system is widely but unevenly distributed in the brain and has both ligands and receptors located in neurons.^{3,4}

CHEMOKINES

A family of small (8-12 kDa) proteins found in the brain and the periphery, chemokines are involved in cellular migration and intercellular communication. Most chemokine ligands act on more than one receptor, although a few, such as CXCL12 and CX3CL1 have specificity for only one.^{5,6} Chemokines and their G protein-coupled receptors are located in both microglia and neurons in such brain areas as the hypothalamus, nucleus accumbens, limbic system, hippocampus, thalamus, cortex, olfactory bulbs, and cerebellum.^{3,7} Human neurons express multiple functional chemokine receptors and ligands.⁸ Neural progenitor cells express chemokine receptors.⁹ Among the chemokines having selective distribution in brain neurons and glia are CCL2/MCP-1, CXCL12/SDF-1 α , CX3CL1/fractalkine, CXCL10/IP10, CCL3/MIP-1 α , and CCL5/RANTES. (It should be noted that the official nomenclature is the first designation of each pair, with the final "L" denoting "ligand." The receptors are designated in a similar fashion, with the final letter "R"). As with neurotransmitter and neuropeptide systems, the differential distribution offers a clue as to possible functional roles.

SUMMARY OF EVIDENCE FOR CHEMOKINES IN THE CENTRAL NERVOUS SYSTEM

Several reports have contributed to the hypothesis that chemokines play a major role in brain function. These include the following:

1. Receptors for several of the chemokines are expressed by brain neurons.^{3,10}
2. Chemokines and their receptors are located selectively in areas throughout the brain.^{4,7,10}
3. Chemokines show selective distribution in brain neurons and glia.^{5,11}
4. Individual neurons appear to co-express multiple functional chemokine receptors and chemokines.^{8,10}
5. CCR5 and CXCR4 in neurons are localized in the perikaryon in a vesicular or granular pattern and extend into the dendritic processes and axons.¹²
6. CXCR4 coexists with neurotransmitters in neurons in the caudate putamen and substantia nigra.¹⁰ Coexistence with neurotransmitters may indicate an interaction of the 2 systems, especially if there is corelease.

Additional reports provide information supporting the idea of a functional role for chemokines in the brain:

1. Chemokines are involved in neuronal development.⁶
2. CXCL12 reduces the amplitude of evoked excitatory postsynaptic currents in Purkinje neurons in a dose-dependent manner.¹³
3. Functional chemokine receptors on astrocytes can release glutamate, which affects neuronal excitability.¹⁴
4. CXCR4 appears to be involved in the induction of neuronal apoptosis.¹⁵
5. CXCR4-mediated signal transduction may be involved in neuronal dysfunction during HIV-1-associated dementia.¹⁶
6. Chemokine receptor activation in neurons induces calcium transients and modulates ion channel activity,¹⁴ indicating that there may be release of transmitters.
7. CXCL12 may be involved in neuronal communication,¹⁷ and even cholinergic and dopaminergic neurotransmission.¹⁰

HOW CHEMOKINES INTERACT WITH OPIOIDS AND CANNABINOID: HETEROLOGOUS DESENSITIZATION

Most reports involved with chemokines in the brain focus on their role in inflammatory processes and in neurogenesis.

The discovery that there is heterologous desensitization between opioids and the chemokines *in vitro*,^{18,19} along with anatomical and immunohistochemical evidence about the chemokine system in the brain, led to a study designed to determine if such desensitization occurred *in vivo*. The concept of heterologous desensitization is illustrated in Figure 1, using opioids, cannabinoids, and chemokines as examples. *In vitro* evidence has demonstrated that the heterologous desensitization process is bidirectional, and chemokine receptor activation was shown to inactivate opioid receptor activity *in vitro*.^{18,20,21} Of potential functional significance was the finding that cross-desensitization of CCR5 by opioids resulted in a decreased susceptibility to R5, but not X4 strains of HIV-1.²²

To ascertain whether the *in vitro* findings had physiological relevance, *in vivo* experiments were conducted in male Sprague-Dawley rats. The aim was to determine if chemokine administration into the brain would block or diminish the antinociceptive effect of an opioid without producing an effect of its own. In these studies, the measure of analgesic response is the ability of a drug to increase the threshold to a noxious stimulus, in this case, exposure of the rat's tail to a cold water bath at -3°C . The end point is the rat's removal or flicking of its tail.²³ There is a cutoff time to prevent damage to the tail and the calculation is the percentage of the maximum possible analgesic response.

Animals were administered a range of doses of CXCL12, CCL5, or CX3CL1 directly into the periaqueductal gray (PAG), the area primarily involved in the antinociceptive effects of opioids, 30 minutes prior to morphine or D-Ala², NMe-Phe⁴-Glyol⁵ (DAMGO), a selective agonist at the μ -opioid receptor. The chemokines blocked the expected analgesic response to the opioids.¹⁸ The effects of the chemokines on opioid analgesia were dose-related and could be seen with doses as low as 1 ng. In another study, rats were given a PAG injection of a chemokine or saline before subcutaneous (sc) injection of 8 mg morphine (Figure 2A). Pretreatment with CCL5/RANTES (100 ng) 30 minutes before morphine administration significantly reduced the antinociceptive effect of morphine. In a similar fashion,

Figure 2. Antagonistic effect of chemokines on antinociception induced by sc morphine: (A) 30-minute pretreatment with RANTES injected into PAG ($n = 7-9$); (B) 30-minute pretreatment with SDF-1 injected into PAG ($n = 3-5$). MPA indicates maximum possible analgesia; SDF, SDF-1 α . Each point represents the mean $\pm$ SE.

CXCL12/SDF-1 α (100 ng), 30 minutes before 8 mg/kg morphine, significantly reduced the antinociceptive effect (Figure 2B). The lack of a total blockade is likely because the chemokines were injected directly into the PAG, whereas the morphine was sc. Since morphine also has analgesic actions that are outside the PAG, this is to be expected. Indeed, when both morphine and the chemokine are given into the PAG, no antinociception is seen (unpublished results, Chen et al, 2005).

The next question we explored was the time course of the desensitization. It was found that if the chemokine was given at the same time as the opioid, there was a marked attenuation of the expected opioid analgesia, but not a complete block (unpublished observations, Chen et al, 2005). If the chemokine was administered 60 minutes prior to the opioid, analgesia was only partially blocked, and if the interval was increased to 2 hours, the ability of CCL5 to desensitize the opioid receptor was abolished. Of interest was the finding that if the time interval was 2 hours or more and the chemokine was readministered, the blockade of the

Figure 1. Illustration of Heterologous Desensitization.

analgesic activity of DAMGO was restored.¹⁸ Several possibilities exist as to why there was a restoration of the blockade. For example, the process might involve a reversible opioid receptor blockade, or the release of the ligand from the receptor and its subsequent metabolism. The blockade could then be reinstated with a second administration of the ligand, resulting in a recombination of receptor and ligand. It is also possible that new receptors are expressed on the cell surface, permitting additional ligand to bind. Formal proof regarding mechanisms has not been obtained, but we have found that there is no detectable internalization of the chemokine or opioid receptor following cross-desensitization in either primary leukocytes or in several leukocyte and nonleukocyte cell lines.^{18,24} It has also been shown that the receptor is phosphorylated and that heterodimers form.²⁴ It may be that the phosphorylation is reversible, as it is with many other types of receptors. Both RANTES and SDF-1 show a similar pharmacokinetic pattern.

The experiments cited above demonstrated that the μ -opioid receptor could be desensitized by chemokines. D-Pen², D-Pen⁵-enkephalin (DPDPE), a selective δ agonist, and dynorphin A 1-17, the endogenous opioid that is a selective κ agonist, were used to determine if the δ -opioid and the κ -opioid receptors could also be cross-desensitized in vivo. As with the μ -opioid receptor, both the δ - and κ -opioid receptors were desensitized by CCL5 and CXCL12, indicating that there was a desensitization of all 3 types of opioid receptor by the CCR5 and CXCR4 chemokine receptors¹⁸ (and unpublished results from our laboratories, 2005). These receptors are expressed on neurons in various areas of the brain, including the PAG.¹⁰ In order to confirm that the chemokines act via their respective receptors, it is necessary to use a selective chemokine receptor antagonist. AMD 3100, an antagonist at the receptor for CXCL12, was tested. As expected, it prevented the desensitization of DAMGO, thus providing proof that the chemokine desensitization of the μ -opioid receptor was mediated via the chemokine receptor (unpublished observations, Chen et al, 2005).

Like CXCL12, CX3CL1 acts on a single receptor, has no antinociceptive action when administered into the PAG, and is expressed in the brain. In fact, it is the only chemokine reported to be expressed in higher concentrations in the central nervous system (CNS) than in the immune system and peripheral tissues.⁶ CX3CL1, like CXCL12 and CCL5, was able to block the antinociceptive action of μ -, κ -, and δ -opioid receptor agonists (unpublished observations, Chen et al, 2005).

Receptors for CCL5, CXCL12, and CX3CL1, the chemokines that produced desensitization of the opioid receptors, have all been found in the PAG. However, CCR2 is a chemokine receptor not found in the PAG at a significant level.⁴ As a consequence, injections of CCL2, the ligand for CCR2, into the PAG did not induce the cross-desensitization of the opioid receptors.

The in vitro results and the anatomical-type studies reported above suggest the importance of the chemokine system in the brain. Recent in vivo experiments indicate strongly that the chemokine system is important in brain function. Among those findings reported in abstracts and presented at meetings (manuscripts in preparation) are as follows:

1. CXCR4, CCR5, and CX3CR1 induce heterologous desensitization of μ -, κ -, and δ -opioid receptors in the PAG as demonstrated by effects on analgesia.
2. The desensitization is time- and dose-related, lasts for 60 to 90 minutes, and is reversible for μ -opioid receptors¹⁸ and for δ - and κ -opioid receptors.
3. CXCR4 and CCR5 also desensitize cannabinoid receptors for analgesia, while CX3CR1 does not.

HYPOTHESIS

The systems of neurotransmitters and neuropeptides in the brain are accepted as playing the major role in the functioning of the brain in maintaining homeostasis and reacting to perturbations of that homeostasis. *We propose that the endogenous chemokine system in the brain, consisting of ligands and receptors, is a third major system of the brain.* It is the third leg in the stool that supports brain function.

The chemokines have many of the characteristics discussed above under neuroactive compounds. They have important actions in the brain and they have both direct and indirect actions on neurons. In fact, Bajetto et al¹⁵ suggested that chemokines might act as neuromodulators, and Tran and Miller²⁵ questioned whether chemokines might play some unexpected role in the physiology of the normal brain. Given the evidence that has accumulated in the past 5 to 7 years, it is reasonable to use the above hypothesis relating to the chemokine system in the brain to begin to make testable predictions.

The evidence cited above indicates that the chemokine system in the brain may well play a vital role in the functioning of the brain under both homeostatic and perturbed situations. Figure 3 illustrates the concept being proposed in this review. It is known that glia can produce and release chemokines and that chemokines and their receptors are present in neurons. We are proposing that, in addition to their accepted role in glia-to-glia and glia-to-neuron communication, the chemokines function in neuron-to-glia communication. Even more important, perhaps, is our hypothesis that neuron-to-neuron communication, in many cases, involves the chemokine system of ligands and receptors. The neuropeptide and neurotransmitter systems function to transmit information between neurons, and we propose that chemokines also have this function. The fine control exercised by the brain under both normal and perturbed conditions is consonant with the idea of the chemokine system serving as an additional system of

Figure 3. Communication pathways in the nervous system.

interacting molecules to explain how the neuronal system achieves such magnificent control of discrete physical, behavioral, and chemical action. It also allows one to anticipate new approaches to treat perturbations in homeostasis and enhance brain activity in nonpathological states.

POTENTIAL THERAPEUTIC SIGNIFICANCE

Several novel potential therapeutic applications can be envisioned by coupling the capacity for heterologous desensitization of the opioid and chemokine systems with knowledge about the neuronal function of the chemokine system. The *in vivo* finding that activation of the chemokine receptor CXCR4 or CCR5 in the PAG of the brain results in a rapid cross-desensitization of both the μ - and δ -opioid receptors,^{18,20} depressing the analgesic function of the opioid receptors and enhancing perception of pain, has great implications. In situations where there is an elevation of chemokine levels in the brain (including most neuroinflammatory diseases such as multiple sclerosis and HIV encephalitis), a resulting loss of opioid receptor function could lead to greater sensitivity to painful stimuli.^{18,26} Findings such as these, coupled with other evidence cited above about the chemokine system in the brain suggest the following therapeutic opportunities:

1. Alter treatment of pain associated with neuroinflammatory states by blocking heterologous desensitization of opioid receptors that may account for diminished efficacy of opioids in inflammatory pain.
2. Decrease certain unwanted side effects of opioids by chemokine desensitization of selected opioid pathways after determining neuronal localization of chemokine receptors.

3. Desensitize chemokine receptors that serve as coreceptors for entry of HIV in the CNS or periphery, resulting in reduced HIV susceptibility. The cross-desensitization of CCR5 and CXCR4 by other G protein-coupled receptors, including the opioid receptors, has been shown to result in the loss of HIV coreceptor function.²²
4. Use the effects of chemokines to modify effects of drugs on behavior by altering the interactions of the neurotransmitter and neuropeptide systems involved in the behavioral effects of drugs abused or used therapeutically to treat mental disorders.
5. Bring new approaches to prevention and treatment of neurodegenerative diseases through blocking or enhancing the actions of chemokines.

OPEN QUESTIONS AND RESEARCH DIRECTIONS

As this review has put forth the postulate that chemokines function as transmitters in neuron-to-neuron, glia-to-neuron, and neuron-to-glia communication, it is vital that experiments be conducted to add to the body of information required to establish this role in the brain. Because the chemokine system has only recently been found to be involved in brain function, much remains to be discovered. As examples, the mechanism of release from neurons, the storage and the inactivation mechanisms, and their presence at excitatory or inhibitory synapses are not known. In addition, there are the questions as to whether the system is both pre- and post-synaptic, and whether the receptors endocytose. Little information exists as to specific functional systems in the brain linked to individual chemokines. However, despite the fact that neuropeptides have been known for over 30 years, not all the criteria have as yet been met¹ to allow neuropeptides to be called neurotransmitters. Nevertheless, there is general acceptance of their importance in interneuronal communication. The evidence that exists is consistent with the hypothesis that the chemokine system acts as a major system in the brain to facilitate or to be an actual chemical network of ligands and receptors that transmits information in a manner analogous to neurotransmitters and neuropeptides.

CONCLUSIONS

We propose that the endogenous chemokine system in the brain acts in concert with the neurotransmitter and neuropeptide systems to govern brain function. The chemokines are postulated to function as transmitters in neuron-to-neuron, glia-to-neuron, and neuron-to-glia communication. In addition, the system can alter the actions of neuronally active pharmacological agents such as opioids and cannabinoids and interact with neurotransmitter systems. Much remains to be discovered about the chemokine

system in the brain. By understanding this system, therapeutic possibilities range from altering treatment of neuro-inflammatory states to interfering with entry of HIV into cells to new treatment of degenerative diseases to modifying treatment of mental disorders to diminishing unwanted side effects of opioids.

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