Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres

Research Report

CXCL12 sensitizes vago-vagal reflex neurons in the dorsal medulla

*Richard C. Rogers, Edouard Viard, Gerlinda E. Hermann**

Autonomic Neuroscience Laboratory, Pennington Biomedical Research Center, Louisiana State University System, 6400 Perkins Rd, Baton Rouge, LA 70808, United States

ARTICLE INFO

Article history:
Accepted 15 November 2012
Available online 23 November 2012

Keywords:
Hindbrain
Chemokines
Gastric
Vagus
Single unit

ABSTRACT

Previous studies from our laboratory illustrated the potential for stromal cell-derived factor one [CXCL12; also referred to as SDF-1] to act on its receptor [CXCR4] within the dorsal vagal complex [DVC] of the hindbrain to suppress gastric motility (Hermann et al., 2008). While CXCR4 receptors are essential for normal brain development, they also play a critical role in the proliferation of the HIV virus and initiation of metastatic cell growth in the brain. Anorexia, nausea, and failed autonomic regulation of gastrointestinal function are significant causes of morbidity and are contributory factors in the mortality associated with these disease states. The implication of our previous study was that CXCL12 caused gastric stasis by acting on gastric reflex circuit elements in the DVC. This hindbrain complex includes vagal afferent terminations in the solitary nucleus, neurons in the solitary nucleus (NST) and visceral efferent motorneurons in the dorsal motor nucleus (DMN) that are responsible for the regulation of digestive functions from the oral cavity to the transverse colon. In the current study, in vivo single-unit neurophysiological recordings from physiologically-identified NST and DMN components of the gastric accommodation reflex show that while injection of femtomole doses of CXCL12 onto NST or DMN neurons has no effect on their basal activity, CXCL12 amplifies the effect of gastric vagal mechanosensory input to activate the NST and, in turn, inhibit DMN motor activity.

1. Introduction

Chemokine receptors (e.g., CXCR4) in the brain are emerging as pleiotropic regulators of brain function in normal and pathological conditions (Banisadr et al., 2002, 2003). Several lines of evidence have shown that successful brain development is critically dependent on the CXCR4 receptor (Li and Pleasure, 2005; Lu et al., 2002). This agonist (CXCL12) and receptor (CXCR4) combination is also involved in neurological disorders associated with multiple sclerosis, Alzheimer's, and Parkinson's diseases (Glabinski et al., 2000; Ransohoff et al., 1996). The CXCL12/CXCR4 interaction also appears to be critical in the initiation and maintenance of glial metastasis (Gabuzda and Wang, 2000). Furthermore, the CXCR4 receptor may serve as the portal of entry by the HIV virus into neurons and glia in acquired immune deficiency syndrome (Gabuzda and Wang, 2000). CXCL12 and CXCR4 are also implicated in neural, glial and vascular remodeling after ischemic brain

Abbreviations: DMN, dorsal motor nucleus of the vagus; DVC, dorsal vagal complex; GAR, gastric accommodation reflex; nL, nanoliter; NST, nucleus of the solitary tract; TNF, tumor necrosis factor

*Corresponding author. Fax: +1 225 763 0273.
E-mail address: hermange@pbrc.edu (G.E. Hermann).

0006-8993/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2012.11.022

injury (Wang et al., 2012). While CXCL12 is constitutively expressed at low-levels in the CNS, hypoxia significantly activates the chemokine's expression and release from astrocytes (Li and Ransohoff, 2008).

CNS neuroinfection, metastasis, and ischemic stroke are also accompanied by a morbidity syndrome variously comprised of nausea, anorexia, emesis and failed gastrointestinal transit (Cheema et al., 2001; Frank et al., 1989; Kaufman et al., 1986; Plata-Salaman, 2000; Schaller et al., 2006). This observation suggests that a site of action of CXCL12 may be within the neural circuitry involved in the control of gastric function. The medullary circuits responsible for coordinating autonomic reflex control of the gut are contained within the dorsal vagal complex [DVC] of the hindbrain. The DVC is composed of the nucleus of the solitary tract [NST], which receives the general visceral afferent input from the afferent vagus, and the dorsal motor nucleus of the vagus [DMN], which is the principal source of parasympathetic efferent control over the gastrointestinal tract. The NST is an important organizer and processor of visceral afferent activity and it regulates gastrointestinal function via vago-vagal reflex connections with the DMN [see (Rogers and Hermann, 2012) for review]. The NST is also critically important for the production of emesis (Andrews and Horn, 2006). Indeed, the perception of nausea associated with emesis may be the awareness of the profound gastric relaxation that precedes the emetic act (Andrews et al., 1990; Andrews and Horn, 2006; Hornbuckle and Barnett, 2000; Miller, 1999; Wolf, 1943).

Our previous study (Hermann et al., 2008) supported a connection between the CXCR4 receptor in the DVC and modulation of gastric function. Both visceral sensory NST neurons and parasympathetic efferent DMN neurons exhibited strong immunohistochemical labeling for the CXCR4 receptor. Additionally, application of CXCL12 to the DVC evoked a reduction in gastric motility paired with increases in cFOS activation of cells in the NST (Hermann et al., 2008). The implication of those results was that CXCL12 may cause an increase in the sensitivity of gastric vago-vagal reflexes by acting on the neurons in NST and/or the DMN; thus explaining the gastroinhibition caused by CXCL12. Therefore, the present study was designed to investigate the effects of CXCL12 on NST and DMN neurons that are first physiologically-identified as belonging to a circuit responsible for controlling gastric relaxation.

2. Results

2.1. *CXCL12 effects on identified gastric reflex NST and DMN neurons*

As we have reported earlier (McCann and Rogers, 1992; Viard et al., 2012), gastric-NST neurons are essentially silent unless driven by afferent input. In contrast, gastric-DMN neurons are spontaneously active and this activity is suppressed by afferent activity (Fig. 1A). For example, mild distension (0.5 ml) of the antral stomach with the gastric balloon evokes a crisp train of action potentials in gastric-NST neurons while eliciting a "mirror image" response in gastric-DMN neurons (Fig. 2B), i.e., a significant reduction in ongoing activity time-locked to the distension (i.e., GAR response). The GAR response was determined under both basal and experimental

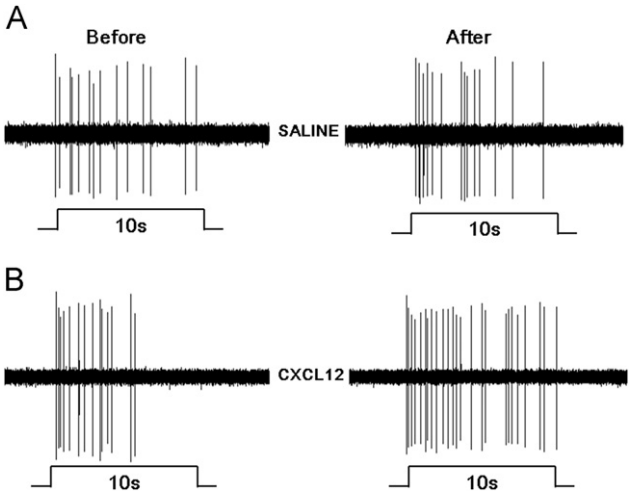

Fig. 1 – An example of raw neurophysiological data from a gastric-NST neuron responding to gastric distension. (Note: Demonstration of verification that data are obtained from the same, individual neuron is seen in Fig. 3). Gastric-NST neurons are typically quiescent until stimulated. In these experiments, 0.5 ml distension of the antral balloon for 10 s was used to activate gastric-NST neurons. Each identified neuron served as its own control. (A) Microinjection of 1 nL saline into the NST did not affect the responsiveness of gastric-NST neurons to the antral distension stimulus. (B) Microinjection of 1 nL CXCL12 [10 femtomole total dose] increased the responsiveness of the identified gastric-NST neurons. Ten second scale bars in A and B denote onset and offset of gastric distension. Quantitated results from all NST neurons examined are presented in Fig. 3A.

condition for each identified neuron, therefore each cell served as its own control (Fig. 1B, Fig. 2C).

Note that proof of single unit recording cannot be made from the slow spike train trace used to evaluate the neuron's response to the GAR (e.g., Figs. 1 and 2) as the displayed spike amplitudes may appear to vary as a result of low sample rate aliasing. Therefore, to verify that neurophysiological data were obtained from the same, identified, individual neuron, spike records were re-analyzed at a faster rate to show multiple, superimposed spikes obtained before, during, and after the period of gastric distension. Based on the uniformity of the overlapping waveforms (Fig. 3), this method can clearly differentiate between single units such as the NST cell shown in Fig. 1 and the DMN cell presented in Fig. 2.

The percentage change in response to the same stimulus under these two conditions represents the "relative sensitization" of the neuron as a consequence of its exposure to agonist and/or antagonist (Fig. 4). Nano-injections of CXCL12 [10femtomoles] had no effect on the basal activity of either gastric-NST or -DMN neurons; e.g., see Fig. 2A. One-way analysis of variance of the relative sensitization scores of either gastric-NST or -DMN neurons were statistically significant [DMN: $F_{3,55}=7.9$; $p=0.0002$; NST: $F_{3,44}=5.2$; $p=0.004$]. Dunnett's post hoc tests using the "saline" group as the point of comparison revealed that both gastric-NST and -DMN neurons were significantly sensitized by CXCL12 ($p<0.05$; Fig. 4). Note that the CXCL12 antagonist, AMD3100, had no effect of its own