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A role for CXC chemokines and their receptors in stress axis regulation of common carp

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ABSTRACT

Although chemokines mainly function to activate leukocytes and to direct their migration, novel evidence indicates non-immune functions for chemokines within the nervous and endocrine systems. These include development of the nervous system, neuromodulation, neuroendocrine regulation and direct neurotransmitter-like actions. In order to clarify a potential role for chemokines and their receptors in the stress response of fish, we studied changes in the expression patterns of CXC ligands and their receptors in the stress axis organs of carp, during a restraint stress procedure.

We showed that stress down-regulated the gene expression of CXCL9-11 (CXCb1 and CXCb2) in stress axis organs and up-regulated expression of CXCR4 chemokine receptor in NPO and pituitary. Moreover, upon stress, reduced gene expression of CXCL12a and CXCL14 was observed in the head kidney.

Our results imply that in teleost fish, CXC chemokines and their receptors are involved in neuroendocrine regulation. The active regulation of their expression in stress axis organs during periods of restraint indicates a significant role in the stress response.

1. Introduction

Chemokines are small chemotactic cytokines, initially described as factors activating leukocytes and directing their migration/chemotaxis. Chemokines are divided into 3 categories: i) homeostatic chemokines – mainly responsible for maintenance of homeostasis and lymphoid organ homing, ii) pro-inflammatory chemokines – inducing migration of leukocytes towards the focus of inflammation and iii) dual function chemokines, involved in both homeostatis and inflammation (Gerard and Rollins, 2001). Another chemokine division is based on their structure, specifically the position of the conserved cysteine-motif at the N-terminal end of the protein. Based on this 4 families are defined: CXC, CC, CX3C, and C (Zlotnik et al., 2006). The biological effects of chemokines are mediated through seven trans-membrane domain G-protein coupled receptors (GPCRs) and, according to the chemokine group they preferentially bind, they are divided into: CXCR.CCR, CXC3R and CR (Rossi and Zlotnik, 2000). To date, over 50 chemokines and 20 chemokine receptors have been characterized in mammals (Allen et al., 2007), while teleost fish appeared to have an even larger chemokine repertoire, with 111 chemokines identified in the zebrafish genome (Palevitch et al., 2010; Nomiyama et al., 2008).

CXC ligands form one of the best known family of chemokines which is further subdivided into two groups: i) chemokines (CXCL1-3, 5–8 and 15) with an ELR (Glu-Leu-Arg) motif preceding the first cysteine, responsible mainly for activation and direction of neutrophils and ii) CXC chemokines lacking this motif (e.g. CXCL4 and CXCL9-11), which in mammals do not attract neutrophils and act mainly on monocytes and lymphocytes (Rossi and Zlotnik, 2000). Moreover, the ELR + CXC chemokines induce angiogenesis whereas ELR- CXC chemokines are angiostatic (Zhang et al., 2016). In mammals 17 CXC chemokines and 7 CXCR were described (Zhang et al., 2016). Recent studies demonstrate that several genes for CXC ligands and receptors exist also in teleost fish. For example, in common carp genes for seven CXCLs (two CXCL8-like: CXCa/CXCL8_L1, CXCL8_L2, two CXCL9-11-like: CXCb1 and CXCb2, two CXCL12: CXCL12a and CXCL12b and one CXCL14) and four CXCRs (CXCR1-CXCR4) were reported (Verburg-van Kemenade et al., 2013). Among the CXC ligands, only two homeostatic chemokines, CXCL12 and CXCL14, mainly involved in development, show clear orthology to mammalian counterparts. The pro-inflammatory (CXCL8 and CXCb) chemokines are rather functional homologs than true orthologs to mammalian CXCL8 and CXCL9-11, respectively (van der Aa et al., 2012a,b, 2010). We and others showed that the CXCL8 related genes usually show increased transcription during early stages of infection/inflammation. In phagocytes they are

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upregulated by inflammatory stimuli like LPS (Abdelkhalek et al., 2009; Laing et al., 2002; van der Aa et al., 2010). Moreover, both *in vitro* and *in vivo* recombinant CXCL8_L1 and CXCL8_L2 induce migration of phagocytes (van der Aa et al., 2010). In contrast, IFN-γ-inducible CXCb chemokines are chemoattractive for both lymphocytes and phagocytes (van der Aa et al., 2010). These data are largely in line with studies in mammals, suggesting that many of the immune functions of the teleost CXC chemokines are relatively well conserved.

Interestingly, novel evidence indicates non-immune functions for chemokines within the nervous and endocrine systems, including development of the nervous system, neuromodulation, neuroendocrine regulation and direct neurotransmitter-like actions [for review see Ślusarczyk et al., 2016 and Stuart et al., 2015]. For example, several CXC chemokines (i.e., CXCL1, CXCL10, CXCL12) have been identified as novel regulators of the hypothalamic–hypophysial axis (Grizzi et al., 2015) while CXCL12-CXCR4 were designated to be critical factors supporting growth and survival of neurons and glia (Barbieri et al., 2007). In mammals, CXCL8 is expressed both in the paraventricular nucleus, where the corticotropin releasing factor (CRF) is produced, and in the hippocampus, where negative feedback to the hypothalamus-pituitaryadrenal axis (HPA) is generated. Therefore, CXCL8 is hypothesized to be of physiological importance for the stress response (Philip and Gold, 1992). Moreover, a role in neuroendocrine regulation is corroborated by the analysis of the evolution of the CXC family of chemokines and by quantification of their profile of expression in different organs/tissues in lower vertebrates. Although most chemokines developed relatively recently and in association with the development of an immune system, ancient chemokines, such as CXCL12 and CXCL14, existed prior to immune system development and are involved in the development of the nervous system (Huising and Stet, 2003).

Analogous to the situation in higher vertebrates, the stress response in fish is characterized by activation the hypothalamo-pituitary-interrenal axis (HPI). Its activity starts in the hypothalamic nucleus preopticus (NPO), which releases CRF. CRF stimulates the pituitary pars distalis (PD) to secrete ACTH, which is cleaved from the pro-hormone pro-opiomelanocortin (POMC). ACTH in turn stimulates the release of cortisol from the interrenal cells in the head kidney (Wendelaar Bonga, 1997; Mosconi et al., 2002; Piccinetti et al., 2017). Common stressors encountered in fish include physical and mental trauma associated with capture, transport, handling, and crowding; malnutrition; variations in water temperature, oxygen, and salinity (Harper and Wolf, 2009).

In order to clarify a potential role of chemokines and their receptors in the stress response of fish we studied changes in the expression patterns of CXC ligands and their receptors in the stress axis organs of carp during a restraint stress procedure.

2. Materials and methods

2.1. Animals

Prior to the experiments fish (common carp, *Cyprinus carpio* L., 100 g body weight, from the Department of Ichthyobiology and Aquaculture, Polish Academy of Science, Golysz, Poland) were adapted for four weeks at 20 °C in recirculating tap water in the Animal Facilities of the Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland. Fish were kept in separated equally positioned identical tanks (volume 375 l, flow rate 4 l/min, density 45 fish/tank, 60 g/l) and all unnecessary interferences were avoided. In order to avoid additional stress and/or differences in handling, all samplings were performed by the same person and at the same time of the day (at 9.00 am). Fish were cultivated and fed as described previously (Pijanowski et al., 2015).

All animals were handled in strict accordance with good animal practice and procedures were approved by the Local Ethical Committee (license number 292/2017).

2.2. Stress model and organ isolation

Restraint (24 h) was given by netting fish and suspending the nets with the fish in identical tanks as described previously (Metz et al., 2006; Pijanowski et al., 2015). Briefly, fish were stressed by confinement of one fish in a net in their own aquarium while maintaining full contact with the water. During the stress challenge fish were not fed. After 24 h restraint, the experimental group was transferred all at once to a tank with 0.2 g l^{-1} Tricaine methane sulphonate (TMS, Sigma-Aldrich, St. Louis, MO, USA) buffered with 0.4 g l^{-1} $NaHCO_3$ (POCH, Gliwice, Poland), resulting in rapid (< 1 min) and deep anesthesia prior to sampling. A control group was housed in an identical tank but left undisturbed. Control fish were sampled following rapid netting and anesthesia, immediately before sampling of the experimental group. Every experiment was performed independently 2 times with at least 3 fish per group every time. Finally, 7 control and 9 stressed fish were used for analysis.

Nucleus preopticus (NPO), pituitary gland and head kidney (HK) were carefully removed as described previously by Metz and co-workers (Metz et al., 2006). Organs were submerged in RNAlater® Stabilization Reagent (Qiagen GmbH, Hilden, Germany) and stored at 4 °C.

2.3. Gene expression

Total RNA extraction, DNase I digestion, first-strand cDNA synthesis, and Real-time quantitative PCR (qRT-PCR) were conducted as previously reported (Pijanowski et al., 2015).

Carp-specific primers (5′–3′) for gene expression of CXC chemokines and CXC receptors were used (Chadzinska et al., 2014; Huising et al., 2004; van der Aa et al., 2012a, 2010). The 40S ribosomal protein s11 gene served as an internal standard. Accessions numbers and primer sequences are described in Supplementary materials (Table S1). All RT-qPCR reactions were performed with the Applied Biosystems StepOne™ Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) using SYBR® Select Master Mix as follows: preheating at 50 °C for 2 min, denaturation at 95 °C for 2 min, and 40 cycles of amplification and quantification (15 s at 95 °C and 60 s at 60 °C), followed by melt curve analysis (ramp + 0.5 °C).

Relative gene expression of target genes in control fish was calculated using the $2^{-\Delta CT}$ method initially normalized against 40S ribosomal protein s11 gene expression (the internal control). Relative expression of target genes in stressed fish was also normalized to that of 40S ribosomal protein s11 using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

2.4. Statistical analysis

Data were expressed as mean ± standard deviation (SD) and significance of differences was determined using T test. For all tests significance level of $p < 0.05$ was used.

3. Results and discussion

All *cxcl* and *cxcr* genes that were studied showed constitutive expression in the stress HPI (hypothalamus-pituitary-interenal) axis organs (Table 1). Not surprisingly the majority of CXC ligand and receptor genes (except CXCb ligands and their putative receptor CXCR3, CXCL12b and CXCL14) showed the highest expression in the head kidney, which in teleost fish comprises the interrenal tissue with cortisol producing cells and the adrenalin-producing chromaffin cells, as well as the major hematopoietic tissue (Verburg-van Kemenade et al., 2017). Therefore, we assume that high expression of chemokines and their receptors in the head kidney is mainly serving the important immune function of this organ. Similarly, in the previous studies of us and others in common carp, constitutive expression of *cxcl12a*, *cxcl8_l1* and *cxcl8_l2*, and both *cxcb* genes was observed mainly in the lymphoid

Table 1
Constitutive gene expression of CXC chemokines and their receptors in the NPO (nucleus preopticus), pituitary gland and the head kidney of common carp. Gene expression was determined by quantitative RT-PCR and expressed relatively to expression of the 40S ribosomal protein s11 gene. Averages and SD (n = 7).

Gene	NPO	Pituitary	Head kidney
cxcl8_l1	0.0019 ± 0.0006	0.0003 ± 0.00009	0.1094 ± 0.0647
cxcl8_l2	0.0001 ± 0.00003	0.0010 ± 0.0006	0.0035 ± 0.0012
cxcb1	0.0080 ± 0.0046	0.0008 ± 0.0003	0.0075 ± 0.0029
cxcb2	0.0546 ± 0.0212	0.0253 ± 0.0228	0.0059 ± 0.0017
cxcl12a	1.6950 ± 0.4987	0.0560 ± 0.0118	14.4461 ± 6.1316
cxcl12b	0.6487 ± 0.3056	0.0033 ± 0.0007	0.0336 ± 0.0179
cxcl14	0.2865 ± 0.0519	0.3090 ± 0.1050	0.0020 ± 0.0011
cxcr1	0.0008 ± 0.0007	0.0002 ± 0.00009	0.0130 ± 0.00581
cxcr2	0.00038 ± 0.0001	0.0002 ± 0.00006	0.0035 ± 0.00151
cxcr3	0.0466 ± 0.01131	0.0145 ± 0.0062	0.0049 ± 0.0020
cxcr4	0.0157 ± 0.00641	0.0038 ± 0.001	0.0599 ± 0.0350

organs (head kidney, trunk kidney, spleen, thymus) and tissues associated with the immune response such as gills, gut and skin (Abdelkhalek et al., 2009; Huising et al., 2004; van der Aa et al., 2012a; Verburg-van Kemenade et al., 2017).

It is striking that the expression of CXCL12a was very high, not only in the head kidney, but also in the NPO (14-fold and two-fold the gene expression of the 40s11 ribosomal protein, respectively). While the ratio of CXCL12a expression was highest in head kidney over the NPO, the highest expression of the CXCL12b gene was measured in the NPO. The putative CXCL12 receptor CXCR4 showed the highest gene expression in the head kidney, although its expression in the NPO was not negligible. Moreover, the second homeostatic CXC chemokine – CXCL14, showed high gene expression in the NPO and the pituitary and a very low expression rate in the head kidney. Similar results indicating high constitutive expression of the CXCL12a gene in the brain of common carp, were previously obtained by Huising et al. (2004). However, in this study chemokine expression was measured in the whole brain tissue, not in the isolated NPO and pituitary. In the same study, constitutive expression of CXCL12b and CXCL14 was confirmed for brain. Moreover, in rats, constitutive expression of CXCL12 was found in the neurohypophysis, adenohypophysis and hippocampus (Rostène et al., 2011). CXCL12 has been identified mostly, but not exclusively, in ACTH-expressing pituitary cells (Barbieri et al., 2007; Rostène et al., 2011) and therefore it has been hypothesized that the CXCR4/CXCL12 axis contributes to the paracrine regulation of pituitary hormone secretion.

It must be mentioned that disruption of either the CXCR4 or the CXCL12 gene in mice, causes lethality during late gestation and that mutant embryos have a number of defects including failure of hematopoietic colonization in the bone marrow and abnormal migration of cerebellar neurons [e.g. Miller et al., 2008]. CXCL12 and CXCR4 were moreover found to be preferably associated with the regulation of the movement of less differentiated cells than with the chemotaxis of any particular cell type. For example, in adult rodents and humans, CXCR4 is unique among chemokine receptors by functioning in the most undifferentiated hematopoietic precursors [for review see Kim and Broxmeyer, 1999] and CXCR4 and CXL12 are critical factors supporting quiescence and bone marrow retention of hematopoietic stem cells (Zhang et al., 2016). In carp, the high expression of the CXCL12a and CXCR4 genes in the head kidney, in fish the homolog of the mammalian bone marrow, indicates a similar, evolutionary conserved, function. Furthermore, during later stages of embryonic development in mice, CXCR4 expression was observed in the germinal layer of the neuronal system, that lines the ventricular space (McGrath et al., 1999). It is this cell layer that contains the neuronal stem cells, capable of multipotential regeneration (Johansson et al., 1999). CXCR4 was also found in the developing hypothalamus and thalamus of mice (McGrath et al., 1999). Moreover, both in mice and in zebrafish, expression of CXCR4 in the hypothalamus was required for CXCL12-directed migration of gonadotropin-releasing hormone (GnRH) neurons from the olfactory system to the hypothalamus and regulation of reproduction (Palevitch et al., 2010; Schwarting et al., 2006; Toba et al., 2008).

In carp, high constitutive expression of CXCL12a, CXCL12b and CXCR4 receptor in the NPO and pituitary, implies a role in proper functioning of the neuroendocrine system.

CXCL14 is the second ancient “homeostatic” chemokine of which orthologs have been cloned in humans, rodents, amphibians and in fish (Huising et al., 2004; Huising and Stet, 2003). In contrast to the very large number of papers published on the effects of CXCL12, very little is known about CXCL14. This may partly be due to the fact that CXCL14 is one of the few chemokines for which its receptor has not yet been identified. Here we found higher *cxcl14* expression in the NPO and pituitary than in the head kidney (Table 1). Also, in mammals the central nervous system was indicated as a major site of CXCL14 expression (Hromas et al., 1999; Park et al., 2009; Sleeman et al., 2000). However only a single publication has dealt with the localization of CXCL14 in the brain in any detail. These results suggested that, as for CXCL12, neurons may be the primary cells expressing CXCL14 in the brain (Schmid et al., 2009). Moreover, as is the case for CXCL12, it was suggested that CXCL14 may play a role in neural development (Li and Ransohoff, 2008; Tran and Miller, 2003), although neural defects have not been reported to date in CXCL14 knockout mice (Meuter et al., 2007; Nara et al., 2007).

Next to the homeostatic CXC chemokines, in the NPO and pituitary we also observed constitutive expression of genes encoding pro-inflammatory CXC8 and CXCb chemokines and their putative receptors: CXCR1-2 and CXCR3, respectively. However, expression levels of these genes did not approach those of *cxcl12* or *cxcl14*. Interestingly, both *cxcb* genes showed the highest expression in the NPO and expression of *cxcb2* was also high in the pituitary. In accordance to these data, the expression profile of the putative CXCb receptor – CXCR3 was highest in the NPO, slightly lower in the pituitary and lowest in the head kidney (Table 1). In contrast, previous studies did not show expression of CXCL8_L1 (CXCa) and CXCb1 mRNA in the whole brain tissue (Abdelkhalek et al., 2009; Huising et al., 2004), while expression of CXCR3 in this tissue was comparable to this observed in the head kidney (Chadzinska et al., 2014). CXCR1 and CXCR2 gene expression in the whole brain of common carp was significantly lower than that measured in the head kidney (Huising and Stolte, 2003). Similar studies in rodents found constitutive expression of the CXCL8 gene in the paraventricular nucleus and in the hippocampus (Philip and Gold, 1992). Moreover, constitutive expression of the CXCL8 receptor - CXCR2 was identified in human pituitary cells (Tecimer et al., 2000). But CXCL8 is not the only CXCR2 ligand that may be involved in the regulation of the pituitary function. For example, cytokine-induced neutrophil chemoattractant CINC (the rat counterpart of the human CXCL1 chemokine, a member of the interleukin-8 family) was reported to be expressed in the PVN and the posterior pituitary in rat (Sakamoto et al., 1996). Moreover, Sawada et al. (1994a,b) found that in pituitary cells *in vitro,* this chemokine up-regulated the secretion of IL-6 and prolactin, while it suppressed the basal secretion of luteinizing hormone and follicle-stimulating hormone.

As mentioned before, in teleosts, CXCb chemokines are related to mammalian the IFN-γ-inducible CXCL9-11 chemokines. Recent studies confirmed that also CXCL10 is expressed in dendritic cells in the anterior lobe of the rat pituitary gland, while its receptor CXCR3 is expressed in ACTH-producing cells of this organ (Horiguchi et al., 2014).

In the present study, stress did not affect gene expression of CXCL8_L1 and L2, CXCL12b and CXCR1-3 receptors (data not shown) in the stress axis organs (NPO, pituitary, head kidney) but it reduced expression of CXCb genes in all stress axis organs (Fig. 1A and B). Moreover, stress reduced the expression of the *cxcl12a* (Fig. 1C) and

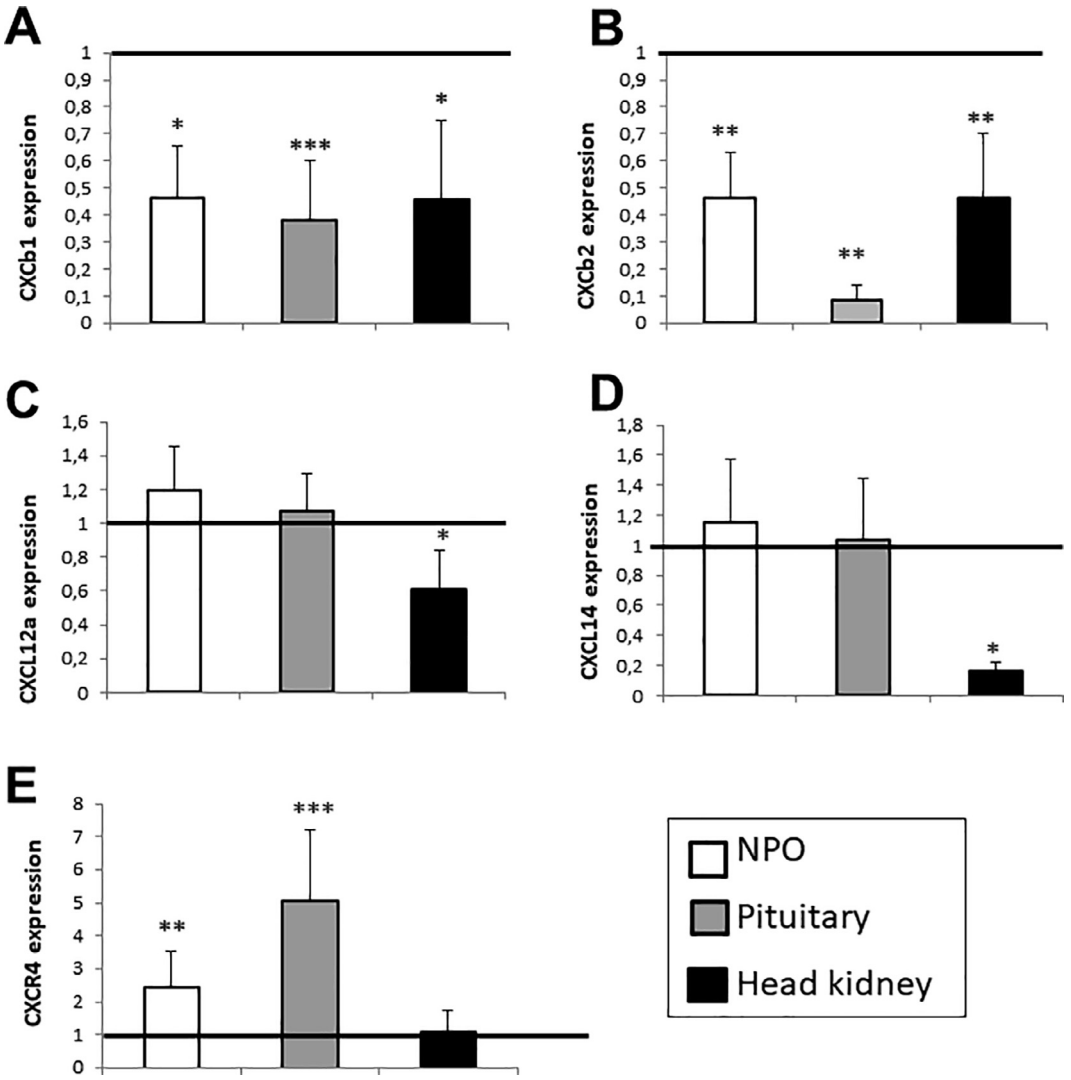

Fig. 1. Restraint (24 h) stress-induced changes in the expression of CXC ligands (A-D) and CXCR4 receptor (E) in the nucleus preopticus (NPO), pituitary gland (PT) and head kidney (HK). Gene expression was determined by quantitative RT-PCR and expressed as fold change between the level of expression in control (solid line = 1) and the experimental samples and standardized for the housekeeping gene 40S ribosomal protein s11 expression. Averages and SD (n = 7–9). Asterisks indicate statistically significant differences (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$) between control unstressed and stressed animals.

cxcl14 (Fig. 1D) genes in the head kidney.

Most probably, stress-induced reduction of gene expression of CXCb, CXCL12a and CXC14 in the head kidney is related to changes in leukocyte redistribution. As shown previously, stress induces a decrease of neutrophils in the head kidney which is associated with their migration into circulation [e.g. Pijanowski et al., 2015]. Moreover, we indicated that, next to monocytes/macrophages and lymphocytes, the head kidney granulocytes may be the source of both CXCb chemokines (van der Aa et al., 2012a). Therefore stress-induced massive reduction of the number of neutrophils in the head kidney is the cause of a significantly lower expression of CXC chemokines in this organ.

Interestingly, in NPO and pituitary, restraining down-regulates the gene expression of both CXCb chemokines, while it up-regulates the expression of the CXCR4 receptor (Fig. 1A, B and E).

According to our knowledge this is the first evidence of stress-induced changes in gene expression of the CXC chemokines and their receptors in teleost fish. Before, the implication of chemokines in the stress responses was reported for rodents. For example, during immobilization stress CXCL1 mRNA expression increased in the parvocellular and magnocellular subdivision of the PVN, but not in the supraoptic nucleus. Moreover, in response to immobilization stress the immunostaining intensity of CXCL1 raised in the posterior pituitary together with an increase in serum levels of CXCL1. This suggests that, CXCL1, synthesized in the PVN, could be axonally transported via the median eminence and released into the peripheral blood from the hypothalamo-neurohypophysial system (Sakamoto et al., 1996). Furthermore, secretion of CXCL8, the second CXCR2 ligand, is under control of steroids through a feedback mechanism. This supports a physiological role of this chemokine in the regulation of ACTH secretion (Philip and Gold, 1992). Most importantly, Horiguchi and co-workers (Horiguchi et al., 2016) found that ligands of CXCR3 may be involved in the regulation of the stress response as *in vitro,* the CXCR3 agonist significantly stimulated the expression of proopiomelanocortin and inhibited the CRF-induced ACTH release. Therefore, the stress-induced down-regulation of the CXCL-9-11-like *cxcb* expression that we now found in carp, reveals that also in fish, IFN-γ-inducible chemokines and their receptors may regulate the stress response.

Altogether, our results imply that in teleost fish, CXC chemokines and their receptors also function in neuroendocrine regulation, thus revealing a phylogenetical conservation of this phenomenon. The active regulation of their expression in stress axis organs during periods of restraint stress indicates a noteworthy role in the stress response.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.05.004.

References

Abdelkhalek, N.K., Komiya, A., Kato-Unoki, Y., Somamoto, T., Nakao, M., 2009. Molecular evidence for the existence of two distinct IL-8 lineages of teleost CXC-chemokines. Fish Shellfish Immunol. 27 (6), 763–767. https://doi.org/10.1016/j.fsi.2009.08.004E.

Allen, S.J., Crown, S., Handel, T.M., 2007. Chemokine: receptor structure, interactions, and antagonism. Annu. Rev. Immunol. 25, 787–820.

Barbieri, F., Bajetto, A., Porcile, C., Pattarozzi, A., Schettini, G., Florio, T., 2007. Role of stromal cell-derived factor 1 (SDF1/CXCL12) in regulating anterior pituitary function. J. Mol. Endocrinol. 38 (3), 383–389.

Chadzinska, M., Golbach, L., Pijanowski, L., Scheer, M., Verburg-van Kemenade, B.M., 2014. Characterization and expression analysis of an interferon-γ2 induced chemokine receptor CXCR3 in common carp (Cyprinus carpio L.). Dev. Comp. Immunol. 47 (1), 68–76. https://doi.org/10.1016/j.dci.2014.07.008.

Gerard, C., Rollins, B.J., 2001. Chemokines and disease. Nat. Immunol. 2 (2), 108–115.

Grizzi, F., Borroni, E.M., Vacchini, A., Qehajaj, D., Liguori, M., Stifter, S., Chiriva-Internati, M., Di Ieva, A., 2015. Pituitary adenoma and the chemokine network: a systemic view. Front. Endocrinol. (Lausanne) 6, 141. https://doi.org/10.3389/fendo.2015.00141.

Harper, C., Wolf, J.C., 2009. Morphologic effects of the stress response in fish. ILAR J. 50, 387–396.

Horiguchi, K., Fujiwara, K., Tsukada, T., Yoshida, S., Higuchi, M., Tateno, K., Hasegawa, R., Takigami, S., Ohsako, S., Yashiro, T., Kato, T., Kato, Y., 2016. CXCL10/CXCR3 signaling mediates inhibitory action by interferon-gamma on CRF-stimulated adrenocorticotropic hormone (ACTH) release. Cell Tissue Res. 364 (2), 395–404. https://doi.org/10.1007/s00441-015-2317-2.

Horiguchi, K., Fujiwara, K., Higuchi, M., Yoshida, S., Tsukada, T., Ueharu, H., Chen, M., Hasegawa, R., Takigami, S., Ohsako, S., Yashiro, T., Kato, T., Kato, Y., 2014. Expression of chemokine CXCL10 in dendritic-cell-like S100β-positive cells in rat anterior pituitary gland. Cell Tissue Res. 357 (3), 757–765. https://doi.org/10.1007/s00441-014-1864-2.

Hromas, R., Broxmeyer, H.E., Kim, C., Nakshatri, H., Christopherson 2nd, K., Azam, M., Hou, Y.H., 1999. Cloning of BRAK, a novel divergent CXC chemokine preferentially expressed in normal versus malignant cells. Biochem. Biophys. Res. Commun. 255 (3), 703–706.

Huising, M.O., van der Meulen, T., Flik, G., Verburg-van Kemenade, B.M., 2004. Three novel carp CXC chemokines are expressed early in ontogeny and at nonimmune sites. Eur. J. Biochem. 271 (20), 4094–4106.

Huising, M.O., Stolte, E., Flik, G., Savelkoul, H.F., Verburg-van Kemenade, B.M., 2003. CXC chemokines and leukocyte chemotaxis in common carp (Cyprinus carpio L.). Dev. Comp. Immunol. 27 (10), 875–888.

Huising, M.O., Stet, R.J., Kruiswijk, C.P., Savelkoul, H.F., Lidy Verburg-van Kemenade, B.M., 2003. Molecular evolution of CXC chemokines: extant CXC chemokines originate from the CNS. Trends Immunol. 24 (6), 307–313.

Johansson, C.B., Svensson, M., Wallstedt, L., Janson, A.M., Frisén, J., 1999. Neural stem cells in the adult human brain. Exp Cell Res. 253 (2), 733–736.

Kim, C.H., Broxmeyer, H.E., 1999. Chemokines: signal lamps for trafficking of T and B cells for development and effector function. J. Leukoc. Biol. 65 (1), 6–15.

Laing, K.J., Zou, J.J., Wang, T., Bols, N., Hirono, I., Aoki, T., Secombes, C.J., 2002. Identification and analysis of an interleukin 8-like molecule in rainbow trout Oncorhynchus mykiss. Dev. Comp. Immunol. 26 (5), 433–444.

Li, M., Ransohoff, R.M., 2008. Multiple roles of chemokine CXCL12 in the central nervous system: a migration from immunology to neurobiology. Prog. Neurobiol. 84 (2), 116–131. https://doi.org/10.1016/j.pneurobio.2007.11.003.

Licinio, J., Wong, M.L., Gold, P.W., 1992. Neutrophil-activating peptide1/interleukin-8 mRNA is localized in rat hypothalamus and hippocampus. Neuroreport 3 (9), 753–756. https://doi.org/10.1097/00001756-199209000-00008. PMID: 1421131.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25 (4), 402–408.

McGrath, K.E., Koniski, A.D., Maltby, K.M., McGann, J.K., Palis, J., 1999. Embryonic expression and function of the chemokine SDF-1 and its receptor, CXCR4. Dev Biol. 213 (2), 442–456.

Metz, J.R., Huising, M.O., Leon, K., Verburg-van Kemenade, B.M., Flik, G., 2006. Central and peripheral interleukin-1beta and interleukin-1 receptor I expression and their role in the acute stress response of common carp Cyprinus carpio L. J. Endocrinol. 191 (1), 25–35.

Meuter, S., Schaerli, P., Roos, R.S., Brandau, O., Bösl, M.R., von Andrian, U.H., Moser, B., 2007. Murine CXCL14 is dispensable for dendritic cell function and localization within peripheral tissues. Mol. Cell Biol. 27 (3), 983–992.

Miller, R.J., Banisadr, G., Bhattacharyya, B.J., 2008. CXCR4 signaling in the regulation of stem cell migration and development. J. Neuroimmunol. 198 (1–2), 31–38. https://doi.org/10.1016/j.jneuroim.2008.04.008.

Nara, N., Nakayama, Y., Okamoto, S., Tamura, H., Kiyono, M., Muraoka, M., Tanaka, K., Taya, C., Shitara, H., Ishii, R., Yonekawa, H., Minokoshi, Y., Hara, T., 2007. Disruption of CXC motif chemokine ligand-14 in mice ameliorates obesity-induced insulin resistance. J. Biol. Chem. 282 (42), 30794–30803.

Nomiyama, H., Hieshima, K., Osada, N., Kato-Unoki, Y., Otsuka-Ono, K., Takegawa, S., Izawa, T., Yoshizawa, A., Kikuchi, Y., Tanase, S., Miura, R., Kusuda, J., Nakao, M., Yoshie, O., 2008. Extensive expansion and diversification of the chemokine gene family in zebrafish: identification of a novel chemokine subfamily CX. BMC Genomics 9, 222. https://doi.org/10.1186/1471-2164-9-222.

Olivotto, I., Mosconi, G., Maradonna, F., Cardinali, M., Carnevali, O., 2002. Diplodus sargus interrenal-pituitary response: chemical communication in stressed fish. Gen. Comp. Endocrinol. 127, 66–70.

Palevitch, O., Abraham, E., Borodovsky, N., Levkowitz, G., Zohar, Y., Gothilf, Y., 2010. Cxcl12a-Cxcr4b signaling is important for proper development of the forebrain GnRH system in zebrafish. Gen. Comp. Endocrinol. 165 (2), 262–268. https://doi.org/10.1016/j.ygcen.2009.07.001.

Park, B.Y., Hong, C.S., Sohail, F.A., Saint-Jeannet, J.P., 2009. Developmental expression and regulation of the chemokine CXCL14 in Xenopus. Int. J. Dev. Biol. 53 (4), 535–540. https://doi.org/10.1387/ijdb.092855bp.

Piccinetti, C.C., Grasso, L., Maradonna, F., Radaelli, G., Ballarin, C., Chemello, G., Evjemo, J.E., Carnevali, O., Olivotto, I., 2017. Growth and stress factors in ballan wrasse (Labrusbergylta) larval development. Aquac. Res. 48, 2567–2580.

Pijanowski, L., Jurecka, P., Irnazarow, I., Kepka, M., Szwejser, E., Verburg-van Kemenade, B.M., Chadzinska, M., 2015. Activity of the hypothalamus-pituitary-interrenal axis (HPI axis) and immune response in carp lines with different susceptibility to disease. Fish Physiol. Biochem. 41 (5), 1261–1278. https://doi.org/10.1007/s10695-015-0084-3.

Rossi, D., Zlotnik, A., 2000. The biology of chemokines and their receptors. Annu. Rev. Immunol. 18, 217–242.

Rostène, W., Guyon, A., Kular, L., Godefroy, D., Barbieri, F., Bajetto, A., Banisadr, G., Callewaere, C., Conductier, G., Rovère, C., Mélik-Parsadaniantz, S., Florio, T., 2011. Chemokines and chemokine receptors: new actors in neuroendocrine regulations. Front. Neuroendocrinol. 32 (1), 10–24. https://doi.org/10.1016/j.yfrne.2010.07.001.

Sakamoto, Y., Koike, K., Kiyama, H., Konishi, K., Watanabe, K., Tsurufuji, S., Bicknell, R.J., Hirota, K., Miyake, A., 1996. A stress-sensitive chemokinergic neuronal pathway in the hypothalamo-pituitary system. Neuroscience 75 (1), 133–142.

Sawada, T., Koike, K., Kanda, Y., Sakamoto, Y., Nohara, A., Ohmichi, M., Hirota, K., Miyake, A., 1994a. In vitro effects of CINC/gro, a member of the interleukin-8 family, on hormone secretion by rat anterior pituitary cells. Biochem. Biophys. Res. Commun. 202 (1), 155–160.

Sawada, T., Koike, K., Sakamoto, Y., Nohara, A., Kanda, Y., Zhang, Z.X., Ohmichi, M., Yamaguchi, M., Hirota, K., Miyake, A., 1994b. In vitro effects of CINC/gro, a member of the interleukin-8 family, on interleukin-6 secretion by rat posterior pituitary cells. Biochem. Biophys. Res. Commun. 200 (2), 742–748.

Schmid, C.D., Melchior, B., Masek, K., Puntambekar, S.S., Danielson, P.E., Lo, D.D., Sutcliffe, J.G., Carson, M.J., 2009. Differential gene expression in LPS/IFNgamma activated microglia and macrophages: in vitro versus in vivo. J. Neurochem. 109 (Suppl 1), 117–125. https://doi.org/10.1111/j.1471-4159.2009.05984.x.

Schwarting, G.A., Henion, TR., Nugent, JD., Caplan, B., Tobet, S., 2006. Stromal cell-derived factor-1 (chemokine C-X-C motif ligand 12) and chemokine C-X-C motif receptor 4 are required for migration of gonadotropin-releasing hormone neurons to the forebrain. J. Neurosci. 26 (25), 6834–6840.

Sleeman, M.A., Fraser, J.K., Murison, J.G., Kelly, S.L., Prestidge, R.L., Palmer, D.J., Watson, J.D., Kumble, K.D., 2000. B cell- and monocyte-activating chemokine (BMAC), a novel non-ELR alpha-chemokine. Int. Immunol. 12 (5), 677–689.

Ślusarczyk, J., Trojan, E., Chwastek, J., Głombik, K., Basta-Kaim, A., 2016. A Potential contribution of chemokine network dysfunction to the depressive disorders. Curr. Neuropharmacol. 14 (7), 705–720.

Stuart, M.J., Singhal, G., Baune, B.T., 2015. Systematic review of the neurobiological relevance of chemokines to psychiatric disorders. Front. Cell Neurosci. 9, 357. https://doi.org/10.3389/fncel.2015.00357.

Tecimer, T., Dlott, J., Chuntharapai, A., Martin, A.W., Peiper, S.C., 2000. Expression of the chemokine receptor CXCR2 in normal and neoplastic neuroendocrine cells. Arch. Pathol. Lab. Med. 124 (4), 520–525.

Toba, Y., Tiong, J.D., Ma, Q., Wray, S., 2008. CXCR4/SDF-1 system modulates development of GnRH-1 neurons and the olfactory system. Dev. Neurobiol. 68 (4), 487–503. https://doi.org/10.1002/dneu.20594.

Tran, P.B., Miller, R.J., 2003. Chemokine receptors: signposts to brain development and disease. Nat. Rev. Neurosci. 4 (6), 444–455.

van der Aa, L.M., Chadzinska, M., Derks, W., Scheer, M., Levraud, J.P., Boudinot, P., Lidy Verburg-van Kemenade, B.M., 2012a. Diversification of IFNγ-inducible CXCb chemokines in cyprinid fish. Dev. Comp. Immunol. 38 (2), 243–253. https://doi.org/10.1016/j.dci.2012.05.005.

van der Aa, L.M., Chadzinska, M., Golbach, L.A., Ribeiro, C.M., Lidy Verburg-van Kemenade, B.M., 2012b. Pro-inflammatory functions of carp CXCL8-like and CXCb chemokines. Dev. Comp. Immunol. 36 (4), 741–750. https://doi.org/10.1016/j.dci.2011.11.011.

van der Aa, L.M., Chadzinska, M., Tijhaar, E., Boudinot, P., Verburg-van Kemenade, B.M., 2010. CXCL8 chemokines in teleost fish: two lineages with distinct expression profiles during early phases of inflammation. PLoS One 5 (8), e12384. https://doi.org/10.1371/journal.pone.0012384.

Verburg-van Kemenade, B.M.L., Cohen, N., Chadzinska, M., 2017. Neuroendocrine-

immune interaction: evolutionarily conserved mechanisms that maintain allostasis in an ever-changing environment. Dev Comp Immunol. 66, 2–23. https://doi.org/10.1016/j.dci.2016.05.015.

Verburg-van Kemenade, B.M., Van der Aa, L.M., Chadzinska, M., 2013. Neuroendocrine-immune interaction: regulation of inflammation via G-protein coupled receptors. Gen. Comp. Endocrinol. 188, 94–101. https://doi.org/10.1016/j.ygcen.2012.11.010.

Wendelaar Bonga, S.E., 1997. The stress response in fish. Physiol. Rev. 77, 591–625.

Zhang, Y., Dépond, M., He, L., Foudi, A., Kwarteng, E.O., Lauret, E., Plo, I., Desterke, C., Dessen, P., Fujii, N., Opolon, P., Herault, O., Solary, E., Vainchenker, W., Joulin, V., Louache, F., Wittner, M., 2016. CXCR4/CXCL12 axis counteracts hematopoietic stem cell exhaustion through selective protection against oxidative stress. Sci. Rep. 6, 37827. https://doi.org/10.1038/srep37827.

Zlotnik, A., Yoshie, O., Nomiyama, H., 2006. The chemokine and chemokine receptor superfamilies and their molecular evolution. Genome Biol. 7, 243.